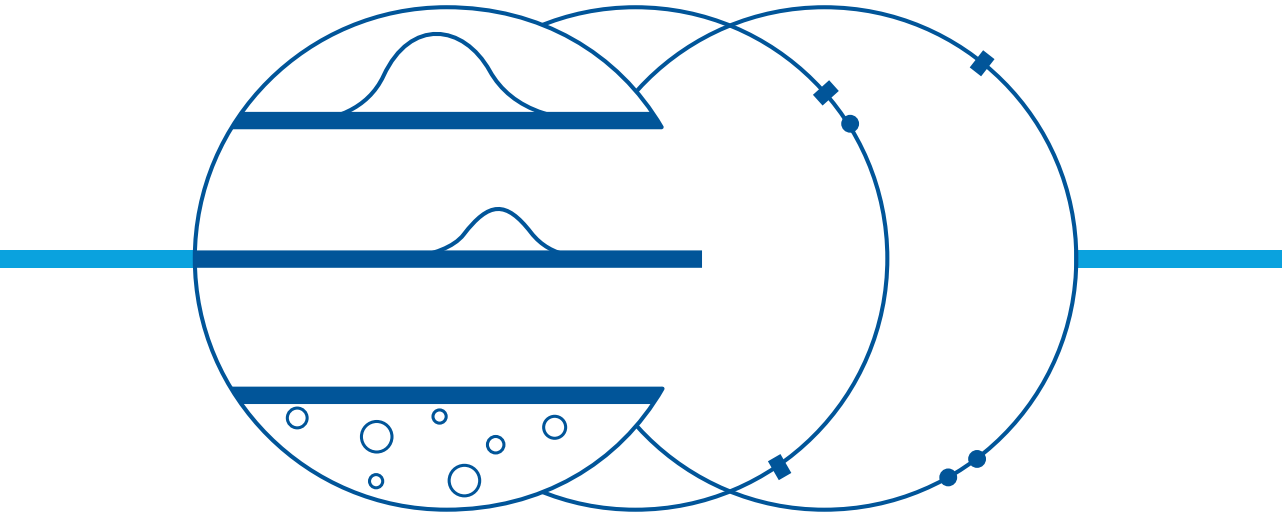
Central serous chorioretinopathy : from pathogenesis to treatment

Dijk, E.H.C. van

CENTRAL SEROUS CHORIORETINOPATHY: FROM PATHOGENESIS TO TREATMENT



ELON HENDRIK CORNELIS VAN DIJK

CENTRAL SEROUS CHORIORETINOPATHY: FROM PATHOGENESIS TO TREATMENT

by
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ABBREVIATIONS

aCSC	acute central serous chorioretinopathy
ACTH	adrenocorticotropin
AE	adverse event
AMD	age-related macular degeneration
ANA	antinuclear antibody
AP50	alternative pathway activity
ARA	antiretinal antibody
ARFGEF1	adenosine diphosphate ribosylation factor guanine nucleotide exchange factor 1
ARMS2	age-related macular degeneration susceptibility 2
AS	Apathy Scale
BCVA	best-corrected visual acuity
BM	Bruch's membrane
BMI	body mass index
bp	base pair
C4B	complement factor 4B
cCSC	chronic central serous chorioretinopathy
CD46	cluster of differentiation 46
CDH5	cadherin 5
CEC	choroidal endothelial cell
CFH	complement factor H
CFHR1/4	complement factor H related 1/4
chr	chromosome
CI	confidence interval
CM	cutaneous melanoma
CNV	choroidal neovascularisation
CP50	classical pathway activity
CRT	central retinal thickness
CS	Cushing's syndrome
CSC	central serous chorioretinopathy
CT	choroidal thickness
DAPP-SF	Dimensional Assessment of Personality Pathology short form
DARA	diffuse atrophic retinal pigment epithelium alterations
DSMB	Data Safety Monitoring Board
EDI	enhanced-depth imaging
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
ELM	external limiting membrane
EOG	electro-oculography

EPACTS	efficient and parallelizable association container toolbox
eQTL	expression quantative trait locus
ERG	electroretinography
ERM	epiretinal membrane
ETDRS	Early Treatment of Diabetic Retinopathy Study
EZ	ellipsoid zone
FA	fluorescein angiography
FAF	fundus autofluorescence
FITC	fluorescein isothiocyanate
Freq	frequency
GATA5	GATA binding protein 5
GCL	ganglion cell layer
GEE	generalised estimating equations
GR	glucocorticoid receptor
GTEx	genotype-tissue expression
GTP	guanosine triphosphate
GWAS	genome-wide association study
Hg	human genome
HPA	hypothalamic-pituitary-adrenal
HRC	haplotype reference consortium
HSML	high-density subthreshold micropulse laser
ICGA	indocyanine green angiography
IIF	indirect immunofluorescence
ILM	internal limiting membrane
INL	inner nuclear layer
IPL	inner plexiform layer
IRR	infrared reflectance
IS	Irritability Scale
ISCEV	International Society for Clinical Electrophysiology of Vision
IZ	interdigitation zone
KCNT2	potassium sodium-activated channel subfamily t member 2
LP50	mannose-binding lectin pathway
MAF	minor allele frequency
MAGMA	multi-marker analysis of genomic annotation
MAPK	mitogen-activated protein kinase
MEGF6	multiple epidermal growth factor like domains 6
MEK	mitogen-activated protein kinase kinase
mir-29b-2	micro ribonucleic acid 29b-2
MR	mineralocorticoid receptor
mSC	midnight salivary cortisol

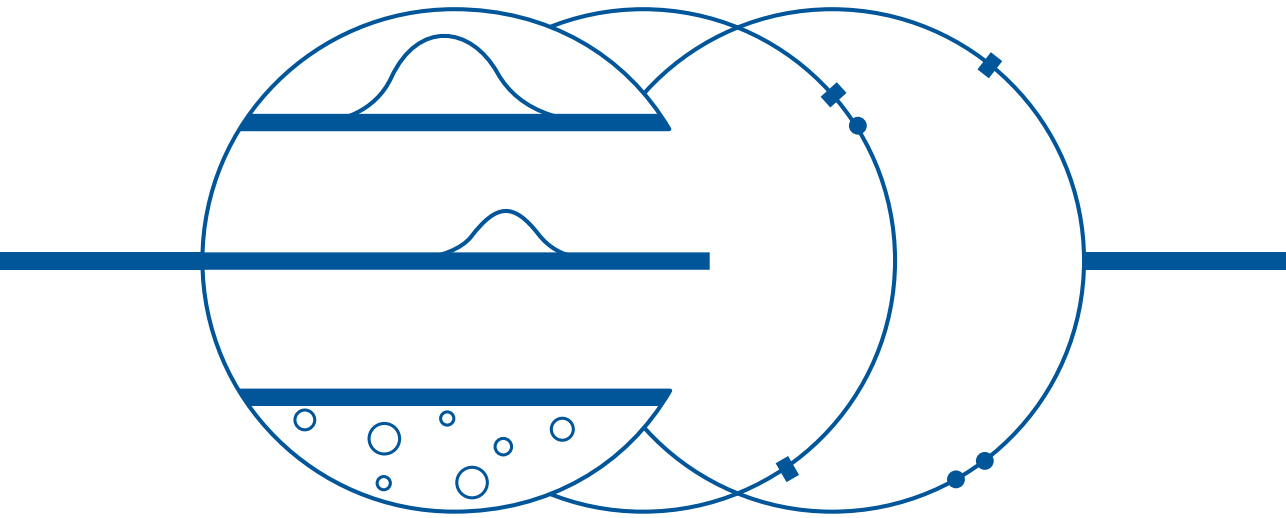
MSigDB	molecular signatures database
NA	not annotated
nAMD	neovascular age-related macular degeneration
NBS	Nijmegen Biomedical Study
NEI-VFQ25	National Eye Institute Visual Function Questionnaire
NR3C2	nuclear receptor subfamily 3 group C member 2
NS	not significant
OCT	optical coherence tomography
ONL	outer nuclear layer
OPL	outer plexiform layer
OR	odds ratio
OS	outer segments
PA	primary aldosteronism
PBS	phosphate-buffered saline
PCRD	posterior cystoid retinal degeneration
PCV	polypoidal choroidal vasculopathy
PDT	photodynamic therapy
PED	pigment epithelial detachment
PITPNC1	phosphatidylinositol transfer protein cytoplasmic 1
PPE	pachychoroid pigment epitheliopathy
PSS	Perceived Stress Scale
RAF	rapidly accelerated fibrosarcoma
RAT	rat sarcoma
RORA	retinoic acid receptor-related orphan receptor alpha
RPE	retinal pigment epithelium
RVO	retinal vein occlusion
SAE	serious adverse event
SBT	second band thickness
SD	standard deviation
SD-OCT	spectral-domain optical coherence tomography
SE	standard error
SFCT	subfoveal choroidal thickness
SNP	single nucleotide polymorphism
SRF	subretinal fluid
TBS	tris-buffered saline
TNFRSF10A	tumor necrosis factor receptor superfamily member 10a
UCS	Utrecht Coping Scale
UFC	urinary free cortisol
UM	uveal melanoma
UTR	untranslated region

VEGAS2	versatile gene-based association study 2
VEGF	vascular endothelial growth factor

CONTENTS

1

INTRODUCTION



THE HUMAN EYE

The eye provides organisms the ability to process visual details. After the entering of light through the cornea, the lens focusses the light waves on the central retina (Figure 1). In the photosensitive cells of the retina, the photoreceptors, this initiates a cascade of chemical and electrical events – the phototransduction cascade, which eventually leads to formation of nerve impulses. These impulses are sent to the brain through the fibers of the optic nerve, which results in visual images and interpretation.

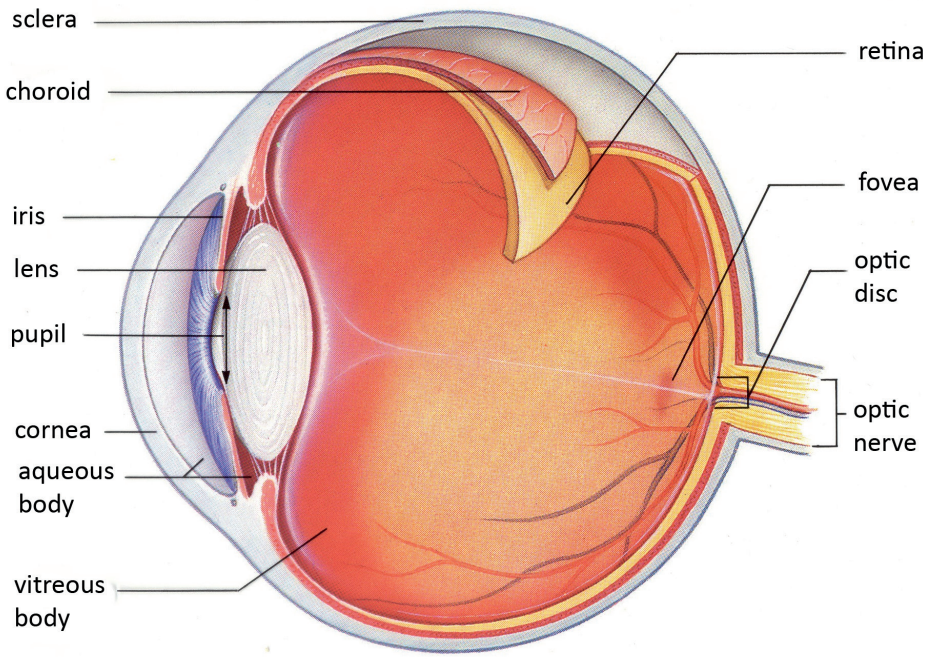


Figure 1. Anatomy of the human eye

Neurosensory retina

The neurosensory retina has a complex structure, and its different neuronal layers are cross-connected by synapses and supporting cells. Eight layers, that are arranged in a highly organised manner, can be distinguished (Figure 2). The outer nuclear layer (ONL) consists of the nuclei of the photoreceptors, which are the light-sensitive cells that absorb photons, which leads to a change in their cell membrane potential. This electrochemical signal is propagated by the cell membrane. Communication with higher-order cellular structures from the retina is induced via synapses, and the information is sent to the visual cortices, via interneurons and the optic nerve. In the visual cortices, the simple visual input is processed into complex visual images and their interpretation. The interconnection between the ONL and the inner nuclear

layer (INL), in which the cell bodies of amacrine, bipolar, horizontal, and Müller cells can be found, is described as the outer plexiform layer (OPL). The ganglion cell layer (GCL) contains the nuclei of the ganglion cells, and is interconnected with the INL with synapses of the inner plexiform layer (IPL).

The external limiting membrane (ELM) is formed by junctional complexes between Müller cells and photoreceptors, whereas the internal limiting membrane (ILM) is the layer of the retina that is closest to the vitreous cavity. The nerve fiber layer lies beneath the ILM. The blood vessels of the central retinal artery deliver blood to the inner layers of the retina, via an extensive network of capillaries from the nerve fiber layer to the INL. The inner blood-retina barrier is formed by the tight junctions of the endothelial cells in the retinal vessels, which is important to maintain retinal homeostasis.

Until now, 3 types of photoreceptors have been identified: rods, cones, and photosensitive retinal ganglion cells.[1] Three types of cone photoreceptors are responsible for vision in bright light: the S ('blue') cones, the M ('green'), and the L ('red') cones, which are most sensitive to short, medium, and long wavelength light, respectively.[2, 3] Rods enable contrast vision and vision in dim light. In the macula (or *macula lutea*, yellow spot), which is the central part of the retina and is encircled by the temporal vascular arcades, both rods and cones can be found. Impaired function of this part of the retina generally leads to blurred vision and loss of color and contrast vision. The center of this area with a diameter of 5-6 mm is the 1.5 mm wide fovea (or *fovea centralis*), in which only cones are present. In the foveal center with a diameter of 0.35 mm (*foveola*) and the highest concentration of cones, the INL and the GCL are laterally displaced, leading to a depression: the foveal pit.[4, 5] In the peripheral retina, mainly rods can be found.[6]

Retinal pigment epithelium

The retinal pigment epithelium (RPE) has several functions. Melanin in the RPE protects the retina by the absorption of scattered light.[8] The tight junctions of the lateral surfaces of the RPE cells form the outer blood-retina barrier, which together with the inner blood-retina barrier prevent unwanted sub- and intraretinal fluid leakage, and closely regulate the transport of ions and metabolites between choriocapillaris and neuroretina, and vice versa. The RPE also produces factors such as vascular endothelial growth factor (VEGF), complement-associated factors, and tissue inhibitor of metalloproteinase-3, that are involved in numerous processes in human body.[9, 10] This layer is also involved in maintenance of the extracellular matrix and coordination of the immune response.[11]

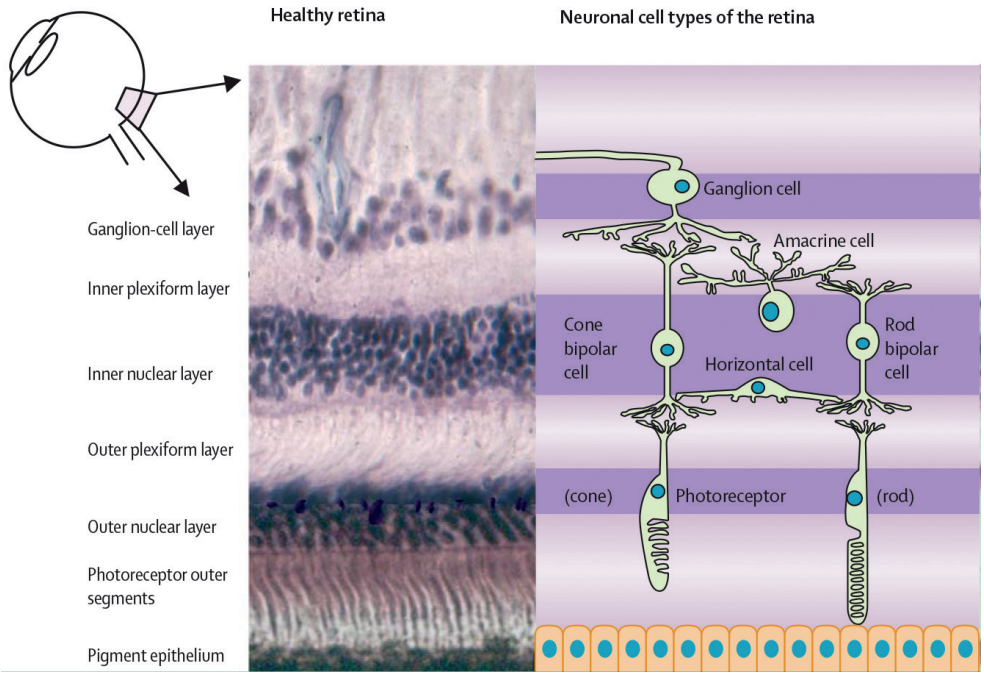


Figure 2. Schematic presentation of the human retinal layers (Hartong DT et al, 2006[7])

Moreover, there is a close anatomical and functional relationship between the RPE monolayer and the photoreceptors: the distal photoreceptor outer segments are surrounded by a large number of RPE microvilli, which facilitates the regeneration of the rod and cone outer segments by the RPE.

Choroid

Bruch's membrane is considered the innermost layer of the choroid, and beneath this membrane the choriocapillaris, 2 vascular layers, and the suprachoroid can be found (Figure 3). These 5 layers are all part of the choroid, and consist of blood vessels, fibroblasts, immune competent cells, melanocytes, and supporting tissue.[12] Bruch's membrane is involved in the regulation of molecule exchange between retina and general circulation, and is rich in elastin and collagen. The Bruch's membrane thickens with age and is involved in the onset and progression of several diseases such as age-related macular degeneration (AMD).[13, 14] The choriocapillaris contains a dense capillary network, whereas small feeding arteries and arterioles (Sattler's layer) and larger vessels (Haller's layer) can be detected in the 2 vascular layers beneath. The suprachoroid sets the boundary of the choroid.[12]

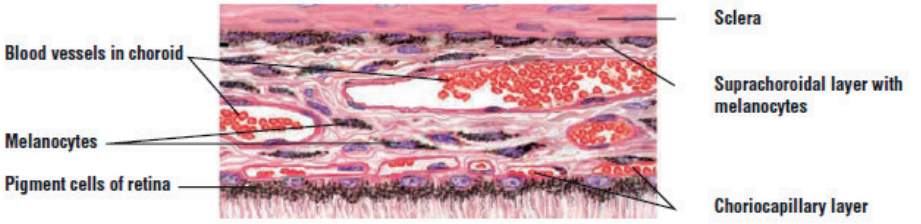


Figure 3. Schematic presentation of the human choroidal layers

The fenestration of the capillaries of the choriocapillaris allows the diffusion of small molecules, whereas the underlying larger choroidal blood vessels of Haller's and Sattler's layer do not have these fenestration. The choriocapillary vascular fenestrations allow fluorescein, used during fluorescein angiography (FA), to diffuse into the extravascular space, while the opposite holds for larger molecules such as indocyanine green.[15, 16]

To be able to provide sufficient oxygen and nutrition to the retina and to remove its waste products, the highest rate of blood flow in the human body is found in the choroidal circulation.[17] With this high flow thermal energy from light absorption can also be dissipated, leading to stabilisation of tissue temperature.[18, 19] Moreover, the choroid secretes growth factors.[12] It also produces complement factors, that are of major importance in controlling intraocular inflammation.[20, 21] Within the choroid, the highest rate of blood flow can be seen in the choriocapillaris. Additionally, differences between macula and periphery exist: blood flow is higher in the macula, which can be explained by its more efficient lobular structure, which operates independently from other parts of the macula. This differs from the ladder-like structure of the peripheral choriocapillaris.[22, 23]

With increasing age, choroidal thickness, choriocapillary blood flow, density, and diameter decrease.[13] Moreover, refraction error correlates with choroidal thickness: myopic patients have a thinner choroid than hyperopic patients.[24, 25]

CLINICAL EVALUATION OF THE RETINA AND CHOROID

Functional evaluation of the retina

Visual acuity measurement

Visual acuity is assessed in a standardised manner: at standard distances and standard illumination, either letters or numbers, or figures of known visual angle are shown.

Electro-oculography

The function of the RPE monolayer can be assessed using electro-oculography (EOG). The resting potential of the RPE decreases during several minutes of dark adaptation ('dark trough'), after which it increases after switching on the light ('light peak'). The outcome of EOG assessment is the Arden ratio, dividing the light peak by the dark trough. The International Society for Clinical Electrophysiology of Vision has published standardised test conditions, for a better comparison of several EOG measurements.[26]

Electroretinography

During electroretinography (ERG), the photoreceptors are examined after exposure to a light stimulus. The function of the rod and cone photoreceptors is assessed separately during the scotopic (dark-adapted) and photopic (light-adapted) phase, respectively. Scotopic responses represent maximal rod sensitivity to light, which is in contrast with the photopic responses that reflect cone function. Moreover, during the exposure to a flash light in scotopic conditions 2 more dark-adapted measurements can be performed: the photoreceptors respond with the maximal response, whereas the ganglion, bipolar, and amacrine cells also contribute to the oscillatory responses.[27, 28] For ERG, standard conditions from the International Society for Clinical Electrophysiology of Vision are available as well.[26]

Perimetry

Testing of the peripheral visual field can be performed with kinetic perimetry, by using the Goldmann perimeter. During this examination, moving stimuli of various strengths are brought into the visual field, leading to the detection of the outer borders, within which specific stimuli are visible for the patient. More precise testing of the central visual field can be performed by the use of static perimetry, with the Humphrey visual field analyser. During proper fixation to a central spot, light of various intensities appears at non-moving positions, and possible defects can be detected.

Microperimetry evaluates both retinal function and anatomy, and has the ability to observe the fundus during examination, while eye tracking and fixation stability are documented. Microperimetry is able to record retinal sensitivity in both mesopic (for light adapted eyes) and scotopic conditions (for dark adapted eyes).[29, 30]

Retinal imaging

Ophthalmoscopy

Ophthalmoscopy can provide information on the aspect of the retina. Indirect ophthalmoscopy is most often used by ophthalmologists, preferably through well-dilated pupils. An overview of the retina may be obtained using a +20 diopter lens and an indirect ophthalmoscope. During slit lamp examination with a +90, +78, or a +60 diopter lens, a more detailed examination of the

macula, optic disc, and (mid-)peripheral retina can be performed. Retinal abnormalities can be recorded by taking color fundus photographs over time (Figure 5A and 5C).

Optical coherence tomography

Optical coherence tomography (OCT) is able to provide high-resolution cross-sectional images of the retinal layers (Figure 4). A light is split into 2 beams by a splitter, which directs the first beam to the tissue and the second beam to the reference mirror. Images can be obtained by combining the returning beams, and contrasts between the different retinal layers occur due to differences in light reflectivity.[31]

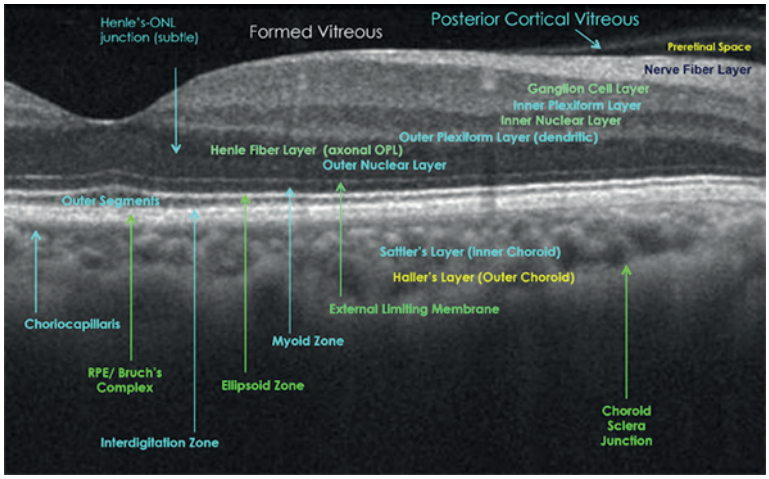


Figure 4. Optical coherence tomography scan of the normal retina and choroid (Spaide et al, 2012[32])

Enhanced-depth and swept-source OCT imaging

Enhanced-depth imaging OCT (EDI-OCT) can provide more detailed images of the choroid, due to the fact that the objective lens is placed closely to the eye. Using EDI-OCT, backscattering of light can be prevented, leading to an increase in sensitivity.[33, 34]

In contrast with the superluminescent diode laser that is used in conventional OCT, swept-source OCT is performed with a short-cavity swept laser. It is less distracting for patients, since this light is invisible. Moreover, swept-source OCT scanning can achieve a higher imaging speed and provides the ability to obtain the clearest images of deep ocular structures to date, because of the long wavelength and swept-source technology that are used.

Optical coherence tomography angiography

Optical coherence tomography angiography is a non-invasive technique that can be used to visualise capillary blood flow, using motion contrast. Since repeated scans within the same area have to be obtained, the imaging speed and retinal coverage are still limited. Moreover, direction of flow and low flow phenomena cannot be determined reliably by currently available optical coherence tomography angiography imaging systems. However, three-dimensional images are obtained, on which the several retinal and choroidal (vascular) layers are visible, while avoiding the disadvantage of using intravenous injection of dyes such as fluorescein.[35, 36] The exact role of optical coherence tomography angiography in clinical practice still has to be determined, also because of the fact that image artifacts occur frequently.[37]

Fundus autofluorescence imaging

Distribution of the autofluorescent compound lipofuscin in the RPE, as a reflection of metabolic activity, can be assessed non-invasively with fundus autofluorescence (FAF) imaging (Figure 5B and 5D).[38, 39] After stimulation by light with an excitation (short) wavelength, intrinsic autofluorescence is sent out by the RPE cells and is captured by the imaging device. Lipofuscin is formed as a degradation product during the process of phagocytizing and processing of photoreceptor outer segments by the RPE.[40, 41] Extensive and focal accumulation of lipofuscin is pathologic, and can lead to hyperautofluorescence on FAF. Hypoautofluorescence can be detected in case of either RPE atrophy or signal blockage.[38] Since FAF is an imaging technique that provides functional information on the RPE, its use can be of added value in many retinal diseases.

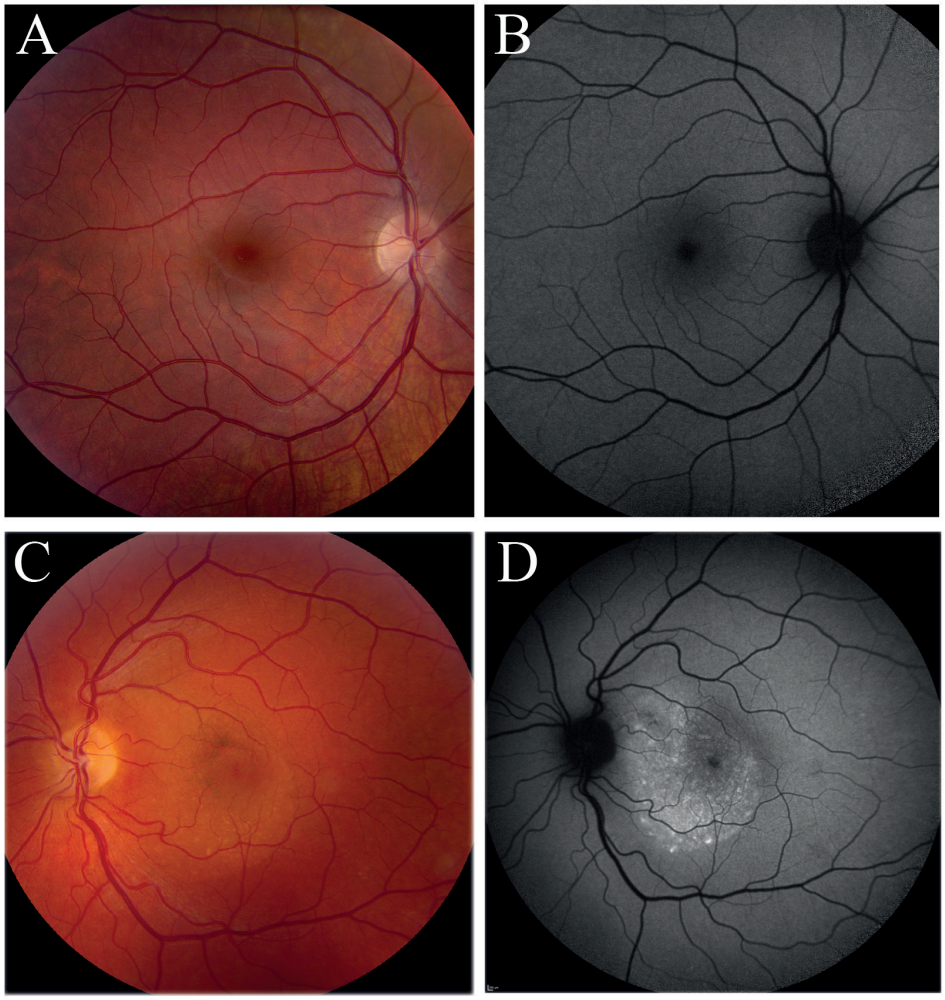


Figure 5. Color fundus photography (A) and fundus autofluorescence (B) images of the healthy fundus of the right eye in the author of this thesis, and color fundus photography (C) and fundus autofluorescence (D) images of the left eye in a patient with central serous chorioretinopathy

Fluorescein angiography

Small molecules can pass the large fenestrated choroidal capillaries, which is used in FA.[15, 16] Fluorescein normally remains inside the blood vessels of the retina which constitute the inner blood-retinal barrier, and does not diffuse through a healthy RPE monolayer, because its tight junctions form the outer blood-retinal barrier. Thus, FA can be used for the assessment of this vasculature (Figure 6A and 6C). The sodium fluorescein dye can be administered either intravenously or orally. A variable degree of hyperfluorescence or hypofluorescence may be observed, depending on the nature and location of abnormalities.

Indocyanine green angiography

Indocyanine green angiography (ICGA) has become the gold standard to image the choroidal vasculature, after its introduction in the early 1990s.[42] After intravenous injection of indocyanine green, these relatively large molecules allow imaging of choroidal vessels, since the fenestrations of the choriocapillaris do not allow its diffusion.[43] In contrast with FA, in which 60-75% of the used blue green light (500 nm) is absorbed by the RPE and choroid, ICGA can be used to visualise sub-RPE structures (Figure 6B and 6D).

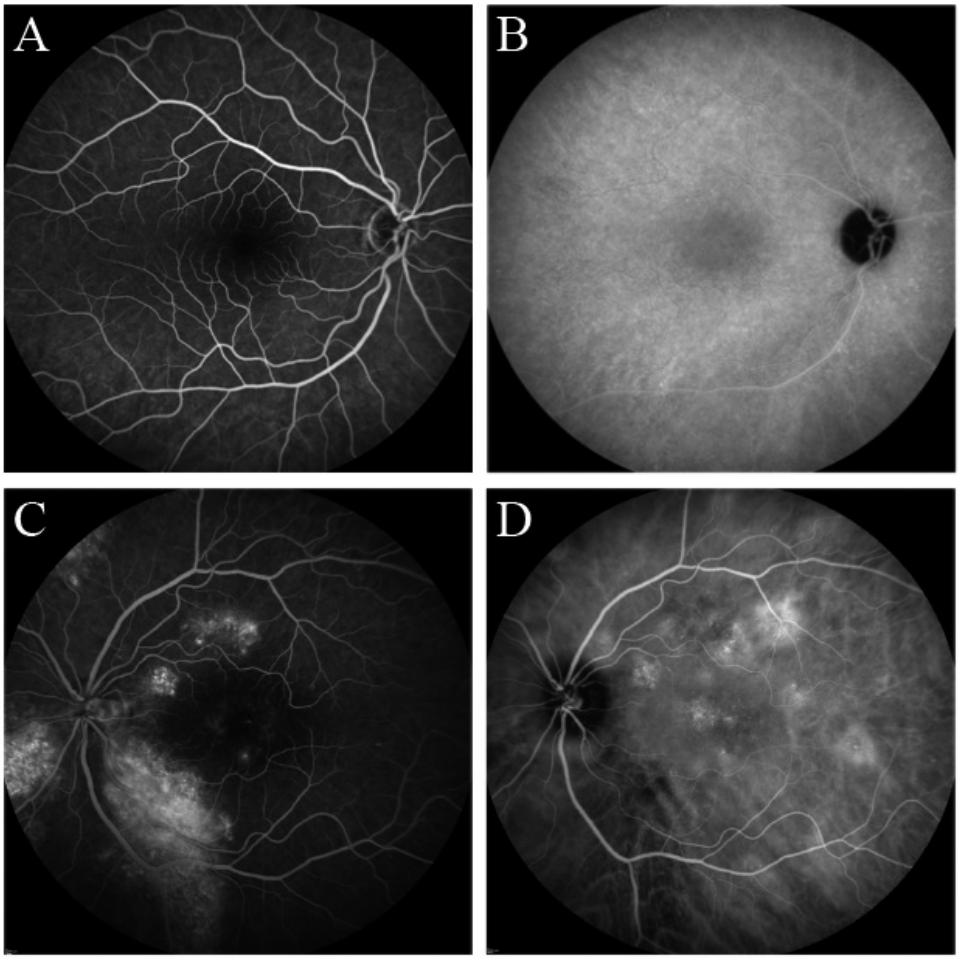


Figure 6. Fluorescein angiography (FA; A) and indocyanine green angiography (ICGA; B) images of the right eye in a healthy individual, and FA (C) and ICGA (D) images of the left eye in a patient with central serous chorioretinopathy

CENTRAL SEROUS CHORIORETINOPATHY

Central serous chorioretinopathy (CSC) has been estimated to be the fourth most frequently occurring maculopathy associated with fluid leakage, after AMD, diabetic retinopathy, and retinal vein occlusion. This chorioretinal disease was presumably first described already in 1866 by the renowned German ophthalmologist Von Graefe.[44] The characteristic trait of the disease is a localised neurosensory retinal detachment, which is caused by an accumulation of serous fluid under the neuroretina. Ophthalmoscopy reveals a subtle retinal elevation in the macular region, with an absent or attenuated foveal reflex. The subretinal fluid (SRF) leakage results from dysfunction of the RPE outer blood-retina barrier, which is most probably induced by a primary congestion, hyperpermeability, and thickening of the choroid (pachychoroid).[45-50]

CSC patients usually present with complaints of decreased visual acuity, metamorphopsia, loss of color and/or contrast vision. These complaints mainly occur when the SRF reaches the macula, which is presumed to be more vulnerable to develop CSC due to both the specific lobular structure and the relatively high flow rate of the underlying choroid at this location.[51] Spontaneous resolution of SRF occurs in more than 90% of patients.[52] However, up to 50% of patients can experience recurrent or chronic episodes of CSC.[53] A prolonged neuroretinal detachment can cause irreversible vision loss due to photoreceptor atrophy. Moreover, long-lasting CSC can be complicated by intraretinal fluid accumulation (posterior cystoid retinal degeneration), as well as by choroidal neovascularisation (CNV) and/or polypoidal choroidal vasculopathy (PCV).[54, 55] Early diagnosis and treatment is desirable to improve the visual outcome and quality of life in CSC. After all, long-term follow-up studies have shown that the natural course of the disease often results in vision loss and decreased vision-related quality of life.[53, 56-58] In most CSC patients, SRF occurs unilaterally. However, a significant increase in choroidal thickness in both affected eyes and non-affected fellow eyes has been described in CSC, supporting the hypothesis that CSC is a bilateral but asymmetrical disorder.[46, 59, 60]

Epidemiology and risk factors

The mean annual incidence of CSC is 9.9 per 100,000 males and 1.7 per 100,000 females.[60] Since asymptomatic CSC can be diagnosed by the presence of extramacular SRF, as observed in contralateral eyes and family members of CSC patients, these incidence rates are considered to be an underestimation.[61, 62] The CSC diagnosis is most often established in middle-aged patients, who are still professionally active. Reported male-to-female ratios are up to 8:1.[49, 63, 64] CSC is considered to be a multifactorial disease. The most pronounced risk factor for the disease is corticosteroid use, with reported odds ratios of up to 37.[65] Both exogenous, independent of the dosage and route of administration, and endogenous hypercortisolism (Cushing's syndrome) have been described to increase the risk to develop CSC.[46, 50, 66-68] Moreover, an association between stress,[69, 70] anxiety,[71] type A personality pattern (described as outgoing,

ambitious, organised, sensitive, impatient, anxious, and proactive),[72] shift work,[73, 74] and cardiovascular diseases and CSC has been suggested.[75] Genetic factors appear to play a role in the pathogenesis of CSC. Familial CSC has been described, although familial occurrence is uncommon.[62, 76-78] Moreover, ethnic differences in the prevalence of CSC have been reported.[63] Single nucleotide polymorphisms in the *age-related maculopathy susceptibility 2, complement factor H*, and *cadherin 5* genes and copy number variations in the *complement component 4B* gene have been associated with CSC in Caucasians,[79-81] and in Asians this *complement factor H* association was also identified.[82] Factors from these studies have been described to be either risk-conferring or protective.[79, 80, 82]

Negative visual prognostic factors for CSC are the appearance of subretinal deposits, which can arise out of abnormal outer segments of the photoreceptors, the disruption of the external limiting membrane, the disruption of the junction between inner and outer photoreceptor segments (ellipsoid zone), and the thinning of the outer nuclear layer.[83-85]

Subtypes of central serous chorioretinopathy: acute versus chronic CSC

Two main subtypes of CSC are generally distinguished: acute CSC (aCSC; Figure 7A-E) and chronic CSC (cCSC; Figure 7F-J). Whether these subtypes are a continuum or separate entities is currently unclear.[46, 47, 49, 63, 86-88] The progression of a single aCSC episode to cCSC appears to be relatively rare.[89] Differentiation between the 2 subtypes is most often based on the duration of the presence of SRF. Patients in which SRF has appeared less than 4 to 6 months ago, has been referred to as aCSC cases. In cCSC, SRF is present during a period of more than half a year.[46, 47, 49, 63, 86-88] Moreover, a distinction between aCSC and cCSC can be made on the basis of the outcome of multimodal imaging, either with or without keeping duration of disease in mind.

Subtypes of central serous chorioretinopathy: aCSC

In aCSC, patients generally present with an acute onset of central vision loss.[90] Compared to cCSC, in aCSC the male preponderance is even more pronounced and the age at presentation is younger.[45]

SRF is present on OCT, together with relatively subtle RPE changes and an increased choroidal thickness in most patients.[45] On FAF, abnormalities in aCSC can change over time. Hypoautofluorescent changes have been described to occur at the area of leakage on FA,[91] whereas hyperautofluorescent abnormalities could be found at the location of the neuroretinal detachment after a prolonged period.[63, 92] Within several months after resolution of SRF, FAF abnormalities disappear.[63] On FA, the phenomena of 'smokestack leakage', an ascending area of hyperfluorescence with a subtle lateral diffusion, and of leakage of an 'ink-blot pattern', a gradual expansion of a round hyperfluorescent area from a central pinpoint of leakage, are typical for aCSC.[63] Underlying choroidal abnormalities can be visualised with ICGA. Caused

by a delay in filling of the vasculature, hypofluorescent areas occur during the early phase of ICGA.[48, 93] Midphase ICGA reveals the typical choroidal vascular hyperpermeability, presenting as hyperfluorescent areas with blurred borders.[88, 94] In the late phase ICGA, either similar or decreased hyperfluorescent areas may be observed, with or without the occurrence of a hyperfluorescent ring.[95]

Spontaneous resolution of SRF in aCSC patients is common.[63] The disease has a good prognosis, both for retinal anatomy and long-term visual function.[96] In some patients, permanent color discrimination and visual field defects may remain.[97, 98]

Subtypes of central serous chorioretinopathy: cCSC

In cCSC, patients gradually develop visual complaints in most cases, and the presenting visual acuity is worse than in aCSC patients.[88] Bilateral involvement, which has been reported in up to 40% of all CSC cases, occurs more often in cCSC.[99] Moreover, corticosteroid use has particularly been associated with cCSC.[87]

Due to the severe choroidal abnormalities and RPE dysfunction in cCSC, SRF is usually present for a prolonged period. Areas of RPE hypopigmentation that extent inferiorly from the macula (gravitational tracts), which are formed by SRF sinking toward the inferior fundus and causing atrophic RPE changes, could be present on multimodal imaging and are pathognomonic for the disease.[63, 89, 100] On OCT, abnormal outer segments of the photoreceptors can be observed within the pocket of SRF accumulation, most probably caused by the absent apposition between photoreceptors and RPE. An increased choroidal thickness can also be present. In general, RPE abnormalities in cCSC are diffuse and widespread.[101] Moreover, the occurrence of photoreceptor atrophy and intraretinal fluid (posterior cystoid retinal degeneration) has been described in longstanding cCSC.[102] Pigment epithelial detachments (PEDs) can be detected in 53-100% of CSC affected eyes, and occur more often in cCSC.[103-106] In cCSC, irregular patterns of both hyper- and hypoautofluorescent changes can be observed on FAF.[39, 107] In comparison with aCSC, abnormalities on both FA and ICGA are relatively widespread.[91]

Diagnosing cCSC in older patients is challenging, since it could resemble AMD or could be complicated by a CNV.[108-110] The performance of ICGA is mandatory to be able to distinguish between these entities.[42, 88] Chronic CSC frequently leads to irreversible vision loss, despite the availability of several treatment options.[45-47, 49, 55, 63, 111, 112]

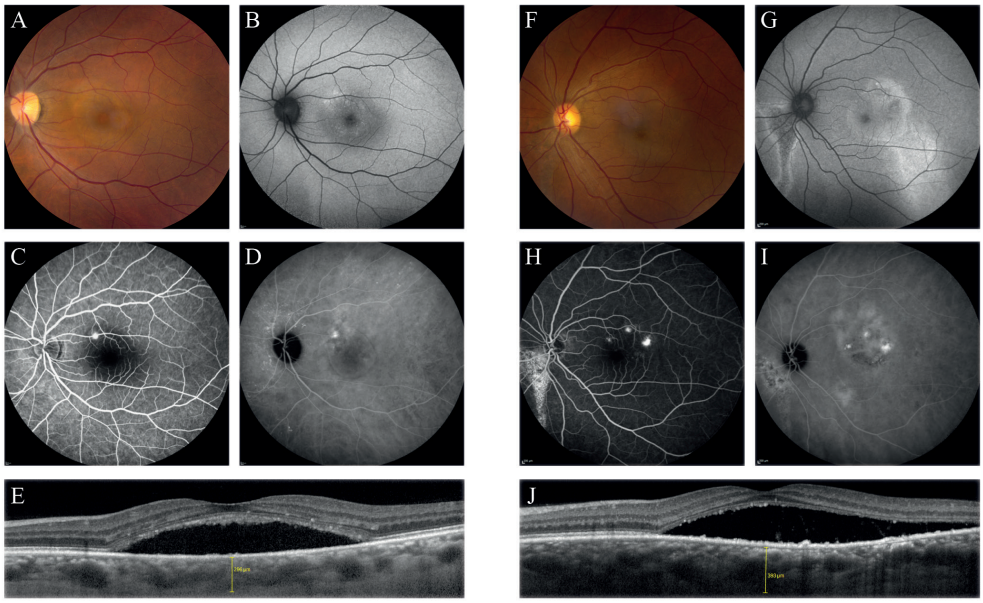


Figure 7. An overview of characteristics on multimodal imaging that can be observed in acute (**A-E**) and chronic (**F-J**) central serous chorioretinopathy (CSC).
(**A-E**) Multimodal imaging of the left eye in a 43-year-old male patient with acute CSC. Fundus photography (A) showed a well-demarcated subretinal fluid (SRF) accumulation in the macula, corresponding with hypo- and hyperautofluorescent changes on fundus autofluorescence imaging (FAF; B). On fluorescein angiography (FA; C), a hot spot of leakage was seen superonasally to the fovea. Indocyanine green angiography (ICGA; D) revealed a larger area of hyperfluorescent changes, compared to FA abnormalities. On optical coherence tomography (OCT; E), a dome-shaped accumulation of SRF was observed. The subfoveal choroidal thickness (SFCT) was 296 µm. (**F-J**) Multimodal imaging of the left eye in a 48-year-old male patient with chronic CSC. On fundus photography (F), a SRF collection could be observed, and alterations of the retinal pigment epithelium (RPE) were present in the macula. FAF (G) showed extensive hyperautofluorescent changes within the temporal vessel arcades, with mainly hypoautofluorescent changes inferiorly to the optic disc. FA (H) revealed 3 hot spots of leakage superiorly to the fovea, and a window defect inferiorly to the optic disc. Moreover, RPE alterations were observed. On ICGA (I), hypofluorescent changes were present, and these were surrounded by extensive hyperfluorescent abnormalities. OCT (J) showed a SRF accumulation and RPE abnormalities, typical for chronic CSC. SFCT was 393 µm in this patient.

PATHOGENESIS OF CENTRAL SEROUS CHORIORETINOPATHY

Despite the identification of several risk factors and the detection of primary abnormalities in the choroid and RPE, the exact pathogenesis of CSC is still unclear.[47]

CSC is most probably of choroidal origin. Ischemia, stasis, and inflammation of the inner choroid have been suggested to cause choroidal hyperpermeability and thickening (pachychoroid), which can be observed most clearly on midphase ICGA.[45-49] The outer choroid mainly contributes to the increased choroidal thickness.[113, 114] This combination of abnormalities leads to focal damage of the RPE outer blood-retina barrier, due to mechanical stress, RPE atrophy as a result of hypoperfusion of the choriocapillaris, decreased RPE adhesion, and changes in ion and water transport.[45, 115] When a focal disruption occurs in the RPE outer blood-retinal barrier, water, electrolytes, and proteins can leak into the subretinal space.[63] Moreover, choroidal abnormalities and an increased choroidal thickness have been described in more than half of the fellow eyes of CSC patients, in which no visual complaints were present.[46, 59, 60, 95, 116] Thus, abnormalities in both the choroid and RPE can often already exist before SRF occurs in CSC patients.

Changes in the choroidal blood flow autoregulation may be an underlying factor, since an increase in intraocular perfusion pressure, calculated from intraocular pressure and mean arterial pressure, was found during exercise of CSC patients.[117] The association of CSC with several systemic diseases also indicates the existence of either a molecular effect on choroidal vessels or a global dysregulation of this vasculature.[118] Contrary to healthy controls, an increase in choroidal thickness was noted in CSC patients when transitioning from vertical to horizontal position, during which the perfusion pressure rises. Since the choroidal circulation is controlled by the sympathetic nervous system, a role of this system in the occurrence of CSC has been suggested.[118]

Hormonal factors may also be involved in the pathogenesis of CSC, since corticosteroid use and male gender are the most important risk factors for CSC. Moreover, stress and shift work have been associated with CSC.[69, 70, 73, 74] CSC risk factors pregnancy and type A personality have also been found, and have been linked to alteration of glucocorticoid metabolism.[119-121]

Still, the pathogenesis of CSC is a mystery, with numerous questions to be answered.

TREATMENT OPTIONS IN CENTRAL SEROUS CHORIORETINOPATHY

Several treatment options have emerged in an attempt to accelerate resolution of SRF accumulation, and to improve visual outcome in CSC patients. However, there is no evidence-based consensus on the optimal type and timing of treatment in CSC, due to the lack of large prospective randomised controlled trials.[45]

At first presentation, the relationship between possible corticosteroid use and CSC has to be addressed both for aCSC and cCSC patients.[63] Discontinuation of the use of this medication should be encouraged.[122] Moreover, signs of or symptoms related to Cushing's syndrome (primary hypercortisolism) have to be drawn attention to.

Spontaneous resolution of SRF in aCSC patients is common, and prognosis in terms of retinal anatomy and visual function is favorable.[63, 96] For aCSC, the usual initial treatment approach is observation. In cCSC, an early treatment is desirable to try to improve the visual outcome and quality of life, especially when foveal SRF is present. After all, long-term follow-up studies have shown that the natural course of the disease often results in visual loss. [49, 53, 55, 57, 58, 89, 96, 123-125] Numerous types of treatment have been and are still prescribed to cCSC patients, out of which photodynamic therapy (PDT) with reduced settings, high-density subthreshold micropulse laser (HSML) treatment, and oral administration of mineralocorticoid receptor antagonists (either eplerenone or spironolactone) seem to lead to the most beneficial effect.[45, 111]

Conventional laser

The use of focal (argon) laser photocoagulation has been performed in CSC, because of the hypothesis of its 'sealing' effect on RPE defects and stimulation of the pump function of RPE cells.[45, 126] During this treatment, a low-intensity green or yellow laser induces tissue coagulation, with a beam that is focused on the RPE. Despite the fact that it has been shown that conventional laser treatment of leakage points on FA does not result in a better visual outcome and a lower recurrence rate, a reduction in time to complete resolution of SRF has been described, mainly in aCSC patients. [49, 127] However, conventional laser is a treatment with a relatively high risk of complications compared to HSML and half-dose PDT, including visual loss, scotoma, decreased color vision, decreased contrast sensitivity, and CNV.[45, 49, 63, 127, 128]

Photodynamic therapy

PDT is thought to lead to short-term hypoperfusion of the choriocapillaris and to long-term remodelling of choroidal vasculature, which results in reduction of leakage of fluid to under the neuroretina.[129] This treatment first requires the intravenous administration of the light sensitive substance verteporfin, followed by the administration of non-thermal red light into the affected eye. Once verteporfin is activated by light in the presence of oxygen, highly reactive, short-lived

singlet oxygen and reactive oxygen radicals are generated. Verteporfin appears to accumulate preferentially in abnormal neovascularisation, but also in choroidal vasculature. The latter mechanism is of special interest in treatment of CSC, as the disease primarily affects the choroidal circulation, resulting in multifocal areas of choroidal vascular hyperpermeability that may finally result in accumulation of SRF.[129-131] Because of the more extensive abnormalities on ICGA compared to FA, in most CSC patients the area to be treated is ICGA-based.[129, 132]

The PDT strategies that are generally used for CSC are either with half the dose (3 mg/m²) of verteporfin in which full-fluence (energy; 50 mJ/cm²) and full-time (83 seconds) of laser treatment are used, or half-fluence with full-dose verteporfin and full-time, or half the treatment time using the full-dose of verteporfin and the full-fluence level, as compared to the original protocol that has been developed for the treatment of neovascular AMD.[133] The introduction of half-dose PDT led to a reduction in the likelihood of the occurrence of adverse events associated with the use of this medication.[134] Resolution of SRF can be achieved with half-dose PDT in 62-100% of cCSC patients.[134-136] Comparable treatment results have been found after either half-fluence or half-time PDT, which may be explained by the reciprocal phenomenon, requiring a minimum of both drug dose and light energy.[135-138] However, lowering the verteporfin dose to one-third may be less effective in terms of SRF resolution.[139]

To date, PDT has emerged as the treatment of choice for CSC, based on the literature that is currently available. [49, 127] A relatively large body of well-documented retrospective studies indicates that half-dose PDT is able to yield positive functional and anatomic outcomes, while at the same time reducing the potential adverse events associated with conventional PDT with full-dose verteporfin. Moreover, a decrease in choroidal hyperpermeability and in subfoveal choroidal thickness has been found after PDT.[140] In a long-term follow-up study with a mean follow-up of 58 months, SRF remained absent in 93% of patients and visual acuity increase remained significant after half-dose PDT treatment, when this therapy was performed before a possible deterioration of visual complaints.[141] However, randomised controlled trials were lacking until our group started working on this subject, and commenced a trial in which half-dose PDT was compared to the other treatment modality that is often prescribed to CSC patients.

High-density subthreshold micropulse laser treatment

HSML is a treatment option for a wide range of retinal diseases.[142] For CSC, it has been reported to lead to a resolution of SRF in 41-58% of patients.[143, 144] During this procedure, for which no intravenous infusion of a light sensitive substance is required, light that does not induce damage to the neuroretina is used. Since the temperature of the RPE remains below the protein-denaturation-threshold, neither damage nor a clinically visible endpoint is induced. Compared to treatment with a suprathreshold laser, more energy can thus be delivered to the RPE, which leads to activation of RPE cells. The energy is transferred within an 'envelope'

of a width of 0.1 to 0.5 seconds, containing repetitive laser pulses with a length of 100 to 300 µs. This leads to transient retinal swelling. However, after absorption of heat energy by the RPE, recovery occurs between pulses. It has been suggested that a gain in treatment density by increasing the number of HSML spot applications maximises treatment effect.[145, 146] The exact mechanism of action of HSML is still to be unraveled, but regulation of factors mediated by RPE cells has been hypothesised.[142] Treatment is performed by using one of the following laser wavelengths: 810 nm, 577 nm, and 527 nm.[147-149] The 810 nm laser mainly affects the choroid, and relatively spares the inner neurosensory retina, which is in contrast with the other wavelengths.[150] Other settings of the laser (duty cycle, power, diameter, and pulse duration), the determination of 'no visible retinal damage' after a test shot, the density of laser spots, and the extent of underlying abnormalities to be treated is also variable, when comparing applied treatments within different studies. For HSML, it is challenging not to undertreat, since the ophthalmologist usually does not see a retinal effect after having performed treatment.[150]

Compared to conventional argon green laser, HSML treatment has been described to lead to faster visual recovery and better contrast vision.[151]

Intravitreal anti-vascular endothelial growth factor treatment

A possible role for the intravitreal injection of VEGF antibodies to reduce choroidal hyperpermeability and ischemia of the choriocapillary has been suggested, despite the fact that an increase in intraocular VEGF levels has not been found in CSC patients.[152, 153] Moreover, half-fluence PDT has been described to lead to complete resolution of SRF in a significant higher percentage of patients, compared to intravitreal ranibizumab (Lucentis®) injections.[154] Complete resolution of SRF has only been described in up to 50% of CSC patients treated with intravitreal injections with anti-VEGF medication.[155, 156] In cases of a clearly identified CNV complicating CSC, therapeutic effect of bevacizumab (Avastin®), ranibizumab, and aflibercept (Eylea®) has been shown in retrospective studies.[157-159] Neovascularisation and vascular permeability of the choroid can be reduced using anti-VEGF treatment.[157-159]

Systemic treatment

Based on the different pathophysiological mechanisms that have been believed to play a role in CSC, an extensive selection of oral medication has been administered to this patient group: mineralocorticoid receptor antagonists (eplerenone, spironolactone),[160-168] carbonic anhydrase inhibitors (acetazolamide),[169, 170] proton pump inhibitors (omeprazole) and antibiotics (metronidazole, clarithromycin, amoxicillin) for the treatment of *Helicobacter pylori* infection,[171] anti-platelet aggregation medication (aspirin),[172] steroid synthesis inhibitors/glucocorticoid receptor antagonists (ketonazole, mifepristone, finasteride, rifampicin),[173-178] and anti-adrenergic medication (propranolol, nadalol).[179] Out of all these therapeutic classes, only the first 3 have been found to lead to a positive effect in terms of SRF resolution.

Treatment of CSC with the mineralocorticoid receptor antagonists spironolactone and eplerenone is the most promising of these, and has recently become more commonplace. The use of this type of medication is based on the involvement of the mineralocorticoid receptor and of choroidal hyperpermeability in CSC pathogenesis.[121, 180] However, the effect of these on central macular and choroidal thickness and on complete and durable resolution of SRF after (cessation of) therapy is unclear, and randomised controlled trials are currently lacking.[165, 166, 181]

The broad spectrum of treatments used and advocated indicates that treatment of CSC is still subject of controversy and treatment results are suboptimal. Significant progress has to be made in discovering disease mechanisms and research on available treatments in the quest for the optimal therapeutic regimen for CSC, to induce a prompt and complete resolution of SRF.

DIFFERENTIAL DIAGNOSIS OF CENTRAL SEROUS CHORIORETINOPATHY

A combination of findings on multimodal imaging leads to the establishment of CSC diagnosis. However, several other diseases can have similar characteristics. SRF is the most distinctive for CSC, but can occur in a broad range of maculopathies. Moreover, typical CSC findings such as hot spots of leakage on FA, hyperfluorescent changes on ICGA, and RPE changes also cannot exclusively be detected in CSC patients. To be able to inform patients correctly about diagnosis, prognosis, and therapeutic options, it is vital to exclude the existence of the diseases that are described below.

Age-related macular degeneration

Since both CSC and AMD (Figure 8A-D) can present with RPE changes, and since CSC can be complicated by CNV and PCV, specific attention should be paid to signs of AMD.[114, 182, 183] However, in typical CSC no drusen are present, and CSC patients are usually below the age of 55 at onset.[45-47, 63] The presence of subretinal fibrosis, hemorrhages, and hard exudates should raise suspicion for CNV. In most AMD cases, drusen are also present in the fellow eye. No hot spots of leakage are present on FA. In cases of a CNV, a well-demarcated neovascular membrane can be observed on ICGA.[184] OCT angiography can be very helpful in detecting subtle cases of CNV, both in AMD and in CSC cases complicated by CNV.

Polypoidal choroidal vasculopathy

Both CSC and PCV (Figure 8E-H) have been associated with a thickened choroid.[185] On fundoscopy, polyps in PCV can be seen as pink subretinal structures. A branching vascular network and hyperfluorescent nodules on early phase ICGA can be detected, often surrounded by a hypofluorescent halo.[186] Serous and hemorrhagic PEDs are typical for PCV. The following findings on OCT can help in establishing its diagnosis: correspondence of the PED with lesions

on ICGA, accumulation of highly-reflective debris in the PED, and the presence of intraretinal fluid.[45, 187] Moreover, lesions are frequently accompanied by a neuroretinal detachment. PCV can present as an isolated disease, but its presence has also been described as a complication of either AMD or CSC.[182, 183] The first treatment option for PCV is a combination of full-dose PDT and intravitreal anti-VEGF injections.[186, 188, 189]

Both CSC and PCV are part of the 'pachychoroid clinical spectrum', in which an increased choroidal thickness is associated with dilatation of the outer choroidal vessels.[113, 114, 190] At these areas of thickening, the inner choroid is relatively thin, due to either primary atrophy of the choriocapillaris or compression by the outer choroid.[45, 104]

Pachychoroid pigment epitheliopathy and pachychoroid neovasculopathy

Pachychoroid pigment epitheliopathy, characterised by RPE changes with reduced fundus tessellation,[190] and pachychoroid neovasculopathy, known as the occurrence of a type 1 CNV over zones of choroidal thickening, are other diseases which are part of the pachychoroid clinical spectrum.[108] For pachychoroid pigment epitheliopathy, it has been hypothesised that the RPE is still able to cope with the fluid overload, resulting in either subtle RPE changes or small PEDs at overlying zones of either pachychoroid or dilated choroidal vessels.[115] When the RPE cannot deal with this overload, CSC can occur in cases of focal breakdown of the outer blood-retina barrier. In patients in whom this subsequently leads to a damaged Bruch's membrane, this could result in a type 1 CNV.[114] Besides the use of FA and ICGA to distinguish these entities, OCT and OCT angiography have been shown to be of importance in the distinction.[191, 192]

Dome-shaped maculopathy and inferior staphyloma associated with tilted disc syndrome

In dome-shaped maculopathy, an inward macular deviation occurs in (highly) myopic eyes,[193, 194] and a central accumulation of SRF is present in half of the eyes.[195] An inferior staphyloma associated with tilted disc syndrome (Figure 8I-L) can be recognised most easily on a vertical OCT scan: the axis of the optic disc is oblique, an inferonasal crescent is present, and retinal vessels are either reversed or mirrored from the normal position.[196]

Since findings on OCT, FA, and ICGA are similar to findings in CSC patients, especially in patients with a small refractive error the distinction between diseases is challenging. Both dome-shaped maculopathy and inferior staphyloma associated with tilted disc syndrome show a poor therapeutic response to for example PDT and intravitreal anti-VEGF injections, but spontaneous resolution of SRF may occur.[197]

Cavitary optic disc anomalies

Optic disc pit (Figure 8M-P) is observed after an imperfect closure of the superior edge of the embryonic fissure, which leads to focal congenital abnormalities in the optic nerve head.

Communication between the subretinal space, the vitreous cavity, and the subarachnoid space occurs, and abnormal gradient fluctuations between intraocularly and intracranially are present. The anomalies can remain asymptomatic, but when a serous retinal detachment occurs, patients usually start to experience visual complaints. In many patients intraretinal fluid also occurs, which could give rise to a schisis-like clinical picture.[45, 63]

On fundoscopy, the optic disc is usually relatively large and contains a round or oval pit, that can vary in size and color. Compared to CSC, no hot spots of leakage on FA and no hyperfluorescent changes on ICGA are observed in these patients.[63]

Choroidal hemangioma

Choroidal hemangioma (Figure 8Q-T) is a benign choroidal tumor, which can be seen as an orange-red coloration on fundoscopy. Since the tumor usually also appears in the macular region and an increased choroidal thickness and a serous retinal detachment can be observed, the distinction between hemangioma and CSC can be challenging. However, a hot spot of leakage is usually not present in hemangioma. Early hyperfluorescence on ICGA (filling of the tumor vessels) is most often observed for choroidal hemangioma, with a 'wash out phenomenon' during later phases.[45, 198] The most effective treatment option for choroidal hemangioma is PDT with full settings.[199]

Choroidal nevus, choroidal melanoma, and lymphoma

Choroidal nevus and choroidal melanoma are relatively easy to differentiate from CSC, due to findings on fundoscopy and ultrasonography. A brown dome-shaped subretinal mass is the most typical presentation of both a choroidal nevus and choroidal melanoma; the degree of pigmentation can vary. Several clinical findings increase the risk of the presence of a melanoma: SRF, visual complaints, orange pigment on the tumor surface, tumor margin close to the optic disc, and tumor thickness of more than 2 mm.[200, 201] Primary B-cell lymphoma of the choroid may also be mistaken for CSC.

Vogt-Koyanagi-Harada disease

Vogt-Koyanagi-Harada disease is an acute exudative pigment epitheliopathy, in which a relatively extensive serous detachment of the neuroretina can also occur. However, extraocular manifestations, such as pleiocytosis (an abnormally large number of lymphocytes in the cerebrospinal fluid), poliosis (an absence of melanin in hair), vitiligo, alopecia, and dysacusis are present in many patients. Compared to CSC, RPE lesions are more numerous and relatively leaky. A distinction with CSC can also be made based on the response to glucocorticoid treatment: a therapeutic response can usually be observed in Vogt-Koyanagi-Harada disease after systemic steroid treatment, whereas such treatment would lead to worsening of the clinical picture of CSC.[202]

Serous retinopathy associated with mitogen-activated protein kinase kinase (MEK) inhibition

A MEK-associated serous retinopathy (Figure 8U-W), resembling CSC, has been described to occur as a side effect of treatment with several MEK inhibitors for metastatic cancer.[203, 204] This time-dependent, reversible retinopathy usually does not lead to visual complaints, and its occurrence is usually bilateral and fairly symmetrical. Additional multimodal imaging does not reveal findings characteristic for CSC: on OCT no RPE changes and no pachychoroid can be found, and FA does not show hot spots of leakage.[205, 206]

Best vitelliform macular dystrophy

A foveal vitelliform lesion similar to a central neuroretinal detachment in CSC can be observed in patients with Best vitelliform macular dystrophy. Lesions, that often occur bilaterally, typically resemble an egg yolk at a certain point in time. The dystrophy is caused by mutations in the *bestrophin 1* gene, and its mode of inheritance is either autosomal-dominant or autosomal-recessive. The EOG is abnormal in these patients, and multifocal ERG may also reveal abnormalities.[207, 208]

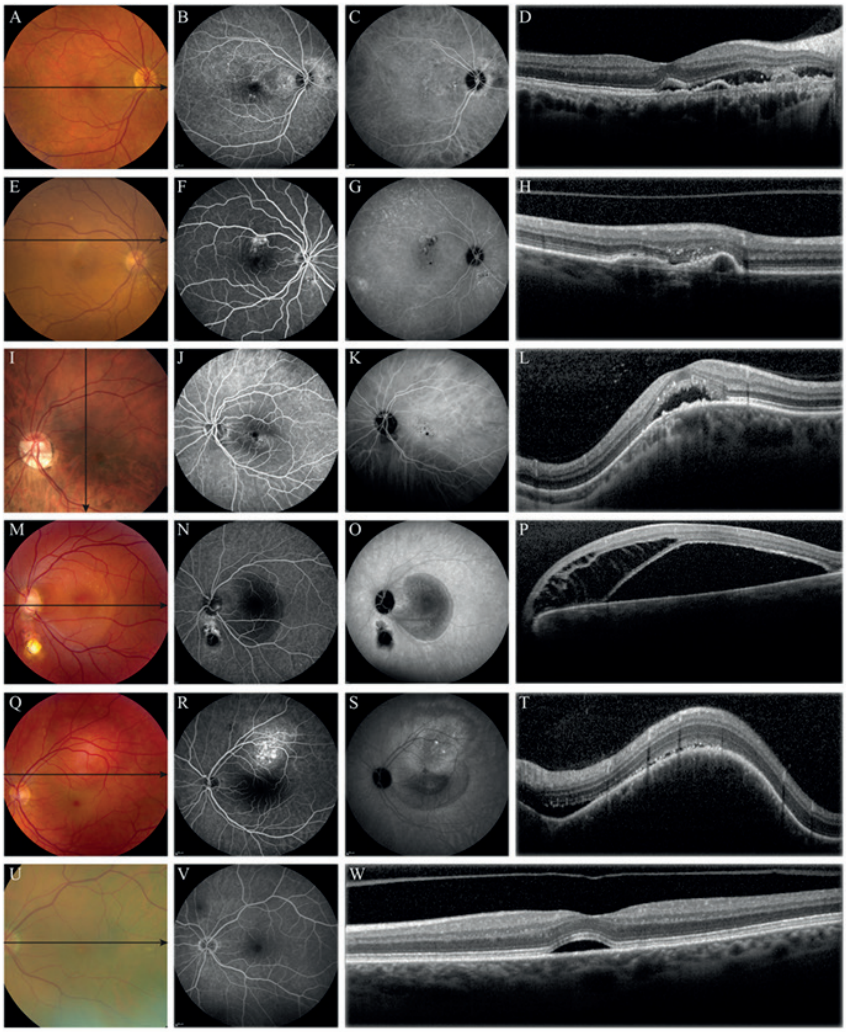


Figure 8. An overview of the findings on multimodal imaging in patients with diseases that have to be taken into account for the differential diagnosis of central serous chorioretinopathy (CSC). (**A-D**) Multimodal imaging of the right eye in a 65-year-old woman with age-related macular degeneration. On fundus photography (A), small yellowish drusen were present in the macula. Fluorescein angiography (FA; B) revealed hyperfluorescent changes, without the presence of a hot spot of leakage. Indocyanine green angiography (ICGA; C) showed relatively subtle hyper- and hypofluorescent changes. On optical coherence tomography (OCT; D), a shallow accumulation of subretinal fluid (SRF) was present, with several detachments of the retinal pigment epithelium (RPE). (**E-H**) Multimodal imaging of the right eye in a 68-year-old woman with polypoidal choroidal vasculopathy (PCV). On fundus photography (E), multifocal RPE alterations were observed in the macula, with a pink lesion superiorly. FA (F) showed hyperfluorescent changes inferiorly to the optic disc and superiorly to the fovea, with several hot spots of leakage. On ICGA

(G), a well-demarcated hyperfluorescent polyp was visible, surrounded by a hypofluorescent zone and with a branching vascular network attached to it. An OCT scan (H) revealed a minimal amount of SRF and RPE detachments with underlying hyperreflectivity. (**I-L**) Multimodal imaging of the left eye in a 48-year-old woman with a serous maculopathy associated with inferior staphyloma/tilted disc syndrome. Fundus photography (I) showed mild-atrophic RPE abnormalities, together with an optic disc tilted inferiorly. Both on FA (J) and ICGA (K) hyperfluorescent changes were visible, without the presence of a hot spot of leakage on FA and/or signs of either PCV or a choroidal neovascularisation on ICGA. On a vertical OCT scan (L), the dome-shaped maculopathy was observed on the border of the transition zone from thinner to thicker choroid. (**M-P**) Multimodal imaging of the left eye in a 37-year-old man with an optic disc pit. On fundus photography (M), a central serous SRF accumulation was visible, together with a microcoloboma inferiorly to the optic disc. FA (N) did not show abnormalities within the temporal vascular arcades, and on ICGA (O) no hyperfluorescent changes were observed, that would have been characteristic for CSC. An OCT scan (P) revealed an accumulation of SRF, which seemed to be connected with the optic disc. Moreover, intraretinal fluid resembling retinoschisis was present, which was presumed to have occurred secondary to the optic disc pit. (**Q-T**) Multimodal imaging of the left eye in a 37-year-old man with a choroidal hemangioma. Fundus photography (Q) showed a red, elevated lesion superiorly to the fovea. On FA (R), hyperfluorescent changes superiorly to the fovea were visible, without a hot spot of leakage. ICGA (S) revealed corresponding hyperfluorescent changes, and the border of the SRF accumulation could be clearly visualised. A typical choroidal mass, SRF, and RPE changes were seen on OCT (T). (**U-W**) Multimodal imaging of the left eye in a 55-year-old woman with a serous retinopathy associated with mitogen-activated protein kinase kinase inhibition. Fundus photography (U) in this patient showed subtle yellowish macular abnormalities. FA (V) did not reveal any abnormalities, and a SRF accumulation was present on OCT (W).

AIMS AND OUTLINE OF THIS THESIS

The aim of this thesis is to increase the understanding of genetic and clinical aspects of CSC, a common and mysterious chorioretinal disease. Moreover, more insight into the optimal treatment for CSC is provided, since this thesis includes the first randomised controlled trial assessing the outcome of half-dose PDT and HSML treatment. As CSC mainly occurs in middle-aged professionally active patients, and can lead to progressive decline in visual acuity and vision-related quality of life, gaining knowledge on the disease and its treatment is of great importance.

Chapter 1 is the general introduction of this thesis, and provides the reader with information on the basic aspects of retinal and choroidal anatomy and function. Moreover, information on the clinical evaluation of the retina and choroid is provided. In addition, the general aspects of CSC are described, together with its treatment options and differential diagnosis.

Chapter 2 assesses the genetics and pathophysiology of CSC. It evaluates the clinical and phenotypical characteristics of patients with familial CSC. Moreover, it includes a genetic study in which the association of functional variants and haplotypes in the glucocorticoid receptor

and the mineralocorticoid receptor with cCSC has been tested. Outcome of the first genome-wide association study of cCSC has also been included.

Chapter 3 reports the outcome of the analysis of systemic abnormalities in CSC patients. A detailed endocrinological phenotyping of CSC patients, thereby assessing the presence of Cushing's syndrome, has been included. In this chapter, personality traits, psychological morbidity, and coping strategies of patients with CSC were also compared to Dutch population reference data, and data from patients with Cushing's disease. Moreover, in this chapter, the presence of either activation or inhibition of the complement system and the presence of antiretinal antibodies has been assessed.

Chapter 4 describes the outcome of extensive retinal phenotyping of patient groups that could be at risk to develop CSC: patients with primary hyperaldosteronism (Conn's syndrome) and patients who chronically used low-dose corticosteroids after a renal transplantation. Moreover, a case series in which CSC appeared to be the presenting symptom of Cushing's syndrome has been included in this chapter.

In **Chapter 5**, the differential diagnosis of CSC is discussed: both the serous retinopathy that could occur during the use of the MEK inhibitor binimetinib and the MEK inhibitor pimasertib are part of this chapter. Moreover, the underlying mechanism of the binimetinib-associated serous retinopathy is investigated.

Chapter 6 presents the results of the first randomised controlled trial for cCSC, the PLACE trial. In this study, half-dose PDT is compared to HSML treatment, both on retinal and functional outcome parameters. Moreover, the clinical and phenotypical outcome of half-dose PDT in patients with severe visual complaints, but in whom SRF was solely present outside the foveal area is reported.

In **Chapter 7**, the most important findings from the studies described in this thesis are summarised and placed in a broader perspective.

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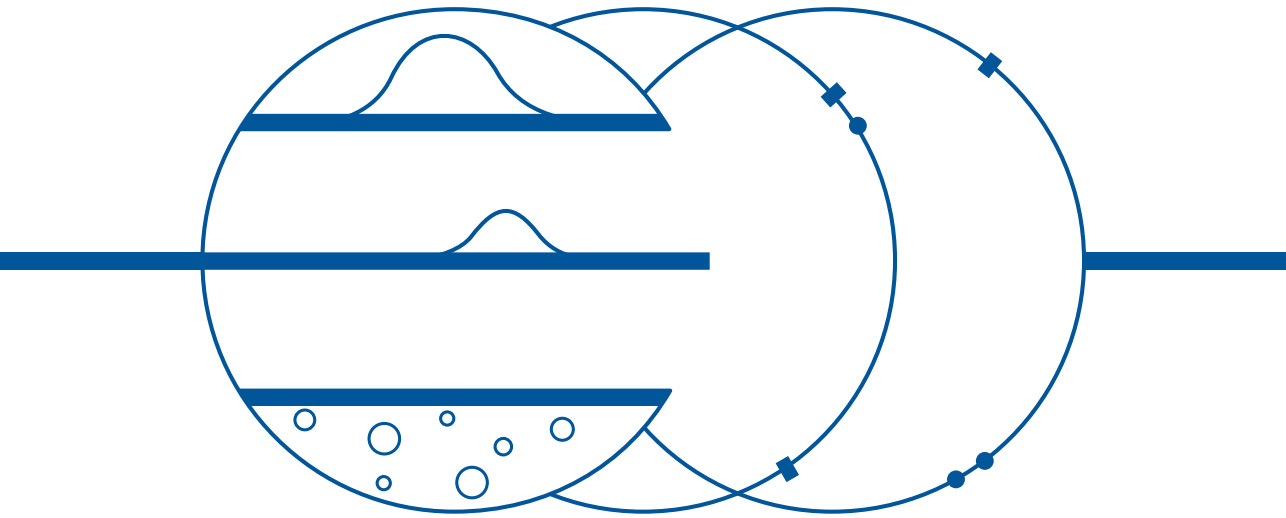
2

GENETICS AND PATHOPHYSIOLOGY OF CENTRAL SEROUS CHORIORETINOPATHY



2.1

FAMILIAL CENTRAL SEROUS CHORIORETINOPATHY

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ABSTRACT

Purpose: To assess ophthalmological characteristics in patients and unaffected individuals in families with multiple members affected by central serous chorioretinopathy (CSC), both at presentation and long-term follow-up.

Methods: In 103 subjects from 23 families, with at least 2 affected CSC cases per family, prospective extensive ophthalmological examination was performed, including best-corrected visual acuity, indirect ophthalmoscopy, digital color fundus photography, optical coherence tomography (OCT), fundus autofluorescence, and fluorescein angiography imaging. From these, 24 individuals from 6 families had undergone extensive ophthalmological examination in either 1994 or 1995 and were followed up in this study.

Results: Subretinal fluid accumulation on OCT and/or hot spots of leakage on fluorescein angiography indicative of CSC were detected in 45 of 103 phenotyped subjects (44%). Findings suggestive of CSC, but without the presence of subretinal fluid on OCT and/or hot spots of leakage on fluorescein angiography, were observed in an additional 27 family members (26%). In 4 out of 17 previously non-affected subjects (24%) from the 24 individuals that were followed up after more than 20 years we found more severe abnormalities.

Conclusion: Extensive ophthalmological phenotyping resulted in the detection of (suggestive) CSC in 52% of family members of CSC patients. Genetic factors may play an important role in these specific CSC cases. Moreover, during follow-up progressive disease can occur in a noteworthy number of patients.

INTRODUCTION

Central serous chorioretinopathy (CSC) is a chorioretinal disease characterised by a detachment of the neurosensory retina due to serous subretinal fluid (SRF). The disease is hypothesised to occur as a result of hyperpermeability and thickening of the choroid (pachychoroid), and subsequent damage to the retinal pigment epithelium (RPE).[1-5] Exposure to exogenous corticoids is the most important risk factor for CSC, with odds ratios of up to 37.[6-8] Genetic risk and protective factors have also been identified in chronic CSC, although the exact pathogenetic mechanism of the disease is still unclear.[9-13] Single nucleotide polymorphisms (SNPs) in the *age-related maculopathy susceptibility 2* (*ARMS2*), *complement factor H* (*CFH*), *cadherin 5* (*CDH5*), and *nuclear receptor subfamily 3 group C member 2 (NR3C2,* mineralocorticoid receptor) genes, and the absence of the *complement component 4B* (*C4B*) gene have been associated with an increased risk for CSC in Caucasians.[10-13] The risk-conferring *CFH* association was also observed in an Asian cohort.[9] Additionally, the presence of 3 copies of the *C4B* gene and several other SNPs in the *CFH* gene were associated with a protective effect for CSC.[11, 13] Familial occurrence of CSC has been described previously, but its occurrence appears to be rare.[14-17] A recent study described a thickened choroid (>395 μm) in 50% of eyes from family members of CSC patients, suggestive of an autosomal-dominant inheritance pattern for pachychoroid.[18]A previous study in which ophthalmological imaging was performed in at least 2 members per family described multiple affected family members in 52% of 27 families and fundus lesions suggestive of CSC in 44% out of 80 screened unaffected relatives.[15] That work, performed over 20 years ago, did not address whether any progression was observed in the unaffected family members that had suspected lesions. In our current study, through extensive ophthalmological imaging in newly recruited families with multiple members affected by CSC, we aimed to corroborate earlier observations on the occurrence of suspected lesions in unaffected family members. Moreover, we followed up a number of individuals with reported suspected lesions over 20 years ago in order to ascertain whether these individuals demonstrated any sign of progression of the disease.

MATERIALS AND METHODS

Subject selection

In this multicenter prospective cohort study, 103 subjects from 23 families including probands were phenotyped either at the department of Ophthalmology of the Radboud University Medical Center (Nijmegen, the Netherlands), the Leiden University Medical Center (Leiden, the Netherlands), or the Rotterdam Eye Hospital (Rotterdam, the Netherlands). All patients were recruited at the outpatient clinic of the participating hospitals, after the proband had reported a history of having additional family members affected by CSC. Thirty subjects (from 6 families)

out of 103, were previously phenotyped at the department of Ophthalmology of the Leiden University Medical Center, before the era of OCT and multimodal imaging, using only indirect ophthalmoscopy and fluorescein angiography (FA), and were invited for a long-term follow-up visit. At the previous visit, findings characteristic for CSC were seen in 9 subjects (30%) and a suspicion of CSC in 16 subjects (53%).[15] To update and extend the available ophthalmological information for these patients, we were able to include 24 of these 30 subjects for new imaging. Five additional members of these families were also recruited and studied. Another 72 members from 17 other families, in which the proband reported either a possible or a confirmed CSC diagnosis in 1 or more additional family members were invited for phenotyping (Figure 1).

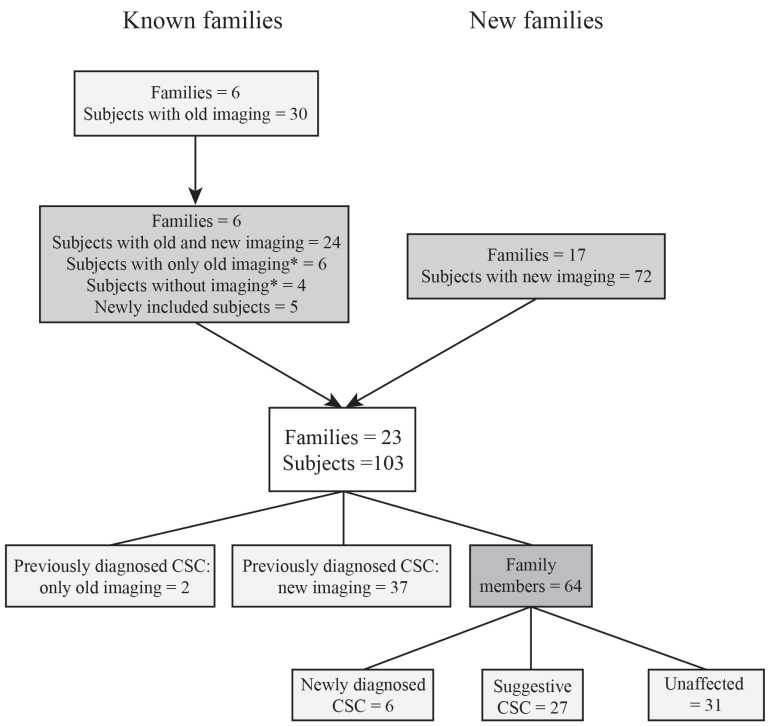

Figure 1. Outline of selection of patients with central serous chorioretinopathy who underwent ophthalmological phenotyping

* Family members who were excluded for ophthalmological analysis, except for the 2 family members in whom CSC had been previously diagnosed.

In total, prior to ophthalmological phenotyping for this study, in 39 subjects (30 males, 9 females) out of the 23 families the diagnosis of CSC had already been confirmed. Out of these 39 patients, 37 patients could be phenotyped again for this study. Two patients with confirmed

diagnosis of familial CSC could not be invited for new phenotyping, but were included in the analysis. Subjects were included from April 2011 to August 2016. Written informed consent for the enrollment was obtained from all subjects. The study adhered to the tenets of the Declaration of Helsinki. Approval of the institutional review board and the ethics committee was obtained for all centers involved.

Ophthalmological imaging

Subjects included in this study received an extensive ophthalmological examination. First, an Early Treatment of Diabetic Retinopathy Study (ETDRS) best-corrected visual acuity (BCVA) measurement was performed. A previously published method was used to convert Snellen BCVA to ETDRS BCVA, when this was not available.[19] Next, pupils were dilated with 1% tropicamide and 5% phenylephrine. Subsequently, indirect ophthalmoscopy and digital color fundus photography (Topcon Corp., Tokyo, Japan) were performed. Using the spectral-domain optical coherence tomography (OCT) device (Spectralis HRA+OCT; Heidelberg Engineering, Dublin, CA, USA) regular OCT, enhanced-depth imaging (EDI-) OCT, fundus autofluorescence, and FA images were obtained. FA imaging was performed at 10, 15, 20, 25, and 30 minutes after oral administration of 10 milliliters of 20% fluorescein, after a subject had fasted for at least 3 hours. When treatment or follow-up was necessary on the basis of the obtained images, subjects were invited for a regular visit to the outpatient clinic. An experienced retina specialist (CJFB) assessed the obtained images in a blinded manner.

Subjects were categorised as having CSC when serous SRF could be detected on OCT and when ≥1 hot spot of leakage or diffuse leakage in combination with irregular RPE window defects was present on FA. No signs of either polypoidal choroidal vasculopathy or age-related macular degeneration, or other atypical findings could be present. In subjects who could not be reinvited for phenotyping and for whom OCT images were not available, a description of a serous neurosensory detachment on ophthalmoscopy in combination with FA abnormalities was considered confirmative for the diagnosis of CSC. Based on multimodal imaging, a diagnosis of a clinical picture suggestive of a CSC background (suggestive CSC) could be established in subjects who did show RPE abnormalities either typical for CSC without SRF on OCT or typical for pachychoroid pigment epitheliopathy.[5]

Choroidal thickness (CT) was defined as the distance from the outer part of the hyperreflective RPE layer to the hyperreflective line of the inner surface of the sclera, and was assessed manually using the caliper tool provided by the Spectralis HRA+OCT machine in subjects for whom EDI-OCT was available. Subjects with a CT of >395 μm were considered to have pachychoroid.[18]

Statistical analysis

Statistical analyses were performed in IBM SPSS Statistics (version 23.0; IBM Corp., Armonk, NY, USA) and GraphPad Prism (version 5.03; GraphPad Software Inc., La Jolla, CA, USA). The distribution of the ETDRS BCVA and CT values of affected and unaffected eyes, and eyes in which suggestive CSC was found at moment of diagnosis were analysed with the D'Agostino-Pearson omnibus normality test. Non-parametric tests were used for further analysis, when at least 1 of the compared groups was not normally distributed. The CT values of affected and unaffected eyes, and eyes with suggestive CSC at moment of diagnosis were compared using a Kruskal-Wallis test with Dunn's post-hoc comparison adjustment. ETDRS BCVA of affected eyes at moment of diagnosis and at follow-up was compared using Wilcoxon's matched-pairs signed-rank test. P-values <0.05 were considered to be statistically significant.

RESULTS

In total, 23 families with multiple individuals diagnosed with CSC were included in this study, resulting in the inclusion of 103 subjects (Figure 1). This included 39 previously diagnosed CSC patients and 64 undiagnosed family members, who were extensively phenotyped. Families were of variable size, with a range of 2 to 10 family members participating in the study (Figure 2). In 6 families (26%) CSC could be diagnosed in multiple generations, with a father-to-son transmission in 3 families. Reduced penetrance seemed to be present in 2 families, and possible maternal transmission was found in 1 family (data not shown).

Characteristics of previously diagnosed CSC patients

Thirty-nine patients (30 males, 9 females), who were previously diagnosed with CSC, were phenotyped in this study. The age at diagnosis of these patients was 49 ± 12 years (Table 1). The median ETDRS BCVA of the 63 affected eyes at diagnosis was 80 letters (20/25; Q1:70-Q3:85) and 85 (20/20; Q1:81-Q3:89) in the 15 unaffected eyes. The median ETDRS BCVA of the 56 affected eyes, for which new ophthalmological phenotyping could be performed after a median follow-up of 38 months (Q1:11-Q3:160), was 80 letters (20/25; Q1:68-Q3:85) and 84 (20/21; Q1:78-Q3:89) in the 12 unaffected eyes, which did not differ from the ETDRS BCVA at the moment of CSC diagnosis.

In 14 of the 39 patients (36%), a history of hypertension was present and in a total of 16 patients (41%) cardiovascular diseases had been previously diagnosed. Three patients were clinically diagnosed with depression. One patient was diagnosed with Cushing's disease, after she had presented with CSC, and 3 patients were previously known with amblyopia. A total of 4 patients (10%) reported steroid use within a year before the first diagnosis of CSC, out of whom 3 patients had received a renal transplant, for which low-dose steroids were prescribed at the moment of ophthalmological phenotyping.

Table 1. Clinical characteristics of different subgroups

	Known CSC	New CSC	Suggestive CSC	Unaffected	Total
Number of patients	39	6	27	31	103
% Males	77%	50%	44%	42%	56%
Mean age ± SD (years)	49 ± 12	48 ± 15	53 ± 9	52 ± 11	51 ± 11
History of cardiovascular diseases	41%	50%	52%	36%	43%
History of depression	8%	0%	0%	0%	3%
Hypertension	36%	33%	44%	23%	34%
Steroid use <1 year before diagnosis (%)	4/39 (10%)	0	5/27 (18%)	7/31 (23%)	16/103 (16%)

Abbreviations: CSC: central serous chorioretinopathy; SD: standard deviation.

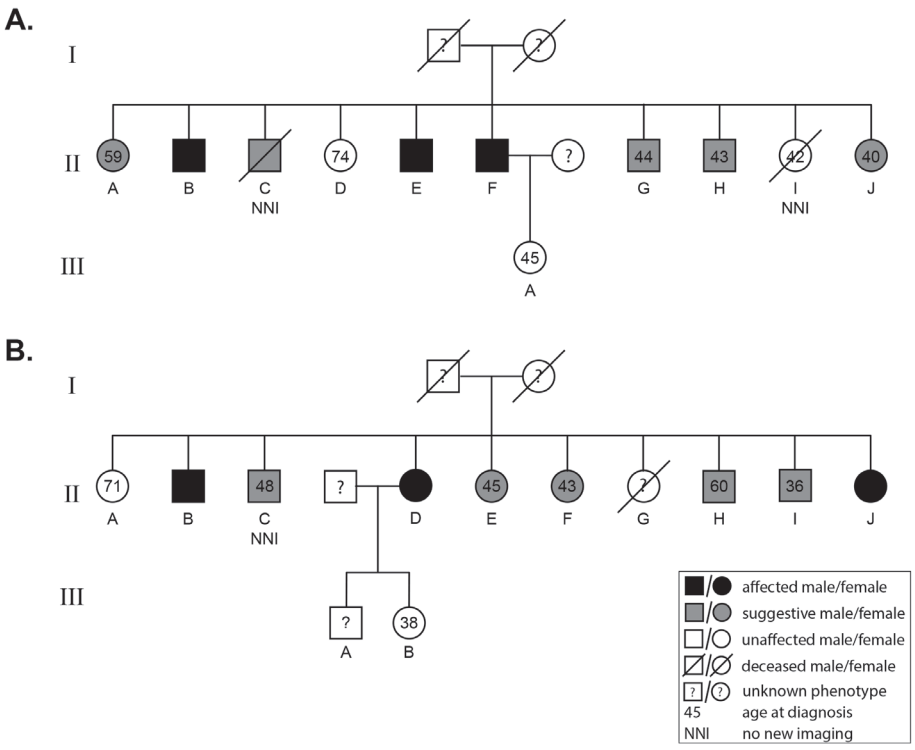

Figure 2. Pedigrees of 2 central serous chorioretinopathy families with multiple affected family members All family members were phenotyped again for this study with the exception of the individuals indicated with NNI (no new images).

Out of the 39 patients, 23 had previously received treatment in a total of 27 eyes: 18 eyes received photodynamic therapy, 11 eyes underwent high-density subthreshold micropulse laser, and 6 eyes were treated with focal thermal laser. In 8 other eyes of 7 patients intravitreal injections with either bevacizumab (7 eyes) or ranibizumab (1 eye) were performed, because of either the suspicion or the presence of a choroidal neovascularisation. Out of these 8 eyes, 2 eyes of 2 different patients exclusively received intravitreal anti-vascular endothelial growth factor injections, after which SRF disappeared in 1 eye. The remaining 6 eyes also received other types of treatment, after which SRF leakage disappeared in 5 eyes (83%). Thirteen eyes had received treatment with a single treatment modality, which lead to complete resolution of SRF in 8 eyes (62%), whereas 14 eyes were treated with multiple treatment modalities, leading to an absence of SRF in 10 eyes (71%). The performed treatments and outcome of these treatments have been summarised in Table 2.

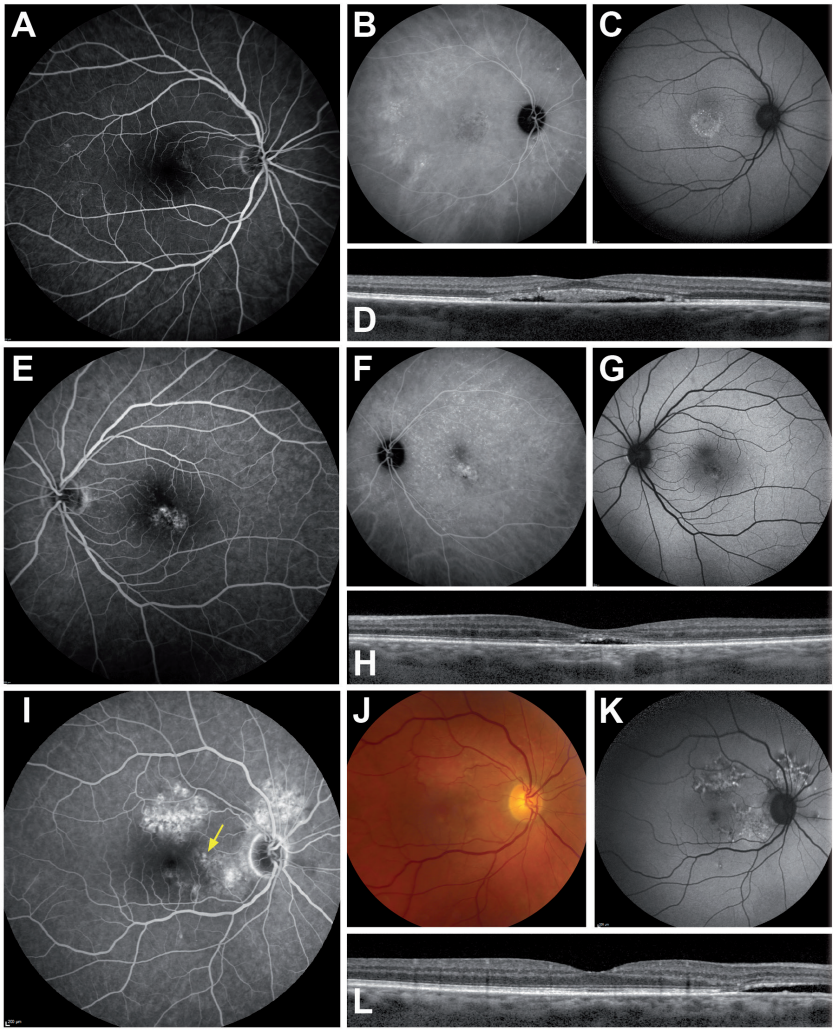

Figure 3. Spectrum of observed phenotypes in previously diagnosed patients with central serous chorioretinopathy

A-D. Multimodal imaging of the right eye of a 40-year-old female patient with unilateral chronic central serous chorioretinopathy (CSC). On intravenous fluorescein angiography (FA; A), some hyperfluorescent changes, but no clear hot spot of leakage could be detected. Indocyanine green angiography (ICGA; B) showed areas of hyperfluorescent changes, which were larger compared to changes on FA. Fundus autofluorescence (FAF; C) imaging revealed hyperautofluorescent changes in the fovea. The enhanced-depth imaging optical coherence tomography (EDI-OCT; D) scan showed a foveal accumulation of subretinal fluid (SRF), with the presence of debris within the SRF. Moreover, dilated vessels in Haller's layer (pachyvessels) were present, and the subfoveal choroidal thickness (CT) was 513 µm.

E-H. Multimodal imaging of the left eye of a 55-year-old male patient with unilateral chronic CSC. Intravenous FA (E) revealed foveal hyperfluorescent changes with a hot spot of leakage. ICGA imaging (F) showed diffuse zones of hyperfluorescent changes, without signs of either polypoidal choroidal vasculopathy or a choroidal neovascularisation. On FAF (G), mostly hypoautofluorescent changes were present foveally. On EDI-OCT (H), a foveal SRF accumulation and pachyvessels could be detected. Subfoveal CT was 420 µm. **I-L.** Multimodal imaging of the right eye of a 76-year-old male patient with bilateral chronic CSC. Oral FA (I) revealed multiple hyperfluorescent areas, with a possible hot spot of leakage nasally of the fovea (arrow). Fundus photography (J) showed multiple areas of alterations of the retinal pigment epithelium. On FAF (K), both hyper- and hypoautofluorescent changes were present. On EDI-OCT (L), SRF could be observed nasally of the fovea. A large pachyvessel could be found below the area of leakage on FA. Subfoveal CT was 245 µm, at that moment.

Table 2. Performed treatments for central serous chorioretinopathy and outcome of these treatments, in 23 treated probands

Type of treatment(s)	Number of patients	Number of eyes	No SRF (leakage) at final follow-up
Treatment with one treatment modality	**13**	**13**	**8/13 (62%)**
Focal thermal laser	2	2	2/2 (100%)
HSML	4	4	2/4 (50%)
Intravitreal bevacizumab injections	2	2	1/2 (50%)
PDT	5	5	3/5 (60%)
Treatment with multiple treatment modalities	**14**	**14**	**10/14 (71%)**
Focal thermal laser + intravitreal bevacizumab injections + PDT	1	1	1/1 (100%)
Focal thermal laser + intravitreal bevacizumab injections	1	1	0/1 (0%)
Focal thermal laser + PDT	2	2	2/2 (100%)
HSML + PDT	6	6	4/6 (67%)
HSML + intravitreal ranibizumab injections + PDT	1	1	0/1 (0%)
Intravitreal bevacizumab injections + PDT	2	3	3/3 (100%)

Abbreviations: HSML: high-density subthreshold micropulse laser; PDT: photodynamic therapy; SRF: subretinal fluid.

A wide range of phenotypical characteristics typical for CSC could be detected on multimodal imaging of the 39 previously diagnosed CSC patients (Figure 3). Either bilateral SRF on OCT or bilateral hot spots of leakage on FA was detected in 24 patients (62%). Until the moment of phenotyping for this study a recurrence of SRF leakage had appeared in 15 patients (38%). On multimodal imaging, a unilateral epiretinal membrane could be observed in 2 patients, choroidal folds were found in both eyes of 1 patient, and cuticular drusen were observed bilaterally in another patient. Median CT in all 48 eyes of 25 patients, for whom EDI-OCT was available, was 323 µm (Q1: 275.5-Q3:365.5). Median CT in the 38 affected eyes was 325 µm (Q1:279-Q3:361), whereas median CT in the 10 unaffected eyes was 287 µm (Q1:247-Q3:372). Pachychoroid could be detected in 6 eyes of 5 patients.

Phenotyping of family members

Sixty-four family members (28 males, 36 females), in whom no prior CSC was diagnosed, were phenotyped for this study. Medical history of these subjects revealed hypertension in 22 subjects (34%) and other cardiovascular diseases in 9 other subjects (14%; total 48%). One subject had received 2 panretinal laser treatments because of a central retinal artery occlusion with secondary ischemia. Twelve of these screened family members (19%) reported steroid use within a year before screening. No link of the outcome of phenotyping with either steroid use or medical history could be detected.

Based on serous SRF on OCT and corresponding abnormalities on multimodal imaging, 6 screened family members (9%) were diagnosed with CSC. Mean age at diagnosis of these 3 males and 3 females was 48 ± 15 years (Table 1), and median ETDRS BCVA was 84 letters (20/21; Q1:80-Q3:85) in the 7 affected eyes, and 85 (20/20; Q1:71-Q3:89) in the 5 unaffected eyes (Table 3). Moreover, multimodal imaging showed RPE changes suggestive of CSC in 27 screened subjects (42%). The mean age at diagnosis was 53 ± 9 years for these 27 suggestive CSC subjects, and 44% of these subjects were male (Table 1).

Table 3. Ophthalmological characteristics of different subgroups

	Known CSC	New CSC	Suggestive CSC	Unaffected	Total
Affected subjects	**39**	**6**	**27**	**31**	**103**
Amblyopia	3	-	1	1	5
Bilateral choroidal folds	1	1	1	-	3
Bilateral cuticular drusen	1	-	2	-	3
Central retinal artery occlusion	-	-	1	-	1
Glaucoma	1	-	-	-	1
Ocular hypertension	1	1	-	-	2
Unilateral epiretinal membrane	2	1	2	-	5
Unaffected eyes					
Number of eyes with available BCVA/CT	15/10	5/4	10/10*	62/60	92/84
Median BCVA (ETDRS) (Q1-Q3)	85 (81-89)	85 (71-89)	87 (73-90)	89 (82-91)	88 (81-90)
Median BCVA (Snellen equivalent)	20/20	20/20	20/18	20/17	20/17
Median CT in µm (Q1-Q3)	287 (247-372)	339 (268-386)	270 (252-332)	277 (227-334)	280 (240-335)
Eyes with suggestive CSC					
Number of eyes with available BCVA/CT	-	-	43/41	-	43/41
Median BCVA (ETDRS) (Q1-Q3)	-	-	89 (85-91)	-	89 (85-91)^{ns}
Median BCVA (Snellen equivalent)	-	-	20/17	-	20/17
Median CT in µm (Q1-Q3)	-	-	272 (237-337)	-	272 (237-337)^{ns}
Affected eyes					
Number of eyes with available BCVA/CT	63/38	7/6	-	-	70/44
Median BCVA (ETDRS) (Q1-Q3)	80 (70-85)	84 (80-85)	-	-	80 (70-85)†
Median BCVA (Snellen equivalent)	20/25	20/21			20/25
Median CT in µm (Q1-Q3)	325 (279-361)	320 (296-376)	-	-	325 (282-360)‡

Abbreviations: BCVA: best-corrected visual acuity; CSC: central serous chorioretinopathy; CT: choroidal thickness; ETDRS: Early Treatment of Diabetic Retinopathy Study, ns: non-significant compared to unaffected eyes.

*: one eye was excluded due to poor visual acuity after a central retinal artery occlusion; p-value compared to unaffected eyes: ‡ <0.05; † <0.001.

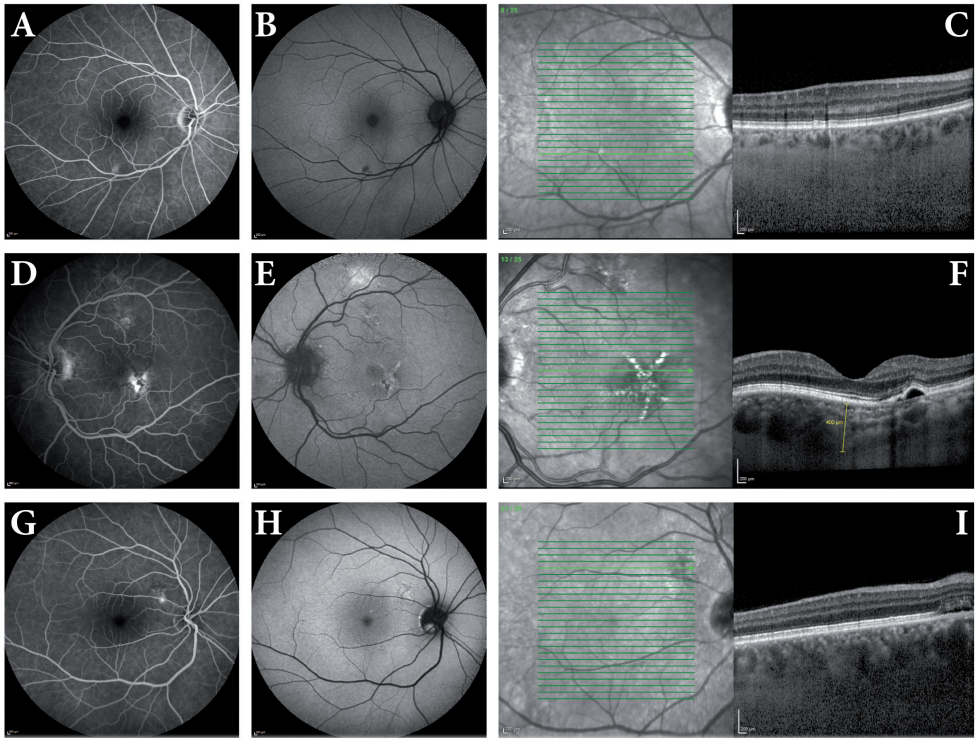

Figure 4. Spectrum of observed phenotypes in family members of central serous chorioretinopathy patients
Various phenotypes resembling central serous chorioretinopathy (CSC) were observed in family members of CSC patients.

A-C. Multimodal imaging of the right eye of a 61-year-old female patient. On oral fluorescein angiography (FA; A) and fundus autofluorescence (FAF; B) imaging, subtle hyper(auto)fluorescent changes suggestive of CSC could be detected inferior of the fovea. Moreover, optical coherence tomography (OCT; C) showed subtle retinal pigment epithelium abnormalities, and a subfoveal choroidal thickness (CT) of 247 µm. Similar hyperfluorescent lesions on FA and FAF could be detected in the left eye of this patient.

D-F. Multimodal imaging of the left eye of a 60-year-old female patient. On oral FA (D) diffuse areas of hyperfluorescence were found without an obvious hot spot of leakage, which was considered to be suggestive of CSC. Both hyper- and hypoautofluorescent changes were present on FAF (E), with an area of likely prior subretinal fluid (SRF) superiorly. On OCT (F), a detachment of the retinal pigment epithelium could be detected temporally of the fovea, without the presence of serous SRF. Subfoveal CT was 400 µm in this eye, and dilated vessels in Haller's layer (pachyvessels) and bilateral choroidal folds were also present in this patient.

G-I. Multimodal imaging of the right eye of a 60-year-old female patient. On oral FA (**G**), a characteristic hot spot of leakage superonasally of the fovea could be detected, with a surrounding area of hyper(auto) fluorescence on FA and FAF (H). On OCT (I), unilateral SRF could be detected superonasally of the fovea. A pachyvessel was present below the SRF, and the subfoveal CT in this eye was 528 µm.

The extent of RPE changes on fundus photography and corresponding either hyper- or hypoautofluorescent changes on fundus autofluorescence imaging in suggestive CSC patients was variable. Abnormalities on FA ranged from subtle hyperfluorescent RPE changes to diffuse areas of hyperfluorescence, but without the presence of SRF leakage (Figure 4). On OCT, either subtle or obvious RPE alterations were present, but without SRF (Figure 4). CT was variable among the suggestive CSC patients, but in only 1 of these patients bilateral pachychoroid was present.

Within the group of screened family members without a previous history of CSC multimodal imaging revealed bilateral cuticular drusen in 2 subjects, bilateral choroidal folds in 2 subjects, a unilateral macular epiretinal membrane in 3 subjects, a macular cystoid lesion in 1 eye, a lamellar macular hole in 1 eye, and findings characteristic for a previous vascular occlusion in 1 eye.

Choroidal thickness in CSC affected and suggestive CSC patients and unaffected individuals

CT data of CSC affected, CSC suggestive, and unaffected eyes in the 3 subgroups were combined (Table 3). CT measurements on EDI-OCT images of 44 affected eyes of patients in whom CSC had been diagnosed showed a median subfoveal CT of 325 µm (Q1:282-Q3:360), while 41 eyes of suggestive CSC patients with both detected abnormalities and available EDI-OCTs, had a median CT of 272 µm (Q1:237-Q3:337), and the 84 unaffected eyes showed a CT of 280 µm (Q1:240-Q3:335). There was a significant difference in CT between affected and both the unaffected and suggestive CSC eyes ($p<0.05$). No significant difference was found between unaffected and suggestive CSC eyes (Table 3).

Pachychoroid could be detected in 18 eyes in 12 family members in both the affected (6 patients, 8 eyes), suggestive CSC (2 patients, 4 eyes), and unaffected (4 family members, 6 eyes) subgroups.

Follow-up of individuals who received ophthalmological phenotyping in 1994-1995

Out of the 30 patients who were previously phenotyped at the department of Ophthalmology of the Leiden University Medical Center,[15] 24 patients could be included for new imaging, which was performed at a median follow-up of 20.8 years (Q1: 20.7-Q3:21.2) after the original phenotyping. In 7 out of these 24 patients, the CSC diagnosis had already been established at baseline. In 4 out of the remaining 17 patients (24%) the outcome of new phenotyping led to a diagnosis of more severe abnormalities. Namely, 2 patients out of 13 patients (15%) in whom RPE alterations were previously observed proved to have active CSC at the moment of new imaging. Moreover, in 1 out of 4 previously non-affected subjects (25%) findings suggestive of CSC were found during our phenotyping, and in another non-affected subject (25%) active CSC was found.

DISCUSSION

Based on the results of this extensive phenotyping, occurrence of CSC and RPE abnormalities suggestive of CSC appears to be relatively common in families with CSC. Family members of CSC patients could therefore be at risk to develop CSC. Within this study, SRF on OCT and/or hot spots of leakage on FA could be detected in 45 of the 103 included subjects (44%), a CSC suspicion in 27 others (26%). In 31 subjects (30%) no signs of CSC were present. The 45 individuals affected by CSC included 6 previously undiagnosed family members of diagnosed CSC patients. Moreover, in 24% of patients who were invited for a long-term follow-up visit after phenotyping more than 2 decades ago, either progression or new occurrence of CSC could be detected.

Active CSC was found only in a minority of patients in whom findings characteristic for CSC could be detected during ophthalmological screening. Based on the outcome of current ophthalmological screening, none of the screened patients in whom CSC was newly diagnosed required treatment for active CSC. In this study, the range of ophthalmological abnormalities in subjects who were diagnosed as having suggestive CSC was wide (Figure 4) and the clinical relevance of RPE abnormalities suggestive of CSC remains to be elucidated, as similar findings have been described not to be present in healthy controls.[20] Therefore, the exact relevance of the suggestive CSC diagnosis is unclear, as is the exact overlap between suggestive CSC and CSC.[5] The diagnosis of suggestive CSC has features similar to the previously described pachychoroid pigment epitheliopathy, for which either unilateral or bilateral reduced fundus tessellation with overlying RPE changes on fundoscopy and abnormalities on fundus autofluorescence have been described.[5] However, in contrast with pachychoroid pigment epitheliopathy an increased subfoveal CT was not seen in all patients with suggestive CSC.[5] In this study, either unilateral or bilateral pachychoroid could be detected in only 6 of the 61 screened family members (10%), for whom EDI-OCT imaging was available. Pachychoroid, using a CT cut-off point of 395 µm, occurred both in CSC affected, CSC suggestive, and unaffected screened family members.[18] Nevertheless, the CT of affected eyes of CSC patients was significantly higher compared to all eyes of unaffected individuals (data not shown). More recently, both the maximal CT and pathologically dilated outer choroidal vessels (in Haller's layer) have been described to be even more typical than CT itself for various diseases within the pachychoroid spectrum including pachychoroid pigment epitheliopathy, CSC, and pachychoroid neovasculopathy.[21, 22] These abnormalities in the choroid, together with a loss in choriocapillary volume, could also be found in our patients (Figure 3 and Figure 4), addressing the importance of the previously reported pachychoroid spectrum.[5, 21, 22]

The underlying pathogenesis of familial clustering of CSC and CSC-like RPE abnormalities remains obscure. Age at CSC diagnosis, gender, steroid use, response to different CSC

treatments, medical history, and presence of choroidal neovascularisation do not differ when comparing the current familial CSC cohort to the occurrence of these factors in previously described non-familial CSC patient cohorts.[1-3, 23-26] This leads to a challenge in recognising family members who may be at risk for developing active CSC, since we did not find phenotypic characteristics in the probands that could be indicative for a familial occurrence of CSC. However, both the percentage of recurrent and/or bilateral CSC among the probands in the CSC families in our study was relatively high, compared to sporadic CSC patients in available literature.[27] The presence of these signs of a relatively severe CSC could lead to a higher risk for family members to develop CSC.

CSC is thought to be a complex, multifactorial disease, in which both environmental and genetic factors play a role, and its inheritance pattern is currently unknown. In our study, most of the affected family members involved sibships, but in some families CSC could be detected in several generations. In most families the mode of inheritance appears to be autosomal-dominant (data not shown). The possible autosomal-dominant inheritance pattern is in line with a recent study on the familial occurrence of pachychoroid, in which this pattern has been suggested.[18] However, the lack of large families with data from multiple generations could lead to an unreliable determination of the inheritance pattern. Ophthalmological phenotyping in multiple generations was only performed when members of the younger generation reported a possible history of CSC or were within the expected age range to develop CSC. Our findings could therefore be an underestimation of the actual occurrence of clinical findings characteristic for CSC in multiple generations.

Based on the familial occurrence of CSC, it has already been suggested that genetic factors might play an important role in CSC. This hypothesis is supported by studies on common genetic variants (SNPs) that have previously been associated with sporadic CSC.[9-11] SNPs in the *ARMS2*, *CDH5*, and *CFH* genes and copy number variations in the *C4B* gene involved in extracellular matrix, cell adhesion, and complement system, respectively, have already been associated with CSC.[9-11] The detection of (suggestive) CSC in 52% of family members of CSC patients reported in this study may indicate that there is also a role for more severe rare genetic variants in CSC. This has already been described in families with age-related macular degeneration, a disease that shows overlapping features with CSC.[28]

In conclusion, CSC and lesions that could be predisposing to CSC can cluster in families, which may indicate that family members of CSC patients are at risk to develop the disease. However, many of these individuals may remain asymptomatic. During a follow-up at which multimodal imaging is performed, progression of disease can occur in a noteworthy number of patients. Comparing next generation genetic sequencing data of both affected and unaffected family members, ideally from multiple generations, may shed a light on which genetic factors are

involved in familial CSC. In future studies, a combination of extensive phenotyping and in-depth genotyping can provide new clues on the etiology of CSC.

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2.2

ASSOCIATION OF A HAPLOTYPE IN THE *NR3C2* GENE, ENCODING THE MINERALOCORTICOID RECEPTOR, WITH CHRONIC CENTRAL SEROUS CHORIORETINOPATHY

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ABSTRACT

Importance: Chronic central serous chorioretinopathy (cCSC) is a chorioretinal disease with unknown disease etiology. The glucocorticoid receptor and the mineralocorticoid receptor, 2 glucocorticoid-binding receptors, might be involved in the pathogenesis of cCSC.

Objective: To assess the association of functional variants and haplotypes in the glucocorticoid receptor (*NR3C1*) and mineralocorticoid receptor genes (*NR3C2*) with cCSC.

Design: Case-control genetic association study. Selected variants in *NR3C1* (rs56149945, rs41423247, rs6198) and *NR3C2* (rs2070951, rs5522) were genotyped using KASP genotyping.

Setting: General community, 3 referral university medical centers, outpatient care.

Participants: Three hundred thirty-six cCSC patients and 1314 unaffected controls.

Main outcome measure: Genetic associations of 3 *NR3C1* variants and 2 *NR3C2* variants with cCSC.

Results: After correction for multiple testing, rs2070951 in the *NR3C2* gene was significantly associated with cCSC (p=0.004, OR=1.29, 95% confidence interval (CI) [1.08-1.53]). Moreover, the GA haplotype of single nucleotide polymorphisms rs2070951 and rs5522 in *NR3C2* conferred risk for cCSC (p=0.004, OR=1.39, 95% CI [1.15-1.68]), whereas the CA haplotype decreased risk for cCSC (p<0.001, OR=0.72, 95% CI [0.60-0.87]). Three known variants in *NR3C1* that alter the activity of the glucocorticoid receptor (rs56149945, rs41423247, rs6198) were not associated with cCSC.

Conclusions and Relevance: In this study, the variant rs2070951 and the GA haplotype in *NR3C2* are associated with an increased risk for cCSC. This is the first genetic study supporting a possible role for the mineralocorticoid receptor in the pathogenesis of cCSC. Since these haplotypes have previously been associated with perceived stress, this study provides a clue bridging clinical risk factors for cCSC to underlying genetic associations.

INTRODUCTION

In central serous chorioretinopathy (CSC), it has been suggested that dysfunction of the retinal pigment epithelium (RPE) due to congestion, thickening, and hyperpermeability of the underlying choroid leads to subretinal fluid accumulation with an associated detachment of the neuroretina.[1-5] The exact etiology of the disease is currently unknown, but clinical associations point towards an involvement of steroid signaling. Endogenous hypercortisolism (Cushing's syndrome), exogenous glucocorticoid exposure, and possibly stress and type A personality are associated with CSC.[6-11] It has been hypothesised that the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR), 2 glucocorticoid-binding receptors, may also be involved in the pathogenesis of CSC.[2]

The involvement of the MR in the pathogenesis of CSC has been suggested based on the results of studies in rats, in which choroidal findings similar to those seen in CSC occurred after intravitreal injection of either corticosterone or aldosterone.[12] MR involvement was further supported by ophthalmological findings in patients with primary hyperaldosteronism (Conn's syndrome).[13] Moreover, studies evaluating the administration of MR antagonists in patients have shown possible beneficial effects.[12, 14-17] However, clinical results were variable and non-permanent, and no prospective randomised placebo-controlled trials have been performed to date to study the role of MR antagonists in CSC treatment.[2, 16]

The GR is the most widely expressed cortisol receptor in the body, it regulates metabolism and the cardiovascular system, and it plays a role in immune suppression and stress response.[18] As stress and both exogenous and endogenous hypercortisolism may be involved in the etiology of CSC,[4, 7, 10, 11] the GR may also be an interesting player in the pathogenesis of CSC.

There are several genetic variants in the genes encoding the MR and GR that are known to alter the MR and GR protein activity.[19-26] The MR is encoded by the *NR3C2* gene, consisting of 10 exons, with 2 alternative 5'-UTR exons 1α and 1β allowing tissue-specific promoter activation.[27] The GR is encoded by the *NR3C1* gene, consisting of 10 exons of which 1-9α are translated into the functional GRα receptor.[22] In this study we assessed whether genetic variants in *NR3C2* (rs2070951 and rs5522) and *NR3C1* (rs56149945, rs41423247, and rs6198) are associated with cCSC.

MATERIALS AND METHODS

We included 336 cCSC patients in this study. Phenotyping of cCSC patients was performed by an experienced retina specialist (CJFB) and was based on a complete ophthalmological examination, including fundoscopy, optical coherence tomography (OCT), fluorescein angiography (FA), and indocyanine green angiography (ICGA). The patients showed the most typical clinical cCSC characteristics (serous subretinal fluid affecting the fovea on OCT, with a disease period of >3 months, ≥ 1 area of 'hot spot' leakage or diffuse leakage in combination with irregular RPE window defects on FA, and corresponding hyperfluorescence on ICGA), described as phenotypic subgroup 1 in a previous paper on genetic associations in cCSC.[28] Patients with high myopia, evidence of choroidal neovascularisation, polypoidal choroidal vasculopathy, and other atypical findings were excluded. For this study, neither previous nor current steroid use was considered an exclusion criterion.The patient cohort consisted of 234 patients from the Radboud University Medical Center (Nijmegen, the Netherlands), 72 patients from the Leiden University Medical Center (Leiden, the Netherlands), and 30 patients from the University Hospital of Cologne (Cologne, Germany). Unaffected individuals enrolled in the European Genetic Database (EUGENDA; www.eugenda.org) were used as controls (n=1314). Controls had no signs of CSC and age-related macular degeneration, when evaluated by multimodal imaging. The study adhered to the tenets of the Declaration of Helsinki, and was approved by the institutional review boards and the ethics committees of all centers involved. Written informed consent was received from all participants.

Genotyping of selected variants was performed using KASP assays (LGC Genomics; Berlin, Germany) according to manufacturer's instructions. Specific primers with FAM and VIC labels were designed per variant (*NR3C1*: rs6198, rs5614994, rs41423247; *NR3C2*: rs5522, rs2070951) and PCR conditions per primer pair were provided by LGC. Data was read out with the 7900HT Fast Real-Time PCR system (Applied Biosystems by Life Technologies, Austin, TX, USA) and was analysed with SDS (version 2.4, Applied Biosystems).

In IBM SPSS Statistics (version 22; SPSS Inc., Chicago, IL, USA), Pearson's Chi-square test was used to compare both the genotype and allele frequencies between cases and controls. Bonferroni correction for multiple testing was performed for 5 variants and p-values <0.01 were considered statistically significant. Logistic regression was performed for the associated rs2070951 variant with Firth's bias-corrected likelihood ratio test implemented in EPACTS (Efficient and Parallelizable Association Container Toolbox, http://genome.sph.umich.edu/wiki/EPACTS, v3.2.6), correcting for gender.[29]

Table 1. Demographic characteristics of the cCSC patients and controls

	n	Mean age ± SD (years)	Males (%)
cCSC cases			
Nijmegen	234	52 ± 9	188 (80%)
Cologne	30	50 ± 9	24 (80%)
Leiden	72	52 ± 10	62 (86%)
Total	336	52 ± 10	274 (81,5%)
Controls	1314	70 ± 7	549 (42%)

Abbreviations: cCSC: chronic central serous chorioretinopathy; SD: standard deviation

Using a haplotype analysis, the combined effect of the 2 variants in *NR3C2* was assessed. Haplotype analysis was performed using R (version 3.0.2) using the haplo.stats package (version 1.6.8). The 2 most frequent haplotypes were separately used as a reference in the haplo.cc command, to determine odds ratios (ORs) for both haplotypes. A logistic regression analysis (haplo.glm) including gender and haplotypes was performed using the most common haplotype as a reference. Only haplotypes with a frequency of >5% are shown. P-values <0.05 were considered to be statistically significant. Power analysis was performed with CaTS (version 0.0.2), using a multiplicative model in a joint analysis.[30] The power per variant was calculated based on the minor allele frequency in controls, a disease prevalence of 0.0001, and a variable genotype relative risk (1-2.6), and the graph was created with Graphpad Prism (version 5.03, Graphpad Software, La Jolla, California, USA,).

RESULTS

The demographic characteristics of the patients and controls enrolled in this study are summarised in Table 1. All described variants were in Hardy-Weinberg equilibrium, both for controls and CSC patients. No statistically significant associations between cCSC and variants in the *NR3C1* gene (rs56149945, rs41423247, rs6198) were found (Table 2). After correction for multiple testing (p<0.01), a significant association between cCSC and the rs2070951 variant in the *NR3C2* gene was observed (p=0.004, OR=1.29, 95% confidence interval (CI) [1.08-1.53]). No association between the variant rs5522 in *NR3C2* and cCSC was found (Table 2).

Table 2. Association analysis of variants in NR3C1 and NR3C2 in cCSC patients

	Gene	Location	Major/ Minor allele	MAF controls	MAF patients	Genotype p-value	Allelic p-value	OR (95% CI)
rs56149945	*NR3C1*	Exon 2	A/G	0.0415	0.0357	0.37	0.50	0.86 (0.55-1.34)
rs41423247	*NR3C1*	Intron 2	C/G	0.360	0.390	0.16	0.15	1.14 (0.95-1.35)
rs6198	*NR3C1*	Exon 9 UTR	A/G	0.169	0.158	0.334	0.48	0.92 (0.73-1.16)
rs2070951	*NR3C2*	c.-2	C/G	0.464	0.527	0.008	0.0040	1.29 (1.08-1.53)
rs5522	*NR3C2*	Exon 2	A/G	0.129	0.137	0.84	0.57	1.08 (0.83-1.38)

Abbreviations: cCSC: chronic central serous chorioretinopathy; CI, confidence interval; MAF: Minor allele frequency; OR: odds ratio; UTR: untranslated region
Bonferroni correction for multiple testing was performed for 5 variants and p-values < 0.01 were deemed significant

Haplotype analysis of the *NR3C2* SNPs rs2070951 and rs5522 showed a significant association with a decreased risk for cCSC for the CA haplotype (p<0.001, OR=0.72, 95% CI [0.60-0.87]) and an increased risk for the GA haplotype (p=0.004, OR=1.39, 95% CI [1.15-1.68]) (Table 3). To account for potential confounding effects of gender between cases and controls we corrected for this factor in a logistic regression model. When including this variable in the model, the association of rs2070951 was independent of gender (p=0.009, OR=1.28, 95% CI [1.16-1.41]). Similarly, when correcting for gender in the haplotype analysis, setting the most common haplotype (GA) as reference, the association of the CA haplotype remained significantly associated with cCSC (p=0.002, OR=0.73, 95% CI [0.66-0.81]).

A multiplicative model was used to calculate the power of the study, since this model produces a genetic relative risk score that is an estimation of the OR of an allelic model.[31] For each variant the power of detecting an association was calculated for the current cohort size. For all variants genotype relative risks <2 could be detected with 80% power using this cohort. The detection limits of the genetic relative risk at 80% power were 1.35, 1.36, 1.46, 1.52, and 1.95 for rs2070951, rs41423247, rs6198, rs5522, and rs56149945, respectively (Figure 1).

Table 3. Haplotype analysis of *NR3C2* in cCSC patients

Haplotype	rs2070951	rs5522	p-value	Freq Controls	Freq Patients	OR (95% CI)		OR (95% CI)	
H1	C	A	0.00069	0.408	0.336	Base	NA	Protective	0.72 (0.60-0.87)
H2	C	G	0.54	0.127	0.137	NS	1.32 (1.00-1.75)	NS	0.95 (0.73-1.24)
H3	G	A	0.0037	0.463	0.527	Risk	1.39 (1.15-1.68)	Base	NA

Abbreviations: cCSC: chronic central serous chorioretinopathy; CI: confidence interval; Freq: frequency; NA: not annotated; NS: not significant; OR: odds ratio

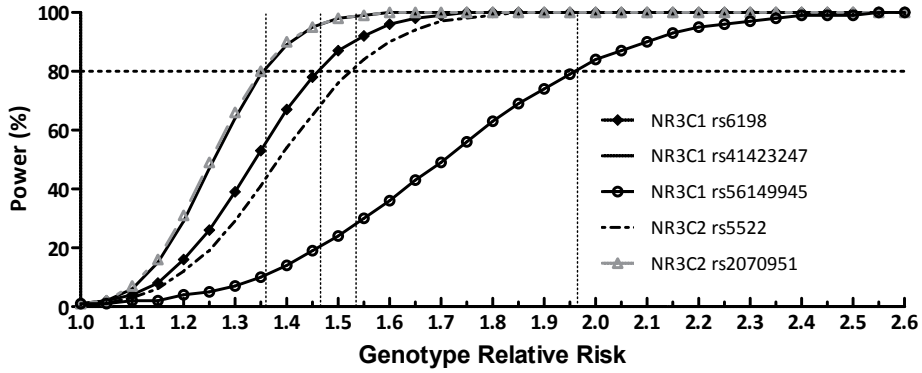

Figure 1. Power calculation for each variant analysed in a cohort of 336 chronic central serous chorioretinopathy patients.
For each variant the power to detect a certain genotype relative risk was assessed with CaTS using a multiplicative model, with minor allele frequency in controls and disease frequency of 0.0001 as input. The 80% power detection limits per variant were 1.35, 1.36, 1.46, 1.52, and 1.95 for rs2070951, rs41423247, rs6198, rs5522, and rs56149945, respectively.

DISCUSSION

In this study, we analysed a possible association of 3 known functional variants in *NR3C1* and 2 known functional variants in *NR3C2* with cCSC. The rs2070951 variant in *NR3C2* was significantly associated with cCSC, whereas the rs5522 variant was not associated with cCSC. The *NR3C2* CA and GA haplotypes were both significantly associated with cCSC, with a protective and a risk-conferring effect, respectively. Odds ratios of the associated *NR3C2* variant and the haplotypes were similar to previously described associations for the *CFH* and *ARMS2* genes, and lower compared to the previously described associations in the *C4* and *CDH5* genes.[28, 32-34] The 3 variants in the *NR3C1* gene (encoding the GR) were not associated with cCSC, which may suggest that MR functionality is more relevant than GR functionality in cCSC disease etiology. However, a larger cohort size is required to exclude the involvement of the 4 variants that were not associated with cCSC in this study.

An abnormal response to the administration of corticosteroids in a subset of (chronic) CSC patients is the strongest risk factor for the disease with described ORs of up to 37.[9] However, the precise mechanism of action of steroids in cCSC disease pathogenesis is unknown. One study showed that both mineralocorticoids and glucocorticoids can activate the MR on choroidal endothelial cells in a rat model.[12] In this animal model, MR activation resulted in vessel dilation via upregulation of the endothelial vasodilatory calcium-dependent potassium channel KCa2.3,[2, 12] producing choroidal thickening that is also commonly observed in cCSC

patients.[2] The MR is also present on RPE cells, and clearance of retinal fluid through the RPE towards the choriocapillaris may be influenced by differences in MR haplotypes.[35] Additionally, on Müller glial cells the MR regulates water homeostasis in the eye, and dysregulation of this mechanism may contribute to the intraretinal fluid observed in a subset of cCSC patients.[2, 36] However, direct GR overactivation without MR involvement seems to be sufficient to induce (chronic) CSC in some patients, since synthetic glucocorticoids with strong selectivity for GR over MR have also described to be a risk factor for the disease.[7, 9]

Both variants in *NR3C2* tested in this study influence the transactivational capacity of the MR after exposure to both cortisol and dexamethasone,[21] and have been shown to affect salivary cortisol levels, especially during the morning cortisol awakening peak.[9, 21, 37] Strikingly, the rs2070951 G-variant, which is associated with cCSC in this study, leads to lower expression of MR and reduced transactivation. One study found that male carriers of the rs2070951 GG genotype in *NR3C2* had a higher systolic blood pressure.[20] This is particularly relevant in the context of cCSC as hypertension is a described risk factor for the disease.[38, 39] The effect of this genetic variant on systolic blood pressure was only observed in male patients, which is interesting since cCSC is much more common in men than in women.[40] In our dataset, the association for rs2070951 was also observed only in male cCSC patients when the data was stratified for gender (p-value in males: 0.020 versus p-value in females: 0.309, data not shown). However, this is likely due to the limited number of female cCSC patients included in the analysis, and based on the current data we therefore cannot definitively conclude that gender differences exist in this genetic association.

Haplotypes in *NR3C2* have previously been associated with differences in perceived chronic stress,[26] another postulated risk factor for cCSC.[11, 41-43] We found that the haplotype (GA) of SNPs rs2070951 and rs5522, which has been previously associated with increased susceptibility to stress,[26] confers risk for developing cCSC in our cohort. The haplotype (CA) that was associated with an optimistic attitude with tendency to recover from or adjust easily to misfortune or change,[44] was protective for the development of cCSC. This could indicate that in cCSC patients with the GA haplotype, both the MR(-mediated pathways) and chronic stress are of significant importance, whereas other not yet identified factors could play a bigger role in cCSC patients with the CA haplotype. Additionally, there is a likelihood that patients with the different haplotypes carry additional unknown genetic variants that might also contribute to an increased or decreased cCSC risk.

Clinical studies that tested the potential of MR antagonists in the treatment of cCSC have yielded mixed results.[14-17] Our findings may partly explain this variable response to MR antagonists, because carriers of different MR haplotypes may respond differently to MR antagonists. The results of our study may lead to the stratification of cCSC patients into subgroups, based on

MR haplotype. Treatment of these stratified patient subgroups with MR antagonists could result in group-specific effects. In patients with the CA haplotype, other (thus far unknown) factors could contribute to the development of CSC to a greater extent. The results of this study may therefore indicate that a more personalised treatment approach in cCSC may be useful. Further studies on the response to treatment in cCSC patients with different MR genotypes are needed to test this hypothesis.

In conclusion, in this study rs2070951 in the *NR3C2* gene, encoding the MR receptor, is significantly associated with cCSC. Additionally, haplotypes of *NR3C2* that have previously been associated with perceived stress also associate with cCSC in this study, which may be a first clue bridging clinical risk factors for cCSC to underlying genetic associations. Functional effects of this variant and the associated haplotype in the MR gene may contribute to the disease mechanisms of cCSC.

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2.3

GENOME-WIDE ASSOCIATION STUDY UNDERLINES THE ROLE OF THE COMPLEMENT SYSTEM IN CHRONIC CENTRAL SEROUS CHORIORETINOPATHY

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ABSTRACT

Purpose: In this study, we performed the first genome-wide association study (GWAS) on chronic central serous chorioretinopathy (cCSC) in order to discover genetic loci associated with the disease and to better understand the etiology of cCSC.
Design: Case-control study.

Participants: 521 cCSC patients and 3,577 population controls.

Methods: We performed a GWAS on cCSC and analysed haplotypes of the complement factor H (*CFH*) gene. Additionally, pathway analyses were performed with MAGMA and VEGAS2, and the effect of genetic variants on gene expression was evaluated with PrediXcan.

Main outcome measurements: Associations of single nucleotide polymorphisms, haplotypes, genetic pathways, and predicted gene expression with cCSC.

Results: One locus on chromosome 1 at the *CFH* gene reached genome-wide significance and was associated with an increased risk of cCSC (rs1329428, $p=3.12x10^{-11}$, odds ratio (OR) = 1.57). The *CFH* haplotypes H1 and H3 were protective for cCSC, whereas haplotypes H2, H4, H5, and the aggregate of rare *CFH* haplotypes conferred increased risk. Pathway analyses showed a significant involvement of the complement cascade and *ARF* pathway in cCSC. Using PrediXcan, we identified significant changes in predicted expression of complement genes *CFH,* complement factor H related 1 (*CFHR1*), complement factor related 4 (*CFHR4*), and membrane cofactor protein (*MCP/CD46*). Additionally, the genes potassium sodium-activated channel subfamily T member 2 (*KCNT2*) and tumor necrosis factor receptor superfamily member 10a *(TNFRSF10A)* were differentially expressed in cCSC patients.

Conclusions: In this first GWAS on cCSC, we identified a locus on chromosome 1 at the *CFH* gene that strongly associated with cCSC and we report novel protective and novel risk-conferring haplotypes in this gene. Pathway analyses were enriched for complement genes and gene expression analysis suggests a role for *CFH, CFHR1, CFHR4, CD46, KCNT2,* and *TNFRSF10A* in the disease. Taken together, our results strongly underscore the importance of the complement pathway in the etiology of cCSC.

INTRODUCTION

Central serous chorioretinopathy (CSC) is characterised by subretinal fluid accumulation between the neuroretina and the retinal pigment epithelium (RPE).[1-3] Patients usually present with clinical symptoms of metamorphopsia and central vision impairment.[4, 5] Although acute CSC can show spontaneous resolution within 3 months, patients with chronic CSC (cCSC) have prolonged presence of fluid accumulation with progressive loss of vision, permanent RPE alterations and a declined quality of life.[4] Generally, CSC occurs more frequently in males (9.9:100,000) compared to females (1.7:100,000) and often presents at an age where patients are still professionally active.[6] The exact etiology of cCSC is unknown, but clinical and experimental studies have implicated dysfunction of the RPE and hyperpermeability of the choroid in CSC.[4] The disease has been associated with the use of corticosteroids, stress, and type A personality. Reports of familial occurrences of cCSC support a genetic component for the disease.[1, 3, 4, 7-11] Genetic studies on cCSC are limited to candidate gene approaches; several associations have been reported with single nucleotide polymorphisms (SNPs) in the *age-related macular degeneration susceptibility 2* (*ARMS2*), *cadherin 5* (*CDH5*), *complement factor H* (*CFH*) and *nuclear receptor subfamily 3 group C member 2* (*NR3C2*) genes, as well as copy number variations in the *complement factor 4B* (*C4B*) gene.[12-16]

Unbiased genome-wide association studies (GWAS) have identified hundreds of genomic loci implicated in complex diseases such as age-related macular degeneration (AMD), myopia, and glaucoma.[17] Such approaches have shed light on the genetic architecture of these diseases, however, establishing functional links between identified loci and disease remains challenging. Besides having direct effects on protein function or structure, SNPs can also influence the expression of nearby (cis) or distal (trans) genes. Such regulatory genetic variants are called expression quantitative trait loci (eQTL).[18] Several eQTL databases, linking genotype information to tissue expression of genes, have been established. One of the largest of these projects is the genotype-tissue expression (GTEx) project in which expression profiles of 44 tissues (version 6) of 449 donors have been collected.[18] These eQTL databases offer functional information on SNPs identified in GWAS, contribute to a better understanding of the studied disease trait and can be used to predict expression in genotyped samples.[19]

In this study, in order to identify new cCSC disease loci and to increase our knowledge on the etiology of the disease, we performed the first cCSC GWAS using 521 cCSC patients and 3,577 population controls. Additionally, we performed pathway analyses to discover new pathways implicated in cCSC. Using publicly available eQTL data we aimed to identify new candidate genes predicted to be differentially expressed in cCSC patients compared to controls.

MATERIALS AND METHODS

Study participants

For all subjects genomic DNA was extracted from blood using standard procedures. In total, 546 cCSC patients recruited from the outpatient clinics of the Radboud University Medical Center, Nijmegen, the Netherlands (n=319), University Hospital of Cologne, Cologne, Germany (n=74), and Leiden University Medical Center, Leiden, the Netherlands (n=153) were included. Grading of all patients was performed by an experienced retinal specialist (CJFB), and was based on extensive ophthalmological examination including fundoscopy, spectral-domain optical coherence tomography, fluorescein angiography, and indocyanine green angiography. Diagnosis of cCSC was based on subgroups 1 and 2 that were previously described.[13] Patients that were included in this study showed the presence of serous fluid on optical coherence tomography in ≥ 1 eye, either unilateral or bilateral RPE irregularities with ≥ 1 hot spots of leakage on fluorescein angiography, and corresponding hyperfluorescence on indocyanine green angiography.[13] Patients diagnosed with acute CSC as recognised by a focal leakage spot (ink blot) or a smokestack pattern on fluorescein angiography, patients with duration of disease of less than 3 months, and patients in whom evidence of another explanatory diagnosis or complication was present, such polypoidal choroidal vasculopathy and/or choroidal neovascularisation, were excluded.

Genotyping data of 3,654 controls was obtained from the Nijmegen Biomedical Study, a population-based survey conducted by the Department for Health Evidence and the Department of Laboratory Medicine of the Radboud University Medical Center. In this study, 21,756 age- and gender-stratified randomly selected inhabitants of the municipality of Nijmegen received an invitation to complete a postal questionnaire on lifestyle and medical history, and to donate an 8.5 ml blood sample in a serum separator tube and a 10 ml EDTA blood sample. The response rate to the questionnaire was 43% (n=9,350), and 69% (n=6,468) of the responders donated blood samples (http://www.nijmegenbiomedischestudie.nl/).[20]

The mean age of cCSC patients and controls was compared with a Mann-Whitney U test, and the gender distribution was compared with a Chi-square test using IBM SPSS Statistics (version 22, SPSS Inc., Chicago, IL, USA). For both test values of $p<0.05$ were deemed significant. This study was carried out in accordance with the tenets of the Declaration of Helsinki and was approved by the local ethics committees of the Radboud University Medical Center, Leiden University Medical Center, and University Hospital of Cologne. Written informed consent was obtained for all subjects involved in the study.

Genome-wide association and haplotype analyses

Genotyping was performed with the OmniExpress-12 or -24 chips and data was imputed with the Haplotype Reference Consortium release 1.1.2016. After stringent quality control, 521 cCSC patients and 3,577 Nijmegen Biomedical Study controls remained for analysis carrying 11,261,291 autosomal and 265,428 X-chromosomal SNPs. Single-variant association analysis of whole-genome SNP genotypes was performed, using the Firth's bias-corrected likelihood ratio test implemented in EPACTS (version 3.2.6, http://genome.sph.umich.edu/wiki/EPACTS), correcting for gender and the first 2 components of the principal component analysis.

Haplotype analysis, combining multiple variants in the *CFH* gene, was performed with the haplo.stats (version 1.7.7) package in R. The SNPs described by Hageman et al. and the *CFH* SNPs that were previously associated with cCSC were used as input.[12, 13, 21] Haplotypes with a frequency >1% were analysed individually, and rare haplotypes (frequency <1%) were aggregated into 1 group. The haplo.glm command was used to obtain the association results of the haplotypes with cCSC, correcting for gender and the first 2 principal components. The analysis was performed using the most common protective haplotype (H1) and cCSC risk carrying haplotype (H2) as references. The frequency of the haplotypes in cases and controls was obtained using the haplo.cc command.

Pathway and PrediXcan analysis

GWAS summary statistics were used to perform competitive gene-set analysis in order to identify pathways associated with cCSC using 2 different programs; MAGMA (version 1.06, https://ctg.cncr.nl/software/magma) and VEGASv2 (version 2, https://vegas2.qimrberghofer.edu.au/vegas2v2).[22, 23] Additionally, PrediXcan (https://github.com/hakyim/PrediXcan) was used to predict gene expression levels based on publicly available eQTL data of GTEx.[24] PrediXcan was performed on all 44 provided GTEx tissues using genetic variants in 17,742 genes. We selected the genes that were significantly associated in at least 1 tissue after Bonferroni correction for multiple testing ($p<0.05/17742$ genes$=2.21\times10^{-6}$) and were nominally associated ($p<0.05$) in at least 50% of the tissues they were expressed in.

Power calculation

Power analysis was performed implementing the effective sample size calculation for alleles [$n_{eff}=4/(1/n_{cases}+1/n_{controls})$][25] in an altered version of the pwr (version 1.2.1) R package provided by Dr. L.G. Fritsche (https://github.com/ilarsf/gwasTools). Power calculation input parameters for the current study were 521 cases and 3,577 controls, $\alpha=5\times10^{-8}$ and $\alpha=1\times10^{-6}$. Top variants were plotted according to their odds ratio (OR) and their MAFs in controls.

RESULTS

Genome-wide association analysis

After quality control, a total of 521 cCSC patients and 3,577 controls (Table 1) were analysed for 11,261,291 autosomal and 265,428 X-chromosomal SNPs. Because of the significant difference in gender between cCSC patients and controls ($p=1.78x10^{-50}$; Table 1,), gender was included as a covariate in the analysis along with 2 principal components of the ancestry analysis.

Table 1. Demographic characteristics of the GWAS cohort

	Discovery cohort		
	n	**Median age in years + Interquartile range**	**Males (%)**
cCSC patients			
Nijmegen	307	51 (15)	242 (79%)
Cologne	71	51 (16)	55 (78%)
Leiden	143	51 (15)	123 (86%)
Total	521	51 (15)	420 (81%)
Controls			
NBS	3577	52 (34)	1630 (46%)
P-value cCSC patients vs Controls		0.192	1.78E-50

Abbreviations: cCSC: chronic central serous chorioretinopathy; GWAS: genome-wide association study; NBS: Nijmegen Biomedical Study.
Age difference between cCSC patients and controls was compared with a Mann-Whitney U test, and gender differences were compared with a chi-square test.

The GWAS identified 20 SNPs that reached genome-wide significance ($p<5.0x10^{-8}$) that all resided at 1 locus on chromosome 1 in the *CFH* gene (lead variant rs1329428, $p=3.12x10^{-11}$; Figure 1, Table 2). Additionally, 6 suggestive signals ($p<1x10^{-6}$) were found on chromosomes 1, 8, 9, 15, 17, and 20 (Figure 1, Table 2). Conditioned analysis of the lead SNP on chromosome 1 did not reveal any other independent signal in the *CFH* gene (data not shown), but did retain the suggestive signal at the *CD46* gene on chromosome 1 (data not shown). This study had sufficient power to detect common variants with OR>1.5, and had more than 80% power to detect the lead variant (data not shown).

Haplotype analysis

To further characterise the association at the *CFH* gene, haplotype analysis was performed. A logistic model corrected for gender and 2 principal components was performed using either the most common cCSC protective (H1) haplotype or the most common cCSC risk-conferring (H2)

haplotype as reference (Table 3). The H2, H4, and H5 haplotypes, all containing the minor allele of the lead variant, were significantly associated with an increased risk of cCSC. The H1 and H3 haplotypes, containing the major allele of the lead variant, were protective. Additionally, the aggregate of rare haplotypes also increased the risk of cCSC. All associations were significant after Bonferroni correction for multiple testing for 10 tests (p<0.005).

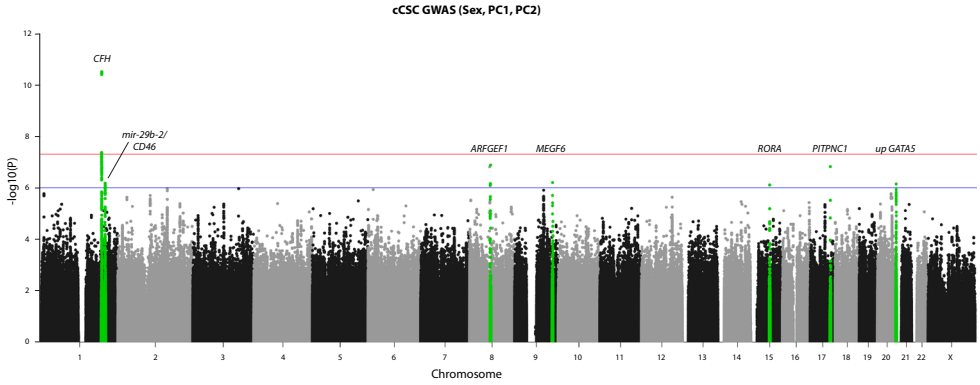

Figure 1. Manhattan plot of genome-wide association study on 521 cCSC patients and 3,577 population controls.

Genome-wide association analysis was performed correcting for gender and 2 principal components. The genome-wide significance line is depicted in blue ($p=5x10^{-8}$), whereas the suggestive line is depicted in red ($p=1x10^{-6}$). Loci with variants that reach the suggestive or genome-wide significance association threshold are depicted in light green.

Gene-set/Pathway analysis

Using the GWAS summary statistics we performed competitive gene-set analysis using 2 programs. MAGMA identified 3 pathways that were significantly associated with cCSC after correction for multiple testing (Table 4). Two of the pathways are implicated in the complement cascade, and 1 is the *ARF* pathway. VEGAS2 identified the same reactome pathways of the complement cascade. However, these findings were not significant after correction for multiple testing ($p<1x10^{-5}$;Table 4).

PrediXcan analysis

For each individual we predicted the effect of genotypes on expression levels of 17,742 genes in 44 different GTEx (version 6) tissues using PrediXcan. The predicted gene expression of genes at the *CFH* locus (*CFH, CFHR1, CFHR4* and *KCNT2*) was significantly different between cCSC patients and controls after correction for multiple testing ($p<2.21x10^{-6}$). Additionally, we observed altered predicted expression of the *CD46* and *TNFRSF10A* genes (Table 5).

Table 2. Top hits in cCSC GWAS study

Top-SNP	chr	bp (hg19)	Major/Minor allele	Gene	MAF cCSC (n=521)	MAF controls (n=3577)	P-value	OR (95% CI)
rs1329428	1	196702810	C/T	CFH	0.543	0.434	3.12E-11	1.57 (1.38-1.80)
rs561753392	8	68131981	C/T	ARFGEF1	0.0070	0.0005	1.33E-07	42.25 (9.55-186.89)
rs76766498	17	65418162	A/G	PITPNC1	0.021	0.005	1.51E-07	5.25 (2.94-9.48)
rs118083394	9	123367552	C/T	MEGF6	0.014	0.002	6.28E-07	8.36 (3.67-19.06)
rs4844392	1	207991209	C/G	mir-29b-2/CD46	0.159	0.230	6.69E-07	0.64 (0.54-0.77)
rs2379120	20	61030580	T/A	upstream GATA5	0.218	0.300	7.14E-07	0.67 (0.57-0.79)
rs541395042	15	61175324	C/T	RORA	0.006	0.00	7.84E-07	206.11 (4.50-9436.68)

Abbreviations: *ARFGEF1*: adenosine diphosphate ribosylation factor guanine nucleotide exchange factor 1; bp: base pair; cCSC: chronic central serous chorioretinopathy; *CD46*: cluster of differentiation 46; *CFH*: complement factor H; chr: chromosome; CI: confidence interval; *GATA5*: GATA binding protein 5; GWAS: genome-wide association study; hg: human genome; MAF: minor allele frequency; *MEGF6*: multiple epidermal growth factor like domains 6; *mir-29b-2*: micro ribonucleic acid 29b-2; OR: odds ratio; *PITPNC1*: phosphatidylinositol transfer protein cytoplasmic 1; *RORA*: retinoic acid receptor-related orphan receptor alpha.

Table 3. Haplotype analysis of the *CFH* gene

Haplotype	rs3753394	rs529825	rs800292	rs3766404	rs1061170	rs203674	rs3753396	rs2284664	**rs1329428**	rs1065489	Freq. Controls	Freq. cCSC patients	P-value Corrected	OR (95% CI)	P-value Corrected	OR (95% CI)
H1	C	G	G	T	C	G	A	C	**C**	G	0.339	0.273	REF	REF	**2.18E-06**	0.64 (0.53-0.77)
H2	C	A	A	T	T	T	A	T	**T**	G	0.221	0.274	**2.18E-06**	1.57 (1.30-1.89)	REF	REF
H3	T	G	G	T	T	T	G	C	**C**	T	0.135	0.094	0.204	0.85 (0.66-1.09)	**2.49E-06**	0.54 (0.42-0.70)
H4	C	G	G	C	T	T	A	C	**T**	G	0.115	0.128	**2.49E-03**	1.43 (1.13-1.80)	0.442	0.91 (0.72-1.15)
H5	T	G	G	T	T	T	A	C	**T**	G	0.052	0.078	**4.61E-05**	1.80 (1.36-2.39)	0.343	1.15 (0.86-1.53)
H6	T	G	G	T	T	G	A	C	**C**	G	0.030	0.025	0.492	1.17 (0.75-1.81)	0.191	0.74 (0.48-1.16)
H7	T	G	G	T	C	G	A	C	**C**	G	0.027	0.018	0.485	0.82 (0.48-1.42)	0.021	0.53 (0.30-0.91)
H8	T	G	G	C	T	T	A	C	**T**	G	0.022	0.027	0.048	1.58 (1.00-2.48)	0.972	1.01 (0.64-1.59)
H9	C	A	A	T	T	T	G	C	**C**	T	0.017	0.017	0.418	1.25 (0.73-2.17)	0.421	0.80 (0.46-1.38)
RARE	*	*	*	*	*	*	*	*	*	*	0.042	0.066	**4.59E-05**	1.99 (1.43-2.77)	0.163	1.27 (0.91-1.77)

Abbreviations: cCSC: chronic central serous chorioretinopathy; *CFH*: complement factor H; CI: confidence interval; Freq: frequency; OR: odds ratio; REF: reference.

Bonferroni correction for multiple testing for 10 haplotypes: 0.05/10=0.005; Analysis was performed correcting for gender and the first 2 principal components.

Table 4. Competitive gene-set analysis MAGMA and VEGAS2

	Pathway name	Genes (n)	Beta	Beta (se)	Nominal P-value	Emperical corrected P-value
MAGMA	reactome_regulation_of_complement_cascade	13	1.29	0.265	5.49E-07	8.90x10-4*
	reactome_complement_cascade	29	0.786	0.179	5.56E-06	7.37x10-3*
	biocarta_arf_pathway	17	0.848	0.194	6.49E-06	8.65x10-3*
VEGAS2	reactome: R-HSA-977606_Regulation_of_Complement_cascade	13	NA		1.03E-07	1.60x10-5
	reactome: R-HSA-166658_Complement_cascade	22			6.09E-07	4.20x10-5

Abbreviations: MAGMA: multi-marker analysis of genomic annotation; NA: not annotated; se: standard error; VEGAS2: versatile gene-based association study 2.

* significant after correction for multiple testing

MAGMA nominal significance threshold is $p<4.12x10^{-5}$ and VEGAS2 empirical significance threshold is $p<1x10^{-5}$

DISCUSSION

In this first unbiased genetic association study on cCSC, we identified a locus for cCSC on chromosome 1, at the *CFH* gene, that had previously been described in 2 targeted candidate gene studies.[12, 13] We discovered novel protective and risk haplotypes in *CFH* and found evidence for involvement of rare *CFH* haplotypes in the disease. Moreover, using gene-set analysis and publicly available expression databases, we uncovered additional evidence for altered regulation of the complement system in cCSC and identified novel candidate genes and pathways implicated in this disease.

So far, the association of cCSC with the *CFH* gene is the only genetic association for cCSC that has been replicated in multiple studies.[12, 13, 26] The effect size of the lead-variant (rs1329428) in *CFH* in this unbiased study was higher compared to our previous targeted study, but lower compared to the study by Miki et al. 2014 (OR=1.57, OR=1.47 and OR=1.79, respectively).[12, 13] Additionally, haplotype analysis of the *CFH* locus confirmed a previously reported protective effect of *CFH*-H3,[13] and identified novel associations with the protective *CFH*-H1 and risk carrying *CFH*-H2, -H4, and -H5 haplotypes. The risk associated with the *CFH*-H5 haplotype was higher than the risk caused by the single lead variant alone and higher than any previously reported SNP in *CFH*, with an OR=1.80 for *CFH*-H5 compared to OR=1.57 for rs1329428. Interestingly, an aggregate of all haplotypes with a frequency <1% also showed a higher risk of

cCSC (OR=1.99). Higher ORs of H5 and the rare haplotype aggregate suggest that other (rare) variants in these haplotypes might play a role in cCSC.

The factor H protein, encoded by the *CFH* gene, is able to block the formation of C3-convertases and therefore is an important regulator of the complement system.[27] The *CFH* gene has been widely studied for its role in AMD and, interestingly, variants that confer risk of AMD are protective for cCSC and vice versa.[13] These genetic associations imply that these 2 diseases might have an opposite disease mechanism, with an overactivation of the complement system in AMD,[27] and a reduced activity of the complement system in cCSC.

In the current study, we further substantiated the involvement of the complement system in cCSC. Pathway analysis with MAGMA and VEGAS2 showed associations of 2 gene-sets of the complement system. We used PrediXcan to predict the expression of genes based on the genotype information of our cCSC patients and controls. Unfortunately, no eye- or retina-specific eQTL databases are publicly available, and these tissues are not yet implemented in GTEx nor in any other large eQTL dataset. Therefore as an indicator for general differences, we used all GTEx tissues and observed that the expression of 4 complement genes (*CFH, CFHR1, CFHR4* and *CD46)* was predicted to be significantly different in cCSC patients compared to controls.

All GTEx tissues that express *CFH* showed an upregulation of *CFH*, suggesting that complement system activity may be reduced in cCSC (Table 5). Depending on the tissue, *CFHR1* showed up- or downregulation, indicating that regulation of this gene might be tissue-specific, while C*FHR4* was consistently down-regulated (Table 5). The factor H related (FHR) proteins, encoded by the *CFHR* genes, show sequence similarities to factor H and can compete with factor H for C3b binding, in this manner influencing complement activation.[28] Deletions of *CFHR1* have been found to be protective for AMD and *CFHR4* deletions have been implicated in atypical hemolytic uremic syndrome,[28] but the genes have not previously been associated with cCSC.

Interestingly, we also observed an association with another complement gene independent of the *CFH* locus: *CD46* was identified as one of the subthreshold hits in the GWAS (Table 2) and PrediXcan predicted up-regulation of *CD46* expression (Table 5). The *CD46* gene encodes the membrane cofactor protein (MCP/CD46), a complement inhibitor that blocks all pathways of the complement cascade through inactivation of C3b and C4b.[29, 30] In light of the clinical characteristics of cCSC, where patients present with hyperpermeability of the choroid and with fluid leakage through the RPE, the involvement of CD46 is particularly interesting. CD46 is an important regulator in the maintenance of epithelial cell barrier integrity through interaction with cadherins and integrins.[30, 31] Previous studies have shown that the activation of CD46 leads to a decrease in transepithelial resistance and loss of tight junctions in intestinal cells, and

Table 5. PrediXcan results, genes differentially expressed between cCSC patients and controls

Gene	Tissues expression (n)	Tissues (50%,P<0.05)	Tissues ($P<2.81x10^{-6}$)	P-value	OR (95% CI)
CFHR4	1	1 (100%)	Liver	1.29E-06	0.91 (0.85-0.98)
CD46	25	21 (84%)	Esophagus_Muscularis	1.79E-06	1.05 (1.03-1.07)
			Skin_Not_Sun_Exposed_Suprapubic	2.26E-06	1.32 (1.18-1.48)
			Heart_Atrial_Appendage	2.39E-06	1.16 (1.09-1.24)
			Colon_Sigmoid	2.57E-06	1.07 (1.04-1.09)
TNFRSF10A	18	15 (83%)	Cells_Transformed_fibroblasts	2.41E-06	0.96 (0.94-0.98)
			Adrenal_Gland	2.60E-06	0.95 (0.93-0.97)
CFHR1	12	9 (75%)	Hypothalamus	1.73E-08	0.78 (0.72-0.85)
			Adipose_Subcutaneous	1.85E-08	1.15 (1.1-1.21)
			Liver	1.87E-07	0.95 (0.93-0.97)
			Cells_Transformed_fibroblasts	1.41E-06	0.79 (0.71-0.87)
KCNT2	5	3 (60%)	Esophagus_Gastroesophageal_Junction	1.62E-07	3.7 (2.27-6.04)
			Nerve_Tibial	4.99E-07	1.13 (1.08-1.18)
CFH	5	3 (60%)	Adipose_Subcutaneous	1.17E-06	1.09 (1.05-1.13)
			Nerve_Tibial	1.63E-06	1.1 (1.06-1.14)

Abbreviations: cCSC: chronic central serous chorioretinopathy; *CD46*: cluster of differentiation 46; *CFH:* complement factor H; *CFHR1/4*: complement factor H related 1/4; CI: confidence interval; GTEx: genotype-tissue expression; *KCNT2:* Potassium Sodium-Activated Channel Subfamily T Member 2; OR: odds ratio; *TNFRSF10A:* tumor necrosis factor receptor superfamily member 10a

a decreased membrane adhesion in RPE cells.[30, 31] The predicted increased *CD46* expression in cCSC patients therefore suggests a downregulation of complement activity, similarly as observed for factor H, and in addition destabilization of the integrity of the RPE, one of the main hallmarks of CSC. Together, both the complement- and epithelial regulatory function of CD46 makes it an interesting protein for further study in cCSC.

Taken together, the associations and predicted altered expression of *CD46, CFH, CFHR1*, and *CFHR4* with cCSC imply a reduced activity of the complement system in cCSC. To date, limited information is available on the actual activity of the complement system in cCSC patients. One study did not find obvious systemic alterations in blood complement components of cCSC patients, but this study had limited power and CD46, FHR1, and FHR4 levels were not measured.[32] Combined with previously described associations of *CFH* and *C4B* and the associations described here,[12-14] a central role for the complement system in the disease mechanism of cCSC emerges. Larger studies measuring systemic complement regulators and activation products in cCSC patients, specifically FH, FHR1, FHR4, C4B, and CD46, are warranted.

Besides consolidating the involvement of the complement system, we also identified a new cCSC candidate pathway and 2 new cCSC candidate genes. MAGMA identified an association between cCSC and the *ARF* pathway. This pathway is involved in ribosomal biogenesis, and activation of the pathway leads to termination of ribosomal RNA production and cell cycle arrest.[33] Proper functioning of the ribosomes is of vital important for the retina due to its high energy demand, and dysfunction of the system might have detrimental consequences. However, the exact role of the *ARF* pathway in the cCSC phenotype remains to be determined. PrediXcan showed decreased expression of *TNFRSF10A* and upregulation of *KCNT2*. Involvement of *TNFRSF10A* in cCSC was suggested previously in our candidate gene study of the main AMD loci. We showed a protective effect for a *TNFRSF10A* variant, which was also observed in this study (OR=0.74, p= 1.47×10^{-5}, data not shown) and the largest AMD GWAS (OR=0.90, p=4.5×10^{-11}).[13, 34] *TNFRSF10A* encodes a member of the TNF-receptor superfamily which is activated upon binding of tumor necrosis factor-related inducing ligand and in this manner regulates cell apoptosis.[35] The *KCNT2* gene encodes a potassium sodium-activated outward rectifier channel (KCNT2) with an unknown function, but a role in pain perception has been suggested based on studies in mice.[36] In the eye, the RPE maintains ion- and water balance in the subretinal space by means of a large variety of ion-channels and transporters.[37] As potassium channels play a pivotal role in this process and KCNT2 is expressed in the eye,[38] it might be one of the channels involved in ion- and water balance. As regulators of apoptosis and ion-balance, the *TNFRSF10A* and *KCNT2* genes are interesting candidate genes for cCSC, but their exact role in the eye remains to be elucidated.

In this study, we describe the first GWAS for cCSC and confirmed the association of genetic variants in *CFH* with the disease. Additionally, we identified *CFHR1*, *CFHR4*, *CD46*, *KCNT2*, and *TNFRSF10A* as cCSC candidate genes due to their genetic associations and predicted altered expression. With *CFH*, *CFHR1*, *CFHR4*, and *CD46* being important regulators of the complement cascade, this study strengthens the involvement of the complement system in cCSC. Further work on the expression of the proteins encoded by these genes is warranted. Additionally, the use of next-generation sequencing techniques, such as exome sequencing, will enable the identification of (rare) coding variants influencing protein function of these genes and might provide more insight in the etiology of cCSC.

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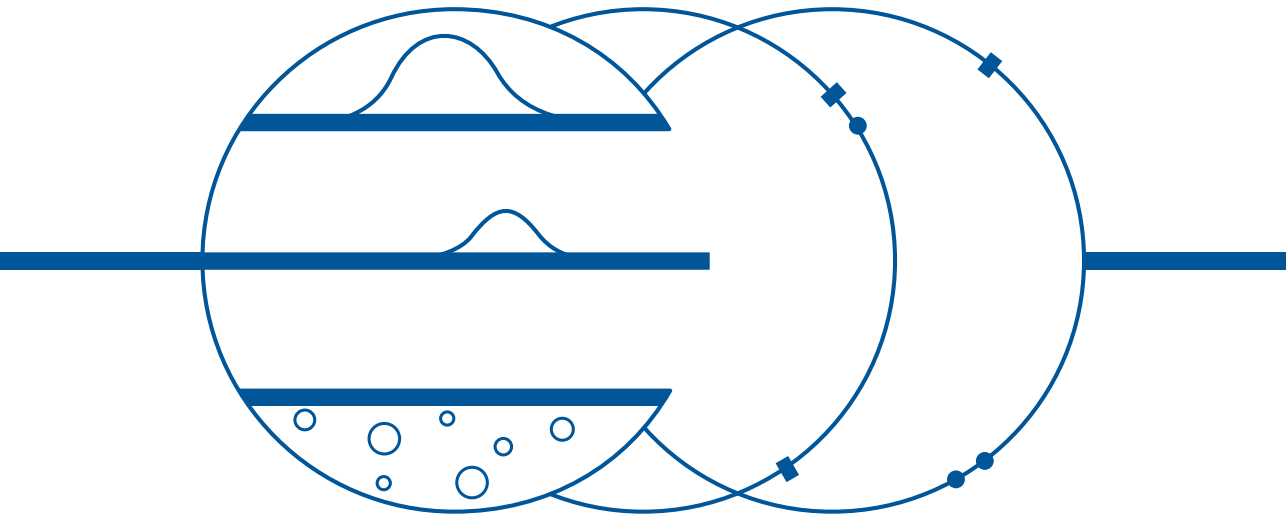
3

SYSTEMIC ABNORMALITIES IN CENTRAL SEROUS CHORIORETINOPATHY



3.1

CUSHING'S SYNDROME AND HYPOTHALAMIC-PITUITARY-ADRENAL AXIS HYPERACTIVITY IN CHRONIC CENTRAL SEROUS CHORIORETINOPATHY

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ABSTRACT

Objective: Central serous chorioretinopathy (CSC), a specific form of macular degeneration, has been reported as presenting manifestation of Cushing's syndrome. Furthermore, CSC has been associated with both exogenous hypercortisolism and endogenous Cushing's syndrome. It is important to know whether CSC patients should be screened for Cushing's syndrome. Although hypothalamic-pituitary-adrenal (HPA) axis hyperactivity in CSC has been suggested, no detailed evaluation of the HPA axis has been performed in a large cohort of CSC patients. This study aimed to investigate whether Cushing's syndrome prevalence is increased among chronic CSC (cCSC) patients and whether detailed endocrinological phenotyping indicates hyperactivity of the HPA axis.

Design: Cross-sectional study.

Patients: 86 cCSC patients and 24 controls.

Measurements: Prevalence of Cushing's syndrome, HPA axis activity.

Results: None of the cCSC patients met the clinical or biochemical criteria of Cushing's syndrome. However, compared to controls, HPA axis activity was increased in cCSC patients, reflected by higher 24 hour urinary free cortisol, and accompanying higher waist circumference and diastolic blood pressure, whereas circadian cortisol rhythm and feedback were not different. Chronic CSC patients did not report more stress or stress-related problems on questionnaires.

Conclusions: No case of Cushing's syndrome was revealed in a large cohort of cCSC patients. Therefore, we advise against screening for Cushing's syndrome in CSC patients, unless additional clinical features are present. However, our results indicate that cCSC is associated with hyperactivity of the HPA axis, albeit not accompanied with perception of more psychosocial stress.

INTRODUCTION

Cushing's syndrome is a rare disease characterised by excessive exposure to cortisol, and is associated with both metabolic and behavioral abnormalities. The clinical manifestation may vary, and in addition to well-known features like facial rounding, truncal obesity, and dorsal fat pad,[1] ophthalmological abnormalities also occur. We recently reported patients who developed visual symptoms caused by chronic central serous chorioretinopathy (cCSC) as presenting manifestation of Cushing's syndrome.[2]

CSC is a relatively common eye disease often affecting the macula, in which choroidal congestion, thickening, and hyperpermeability lead to retinal pigment epithelial damage and cause serous subretinal fluid accumulation. Persistent neuroretinal detachments in untreated cCSC may result in irreversible photoreceptor damage, which may lead to permanent visual loss and decreased quality of life.[3, 4]

The association of CSC with both exogenous steroids and endogenous hypercortisolism has been reported.[2, 5, 6] Although no data are available on the prevalence of CSC in patients treated with corticosteroids, up to 52% of CSC patients in different cohorts reported to use steroids during the active phase of disease.[6, 7] Higher endogenous cortisol levels were reported in 30 patients with acute CSC,[8] and 24 hour urinary free cortisol (UFC) was higher among 16 patients with chronic CSC compared to controls.[9] However, clinical characteristics, circadian tests, and cortisol feedback were not included in these studies, making it impossible to conclude on the prevalence of Cushing's syndrome.

In addition, psychosocial stress has been described in relation to CSC. Different studies reported associations between psychosocial stressful events and CSC, especially in patients with poor coping mechanisms.[10] People with type A personality characteristics have been suggested to be at higher risk of developing CSC.[11]

In view of the suspected relationship between overactivity of the hypothalamic-pituitary-adrenal (HPA) axis and CSC, a relevant question is whether CSC patients should be screened for Cushing's syndrome. Therefore, we conducted a systematic screening for the presence of Cushing's syndrome in a large cohort of cCSC patients, using detailed clinical and biochemical evaluation of the HPA axis, and compared the latter to a control group. Furthermore, perceived stress was evaluated using validated questionnaires.

MATERIALS AND METHODS

Study design

Cross-sectional study with the following key objectives: to assess the prevalence of Cushing's syndrome in cCSC patients, and to assess whether cCSC is associated with hyperactivity of the HPA axis. If this second aim was confirmed, we aimed to explore the association between HPA axis hyperactivity and psychosocial stress in cCSC.

Study population

Eighty-six consecutive cCSC patients, who were followed at the Department of Ophthalmology at our tertiary referral center, were screened. The cCSC diagnosis had been confirmed by fundoscopy, digital color fundus photography (Topcon Corp., Tokyo, Japan), fundus autofluorescence imaging (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany), spectral-domain optical coherence tomography (Spectralis HRA+OCT), fluorescein angiography (Spectralis HRA+OCT), and indocyanine green angiography (Spectralis HRA+OCT), according to current standard.[5, 12-16] Patients diagnosed with acute CSC (focal leakage spot or a smokestack pattern on fluorescein angiography) were excluded.[5, 12-16] No evidence of other retinal diagnoses had to be present.

Other exclusion criteria possibly affecting the evaluation of the HPA axis were: use of corticosteroids/sleep medication prior to the development or during the time course of cCSC, excessive alcohol intake (>21 Units/week), nightshift work or traveling from another time zone in the 6 weeks before evaluation.

We also performed tests for hypercortisolism in a set of gender matched controls. Thirty-eight healthy subjects responded to advertisements. Fourteen were excluded based on criteria described below. A total of 24 healthy gender-matched control subjects were eligible for inclusion (inclusion period: September 2015 to December 2016). Exclusion criteria were: (familial) history of eye diseases/visual problems, psychiatric diseases, or chronic physical diseases possibly influencing endocrinological screening, corticosteroids/antidepressants/sleep medication use, excessive alcohol intake (>21 Units/week), recent weight loss/gain of >10%, and working nightshifts or traveling from another time zone in the 6 weeks before evaluation.

Written informed consent was obtained from all participants and approval of the institutional review board and the ethics committee was obtained (NL50816.058.14).

Endocrinological evaluation

Screening was performed including a detailed medical history, complete physical examination, and biochemical analysis. The physical examination consisted among others of the evaluation of clinical Cushing stigmata and was performed by 2 physicians (FMvH/MBB).

For evaluating HPA axis activity, all 3 commonly available screening tests were performed: UFC in 2 24 hour urine samples, midnight salivary cortisol (mSC), and 1mg dexamethasone overnight suppression test. Healthy controls were subjected to 1 24 hour urine and 1 midnight saliva collection only. In case of deviant test results, participants were re-tested to exclude relevant pathology. The first test results were included in the analysis. Urinary free cortisol (82 patients and all controls) was analysed using an in-house LC-tandemMS method, calibrated using Cerilliant certified reference material C-106, Cortisol 1 mg/ml in methanol. The analytical variation was between 6.5% and 5% for urine cortisol levels between 50 nmol/L and 900 nmol/L. Cortisol levels below 150 nmol/24 hour were considered normal. Serum (81 patients) and salivary cortisol (82 patients and 23 controls) were analysed using a Roche ECLIA Cortisol assay (second generation) on a Modular E170 immunoanalyser (Roche Holding AG, Basel, Switzerland). Analytical variation ranged between 10.1% and 1.9% for serum cortisol levels between 3.6 and 1660 nmol/L and between 14.2% and 2.5% for saliva cortisol levels between 2.6 and 78 nmol/L. Cortisol levels below 1.5 nmol/L could not be determined. In midnight saliva, cortisol levels below 5.7 nmol/L were considered normal. The cut-off limit for the dexamethasone suppression test was 50 nmol/L.[17]

Questionnaires

Perceived Stress Scale (PSS)

The PSS developed by Cohen et al. was designed to measure the intensity of perceived stress, and considers the degree to which individuals experience their lives as unpredictable, uncontrollable, and overloading.[18] The original scale contained 14 items, but its creators refined it to 10 items, of which 4 are positively and 6 are negatively phrased.[19] Items are coded from 0 to 4 and summed to compute a total score. Higher scores indicate greater perceived stress. Scores around 13 on the PSS are considered average, whereas high stress groups have reported scores of approximately 20 points.[19]

Stress thermometer

A visual analogue scale was designed by the authors to measure the amount of stress experienced in the week before evaluation. Individuals rate their amount of stress on a scale from 0 to 10, with 0 indicating 'no stress at all' and 10 indicating 'the highest possible amount of stress'.

Insomnia Severity Index

This 7-item scale assesses self-reports of insomnia symptoms over the last 2 weeks. The items are scored on a scale from 0 to 4. Total scores of 0 to 7 are categorised as 'no insomnia', scores from 8 to 14 are considered to indicate 'subthreshold insomnia', scores from 15 to 21 are indicative of 'moderate insomnia', and scores from 22 to 28 are considered 'severe insomnia'.[20]

Brugha questionnaire on life events

The list to assess the occurrence of stressful events includes 12 life events that were found to have long-term negative effects on most people who experience them. Participants indicate whether certain events have occurred to them during the past year or earlier in their lives.[21]

Statistical analysis

Based on data derived from a recent study of Aranda and colleagues,[22] a power calculation was performed on the difference in 24 hour UFC deemed relevant to detect (20 nmol/24 hours). To detect such a difference (with power 80% and alpha 0.05), a sample size of 54 cCSC patients and 18 healthy controls would suffice.

Data were analysed using SPSS Statistics (version 23; IBM Corp., Armonk, NY, USA). Data were presented as mean and SD, unless mentioned otherwise. The primary analyses comprised: 1. prevalence of Cushing's syndrome in cCSC and 2. comparison of biochemical results between cCSC patients and healthy controls. Because the majority of cCSC patients were males (in line with other cohorts described in literature), a male-only sensitivity analysis was performed. Mean and SD scores for each questionnaire were calculated.

Normality of data was tested using the Shapiro-Wilk test. All normally distributed data were analysed using independent sample t-tests. Data with a non-normal distribution were analysed by means of nonparametric independent sample tests. The 2 groups were compared using a general linear model, correcting for potential confounders such as age, waist-hip ratio, and waist circumference. Associations were assessed using linear regression analyses. The level of significance was set at p=0.05. For the analysis of the questionnaires, the level of significance was set at p=0.01 to correct for multiple testing.

After reassessment of the retinal imaging by 2 independent ophthalmologists, 5 patients considered to have less typical cCSC findings on imaging were excluded from analysis. Moreover, an analysis excluding outliers (n=1) was performed. All results are described below.

Table 1. Clinical characteristics of participants.

	cCSC patients n=86	**Controls** n=24	***P*-value**
Age, in years	48.74 (10.84)	41.08 (13.08)	0.004
Sex, male / female	77 / 9	19 / 5	0.182
Duration of cCSC disease, in years (range)	3.86 (0.17 - 37.06)	-	-
History of hypertension, n (%)	23 (26.7%)	1 (4.2%)	0.023[a]
History of diabetes mellitus, n (%)	6 (7.0%)	0 (0.0%)	0.336
History of dyslipidemia, n (%)	18 (20.9%)	1 (4.2%)	0.068
History of psychiatric disorders[b], n (%)	16 (18.6%)	1 (4.2%)	0.113
History of thromboembolic events, n (%)	0 (0%)	0 (0%)	-
History of cardiac events[c], n (%)	5 (5.9%)	2 (8.3%)	0.648
History of sexual disorders[d], n (%)	19 (22.1%)	1 (4.2%)	0.069
Systolic blood pressure, mmHg	135.41 (16.64)	129.75 (12.41)	0.143
Diastolic blood pressure, mmHg	82.94 (10.30)	77.29 (12.36)	0.006
Body mass index	26.15 (3.59)	24.92 (3.14)	0.096
Waist circumference, cm	92.74 (11.07)	86.42 (9.28)	0.011
Waist-hip ratio	0.95 (0.07)	0.90 (0.06)	0.003
Moon face, n (%)	1 (1.2%)	0 (0.0%)	1.000
Dorsal fat pad, n (%)	1 (1.2%)	0 (0.0%)	1.000
Purple striae, n (%)	0 (0.0%)	0 (0.0%)	-
Muscle weakness, n (%)	3 (3.5%)	0 (0.0%)	1.000
Active skin infections, n (%)	2 (2.3%)	0 (0.0%)	1.000
Hematomas, n (%)	3 (3.5%)	1 (4.2%)	1.000
Ankle edema, n (%)	2 (2.3%)	0 (0.0%)	1.000

Data presented as mean (SD) or as numbers, unless specified otherwise.

[a] Not statistically significant after correction for age

[b] Consisting of depression, anxiety or panic disorder, posttraumatic stress disorder, burn-out, alcohol abuse, and schizophrenia

[c] Consisting of myocardial infarction, endocarditis, and atrial fibrillation

[d] Consisting of impotence, hirsutism, menstrual cycle disorders, and loss of libido

Abbreviations: cCSC: chronic central serous chorioretinopathy; SD: standard deviation.

RESULTS

Baseline characteristics

Eighty-six cCSC patients (77 males) and 24 healthy controls (19 males), were included. The gender distribution was in line with available literature.[5, 15] The mean duration of disease from first diagnosis of cCSC to the time of evaluation was 3.86 years (range, 0.17 - 37.06). Fifty-eight patients had active CSC (presence of subretinal fluid) at the moment of screening.

There was no difference in gender distribution or body mass index between the 2 groups. Patients were 7.5 years older than controls (Table 1).

Clinical evaluation

None of the cCSC patients presented with a combination of clinical signs and symptoms typical for Cushing's syndrome. Hypertension was reported by 27% of patients and 1 control (4%, p=0.023). In addition, cCSC patients had a higher prevalence of other comorbidities, e.g. dyslipidemia and psychiatric disorders (Table 1). Waist circumference, waist-hip ratio, and diastolic blood pressure were higher in patients compared to controls, despite a higher prevalence of ongoing antihypertensive medication use in the patient group. These differences remained significant after adjustment for age. Characteristic Cushing features were rare among cCSC patients.

Hormonal evaluation

Clinical evaluation of patients

None of the cCSC patients had Cushing's syndrome, but several patients demonstrated an abnormally high cortisol value in 1 or more of the screening tests (Table 2). Increased UFC (>150 nmol/24 hour, average of 2 portions) was present in 7 patients, in whom repeated testing revealed normal values. Increased midnight salivary cortisol levels (>5.7 nmol/L, average of 2 portions) was observed in 3 patients, which normalised upon retesting in 2 and persisted to be slightly elevated in 1 patient. Insufficient suppression after dexamethasone administration was observed in 4 patients. Retesting revealed normal test results in 1 patient. In the absence of other biochemical and clinical features of Cushing's syndrome we concluded that the abnormal test results in the other patients were likely due to intervening medication (antidepressants, gonadotropin-releasing hormone analogues, covert stimulant use). Furthermore, normal lipid profiles, no elevated inflammation parameters, and no hypokalemia were detected (data not shown).

Table 2. Biochemical characteristics of participants.

	cCSC patients	Controls	*P*-value
Urinary free cortisol, nmol/24 hours[a]	83.99 (49.04)	51.55 (28.49)	0.000
Detectable midnight salivary cortisol[a,b], %	24.4	60.9	0.002
Serum cortisol after 1mg dexamethasone, μmol/L[a]	0.032 (0.047)	-	-
	cCSC patients with active disease	**cCSC patients with inactive disease**	
Urinary free cortisol, nmol/24 hours[a]	78.44 (38.63)	95.30. (64.76)	0.524
Detectable midnight salivary cortisol[a], %	27.3	18.5	0.428
Serum cortisol after 1mg dexamethasone, μmol/L[a]	0.028 (0.045)	0.031 (0.041)	0.855

Data are presented as mean (SD) or as numbers, unless specified otherwise.

[a] Number of participants:

Urinary free cortisol: 82 cCSC patients (55 patients with active cCSC, 27 patients with inactive CSC) and 24 controls

Midnight salivary cortisol: 82 cCSC patients (55 patients with active cCSC, 27 patients with inactive CSC) and 23 controls

Serum cortisol after 1mg dexamethasone: 55 patients with active cCSC, 26 patients with inactive CSC

[b] > 1.5 nmol/L

Abbreviations: cCSC: chronic central serous chorioretinopathy; SD: Standard deviation.

Comparison with healthy controls

Mean UFC levels were higher in cCSC patients, compared to controls (Figure 1). This difference remained after correction for age (p=0.001), age, and waist-hip ratio (p=0.002), age and co-morbidities (e.g. psychiatric disorders, diabetes mellitus, hypertension, obesity; p=0.011), and when males were evaluated solely (p=0.001).

Nonparametric analysis revealed that non-detectable mSC was present in 76% of cCSC patients compared to 39% of healthy controls (p=0.002), with a similar difference in a male-only analysis (72% versus 33%, p=0.001). Three patients (4%) showed abnormally elevated mSC, compared to 2 controls (9%). The other participants mSC levels were between 1.5 and 5.7 nmol/L.

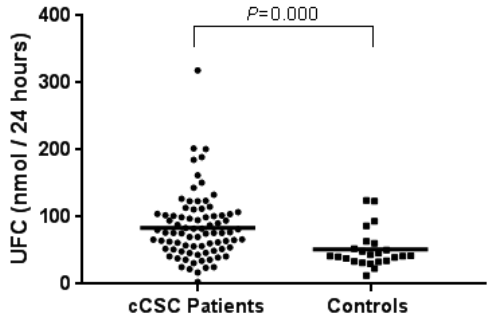

Figure 1. Urinary free cortisol levels in cCSC patients and healthy controls
Data presented as individual values and mean.
Patients: n=82; controls: n=24
Abbreviations: cCSC: chronic central serous chorioretinopathy; UFC: urinary free cortisol

HPA axis at different cCSC disease stages

HPA axis activity was not different between patients with active and inactive cCSC (Figure 2). Mean UFC levels were 78.44 (SD 38.63) versus 95.30 (SD 64.76) respectively (p=0.524), and mSC was detectable in 27% of patients with active disease versus 19% of patients with inactive disease (p=0.386).

The exclusion of 5 atypical cCSC patients or the exclusion of 1 outlier in UFC did not affect any of the described results. Clustered analysis did also not significantly change the aforementioned results (data not shown).

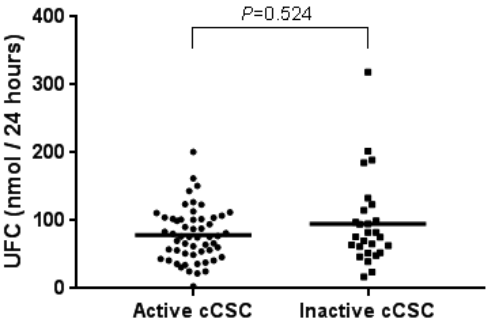

Figure 2. Urinary free cortisol levels in active cCSC patients and inactive cCSC patients
Data presented as individual values and mean.
Patients with active cCSC: n=55; patients with inactive CSC: n=27
Abbreviations: cCSC: chronic central serous chorioretinopathy; UFC: urinary free cortisol

Questionnaire analysis

Perceived Stress Scale

Chronic CSC patients (n=82%) reported a mean total score of 12.95 (SD 5.82, range, 0 - 30). After correction for multiple testing, no significant difference in PSS score was found between cCSC patients with active disease compared to inactive patients (mean 11.89 versus 15.07, p=0.019). Furthermore, no association was found between UFC level and the total score in patients (β=2.04, p=0.032, R^2=0.06).

Stress thermometer

Eighty-three cCSC patients (96%) scored their amount of experienced stress in the week prior to evaluation, reporting a mean score of 4.4 (range, 0 - 10). In addition, no differences were found when active patients were compared to patients with inactive disease, and no association between UFC level and score on this scale was found (β=0.71, p=0.742, R^2=0.001).

Insomnia Severity Index

Total scores were calculated for 83 cCSC patients (mean 6.54, range, 0 - 24). The mean score was categorised as 'no clinical significant insomnia'. When insomnia was scored as a 'yes or no' variable, 11% of patients scored either moderate or severe insomnia. There was no difference in presumed insomnia between patients with active and patients with inactive disease, and no association between UFC levels and Insomnia Severity Index scores was found (β=-.97, p=0.378, R^2=0.010).

Brugha questionnaire on life events

Thirty out of 83 patients (36%) reported serious life events in the past year. Twenty-four hour UFCs were not higher in these patients compared to the patients with no serious life event in the preceding year. Disease activity did not affect the report of serious life events in the past year (p=1.000). The type of life events experienced, however, was different, with patients with active disease reporting more experiences with serious illness or violence of a near relative, whereas patients with inactive disease more often reported the same experiences earlier in live.

Associations of questionnaire outcomes and cortisol

Analyses performed with exclusion of the 5 atypical cCSC patients and analyses with exclusion of the UFC outlier did not significantly change the aforementioned results.

DISCUSSION

This is the first study that systematically evaluated various aspects of the activity of the HPA axis in a large cohort of cCSC patients. Whereas we did not find any case of Cushing's syndrome, the activity of the HPA axis appeared to be increased in cCSC patients, without disruption of circadian rhythm. This was reflected by significantly higher 24 hour UFCs, increased waist circumference, and diastolic blood pressure, but normal midnight salivary cortisol levels. Our study demonstrates that systematic screening of all cCSC patients for the presence of Cushing's syndrome is not indicated.

In the present study, we have screened a large patient cohort in detail, combining all currently available biochemical screening tests with a detailed clinical phenotyping. We found significantly higher 24 hour UFC levels in cCSC patients, albeit within the normal reference range, with preservation of normal diurnal rhythmicity. Elevated UFC levels have been reported previously in a small cohort of acute CSC patients during hospital admission, when compared to patients with acute retinal detachment, [8] and in a small cohort of cCSC patients that were compared to age- and sex-matched controls, [9] though data on demographics of these participants were lacking.

In contrast to earlier studies suggesting an association between cCSC and psychosocial stress, we did not find a clear relationship between cCSC activity and stress, based on the results of 4 stress-questionnaires. In addition, no association was found between HPA axis activity and psychosocial stress. A critical evaluation of the available literature does not support a clear association between cCSC and stress: Conrad et al. demonstrated no increased exposure to critical life events in 30 CSC patients, and reported findings suggestive of difficulties in emotional regulation.[23] Other studies reported an association between stress, severe stressfull events, and CSC, especially in patients with poor coping mechanisms,[10, 24, 25] but the provided information on how stress was measured was very limited, circumstantial, or even absent. Our patients scores (on the PSS) did not differ from reported average scores and were not comparable with scores reported by high stress groups.[19] In addition, our patients with active cCSC reported no difference in experienced life events, insomnia (as an expression of stress) or perceived stress on 2 different scales, indicating that cCSC activity is not associated with psychosocial stress.

Both endogenous hypercortisolism and exogenous administration of corticosteroids are related to CSC.[2, 5, 6, 9] Occurrence of 1 or more episodes of CSC has previously been described in 5% of 60 patients with active endogenous hypercortisolism. All these CSC patients had been diagnosed with pituitary adenoma.[26] Fundus characteristics resembling CSC have also been reported in patients with Cushing's disease.[27] Moreover, in a patient with hypercortisolism due

to adrenocortical carcinoma, bilateral CSC has been found.[28] Several underlying mechamism have been hypothesised. Endogenous hypercortisolism increases platelet aggregation leading to microthrombi and increased blood viscosity, which could be of importance in the pathogenesis of CSC.[29] Hypercortisolism has also been associated with choroidal fragility and hyperpermeability.[30] Moreover, increased transcription of adrenergic receptors has been correlated with CSC.[31] In addition, a role for the mineralocorticoid pathway has been suggested by recent studies in rats and by findings in CSC patients treated with mineralocorticoid receptor antagonists (eplerenone or spironolactone).[15, 32] Both glucocorticoids and mineralocorticoids activate the mineralocorticoid receptor expressed on choroidal endothelial cells. Activation of the mineralocorticoid receptor, via upregulation of the endothelial vasodilatory calcium-dependent potassium channel KCa2.3 by hyperpolarisation of these endothelial cells and of smooth muscle cells, has been suggested to lead to vasodilation.[15] Endogenous hypercortisolism increases platelet aggregation leading to microthrombi and increased blood viscosity, which could be of importance in the pathogenesis of CSC.[29] Hypercortisolism has also been associated with choroidal fragility and hyperpermeability.[30] Moreover, increased transcription of adrenergic receptors has been correlated with CSC.[31] In addition, a role for the mineralocorticoid pathway has been suggested by recent studies in rats and by findings in CSC patients treated with mineralocorticoid receptor antagonists (eplerenone or spironolactone).[15, 32] Both glucocorticoids and mineralocorticoids activate the mineralocorticoid receptor expressed on choroidal endothelial cells. Activation of the mineralocorticoid receptor, via up-regulation of the endothelial vasodilatory calcium-dependent potassium channel KCa2.3 by hyperpolarisation of these endothelial cells and of smooth muscle cells, has been suggested to lead to vasodilation.

Our study also has limitations. The cross-sectional character does not allow drawing conclusions on any causal relationship. Furthermore, a reversed causation (cCSC as a trigger for activation of the HPA axis) seems to be less likely in light of the currently available literature, yet is not ruled out. Also, the number of healthy control subjects recruited via advertisements was limited, and because our study was not powered for the questionnaire outcomes, we did not compare patient and control data. In order to limit the burden of the study in an attempt to gather more control subjects, only 1 24 hour urine sample was collected from healthy controls. Volume and creatinine level analyses led us to conclude that these single samples were collected adequately. Since the difference in UFC between the cCSC patients and controls was big, we did not expect that a second urine sample would alter the results significantly. MSC levels were more often detectable in controls compared to cCSC patients. However, the great majority of mSC levels were within the lower range of normal and with the impossibility to quantify mSC levels below 1.5 nmol/L, no conclusions on higher mSC levels in controls could be drawn. The absence of associations between UFC level and either cCSC activity or outcomes of stress questionnaires in our study may appear to be contradictory to the conclusion that

the HPA axis is more activated in cCSC patients. Nonetheless, one should keep in mind that there is a wide individual variation in normal cortisol levels and in cortisol receptor activation thresholds, leading to different thresholds for the development of cortisol-related symptoms and pathology. Together, this may explain why the HPA axis could still be activated in cCSC patients despite the absence of an association between UFC and cCSC activity or questionnaire outcomes in our patient population.

Although CSC has been described to be a presenting symptom of Cushing's syndrome and these diseases are known to sporadically coexist,[2] our results argue against screening for endogenous hypercortisolism in all cCSC patients. Since the interpretation of the available biochemical screening tests in light of the clinical features is challenging and in order to minimize the risk of false positive test results, screening should be reserved for those cCSC patients in whom clinical signs or symptoms raise suspicion of Cushing's syndrome. Only then patients should be referred to an endocrinologist for evaluation of the HPA axis. In dealing with Cushing's syndrome patients, endocrinologists also need to be aware of the potential coexistence of CSC.

In conclusion, systematic screening of all patients with cCSC for Cushing's syndrome is not indicated. However, the activity of the HPA axis appears to be increased, with preservation of circadian rhythm. Finally, in contrast to earlier ideas, we did not find obvious associations between cCSC, cCSC activity, and psychosocial stress. The observed hyperactivity of the HPA axis confirms the previously reported association between cortisol and CSC and merits further studies to unravel the underlying pathophysiological mechanisms.

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3.2

MALADAPTIVE PERSONALITY TRAITS, PSYCHOLOGICAL MORBIDITY, AND COPING IN CHRONIC CENTRAL SEROUS CHORIORETINOPATHY

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ABSTRACT

Purpose: The pathogenesis of chronic central serous chorioretinopathy (cCSC) is unclear, although an increased hypothalamic-pituitary-adrenal (HPA) axis activity has been described. Despite the proposed greater risk of cCSC in humans with a type A personality, a typical personality profile could not be identified. Associations between psychosocial stress and cCSC were reported, especially in patients with poor coping mechanisms. Similar to cCSC patients, patients with primary hypercortisolism (Cushing's disease) have hyperactivation of the HPA axis. Patients with Cushing's disease demonstrated maladaptive personality traits, psychological morbidity, and less effective coping strategies. This cross-sectional study aimed to assess maladaptive personality traits, psychological morbidity, and coping strategies in patients with cCSC.

Methods: Personality traits, psychological morbidity, and coping strategies of patients with cCSC were compared to Dutch population reference data, and data from patients with Cushing's disease.

Results: Maladaptive personality traits do not occur more often in cCSC patients than in the general population. Only intimacy problems are more prevalent in cCSC patients. No differences in the presence of psychological morbidity was observed between the included groups, but patients with cCSC were shown to make more use of certain coping strategies. These differences regarding coping were partly explained by the personality traits of cCSC patients. Characteristics of cCSC patients were more comparable to features of Cushing's disease patients than to population based data.

Conclusion: No evidence for a higher prevalence of type A personality characteristics in cCSC patients was found, which is in contrast with available literature. The results contribute to the psychological phenotyping of this patient group, and could be of importance for the development of personalised treatment for cCSC.

INTRODUCTION

Central serous chorioretinopathy (CSC) is a specific and relatively common form of macular degeneration in which choroidal congestion, thickening, and hyperpermeability damage the retinal pigment epithelium and subsequently induce serous subretinal fluid accumulation and detachment of the neuroretina. CSC can present as either an acute or a chronic disease (cCSC), and men between the age of 25 and 55 years are particularly affected.[1] Early diagnosis and treatment is desirable to try to improve the visual outcome, since untreated cCSC may result in irreversible photoreceptor damage. Long-term follow-up studies have shown that prolonged presence of subretinal fluid may lead to permanent visual loss and decreased quality of life in the majority of patients.[2-5]

The pathogenesis of CSC is currently unclear, but an association with both exogenous and endogenous steroids has been described to be the most pronounced to date.[2, 6-12] Furthermore, we have recently observed hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis in patients with cCSC.[13] Moreover, both acute and chronic psychosocial stress have been suggested to be a predisposing factor for CSC.[14, 15] It has also been suggested that people with a type A behavior pattern (e.g., competitiveness, agression, hostility) have a higher risk to develop CSC,[16-19] which is in accordance with the findings from a recent meta-analysis concluding that patients with CSC demonstrated significantly more type A behavior characteristics than healthy controls (odds ratio (OR)= 2.53; confidence interval (CI) 1.08-5.96).[20] It has been hypothesised that behavior is linked to CSC by the levels of circulating catecholamines and corticosteroids, since these are higher in people with type A behavioral characteristics compared to type B.[21]

Personality has an effect on coping behavior.[22] Coping encompasses the way people react on a behavioral, cognitive, and emotional level to situations that require adjustments in dealing with possible adverse events.[23] The way one copes has an effect on the amount of stress experienced in a certain situation,[24] and even has been reported to have some effect on the experienced disease severity.[25] Several studies have reported an association between severe psychosocial stressful events and the onset of CSC, especially in patients with poor coping mechanisms.[14, 15] Lahousen and colleagues reported a possible association between the appearance of CSC and a combination of stressful life events and unfavorable coping styles, with patients with acute CSC reporting even more unfavorable stress coping compared to patients with cCSC.[26]

Cushing's disease is a rare disease which is characterised by exposure to elevated cortisol levels. Similar to patients with CSC, patients with Cushing's disease have overactivation of the HPA axis, resulting in exposure to excessive cortisol levels. Patients with Cushing's disease have been demonstrated to have maladaptive personality traits and psychological morbidity, such as somatic arousal, negative affect, irritability, and apathy.[27] Furthermore, less effective

coping strategies have bee detected in patients with Cushing's disease, characterised by more avoiding and less active coping.[28]

Although it is proposed that people with a type A personality are at greater risk of developing CSC,[17] a typical CSC personality profile could not be identified in previous studies, with only the identification of type A behavioral characteristics being at risk.[29] To the best of our knowledge, no reports are available assessing personality traits in patients with CSC. In addition, there are no studies in a large cohort of patients with CSC that have evaluated coping strategies using a specific coping-oriented validated questionnaire by comparing coping strategies of patients with cCSC to coping strategies of reference populations.

Therefore, the primary aim of our study was to assess maladaptive personality traits, i.e. type A personality traits, in patients with cCSC. For this evaluation, we compared personality traits of patients with cCSC to personality traits of Dutch population reference data, but also to personality traits of patients with Cushing's disease. Secondary, this study was aimed to assess psychological morbidity (i.e., apathy, irritability) and coping strategies in patients with cCSC by comparing psychological morbidity and coping strategies of patients with cCSC to psychological morbidity and coping strategies of the same reference groups. Moreover, we aimed to assess the association between personality and coping in patients with cCSC. Considering the recently described hyperactivity of the HPA axis in patients with cCSC,[13] and the previously observed maladaptive personality traits in patients exposed to hypercortisolism (i.e. Cushing's disease) it was hypothesized that patients with CSC would report more maladaptive personality traits, more psychological morbidity (i.e., apathy, irritability), and less effective coping strategies compared to reference data from general population, and would demonstrate a profile similar to patients with Cushing's disease.

MATERIALS AND METHODS

Study design

We conducted a cross-sectional study with the following key objectives: to assess the prevalence of maladaptive traits (i.e. former type A personality) in a large cohort of cCSC patients, to investigate psychological morbidity in the form of apathy and irritability, to assess coping strategies in these patients, and to assess the association between personality traits and coping strategies. Patients were asked to fill out a set of questionnaires on personality characteristics, apathy, irritability, and coping strategies at home, using an online survey. Furthermore, clinical evaluation took place during a single visit to the outpatient Endocrinology clinic of Leiden University Medical Center (Leiden, the Netherlands).

Study population

Eighty-six consecutive cCSC patients above 18 years of age, who were followed at the Department of Ophthalmology at our center, a tertiary referral center for CSC, were invited to complete the questionnaires. The cCSC diagnosis had been confirmed by fundoscopy, digital color fundus photography (Topcon Corp., Tokyo, Japan), fundus autofluorescence (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany), spectral-domain optical coherence tomography (Spectralis HRA+OCT), fluorescein angiography (Spectralis HRA+OCT), and indocyanine green angiography (Spectralis HRA+OCT), according to current standards.[2, 6-8, 30, 31] Patients diagnosed with acute CSC, defined by either a focal leakage spot or a smokestack pattern on fluorescein angiography, were excluded,[2, 6-8, 30, 31] as well as patients in whom evidence of other retinal diagnoses was detected. On multimodal imaging, the following characteristics had to be present within the past 2 years: serous subretinal fluid on optical coherence tomography, ≥1 area of multifocal diffuse leakage or irregular retinal pigment epithelium window defects on fluorescein angiography, and corresponding hyperfluorescence on indocyanine green angiography. The patients also participated in a study on endocrinological evaluation of the HPA axis,[13] for which other exclusion criteria were: the use of corticosteroids or sleep medication prior to the development or during the time course of cCSC, excessive alcohol intake (>21 Units/week), either nightshift work or traveling from another time zone in the 6 weeks prior to evaluation. Clinical evaluation of the patients included a detailed medical history and complete physical examination and was performed by 2 physicians. After reassessment of the retinal imaging by 2 independent ophthalmologists, 5 patients were considered to have less typical cCSC findings on imaging.

Written informed consent was obtained from all participants and approval of the institutional review board and the ethics committee was obtained (NL50816.058.14).

Questionnaires

Dimensional Assessment of Personality Pathology short form (DAPP-SF)

This questionnaire consists of 136 items assessing personality, which are subdivided into 18 subscales: submissiveness, cognitive distortion, identity problems, affective lability, stimulus seeking, compulsivity, restricted expression, callousness, oppositionality, intimacy problems, rejection, anxiousness, conduct problems, suspiciousness, social avoidance, narcissism, insecure attachment, and self-harm. The maximal scores for each subscale differ from 30 to 40, and higher scores indicate more pronounced maladaptive personality traits. No formal cut-off scores for these subscales exist.[32, 33]

Apathy Scale (AS)

The AS of Starkstein was used to assess apathy. The scale consists of 14 questions on a 4-point scale, measuring different features of apathy in the 2 previous weeks. Total scores in a range from 0 to 42 points are calculated, with higher scores indicating greater apathy. A total score of 14 points or more defines apathy.[34, 35]

Irritability Scale (IS)

Irritability was assessed by the IS. This scale consists of 14 items on a 4-point scale, assessing different features of irritability in the 2 previous weeks. Total scores range from 0 to 42 points, with higher scores indicating greater irritability. A total score of 14 points or more defines irritability. [35]

Utrecht Coping Scale (UCS)

The UCS is an established Dutch coping list with well-documented validity and reliability.[36] It contains 47 statements where one indicates whether he/she finds these applicable to him- or herself. This scale assesses the way a person acts to minimize the impact of stressful events, with 7 subscales that represent different coping styles. These subscales include: active, distraction-seeking, avoidance, seeking social support, passive, expression of emotions, and positive reframing. The different items have a 4-point scale ranging from 1 (seldom or never) to 4 (very often). Scores on each subscale are summed to create a total score, with scores of 4 or 5 indicating high use of that specific coping style.[37] Data from an aselect sample of the Dutch railway workers (1493 men, aged between 19 and 65 years) was used as reference data.[38] A cohort of 42 Cushing's disease patients (6 men and 36 women) with a mean age of 54 (±12) years was used for comparison.[28]

Reference data

Outcomes of the questionnaires were compared to reference of an aselect sample of the Dutch population and reference data from patients with Cushing's disease derived from Tiemensma and colleagues.[27, 28] For comparison of DAPP-SF outcomes, reference data from the publisher of this questionnaire is available.[39] The sample used for obtaining these data consisted of 58 men aged 15-34 years, 94 men aged 35-54 years, 146 women aged 15-34 years, and 172 women aged 35-54 years. The sample of patients with Cushing's disease used for comparison consisted of 8 men and 43 women with a mean age of 53 (± 13) years.[27] Concerning the AS, reference data was derived from the healthy control population described by Tiemensma and colleagues,[40] consisting of 35 men and 33 women with a mean age of 59 (± 11) years. No male only reference data was available. The same cohort of patients with Cushing's disease was used for the comparison of the AS scores.[27] For the IS outcome comparison, reference data was also derived from the healthy control population described by Tiemensma and colleagues.[40] No male only reference data was available. For comparison of IS scores between cCSC patients and

patients with Cushing's disease, the same Cushing's disease cohort as described above was used.[27] Data from the aselect sample of the Dutch railway workers was also used as reference data.[38] Moreover, the cohort of Cushing's disease patients was used for comparison.[28]

Statistical analysis

Data was analysed using SPSS Statistics (version 23; IBM Corp., Armonk, NY, USA). Data was presented as mean and standard deviation (SD), unless mentioned otherwise. The primary analyses comprised the comparison of questionnaire outcomes between patients with cCSC and reference data from general population. Secondary analyses comprised the comparison of results between cCSC patients and patients with Cushing's disease. Where possible, depending on the availability of gender specific reference data, also a male only sensitivity analysis was performed (because the majority of cCSC patients were males). Means and SD scores for each questionnaire subscale were calculated. Groups were compared using pooled *t*-tests. The level of significance was set at $P \leq 0.01$ in order to correct for multiple testing. Normality of data was tested using the Shapiro-Wilk test. Correlations were assessed using Pearson's correlation in case of normally distributed data, and data with a non-normal distribution was correlated using Spearman correlation. Only moderate to strong correlations (correlation coefficient of >0.5) were described.

A post-hoc analysis excluding the 5 less typical cCSC patients was also performed. All results are described below.

RESULTS

Baseline characteristics

A total of 86 patients with cCSC (77 males and 9 females) with a mean age of 48.7 years were included. Gender and age distribution of these patients were in accordance with available literature on cCSC.[6, 31] In 58 of the patients with cCSC (67%) subretinal fluid was present at moment of evaluation, indicating active cCSC. The duration of disease in patients with cCSC ranged from 0.17 to 37.06 years (mean 3.86 years). A history of hypertension was reported by 27% of patients, dyslipidemia by 21%, and psychiatric disorders by 19% of patients (Table 1).

Table 1. Clinical characteristics of participants

	cCSC patients n=86
Age, in years	48.74 (10.84)
Sex, male / female	77 / 9
Duration of cCSC disease, in years (range)	3.86 (0.17 - 37.06)
History of hypertension, n (%)	23 (26.7%)
History of diabetes mellitus, n (%)	6 (7.0%)
History of dyslipidemia, n (%)	18 (20.9%)
History of psychiatric disorders[ʃ], n (%)	16 (18.6%)
History of thromboembolic events, n (%)	0 (0%)
History of cardiac events[¥], n (%)	5 (5.9%)
History of sexual disorders*, n (%)	19 (22.1%)

Data is presented as mean (SD) or as numbers, unless specified otherwise.

Abbreviations: cCSC: chronic central serous chorioretinopathy; SD: standard deviation.

[ʃ] Consisting of depression, anxiety or panic disorder, posttraumatic stress disorder, burn-out, alcohol abuse, and schizophrenia

[¥] Consisting of myocardial infarction, endocarditis, and atrial fibrillation

* Consisting of impotence, hirsutism, menstrual cycle disorders, and loss of libido

Personality traits

The DAPP-SF was filled out by 81 (94%) patients with cCSC. Compared to patients with active cCSC (n=54), patients with inactive disease (n=27) reported more affective lability, submissiveness, and social avoidance (p=0.007, p=0.007, and p=0.002 respectively).

Compared to reference data, cCSC patients reported significantly less submissiveness (p=0.000), cognitive distortion (p=0.000), affective lability (p=0.000), stimulus seeking (p=0.000), compulsivity (p=0.003), oppositionality (p=0.000), anxiousness (p=0.000), suspiciousness (p=0.001), social avoidance (p=0.000), narcissism (p=0.000), and insecure attachment (p=0.000) (Table 2 and Figure 1). On the other hand, patients reported more intimacy problems (p=0.000) compared to reference data. Compared to patients with Cushing's disease, patients with cCSC reported less affective lability (p=0.000), cognitive distortion (p=0.005), and oppositionality (p=0.005). On the other hand, patients with cCSC reported more conduct problems (p=0.009) compared to patients with Cushing's disease. No difference was observed between patients with cCSC and patients with Cushing's disease patients on the remaining personality traits.

Table 2. Personality traits in cCSC patients

DAPP-SF	**cCSC patients (n=81)**	**Reference data (n=475)**	***P*-value**
Submissiveness	16.21 (6.64)	19.67 (6.33)	0.000
Cognitive distortion	9.04 (4.34)	12.10 (5.38)	0.000
Identity problems	10.57 (4.68)	12.18 (5.63)	0.015
Affective lability	16.47 (6.86)	21.03 (7.29)	0.000
Stimulus seeking	15.19 (5.36)	18.00 (5.80)	0.000
Compulsivity	21.89 (7.06)	24.22 (6.50)	0.003
Restricted expression	20.91 (5.89)	21.26 (6.49)	0.650
Callousness	17.16 (5.17)	18.84 (5.41)	0.010
Oppositionality	18.95 (7.04)	23.08 (7.20)	0.000
Intimacy problems	20.89 (5.91)	16.93 (5.68)	0.000
Rejection	19.32 (6.67)	20.08 (5.71)	0.281
Anxiousness	12.81 (6.21)	17.84 (5.70)	0.000
Conduct problems	10.84 (4.77)	11.51 (4.38)	0.210
Suspiciousness	12.65 (5.40)	14.96 (5.91)	0.001
Social avoidance	10.99 (5.18)	13.79 (5.53)	0.000
Narcissism	15.73 (5.68)	18.73 (6.16)	0.000
Insecure attachment	10.89 (4.85)	13.74 (5.64)	0.000
Self-harm	7.02 (2.72)	7.98 (4.20)	0.048
	cCSC patients (n=81)	**Cushing's disease patients (n=51)**	***P*-value**
Submissiveness	16.21 (6.64)	19.00 (7.70)	0.029
Cognitive distortion	9.04 (4.34)	11.50 (5.60)	0.005
Identity problems	10.57 (4.68)	13.00 (6.60)	0.015
Affective lability	16.47 (6.86)	21.70 (7.80)	0.000
Stimulus seeking	15.19 (5.36)	16.40 (4.80)	0.191
Compulsivity	21.89 (7.06)	23.80 (6.60)	0.123
Restricted expression	20.91 (5.89)	21.20 (7.30)	0.802
Callousness	17.16 (5.17)	16.10 (4.50)	0.231
Oppositionality	18.95 (7.04)	22.90 (8.80)	0.005
Intimacy problems	20.89 (5.91)	18.80 (6.40)	0.058
Rejection	19.32 (6.67)	17.20 (5.70)	0.063
Anxiousness	12.81 (6.21)	15.30 (6.20)	0.027
Conduct problems	10.84 (4.77)	9.00 (1.80)	0.009
Suspiciousness	12.65 (5.40)	12.60 (5.90)	0.960
Social avoidance	10.99 (5.18)	12.30 (6.30)	0.196
Narcissism	15.73 (5.68)	15.00 (5.50)	0.468
Insecure attachment	10.89 (4.85)	13.30 (6.60)	0.017
Self-harm	7.02 (2.72)	7.30 (2.90)	0.576

Data is presented as mean (SD).

Abbreviations: cCSC: chronic central serous chorioretinopathy; DAPP-SF: Dimensional Assessment of Personality Pathology short form; SD: standard deviation.

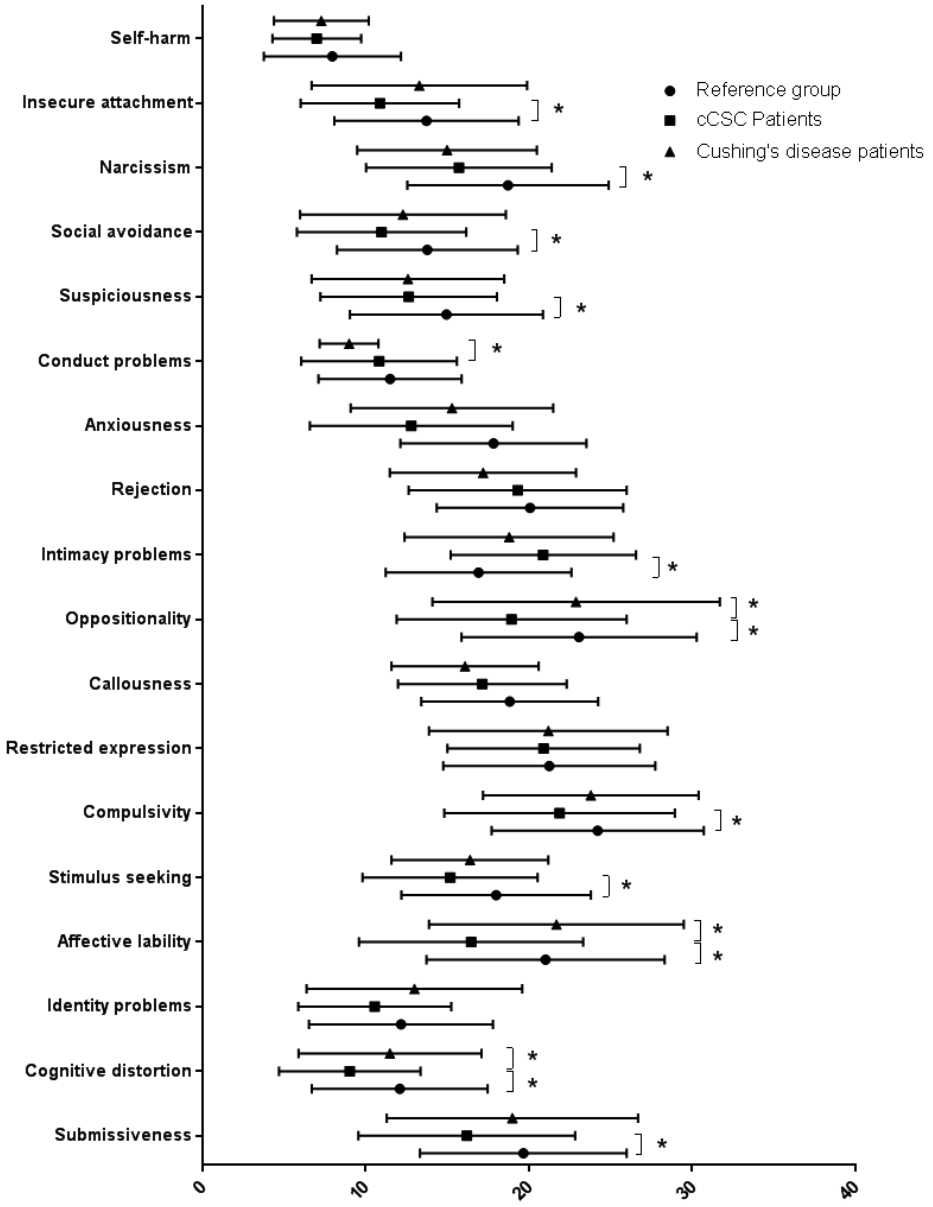

Figure 1. DAPP-SF personality traits in cCSC patients

Abbreviations: cCSC: chronic central serous chorioretinopathy; DAPP-SF: Dimensional Assessment of Personality Pathology short form; SD: standard deviation.

* = statistically significant

Psychological morbidity

The AS was completed by 83 (97%) patients with cCSC (Table 3). Mean patients score was 12.22 (range, 3 - 26). Clinically significant apathy (a score of ≥14) was present in 34.9% of patients with cCSC. No difference was observed in total scores between patients with active cCSC and patients with inactive disease (p=0.256). No differences in reported apathy were found between patients with cCSC and the reference data from the general population, nor to reference data from patients with Cushing's disease (p=0.033 and p=0.011, respectively). Although no significant differences were found, the scores of patients with cCSC were in between the scores of the reference data from the general population and the reference data from patients with Cushing's disease (Figure 2).

Table 3. Apathy and irritability in cCSC patients

	cCSC patients (n=83)	**Reference data (n=68)**	***P*-value**
Apathy	12.22 (4.95)	10.50 (4.80)	0.033
Irritability	9.78 (6.24)	9.50 (5.70)	0.792
	cCSC patients (n=83)	**Cushing's disease patients (n=51)**	
Apathy	12.22 (4.95)	14.80 (6.50)	0.011
Irritability	9.78 (6.24)	11.50 (7.70)	0.154

Data is presented as mean (SD).

Abbreviations: cCSC: chronic central serous chorioretinopathy; SD: standard deviation.

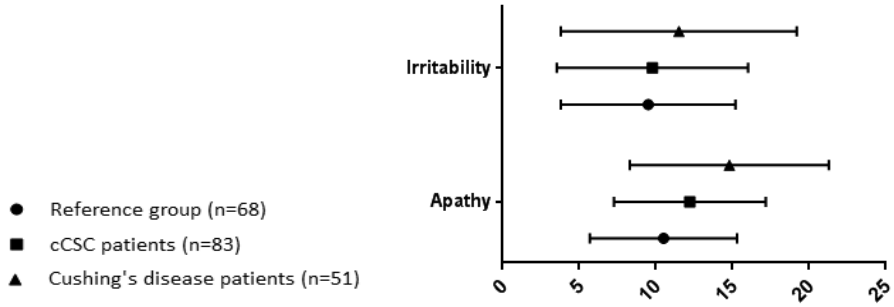

Figure 2. Apathy and irritability in cCSC patients

Data is presented as mean (SD)

Abbreviations: cCSC: chronic central serous chorioretinopathy; SD: standard deviation.

The IS was also completed by 83 (97%) patients with cCSC (Table 3). Mean patient score was 9.8 (range, 0 - 26). Clinically significant irritability (a score of ≥14) was present in 29.3% of patients with cCSC. Total scores did not differ between patients with active cCSC and patients with inactive cCSC (p=0.360). No differences in reported irritability were observed between patients with cCSC and the reference data from the general population (p=0.792), nor to reference data from patients with Cushing's disease (p=0.154). Although no statistically significant differences were found, it could be observed that the scores of patients with cCSC were in between the scores of the reference data from the general population and the reference data from patients with Cushing's disease, which was in line with the outcome of the assessment of apathy (Figure 2).

Table 4. Coping in cCSC patients

	cCSC patients (n=83)	**Reference data (n=1493)**	***P*-value**
Active coping	19.33 (4.08)	18.30 (3.50)	0.010
Seeking distraction	16.10 (3.61)	15.50 (3.60)	0.142
Avoiding	15.54 (3.29)	14.80 (3.30)	0.046
Seeking social support	12.64 (2.67)	11.30 (3.00)	0.000
Passive coping	11.75 (3.29)	10.70 (2.90)	0.002
Expressing emotions	5.71 (1.63)	6.20 (1.70)	0.011
Fostering reassuring thoughts	11.47 (2.54)	11.60 (2.50)	0.645
	cCSC patients (n=83)	**Cushing's disease patients (n=42)**	***P*-value**
Active coping	19.33 (4.08)	17.50 (3.00)	0.012
Seeking distraction	16.10 (3.61)	17.70 (3.00)	0.015
Avoiding	15.54 (3.29)	16.30 (3.00)	0.218
Seeking social support	12.64 (2.67)	13.30 (4.00)	0.274
Passive coping	11.75 (3.29)	12.00 (3.00)	0.676
Expressing emotions	5.71 (1.63)	5.90 (2.00)	0.572
Fostering reassuring thoughts	11.47 (2.54)	12.30 (3.00)	0.107

Data is presented as mean (SD).
Abbreviations: cCSC: chronic central serous chorioretinopathy; SD: standard deviation.

Coping strategies

Eighty-three (97%) patients with cCSC filled in the UCS (Table 4). Patients with inactive disease (n=27) made significantly more use of avoiding compared to cCSC patients with active disease (n=56, p=0.007). Compared to reference data, patients with cCSC reported to use more passive coping strategies and to seek more social support (p=0.000 and p=0.002, respectively). Because the reference data was male only, data from male cCSC patients between 19 and 65 years of age (n= 67) was compared separately. This cCSC patient category also made more use of active coping compared to the reference population (p=0.006), beside the aforementioned seeking

social support (p=0.000) and passive coping (p=0.000). No differences in coping strategies were observed between patients with cCSC and patients with Cushing's disease.

Correlation of coping strategies and personality

Moderate to strong correlations were only found between the coping strategy passive coping and several personality traits. This coping strategy was positively correlated with affective lability (p=0.000, R^2= 0.759), cognitive distortion (p=0.000, R^2= 0.656), identity problems (p=0.000, R^2= 0.675), insecure attachment (p=0.000, R^2= 0.558), oppositionality (p=0.000, R^2= 0.522), social avoidance (p=0.000, R^2= 0.515), and anxiousness (p=0.000, R^2= 0.711).

Post-hoc analysis without patients with less typical cCSC

Analyses performed without the 5 patients with atypical cCSC revealed, in addition to the previously reported results, that patients with inactive cCSC only reported more social avoidance compared to patients with active disease (p=0.006). Furthermore, when outcomes of the patients with cCSC were compared to reference data from the general population, no difference in compulsivity was observed anymore (p=0.011), and less callousness was observed in patients with cCSC (p=0.010). When comparing our patient data with Cushing's disease patients, no difference on conduct problems was observed anymore (p=0.013). On the AS, patients with typical cCSC reported less apathy than Cushing's disease patients (p=0.006), after the exclusion of these 5 patients. When the males and females together were compared to the reference group of the general population, patients with cCSC made more use of the coping styles active coping (p=0.006), seeking social support (p=0.001), and passive coping (p=0.002). When male only data was compared to this group, the same differences were found (p=0.003, p=0.000, and p=0.001, respectively). Excluding the 5 patients with less typical cCSC did not significantly change the rest of the aforementioned results.

DISCUSSION

This is the first study that extensively evaluated maladaptive personality traits, psychological morbidity, and coping strategies in cCSC patients. We demonstrated that patients with cCSC overall did not suffer from more maladaptive personality traits compared to the general population, except for the personality trait intimacy problems. In contrast to earlier ideas, we did not find evidence for a higher prevalence of type A personality characteristics in these patients. Furthermore, on the level of conduct, no more psychological morbidity in the form of either apathy or irritability was observed. However, patients with cCSC were shown to make more use of certain coping strategies (e.g. seeking social support, passive coping, and in males also active coping), in comparison with the general population. Correlation analysis revealed that these differences found on coping strategies were partly explained by personality traits.

In our study, personality traits, psychological morbidity, and coping strategies were assessed in a large cohort of cCSC patients. We did not find evidence for a higher prevalence of type A personality characteristics in these patients, which is in contrast with earlier studies.[17-19] Critical evaluation of the literature revealed that type A personality was mainly assessed using behavioral outcome measures (e.g. Jenkins activity survey) in these studies, while this inventory has been shown not to correlate to personality characteristics and psychopathology.[41] In other studies, type A personality was not strictly defined, and the diagnosis of such a personality could have been based on either the appearance of this term in medical records or a character description of being 'tense', 'high strung' or 'highly ambitious' by either the patient or social environment.[42]

The personality profile of patients with cCSC in our cohort was more comparable to the profile of patients with Cushing's disease than to the general population, since 14 out of the 18 DAPP-SF subscales outcomes of cCSC patients matched with patients with Cushing's disease, where only 8 out of 18 were comparable with the general population. However, patients with cCSC showed less affective lability, cognitive distortion, and oppositionality compared to patients with Cushing's disease, whereas they reported more conduct problems (although the significance of this difference was omitted in the post-hoc analysis excluding patients with less typical cCSC). It was previously demonstrated that patients with cCSC have an activated HPA axis leading to high normal levels of cortisol, which could be an explanation for the findings on personality profile in this study.[13] It is known for patients with Cushing's disease, with appurtenant supraphysiological cortisol levels, to have more maladaptive personality traits compared to healthy people.[27] Our study showed that there also is similarity between patients with cCSC and patients with Cushing's disease regarding personality features, which is in line with the biochemical resemblance.

Furthermore, also on the level of coping strategies, patients with cCSC were more comparable to patients with Cushing's disease than to general population. Compared to general population, patients with cCSC seek more social support. This may be explained by the fact that cCSC results in visual problems with a decreased quality of life,[5] which makes patients more dependent on others. Moreover, more active and passive coping was reported by cCSC patients. Although this seems to be contradictory, these coping styles contain different subitems which can certainly occur together. Knowledge on coping strategies is valuable information, since cCSC is an invalidating, chronic disease and this information can be used in for example designing a self-management training aiming at improving quality of life of this patient group.

The lack of a gender- and age-matched control group can be considered to be a limitation of this study. The matched healthy control group that we recruited for the other study our patients

with cCSC participated in, could unfortunately not be used, since the number of healthy controls could not make statistical power for the comparison of the questionnaire outcomes. However, population based reference data was available and considered to be a worthy alternative, since this is data derived from big population based cohorts. Questionnaire outcomes of healthy controls described in other studies were also used, with their demographics being comparable with the demographics of our patients. The comparison with a cohort of patients with Cushing's disease made it possible to describe personality traits, psychological morbidity, and coping strategies within the whole spectrum of HPA axis functioning.

The results of this study contribute to further psychological phenotyping of patients with cCSC. Using a systematic approach and an extensive number of questionnaires, no evidence for a higher prevalence of type A personality characteristics was found in the patient group. Despite the fact that we could not reveal specific points of action for psychological interventions, we hope to contribute to the clinicians' awareness regarding the personality characteristics and coping strategies of patients with cCSC, leading to a more suitable approach of these patients in the consulting room.

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3.3

SYSTEMIC COMPLEMENT ACTIVATION IN CENTRAL SEROUS CHORIORETINOPATHY

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ABSTRACT

Purpose: A clear link between several variants in genes involved in the complement system and chronic central serous chorioretinopathy (CSC) has been described. In age-related macular degeneration, a disease that shows clinical features that overlap with CSC, both genetic risk factors and systemic activation of the complement system have previously been found. In this case-control study, we assessed whether there is evidence of either systemic activation or inhibition of the complement system in patients with chronic CSC.

Methods: A prospective case-control study of 76 typical chronic CSC patients and 29 controls without ophthalmological history was conducted. Complement activity assays (classical, alternative, and mannose-binding lectin pathway), complement factors 3, 4, 4A, 4B, B, D, H, I, and P, activation products C3d, C5a, and sC5b-C9, and the C3d/C3 ratio were analysed in either serum or plasma. A correction for possible effects of gender, age, body mass index, and smoking status was performed.

Results: In this study, none of the tested variables, including regulation and activation products, proved to be significantly different between the groups. Moreover, no associations with either CSC disease activity or possible CSC related steroid use were observed.

Conclusion: Despite the available literature regarding a possible relationship between chronic CSC and variants in genes involved in the complement system, we did not find evidence of an association of chronic CSC with either systemic complement activation or inhibition.

INTRODUCTION

Central serous chorioretinopathy (CSC) mainly occurs in middle-aged male patients, and may cause irreversible vision loss.[1] CSC originates from dysfunction of the choroid, which shows an increase in permeability and thickness. These choroidal abnormalities and retinal pigment epithelium (RPE) damage lead to breakdown of the outer blood-retinal barrier, with subsequent serous subretinal fluid (SRF) leakage and neuroretinal detachment, often in the macula.[2-6] Although the use of exogenous corticoids is strongly associated with an increased risk of CSC,[7-10] the precise pathogenetic mechanism is unclear.[2-6]

The occurrence of CSC has been described in patients with several inflammatory diseases such as systemic lupus erythematosus and membranoproliferative glomerulonephritis.[11, 12] However, it is still unknown whether either one or both of these immune-mediated diseases or, alternatively, the prescribed glucocorticoid treatment leads to CSC in these patients.[13] Genetic factors may also play a part in the pathogenesis of CSC. Earlier reports on the familial occurrence of CSC have been published and associations between CSC and genetic variants in the *complement factor H* (*CFH*) gene, part of the innate immune system, have been found in several chronic CSC (cCSC) cohorts of diverse ethnic origins.[4, 14-18] Factor H, produced by both the choroidal and RPE cells, and critical in controlling local intraocular inflammation, is responsible for downregulation of the activation of the complement alternative pathway.[19-21] In the *CFH* gene, the single nucleotide polymorphisms (SNPs) have been observed to be either protective or risk conferring.[14-16] A recent study, demonstrating a possible involvement of the complement system in CSC, reported the absence of *complement component 4B* (*C4B*) gene copies to be associated with an increase in the risk of developing CSC, whereas the presence of 3 *C4B* copies is reported to be protective for CSC.[22]

In age-related macular degeneration (AMD), a disease with features overlapping with CSC,[15, 23] the Tyr402His amino acid substitution in the *CFH* gene has been shown to be strongly associated with the development of disease.[24] Moreover, a recently published study detected opposing effects of alleles in the *CFH* gene within a CSC and AMD patient group: genetic variants in *CFH* that led to an increased risk of AMD were protective for CSC, and vice versa.[15] In AMD, variants in *C3*, *CFB*, and *C2* have also been described to affect the risk of the disease and its progression.[25-27] Furthermore, in comparison with a control group systemic activation of the complement system has been detected in multiple AMD patient cohorts.[28, 29] Both in patients and in controls this activation showed a correlation with specific AMD risk alleles in complement genes, including the Tyr402His variant in *CFH*.[29]

Therefore, analogous to AMD, the association of complement gene SNPs in CSC may also gesture at a role for either increased or decreased systemic complement system activity in

the pathogenesis of CSC. To assess systemic activation of the complement system in cCSC patients, we performed the first case-control study in this patient group and analysed whether there are differences in complement activation for several CSC subgroups.

MATERIALS AND METHODS

Study population

Seventy-eight cCSC patients who visited the Department of Ophthalmology at Leiden University Medical Center, the Netherlands, were included in this study. The study was powered based on previous reports on the C3d/C3 ratio as a measure of complement activation in AMD; with the current sample size we had >80% power to detect the previously observed effects.[28-30] Chronic CSC diagnosis was confirmed by fundoscopy, digital color fundus photography (Topcon Corp., Tokyo, Japan), fundus autofluorescence (Spectralis HRA+ optical coherence tomography (OCT); Heidelberg Engineering, Heidelberg, Germany), spectral-domain OCT (Spectralis HRA+OCT), fluorescein angiography (FA; Spectralis HRA+OCT), and indocyanine green angiography (Spectralis HRA+OCT), based on current knowledge from literature. All of the following characteristics had to be present: serous SRF on OCT, ≥1 area of multifocal diffuse leakage or irregular RPE window defects on FA, and corresponding hyperfluorescence on indocyanine green angiography. In all patients, either SRF or intraretinal fluid on OCT had to have been present less than 2 years ago. Patients diagnosed with acute CSC as recognised by a focal leakage spot (ink blot) or a smokestack pattern on FA, patients with duration of disease of less than 3 months, and patients in whom either polypoidal choroidal vasculopathy or a choroidal neovascularisation or (signs of) AMD were present, were excluded.[2-5, 13, 28, 29, 31] Since the administration of corticosteroids can affect both the innate and adaptive immune system and can influence choroidal vascular permeability in male CSC patients by cadherin 5 downregulation, patients who used corticosteroids less than 1 year before diagnosis were also analysed separately in our study.[32, 33] Patients in whom the presence of SRF was confirmed on OCT at the day of blood puncture for this study, indicating active disease, were also analysed separately, to assess the possible influence of CSC disease activity on systemic complement activation. None of the patients had a history of either systemic autoimmune diseases associated with complement activation (systemic lupus erythematosus, ANCA-associated vasculitis, systemic sclerosis, rheumatoid arthritis) or with any (familial) ocular disease. Thirty-two matched controls without ophthalmological history were recruited at the outpatient clinic of the Department of Ophthalmology at Leiden University Medical Center.

Clinical data including demographics (age, gender, and ethnicity), body mass index (BMI), smoking, medical history, and use of both steroids and immunosuppressive medication were obtained, both for patients and controls (Table 1). For all patients, clinical information regarding

CSC was collected. Written informed consent was obtained from all subjects before enrollment in this study. The study adhered to the tenets of the Declaration of Helsinki. Approval of the institutional review board and the ethics committee of Leiden University Medical Center were obtained (NL50816.058.14). Subjects were included in this study from June 2015 to April 2016.

Table 1. Demographic characteristics of the study population

Variable	Patients (n=76)	Controls (n=29)	OR (95% CI)	P-value
Male gender (n [%])	70 (92%)	26 (90%)	1.78 (0.34-9.3)	0.50
Non-smoker (n [%])	35 (46%)	21 (73%)	reference	
Past smoker (n [%])	30 (39%)	2 (7%)	8.66 (1.85-40.6)	0.01
Current smoker (n [%])	11 (14%)	3 (10%)	2.47 (0.60-10.1)	0.21
Age (mean [SD], (in years))	49.2 (11.2)	43.0 (11.2)	1.06 (1.00-1.11)	0.045
Body mass index (mean [SD])	25.6 (3.30)	25.3 (4.36)	0.96 (0.84-1.10)	0.57

Only patients in whom all covariates were available were included in this study.
Abbreviations: CI: confidence interval; OR: odds ratio; SD: standard deviation.

Complement measurements

After blood drawing, ethylenediaminetetraacetic acid (EDTA) samples were placed on ice and centrifuged (10 minutes at 1083 g at 4°C). These plasma samples were stocked in a -80°C freezer within 1 hour after collection. The activation products C5a and sC5b-C9 were measured in plasma samples using validated enzyme-linked immunosorbent assay kits (Hycult Biotech, Uden, the Netherlands).

After coagulation at room temperature for 1 hour, serum samples were centrifuged (10 minutes at 1083 g at 4°C) and aliquots were immediately placed in the -80°C freezer. These serum samples were used to quantify the complement activity of the classical pathway (CP50), the alternative pathway (AP50), and the mannose-binding lectin pathway (LP50) with the Wieslab kit (Euro-diagnostics, Malmö, Sweden). In addition, complement factors 3, 4, B, D, H, I, P, the activation product C3d, and the C3d/C3 ratio (parameter of activation of complement alternative pathway factor 3) were analysed in these sera, according to previously described measurement techniques.[29] Moreover, complement factors 4A and 4B were assessed separately, according to a protocol that has been previously published.[34]

Statistical analysis

Statistical analyses were performed in R (R Foundation for Statistical Computing, Vienna, Austria). Since the information for the covariates age, gender, BMI, and smoking was not complete for 5 subjects, 105 subjects (76 patients, 29 controls) could be included in the statistical tests. Baseline and clinical characteristics, and values of systemic complement

activation of both cases and controls were described by using standard descriptive statistics. Mean differences between the case and control group were assessed using a linear regression model, where correction for the covariates was performed. A role for possible CSC related steroid use and CSC disease activity was also assessed by using a linear regression model, for which again was corrected for the previously mentioned covariates.

Two-sided p-values of <0.05 were considered to be statistically significant. The Bonferroni correction was performed for the tests comparing different patient groups and control subjects, since adjusting was required for the 16 tests that were done.

RESULTS

Patient characteristics

Since information regarding all covariates was available for 76 patients, only the assessments of these cCSC patients could be taken into account. The mean age of these patients (70 males, 6 females) was 49 ± 11 years (range, 25 - 83 years). Sixty patients (79%) were Caucasian. Either bilateral SRF on OCT or bilateral hot spots of leakage on FA either was or had been present in 28 patients (37%), and until blood puncture for this study a recurrence of CSC had been diagnosed in 33 patients (43%). Medical history of 12 patients (16%) revealed hypertension, and in 3 other patients (4%) other cardiovascular diseases had been previously diagnosed. Three patients (5%) reported that they were previously clinically diagnosed with a depression. In 3 patients (5%) a burn-out had been diagnosed, whether in 2 other patients (3%) this was the case for post-traumatic stress disorder. None of the included patients reported the use of immunosuppressive medication. Fifteen cCSC patients (20%) had used corticosteroids less than 1 year before diagnosis, and in 23 patients (30%) the presence of SRF on OCT was confirmed at the day of blood puncture for this study, indicating active disease.

Control characteristics

The mean age of the 29 control subjects (26 males, 3 females; 83% of Caucasian ethnicity), in whom no ophthalmological diseases had been diagnosed before and from whom covariates were available, was 43 ± 11 years (range, 24 - 52 years), which was significantly lower compared to the patient group (p=0.04). When comparing the patients and controls, no differences regarding gender, BMI, and current smoking could be detected. Three controls (10%) were previously diagnosed with hypertension, and 2 others (7%) were known with other cardiovascular diseases. Two controls (7%) reported that a depression had been diagnosed in their medical history, whereas 3 others (10%) reported the diagnosis of a burn-out. Four controls (14%) reported the previous use of steroids.

Complement levels

After the Bonferroni correction had been performed and covariates had been taken into account (Table 1), no significant differences were detected between CSC patients and the control group for the classical, alternative, and mannose-binding lectin pathway. Moreover, no significant differences were found for complement factors 3, 4, 4A, 4B, B, D, H, I, and P, activation products C3d, C5a, and sC5b-C9, and the C3d/C3 ratio (Table 2). To detect possible pathophysiological differences between patient groups, dividing CSC patients in groups based on a possible relationship with either steroid use (Table 3) or CSC disease activity at the day of blood puncture (Table 4), also did not lead to differences in either activation or inhibition of the complement system comparing patients and controls.

Table 2. Mean complement activities and concentrations in chronic central serous chorioretinopathy (CSC) patients and controls

Complement activity/protein (units)	Chronic CSC (n=76), mean (SD)	Controls (n=29), mean (SD)	P-value	Adjusted p-value	Normal laboratory values
Classical pathway activity (CP50) (%)*	101.2 (4.23)	102.2 (2.85)	0.05	0.81	>74
Alternative pathway activity (AP50) (%)*	89.8 (18.6)	90.6 (11.7)	0.15	1.00	>39
Mannose-binding lectin pathway (LP50) (%)*	68.7 (41.0)	65.1 (44.4)	0.95	1.00	>10
C3 (mg %)	126.6 (24.4)	122.6 (20.2)	0.95	1.00	90-200
C4 (mg %)	26.3 (8.06)	24.2 (7.06)	0.13	1.00	9.5-41.5
C4A (µg/ml)	299.7 (165.7)	335.7 (151.9)	0.17	1.00	NA
C4B (µg/ml)	115.2 (34.6)	97.3 (26.6)	0.05	0.82	NA
CFB (mg %)	17.3 (3.74)	16.4 (2.89)	0.41	1.00	13-22
CFD (µg/ml)	2.75 (0.62)	2.68 (0.68)	0.78	1.00	NA
CFH (mg %)	21.6 (3.43)	21.0 (3.54)	0.79	1.00	19-26
CFI (mg %)	45.9 (7.78)	46.0 (7.39)	0.64	1.00	NA
CFP (µg/ml)	23.1 (6.45)	24.1 (4.25)	0.73	1.00	17.1-27.7
C3d (µg/ml)	2.61 (0.97)	2.76 (1.49)	0.56	1.00	NA
C5a (ng/ml)	5.32 (12.6)	3.16 (3.35)	0.22	1.00	NA
C5b-C9 (Au/ml)	0.70 (0.18)	0.70 (0.18)	0.47	1.00	NA
C3d/C3 ratio	0.21 (0.09)	0.23 (0.13)	0.73	1.00	NA

Only patients in whom all covariates were available were included in this study.

* Determined by ELISA, and presented as a percentage of the standard in the kit.

Abbreviations: ELISA: enzyme-linked immunosorbent assay; NA: not available; SD: standard deviation.

Table 3. Mean complement activities and concentrations in chronic central serous chorioretinopathy (CSC) patients and controls. Chronic CSC patients were divided into 2 groups: patients who had used steroids within 1 year before diagnosis versus patients who had not used steroids within 1 year before diagnosis

Complement activity/protein (units)	Chronic CSC, steroid related (n=15), mean (SD)	Chronic CSC, non-steroid related (n=61), mean (SD)	Controls (n=29), mean (SD)	P-value	Adjusted p-value
Classical pathway activity (CP50) (%)*	102.4 (3.85)	101.0 (4.30)	102.2 (2.85)	0.08	1.00
Alternative pathway activity (AP50) (%)*	87.9 (21.3)	90.2 (18.0)	90.6 (11.7)	0.29	1.00
Mannose-binding lectin pathway (LP50) (%)*	68.1 (46.0)	68.8 (40.1)	65.1 (44.4)	0.99	1.00
C3 (mg %)	126.8 (25.8)	126.5 (24.3)	122.6 (20.2)	1.00	1.00
C4 (mg %)	29.0 (8.55)	25.7 (7.87)	24.2 (7.06)	0.15	1.00
C4A (µg/ml)	303.6 (131.4)	298.7 (174.0)	335.7 (151.9)	0.38	1.00
C4B (µg/ml)	116.6 (41.0)	114.9 (33.2)	97.3 (26.6)	0.14	1.00
CFB (mg %)	16.9 (4.55)	17.3 (3.55)	16.4 (2.89)	0.63	1.00
CFD (µg/ml)	2.73 (0.44)	2.75 (0.65)	2.68 (0.68)	0.92	1.00
CFH (mg %)	22.3 (4.46)	21.5 (3.14)	21.0 (3.54)	0.80	1.00
CFI (mg %)	43.0 (9.24)	46.6 (7.30)	46.0 (7.39)	0.42	1.00
CFP (µg/ml)	21.7 (6.84)	23.5 (6.36)	24.1 (4.25)	0.61	1.00
C3d (µg/ml)	2.40 (0.66)	2.66 (1.03)	2.76 (1.49)	0.34	1.00
C5a (ng/ml)	2.09 (0.75)	6.11 (14.0)	3.16 (3.35)	0.01	0.22
C5b-C9 (Au/ml)	0.64 (0.15)	0.71 (0.18)	0.70 (0.18)	0.15	1.00
C3d/C3 ratio	0.20 (0.08)	0.22 (0.09)	0.23 (0.13)	0.75	1.00

Only patients in whom all covariates were available were included in this study.

* Determined by ELISA, and presented as a percentage of the standard in the kit.

Abbreviations: ELISA: enzyme-linked immunosorbent assay; SD: standard deviation.

Table 4. Mean complement activities and concentrations in chronic central serous chorioretinopathy (CSC) patients with subretinal fluid at the day of blood taking, compared to controls

Complement activity/protein (units)	Active chronic CSC (n=22), mean (SD)	Controls (n=29), mean (SD)	P-value	Adjusted p-value
Classical pathway activity (CP50) (%)*	101.2 (3.10)	102.2 (2.85)	0.19	1.00
Alternative pathway activity (AP50) (%)*	85.2 (19.3)	90.6 (11.7)	0.12	1.00
Mannose-binding lectin pathway (LP50) (%)*	71.5 (34.3)	65.1 (44.4)	0.43	1.00
C3 (mg %)	127.4 (26.2)	122.6 (20.2)	0.51	1.00
C4 (mg %)	27.4 (8.86)	24.2 (7.06)	0.07	1.00
C4A (µg/ml)	252.1 (135.5)	335.7 (151.9)	0.04	0.62
C4B (µg/ml)	107.7 (36.5)	97.3 (26.6)	0.31	1.00
CFB (mg %)	17.2 (4.21)	16.4 (2.89)	0.57	1.00
CFD (µg/ml)	2.61 (0.51)	2.68 (0.68)	0.89	1.00
CFH (mg %)	21.3 (3.43)	21.0 (3.54)	1.00	1.00
CFI (mg %)	44.8 (9.51)	46.0 (7.39)	0.95	1.00
CFP (µg/ml)	23.39 (6.67)	24.1 (4.25)	0.76	1.00
C3d (µg/ml)	2.64 (0.95)	2.76 (1.49)	0.83	1.00
C5a (ng/ml)	5.72 (16.6)	3.16 (3.35)	0.43	1.00
C5b-C9 (Au/ml)	0.68 (0.22)	0.70 (0.18)	0.57	1.00
C3d/C3 ratio	0.21 (0.09)	0.23 (0.13)	0.84	1.00

Only patients in whom all covariates were available were included in this study.

* Determined by ELISA, and presented as a percentage of the standard in the kit.

Abbreviations: ELISA: enzyme-linked immunosorbent assay; SD: standard deviation.

DISCUSSION

To the best of our knowledge, we conducted the first case-control study on systemic complement activation in cCSC patients. Although the study was sufficiently powered to detect differences in the C3d/C3 ratio as previously reported in AMD,[28, 29] no association was found between CSC and both complement activation and inhibition, which suggests that the effect size of C3d/C3 in CSC is either smaller, or absent. Moreover, when dividing the patients into subgroups based on either possible CSC related steroid use or CSC disease activity, no differences were detected either between several patient groups or between patients and controls for the sample sizes that were included in this study.

The outcome of our study differs from findings in AMD, a disease that shows overlapping features with CSC and in which systemic activation of the complement system has been found.[15, 23, 28, 29] In a recent study assessing SNPs in complement genes in both AMD and CSC patients, opposing effects were observed for genetic associations of the *CFH* gene, suggesting that the complement system is involved in CSC, although the direction of the effect remained uncertain.[15] The lack of any association in our study may be a consequence of the fact that the effect sizes for genetic associations of *CFH* in CSC were weaker compared to AMD.[15, 28, 29] Variables known to influence activation of the complement system, such as age, gender, BMI, and smoking, were taken into account during statistical analysis of data from our study.[28, 29]

Since steroid use has been described to be the most pronounced risk factor for CSC, and the etiology in patients with previously reported CSC related steroid has been described to differ from non-steroid associated disease, both groups were also analysed separately.[9, 33] Our results indicate that there is no clear role for complement activation in either of these patient groups. From all patients, in whom SRF had to have been present on OCT within the last 2 years, patients with active disease at the day of blood puncture were also analysed separately. Even in cases with active CSC at the time of systemic complement analysis, no abnormalities in complement factors were detected in this study, which would indicate that systemic complement dysfunction does not play a significant role in active CSC as well.

Despite the available literature regarding a possible relationship between cCSC and variants in genes involved in the complement system, an association between cCSC and either systemic complement activation or inhibition was not found in this study. However, for several complement components the number of included patients and controls could have led to underpowered results. In future studies, the exact role of the complement system in CSC remains to be elucidated. Since previously reported genetic associations clearly suggest involvement of the complement system in CSC, the functional translation of these findings and their contribution to the disease mechanism should be the focus of future investigations.

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3.4

ANTIRETINAL ANTIBODIES IN CENTRAL SEROUS CHORIORETINOPATHY: PREVALENCE AND CLINICAL IMPLICATIONS

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ABSTRACT

Purpose: To investigate the possible role of autoimmune reactions directed against retinal tissue in central serous chorioretinopathy (CSC), by analysing the presence of serum antiretinal antibodies (ARAs) and establishing their clinical relevance.

Methods: Sixty-three CSC patients were included and clinical characteristics were collected. Serum samples of all CSC patients, 101 uveitis patients, and 60 healthy donors were analysed for the presence of ARAs by indirect immunofluorescence. Furthermore, all CSC serum samples were analysed on Western blot. Correlations between laboratory findings and clinical features of CSC were determined by logistic regression.

Results: ARAs were present in 54% of the CSC patients, in 46% of uveitis patients ($p=0.153$), and in 17% of healthy controls ($p<0.001$). The majority of ARAs in CSC were directed against photoreceptors (27%), which occurred significantly more often compared to uveitis patients (15%, $p=0.039$) and to healthy controls (5%, $p=0.003$). No associations between clinical CSC characteristics and the presence of ARAs were found.

Conclusion: Serum ARAs are present in more than half of the CSC patients, and especially ARAs directed against photoreceptors were detected more frequently compared to both healthy controls and uveitis patients. Further research is warranted to unravel the role of ARAs in the pathogenesis of CSC.

INTRODUCTION

Central serous chorioretinopathy (CSC) is a specific and relatively common early-onset chorioretinal disease that primarily affects the macula. In CSC, a subretinal fluid (SRF) leakage through a dysfunctional retinal pigment epithelium (RPE) leads to detachment of the neuroretina.[1-3] A prolonged neuroretinal detachment in the macula causes permanent central visual loss due to photoreceptor atrophy.[1, 2] Such a loss of visual acuity, sometimes associated with image distortion and loss of color and contrast vision, may have a high impact on a patient's personal and professional life. Early diagnosis and treatment is desirable to try to improve the visual outcome and quality of life.[1, 3-12]

The exact pathogenesis of CSC is currently obscure; presumably CSC occurs due to dysfunction of the RPE with hyperpermeability, swelling, and leakage of the underlying choroid.[3, 13] Moreover, the optimal treatment for CSC is unknown.[14] CSC is up to 6 times more common in men (estimated mean annual age-adjusted incidence: 9.9 per 100,000) compared to women (estimated incidence: 1.7 per 100,000).[15] CSC is associated with the use of steroid containing medication, with odds ratios up to 37.1, as well as with endogenous hypercortisolism.[16-19] Familial occurrence of CSC has been described and recent studies have found evidence of genetic associations in CSC patients, including genetic polymorphisms in the *CFH* gene, the *ARMS2* gene, the *C4b* gene, and the *CD5* gene.[20-27]

Although little is known about the exact cause of CSC, a role of the immune system via the complement system has been suggested based on associations found in complement-related genes. Recent evidence suggests a role for antiretinal antibodies (ARAs) in uveitis and age-related macular degeneration.[28-30] A systematic study on presence and the possible role of ARAs in CSC is currently lacking.

We hypothesize that damage to the RPE outer blood-retinal barrier in CSC may result in a secondary formation of ARAs, which may affect the clinical course of CSC. In this study, we set out to investigate the presence of serum ARAs in CSC patients and to analyse a possible correlation of ARAs with the clinical characteristics of CSC.

MATERIALS AND METHODS

Patient and data selection

In this study, we included 63 Caucasian patients with chronic CSC who visited the outpatient clinic of the Department of Ophthalmology of the Leiden University Medical Center, Leiden, the Netherlands. The diagnosis of chronic CSC was based on ophthalmic examination and multimodal imaging, including fundoscopy, optical coherence tomography (OCT) using either the spectral-domain OCT or the Cirrus OCT device, fundus autofluorescence, fluorescein angiography (FA), and indocyanine green angiography. The diagnosis of chronic CSC was based on the presence of all of the following criteria: serous SRF on OCT, ≥1 area of multifocal diffuse leakage or irregular RPE window defects on FA, and corresponding hyperfluorescent areas on indocyanine green angiography. Patients diagnosed with acute CSC were excluded from this study, since it is currently unclear if acute CSC and chronic CSC represent a continuum or are separate disease entities. Patients with evidence of other retinal diagnoses, choroidal neovascularisation, and/or polypoidal choroidal vasculopathy were also excluded. Clinical data of CSC patients were collected from medical data files and included patient demographics (age, gender, and (family) history) and ocular characteristics comprising stage, duration, activity and laterality of CSC, use of steroids or immunosuppressive medications, presence of intraretinal fluid, treatment for CSC, and central retinal thickness (distance from the outer part of the ellipsoid zone to the inner part of the internal limiting membrane; CRT). The CRT of the affected eye was selected; if both eyes were affected the right eye was included. Diffuse CSC was characterised by the presence of >5 disc areas of hyperfluorescent RPE changes or leakage on FA. Serum samples of 101 patients with uveitis (intermediate uveitis, posterior uveitis or panuveitis) were used as disease controls, since a higher prevalence of ARAs in serum in this cohort has been described previously.[31] Serum of 60 blood bank donors (gender and age unknown) was used as (presumed) healthy controls. The study adhered to the tenets of the Declaration of Helsinki. Approval of the ethics committee and institutional review board was obtained.

Detection of ARAs using indirect immunofluorescence (IIF)

Initial screening of sera for ARAs was performed as described previously.[31] In short, cryosections of primate retinal tissue generated by Euroimmun (Lubeck, Germany) were left unfixed and incubated with 1:100 diluted serum for 30 minutes at room temperature. Sections were washed in stagnant phosphate-buffered saline (PBS) and incubated with goat-anti-human IgG conjugated with fluorescein isothiocyanate (FITC) for 30 minutes at room temperature. Thereafter, sections were washed in stagnant PBS and embedded. The positive control consisted of retinal tissue incubated with 1:100 diluted serum of an anti-nuclear antibody

(ANA) positive patient; for the negative controls we used incubation with PBS and 1:100 diluted serum of a healthy control.

Detection of ANA using IIF

All sera showing staining of the retinal nuclear layers (outer/inner nuclear layer, ganglion cell layer) on IIF were subsequently analysed for the presence of ANAs. ANA detection was performed by IIF using HEp-2 cells (Inova, San Diego, CA, USA), as described before.[31] In summary, HEp-2 cells were incubated with 1:80 diluted serum for 30 minutes at room temperature. After washing in PBS with continuous stirring, slides were incubated for another 30 minutes with FITC-conjugated goat anti-human IgG with propidium iodide (Inova). Subsequently, slides were washed and embedded.

Evaluation of IIF results

All slides were evaluated with a fluorescence microscope (20x magnification) by 2 independent observers. Specific retinal layers (ganglion cell layer, inner plexiform layer, inner nuclear layer, outer plexiform layer, outer nuclear layer, and rods and cones layer) were evaluated for the presence of fluorescent staining including intensity of the staining. When both ANA (on HEp-2-cells) and ARA (on primate retinal tissue) had an equivalent intensity of staining, a sample was scored as 'unknown' because this combination does not allow a proper discrimination between the presence and absence of ARAs, by possible masking due to ANAs. These samples were excluded from the final analyses.

Western blot analysis

All CSC patients were evaluated for the presence of ARAs using Western blot analysis. Healthy human retinal protein extract was obtained after retina tissue homogenization in PBS. Tissue fragments were removed by centrifugation and the supernatant was frozen at -80°C until use. Retinal tissue extract was fractionated by SDS-polyacrylamide gel electrophoresis and separated proteins were subsequently transferred to nitrocellulose membranes. The membranes were blocked by incubation with 5% non-fat dry milk and incubated with serum of CSC patients or with appropriate control serum at a dilution of 1:100 in 5% non-fat dry milk in tris-buffered saline (TBS) overnight. After multiple washes with 0.1% TBS, membrane bound human IgG was identified by horseradish peroxidase-conjugated F(ab') 2 goat anti-human IgG (Thermo Fisher Scientific, Waltham, MA, USA) at a dilution of 1:5000 in 5% non-fat dry milk in TBS-Tween. Reactivity was visualised using enhanced chemiluminescence.

Statistics

First, descriptive analyses were performed to obtain information on the characteristics of the CSC patients. Second, logistic regression with correction for age and gender was employed to evaluate if the presence of ARAs is associated to CSC. Finally, logistic regression with

correction for age and gender was used to identify any clinical characteristics that were possibly associated with the presence of ARAs. P<0.05 was considered statistically significant. All analyses were performed with IBM SPSS Statistics (version 21; SPSS Inc., Chicago, IL, USA).

RESULTS

Patient characteristics

All patient characteristics are shown in Table 1. A total of 63 chronic CSC patients (56 male, 7 female), with a mean age of 51 ± 9 years (range, 31 - 72 years) was included in this study. The median duration of CSC from first diagnosis to the time of blood collection was 585 days (range, 3 - 7832 days). Diffuse CSC was present in 11/62 patients (18%; data not available for 1 patient). In 38/48 CSC patients (79%; data not available for 15 patients) SRF was present at the moment of blood collection, indicating active CSC. The mean CRT was 134 ± 37 µm (range, 57 - 226 µm). Previous to blood collection, no treatment for CSC was given in 35/63 patients (56%), whereas 13/63 patients (21%) had received either micropulse or focal laser treatment and 10/63 patients (16%) had received photodynamic therapy.

ARAs on IIF in CSC patients

The IIF results are summarised in Table 2. Positive staining of the retina on IIF was present in 35/63 CSC patients (56%). After adjustment for retinal staining due to ANA, the presence of ARAs was confirmed in 32/59 CSC patients (54%). Among different staining patterns observed, staining of the photoreceptors was the most prevalent pattern in CSC patients (17/63; 27%; Figure 1). Other observed staining patterns in serum of CSC patients included staining of nuclear layers (n=8), the inner plexiform layer (n=2), fluorescence between the inner nuclear layer and the outer plexiform layer (n=7), and/or fluorescence between the outer nuclear layer and the photoreceptors (n=3). Nine CSC patients had a combination of different staining patterns on IIF.

The prevalence of ARAs in CSC patients was higher than in healthy controls (10/59; 17%; p<0.001). In addition, staining of the photoreceptors occurred in a lower percentage of healthy controls (5%) compared to CSC patients (p=0.003). In patients with uveitis, any positive staining of the retina (after correction for ANA presence) was observed in 39/84 patients (46%), which was not significantly different from CSC patients (p=0.153). In contrast, specific staining of the photoreceptors in uveitis patients (15%) was less prevalent compared to CSC patients (p=0.039). No significant differences in the prevalence of staining of other specific retinal layers between CSC patients and the 2 control groups were observed.

Table 1. Clinical characteristics of 63 patients with central serous chorioretinopathy at the moment of blood collection

Patient characteristics	
Male-to-female ratio	8:1
Mean age (SD), in years	51 (9)
Median duration of CSC (min-max), in days*	585 (3-7832)
CSC characteristics	**Number (%)**
Stage †	
- Focal CSC	51/62 (82%)
- Diffuse CSC	11/62 (18%)
Active CSC †,‡	38/48 (79%)
Bilateral CSC during follow-up ‡	32/63 (51%)
Recurrent CSC †, ‡	26/62 (42%)
Familial CSC	2/63 (3%)
Presence of intraretinal fluid during follow-up	10/63 (16%)
Mean central retinal thickness (SD), in micrometers †	134 (37) (46 patients)
Previous treatment for CSC	
- Photodynamic therapy	10/63 (16%)
- Micropulse or focal laser treatment	13/63 (21%)
- Other treatments or combinations of treatment	5/63 (8%)
- No treatment	35/63 (56%)
Systemic corticosteroids medication	
- Never	33/63 (52%)
- During diagnosis of CSC	30/63 (16%)
- <3 months before the diagnosis of CSC	7/63 (11%)
- After or >3 months before the diagnosis of CSC	13/63 (21%)
Use of systemic corticosteroids at the moment of blood collection	9/63 (14%)
Use of systemic immunosuppressive medication (excluding steroids) †	1/49 (2%)
Comorbidities †	
- Autoimmune diseases	3/59 (5%)
- Malignancies	4/59 (7%)

* Duration of CSC: interval between the initial diagnosis of CSC and the moment of blood collection

† Data not available for all patients

‡ Presence of subretinal fluid is considered to be the indication of 'active' CSC

Abbreviations: CSC: central serous chorioretinopathy; SD: standard deviation.

Table 2. Presence of antiretinal antibodies in patients with central serous chorioretinopathy and control groups

	CSC (n=63)	Uveitis (n=101)	P-value	Odds ratio (95% CI)	Healthy controls (n=60)	P-value*	Odds ratio (95% CI)*
Staining of any retinal layer(s)	32/59 (54%)**	39/84 (46%)	0.153	1.806 (0.803-4.063)	10/59 (17%)	<0.001	5.807 (2.479-13.606)
Staining of photoreceptors	17/63 (27%)	15/101 (15%)	0.039	2.864 (1.054-7.786)	3/60 (5%)	0.003	7.022 (1.938-25.442)

* Data not adjusted for age and gender

** In 4 patients, both antinuclear antibodies and antiretinal antibodies were present, and these patients were therefore excluded from final analysis. Exclusion of these samples did not affect the results of the statistical analysis.

Abbreviations: CI: confidence interval; CSC: central serous chorioretinopathy.

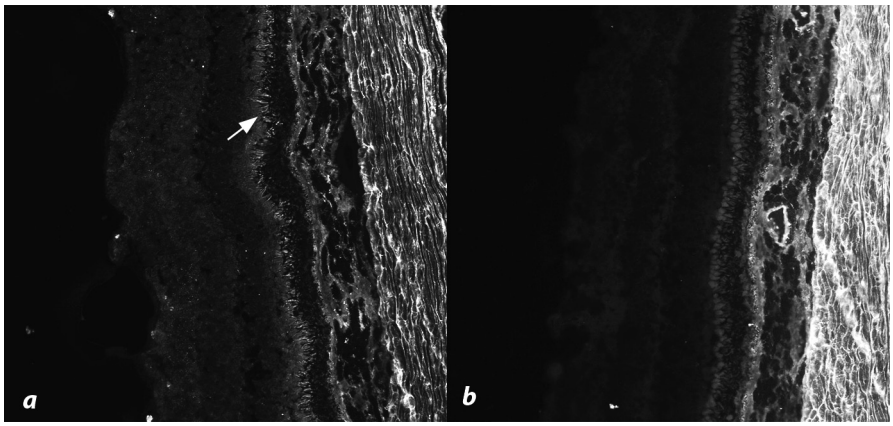

Figure 1. Staining patterns on indirect immunofluorescence from serum of patients with central serous chorioretinopathy
Serum of central serous chorioretinopathy patients was tested for the presence of antiretinal antibodies using indirect immunofluorescence with primate retinal tissue. Presence of antiretinal antibodies was visualised by labelling with fluorescein isothiocyanate (FITC). **A**, staining of the photoreceptors (arrow); **B**, no retinal staining (absence of antiretinal antibodies).

Clinical characteristics of CSC in relation to presence of ARAs on IIF

The presence of ARAs on IIF was higher in the 6 female CSC patients (100%) compared to the 57 male CSC patients (46%; p=0.024; Table 3). Staining of the photoreceptors in the samples of female and male CSC patients (57% versus 23%) did not differ (p=0.078). All other clinical characteristics of CSC (including age at onset, duration of CSC, stage of CSC, activity of CSC, unilateral versus bilateral CSC, recurrence of CSC, familial occurrence of CSC, CRT, presence of intraretinal fluid, previous CSC treatment, and systemic steroid use during confirmation of the diagnosis of CSC) were not significantly associated with the presence of ARAs or specific staining of the photoreceptors. Masked assessment of the OCT images of the 17 CSC patients with photoreceptor staining on IIF and of 17 CSC patients without photoreceptor staining on IIF (randomly selected) showed no remarkable differences in signs of retinal damage.

Table 3. Presence of antiretinal antibodies in relation to clinical characteristics of central serous chorioretinopathy

	Staining of any retinal layer(s) (n=32/59)	P-value	Staining of photoreceptors (n=17/63)	P-value
Gender				
Male	26/53 (49%)	0.018 †	13/56 (23%)	0.078 †
Female	6/6 (100%)*		4/7 (57%)	
Mean age (SD), in years	51 (9)	0.675 †	50 (9)	0.739 †
Median duration of CSC (min-max), in days ‡	261 (3-7832)	0.299	530 (3-6570)	0.446
Stage**				
Focal CSC	23/47 (47%)	0.066	13/51 (26%)	0.405
Diffuse CSC	8/11 (73%)		3/11 (27%)	
Activity, ††**				
Active	20/36 (56%)	0.782	12/38 (32%)	0.573
Not active	4/9 (44%)		2/10 (20%)	
Laterality of SRF during follow-up				
Unilateral	15/30 (50%)	0.496	9/31 (29%)	0.645
Bilateral	17/29 (59%)		8/32 (25%)	
Recurrence**				
Recurrent CSC	11/24 (46%)	0.393	5/26 (19%)	0.379
First CSC episode	21/34 (62%)		12/36 (33%)	
Familial				
Familial CSC	1/2 (50%)	0.949	1/2 (50%)	0.454
Non-familial CSC	31/57 (54%)		16/61 (262%)	
Mean central retinal thickness (SD), in micrometers**	125 (32) (24 patients)	0.091	121 (39) (14 patients)	0.080
Intraretinal fluid during follow-up				
Present	7/9 (78%)	0.222	4/10 (40%)	0.288
Absent	25/50 (50%)		13/53 (25%)	
Treatment: PDT				
PDT	6/10 (60%)	0.665	3/10 (30%)	0.804
No(t solely) PDT	26/49 (53%)		14/53 (26%)	

Treatment: (focal and/or micropulse) laser				
Laser treatment	7/13 (54%)	0.956	6/13 (46%)	0.053
No(t solely) laser treatment	25/46 (54%)		11/50 (22%)	
Use of corticosteroids				
During or < 3 months before CSC diagnosis	25/49 (51%)	0.674	12/53 (23%)	0.138
No current use	7/10 (70%)		5/10 (50%)	

* After the study was completed, 3 additional samples from female patients with CSC were analysed and all showed absence of ARAs, resulting in an ultimate percentage positive ARAs among females of 67% (6/9), which is not distinct from male CSC patients (26/53; 49%).

† Determined by chi-square test

‡ Duration of CSC: from CSC diagnosis to the moment of blood collection

** Data not available for all patients

†† Presence of subretinal fluid is considered to be the indication of 'active' CSC

Abbreviations: ARA: antiretinal antibody; CSC: central serous chorioretinopathy; PDT: photodynamic therapy; SD: standard deviation; SRF: subretinal fluid.

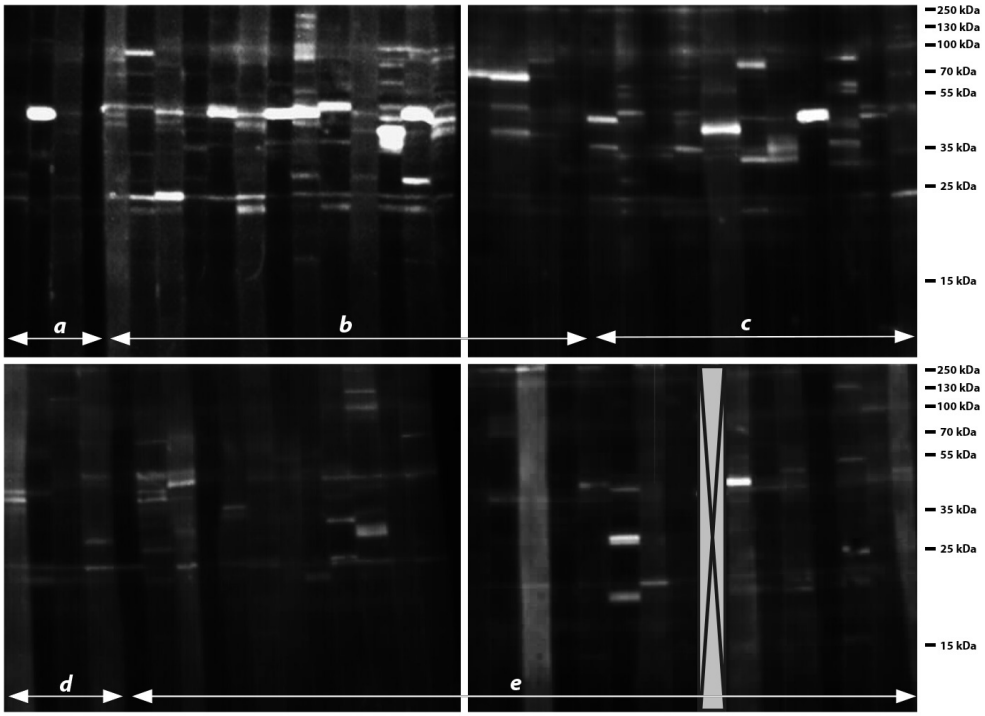

Figure 2. Reactivity on Western blot from serum of patients with central serous chorioretinopathy
Serum of patients with central serous chorioretinopathy was evaluated for the presence and approximate size of antiretinal antibodies using Western blot analysis with retinal protein extract. The results are sorted based on staining patterns on indirect immunofluorescence: **A**, nuclear staining (antinuclear antibody (ANA) negative); **B**, photoreceptor staining; **C**, diverse staining patterns; **D**, nuclear staining (ANA positive); **E**, no staining.

ARAs on Western blot in patients with CSC

Multiple ARAs were observed in the majority of CSC patients (Figure 2). The most abundant ARA reactivity on Western blot was observed with those CSC patients showing staining of the photoreceptors on IIF, when compared to patients without retinal staining patterns as well as to patients with other staining patterns on IIF. The most prevalent ARAs on Western blot in CSC patients showing staining of the photoreceptors on IIF had molecular weights of approximately 24 kDa and 45 kDa. However, these antibodies were not entirely specific for samples with photoreceptor staining, since they were also present in CSC patients showing no staining of the photoreceptors on IIF, and even in patients with no staining at all.

DISCUSSION

We show that serum ARAs were present in 54% of patients with chronic CSC and in 17% of healthy controls. No significant difference in the prevalence of overall ARAs in serum was found between CSC patients and uveitis patients (46%), although staining of the photoreceptors occurred more often in CSC patients (27%) than in uveitis patients (15%). No differences in the presence of ARAs were observed when CSC patients were subdivided into specific subgroups based on clinical characteristics.

The multiple staining patterns on IIF in individual CSC patients indicate the presence of diverse ARAs, which was confirmed by Western blot analysis. Also, within patients who were solely showing staining of the photoreceptors on IIF, Western blot indicated the presence of diverse antibodies directed against the retina. This suggests that diverse retinal antibodies are associated with photoreceptor staining.

The reason for the higher prevalence of ARAs in CSC, especially those directed against photoreceptors, and their clinical significance is currently not clear. Possibly, chronic retinal damage caused by the presence of SRF accumulation, associated with a breakdown of the RPE (which normally constitutes the outer blood-retinal barrier), may trigger formation of ARAs through an inflammatory reaction of the immune system.[32] In the past, ARAs have been shown in autoimmune retinopathy, in which ARAs are suggested to have pathogenic properties. Elevated serum ARAs were also observed in other chorioretinal diseases (e.g. uveitis, macular degeneration, retinitis pigmentosa) as well as in the healthy population.[28, 29, 31, 33-44][32] Here, an immune response to ocular tissue damage has been previously suggested to play a role in aggravation of the various retinal diseases.[42, 45] Similarly, autoreactive immunologic responses have been found in patients with proven infectious uveitis, in which the primary cause (infection) presumably incited the secondary formation of antibodies, possibly due to tissue damage.[46] Interestingly, recent genetic studies in CSC have found an association with genetic variants in the complement system, an essential part of the innate immune system.[25, 47] However, it is unclear if there is a primary role of the immune system in the pathogenesis of CSC. The mere presence of serum autoantibodies does not necessarily indicate an autoimmune basis of the disease. Moreover, ARAs were not found in all patients and exhibited a wide variety.

The presence of ARAs in serum of CSC patients might be hypothetically explained by their immune predisposition. CSC can occur in patients with various immune-mediated diseases including membranoproliferative glomerulonephritis and systemic lupus erythematosus.[48, 49] It is unclear whether CSC in these patients is only caused by glucocorticoid treatment prescribed for these conditions or if it is also influenced by the presence of inflammatory disease.[22] Another potential systemic mechanism in the development of ARAs could be the

mimicry between ocular antigens and microbial proteins, brought up by a previous systemic infection.[50] However, the presence of antecedent infectious disorders in CSC has not previously been assigned as its cause. A higher prevalence of Helicobacter pylori infections in CSC patients has been described, although a firm association has never been proven.[51-53] Moreover, autoimmune responses would be rather expected in a female-dominated disease. In CSC, particularly men between the age of 25 and 55 are affected, analogous to the phase of life in which highest androgen levels can be detected, suggesting a possible role of this hormone within the development of CSC. In our study, the role of the immune system in CSC seemed to be most important in female CSC patients, as ARAs occurred in all 6 initially included female CSC patients. However, after our study was completed, we had the possibility to analyse 3 extra samples from female patients with CSC. All 3 samples showed absence of ARAs on IIF, resulting in similar occurrences of ARAs in male and female CSC patients (Table 3).

So far it is unclear whether ARAs are involved in the pathogenesis of CSC or whether their presence represents a secondary epiphenomenon. As CSC is mainly a disease of the choroid and RPE, experiments assessing antibodies directed against the choroid and RPE could give more insight to the pathogenesis.[1-3, 13] The assessment of intraocular ARAs might help to clarify the possible pathogenic role of ARAs.[31]

In conclusion, serum ARAs are more common in CSC patients than in healthy controls. No clear association between the presence of ARAs and clinical CSC characteristics could be identified in the current cohort. To unravel the possible involvement of autoimmune reactivity in the pathogenesis of CSC, further research is warranted.

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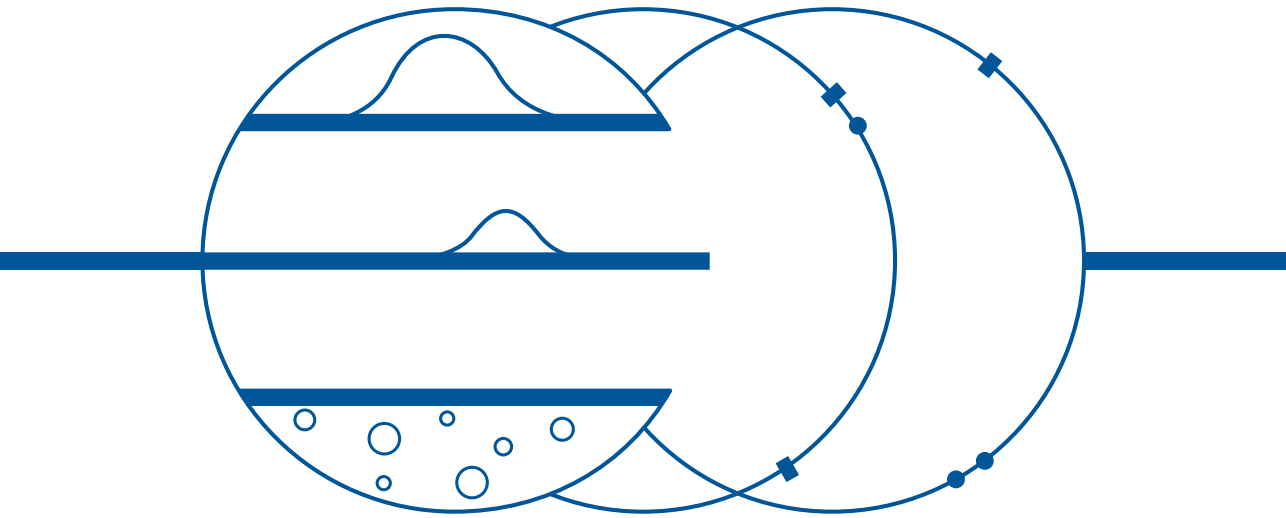
4

STEROIDS AND CENTRAL SEROUS CHORIORETINOPATHY



4.1

CENTRAL SEROUS CHORIORETINOPATHY IN PRIMARY HYPERALDOSTERONISM

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ABSTRACT

Purpose: To describe ophthalmological characteristics of 13 patients with primary hyperaldosteronism (PA).

Methods: Cross-sectional study. All patients underwent extensive ophthalmological examination.

Results: Thirteen PA patients (9 male, 4 female) were diagnosed with arterial hypertension for 11.0 ± 11.2 years. Ophthalmological imaging revealed macular serous subretinal fluid (SRF) on optical coherence tomography (OCT) in 2 patients (15%). In 1 of these patients bilateral chronic central serous chorioretinopathy (CSC) with polypoidal choroidal neovasculopathy was diagnosed, which was effectively treated with full-dose photodynamic therapy. In the other patient with SRF and bilateral diffuse hyperfluorescent areas on fluorescein angiography, the SRF had decreased spontaneously after 6 weeks of follow-up. In 5 of the remaining patients (38%) retinal pigment epithelium alterations resembling findings characteristic for CSC were seen on multimodal imaging. The mean subfoveal choroidal thickness was 290.2 ± 65.0 µm.

Conclusions: Retinal abnormalities resembling (subclinical) CSC are common in patients with PA. These findings indicate that mineralocorticoid-mediated pathways are involved in the pathogenesis of CSC. In CSC patients with hypertension of unknown origin a diagnosis of PA should be considered.

INTRODUCTION

Central serous chorioretinopathy (CSC) is a chorioretinal disease, characterised by a neuroretinal detachment due to the presence of serous subretinal fluid (SRF), as a result of dysfunction of the retinal pigment epithelium (RPE), and hyperpermeability and thickening of the underlying choroid. The exact pathogenetic mechanism of CSC still has to be unraveled.[1-5] CSC is more common in men, and presents with complaints such as vision loss, image distortion, and loss of color and contrast vision.[2-4, 6, 7] Genetic risk factors may also play a role in CSC, possibly via the complement system.[8-10] Exposure to both endogenous (endogenous hypercortisolism/Cushing's syndrome) and exogenous corticoids is strongly associated with CSC.[11-14] However, the pathogenetic explanation for the link between CSC and corticosteroids is currently unclear. In the pathogenesis of CSC both the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR), a receptor with equal affinity for glucocorticoids and for mineralocorticoids such as aldosterone, have been postulated to play a role.[2]

In a rat model, overstimulation of the MR resulted in choroidal changes resembling findings in CSC patients.[15] Several pilot studies have indicated that there may be a possible therapeutic role for the MR antagonists spironolactone and eplerenone in the treatment of CSC.[16-19] However, the effect of these antagonists on central macular and choroidal thickness and on complete and durable resolution of SRF after cessation of therapy is unclear, and randomised controlled trials are currently lacking.[18-20] Although the increased incidence of CSC in patients with endogenous hypercortisolism is well-known,[14, 21, 22] no studies have been published on the occurrence of CSC in patients with primary aldosteronism (PA, primary hyperaldosteronism) thus far.

PA is characterised by the overproduction of aldosterone by 1 or both of the adrenal glands, independent of its normal regulator angiotensin II. Unilateral adrenal adenoma (Conn's disease) and bilateral adrenal hyperplasia account for more than 90% of PA cases. For these diseases laparoscopic adrenalectomy and MR antagonists such as spironolactone and eplerenone are recommended, respectively. PA due to both adrenal and non-adrenal aldosterone-producing tumors, primary unilateral adrenal hyperplasia, and familial hyperaldosteronism occurs less frequently.[23]

Although the association between endogenous hypercortisolism and CSC is well-established,[14, 21, 22] and there may be a role for MR and treatment with MR antagonists in CSC, it is unknown if PA is also associated with CSC-like abnormalities. Therefore, in this study we examined patients with PA to detect clinical findings indicative of (subclinical) CSC.

MATERIALS AND METHODS

Patient selection

In this cross-sectional study, 13 patients with PA were referred to the Department of Ophthalmology of the Leiden University Medical Center for ophthalmological analysis. PA patients in whom diagnosis had been established between May 2008 and August 2015 were asked to take part in this study. Both patients who already received either surgery or medication because of the hyperaldosteronism and patients who had not received any PA treatment were included in this study. One patient with a pituitary adenoma, influencing the hypothalamic-pituitary-adrenal axis, was excluded, because of a known relationship of this disease with CSC.[14, 21, 22] Written informed consent for the enrollment was obtained from all subjects. The study adhered to the tenets of the Declaration of Helsinki. Approval of the institutional review board and the ethics committee was obtained (NL50816.058.14).

Ophthalmological imaging

All patients received complete ophthalmic examination, including Early Treatment of Diabetic Retinopathy Study (ETDRS) best-corrected visual acuity (BCVA) measurement. After this measurement, pupils were dilated by using 1% tropicamide and 5% phenylephrine. Indirect ophthalmoscopy and digital color fundus photography (Topcon Corp., Tokyo, Japan) were performed, and optical coherence tomography (OCT), enhanced depth imaging (EDI-)OCT, fundus autofluorescence, and oral fluorescein angiography (FA) images were obtained, using spectral-domain OCT (Spectralis HRA+OCT; Heidelberg Engineering, Dublin, CA, USA). EDI-OCT was used to measure subfoveal choroidal thickness (CT). Images were evaluated by an experienced retina specialist (CJFB). Oral FA was performed after 10 milliliters of 20% fluorescein was administered orally after a fasting period of at least 3 hours, and photos were taken at 10, 15, 20, 25, and 30 minutes after administration. When ophthalmological imaging revealed findings requiring treatment and/or follow-up based on the judgment of the treating ophthalmologist, these visits were scheduled at our outpatient clinic. Based on the results of the ophthalmological screening, indocyanine green angiography using the spectral-domain OCT could also be performed if deemed necessary.

RESULTS

Patient characteristics

The mean age of the 13 PA patients (9 male, 4 female) was 55.1 ± 12.0 years (range, 36 - 74). All patients had arterial hypertension, which is the most prominent clinical sign of PA.[23] Hypertension was diagnosed 11.0 ± 11.2 years (range, 0.5 - 43) before ophthalmological evaluation. No significant difference between the patients with and without ophthalmological abnormalities could be detected regarding both patient age and duration of pre-existing hypertension. At the moment of PA presentation, hypertension was treatment-resistant (need of prescription of >2 antihypertensive agents) in 8 patients (62%). At the time of ophthalmological phenotyping 2 patients still had hypertension despite antihypertensive treatment. Hypokalemia, a sign of relatively severe PA,[24] developed either spontaneously or after the use of diuretics and always in combination with hypertension,[23] was a presenting symptom of PA in 10 patients (77%). Obstructive sleep apnea syndrome was previously diagnosed in 1 patient. No other diseases known to be possibly associated with CSC were present in the included patients.

At the time of the visit to the department of Ophthalmology 3 patients were on chronic spironolactone treatment and 3 patients used eplerenone as antihypertensive treatment, previously prescribed by the endocrinologist for a mean duration of 2.2 years (range, 10 weeks - 7 years). One patient reported the current local nasal daily use of the steroid fluticasone, because of allergic rhinitis. Two other patients reported a previous short course of oral steroid treatment in 2012 and cutaneous steroid treatment in 2013, respectively. No patients reported the use of either sildenafil or tadalafil.

PA due to unilateral adrenal adenoma was diagnosed in 7 patients; bilateral adrenal hyperplasia was detected in 3 patients. In 1 patient the cause of PA was not found. Six patients had undergone unilateral laparoscopic adrenalectomy, at the time of the ophthalmological evaluation. Another patient was already scheduled for surgery, and was prescribed eplerenone and nifedipine at the moment of ophthalmological analysis. After surgery, pathological analysis revealed hyperplasia of the excised adrenal gland. The last patient was scheduled to receive adrenal vein sampling, to detect the origin of PA. Eleven out of the other 12 patients were treated with antihypertensive drugs, including the 3 patients who used spironolactone and 3 patients who received eplerenone for this purpose. Clinical characteristics of the patients are summarised in Table 1.

Table 1. Clinical characteristics of patients with primary hyperaldosteronism

Patient	Age	Gender	Type PA (type of surgery)	Systemic presentation of PA	Current medication
1	72	M	adrenal adenoma (-)	hypertension	amlodipine, enalapril, phenprocoumon, propafenone, spironolactone
2	64	F	unknown origin (-)	therapy-resistant hypertension	colecalciferol/ calcium carbonate, levothyroxine, metoprolol, spironolactone
3	59	M	adrenal adenoma (laparoscopic adrenalectomy)	therapy-resistant hypertension, hypokalemia	colecalciferol, doxasozin, furosemide, nifedipin, omeprazole
4	62	F	adrenal hyperplasia (-)	hypertension, hypokalemia	amlodipine, colecalciferol/ calcium carbonate, melatonin, simvastatin, potassium chloride, spironolactone
5	42	M	adrenal adenoma (laparoscopic adrenalectomy)	therapy-resistant hypertension, hypokalemia	amlodipine, calcium carbonate, metoprolol, perindopril
6	41	M	adrenal adenoma (laparoscopic adrenalectomy)	hypertension, hypokalemia	nifedipine
7	50	M	adrenal adenoma (laparoscopic adrenalectomy)	therapy-resistant hypertension, hypokalemia	barnidipine
8	36	F	adrenal adenoma (laparoscopic adrenalectomy)	hypertension, hypokalemia	-
9	58	M	adrenal hyperplasia (laparoscopic adrenalectomy)	therapy-resistant hypertension, hypokalemia	amlodipine, enalapril
10	74	M	adrenal adenoma (-)	therapy-resistant hypertension, hypokalemia	amlodipine, eplerenone, losartan
11	48	M	adrenal hyperplasia (laparoscopic adrenalectomy)	hypertension, hypokalemia	eplerenone, metformin, nifedipine
12	58	M	adrenal hyperplasia (-)	therapy-resistant hypertension, hypokalemia	acetylsalicylic acid, barnidipine, diclofenac, eplerenone, fluticasone, perindopril, potassium chloride
13	45	F	unknown origin (scheduled for adrenal vein sampling)	therapy-resistant hypertension	amlodipine, hydrochlorothiazide

Abbreviations: PA: primary hyperaldosteronism; treatment-resistant hypertension: need of prescription of >2 antihypertensive agents.

Ophthalmic characteristics

Ophthalmologic history taking revealed a 64-year-old female patient who reported unilateral blurred vision and metamorphopsia. This patient had been visiting another hospital since 2012 because of 'early age-related macular degeneration'. Ophthalmological history revealed that 2 other patients had been previously diagnosed with venous hemi-occlusion, for which scatter laser coagulation treatment was performed. Another patient was previously diagnosed with a choroidal nevus. Ophthalmic family history was unremarkable except for 1 PA patient who reported a mother who was previously diagnosed elsewhere with age-related macular degeneration. Mean ETDRS BCVA of the 26 eyes was 89.0 ± 6.5 letters (range, 67 - 99), with a mean spherical equivalent of the manifest refraction of 0.19 ± 2.39 diopters (range, -6.25 to +5).

On OCT, fovea-involving serous SRF was detected in 2 of 13 patients (15%; a 64-year-old female and a 74 year-old male patient). Imaging of the female patient, to whom spironolactone 50 mg thrice daily was being prescribed since 2008, showed signs of bilateral chronic CSC (Figure 1A-I and 1K-L). On FA, hot spots of leakage were detected in the right eye (Figure 1E). As part of standard clinical care, she underwent indocyanine green angiography, which showed polypoidal choroidal vasculopathy on the border of an area of a possible occult neovascularisation (Figure 1F). The patient was scheduled for full-dose photodynamic therapy, leading to disappearance of SRF at the evaluation visit 6 weeks later (Figure 1J). At the follow-up visit 3 months after PDT, SRF had disappeared. Ophthalmological imaging in the male patient (Figure 1M-T and IV), who was using eplerenone 100 mg twice daily since 2012 and in whom bilateral diffuse hyperfluorescent areas were detected on FA (Figure 1Q-R) showed serous SRF (Figure 1S). The SRF had decreased at the scheduled visit 6 weeks later (Figure 1U). OCT imaging revealed outer photoreceptor/RPE changes reminiscent of changes in chronic CSC in 5 of the remaining patients (38%), which was supported by corresponding hyperfluorescent changes on FA (Figure 2A-T). Out of these patients, only 1 patient was using spironolactone since 10 weeks. Fundus autofluorescence showed variable mild hypo- and hyperautofluorescence of the lesions. The blood pressure of 1 of these patients was not adequately controlled. In the other hypertensive patient no ophthalmological pathology could be detected. Ophthalmological phenotyping of the 3 patients who reported current or previous steroid use did not reveal any abnormalities. In all 13 patients, mean subfoveal CT on EDI-OCT was 290.2 ± 65.0 µm (range, 123 - 377). Ophthalmological characteristics of the patients are summarised in Table 2.

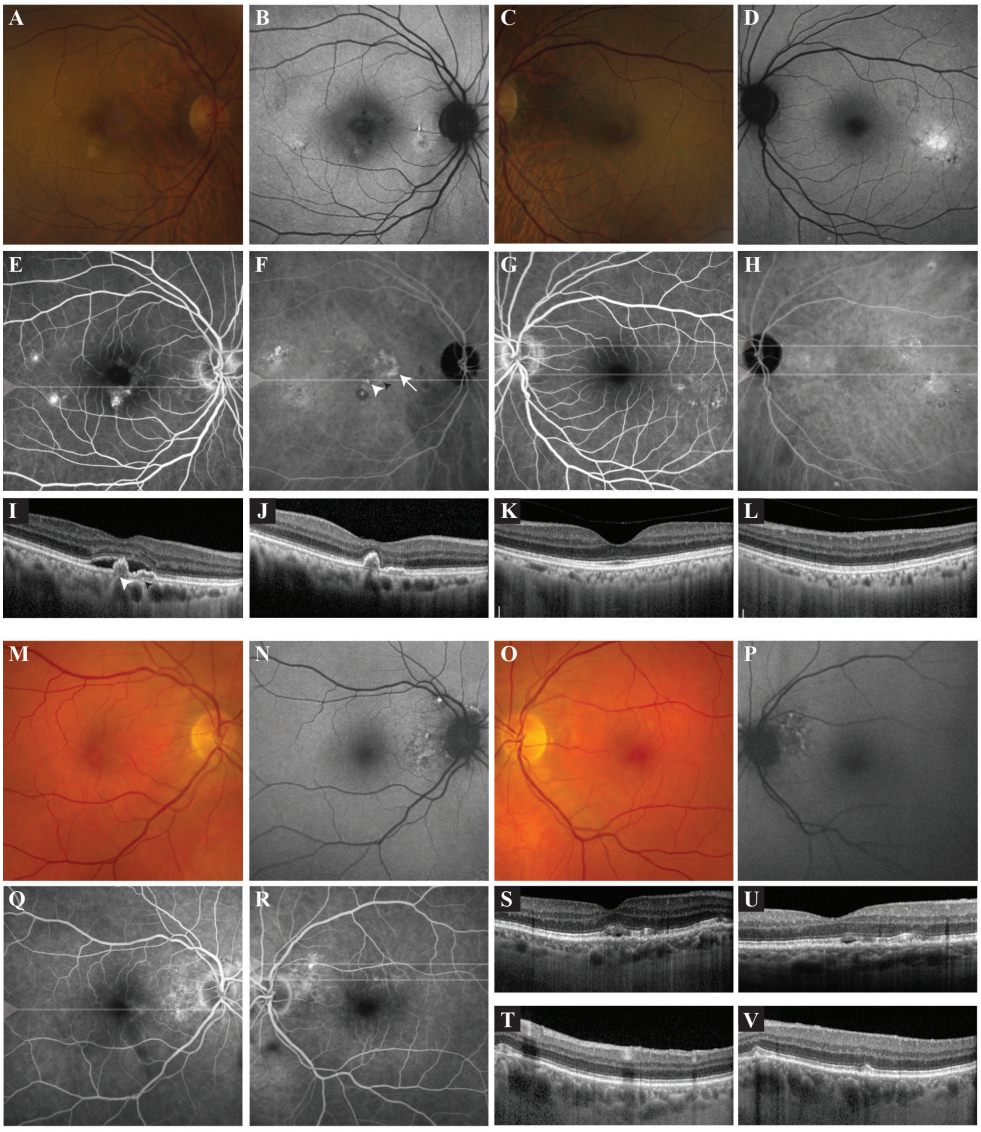

Figure 1. Ophthalmological imaging of 2 patients with primary hyperaldosteronism in whom foveal subretinal fluid (SRF) was detected at screening.

A-L. Multimodal imaging of a 64-year-old female with primary hyperaldosteronism. Fundus photography of the right eye (A) revealed central irregular yellowish changes in the fovea and milder focal pigmentary changes in the temporal macula, with multifocal autofluorescence changes on fundus autofluorescence (FAF; B). Fundus photography of the left eye (C) also showed mild retinal pigment epithelium (RPE) abnormalities, with both hypo- and hyperautofluorescent changes on FAF temporally in the macula (D). On fluorescein angiography (FA) of the right eye (E) 2 hot spots of leakage were present in the temporal macula, as well as a hyperfluorescent spot inferior to the fovea. Indocyanine green angiography (ICGA)

revealed a lesion suspicious for polypoidal choroidal vasculopathy in the right eye (white arrowhead), possibly on the border of an area of an occult neovascularisation (white arrow) (F). Hyperfluorescent areas compatible with chronic central serous chorioretinopathy (CSC) on ICGA of the left eye (H) were larger as compared to the areas on FA (G). Optical coherence tomography (OCT) revealed foveal SRF in the right eye (I), with the presence of a round, dome-shaped RPE detachment (large white arrowhead in I) corresponding to the small round polyp on ICGA (white arrowhead in F), in association with a more shallow RPE detachment ("double layer sign", small black arrowhead in I) that may correspond to the possible occult neovascularisation seen on ICGA (small black arrowhead in F). Moreover, extrafoveal RPE alterations were found on OCT. At baseline, subfoveal choroidal thickness (CT) was 235 µm in the right and 206 µm in the left eye. The patient underwent full-dose photodynamic therapy in the right eye, refusing anti-vascular endothelial growth factor treatment as an additive treatment. At the evaluation visit 6 weeks after therapy, the SRF in the right eye (J) had disappeared, with the RPE detachments still present. No foveal SRF was present on OCT in the left eye (K; lower scan in G and H). An extrafoveal OCT of this eye (L; upper scan in G and H) revealed RPE changes temporally in the macular region. At the evaluation visit, subfoveal CT in the right eye had decreased to 220 µm.

M-V. Multimodal imaging of a 74-year-old male patient with primary hyperaldosteronism. Fundus photography of the right eye (M) showed mild foveal and extrafoveal RPE abnormalities, and mild irregular peripapillary changes on FAF (N). Fundus photography of the left eye (O) revealed mainly extrafoveal RPE changes, with the same picture on FAF (P) as in the right eye. FA of the right (Q) and left eye (R) showed multifocal hyperfluorescent areas. The foveal OCT scan of the right eye (S) showed shallow serous SRF and RPE and outer photoreceptor changes suggestive of CSC, whereas the foveal OCT scan of the left eye was normal. Extrafoveal OCT scanning in the left eye (T and V; upper and lower scan in R) showed RPE/outer photoreceptor changes without SRF, despite the possible hot spot of leakage on FA. At screening, subfoveal CT was 232 µm in the right eye and 274 µm in the left. On OCT, foveal SRF in the right eye (U) had decreased after 6 weeks of follow-up.

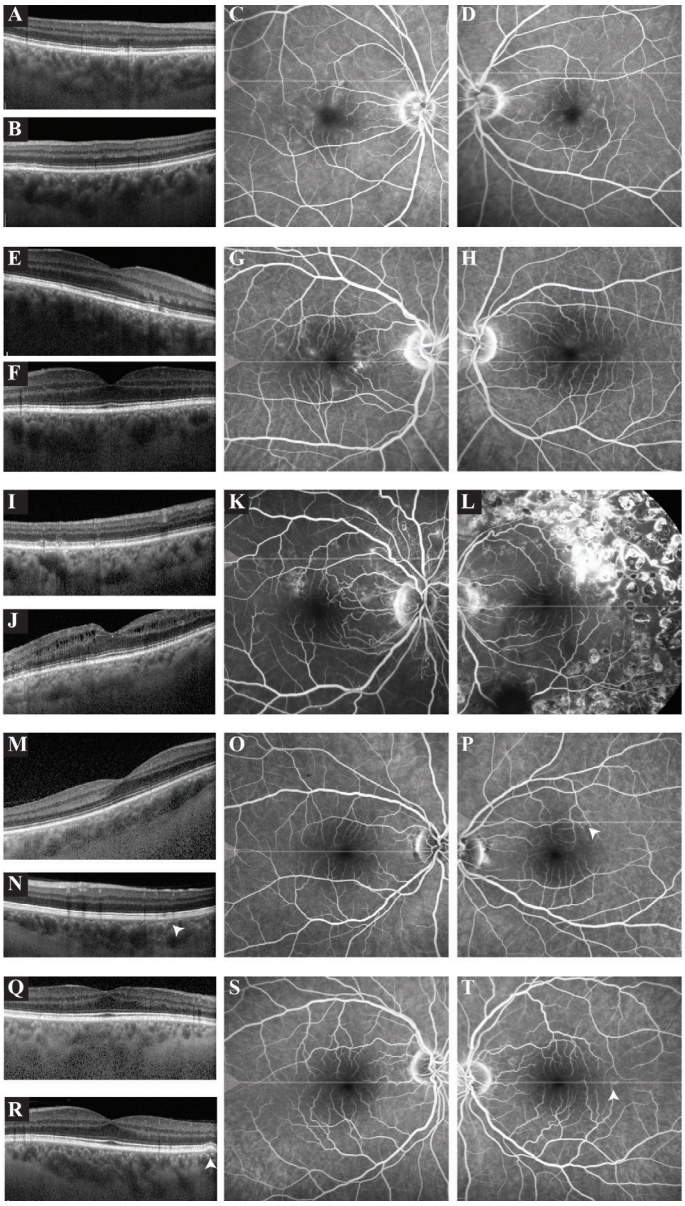

Figure 2. Ophthalmological imaging of 5 patients with primary hyperaldosteronism in whom retinal pigment epithelium (RPE) changes suggestive of subclinical central serous chorioretinopathy (CSC) were detected at screening.

A-D. Extrafoveal optical coherence tomography (OCT) scanning of the right (A) and the left eye (B) of this 72-year-old male patient revealed mild outer retinal changes. Mild diffuse macular hyperfluorescent changes were detected on fluorescein angiography (FA) of the right (C) and the left eye (D).

E-H. On the foveal OCT scan of this 50-year-old male patient, subtle outer retinal changes were found in the right eye (E), with a normal OCT in the left eye (F). FA of the right eye (G) showed diffuse hyperfluorescent changes, and mild hyperfluorescence in the macula of the left eye (H).
I-L. Extrafoveal OCT scan of the right eye (I) of a 59-year-old male patient revealed mild hyperreflective focal irregularities on the photoreceptor/RPE level. On the foveal OCT scan of the left eye (J) intraretinal fluid was observed. FA of the right eye (K) of this patient showed multifocal irregular hyperfluorescent changes, with some peripapillary indistinct focal hyperfluorescent areas suggestive of small leaking hot spots, despite an absence of serous SRF on OCT. FA of the left eye (L) showed scars of previous scatter laser treatments performed elsewhere for a retinal venous occlusion.
M-P. OCT scan (M) and FA of the right eye (O) of this 36-year-old female patient revealed no abnormalities. OCT scanning of the area superior to the macula of the left eye (N) revealed mild outer retinal changes, with corresponding mildly hyperfluorescent changes on FA (P; arrowhead in N and P).
Q-T. OCT scan (Q) and FA of the right eye (S) of a 58-year-old male patient showed no abnormalities. Foveal OCT scanning of the left eye (R) revealed a small RPE detachment, with corresponding mildly hyperfluorescent changes on FA (T; arrowhead in S and T).

Table 2. Ophthalmological characteristics of patients with primary hyperaldosteronism

Patient	Steroid use	History of chorioretinal disease	BCVA OD	BCVA OS	Sph eq OD	Sph eq OS	OCT OD	CT OD (µm)	OCT OS	CT OS (µm)	FAF OD	FAF OS	FA OD	FA OS	Figure
1	-	choroidal nevus (right eye)	91	91	+1,75	+1,75	outer retinal changes	367	outer retinal changes	356	-	-	diffuse macular hyperfluorescent changes	diffuse macular hyperfluorescent changes	2A-2D
2	-	"early age-related macular degeneration" (right eye)	67	77	−0,25	−0,75	PCV, foveal SRF, RPE detachments, RPE alterations	235	RPE alterations	206	multifocal FAF changes	multifocal FAF changes	temporal hot spots of leakage, hyperfluorescent spot under the fovea	hyperfluorescent changes	1A-1L
3	-	venous hemi-occlusion (left eye)	98	91	+2,00	+2,50	mild hyperreflective focal irregularities	284	intraretinal fluid	281	-	-	diffuse hyperfluorescent changes, with a possible small hot spot of leakage	scars of previous scatter laser treatments	2I-2L
4	-	-	89	85	−1,00	−1,75	-	287	-	268	-	-	-	-	
5	cutaneous (2013)	-	88	91	−6,25	−5,50	-	123	-	128	-	-	-	-	
6	-	-	95	93	−1,25	−1,50	-	305	-	302	-	-	-	-	
7	-	-	84	93	+5,00	+2,50	outer retinal changes	341	outer retinal changes	351	focal FAF changes	-	diffuse hyperfluorescent changes	mild macular hyperfluorescent changes	2E-2H
8	-	-	89	90	0	+0,25	-	357	outer retinal changes	315	-	-	-	hyperfluorescent changes	2M-2P
9	-	-	89	86	+0,50	+0,50	-	377	RPE detachment	372	-	-	-	hyperfluorescent changes	2Q-2T
10	-	-	86	94	+3,00	+2,25	foveal SRF, RPE alterations	232	RPE alterations	274	peripapillary FAF changes	peripapillary FAF changes	hyperfluorescent changes	hyperfluorescent changes, with a possible hot spot	1M-1V
11	oral (2012)	-	84	85	−0,25	−0,25	-	306	-	289	-	-	-	-	
12	intranasal (current)	-	99	93	+1,00	+1,50	-	292	-	302	-	-	-	-	
13	-	-	92	93	−0,50	−0,25	-	306	-	289	-	-	-	-	

Abbreviations: BCVA: best-corrected visual acuity; CT: subfoveal choroidal thickness; FA: fluorescein angiography; FAF: fundus autofluorescence; OCT: optical coherence tomography; OD: right eye; OS: left eye; PA: primary hyperaldosteronism; PCV: polypoidal choroidal vasculopathy; PDT: photodynamic therapy; RPE: retinal pigment epithelium; Sph eq: spherical equivalent of the manifest refraction; SRF: subretinal fluid.

DISCUSSION

In this study, 7 out of 13 PA patients (54%) had a variable degree of outer retinal and RPE changes on multimodal imaging. Two of these patients (29%) had serous SRF leakage in the macula, including a patient with CSC-like changes and unilateral polypoidal choroidal vasculopathy (Figure 1A-L), and a patient with unilateral CSC with subfoveal fluid and bilateral RPE changes resembling those seen in chronic CSC (Figure 1M-V). Despite the fact that the latter patients used MR antagonists at the time of ophthalmological evaluation the abnormalities and serous SRF leakage were present. In the 5 other patients retinal abnormalities that could be characteristic of (subclinical) CSC were seen. Although we observed RPE abnormalities and no history of SRF in these patients, mean subfoveal CT in the current case series of PA patients was comparable with the findings in healthy volunteers at the time of evaluation.[25, 26] Despite the fact that mean subfoveal CT in CSC patients is usually increased compared to healthy subjects, several authors have described that this is not the case in all CSC patients.[27-29] The small number of included patients and the relatively high age of these possible CSC patients could also have contributed to the normal subfoveal CT in this study.[30]

Based on the results of this study, aldosterone and the MR-mediated pathway may play a role in the pathogenesis of CSC-like changes in patients with PA. Aldosterone and cortisol are adrenal cortical steroid hormones that exert their actions via the MR and GR. It is unclear how aldosterone and the MR may be linked to the pathogenesis of CSC. The MR is present in the choroid, the RPE, and neurosensory retina, as observed in humans, rats, and monkeys.[2, 15, 31-33] Aldosterone stimulation of the MR may affect the choroidal vascular system through various effects, such as induction of oxidative stress, inflammation, hypertrophic remodeling, fibrosis, and endothelial dysfunction.[34, 35] The 2 physiological ligands aldosterone and cortisol are able to bind to the MR with similar affinity. In epithelial tissues and the choroid, MR acts as a physiological aldosterone receptor. It is protected from being activated by glucocorticoids by the enzyme 11ß-hydroxysteroid dehydrogenase type 2, which metabolizes cortisol into inactive cortisone.[36] Like the MR, this enzyme is present in the choroid, RPE, and neurosensory retina.[31-33] Without the conversion, MR acts as a cortisol receptor, as blood levels of cortisol are much higher compared to aldosterone levels, even when binding of cortisol to corticosteroid binding globulin is taken into account.[37] The effect of PA suggests either overactivation of aldosterone-preferring MRs or occupancy of cortisol-preferring MRs, for example during periods in the circadian cycle when cortisol is normally very low. In a rat model, MR overexpression increased CT, and resulted in dilation of choroidal vessels, focal disruption of RPE tight junctions, and RPE detachments.[2] Preliminary clinical studies also indicate that eplerenone and spironolactone have a beneficial effect on CSC, although their exact role in the treatment of CSC is somewhat controversial due to possible variable and temporary treatment effects and a lack of randomised controlled trials.[17-20] Furthermore, there

does not seem to be a marked effect of these treatments on CT.[19] Apart from a possible direct role of the MR pathway in the pathogenesis of retinal abnormalities in this PA patient cohort, effects of prolonged hypertension (which was present in all patients) may have also played a role. Moreover, MR antagonists were prescribed to both patients in whom macular SRF was detected at the moment of ophthalmological phenotyping. The relatively severe PA in the patient group that received the ophthalmological phenotyping could also have contributed to the results of this study.[24]

In conclusion, findings suggestive of (subclinical) CSC were detected in most patients with PA. Ophthalmologists who treat CSC patients with hypertension of unknown origin should thus have a high index of suspicion for PA, in particular because of the underestimated incidence of PA. In dealing with PA patients, clinicians also need to be aware of the potential coexistence of CSC, which may remain subclinical.

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4.2

SPECTRUM OF RETINAL ABNORMALITIES IN RENAL TRANSPLANT PATIENTS USING CHRONIC LOW-DOSE STEROIDS

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ABSTRACT

Purpose: To assess the ophthalmological characteristics of asymptomatic patients with a renal transplant on chronic low-dose steroids for at least last 2 years prior to examination.

Methods: Cross-sectional study. All patients underwent an extensive ophthalmological examination.

Results: Of the 37 included patients (25 male, 12 female; 59 ± 11 years (range, 38 - 77)) ophthalmological phenotyping revealed abnormalities in 22 patients (59%). Findings characteristic for (subclinical) central serous chorioretinopathy were detected in 10 patients (27%), including 2 patients with serous subretinal fluid in the macula. An epiretinal membrane of the macula was present in 6 patients (16%). Mean subfoveal choroidal thickness was significantly increased in patients with ophthalmological abnormalities, in comparison with patients without abnormalities.

Conclusions: Retinal abnormalities are common in the majority of renal transplant patients using chronic low-dose steroids. These retinal changes may be associated with the renal disease and/or the effect of chronic steroid use on the choroid and retina.

INTRODUCTION

For end-stage solid organ disease, transplantation is a therapeutic option in a noteworthy number of patients. Abnormal ophthalmological findings after several types of transplantation occur in more than 50% of patients,[1-3] and are caused by the combination of pre-existing disease and immunosuppressive regimen.[4] Fortunately, vision-threatening conditions are seldom observed after long-term follow-up.[5] Up to 45% of the transplant patients develop typical steroid-induced bilateral posterior subcapsular cataract within the first year after transplantation.[1] Bilateral, open-angle glaucoma is detected in up to 7% of patients within the first year after transplantation, which may be caused by the use of systemic steroids.[1] Little is known about transplantation complications in the posterior segment of the eye. Documented retinal complications are of infectious, hemorrhagic, or microvascular origin and have been described after bone marrow transplantation preceded by total body irradiation.[4] Moreover, optic disc edema and pigmentary changes have been found to occur after heart transplantation.[6] The kidney is the most commonly transplanted organ worldwide. Little is known about retinal abnormalities in renal transplant patients. Some renal diseases that often require renal transplantation, for example complement-associated diseases like dense deposit disease, are known to be associated with retinal abnormalities such as drusen and macular degeneration.[7] Use of steroids as is common after transplantation, is known to be a major risk factor associated with central serous chorioretinopathy (CSC). This is a form of macular degeneration caused by an accumulation of serous subretinal fluid (SRF) due to choroidal and retinal pigment epithelium (RPE) abnormalities.[8-11] Previous steroid intake has been described in up to 52% of CSC patients, and odds ratios of up to 37 for CSC have been found for steroid using subjects.[8, 12, 13]

In the current study, we performed an extensive cross-sectional ophthalmological phenotyping in renal transplant patients on low-dose steroids for at least 2 years prior to examination.

MATERIALS AND METHODS

Patient selection

In this cross-sectional study, 37 sequential outpatient renal transplant patients who chronically used oral low-dose steroids for at least 2 years prior to examination and who did not have visual complaints were invited to take part in this study conducted at the Department of Ophthalmology of the Leiden University Medical Center for ophthalmological analysis. Patients who were previously diagnosed with either retinal abnormalities or systemic disorders that may cause persistent retinal damage (for example uncontrolled diabetes mellitus or uncontrolled hypertension) were excluded. Written informed consent was signed by all subjects. The study

adhered to the tenets of the Declaration of Helsinki, and was approved by the institutional review board and the ethics committee (NL50816.058.14). Clinical information was collected from the patients' charts. Patients were included in this study from February 2016 to October 2016.

Ophthalmological imaging

Patients underwent an extensive ophthalmic assessment, including Early Treatment of Diabetic Retinopathy Study (ETDRS) best-corrected visual acuity (BCVA) measurement, and indirect ophthalmoscopy. After administration of 1% tropicamide and 5% phenylephrine drops, digital color fundus photography of the central and peripheral retina (Topcon Corp., Tokyo, Japan), optical coherence tomography (OCT), enhanced depth imaging (EDI-) OCT, fundus autofluorescence, and oral fluorescein angiography (FA) images were made with the spectral-domain OCT (Spectralis HRA+OCT; Heidelberg Engineering, Dublin, CA, USA). Subfoveal choroidal thickness (SFCT; distance from the outer part of the hyperreflective RPE layer to the hyperreflective line of the inner surface of the sclera) was measured on EDI-OCT. For the oral FA, patients had to ingest 10 milliliters of 20% fluorescein after a fasting period of at least 3 hours. Oral FA images were obtained at 10, 15, 20, 25, and 30 minutes after the administration of fluorescein. Peripheral images were acquired between 25 and 30 minutes after ingestion. When the evaluation of ophthalmological imaging by an experienced retina specialist (CJFB) led to findings requiring treatment and/or follow-up, these examinations and/or visits were scheduled at the outpatient clinic of the Department of Ophthalmology of Leiden University Medical Center.

Statistical analysis

For statistical analyses, descriptive statistics were used in SPSS Statistics (version 23; IBM Corp., Armonk, NY, USA). Independent *t* tests were used to compare the patient group in whom ophthalmological abnormalities could be detected to the other patients for: need for and total duration of dialysis before transplantation, reason of (re)transplantation, time from the first renal transplantation (and start of steroid use) until phenotyping, number of rejection therapies, type of immunosuppressive drugs at the moment of phenotyping, age of the patient at the moment of transplantation and at phenotyping, and donor age at the moment of transplantation. Moreover, independent *t* tests were used to compare the mean SFCT for patients with and without ophthalmological abnormalities. The level of statistical significance was set at $p<0.05$.

RESULTS

Patient characteristics

The mean age at phenotyping of the 37 included patients (25 male, 12 female) was 59 ± 11 years (range, 38 - 77). The mean age at which the first renal transplantation had been performed was 50 ± 12 years (range, 22 - 71). Retransplantation had been performed in 4 patients (11%). Eleven patients received a transplantation prior to dialysis. The total duration of dialysis was 3.2 ± 2.1 years (range, 0.3 - 7.5) in the 24 other patients, for whom this information was available.

Among other immunosuppressant medication, which was used by all patients, all patients started using steroids after their first transplantation. The estimated minimal cumulative prednisolone dose was 4,000 mg and the mean cumulative prednisolone dose was 20,000 mg. At the moment of ophthalmological phenotyping, 25 patients used 5 mg and 12 used 7.5 mg prednisolone once daily. Eleven patients reported the use of other steroid-containing medication during the last year before ophthalmological phenotyping. The medical history of the participants was notable for arterial hypertension in all patients (100%) and diabetes mellitus in 12 patients (32%). Besides the diagnosis of a depression in 3 patients (8%), no other diseases known to be possibly associated with CSC were present.[14] Four patients used a mineralocorticoid receptor antagonist to treat hypertension, a drug which has also been described to be a therapeutic option for CSC.[15] Clinical characteristics of the patients are summarised in Table 1.

Ophthalmic characteristics

At the visit to the Department of Ophthalmology, ETDRS BCVA was 84 ± 11 letters (range, 25 - 96) in the included eyes. Two patients had a history of unilateral amblyopia and 1 patient of recurrent unilateral herpes keratitis. In 60 eyes (79%) ETDRS BCVA was ≥80 letters. During slit-lamp examination, unilateral mature cataract was detected in 1 patient. BCVA ETDRS in this patient improved from 25 to 85 ETDRS letters, after phacoemulsification. Besides the presence of pre-existing primary open-angle glaucoma, no other abnormalities of the anterior segment were found. OCT showed the presence of macular serous SRF in 3 eyes of 2 patients (5%). On FA, hyperfluorescent changes that could fit well within the spectrum of (subclinical) CSC were detected in these eyes, without the presence of a hot spot of leakage (Figure 1A-E). Fundus autofluorescence revealed variable mild hypo- and hyperautofluorescence of the lesions. On OCT imaging a variable degree of outer photoreceptor/RPE changes compatible with subclinical CSC was found in 8 of the remaining patients (22%), which was supported by corresponding hyperfluorescent changes on FA (Figure 1F-J). On OCT imaging, 2 patients had a bilateral epiretinal membrane (ERM) and 4 had a unilateral ERM, resulting in a total of 6 patients (16%) in whom an ERM in the macula was detected (Figure 1W-X). One of the patients with a unilateral ERM also had a macular pseudohole.

Table 1. Clinical characteristics of the 37 phenotyped patients who chronically used low-dose steroids for at least the last 2 years prior to examination

Clinical characteristics	
Number of patients	37
Number of males	25 (68%)
Number of females	12 (22%)
Mean age ± SD (range) at phenotyping, in years	59 ± 11 (38-77)
Mean age ± SD (range) at renal transplantation, in years	50 ± 12 (22-71)
Reason for first transplantation	
Autosomal polycystic kidney disease	8 (22%)
IgA nephropathy	7 (19%)
Unknown origin of disease	6 (16%)
Acute tubular necrosis	2 (5%)
Membranoproliferative glomerulonephritis	2 (5%)
Malignancy	2 (5%)
Reflux nephropathy	2 (5%)
Alport syndrome	1 (3%)
C3 glomerulopathy	1 (3%)
Chronic urinary tract infections	1 (3%)
Hemolytic-uremic syndrome + hypertension	1 (3%)
Medullary cystic kidney disease	1 (3%)
Oxalate nephropathy	1 (3%)
Pauci-immune crescentic glomerulonephritis	1 (3%)
Tubulointerstitial nephritis	1 (3%)
Retransplantation	4 (11%)

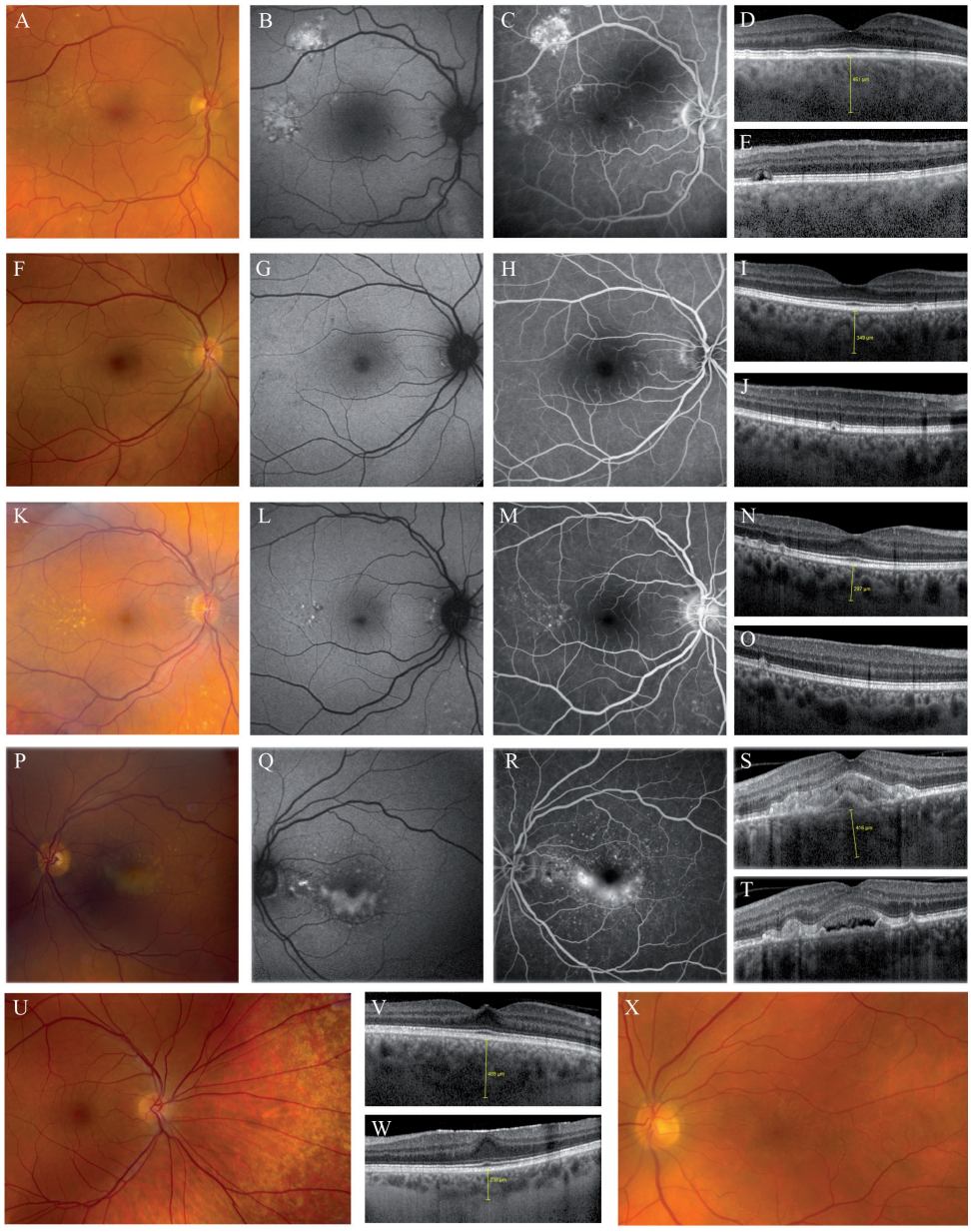

Figure 1. Spectrum of retinal abnormalities in renal transplant patients who chronically used low-dose steroids for at least the last 2 years prior to examination.

A-E. Multimodal imaging of a 58-year-old male patient who received a renal transplant 21 years before phenotyping, because of membranoproliferative glomerulonephritis. Fundus photography of the right eye (A) showed mild foveal and extrafoveal retinal pigment epithelium (RPE) abnormalities, and on fundus autofluorescence (FAF; B) irregular changes were present. Fluorescein angiography (FA) of the right eye (C), which was performed after oral administration of fluorescein, showed multifocal hyperfluorescent areas, but no clear hot spot of leakage. On the foveal optical coherence tomography (OCT) scan of the right eye (D) RPE and outer photoreceptor changes suggestive of central serous chorioretinopathy, and a subfoveal choroidal thickness (SFCT) of 451 µm were found. No subretinal fluid (SRF) was present in the right eye. In the left eye, milder abnormalities were detected on multimodal imaging, but extrafoveal OCT scanning (e) showed SRF nasosuperiorly and RPE changes.
F-J. Multimodal imaging of a 43-year-old male patient who was transplanted 11 years ago, because of reflux nephropathy. On fundus photography of the right eye (F) subtle extrafoveal RPE abnormalities were observed, with corresponding changes on FAF (G). Mild hyperfluorescent changes were found on oral FA (H) of this eye, partly due to staining and partly due to a window defect. The foveal (I) and extrafoveal OCT scans (J) revealed mild outer retinal changes and the SFCT was 349 µm. Moreover, RPE detachments, which could also correspond to age-related (subclinical) small hard drusen, were found. Similar findings were observed in the left eye of this patient.
K-O. Multimodal imaging of a 44-year-old female patient who underwent transplantation surgery 22 years before retinal phenotyping, and had received a transplant because of membranoproliferative glomerulonephritis. Cuticular drusen, known to be associated with membranoproliferative glomerulonephritis type II (dense deposit disease), were detected on fundus photography (K), FAF (L), oral FA (M), and both foveal (N) and extrafoveal OCT images (O) of the right eye. SFCT was 297 µm at this visit. In the left eye, identical findings were detected.
P-T. Multimodal imaging of a 63-year-old male patient who received a renal transplant 6 years before ophthalmological phenotyping, because of IgA nephropathy, showed findings characteristic for bilateral non-exudative age-related macular degeneration and secondary vitelliform-like lesions. On fundus photography of the left eye (P), yellow-white lesions and confluent drusen were seen in the macula, together with cuticular drusen and RPE alterations peripherally. On FAF (Q) hyperfluorescent changes with an irregular border were seen in the macular area. Oral FA (R) revealed cuticular drusen and irregular hyperfluorescent changes foveally and perifoveally, which could be characteristic for the vitelliform-like lesions. Foveal (S) and extrafoveal OCT images (T) showed drusen, and a hyperreflective subretinal accumulation, that was most pronounced foveally. Comparable lesions were found in the other eye of this patient.
U-V. In a 37-year-old male patient, who had received a renal transplant for Alport's disease 7 years before retinal phenotyping, fundus photography of the right eye (U) showed multiple, small yellow-white lesions in the midperiphery of the fundi, known to be associated with this disease. Foveal OCT scanning (V) showed some traction of unknown origin, and a SFCT of 489 µm. Comparable lesions were found in the left eye.
W-X. In a 62-year-old female patient, who was previously diagnosed with autosomal dominant polycystic kidney disease and was transplanted 19 years ago, a unilateral foveal epiretinal membrane was detected on OCT (W). SFCT was 238 µm, at that visit. The epiretinal membrane was also observed on fundus photography (X).

Cuticular drusen, which are associated with membranoproliferative glomerulonephritis type II (dense deposit disease), were seen in 1 patient with this disease (Figure 1K-O), and multiple, small yellow-white lesions in the midperiphery of the fundi, which are associated with Alport's disease, were seen in 1 patient with this diagnosis (Figure 1U-V).[16, 17] Bilateral intermediate age-related macular degeneration with soft drusen and secondary vitelliform-like lesions was found in 1 patient (Figure 1P-T), whereas bilateral RPE detachments in combination with drusenoid lesions were detected in another patient. In single patients a unilateral RPE detachment, an old unilateral retinal venous occlusion, and unilateral RPE atrophy was detected. In patients with (subclinical) CSC, mean SFCT on EDI-OCT was 286 ± 82 µm (range, 111 - 451). In all eyes with retinal abnormalities, mean SFCT on EDI-OCT was 301 ± 88 µm (range, 111 - 489), and mean SFCT in all eyes of patients with either unilateral or bilateral abnormalities was 292 ± 84 µm (range, 111 - 489). These CTs differed from the eyes of patients in whom no abnormalities were detected (232 ± 108 µm (range, 98 - 571); p=0.071, p=0.01, and p=0.006, respectively). None of the clinical characteristics of the included patients could be linked with either the presence or absence of ocular abnormalities. Treatment was not needed for the retinal abnormalities that were detected in this study. For the patient with macular pseudohole and for the patient with maculopathy with secondary vitelliform-like lesions, additional follow-up visits were scheduled. The retinal findings of the patients are summarised in Table 2.

Table 2. Reason for transplantation of the 22 phenotyped patients with retinal abnormalities who chronically used low-dose steroids for at least the last 2 years prior to examination

Retinal abnormality	Reason for transplantation (number of patients)
Bilateral findings characteristic for CSC*, bilateral SRF	Membranoproliferative glomerulonephritis (1)
Bilateral findings characteristic for CSC*, unilateral SRF	Unknown origin of disease (1)
Bilateral findings characteristic for CSC*, no SRF	Acute tubular necrosis after surgery (1), ADPKD (1), reflux nephropathy (1), tubulointerstitial nephritis (1)
Unilateral findings characteristic for CSC*, no SRF	Chronic urinary tract infections (1), hemolytic-uremic syndrome AND hypertension (1), IgA nephropathy (1)
Unilateral findings characteristic for CSC*, no SRF; AND a bilateral epiretinal membrane	ADPKD (1)
Bilateral epiretinal membrane	Unknown origin of disease (1)
Unilateral epiretinal membrane	ADPKD (2), pauci-immune crescentic glomerulonephritis (1)
Unilateral epiretinal membrane AND a unilateral macular pseudohole AND bilateral choroidal folds	Nephrosclerosis AND neoplasm (1)
Bilateral cuticular drusen, known for membranoproliferative glomerulonephritis	Membranoproliferative glomerulonephritis (1)
Bilateral dry age-related macular degeneration AND secondary vitelliform lesions AND cuticular drusen	IgA nephropathy (1)
Bilateral peripheral yellow-white lesions, known for Alport syndrome	Alport syndrome (1)
Bilateral retinal pigment epithelium detachments AND drusenoid lesions	Oxalate nephropathy (1)
Unilateral old venous occlusion	Unknown origin of disease (1)
Unilateral retinal pigment epithelium atrophy	Unknown origin of disease (1)
Unilateral retinal pigment epithelium detachment	ADPKD (1)
No abnormalities	Acute tubular necrosis after surgery (1), ADPKD (3), C3 glomerulopathy (1), IgA nephropathy (5), medullary cystic kidney disease (1), nephrosclerosis AND neoplasm (1), reflux nephropathy (1), unknown origin of disease (2)

* Outer photoreceptor/retinal pigment epithelium changes on OCT, reminiscent of changes in chronic CSC, with corresponding hyperfluorescent changes on FA

Abbreviations: ADPKD: autosomal dominant polycystic kidney disease, CSC: central serous chorioretinopathy, FA: fluorescein angiography, OCT: optical coherence tomography, SRF: subretinal fluid

DISCUSSION

Retinal abnormalities were found in 22 of 37 renal transplant patients (59%), who were using chronic low-dose steroids for at least 2 years prior to examination and who were not previously known to have chorioretinal diseases. Findings characteristic for (subclinical) CSC were found in 10 patients (27%), and 2 of these patients showed active SRF leakage in the macula. Moreover, in 5 other patients and in a total of 6 patients (16%) an ERM was found. These findings indicate that renal transplant patients could benefit from ophthalmological examination. The frequent occurrence of CSC-like abnormalities may have been caused by steroid use, which has been described to be the most important risk factor.[12, 18, 19] In light of the underlying disease and use of comedication, and the cross-sectional/observational nature of this study, it remains uncertain whether a causal relation exists between the use of steroids and the high prevalence of retinal abnormalities in renal transplant patients. If there is a causal relation, this might be either due to a (previous) short-term high dosage or due to the cumulative effect of a long-term low dosage.

Extensive ophthalmological phenotyping in renal transplant patients using steroids has not been conducted so far. The low prevalence of CSC with active SRF leakage in our cohort (5%) is surprising given the fact that all patients had used high cumulative doses of steroids. This suggests that the susceptibility for developing CSC depends on more risk factors than solely the use of steroids. Still, 22% of patients had retinal abnormalities that may be compatible with subclinical CSC without SRF leakage. In a previous study, in which the authors did not exclude patients with pre-existing ophthalmological diseases and in which no ophthalmological imaging was performed and in which exact definitions of disease were lacking, retinal pigment epitheliopathy (14%), CSC (3%), retinal vein occlusion (3%), and macular edema (3%) were described.[11] The number of patients with retinal pigment epitheliopathy/CSC in our study seems to be in line with these previously published findings.

For our study, only patients who used steroids for more than 2 years and who did not have a history of either chorioretinal diseases or complaints that could be related to these diagnoses were included. Since a selection based on underlying renal pathology was not performed, patients with various renal diseases were included. In contrast with another study, we did not find a correlation between the outcome of phenotyping and the duration of dialysis before transplantation and time since transplantation.[11] Other clinical characteristics of the included patients could also not be linked to the occurrence of ophthalmological abnormalities. However, compared to patients in whom no ophthalmological abnormalities were found, a significantly increased mean SFCT was observed in patients in whom ophthalmological abnormalities were seen. In CSC patients, the choroid is usually thicker compared to the healthy population, strengthening the relevance of this finding.[20-22] Both the increased CT

and percentage of patients with (subclinical) CSC in this study could thus have been caused by the effect of steroids on the choroid, in cases of administration for a prolonged period and only in a specific patient group with predisposition to develop these abnormalities.[23, 24] However, CT between patients with (subclinical) CSC and patients without ophthalmological abnormalities did not differ significantly, which may be related to the small sample size. Since younger age, shorter axial length, male gender, deeper anterior chamber, thicker lens, flatter cornea, and better BCVA have all been associated with an increase in SFCT, comparison of the measurements in our patients with matched healthy controls to whom low-dose steroids had not been prescribed chronically could have attached significance to our data.[25, 26] Even though we expected to find abnormal ocular findings in patients with chronic kidney disease, given the correlation between nephrological and ophthalmological diseases,[27-29] the prevalence of abnormalities was higher than we had expected beforehand. This may have been caused by the use of other (immunosuppressive) medication, due to hypertension, due to pre-existing renal diseases, or even due to diabetes mellitus.

In conclusion, retinal abnormalities are present in the majority of renal transplant patient who use low-dose steroids chronically. Therefore, an ophthalmological examination including evaluation of the retina is indicated in these patients. However, seeing that the ophthalmological abnormalities were relatively mild and had a low likelihood for causing symptoms and complications, the dosage of prescribed steroids does not have to be lowered or discontinued in this patient group.

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4.3

CHRONIC CENTRAL SEROUS CHORIORETINOPATHY AS A PRESENTING CLINICAL FEATURE OF CUSHING'S SYNDROME

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ABSTRACT

Purpose: To describe 4 patients who were diagnosed with chronic central serous chorioretinopathy (cCSC), which appeared to be the presenting symptom of Cushing's syndrome (CS).

Methods: Retrospective review of charts. All patients received extensive ophthalmological examination and endocrinological analyses.

Results: A 56-year-old male and a 49-year-old female patient were treated because of bilaterally active, therapy-resistant cCSC. The clinical sign indicative for CS leading to referral to the endocrinologist was muscle weakness in the male and plethora in the female. In a 37-year-old female patient known with diabetes mellitus and central obesity bilateral cCSC was diagnosed before CS screening. Another 49-year-old female patient was treated for unilateral cCSC for 4 years. Complaints of fatigue, muscle weakness, central adiposity, and skin atrophy led to referral, and a CS diagnosis due to bilateral macronodular adrenal hyperplasia. In all patients, CS surgery resulted in complete resolution of subretinal fluid. During post-surgical follow-up no reactivation of cCSC was observed.

Conclusions: Chronic CSC can be the principal manifestation of relatively mildly symptomatic, and unrecognised CS. In patients with cCSC, ophthalmologists should have a high index of suspicion for clinical signs of CS that warrant endocrinological analysis. CS surgery can stop active subretinal fluid leakage in cCSC.

INTRODUCTION

Central serous chorioretinopathy (CSC) is a relatively common early-onset eye disease, characterised by an accumulation of leaked serous fluid under the retina, causing a detachment of the neuroretina. This subretinal fluid (SRF) leakage results from dysfunction of the retinal pigment epithelium (RPE), caused by congestion, thickening, and hyperpermeability of the choroid, which implies an important role for choroidal abnormalities as an underlying cause for RPE dysfunction and SRF leakage in CSC.[1-4] Two main subtypes of CSC are generally distinguished: acute CSC (aCSC) and chronic CSC (cCSC). Whether these subtypes are a continuum or separate entities is currently unclear.[1-7] Patients with aCSC present with sudden and marked central vision loss, because of SRF leakage in the macula due to a focal leak in the RPE that is visible as a single hot spot on fluorescein angiography (FA). Acute CSC generally has a favorable prognosis because the SRF often disappears spontaneously within 2 to 3 months, with (near-)normal recovery of vision. In contrast, cCSC is typically not self-limiting and SRF persists for more than 3 months. Patients with the cCSC phenotype have more diffuse multifocal leakage on FA and indocyanine green angiography (ICGA), as well as irregularly distributed widespread RPE changes associated with various degrees of low-grade, more indistinct leakage on angiography. The persistent serous neuroretinal detachments may cause progressive and irreversible photoreceptor damage, resulting in a poorer visual prognosis of cCSC as compared to aCSC.[2, 8, 9] Although the etiology of CSC is largely obscure, clinical observations indicate an association with the use of corticosteroids, hypercortisolism, stress, and possibly with type A personality.[1, 10, 11] The incidence of CSC is approximately 6 times higher in men than in women, suggesting a role for hormonal risk factors, although this male-to-female proportion seems to be less pronounced in cCSC and steroid-related CSC.[1] Recently, single nucleotide polymorphisms in the *complement factor H* gene, the *complement component 4B* gene, and the *ARMS2* gene have been found to be associated with cCSC.[12-14]

Elevated levels of glucocorticoids caused by either endogenous or exogenous mechanisms have been described in association with CSC.[15, 16] Prolonged and excessive exposure to glucocorticoids leads to a typical clinical entity: Cushing's syndrome (CS). Whereas CS secondary to exogenous corticosteroid use is relatively common, endogenous CS is very rare with an incidence of approximately 2 per million per year.[17] Most cases of endogenous hypercortisolism are adrenocorticotropin (ACTH) dependent; not more than 20% of cases are of ACTH independent adrenocortical origin.[18] The term 'Cushing's disease' is reserved for patients with CS due to an ACTH secreting pituitary adenoma. Other causes for ACTH dependent CS include ectopic ACTH producing neuroendocrine tumors, mainly bronchial carcinoids. ACTH independent CS includes adrenal adenoma or macronodular (bilateral) adrenal hyperplasia. There is a broad and variable spectrum of signs of CS including facial rounding ('moon face'), generalised or truncal obesity, muscle weakness, flushing of the face (plethora), and reddish-purple skin

striae. In addition, patients with CS often experience amenorrhea, bruising with no obvious trauma, glucose intolerance/type 2 diabetes mellitus, loss of libido, metabolic syndrome (a combination of abdominal obesity, elevated fasting plasma glucose, hypertension, high serum triglycerides, and low high-density lipoprotein), osteoporosis, neuropsychiatric symptoms, and severe fatigue (Supplementary Table 1). It is well-known that there is an individual variation in glucocorticoid sensitivity, and this may also be the case for different tissues within an individual.

In our tertiary referral center we identified 4 patients with cCSC as a hallmark heralding the diagnosis of CS. In this case series we aimed to describe their clinical characteristics, treatment, and findings during follow-up.

MATERIALS AND METHODS

We retrospective reviewed the charts of 4 patients in whom cCSC appeared to be the first clinically recognised disease entity after which CS was diagnosed. All patients received a complete ophthalmic examination, including measurement of best-corrected visual acuity (BCVA), applanation tonometry, slit-lamp examination, and indirect ophthalmoscopy. A conversion method was used to obtain Early Treatment of Diabetic Retinopathy Study (ETDRS) BCVA.[19] Dilation of pupils was achieved by the administration of topical 0.5% tropicamide and 5% phenylephrine drops. Optical coherence tomography (OCT), using either the Cirrus OCT device (Carl Zeiss Meditec, Dublin, CA, USA) or a spectral-domain OCT (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany), and digital color fundus photography (Topcon Corporation, Tokyo, Japan) were performed in all patients. In all patients fundus autofluorescence and FA were performed with either the spectral-domain OCT or the Topcon device. One patient received an ICGA with the Topcon device, and in 1 patient ICGA was performed using the spectral-domain OCT. In 1 patient OCT angiography with a SD-OCT device (OptoVue RTVue XR Avanti AngioVue, Optovue Inc., Fremont, CA, USA) was performed. Endocrinological analysis for the presence and etiology of CS was performed according to the Endocrine Society clinical guidelines.[20]

RESULTS

Case 1

A 56-year-old male patient was being treated at the department of Ophthalmology for 3 years because of bilaterally active, therapy-resistant cCSC. Progressive muscle weakness led to referral to the department of Endocrinology in October 2013. CS was not considered before.

The patient did not appear typically cushingoid (Figure 1A-C). However, fatigue, hypertension, mood swings, truncal obesity, osteoporosis, and complaints of an easy-bruising skin were present at that time. Laboratory evaluation showed hypercortisolism and a cystoid lesion in the central pituitary gland on magnetic resonance imaging. Bilateral inferior petrosal sinus sampling confirmed the diagnosis of an ACTH secreting pituitary microadenoma.

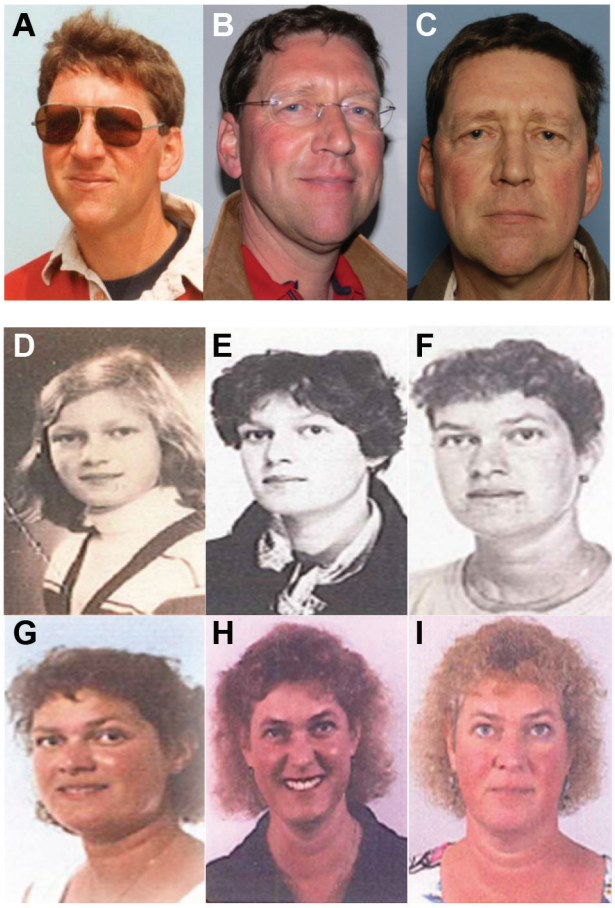

Figure 1. Follow-up photographs of 2 patients with chronic central serous chorioretinopathy.
Follow-up photographs of a 56-year-old man (A-C) and a 49-year-old woman (D-I), who were diagnosed with Cushing's syndrome due to a pituitary adenoma after visiting the department of Ophthalmology because of chronic central serous chorioretinopathy since 2010 and since 2001. The diagnosis of Cushing's disease was established in 2013 and in 2012, respectively.
A-I. The portraits of the male patient were taken in 2003 (A), 2007 (B), and 2013 (C), and those of the female patient in 1974 (D), 1983 (E), 1991 (F), 1996 (G), 2004 (H), and 2010 (I).

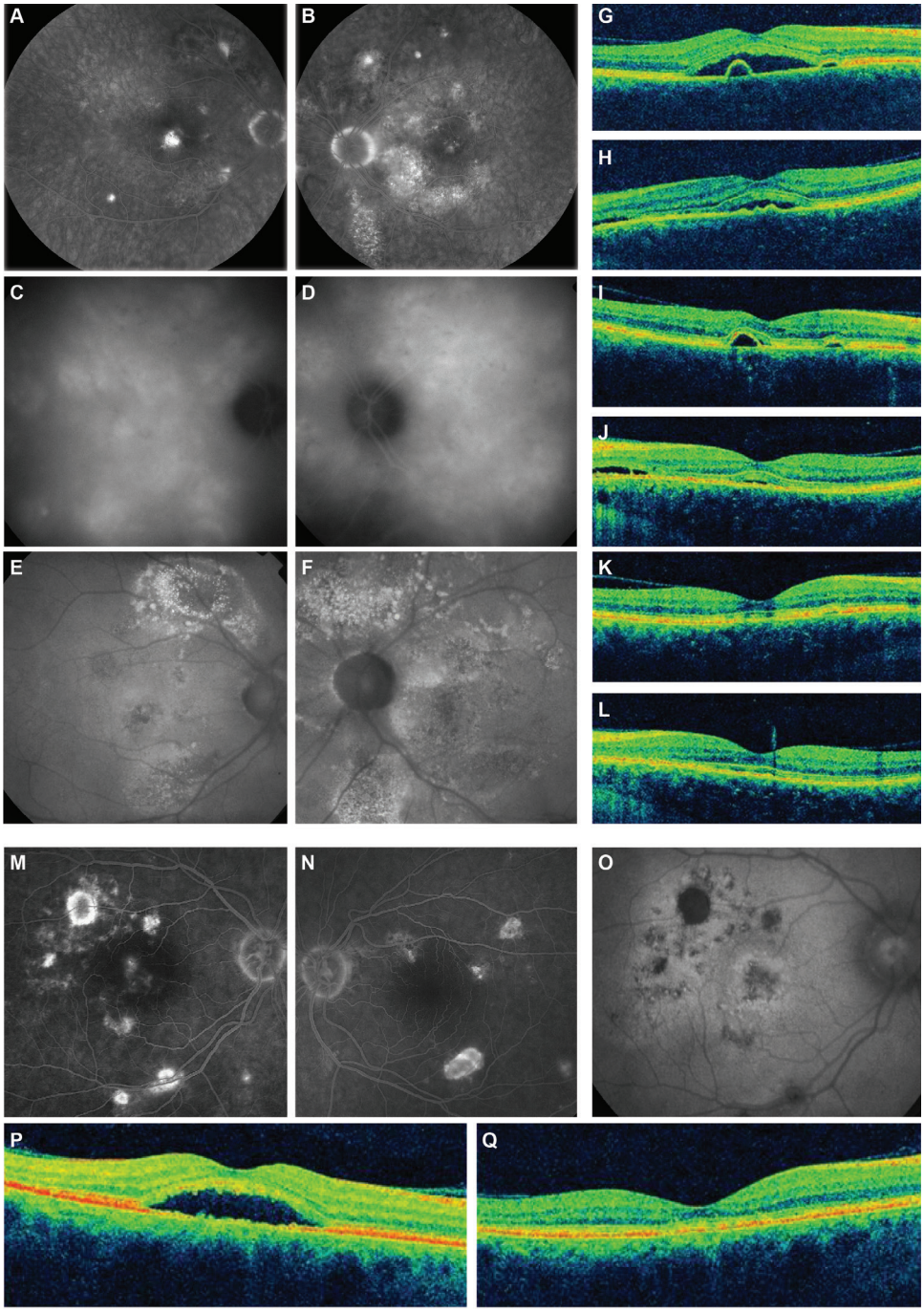

Figure 2. Ophthalmological imaging of the first 2 patients with chronic central serous chorioretinopathy in whom Cushing's syndrome was diagnosed.

Ophthalmological imaging of a 56-year-old male (A-L) and a 49-year-old female patient (M-Q), who were diagnosed with Cushing's syndrome due to a pituitary adenoma after visiting the department of Ophthalmology because of chronic central serous chorioretinopathy (CSC) since 3 and 11 years, respectively. **A-H**. At the time of referral to the endocrinologist, when this 56-year-old patient had already received 1 half-dose photodynamic therapy (PDT) treatment in the right eye, fluorescein angiography (A, B) revealed multiple hot spots of leakage and diffuse retinal pigment epithelium (RPE) window defects typical of chronic CSC in both eyes. Indocyanine angiography showed diffuse hyperfluorescent areas in both eyes (C, D). Fundus autofluorescence (FAF) imaging showed diffuse, irregular, and predominantly hyperautofluorescent changes (E, F). On optical coherence tomography (OCT) leakage of serous subretinal fluid (SRF) caused a foveal neuroretinal detachment in both the right (G) and the left eye (H), with RPE detachments in the right eye. **I-J**, Despite half-dose PDT serous SRF had persisted on OCT in both eyes, at the evaluation visit 5 weeks after treatment. **K-L**. Transsphenoidal resection of a detected pituitary microadenoma resulted in complete disappearance of SRF on OCT in both eyes within 1 month after surgery.
M-P. This 49-year-old patient was known with chronic CSC for 11 years, and previously received multiple focal argon laser treatments in both eyes. Fluorescein angiography revealed atrophic laser scars in both eyes and unilaterally active chronic CSC with 2 parafoveal hot spots of leakage in the right eye (M) and no hot spots in the left eye (N). FAF imaging of the right eye (O) also showed laser scars and diffuse, irregular FAF abnormalities. At that time, serous SRF was present only in the right eye (P). **Q.** This SRF had disappeared 6 weeks after full dose, half-time (42 seconds) PDT.

At the time of referral to the endocrinologist, he complained of vision loss in his right eye. ETDRS BCVA was 78 OD (20/27) and 85 OS (20/20). On OCT serous SRF was present in both eyes (Figure 2G-H), despite having received 1 session of half-dose photodynamic therapy (PDT) in his right eye in December 2011. FA showed diffuse leakage of fluorescein in both eyes (Figure 2A-B), and on ICGA a diffuse hyperfluorescence was observed (Figure 2C-D). The patient received half-dose PDT in both eyes in November 2013. However, it did not result in a permanent complete resolution of SRF, 5 weeks later (Figure 2I-J). The patient underwent successful transsphenoidal resection of the pituitary microadenoma resulting in complete disappearance of the serous SRF within the first month after surgery (Figure 2K-L). No recurrence of serous SRF occurred during a follow-up period of 11 months on physiological

Case 2

A 49-year-old female patient had been visiting our outpatient clinic since March 2004 for recurrent bilateral CSC that had been present since 2001. At the first visit ETDRS BCVA was 90 OD (20/16) and 85 OS (20/20). She received multiple focal argon laser treatments in both eyes, but fluctuating complaints of blurred vision were still present in May 2012. At that time ETDRS BCVA was 79 OD (20/26) and 85 OS (20/20). Fundoscopy revealed an extensive serous detachment of the neurosensory retina of the macula in the right eye (Figure 2Q). FA revealed 2 parafoveal foci of leakage (hot spots) in her right eye (Figure 2N), and no hot spots (Figure 2O) and serous SRF in her left eye. She received half-time PDT in her right eye, which led to disappearance of SRF on OCT in July 2012 (Figure 2S).

Suspicion of CS by the ophthalmologist arose based on clinical signs including progressive plethora (Figure 1D-I) and relatively therapy-resistant hypertension since 2002. She was referred for endocrinological evaluation in November 2012. The diagnosis of CS was established based upon biochemical testing. Magnetic resonance imaging of the pituitary gland showed a right-sided lesion compatible with a microadenoma. In May 2013 she underwent successful transsphenoidal adenectomy with the need for postsurgical hydrocortisone replacement. However, visual symptoms and SRF did not recur. ETDRS BCVA at last visit in July 2013 was 79 OD (20/26) and 89 OS (20/17).

Case 3

A 37-year-old female patient was referred from another hospital in July 2014 because of cCSC of her left eye. At presentation, there was a direct clinical suspicion of CS, because of the presence of central obesity (weight gain of 20 kg in 2 years), diabetes mellitus, involuntary infertility, recurrent infections, and therapy-resistant hypertension. The patient used beclomethasone for these infections. She had complaints of decreased vision of her left eye since February 2014, and was operated for posterior subcapsular cataract in her left eye in May 2014.

In July 2014, she was referred to the outpatient clinic of Endocrinology where the clinical diagnosis of CS was confirmed based upon evident features like a buffalo hump, central obesity, striae, proximal muscle atrophy, a "moon face", supraclavicular fat pads, and hirsutism.

At the first ophthalmological evaluation ETDRS BCVA was 80 OD (20/25) and 68 OS (20/44). Fundoscopy revealed bilateral mild-atrophic irregular RPE alterations and a macular neurosensory detachment in the left eye. OCT showed serous SRF in OS (Figure 3E). FA revealed a focus of leakage superonasally in the macula of the left eye (Figure 3B), and ICGA showed extensive hyperpermeability of the choroid and microdilations of the choriocapillaris of both eyes (Figure 3C-D).

Biochemical screening tests, which were performed after the discontinuation of beclomethasone, indicated ACTH dependent CS. Pituitary imaging showed a microadenoma and, subsequently she started with preoperative medical treatment with metyrapone to reduce cortisol secretion. Within 8 days after the start of metyrapone treatment a complete resolution of serous SRF on OCT was observed. In December 2014 she underwent successful transsphenoidal adenectomy with subsequent hydrocortisone replacement for secondary adrenal insufficiency. Three months after surgery, ETDRS BCVA was 81 OD (20/24) and 76 OS (20/30). On OCT, serous SRF was absent (Figure 3F).

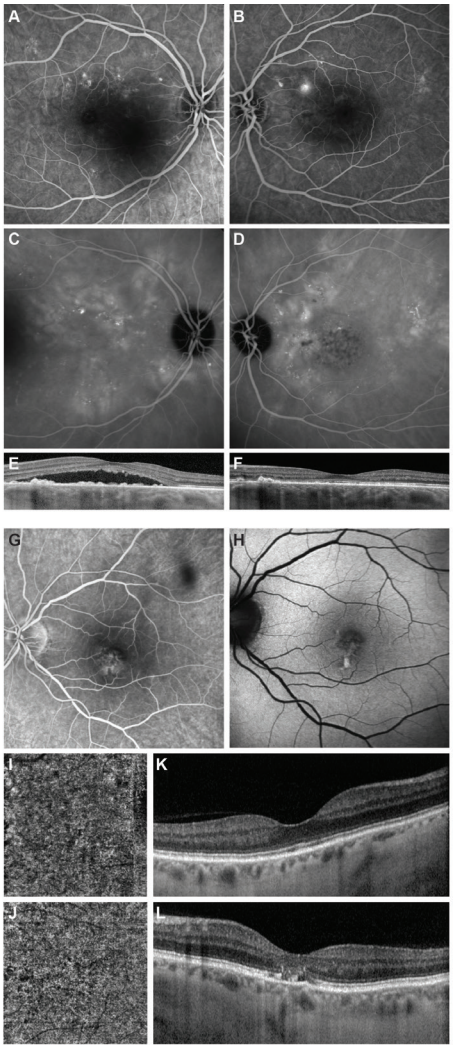

Figure 3. Ophthalmological imaging of the last 2 patients with chronic central serous chorioretinopathy in whom the diagnosis of Cushing's syndrome was established.
Ophthalmic imaging of a 37-year-old female patient , who was diagnosed with chronic central serous chorioretinopathy (CSC) and in whom screening for Cushing's syndrome revealed a pituitary adenoma (A-F), and imaging of a 49-year-old female patient, who was diagnosed with Cushing's syndrome due to bilateral macronodular adrenal hyperplasia, after visiting the department of Ophthalmology because of chronic CSC for 4 years (G-L).
A-E. Fluorescein angiography (FA) of the right eye of this 37-year-old patient (A) showed mild, irregular, and hyperfluorescent detachments of the retinal pigment epithelium and possibly a small hot spot of leakage superotemporally in the macula. In the left eye (B) a hot spot was present in the superonasal macula. Indocyanine green angiography of the right (C) and the left eye (D) revealed extensive indistinct

hyperfluorescent hyperpermeability of the choroid and smaller, more circumscript hyperfluorescent lesions interpreted as microdilations of the choriocapillaris and/or small detachments of the retinal pigment epithelium. On optical coherence tomography (OCT) of the left eye (E) central serous subretinal fluid (SRF) and irregularities of the retinal pigment epithelium were present. **F**. Subfoveal SRF disappeared after Cushing's syndrome was diagnosed, when the patient had discontinued using beclomethasone and started treatment with metyrapone to reduce cortisol secretion.
G-L. At last follow-up visit of a 49-year-old patient, which was scheduled 13 years after bilateral adrenalectomy, FA of the left eye (G) after oral administration of fluorescein revealed a foveal area of hyperfluorescence, but no focal leakage. Lesions on fundus autofluorescence imaging corresponded to the foveal area of hyperfluorescence on FA (H). OCT angiography of the choriocapillaris segment of the right (I) and the left eye (J) did not show pronounced changes. No SRF was present on OCT (K, L).

Case 4

This 49-year-old female patient had been visiting the department of Ophthalmology of several hospitals since October 1996, because of unilaterally active cCSC. The patient had complaints of blurred vision, and fundoscopy revealed the presence of RPE alterations, more prominent in the left eye compared to the right. FA showed unilateral leakage of fluorescein, without the presence of a neovascularisation. ETDRS BCVA in both eyes was 85 (20/20). She had not received any ophthalmological therapeutic intervention during follow-up.

This patient was also known to the endocrinologist since December 1997, because of analysis of osteoporosis. In 2000, several miscarriages from 1973 to 1982, hypertension since 1998, complaints of fatigue, muscle weakness, central adiposity, and skin atrophy rose the suspicion of CS. Biochemical screening confirmed ACTH independent CS. Abdominal CT scanning revealed CS due to bilateral macronodular adrenal hyperplasia.

She underwent bilateral adrenalectomy and received hydrocortisone and fludrocortisone replacement. During follow-up no reactivation of CSC was observed on FA (Figure 3G). At the last visit to the outpatient clinic of Ophthalmology, 13 years after surgery, ETDRS BCVA was 80 OD (20/25) and 81 OS (20/24). On OCT, no serous SRF was present in both eyes (Figure 3K-L).

DISCUSSION

Chronic CSC can be the principal manifestation of CS with relatively subtle clinical signs. Moreover, cCSC can coexist with full-blown – textbook – cases of hypercortisolism. A delay in establishing the diagnosis and treatment of CS can be prevented if ophthalmologists actively pay attention to potential signs and symptoms compatible with hypercortisolism. Treatment of the underlying hypercortisolism can result in complete resolution of SRF leakage in cCSC.

Two patients in this case series had only few clinically evident symptoms pointing to CS: muscle weakness in the first patient, and plethora in the second. In retrospect, the 2 other patients had more physical signs indicating overt CS, but were previously not recognised because of a gradual clinical onset for many years. Such a delay in diagnosis is typical for any rare disease with a gradual, slowly progressive clinical onset, especially when the individual symptoms are non-specific and largely overlap with the highly prevalent metabolic syndrome.[21] Moreover, there is a subgroup of patients with mild autonomous hypercortisolism without the full-blown clinical picture, sometimes referred to as subclinical hypercortisolism.[22]

Episodes of CSC have been described in several patients with an established diagnosis of endogenous hypercortisolism.[23-28] In a previous study, 5% of 60 patients with active Cushing's disease had developed 1 or more episodes of CSC before diagnosis; mostly the patients with high concentrations of plasma cortisol.[23]

Moreover, an indication of the occurrence of hypercortisolism has been found in CSC patients. In 30 aCSC patients, significantly increased cortisol levels in urine and plasma were detected in a pilot study, as compared to a control group with an acute unilateral retinal detachment.[29] In another pilot study with 16 CSC patients 24-hour urinary free cortisol was found to be significantly elevated, as compared to a control group, whereas in 24 aCSC patients 50 percent of patients showed elevated 24-hour urinary free cortisol.[30, 31] These anecdotal reports indicate that subclinical hypercortisolism may be present in some CSC patients. Further research is needed to unravel the exact pathophysiological mechanism of CSC, whether subclinical hypercortisolism may play a role in CSC patients without clinical evidence of CS, or whether these cases represent sequelae of 'stress' with secondary activation of the hypothalamic-pituitary-adrenal axis and concurrent CSC.

In accordance with our findings, a recent case described bilateral CSC as the primary clinical manifestation of a patient with confirmed CS.[32] This patient had hypertension and severe osteoporosis, but none of the classic clinical complaints of CS. Even prior to transsphenoidal adenectomy, bilateral resolution of SRF occurred after low-fluence PDT.[32] In our cases, 1 cCSC patient with SRF responded well to PDT treatment, whereas another patient had persistent SRF despite bilateral PDT. The third patient had SRF resolution after oral metyrapone treatment was started for CS. All patients responded with an absence of SRF after normalization of cortisol exposure.

The described cases underline that increased glucocorticoid exposure can result in CSC. Systemic steroid use increases the risk to develop CSC dramatically, with described odds ratios up to 37.1.[33] CSC appears to occur significantly more often in patients who use steroids after a renal transplantation, possibly due to the use of high-dose corticosteroids, although the underlying disease *per se*, the presence of systemic hypertension or the vascular effects of hemodialysis may also play a role.[34, 35] Bilateral CSC and an evolution of acute to chronic disease may be more common in CSC associated with exogenous hypercortisolism.[23]

The pathophysiological basis of CSC still remains to be elucidated. Changes in regulation of subfoveal choroidal blood flow have been described in CSC patients,[36] and autoregulation of the blood vessels of the choroid might be influenced by elevated cortisol levels. Dysfunctional autoregulation and choriocapillary hyperpermeability and congestion could lead to dysfunction of the outer blood-retinal barrier of the RPE, with subsequent SRF leakage.[15, 16, 23, 37] Moreover, cortisol excess may increase the fragility and permeability of the choriocapillaris, which could lead to choroidal decompensation and to the formation of SRF.[38] Choriocapillary non-perfusion and ischemia has also been found in areas of increased hyperpermeability on ICGA and OCT angiography.[38, 39] Direct damage and reduced regeneration in already injured RPE by cortisol has been described, due to its influence on fibroblastic activity and the formation of extracellular matrix components.[40, 41] Moreover, cortisol is involved in the inhibition of collagen formation, which is the main component of Bruch's membrane.[15, 42] Recent studies indicate that there may also be a role for the mineralocorticoid pathway.[43] This mechanism is suggested on the basis of studies in rats, and findings in several patients who were orally treated with either the mineralocorticoid receptor antagonist eplerenone or spironolactone.[43, 44] Both mineralocorticoids and glucocorticoids could activate the mineralocorticoid receptor on endothelial cells of the choroid, which in turn may lead to vessel dilatation via upregulation of the endothelial vasodilatory calcium-dependent potassium channel KCa2.3 by hyperpolarisation of these endothelial cells and smooth muscle cells.[43]

In conclusion, in patients with cCSC ophthalmologists should have a high index of suspicion for clinical stigmata of CS, a potentially lethal disease with increased morbidity and mortality. Endocrinologists dealing with patients with CS also need to be aware of the potential coexistence of CSC. Visual complaints may be partly neglected in the intensive treatment period with many comorbidities to be addressed by many specialists. As cCSC can be the first and principal manifestation of a subclinical CS, we advocate to refer all cCSC patients with potential signs indicative for CS for endocrinological screening.

Supplementary Table 1. Signs and symptoms of Cushing's syndrome

Generalised	Psychological
Central (truncal) obesity	Cognitive dysfunction
Fatigue	Decreased libido
Increased risk of infections	Depressed mood and anxiety
Glucose intolerance/type 2 diabetes mellitus	Impaired stress responsiveness
Proximal muscle atrophy	Loss of emotional control
Weight gain	
	Reproductive
Bone	Men: erectile dysfunction
Increased fracture risk	Women: irregular or absent menstrual periods
Muscular	Skin
Weakness	Acne
	Excessive hairiness (hirsutism)
Other	Striae
Facial rounding ('moon face')	Thin skin due to atrophy of subcutaneous fat with easy bruisability
Fat accumulation on the back of the neck ('buffalo hump')	
Flushing of the face (plethora)	Vascular
Headache	Increased risk for deep venous thrombosis
	New or worsened hypertension

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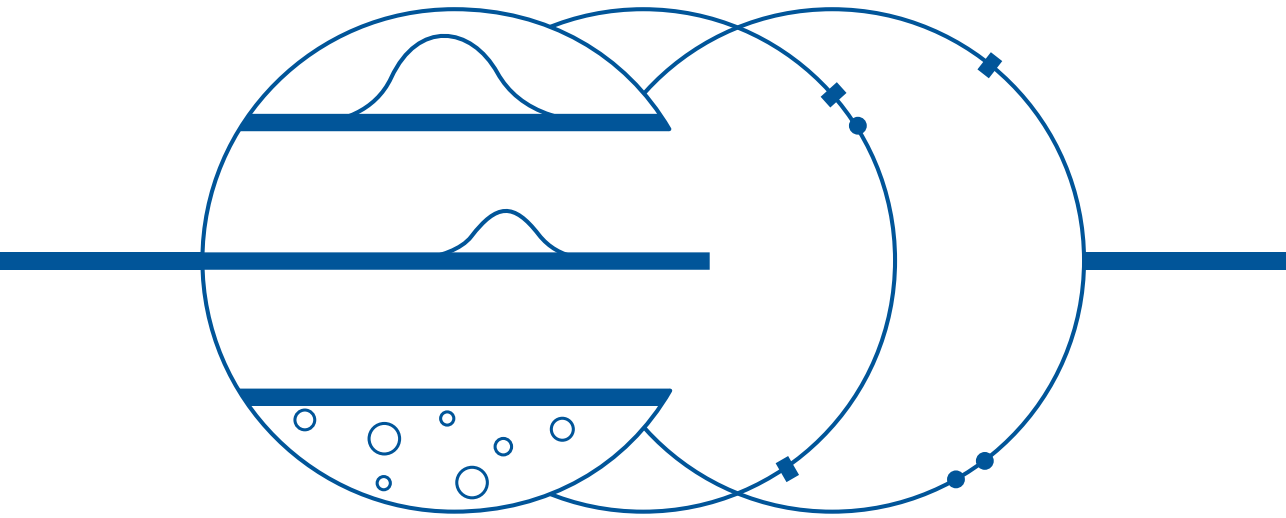
5

DIFFERENTIAL DIAGNOSIS OF CENTRAL SEROUS CHORIORETINOPATHY



5.1

SEROUS RETINOPATHY ASSOCIATED WITH MEK INHIBITION (BINIMETINIB) FOR METASTATIC CUTANEOUS AND UVEAL MELANOMA

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ABSTRACT

Objective: To analyse the clinical characteristics of a serous retinopathy associated with MEK inhibition with binimetinib treatment for metastatic cutaneous melanoma (CM) and uveal melanoma (UM), and to determine possible pathogenetic mechanisms that may lead to this retinopathy.

Design: Prospective observational, cohort-based, cross-sectional study.

Participants: Thirty CM and 5 UM patients, treated with the MEK inhibitor binimetinib (CM) as single agent or a combination of binimetinib and the PKC inhibitor sotrastaurin (UM).

Methods: Extensive ophthalmic examination was performed, including Early Treatment of Diabetic Retinopathy Study (ETDRS) best-corrected visual acuity (BCVA), applanation tonometry, slit-lamp examination, indirect ophthalmoscopy, digital color fundus photography, and optical coherence tomography (OCT). In selected cases, additional examinations were performed, including visual field testing with a Humphrey Field Analyser and electro-oculography (EOG). Blood samples were obtained from 3 CM and 3 UM patients to analyse the presence of autoantibodies against retinal and retinal pigment epithelium (RPE) proteins.

Main Outcome Measures: visual symptoms; visual acuity; fundus appearance; characteristics on OCT, fundus autofluorescence (FAF), EOG.

Results: Six CM (20%) and 2 UM patients (40%) reported visual complaints during the study. The median time until onset of complaints, which were all mild and transient, was 3.5 days (range, <1 hour - 3 weeks). On OCT, subretinal fluid (SRF) was detected in 77% of CM and 60% of UM patients. In the 26 patients with SRF, the fovea was affected in 85%. After the start of the medication, an EOG was performed in 19 eyes of 11 patients: 16 of these eyes (84%) developed SRF on OCT. Fifteen of these eyes (94%) showed an abnormal Arden ratio (<1.65), and 1 eye (6%) showed a subnormal Arden ratio (1.65-1.8). A broad pattern of antiretinal antibodies was found in 3 CM and 2 UM patients tested, while anti-RPE-antibodies were detected in all 6 treated patients.

Conclusions: A time-dependent and reversible serous retinopathy can develop in metastatic CM and UM patients treated with binimetinib. A minority of patients develop visual complaints, which are generally mild and transient. A cause of binimetinib-associated serous retinopathy may be toxicity of medication, but autoantibodies may also be involved.

INTRODUCTION

Cutaneous malignant melanoma (CM) is an increasingly occurring malignant skin tumor. Distant metastases, either via lymphatics or the bloodstream, occur in 15% of patients, with a 5-year survival rate of 15-22%.[1] Although CM represents only 4% of skin cancer cases, it causes 65% of skin-cancer related deaths.[2]

Uveal melanoma (UM) is the most common primary intraocular malignancy in adults. Metastasis is seen in 34% of patients within 10 years after diagnosis, and after detection of metastases, the 2-year survival rate is only 8%.[3] Metastasis in UM occurs purely hematogenous and the liver is involved in 95% of patients. Median survival in these cases is 4 to 6 months, whereas the median survival in cases without liver metastases is 19 to 28 months.[4]
Unfortunately, treatment options for metastatic CM and UM are limited. However, new targeted treatment options include therapies that target the mitogen-activated protein kinase (MAPK) signalling pathway. The MAPK pathway plays a crucial role in intracellular signal transduction and induces transcription of genes, encoding several cellular processes, such as growth, differentiation, migration, inflammation, angiogenesis, and cell death.[5, 6] The MAPK pathway is regulated by several growth factors, including fibroblast growth factor receptors, which are present on the cell surface. The MAPK cascade can be activated by the rat sarcoma (RAS) small guanosine triphosphatase (GTPase), which subsequently activates rapidly accelerated fibrosarcoma (RAF) kinase, enabling the phosphorylation and activation of mitogen-activated protein kinase kinase (MEK) and, finally, extracellular signal-regulated kinase.[7]

Patients with CM or UM can be subdivided on the basis of underlying genetic mutations in the melanoma cells. In 52-86% of UM patients and in more than 66% of CM patients, uncontrolled activation and aberrant signalling of the MAPK pathway can be detected.[8] In CM, *RAS* and *BRAF* gene mutations may cause activation of the MAPK pathway.[9] In UM, mutually exclusive mutations in *GNAQ* and *GNA11* can often be found. These mutations cause a permanently-activated GTP-bound state, leading to the activation of protein kinase C and consequently the MAPK pathway.[8, 9]

Several targeted therapies such as the BRAF-inhibitors vemurafenib and dabrafenib, the MEK inhibitors trametinib, selumetinib, and binimetinib (MEK162), and combinations of BRAF and MEK inhibitors have emerged as novel therapeutic options, especially for metastatic BRAF mutated CM patients.[10-12] In NRAS mutated CM patients MEK inhibition by MEK162 has shown clinical activity.[13]

Retinopathy, described as either a "central serous chorioretinopathy-like event" or an unspecified "chorioretinopathy" or a "bilateral, multifocal, mild and self-limiting retinopathy"

was described in association with binimetinib treatment in patients with advanced or metastatic CM, in 2-65% of patients.[12, 14]

Little is known about the clinical characteristics, outcome, and pathogenesis of MEK inhibitor-associated retinopathy. In this study, we analysed the clinical characteristics of retinopathy associated with MEK inhibition treatment for metastatic CM and UM. In addition, we studied possible pathogenetic mechanisms that may lead to such retinopathy, and we examined a correlation between the development of retinopathy and the tumor response to MEK inhibition treatment in metastatic CM.

MATERIALS AND METHODS

Patient characteristics

Twenty patients from an academic medical center (Radboud University Medical Center, Nijmegen, the Netherlands) and 10 patients from a comprehensive cancer center (The Netherlands Cancer Institute Antoni van Leeuwenhoek; in collaboration with the general hospital 'Onze Lieve Vrouwe Gasthuis', Amsterdam, the Netherlands) were included. These patients had a histologically confirmed, locally advanced and unresectable or metastatic CM, harbouring *BRAF* V600E or *NRAS* mutations, confirmed by a central laboratory.[12] From another academic medical center (Leiden University Medical Center, the Netherlands) 5 patients with liver biopsy-confirmed metastatic UM were included.

Local ethics committee approved the study. Each patient signed informed consent, and the studies were performed in accordance with the Declaration of Helsinki. The clinical trials were registered in the Clinical Trial Registration as numbers NCT01320085 (Nijmegen and Amsterdam) and NCT01801358 (Leiden). Within these trials, patients were included from July 2011 to April 2014.

Treatment

In patients with metastatic CM, the MEK inhibitor binimetinib was administered in a phase II trial setting. Patients received 45 milligrams of binimetinib orally twice a day, continuously for 28 days (defined as a 1 treatment cycle, for scheduling purposes). The patients with metastatic UM received a combination of binimetinib and the PKC-inhibitor sotrastaurin (AEB071) in a phase Ib/II trial setting. Metastatic UM patients started with continuous dosing of either binimetinib 30 or 45 milligrams twice daily for 28 days. They also received sotrastaurin twice daily. Patients continued with the study medication until either the development of disease progression or unacceptable toxicity.

Ophthalmic examinations

All patients received a complete ophthalmic examination, including Early Treatment of Diabetic Retinopathy Study (ETDRS) best-corrected visual acuity (BCVA), applanation tonometry, slit-lamp examination, and indirect ophthalmoscopy. When ETDRS BCVA was not available (in 10 patients), Snellen BCVA was determined and a previously-established conversion method was used to achieve ETDRS values.[15]

Dilation of pupils was achieved by topical administration of 1% tropicamide and 5% phenylephrine drops. Optical coherence tomography, using either spectral-domain optical coherence tomography (OCT (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany)) or the Cirrus OCT device (Carl Zeiss Meditec, Dublin, CA, USA), as well as digital color fundus photography (Topcon Corporation, Tokyo, Japan) were performed at baseline, at either day 15 of treatment cycle 1 (CM) or at 8 and day 22 of treatment cycle 1 (UM), day 2 of each treatment cycle from cycle 2 and at the end of treatment (within 14 days after discontinuation of treatment). In selected cases, enhanced-depth imaging technique (EDI-)OCT, fundus autofluorescence (FAF), and fluorescein angiography (FA) were performed with spectral-domain OCT. In addition, EOG and electroretinography (ERG) were performed according to the guidelines of the International Society for Clinical Electrophysiology of Vision (ISCEV) in selected cases, as well as color vision testing using the Desaturated Panel D-15 test.[16-18]

In 25 patients (20 CM and 5 UM patients) central visual analysis was performed (Humphrey Field Analyser, Carl Zeiss Meditec; 24-2 or 30-2 SITA Standard algorithm). One patient underwent microperimetry using the MAIA microperimeter (CenterVue, SpA, Padova, Italy), examining an area covering the central 15°.

Patients who reported new or worsened visual complaints received an additional ophthalmic examination at their earliest convenience. Additional ophthalmic examinations were performed as clinically indicated. When signs of ocular toxicity occurred, patients received weekly ophthalmologic examinations until the symptoms had resolved or stabilised after at least 6 months of follow-up.

In this study, serous retinopathy was defined as retinal lesions on ophthalmoscopy corresponding to a localised separation of the neuroretina and retinal pigment epithelium (RPE) on OCT.

Dose modification in patients with ophthalmologic symptoms

Adverse events were assessed according to the Common Terminology Criteria for Adverse Events version 4.03.[19] The classification of adverse events into 4 grades and dose changes after the occurrence of adverse events specified in the study protocols, can be found at http://aaojournal.org.

Analysis of autoantibodies in serum

Sera from patients were collected after the start of binimetinib treatment. Sera were examined for antiretinal and anti-RPE autoantibodies by Western blotting using human retinal and RPE proteins extracted from the retina or RPE, dissected from the human eye. Description of the analyses can be found in at http://aaojournal.org.

Safety and efficacy monitoring

During every visit to the outpatient clinic of the department of Medical Oncology, a report was completed. After clinical assessment, dose of study medication could be reduced, when either unacceptable signs of toxicity or disease progression developed. Every 2 treatment cycles, a CT scan was made for evaluation of disease. The main efficacy endpoints of the study were progression free survival and overall survival.

Statistical analysis

Both ETDRS BCVA at the time point of most pronounced subretinal fluid (SRF) on OCT and at final follow-up were compared to EDTRS BCVA at screening, using an independent t-test in IBM SPSS Statistics (version 20.0; SPSS Inc., Chicago, IL, USA). The same statistical test was used to assess the differences in progression free survival and overall survival in CM patients, in whom EOG abnormalities and SRF on OCT were and were not detected.

RESULTS

Patient characteristics

The 30 CM and 5 UM patients (22 male, 13 female) were 56.3 years old (median: 56; range, 32 - 77). The clinical characteristics of the CM and UM patients are summarised in Table 1.

Ophthalmic characteristics

During the period of administration of the study medication 6 CM patients (20%) and 2 UM patients (40%) reported visual complaints. Five patients developed complaints of blurred vision, which resolved within a few hours in 3 patients, and within 1 week in the other 2 patients. In 1 case, these symptoms recurred after 2 weeks. Dark flecks in the visual field were noticed in 2 patients after taking the study medication. One patient developed persistent binocular vertical diplopia 26 days after the start of study medication. Ophthalmic characteristics are summarised in Table 1.

SRF as seen on OCT developed in 22 binocular patients, 3 monocular patients, and 1 functionally monocular patient (a total of 48 eyes); the (converted) median ETDRS BCVA at screening was 86 letters (range, 72 - 97). At final follow-up, the median ETDRS BCVA recorded in 42 eyes was

87 letters (range, 65 - 99). The difference in ETDRS BCVA between final follow-up and baseline was not statistically significant (p=0.586).

Table 1. Clinical and ophthalmic characteristics of cutaneous melanoma (CM) and uveal melanoma (UM) patients, receiving either binimetinib as a single agent (in CM patients) or a combination of binimetinib and sotrastaurin (in UM patients)

	CUTANEOUS MELANOMA	UVEAL MELANOMA
Clinical characteristics		
Number of patients	30	5
Number of males	18/30 (60%)	4/5 (80%)
Number of females	12/30 (40%)	1/5 (20%)
Mean age (years)	55.7	59.8
Median age (years)	56	59
Ophthalmic characteristics		
Number of patients developing visual complaints	6/30 (20%)	2/5 (40%)
Ophthalmoscopy images available during ophthalmological follow-up (patients)	18	5
Transparent to yellowish fundus lesions (eyes)	27/35 (77%)	3/5 (60%)
Single foveal lesion (eyes)	2/27 (8%)	1/3 (33%)
Multifocal lesions (eyes)	25/27 (92%)	2/3 (67%)
Optical coherence tomography (OCT) available during ophthalmological follow-up (patients)	30	5
Subretinal fluid (SRF) on OCT (patients)	23/30 (77%)	3/5 (60%)
Foveal SRF (patients)	19/23 (82%)	3/3 (100%)
Only foveal SRF	2/19 (11%)	1/2 (50%)
Foveal and extrafoveal SRF	17/19 (89%)	2/2 (100%)
Extrafoveal SRF (patients)	21/23 (91%)	0/2 (0%)
Only extrafoveal SRF	4/23 (17%)	0/2 (0%)
Recurrence of SRF (patients)	3/23 (13%)	1/2 (33%)
Infrared reflectance imaging (IRR) available during ophthalmological follow-up (patients)	18	3
Extrafoveal lesions (eyes)	34/36 (94%)	3/3 (100%)
Small, focal extrafoveal lesions (eyes)	32/36 (89%)	2/3 (66%)
Larger, diffuse extrafoveal lesions (eyes)	19/36 (53%)	2/3 (66%)
Fundus autofluorescence (FAF) available during ophthalmological follow-up (patients)	13	3
Subfoveal fluid: increase central FAF, surrounded by a mildly decreased FAF (eyes)	16/26 (61%)	3/3 (100%)
Extrafoveal focal fluid: slight decrease central FAF, surrounded by an increased FAF (eyes)	15/26 (58%)	2/3 (66%)
Extrafoveal diffuse fluid: mildly decreased FAF (eyes)	10/26 (38%)	1/3 (33%)

Electro-oculography (EOG) available during ophthalmological follow-up (patients)	11	4
Abnormal Arden ratio (<1.65) (eyes)	14/22 (64%)	4/5 (80%)*
Subnormal Arden ratio (1.65-1.8) (eyes)	4/22 (18%)*	1/5 (20%)
Normal Arden ratio (>1.8) (eyes)	4/22 (18%)	0/5 (0%)
Full-field electro-retinography (ERG) available during ophthalmological follow-up (patients)	0	2
Decreased B-wave (eyes)		1/2 (50%)
No abnormalities (eyes)		1/2 (50%)
Multifocal electro-retinography (ERG) available during ophthalmological follow-up (patients)	3	0
Decreased responses (eyes)	2/6 (33%)	
No abnormalities (eyes)	4/6 (67%)	

Three of the patients in the study were (functionally) monocular: 2 UM patients due to previous treatment of their UM, and 1 CM patient due to ocular trauma in childhood. One patient had received proton beam irradiation for UM in the past, while another patient had undergone a primary enucleation for a large UM. ***** In 2 patients who received both 30 mg of binimetinib twice daily at the time of testing, and who did not show SRF at any time point during follow-up, we still noticed an abnormal Arden ratio in the only eye of a monocular UM patient and a subnormal ratio in both eyes of a CM patient.

Abbreviations: CM: cutaneous melanoma; EOG: electro-oculography; ERG: electro-retinography; FAF: fundus autofluorescence; IRR: infrared reflectance; OCT: optical coherence tomography; SRF: subretinal fluid; UM: uveal melanoma.

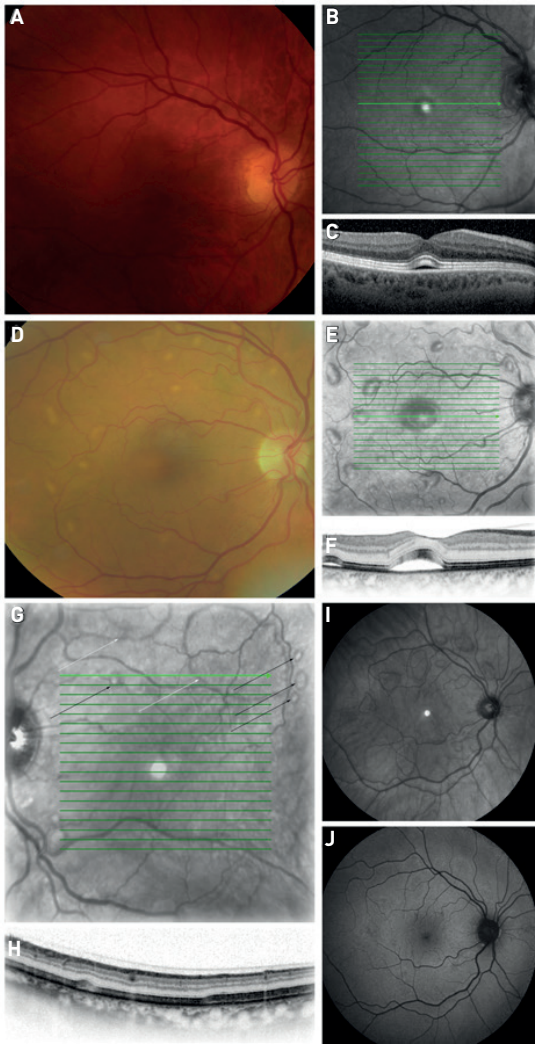

Figure 1. Spectrum of serous retinopathy associated with binimetinib treatment.

A-C. Fundus photograph of a 65-year-old patient with metastatic cutaneous melanoma who developed a central yellowish lesion in the fovea (A). On infrared reflectance (IRR) imaging a lesion of approximately 1/3 disc diameter was found, showing a hyperreflective center, surrounded by a hyporeflective zone (B). On optical coherence tomography (OCT) a dome-shaped accumulation of subretinal fluid (SRF) was detected (C).

D-F. Foveal and extrafoveal yellowish lesions with a vitelliform aspect developing 12 days after the start of binimetinib treatment in a 67-year-old patient with metastatic cutaneous melanoma (D). IRR imaging showed a foveal lesion of approximately 1 optic disc diameter, again showing a hyperreflective center, surrounded by a hyporeflective zone, and multiple smaller lesions scattered throughout the posterior pole with similar reflectance characteristics. These lesions were dome-shaped on OCT (F).

G-H. Multifocal serous retinopathy with smaller round to oval lesions as well as larger, more indistinct and irregular lesions shown on IRR imaging (G), occurring 16 days after the start of binimetinib in a

64-year-old metastatic cutaneous melanoma patient. Lesions were only present outside the fovea. On OCT (scanning plane depicted with light-green line in G) the smaller round/oval lesions corresponded to a dome-shaped accumulation of SRF (H – black arrow), and the larger irregular lesions corresponded to a shallow neuroretinal detachment (H – white arrow). The space between the small and shallow neuroretinal detachments and RPE was filled mainly with hyperreflective material on OCT, presumably corresponding to accumulation of shed photoreceptor outer segment debris.
I-J. Multiple lesions on IRR imaging (I) and the corresponding fundus autofluorescence image (J) in binimetinib-associated serous retinopathy. On fundus autofluorescence, the lesions corresponded to mildly decreased autofluorescence, in some lesions with a border of faintly increased autofluorescence.

Ophthalmoscopy revealed transparent to yellowish vitelliform fundus lesions consisting of shallow subretinal fluid that developed in 27/35 eyes from 18 CM patients (77%) and in 3/5 eyes from 5 UM patients (60%), from whom ophthalmoscopy images were available (Figure 1A, 1D, 2A, and 2D). Patients showed either single or multifocal lesions (Table 1). Residual mild granular RPE changes were seen after the disappearance of SRF, in 7 of 15 follow-up cases (47%).

SRF was detected in 23 of 30 CM patients (77%), and 3 of 5 UM patients (60%)(Figure 1A and 1D). SRF accumulation was bilateral in all CM patients. Also, the onset of SRF accumulation was virtually identical in both eyes of all 22 binocular patients. On OCT, lesions were detected between a few hours and 26 days (median: 14 days) after the start of binimetinib. In 22 of 26 CM/UM patients (85%) who showed SRF on OCT, the SRF affected the fovea.
In all 18 eyes of 10 patients out of a total of 40 eyes of 22 patients (45%) with subfoveal serous SRF on OCT, the aspect of lesions was dome-shaped (Figure 1C and 1F). On infrared reflectance (IRR) imaging, these lesions showed a hyperreflective center surrounded by a hyporeflective zone, with a median size of 0.50 optic disc diameter (mean 0.63; range, 0.33 - 1.5) (Figure 1B and 1E). In all 22 eyes of the other 12 patients (55%) with subfoveal SRF on OCT, smaller, round, and relatively well-demarcated foveal lesions were present, which corresponded to a shallow neuroretinal detachment on OCT with a hyperreflective subretinal accumulation (Figure 2I and 2N).

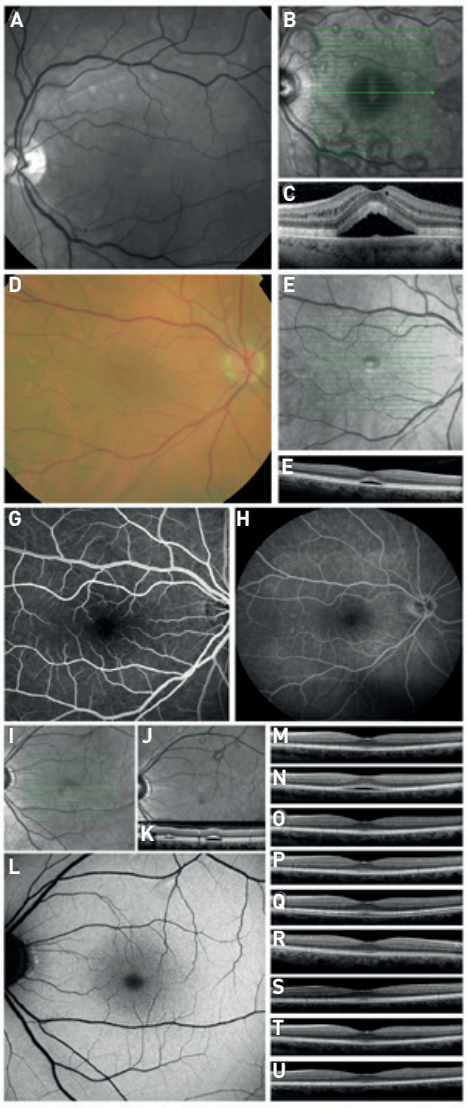

Figure 2. Multimodal imaging illustrating the clinical course of serous retinopathy associated with binimetinib treatment.

A-C. Multifocal, variably-sized serous neuroretinal detachments on red-free fundus photography (A) and infrared reflectance (IRR) imaging (B) in a 64-year-old binimetinib-treated patient with metastatic cutaneous melanoma (CM). Bilateral mild intraretinal fluid accumulation and a dome-shaped serous neuroretinal detachment with subretinal fluid (SRF) accumulation were seen on optical coherence tomography (OCT) (C). The intraretinal fluid appeared 20 days after the start of binimetinib and disappeared in 12 days. The binimetinib dose was tapered 7 days after appearance of the intraretinal fluid. Resolution

of the intraretinal fluid occurred faster than the resolution of the subfoveal SRF, which was still present at ophthalmological follow-up 11 weeks later.

D-H. A 65-year-old metastatic CM patient with yellowish foveal and small, round, and oval vitelliform extrafoveal lesions on fundus photography (D), corresponding to hyporeflective lesions with a moderately hyperreflective center on IRR imaging (E). On OCT (light-green line in E indicating scanning plane), the foveal lesion corresponded to a focal serous neuroretinal detachment (F). On fluorescein angiography (FA), lesions showed hardly any fluorescence changes in the early phase (G), whereas the smaller temporal lesions showed modest fluorescein staining in the late phase of the angiogram, while the foveal lesions remained mostly hypofluorescent (H). No FA abnormalities were present after resolution of serous SRF.

I-U. Evolution of binimetinib-associated serous retinopathy in a 45-year-old patient with metastatic CM. At the baseline examination before the start of binimetinib treatment the OCT scan showed an entirely normal macular structure (M). Within 1 day after the start of binimetinib treatment (45 milligrams twice daily), the patient developed bilateral small, round, and relatively well-demarcated foveal lesions without evidence of extrafoveal lesions (I). These lesions corresponded to a shallow neuroretinal detachment on OCT (N). Binimetinib was stopped to assess the effect of treatment discontinuation on the retinopathy. Five days after the discontinuation of binimetinib a resolution was seen on OCT (O). Study medication was restarted in a lower dose (30 milligrams twice daily), and a shallow unilateral serous neuroretinal detachment reoccurred between 3 (P) and 4 (Q) days after the restart. In addition small, round, and oval extrafoveal lesions (J), which were dome-shaped on OCT (K), gradually developed in these 5 days after restarting binimetinib. Fundus autofluorescence (FAF) of the foveal lesion showed no obvious abnormalities in the fovea and only minor FAF changes corresponding to the focal lesions superior in the macula (L). Despite the fact that binimetinib treatment was continued after this reoccurrence of lesions, the foveal SRF resolved 2 days after the restart of binimetinib (R), and this resolution of the lesion persisted 6 (S), 21 (T), and 43 days later (U). Resolution of the extrafoveal lesions occurred between the 6 and 21 days later.

The median ETDRS BCVA at the time point of most pronounced subfoveal SRF accumulation on OCT was 85 letters (range, 64 - 97). No statistically significant difference (p=0.572) was found between ETDRS BCVA at the time point of most pronounced SRF on OCT and at screening.

Subretinal fluid was also detected extrafoveally on OCT in 22 of 26 patients (85%). Four of 22 patients (18%) showed only extrafoveal lesions (Figure 1G and 1H). The median total number of extrafoveal lesions counted in the posterior pole of 21 CM and UM patients, based on a 30° IRR photograph was 7 (mean: 8.33; range, 0 - 27; 2 eyes without extrafoveal lesions) in 39 eyes. Complete resolution of SRF on OCT within 2 weeks after detection of SRF was observed in 7 of 23 patients (30%) who received ophthalmological follow-up. In 4 of these patients SRF disappeared spontaneously, in 1 patient the SRF disappeared after binimetinib dose reduction, and in 2 patients the study medication was stopped 3 and 6 days before complete resolution of SRF, respectively. After the first detection of SRF, 6 of 14 patients (43%) showed a complete resolution at 2 months of follow-up. In all these patients the resolution occurred spontaneously. Persistent subfoveal and/or extrafoveal SRF for at least 4 months after the first detection of SRF on OCT was seen in 2 of 5 patients who had sufficient ophthalmological follow-up. None of these patients had visual complaints. Mild intraretinal fluid accumulation was present on OCT in 1 CM (Figure 2A- 2C) and 1 UM patient. The intraretinal fluid had disappeared after 12 and 7 days after the first detection, respectively.

In 4 of 26 patients (15%), including 3 CM patients and 1 UM patient, recurrence of SRF was seen after initial resolution. SRF reappeared 9 days (range, 3 - 16) after the restart of treatment. Despite the continuation of medication this SRF showed spontaneous resolution in all patients. One patient with a recurrence had symptoms of blurred vision, which had also occurred at the first detection of SRF. In 3 patients with recurrence of SRF, the location of the SRF was almost similar to the previous episode. In 1 patient with initial bilateral subfoveal SRF, for which the binimetinib was temporarily stopped, unilateral extrafoveal SRF with subfoveal SRF occurred within 1 week after restarting binimetinib (Figure 2I-2U).

FAF imaging was performed during ophthalmological follow-up in 16 patients, 13 of whom had serous SRF, and abnormalities varied from a mild decrease of FAF to slightly increased FAF (Table 1).

One patient with serous foveal and extrafoveal lesions underwent multiple fluorescein angiography examinations during the course of SRF accumulation and after resolution of SRF. Lesions showed moderate hypofluorescence in the late phase of the angiogram (Figure 2D-2H).

Eight patients underwent an EOG before the start of the binimetinib therapy, which showed a normal Arden ratio (>1.8) in 14 eyes of 7 patients, while 1 patient had an abnormal Arden ratio (<1.65) in both eyes without any ophthalmological abnormalities, revealing a pathologically-decreased light rise. During treatment, a subsequent EOG showed a subnormal Arden ratio (1.65-1.8) in 2 eyes of 2 patients (25%) and an abnormal ratio in both eyes of 1 patient (25%) and a normal ratio in the remaining 4 eyes (50%), although 3 of these eyes had either previously or at the time of testing developed SRF. The normal Arden ratios were found in 1 eye of a patient who did not develop SRF on OCT, and in 3 eyes of 2 patients who did develop SRF. In 19 eyes of 11 cases, an EOG was only performed after the start of the binimetinib medication, and 16 of these eyes (84%) had developed SRF. Fifteen of these eyes (94%) with SRF showed an abnormal Arden ratio and 1 eye (6%) showed a subnormal Arden ratio. Color vision testing in 17 patients, including 9 patients with subfoveal SRF on OCT at the time of color vision testing, showed no significant changes in color vision during the time of follow-up. In 22 patients, including 12 patients with subfoveal SRF, central visual field analysis was within normal limits over several treatment cycles. In 1 monocular UM patient, microperimetry using the MAIA microperimeter was performed when SRF was already decreasing, and the patient had no visual complaints. Microperimetry revealed a mildly-decreased retinal sensitivity (Figure 3).

During the period of the administration of study medication, 1 UM and 1 CM patient developed small retinal hemorrhages without signs of retinal vascular occlusion. One CM patient developed papillary edema in both eyes.

Correlation between ophthalmological findings and oncological response to binimetinib treatment in CM patients

In the 30 CM patients the median progression free survival was 77 days (range, 20 - 898) and the median overall survival was 189.5 days (range, 24 - 919). The median overall survival was 193 days (range, 24 - 861) in 23 patients who developed SRF, as compared to 167 days (range, 63 - 919) in 7 patients without SRF. This finding was not statistically significant (p=0.595).

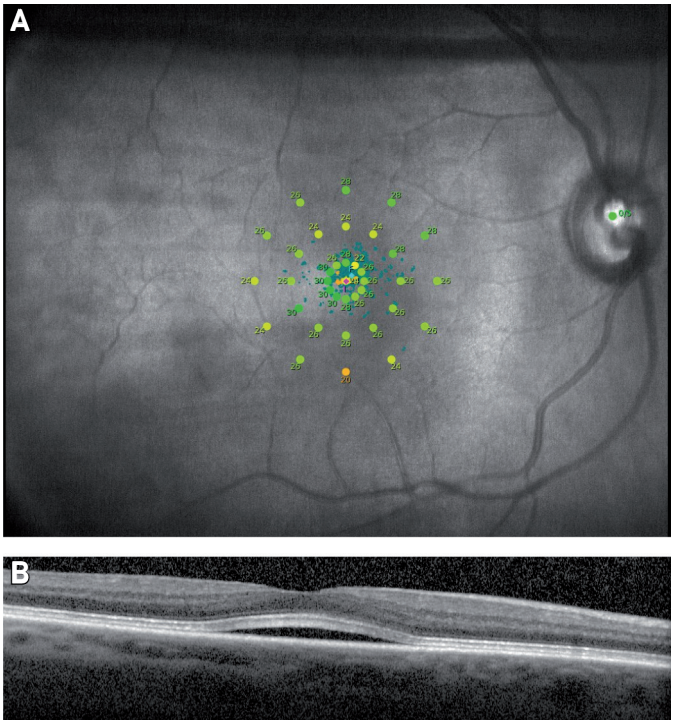

Figure 3. Functional findings in serous retinopathy associated with binimetinib treatment.
This 67-year-old asymptomatic patient with metastatic uveal melanoma (UM), who previously received proton beam radiation therapy in left eye and was currently treated with binimetinib and sotrastaurin, showed mildly decreased retinal sensitivity (**A**) on microperimetry in the area of neuroretinal detachment (**B**). At this moment the patient had no visual complaints. An electro-oculogram was made 1 week before, when the patient reported complaints of blurred vision, showing Arden ratios of 1.68 (in the right eye) and 1.28 (in the left eye). However, the low Arden ratio in the left eye may also be partly due to the previous proton beam radiation therapy.

Autoantibody analysis

Evidence of anti-RPE autoantibodies was found 3 CM and 3 UM patients, and antiretinal antibodies were detected in 3 CM and 2 UM patients. Multiple antiretinal autoantibodies and anti-RPE autoantibodies could be detected in a single patient. Anti-bestrophin antibodies were present in 1 CM and 2 UM patients (Table 2).

Table 2. Analysis of antiretinal, antiretinal pigment epithelium (RPE), and anti-bestrophin antibodies

Patient	Diagnosis	Antiretinal AAbs	Anti-RPE AAbs	Anti-recombinant bestrophin AAbs
58-year-old male	UM	30k, 36k (GAPDH), 40k (aldolase), 46k (enolase)	40k, 46k, 60k	++
55-year-old male	UM	30k, 32k,33k, 36k (GAPDH), 40k (aldolase), 92k	36k, 40k, 118k	+
64-year-old male	UM	negative	52k, 120k	+/-
49-year-old female	CM	136k, 189k	40k, 136k	+++
70-year-old male	CM	28k	37k, 70k	-
48-year-old male	CM	30k, 33k, 36k (GAPDH), 62k, 80k	62k, 70k	+/-

Analysis of antiretinal, antiretinal pigment epithelium (RPE), and anti-bestrophin antibodies in 3 uveal melanoma (UM; 1 female, 2 male) and 3 cutaneous melanoma (CM; 3 male) patients. Of these patients, 2 CM and 2 UM patients showed subretinal fluid on optical coherence tomography, and 2 CM and 3 UM patients showed abnormal Arden ratios on electro-oculography (EOG) at ophthalmological examination during the study. Antiretinal antibodies were detected in 3 CM and 2 UM patients. None of these patients complained of dark adaptation difficulties or night blindness, and full-field electro-retinography was performed in 3 of these patients. Anti-RPE antibodies were detected in all 6 patients. Anti-bestrophin antibodies were detected in 1 CM patient, and 2 UM patients. These anti-bestrophin autoantibodies were analysed, because they were detected previously in a patient with choroidal malignant melanoma in the left eye and a vitelliform macular lesion in the right eye with a markedly abnormal EOG.[24] In 2 patients in whom anti-bestrophin antibodies were detected and in 3 patients in whom antibodies were not detected, EOG did show an abnormal Arden ratio. In 1 patient with anti-bestrophin antibodies, EOG showed a normal Arden ratio in 1 eye and a subnormal Arden ratio in the other eye.

Abbreviations: Aabs: antibodies; CM: cutaneous melanoma; EOG: electro-oculography; RPE: retinal pigment epithelium; UM: uveal melanoma.

DISCUSSION

In this study, serous retinopathy developed in 77% of 30 CM and in 60% of 5 UM patients treated with the MEK inhibitor binimetinib, and affected the fovea in 85% of these cases. However, visual complaints developed in only 23% of patients. Most patients developed complaints of blurred vision or dark flecks in the visual field within a few hours to 3 weeks after starting the study medication, and these complaints generally disappeared within 1 week. In some patients, visual symptoms recurred within a week after restart of the binimetinib. However, the serous subretinal fluid collections in general seemed to have little visual impact: the difference between visual acuity at the time of most pronounced SRF and at screening was insignificant. At the end of study follow-up there was no significant change in visual acuity compared to baseline pre-treatment values.

In binocular patients, lesions were always bilateral and fairly symmetrical. On ophthalmoscopy, foveal and extrafoveal lesions could have a vitelliform aspect corresponding to dome-shaped serous neuroretinal detachments, but some patients had more extensive, irregular shallow neuroretinal detachments. Multifocal areas of SRF occurred frequently outside the fovea, which is an indication of a more extensive retinal/RPE dysfunction. We did not find a clear correlation between the presence or absence of serous retinopathy and the progression free or overall survival of the metastatic CM patients.

Previous studies have described the aforementioned retinal lesions associated with MEK inhibition treatment as central serous-like chorioretinopathy,[12] an eye condition resembling central serous chorioretinopathy,[14] bilateral subfoveal neurosensory retinal detachments,[20] and MEK inhibitor-associated retinopathy.[21] In our study, there was no evidence of choroidal abnormalities or leakage from focal RPE defects in the patients with serous SRF. Instead, we found that the most striking abnormality besides the usually transient serous retinopathy was the profoundly abnormal EOG. This indicates that the primary abnormality is a panretinal dysfunction of the RPE, which can lead to a serous retinopathy supposedly due to failure of the RPE pump. Previous studies and OCT, FA, and FAF findings as in our study did not show visible structural RPE changes or hot spots of leakage that would have been suggestive of central serous chorioretinopathy.[20, 21] Therefore, we suggest to describe the phenotype as *serous retinopathy*. Disappearance of SRF was observed in 43% of patients within 8 weeks after the start of binimetinib treatment, despite continuation of the study medication. A possible reason for this disappearance could be a gradual reduced sensitivity of the RPE to medication. However, the EOG remained abnormal despite SRF resolution, indicating prolonged RPE dysfunction despite SRF resolution.

The pathogenesis of serous retinopathy associated with MEK inhibitor treatment is unclear. Serum samples of all 3 CM and 3 UM patients tested for autoantibodies showed various antiretinal and anti-RPE antibodies. Data on the possible prevalence of these autoantibodies in individuals is scarce.[22] In 1 study, antiretinal autoantibodies were found in 16% of 58 healthy subjects, which was significantly lower compared to patients with macular telangiectasia type 2.[23] Autoantibodies against bestrophin were present in 3 of 6 patients. These autoantibodies were also reported earlier in a patient with choroidal malignant melanoma in the left eye and vitelliform lesions in the right eye.[24] It has been proposed that dysfunction of bestrophin results in abnormal fluid and ion transport by the RPE.[25] It is possible that treatment with binimetinib triggers (tumor) cells to stimulate the generation and/or release antibodies that could play a role in the pathogenesis of serous retinopathy. Moreover, an autoantibody attack against certain RPE epitopes could result in a compromised RPE ion homeostasis, resulting in an abnormal EOG (which reflects changes in the standing potential of the entire RPE), as well as a disturbed RPE pumping function of fluid from the subretinal space, resulting in serous SRF accumulation. A mechanism of anti-RPE antibodies against the RPE protein bestrophin, which is also affected in Best vitelliform macular dystrophy as a result of *BEST1* gene mutations, has been previously described in a metastatic choroidal melanoma patient with vitelliform paraneoplastic retinopathy who also had a pathologically reduced Arden ratio on the EOG.[24, 25] In a metastatic melanoma patient with acute exudative polymorphous vitelliform maculopathy, which may resemble cases with the more extensive multifocal serous retinopathy in our study, anti-RPE antibodies to peroxiredoxin 3 (26-kDa) were found in combination with an abnormal EOG. The autoantibodies became undetectable after the exudation had resolved.[26]

SRF was present on OCT in the 1 CM and 2 UM patients who showed the highest number of different antibodies. Multiple antibodies could have a higher pathogenic potential than a single antibody by enhancing pathology. However, only 1 of the 6 patients tested for autoantibodies had visual complaints. In patients with cancer-associated retinopathy, the presence of anti-α-enolase autoantibodies has been described previously.[27] These anti-α-enolase autoantibodies were found in only 1 of the patients in our study, who did not have visual complaints. In our study, neither a clear relationship between the presence of anti-bestrophin antibodies and EOG, nor a consistent pattern of specific autoantibodies in the tested patients was found. Therefore, the identification of a broad spectrum of antiretinal and anti-RPE antibodies does not necessarily mean that binimetinib-associated serous retinopathy is primarily caused by this autoantibody attack, despite the fact that the presence of these autoantibodies is an indication of autoimmune response.

Bearing this in mind, we hypothesize that direct toxicity of MEK inhibitor treatment to the RPE can also be an important factor in the pathogenesis of binimetinib-associated serous

retinopathy and RPE dysfunction. The potentially rapid onset of visual complaints and serous retinopathy would support direct RPE toxicity as an important contributing factor.

Serous retinopathy associated with MEK inhibitor treatment can be confused with several other clinical entities. Our study indicates that the clinical picture of the retinopathy can be discerned from central serous chorioretinopathy based on the occurrence of complaints within days after administration of the medication, the absence of associated RPE detachments, absence of hot spots on FA, and an abnormal EOG.[21] Melanoma-associated retinopathy occurs most frequently in patients with metastasised CM. Patients with melanoma-associated retinopathy characteristically have a sudden start of photopsia, and may experience night blindness, scotomas, and visual field loss, with rod function being primarily affected.[20, 28] In cancer-associated retinopathy both rod and cone function are affected. An association with autoantibodies against the retinal bipolar cells has been found both in melanoma-associated retinopathy and cancer-associated retinopathy,[29] as well as autoantibodies against a 22-kDa neuronal antigen, a 34-kDa protein in Müller cells, transducin-β, mitofilin, and titin in cases of melanoma-associated retinopathy.[30, 31] Paraneoplastic vitelliform retinopathy resembling Best vitelliform macular dystrophy, acute exudative polymorphous vitelliform maculopathy, and adult-onset foveomacular vitelliform macular dystrophy have also been described in patients with metastatic CM and UM patients.[32-37] In Best vitelliform macular dystrophy, a disease caused by autosomal dominantly inherited *BEST1* gene mutations, which lead to an aberrant bestrophin protein in the RPE, a vitelliform lesion similar to a central serous retinal detachment, is described.[25, 38] An abnormal EOG and a normal full-field ERG are typically found in Best vitelliform macular dystrophy. In autosomal recessive bestrophinopathy, also caused by *BEST1* gene mutations, the full-field ERG can also be abnormal in addition to the abnormal EOG.[39]

In summary, we show that the MEK inhibitor binimetinib is associated with a high incidence of serous retinopathy due to dysfunction of the RPE. Possible pathogenetic mechanisms include anti-RPE and antiretinal autoantibodies and/or direct RPE toxicity of MEK inhibitors, which is subject for further studies. Binimetinib-associated serous retinopathy was time-dependent and reversible despite continuation of study medication. Ophthalmological monitoring is warranted in patients using MEK inhibition treatment, but discontinuation of binimetinib administration generally does not seem necessary because of the relatively low visual impact and transient nature of the associated serous retinopathy. Future studies should assess whether other MEK inhibitors besides binimetinib are also associated with the development of a similar clinical picture.

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5.2

LOSS OF MAPK PATHWAY ACTIVATION IN POST-MITOTIC RETINAL CELLS AS MECHANISM IN MEK INHIBITION RELATED RETINOPATHY IN CANCER PATIENTS

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ABSTRACT

Recently, treatment with mitogen-activated protein kinase kinase (MEK) inhibitors has been shown to be an effective treatment option for metastatic melanoma. Treatment efficacy is dependent on inhibition of mitogen-activated protein kinase (MAPK) related melanoma proliferation. However, targeting of MEK can be accompanied by a time-dependent and reversible serous retinopathy of unknown origin. We analysed the molecular mechanism by which the MEK inhibitor binimetinib may lead to retinopathy, using neuroretina and cell models of retinal pigment epithelium (RPE). Binimetinib inhibited the MAPK pathway while discontinuation of treatment resulted in reactivation. However, cell proliferation was not inhibited correspondingly during binimetinib treatment of ARPE19 cells. Remarkably, post-mitotic neuroretinal tissue displayed a strong MAPK activation that was lost after binimetinib treatment. We propose that binimetinib-associated retinopathy is correlated with inhibition of the MAPK pathway in multiple retinal components. Retinal cells are able to regain the activation after binimetinib treatment, mimicking the reversibility of the retinopathy. Since most retinal cells are non-regenerating, other mechanisms than stimulation of proliferation must be involved.

INTRODUCTION

Inhibitors of mitogen-activated protein kinase (MEK), such as binimetinib, have proven to be an effective treatment for patients with metastatic melanoma.[1] However, serous retinopathy is a common complication of binimetinib.[1-4] Binimetinib-associated serous retinopathy develops in up to 77% of metastatic cutaneous melanoma patients and in 60% of metastatic uveal melanoma patients.[2] Although the central retina (macula) was affected in most patients, only 22% of all patients in our previous clinical study developed visual complaints. Dose reduction or discontinuation of the treatment with binimetinib led to the disappearance of complaints and subretinal fluid (SRF) in most patients. The symptoms recurred in most cases after restarting the treatment (Figure 1A). However, despite continuation of administration of binimetinib in the other patients, resolution of both complaints and lesions occurred.[2] Electro-oculography, which is an indirect measurement of RPE cell function, was found to be abnormal in virtually all cases over a prolonged period. This indicates that binimetinib may induce persistent panretinal RPE cell dysfunction, despite resolution of the SRF.[2, 3]

Other MEK inhibitors such as trametinib, cobimetinib, and RO5126766 can also cause a similar retinopathy. This suggests that the development of retinopathy is not restricted to binimetinib, but related to the class of MEK inhibitors.[4-8] This retinopathy is thereby most likely to be an on-target effect of MEK inhibition and may reflect the treatment efficacy. However, no relationship between occurrence of serous retinopathy and both overall survival and progression-free survival of cutaneous melanoma patients has been found.[2] Despite the fact that activation of the mitogen-activated protein kinase kinase (MAPK) pathway has only been observed in pathologic RPE, binimetinib-associated serous retinopathy occurs in eyes without a history of other ophthalmological diseases.[1] A possible role for the MAPK pathway in the neuroretina has not been described yet. A better understanding of the underlying molecular mechanisms of the treatment enables the improvement of risk estimation of therapeutic effect in non-pathologic tissues. Therefore, we set out to analyse MAPK activation in RPE and neuroretina as a possible mechanism by which binimetinib causes serous retinopathy.

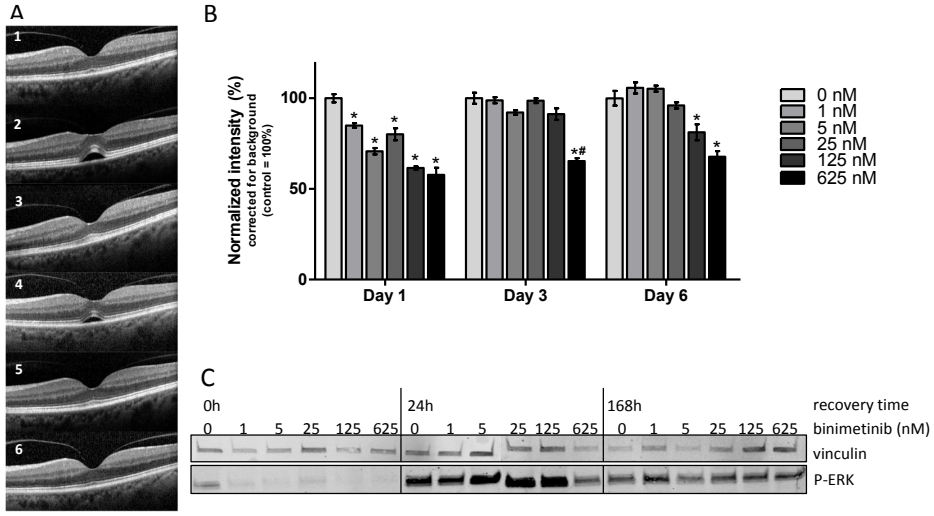

Figure 1. Illustration of the reversibility of binimetinib-associated serous retinopathy

A. Ophthalmic imaging of the retina during binimetinib treatment of a 56-year-old female with metastatic cutaneous melanoma. At initial screening the patient had a normal overall arrangement of the retinal layers (1). Eleven days after start of treatment an accumulation of subretinal fluid (SRF) had developed (2). Besides seeing dark flecks, the patient did not have ocular complaints. Thirty days after treatment started, SRF had disappeared despite continuation of medication (3). After discontinuation and a restart of medication a recurrence of SRF occurred (4), and (permanent) disappearance of SRF could be detected upon discontinuation of treatment due to a bad physical condition of the patient (5, 6).

B. Mild growth inhibition of ARPE19 cells upon increasing concentrations of binimetinib. Bars represent mean with standard error of the mean, control (0 nM) was set to 100%, and the intensities were corrected for background. *p<0.05 compared to control (0 nM), and #p<0.05 compared to treatment with 125 nM.

C. Regain in ERK phosphorylation after 24 and 168 hours of recovery after treatment of ARPE19 cells with binimetinib. Both during treatment and recovery, vinculin expression was stable.

MATERIALS AND METHODS

Cell culture

ARPE19 cells were cultured in order to serve as a positive control, exhibiting MAPK activity.[9] Cells were cultured in DMEM/F-12 plus GlutaMax medium (Life Technologies, Carlsbad, CA, USA) supplemented with fetal calf serum (Greiner Bio-One, Kremsmünster, Austria), penicillin, streptomycin, and glutamine (all Life Technologies) at 37°C with 5% CO_2 at humidified conditions.

To obtain primary neuroretinal tissue, human tumor eyes were used to perform a separation of the neuroretinal layer and the choroid-RPE complex. A healthy part of an enucleated tumor eye was transferred to a CO_2-independent medium (Life Technologies) to harvest the neuroretinal layer. After harvesting, the neuroretina was divided into 4 pieces and transferred to a 0.4 µm Corning Transwell polycarbonate membrane insert (Sigma Aldrich, St. Louis, MO, USA), already prepared with a drop of CO_2-independent medium. Under each insert 2 ml neurobasal-A medium (Life Technologies) with penicillin, streptomycin, glutamine, and B27 supplement (Life Technologies) was added. Before starting the experiment, the tissue cultures were maintained at 37°C in 5% CO_2 at humidified conditions for at least 24 hours to allow the cells to recover from the dissection.[10]

Experiments

An in-cell-western assay was performed to analyse the effect of binimetinib (MW 441.23 g/mole) on cell proliferation of dividing ARPE19 cells. Besides the DMEM/F-12 plus GlutaMax medium with supplements as a control, the binimetinib dosages 1, 5, 25, 125, and 625 nM were tested. Following 24, 72, and 144 hours of treatment, the cells were fixed with 4% formaldehyde and DRAQ5 (Biostatus, Shepshed, UK) was used to stain the fixed cells. Intensity of DRAQ5 fluorescence was quantified with the Licor Odyssey infrared imaging system (LI-COR, Lincoln, NE, USA). In another experiment, ARPE19 cells were treated with binimetinib for 24 hours with the concentrations above. After treatment, binimetinib was replaced by the normal culture medium in order to allow the cells to recover for 24 or 168 hours.

After culturing the primary neuroretinal tissue, the medium was fortified with binimetinib, which was added to the wells under the inserts. The neuroretinal tissue was treated with 5, 25, and 125 nM binimetinib for 24 hours.

Following binimetinib treatment, protein of ARPE19 cells and of the pieces of primary neuroretina was isolated by using mammalian protein extraction reagent buffer supplemented with phosphatase and protease inhibitors (Life Technologies). Ten µg of protein was loaded on a mini-protean TGX gel (4-15%, Bio-Rad, Hercules, California) with 10 wells, followed by transfer to a PVDF (low-fluorescence) membrane using the trans-blot turbo system (Bio-Rad). The primary antibodies used included mouse-anti-phospho-p44/42 ERK antibody (Sigma Aldrich), rabbit-anti-p44/42 ERK antibody (Cell Signalling Technologies, Danvers, MA, USA), mouse-anti-vinculin antibody (Sigma Aldrich), and rabbit-anti-calbindin-D28K antibody (Cell Signalling Technologies). The secondary antibodies used were goat-anti-mouse 800 nm antibody and goat-anti-rabbit 680 nm antibody (LI-COR). The fluorescence intensity was measured using the Licor Odyssey infrared imaging system.

Statistical analysis

Statistical analysis of the results was performed in IBM SPSS Statistics (version 23.0; SPSS Inc., Chicago, IL, USA) using a non-parametric Mann-Whitney test to determine significance between different binimetinib concentrations. P-values below 0.05 were considered to be statistically significant.

RESULTS

Although no significant growth inhibition was observed (Figure 1B), ERK activation in ARPE19 cells decreased with increasing binimetinib concentrations (Figure 1C). Expression of total ERK was not affected by binimetinib treatment (data not shown), but a complete loss of ERK activation was observed.

The analysis of the reversible nature of the binimetinib-associated serous retinopathy in the ARPE19 cell line showed a decrease in ERK activity after 24 hours of binimetinib treatment with increasing concentrations (Figure 1C). Both 24 and 168 hours after discontinuation of binimetinib treatment, an increase in ERK activity was found, compared to immediately after the cessation of binimetinib treatment. The increase in ERK activity was similar for all concentrations of binimetinib (Figure 1C). This experiment showed the ability of ARPE19 to recover from binimetinib treatment within 24 hours.

Primary neuroretinal tissue, unexpectedly, displayed activated ERK (Figure 2A). A reduction in ERK activity was observed in all 7 tested neuroretinas following binimetinib treatment with increasing concentrations (Figure 2A and B). Binimetinib treatment at 25 and 125 nM significantly decreased ERK activity ($p=0.004$ and $p<0.001$, respectively). Calbindin antibody was used to validate the presence of neuroretinal tissue in all 7 tested samples by staining the ganglion cell layer, the inner nuclear layer, and the outer nuclear layer. Calbindin and total ERK expression were not affected by binimetinib treatment (Figure 2A and B).

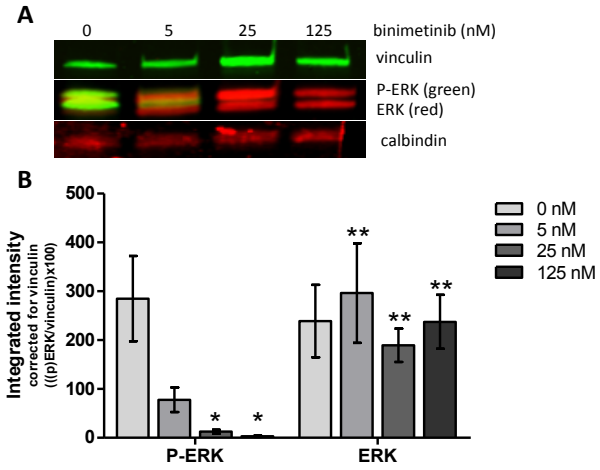

Figure 2. ERK activation in non-regenerative primary neuroretinal tissue

A. Binimetinib reduced ERK activity (P-ERK) in primary neuroretinal tissue. MEK inhibition had no effect on the expression of calbindin, vinculin, and total ERK.

B. In all 7 neuroretinas, a reduction in ERK activity was observed after treatment with binimetinib. The differences in activity after treatment with binimetinib concentrations of 25 and 125 nM, when comparing to 0 nM, were significant ($p=0.004$ and $p<0.001$, respectively). Between the binimetinib concentrations, no significant difference was seen in total ERK expression. The integrated intensities of both ERK and total ERK were corrected for vinculin (([p]ERK/vinculin)*100). Bars represent mean with standard error of the mean. *$p<0.05$ compared to control (0 nM), and **$p>0.05$ compared to control (0 nM).

DISCUSSION

Based on the results of this study, we propose that the binimetinib-associated serous retinopathy represents a class effect of MEK inhibitors and that it therefore is most likely to be an on-target side effect. This leads to the challenging hypothesis that MAPK activation is playing a role in the retina, an essentially non-regenerating tissue.

We analysed MAPK activation in a RPE cell line model and in primary neuroretina to investigate involvement of MAPK inhibition in serous retinopathy. We revealed ERK activation in both ARPE19 and primary neuroretina. ERK activation in ARPE19 cells has been observed before,[11] and gene expression analysis of primary RPE cells has suggested a broad functional role for ERK.[12] ERK activation in neuroretina has not previously been described. Binimetinib treatment inhibits ERK in both the neuroretina and the RPE cell model. This potentially involves MAPK inhibition in the development of MEK-associated serous retinopathy. During recovery from the binimetinib treatment, ERK activation increased in the RPE model, at all concentrations. The

in vitro ability to recover from treatment mirrors the disappearance of the serous retinopathy after treatment discontinuation. Upon resuming binimetinib treatment, serous retinopathy and visual symptoms often recurred. However, in some patients resolution of SRF occurred spontaneously despite continued treatment (Figure 1A).[2] This time-dependent and self-limiting nature of the serous retinopathy suggests redundancy of ERK during prolonged MEK inhibitor treatment.

Binimetinib-treated patients display irreversible electro-oculography abnormalities, reflecting an impaired standing potential of the RPE and RPE dysfunction.[2, 3] Normally, the RPE prevents SRF accumulation by maintaining the outer blood-retinal barrier and by regulating ion channels.[13] Tight junctions between the RPE cell monolayer that enable the RPE to form the outer blood-retinal barrier are regulated by the MAPK pathway.[14] A factor that is involved in this process is the fluid transport channel aquaporin 1, that was specifically shown to be regulated by the MAPK pathway.[15] The prolonged RPE dysfunction together with serous SRF accumulation that is observed in binimetinib treated patients may be explained by these mechanisms.

The clinical phenotype may also originate from abnormalities induced in the neuroretina, as we observed a strong activation of ERK in primary neuroretinal tissue. ERK is a neuroprotective regulator in the neuroretina and could be involved in the maintenance of a normal neuroretina-RPE interaction.[16, 17] ERK activity may also play a role in maintenance of the inner blood-retinal barrier that lines the retinal vasculature, by regulating tight junctions similar to the RPE outer blood-retinal barrier. Only few cases that developed a retinal vein occlusion during MEK inhibitor treatment have been described.[2, 3, 5, 7, 18]

Treatment consisting of both BRAF and MEK inhibitors improves efficacy in treating metastatic melanoma as the addition of a MEK inhibitor neutralises the paradoxical MAPK pathway activation that occurs due to BRAF inhibition.[6] For that reason, combination treatment reduces the risk of tumorigenesis in other tissues, such as the development of squamous cell carcinoma.[19] However, the occurrence of a treatment-associated serous retinopathy increased with this combination, supporting the notion that this adverse event is most likely to be an on-target treatment effect of MAPK targeted drugs.[20]

In conclusion, MEK inhibitors such as binimetinib can cause a rapid-onset serous retinopathy in patients with metastatic melanoma, which is at least partially reversible. In monocular uveal melanoma patients the functional visual impact may be clinically the most relevant. It is essential to unravel the (molecular) origin of these adverse events to be able to prevent such events, for instance by means of a dose reduction in symptomatic patients.

Our findings show that the retinopathy may be caused by reversible suppression of ERK activity in neuroretina and RPE, which could lead to disturbances in the neuroretina-RPE interaction and to the occurrence of SRF. The strong activity of ERK in primary neuroretinal tissue indicates that ERK has other functions besides regulation of cell proliferation, as neuroretina represents post-mitotic neural tissue. Inhibition of retinal ERK activity appears to be a key component in the pathogenesis of MEK-associated serous retinopathy.

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5.3

PIMASERTIB-ASSOCIATED OPHTHALMOLOGICAL ADVERSE EVENTS

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ABSTRACT

Purpose: To analyse ophthalmological adverse events associated with mitogen-activated protein kinase kinase (MEK) inhibition with pimasertib treatment for metastatic cutaneous melanoma (CM).

Methods: In this prospective observational, cohort-based, cross-sectional study, 8 patients treated with the MEK inhibitor pimasertib received a complete ophthalmic examination. This included Early Treatment of Diabetic Retinopathy Study best-corrected visual acuity, visual field testing, color vision testing, slit-lamp examination, applanation tonometry, indirect ophthalmoscopy, digital color fundus photography, and optical coherence tomography (OCT). In selected cases fluorescein angiography was performed.

Results: Serous subretinal fluid (SRF) developed in all patients, within a time frame of 9 to 27 days after the start of treatment. The fovea was involved in 6/8 patients (75%). None of the patients with foveal SRF (excluding a patient who developed a bilateral retinal vein occlusion (RVO)) experienced visual symptoms. SRF decreased or resolved in all patients, despite continuation of study medication in 6/8 patients (75%). Complaints in the CM patient (13%) consisted of experiencing a dark fleck in the inferior part of the visual field of the right eye 1 week after the start of treatment, due to an RVO. Subsequent intravitreal bevacizumab treatment resulted in functional and anatomical improvement.

Conclusions: Patients with metastatic CM who are treated with the MEK inhibitor pimasertib are at high risk of development of ocular adverse events including serous retinopathy and possibly RVO, stressing the need of adequate ophthalmological follow-up including OCT during administration of pimasertib, despite the fact that SRF generally does not lead to ophthalmological complaints.

INTRODUCTION

Spectral-domain optical coherence tomography (OCT) is a sensitive means to detect serous retinopathy, which is defined as retinal lesions on ophthalmoscopy corresponding to a localised separation of the neuroretina and retinal pigment epithelium (RPE).[1] It may occur as an independent entity, but may also be associated with a broad spectrum of other diseases or may follow the use of certain drugs.[2] We and others recently described that serous retinopathy is seen in patients with metastatic melanoma during treatment with mitogen-activated protein kinase kinase (MEK) inhibitors such as binimetinib,[1,3] cobimetinib,[4] trametinib,[5] and RO5126766.[6] Luckily, in many cases vision is not affected by the treatment, but serious problems including retinal vein occlusion (RVO) may occur.[7,8]

As treatment options for metastatic malignant cutaneous melanoma (CM) are scarce, much effort is being put into the development of possible new treatments for this patient group. One of the target pathways for treatment is the mitogen-activated protein kinase (MAPK) signalling (Ras - Raf - MEK - extracellular signal-regulated kinase) pathway. A broad spectrum of cellular processes is coordinated by this pathway. Activation of the MAPK pathway influences processes such as cell differentiation and metabolism, cell migration, and cell death.[9] Although visual symptoms may be absent or relatively mild in patients to whom MEK inhibition is prescribed, OCT imaging has shown that some of the MEK inhibitors induce retinal changes in a high percentage of patients.[1,4,10-12]

Preclinical studies have shown that the new MEK inhibitor pimasertib displays activity against *RAS* and *BRAF* mutated cell lines.[13,14] Moreover, induction of apoptosis in *BRAF* mutated human malignant melanoma cell lines has been described after the administration of a combination of pimasertib and the BRAF inhibitor PLX4032, whereas either drug alone did not.[15] In the first human trial IMP28062, activity against *NRAS* mutated tumors could be detected after prescription of pimasertib. In a recently published report of a patient with metastatic ovarian cancer bilateral multifocal retinal detachments were described 2 days after the start of pimasertib.[16] This patient developed complaints of blurred vision, and treatment was discontinued. Three days after stopping pimasertib treatment, ocular complaints and lesions had disappeared.[16] The occurrence of serous retinal detachments in patients, to whom pimasertib was prescribed, was not further specified in other studies.[17,18] In 1 of these studies, the occurrence of an RVO in a patient was also not discussed in detail.[18] No other ocular adverse events during the prescription of pimasertib have been reported so far.

We hypothesised that this drug may also lead to subretinal fluid (SRF) and wondered whether SRF would occur in only in a sensitive subset of patients, or that it might be a general phenomenon. As patients are in quite a good general condition when using this treatment, we

were able to perform a prospective study of patients using the new MEK inhibitor pimasertib, and included high resolution OCT imaging to examine the macular area in detail.

MATERIALS AND METHODS

Patient characteristics

Eight patients from an academic medical center (Erasmus University Medical Center, Rotterdam, the Netherlands) were included in this study. All patients were diagnosed with a measurable, histologically or cytologically confirmed, locally advanced or metastatic *NRAS* mutated malignant CM. Patients with a medical history of retinal degenerative disease, uveitis, or RVO were excluded. Local ethics committee approved the study. Each patient gave written informed consent, and the study was performed in accordance with the Declaration of Helsinki. The clinical trial was registered in the Clinical Trial Register with number NCT01693068. Patients were included in this trial from September 2013 to June 2014.

Treatment

All patients received pimasertib in a randomised phase II trial, in which the comparator arm was chemotherapy with dacarbazine (intravenous administration at dose of 1000 mg per square meter of body surface area, every 3 weeks). Patients received 60 mg of pimasertib orally twice a day, continuously for 21 days (defined as a 1 treatment cycle, for scheduling purposes). Within this trial 7 patients were primarily randomised to pimasertib, whereas 1 patient made a cross-over to treatment with pimasertib after having developed progressive disease upon chemotherapy with dacarbazine.

Ophthalmic examinations

All patients received complete ophthalmic examination, including Early Treatment of Diabetic Retinopathy Study (ETDRS) best-corrected visual acuity (BCVA) testing, visual field testing, color vision testing using the Desaturated Panel D-15 test, slit-lamp examination, intraocular pressure measurement, indirect ophthalmoscopy, digital color fundus photography (Topcon Corporation, Tokyo, Japan), and OCT using the spectral-domain OCT (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany), before start of the study. Part of these examinations was performed after dilation of pupils by topical administration of 1% tropicamide and 5% phenylephrine drops. Moreover, study protocol instructed the BCVA measurement at every evaluation visit: at the beginning of treatment cycle 2 and at the beginning of every subsequent odd treatment cycle. At these evaluation visits, slit-lamp examination and assessment of the posterior pole using indirect ophthalmoscopy were also prescribed to be executed, together with OCT scanning. When ETDRS BCVA testing was not available, Snellen

BCVA was determined and a previously-established conversion method was used to achieve ETDRS values.[19]

In patients with visual disturbances or abnormal retinal findings on ophthalmological assessments, fluorescein angiography was performed with the spectral-domain OCT. Treatment had to be interrupted when a serous retinal detachment with a decrease in ETDRS BCVA of ≥15 letters occurred. A serous retinal detachment was defined as a localised separation of the neuroretina and RPE on OCT, with an accumulation of SRF between these layers. Restart of treatment could only be scheduled after full resolution of the detachment and full recovery of vision within 2 weeks, after weekly follow-up visits. In case of the occurrence of an RVO the study medication had to be stopped, unless the event resolved within 2 weeks.

Safety and efficacy monitoring

After clinical assessment at the evaluation visits at the department of Oncology, the dose of study medication was reduced or medication was discontinued, when either unacceptable signs of toxicity or disease progression had developed. Subjects with documented tumor progression on the dacarbazine arm could choose to switch to receiving pimasertib.

Statistical analysis

Both ETDRS BCVA at the time point of most pronounced SRF on OCT and at final follow-up were compared to ETDRS BCVA at initial screening, using an independent t-test in IBM SPSS Statistics, version 23.0 (IBM Corp., Armonk, NY, USA).

RESULTS

The 8 CM patients (4 male, 4 female) had a mean age of 62.9 years (median: 64; range, 56 - 67). The clinical patient characteristics are summarised in Table 1.

During this study, 1 patient (13%) developed visual complaints, consisting of experiencing a dark fleck in the inferior part of the visual field of the right eye. These complaints started 1 week after the restart of pimasertib treatment, after a previous discontinuation because of a reversible decrease in left ventricular ejection fraction. In the right eye, ETDRS BCVA had dropped from 95 to 67 letters, while ETDRS BCVA of the left eye had not changed at the time of visual complaints. Indirect ophthalmoscopy and fluorescein angiography led to the diagnosis of a hemi-RVO of the superior temporal vein of the right eye and an asymptomatic mild hemi-RVO of the inferior temporal retinal vein of the left eye (Figure 1A-L).

Table 1. Characteristics of the cutaneous melanoma patients

Clinical characteristics	
Number of patients	8
Number of males	4/8 (50%)
Number of females	4/8 (50%)
Mean age (years)	62.9
Median age (years)	64
Ophthalmic characteristics	
Number of patients developing visual complaints	1/8 (13%)
Ophthalmoscopy images available during ophthalmological follow-up (patients)	8
Transparent to yellowish fundus lesions (eyes)	16/16 (100%)
Single foveal lesion (eyes)	0
Multifocal lesions (eyes)	16/16 (100%)
OCT imaging available during ophthalmological follow-up (patients)	8
SRF on OCT (eyes)	16/16 (100%)
Foveal SRF (eyes)	12/16 (75%)
- Only foveal SRF	0/12
- Foveal and extrafoveal SRF	12/12
Extrafoveal SRF (eyes)	16/16 (100%)
- Only extrafoveal SRF	4/16
- Foveal and extrafoveal SRF	12/16
Infrared reflectance imaging available during ophthalmological follow-up (patients)	8
Extrafoveal lesions (eyes)	16/16 (100%)
Mean number of extrafoveal lesions	5
Median number of extrafoveal lesions	6

Abbreviations: OCT: optical coherence tomography; SRF: subretinal fluid.

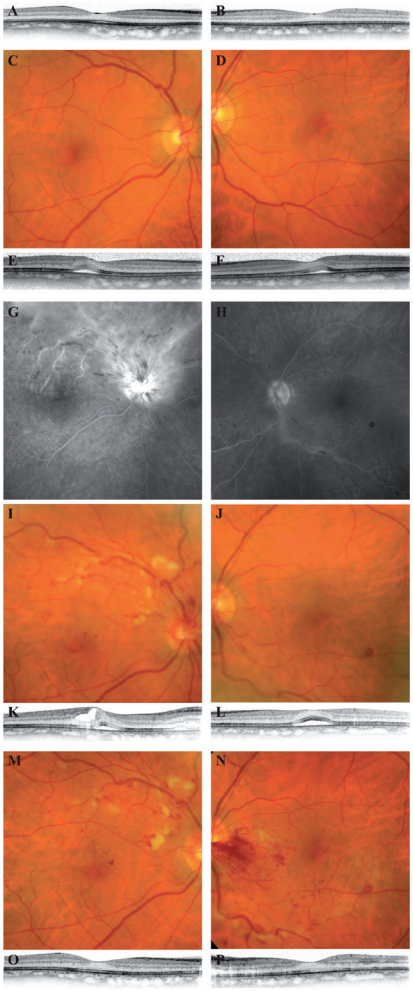

Figure 1. Bilateral retinal vein occlusion associated with pimasertib treatment
A-B, Optical coherence tomography (OCT) scan of the right (A) and left eye (B) of a 60-year-old male patient revealed a normal macular structure at baseline examination. **C-D**, Fundus photography of the right (C) and left eye (D), 41 days after the start of pimasertib treatment, revealed bilateral foveal and extrafoveal yellowish lesions. **E-F**, Foveal OCT scanning of both the right (E) and left eye (F) showed a serous neuroretinal detachment. Treatment was discontinued 22 days later. Nine days after discontinuation, treatment with 45 mg pimasertib twice daily could be restarted and patient attended a follow-up visit 12 days later. At that time, patient experienced a dark fleck in the inferior part of the visual field of the right eye. **G-J**, Fluorescein angiography and fundus photography revealed a hemi-retinal vein occlusion (RVO) in the right (G, I), and left eye (H, J). **K**, OCT of the right eye showed cystoid macular edema and both foveal and extrafoveal neuroretinal detachments were also present. **L**, OCT scanning of the left eye revealed both foveal and extrafoveal neuroretinal detachments. At this time, administration of pimasertib was discontinued. **M-N**, At follow-up 8 days after a single intravitreal injection of bevacizumab, fundus photography of the right (M) and left eye (N) revealed persistent cotton-wool spots and hemorrhages associated with the RVO in

the right eye, and an increase of hemorrhages and cotton-wool spots in the left eye. **O-P**, On an OCT scan during this visit, both cystoid macular edema and SRF had resolved in the right eye (O), and resolution of the neuroretinal detachment had occurred in the left eye (P).

Because of cystoid macular edema, a single intravitreal injection of bevacizumab in the right eye was given to this patient, after which ETDRS BCVA recovered to 89 letters at follow-up visit 8 days later (Figure 1M-P).Because of the need for palliative care, which this patient preferred to receive in another hospital, and because of the good functional and anatomical result of this single injection, no additional follow-up was scheduled.

OCT indicated that SRF developed in 16/16 eyes (100%). None of the patients (excluding the patient with RVO) experienced visual symptoms. Moreover, no measurable significant influence on visual acuity could be detected, as (converted) median BCVA ETDRS was 88 letters (range, 77 - 93) at screening, 87 letters (range, 66 - 98) at the moment of most prominent SRF on OCT, and 89 letters (range, 75 - 98) at the final follow-up visit. Differences in ETDRS BCVA were not statistically significant (p=0.58 (screening versus most prominent SRF), p=0.31 (most prominent SRF versus final follow-up visit), and p=0.51 (screening versus final follow-up visit)). Ophthalmoscopy revealed transparent to yellowish vitelliform lesions at the time that SRF was observed on OCT (Figure 1D, 1K-L, 2A), whereas either no abnormalities or mild RPE changes were observed after disappearance of SRF. The SRF was bilateral and fairly symmetrical (Figure 2K-T). These lesions were seen in all patients at the time of their first OCT after the start of the study medication, which was performed after 9 to 27 days (median: 20). Foveal SRF accumulation was present in 6/8 patients (75%). The center of these lesions was hyperreflective on infrared reflectance imaging, and the lesions were surrounded by a hyporeflective zone. On OCT, SRF was detected extrafoveally in all 8 patients (Figure 1K-L, 2D, 2I-J). The median total number of extrafoveal lesions in the posterior pole, based on a 30° infrared reflectance photograph, was 6 (mean: 5, range, 3 - 13) in the 16 eyes.

At last ophthalmological follow-up at 14 to 126 days (median: 81) after the first appearance of SRF, the SRF had disappeared in both eyes in 5/8 patients (63%). In the patient who received intravitreal bevacizumab, resolution of both cystoid macular edema and SRF had occurred. Administration of pimasertib had been discontinued in this patient. In the other 4 patients, SRF disappeared spontaneously while pimasertib was either still administered according to the original dose (2 patients) or dose of pimasertib was reduced (1 patient), or pimasertib was discontinued because of a decrease in left ventricular ejection fraction (1 patient). Three patients had persistent SRF, during treatment with 60, 60, and 30 mg of pimasertib twice a day, respectively. However, during follow-up the amount of SRF had decreased in all 3 patients. Color vision testing and visual field testing revealed no significant changes, despite macular SRF accumulation during pimasertib treatment. For all patients, information on the occurrence and evolution of SRF, and the possible relationship with orally administered dose of pimasertib has been depicted in Figure 3.

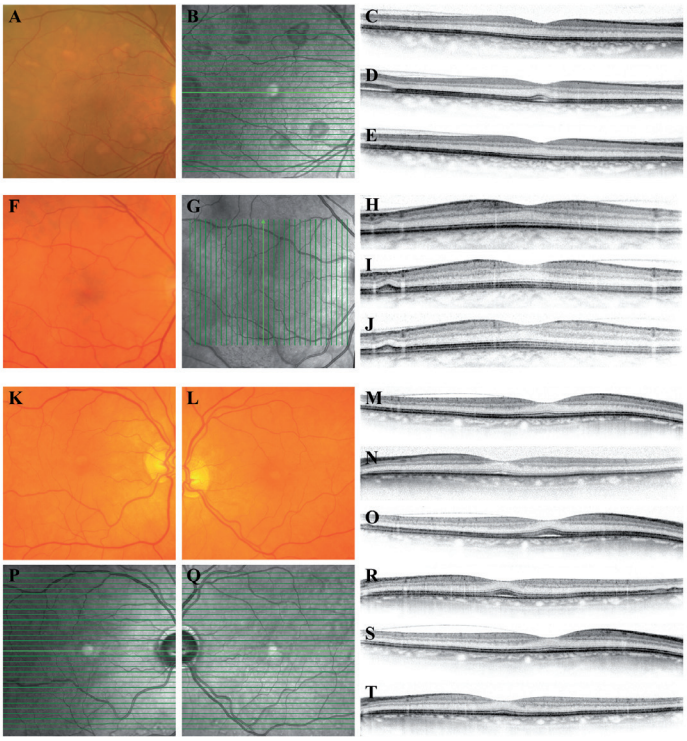

Figure 2. Spectrum of ophthalmological findings in pimasertib-associated serous retinopathy
A, Fundus photography of the right eye of a 67-year-old male patient who developed foveal and extrafoveal yellowish lesions, at 13 days after the start of pimasertib treatment. **B**, Infrared reflectance (IRR) imaging showed hyperreflective foveal and extrafoveal lesions, which were surrounded by a hyporeflective zone. **C**, Optical coherence tomography (OCT) had shown a normal macular structure at baseline. **D**, Thirteen days after the start of study treatment, both foveal and extrafoveal serous neuroretinal detachments on OCT had developed. **E**, At final follow-up, 63 days after the start of the prescription, resolution of lesions had occurred.
F, Fundus photography of the right eye of a 63-year-old female patient showed subtle extrafoveal transparent to yellowish lesions, 27 days after the start of pimasertib treatment. **G**, IRR imaging showed corresponding hyperreflective lesions. **H**, Before treatment, OCT had shown a normal macular structure. **I**, An extrafoveal neuroretinal detachment could be detected on OCT, 27 days after the beginning of pimasertib. **J**, This serous retinopathy was still present at final follow-up 14 days later.
K-L, In a 66-year-old patient, showing pimasertib-associated serous retinopathy, fundus photography revealed both foveal and extrafoveal yellowish lesions, 20 days after the start of pimasertib. **P-Q**, These lesions corresponded to hyperreflective lesions, surrounded by a hyporeflective zone on IRR imaging. **M-N**, At baseline examination, no abnormalities had been found on OCT. **O-R**, Twenty days after the start of treatment, an OCT scan showed a bilateral foveal neuroretinal detachment. **S-T**, Resolution of this detachment was detected, 62 days after discontinuation of treatment.

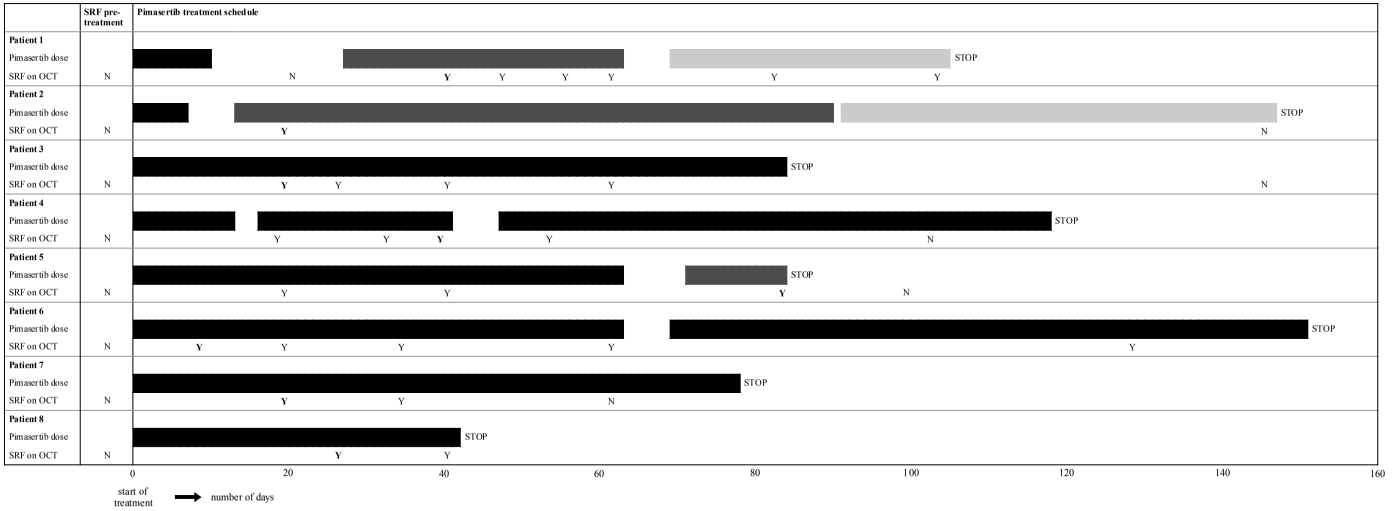

Figure 3. Occurrence and evolution of subretinal fluid over time, and possible relationship with orally administered dose of pimasertib

For all 8 patients included in this study, the prescribed dose of pimasertib and the occurrence of subretinal fluid (SRF) on optical coherence tomography (OCT) over time have been depicted. Colors in the 'pimasertib dose' rows correspond to the following doses: black = 60 mg twice daily, dark grey = 45 mg twice daily, light grey = 30 mg twice daily, white = no treatment. 'STOP' corresponds to end of treatment. 'Y' in the 'SRF on OCT' rows corresponds to presence of SRF on OCT at evaluation, with the maximum amount of SRF over time in bold. 'N' corresponds to the absence of SRF on OCT.

During the period of the administration of study medication in 1 patient who had previously been diagnosed with bilateral diabetic macular edema, (Figure 4A-D) edema had increased unilaterally at ophthalmological follow-up, 9 days after the start of study medication (Figure 4E-F). This patient also developed bilateral foveal SRF. No signs of other ophthalmological diseases were detected during follow-up. Eleven days later, SRF and edema had decreased spontaneously (Figure 4G-H). At final follow-up 106 days later, only minimal unilateral foveal SRF remained (Figure 4I-J). Ophthalmic characteristics of all patients are summarised in Table 1.

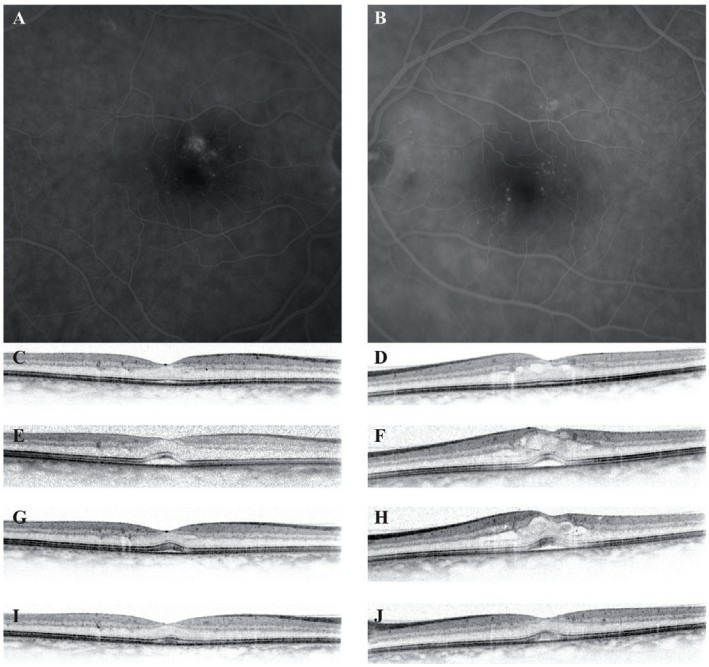

Figure 4. Worsening of diabetic macular edema and occurrence of serous retinopathy associated with pimasertib treatment

A-B, Fluorescein angiography in a 67-year-old male patient with metastatic cutaneous melanoma and diabetes mellitus type 2 before the start of pimasertib treatment, showed bilateral microaneurysms and mild fluorescein leakage. **C-D**, Optical coherence tomography (OCT) at baseline examination showed very mild diabetic macular edema (DME) without other abnormalities (C), and mild DME in the left eye (D). **E**, OCT scanning at 9 days after the start of pimasertib treatment showed a foveal serous neuroretinal detachment in the right eye. **F**, At this moment, the left eye showed both a foveal serous neuroretinal detachment and worsening of the DME on OCT. **G-H**, Eleven days later the amount of serous subretinal fluid had decreased spontaneously in the right (G) and the left eye (H). **I-J**, Despite the fact that this patient still used pimasertib in a dosage of 60 mg twice daily, the neuroretinal detachment had almost resolved in both eyes, together with the DME in the left eye, at final ophthalmological follow-up.

DISCUSSION

Spectral-domain OCT is a sensitive means to detect a typical serous retinopathy in patients treated with MEK inhibitors such as pimasertib. Despite these striking MEK inhibitor-associated retinal abnormalities in all 8 metastatic CM patients, all but 1 patient remained asymptomatic. This patient experienced central vision loss due to bilateral RVO, which responded to intravitreal bevacizumab treatment. Lesions occurred both foveally and extrafoveally in most of the 8 patients, indicating a more extensive dysfunction of the retina and/or RPE that extended beyond the fovea. Serous retinopathy had either disappeared or decreased at the last ophthalmological follow-up in all patients.

The exact pathogenesis of both serous retinopathy and RVO associated with MEK inhibitor treatment is as yet unclear. A time-dependent and reversible serous retinopathy, resembling the clinical picture we present in the current study, was previously described in association with several other MEK inhibitors such as cobimetinib,[4] trametinib,[5] and RO5126766.[6] We described this specific phenotype by a mildly symptomatic, time-dependent, and reversible accumulation of both foveal and extrafoveal serous SRF, with abnormalities on electro-oculography but without any evidence of choroidal abnormalities.[1] This serous retinopathy is most probably related to a class effect of this type of drugs, and thereby an on-target side effect of treatment,[20, 21] although pharmacological differences between several MEK inhibitors exist.[22] We have previously described prolonged abnormalities on electro-oculography in patients with transient binimetinib-associated serous retinopathy, indicating panretinal RPE dysfunction and dysfunction of the RPE pump.[1, 4] In addition, anti-RPE and antiretinal autoantibodies may play a role.[1] In a cell model of RPE and neuroretina, binimetinib administration resulted in inactivation of the MAPK pathway, and discontinuation of administration of the MEK inhibitor binimetinib led to reactivation, mimicking the mild and reversible retinopathy.[23] The occurrence of an RVO, during the prescription of MEK inhibition has also been described previously in up to 5% of patients.[7, 8, 18] MEK inhibitors could influence the vasculature of the retina, leading to both this serous retinopathy and RVO.[1, 24] The RVO that occurred in 1 of our patients could have been associated with the previous reversible decrease in left ventricular ejection fraction in this case, which had been detected 3 weeks before the diagnosis of RVO had been established. Moreover, after oral administration of the MEK inhibitor PD0325901 to rats, retinal gene expression suggested an increase in inflammatory and oxidative stress response, endothelial and blood-retinal barrier damage, and effects on blood coagulation, possibly characteristic for RVO.[25] Effects of oxidative stress and endothelial cell inflammation could lead to vascular hyperpermeability and damage to the blood-retinal barrier.[25] Moreover, an imbalance between thrombosis and fibrinolysis has previously been described to play an active role in the development of RVO.[26, 27]

MEK-associated serous retinopathy has to be discerned from several other retinal diseases. In contrast to central serous chorioretinopathy, in which lesions usually start unilaterally or bilateral asymmetrically, the MEK-associated serous retinopathy is not associated with RPE detachments on OCT, and there are no hot spots of trans-RPE subretinal leakage on fluorescein angiography.[4, 12] In cancer-associated retinopathy, melanoma-associated retinopathy, or non-neoplastic autoimmune retinopathy, symptoms may include a relatively rapid-onset photopsia, night blindness, scotomas, and progressive visual field loss.[28] Interestingly, this spectrum of autoimmune retinopathy is associated with antiretinal and/or anti-RPE antibodies, which we have also described in association with MEK inhibitor-associated serous retinopathy.[1] Hereditary retinal dystrophies such as autosomal dominant inherited Best vitelliform macular dystrophy and autosomal recessive bestrophinopathy usually have an earlier onset in combination with typical fundus lesions and a markedly abnormal electro-oculogram.[29]

In conclusion, we show that serous retinopathy associated with MEK inhibition treatment such as pimasertib is very common. This serous retinopathy generally does not lead to marked ophthalmological complaints,[1] and lesions may resolve despite continuous treatment. Therefore, discontinuation of treatment in most patients with metastatic cancer is not necessary. As ophthalmological complaints may occur in a noteworthy number of patients,[1] and as survival may increase in these patients because of the use of this new type of treatment for metastatic melanoma, performing OCT scanning before, during, and after MEK inhibition treatment may be advisable for monitoring and follow-up of fundus lesions. Another ophthalmological side effect of MEK inhibition is RVO, which can cause visual complaints that requires therapeutic intervention. Especially OCT follow-up is able to precisely monitor such abnormalities even in patients that remain asymptomatic. It is currently unclear if MEK inhibitor-associated serous retinopathy correlates with other clinical parameters of MEK inhibition treatment such as treatment response of metastases.[1] Further studies are needed to unravel the exact pathogenesis and clinical consequences of MEK inhibitor-associated ophthalmological adverse events.

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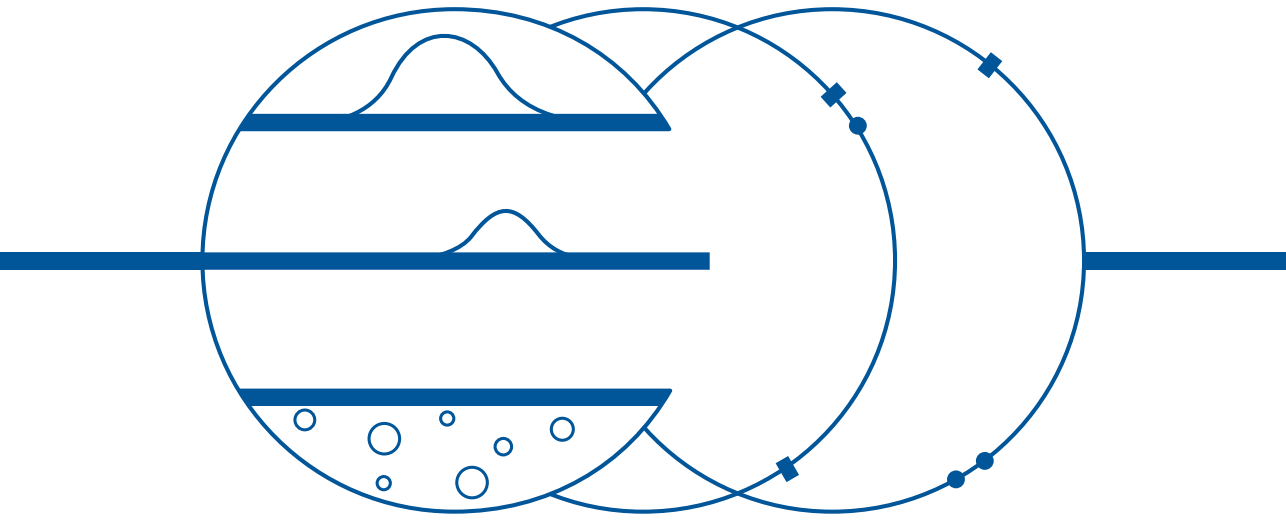
6

TREATMENT OF CENTRAL SEROUS CHORIORETINOPATHY



6.1

HALF-DOSE PHOTODYNAMIC THERAPY VERSUS HIGH-DENSITY SUBTHRESHOLD MICROPULSE LASER TREATMENT IN PATIENTS WITH CHRONIC CENTRAL SEROUS CHORIORETINOPATHY: THE PLACE TRIAL

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ABSTRACT

Purpose: To compare the anatomical and functional efficacy and safety of half-dose photodynamic therapy (PDT) versus high-density subthreshold micropulse laser (HSML) treatment in patients with chronic central serous chorioretinopathy.

Design: Open-label multicenter randomised controlled clinical trial.

Participants: Patients with chronic central serous chorioretinopathy, which had to be confirmed by both clinical characteristics and findings on multimodal imaging.

Materials and methods: Eligible patients were randomised in a 1:1 allocation ratio. Treatment was evaluated during a follow-up visit, and the same treatment was repeated in patients who still presented with subretinal fluid.

Main Outcome Measures: The primary endpoint was the complete disappearance of subretinal fluid at the first evaluation visit at 6 to 8 weeks after treatment. As a secondary outcome measure, we assessed this anatomical result at final evaluation visit at 7 to 8 months after treatment. Other secondary outcomes covered functional improvement and included change in best-corrected visual acuity (measured in Early Treatment of Diabetic Retinopathy study (ETDRS) letters), retinal sensitivity (measured using microperimetry), and vision-related quality of life using a validated questionnaire.

Results: Between November 2013 and September 2016, 179 patients were included: 89 patients were randomly assigned to half-dose PDT, and 90 to HSML treatment. At their first evaluation visit, subretinal fluid had resolved in 51.2% and 13.8% of patients, respectively ($p< 0.001$). At their final evaluation visit, a significantly higher percentage of PDT-treated patients presented with no subretinal fluid (67.2% versus 28.8%; $p<0.001$). Moreover, at first evaluation visit, the PDT-treated patients had a significantly higher increase in best-corrected visual acuity in ETDRS letters (+4.60 ± 6.62 versus +1.39 ± 8.99; $p=0.011$), and a significantly higher increase in retinal sensitivity in dB on microperimetry (+2.01 ± 3.04 versus +0.92 ± 3.65; $p=0.046$); however, the improvement in vision-related quality of life was similar (+2.87 ± 8.35 versus +2.56 ± 7.36, respectively; $p= 0.800$).

Conclusions: Half-dose PDT is superior to HSML for treating chronic central serous chorioretinopathy leading to a significantly higher proportion of patients with complete resolution of subretinal fluid and functional improvement. These findings can be used to develop a best-practice treatment guideline, and may alleviate current reimbursement restrictions for off-label verteporfin use for PDT treatment for this disease.

INTRODUCTION

Central serous chorioretinopathy (CSC) is a relatively common chorioretinal disease associated with a sudden loss of central vision. Although the cause of CSC is currently unknown, it affects primarily middle-aged men and is associated with corticosteroid use, stress, and certain genetic susceptibility factors.[1-3] In CSC, congestion, hyperpermeability, and thickening of the choroid lead to impaired function of the retinal pigment epithelium (RPE), which normally maintains the outer blood-retina barrier. The resulting loss of integrity in this barrier leads to the accumulation of subretinal fluid (SRF), which leads to marked central vision loss, primarily affecting the macula.[1] In acute CSC, sudden-onset loss of central vision occurs due to a single point of leakage in the RPE; such cases tend to resolve spontaneously within several months.[1] However, at least 15% of patients with CSC have chronic SRF accumulation, with associated persistent vision loss and more extensive pathology of the retina and choroid. In these cases of chronic central serous chorioretinopathy (cCSC), persistent SRF can lead to progressive and irreversible damage to photoreceptors, resulting in both a guarded visual prognosis and a decline in quality of life among these relatively young patients.[4-6] Although the efficacy of a variety of treatment modalities for cCSC has been evaluated retrospectively, no evidence-based consensus regarding the optimal clinical management of cCSC currently exists due to a lack of large prospective randomised controlled treatment trials.[1, 7] The 2 most commonly performed treatments for cCSC are photodynamic therapy (PDT) with verteporfin (Visudyne), originally developed to treat age-related macular degeneration,[8] and high-density subthreshold micropulse laser (HSML) treatment. Although both treatments have reported anatomical success rates (i.e. complete resolution of SRF) of 41-100%, and although both treatments have a favorable safety profile,[1, 7, 9-15] there is currently no consensus with respect to which intervention may be more efficacious. For example, in the United Kingdom the National Health Service has not approved either of these treatments for cCSC. Here, we report the results of the PLACE trial, the first prospective multicenter study of patients with cCSC in which we directly compared both safety and efficacy between half-dose PDT and HSML treatment.

MATERIALS AND METHODS

Study design

Half-dose Photodynamic therapy versus high-density subthreshold micropulse LAser treatment in patients with chronic CEntral serous chorioretinopathy (PLACE) trial was an investigator-initiated, open-label, multicenter, randomised controlled treatment trial conducted at 5 academic medical centers in 4 European countries. The study was done in accordance with the tenets of the Declaration of Helsinki, and all participating centers received approval from their

respective institutional review board and ethics committee (NCT01797861). The protocol has been published previously.[16]

Participants

In this trial, we enrolled both male and female patients 18 years of age or older with cCSC, based on both clinical characteristics and findings on multimodal imaging. On multimodal imaging, SRF that affected the fovea visible on spectral-domain optical coherence tomography (OCT) scan (Figure 1A), 1 or more regions of active leakage (hot spots) combined with RPE window defects visible on fluorescein angiography (Figure 1B), and hyperfluorescent changes typical of cCSC visible on indocyanine green angiography (ICGA; Figure 1C) were mandatory to be eligible for inclusion. In addition, SRF on OCT and/or subjective visual complaints related to cCSC had to be present for at least 6 weeks for inclusion in the study. SRF did not need to include the fovea at the first evaluation visit for the patient to be eligible for treatment, provided there was persistent SRF in the macula, which was interpreted as persistent active disease. Study eye exclusion criteria included previous treatment for active CSC in the study eye; evidence of any other diagnosis that could explain SRF or vision loss; best-corrected visual acuity (BCVA) of 20/200 or worse (Snellen equivalent); profound chorioretinal atrophy in the central macular area visible on ophthalmoscopy and OCT; myopia exceeding 6 dioptres; continuous and/or progressive vision loss and/or SRF (visible on OCT) for more than 18 months; absence of active leakage visible on fluorescein angiography; absence of hyperfluorescence on ICGA; presence of soft drusen in the study eye and/or other eye; and signs of choroidal neovascularisation visible on ophthalmoscopy and/or fluorescein angiography/ICGA. Moreover, CSC patients with intraretinal fluid on OCT were not included in the study, since this is often viewed upon as evidence of prolonged disease with more pronounced irreversible structural and functional changes, which may lead to a decreased response to treatment.[17] More importantly, it may be difficult in CSC cases with intraretinal fluid – especially without OCT angiography which was not available at the time of the start of the study – to definitely exclude neovascularisation. Non-ocular exclusion criteria included current topical or systemic treatment with corticosteroids; corticosteroid use within 3 months prior to the initial trial treatment; an anticipated start of corticosteroid treatment within the first 7 to 8 months after the start of the trial period; contraindications for PDT treatment (e.g. pregnancy, porphyria, or severely reduced liver function); and contraindications for fluorescein angiography or ICGA (e.g. known allergies, particularly to shellfish, previous reactions).

Eligible patients were identified and counselled in their respective participating hospitals. Written informed consent was obtained from each subject prior to participation.

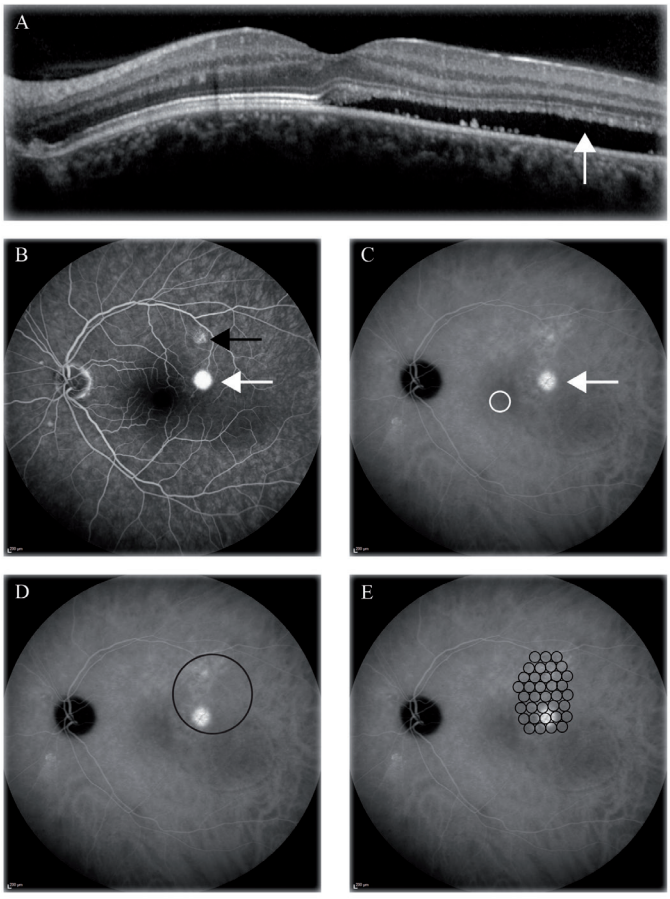

Figure 1. Example images of a 48-year-old male patient with chronic central serous chorioretinopathy, showing the areas that would be treated using half-dose photodynamic therapy and the areas that would be treated using high-density subthreshold micropulse laser

A. An optical coherence tomography scan shows subretinal fluid (arrow). **B**. Fluorescein angiography shows a hot spot of leakage (white arrow) as well as retinal pigment epithelium abnormalities (black arrow). **C**. Indocyanine green angiography shows hyperfluorescent changes in the choroid; the arrow indicates the most evident changes, which extend to more superiorly; the fovea is indicated by the white circle. **D**. The area that would be treated using half-dose photodynamic therapy is indicated by the black circle. **E**. The area that would be treated using high-density subthreshold micropulse laser treatment is indicated by the small black circles. Note that the area depicted for high-density subthreshold micropulse laser treatment is a schematic representation only; the actual non-overlapping adjacent spots relative to the fundus are smaller than depicted here.

Randomisation and masking

Patients were randomly assigned (at a 1:1 ratio) to receive either half-dose PDT or HSML treatment. Randomization was performed using a central computerised procedure using block randomization with alternating block sizes of 4, 5, and 6, with stratification on the including hospitals, and without minimization. We did not take baseline characteristics of our patients into account, before randomization, which can be considered to be standard for this type of study. The department for Health Evidence at the Radboud University Medical Center performed the randomization procedure. Because of the nature of the interventions, neither the clinical staff nor the patients could be blinded with respect to the treatment group.

Procedures

Each patient's demographic characteristics and medical history were documented at their baseline visit. An extensive ophthalmological examination was performed, including Early Treatment of Diabetic Retinopathy Study (ETDRS) BCVA, retinal sensitivity (measured using microperimetry), and vision-related quality of life (measured using the validated National Eye Institute Visual Function Questionnaire (NEI-VFQ25)).[17] Microperimetry was measured using 2 different devices, the MP1 (Nidek Technologies, Padova, Italy) and the Macular Integrity Assessment (CenterVue, Padova, Italy); a previously published conversion method was then applied to obtain values with the same dynamic range.[18] Fundus photography, OCT, fundus autofluorescence, fluorescein angiography, and ICGA imaging were performed by certified medical photographers. After eligibility was determined by a central reading center, patients were randomly assigned to the treatment groups and then received either half-dose PDT or HSML treatment within 3 weeks of randomization. For each treatment modality, the ocular area to be treated was determined by the central reading center based on the hyperfluorescent areas of leakage seen on ICGA, to avoid interobserver variability on the interpretation of the extent these choroidal abnormalities. This approach was used because ICGA-guided therapy targets the primary affected tissue (i.e. the choroid) and decreases the risk of undertreatment, as ICGA generally reveals more extensive abnormalities compared to fluorescein angiography (Figure 1). Care was taken to include the focal area(s) of leakage on fluorescein angiography in the treatment area. All diagnostic and therapeutic devices were maintained and serviced by certified personnel at the participating centers in accordance with the manufacturers' instructions.

Verteporfin was initially developed for PDT-based treatment of neovascular age-related macular degeneration; however, it is now commonly used as an off-label medication for treating cCSC.[7, 8] A safety-enhanced protocol, in which half the dosage of verteporfin is used, has been shown to induce the desired treatment outcome in a similar percentage as after performing PDT using full-dose verteporfin, but reducing the occurrence of adverse events (AEs).[11, 19] Patients in the half-dose PDT group received an intravenous infusion of 3 mg/m^2 verteporfin delivered over a period of 10 minutes. Exactly 15 minutes after the start of this

infusion, a contact lens was positioned on the eye, the treatment spot was centered using predefined criteria for ICGA-guided treatment, and PDT was applied using standard parameters (fluency: 50 J/cm^2; wavelength: 689 nm; and treatment duration: 83 seconds). Where necessary, the fovea was included in the area to be treated. In the HSML-treated group, several adjacent non-overlapping spots were applied during ICGA-guided treatment using an 810 nm diode laser, keeping a distance of 500 µm from the foveal center. To minimise the likelihood of undertreatment, a relatively high power of 1800 mW (which is considered to be within the upper range of subthreshold settings) was applied to a relatively large ICGA-guided treatment area.[20] In addition, the duty cycle was 5%, the frequency was 500 Hz, the exposure time was 0.2 seconds per spot, and the spot size was 125 µm. Because subthreshold treatment was desired, the power of the treatment was reduced in 300 mW increments if any retinal discoloration was visible after a 'test' treatment spot was applied outside the macular area, at the beginning of the procedure. All laser operators in the study underwent formal training and were approved by the chief investigator.

In cases in which SRF was still present within the macular area at evaluation 6 to 8 weeks after treatment (i.e. at the first evaluation visit), the same treatment was repeated, which is often considered to be part of standard care, and a second evaluation visit was scheduled 6 to 8 weeks after this second treatment. All patients underwent a final evaluation visit, 7 to 8 months after the first treatment. At each evaluation visit, the patients underwent complete ophthalmological examination and imaging as described above.

Outcomes

The primary outcome measure was the complete absence of SRF on OCT (anatomical success) at the first evaluation visit (i.e. 6 to 8 weeks after the first treatment). In addition, the following protocol-defined key secondary outcome measures in both the half-dose PDT group and the HSML treatment group were assessed: a complete absence of SRF on OCT at the final evaluation visit; the number of repeat (i.e. second) treatments needed in each treatment arm; and the change in ETDRS BCVA, retinal sensitivity on microperimetry, and vision-related quality of life measured using NEI-VFQ25, from baseline to both first and final evaluation visit.

Adverse events and serious adverse events (SAEs) were reported to the Data Safety Monitoring Board (DSMB), which had the option to terminate the study prematurely if deemed necessary.

Statistical analysis

This randomised controlled trial was designed to be a superiority study. We calculated that a sample size of 156 patients (78 in each group) would be required at the first evaluation visit in order to detect a difference of 22% in treatment success with a power of at least 80%; this calculation was based on previously published retrospective studies regarding PDT and HSML

treatment in cCSC, possible positive publication bias, our own empirical experience, and an interim analysis.[1, 7, 11, 12, 14]

Statistical analysis on the primary outcome measure was performed using a cross-table to assess the relative risk. The key secondary analyses 'complete absence of SRF on OCT at the final evaluation visit' and 'number of repeat treatments needed in each treatment arm' were also performed using a cross-table. Continuous secondary endpoints were analysed using an ANCOVA with baseline and treatment as factors. Categorical secondary variables were analysed using a chi-square test. Changes in the replies to the NEI-VFQ25 are reported using a scale ranging from 0 to 100, as described previously.[17] The PLACE trial was registered internationally with ClinicalTrials.gov (number NCT01797861) and was assigned the EudraCT number 2012-004555-36.

RESULTS

We initially screened a total of 309 patients for eligibility; after applying the inclusion and exclusion criteria, 179 patients (89 patients in the PDT treatment arm and 90 patients in the HSML treatment arm) were determined to be eligible and were enrolled between November 2013 and September 2016 (Figure 2). Eighty patients in each treatment arm received their respective treatment in accordance with the study protocol (Figure 2). Due to the presence of SRF at the first evaluation visit, 108 of the original 160 patients (67.5%) underwent repeat treatment. Six patients who would have required repeat treatment based on the protocol did not receive this second treatment (available at www.aaojournal.org), and 1 patient was excluded from the trial after the first evaluation visit due to corticosteroid use. Two patients did not attend the second evaluation visit, but did attend the final evaluation visit. Nineteen patients did not attend the final evaluation visit. In total, 67 and 66 patients in the half-dose PDT and HSML groups, respectively, were analysed after their final evaluation visit (Figure 2).

The baseline characteristics were balanced between the 2 groups (Table 1). Specifically, there was no significant difference between the half-dose PDT group and the HSML-treated group with respect to BCVA (76.9 ± 8.32 versus 75.9 ± 9.22 ETDRS letters, respectively; p=0.478), retinal sensitivity measured using microperimetry (20.5 ± 4.21 versus 20.3 ± 4.80 dB, respectively; p=0.755), or vision-related quality of life measured using the NEI-VFQ25 (81.2 ± 13.0 versus 83.1 ± 11.5 points, respectively; p=0.158).

At the first evaluation visit, a significantly higher percentage of patients in the PDT-treated group had a complete resolution of SRF (the primary outcome measure) compared to the HSML-treated group (51.2% versus 13.8%, respectively; p<0.001). After HSML treatment, the relative

risk of persistent SRF at the first evaluation visit following HSML treatment was 1.77 (95% confidence interval, 1.39 - 2.52) compared to receiving half-dose PDT. At the final evaluation visit, a significantly higher percentage of patients in the PDT group had a complete resolution of

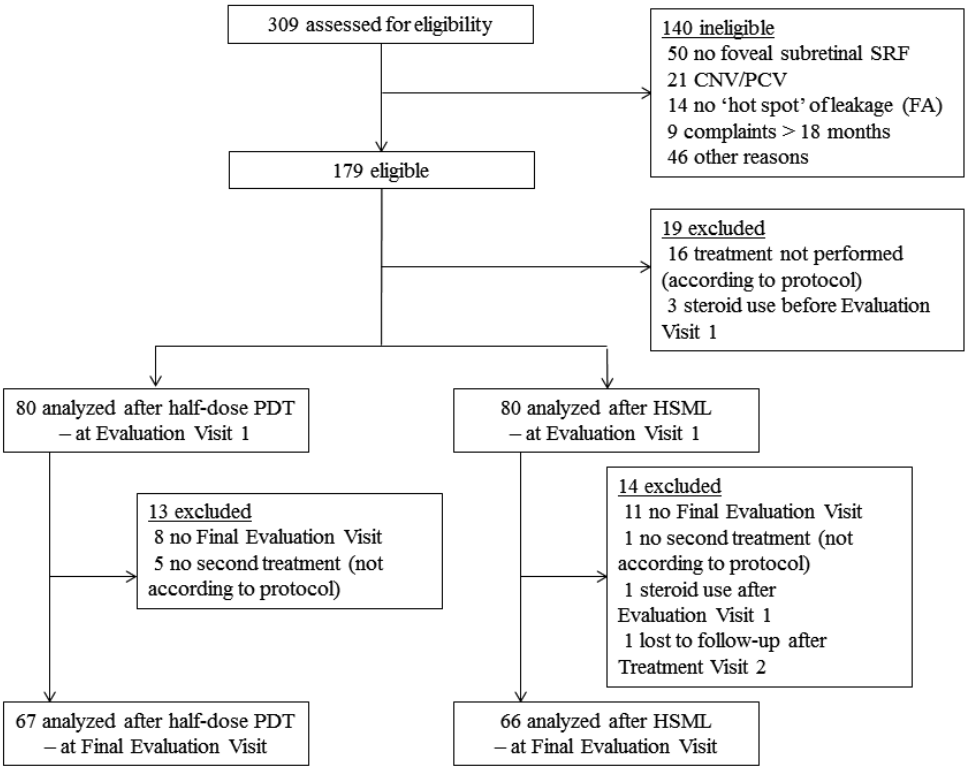

Figure 2. Trial profile for the primary outcome population

Abbreviations: CNV: choroidal neovascularisation; FA: fluorescein angiography; HSML: high-density subthreshold micropulse laser treatment; PCV: polypoidal choroidal vasculopathy; PDT: photodynamic therapy; SRF: subretinal fluid.

Table 1. Baseline characteristics of patients included in assessment of primary outcome measure

	PDT (n = 80)	HSML (n = 80)	P-value
Demographics			
Sex			0.072
female	20 (25%)	11 (13.8%)	
male	60 (75%)	69 (86.3%)	
Age (years)	48.9 ± 8.9	48.6 ± 8.3	0.819
Ethnicity			
Caucasian	71 (88.8%)	70 (87.5%)	0.807
other	9 (11.2%)	10 (12.5%)	
Clinical characteristics			
Duration of visual complaints (months)	6 (3.76-11)	6 (4-9.75)	0.796
Stress during month before visual complaints started measured with the Cohen stress questionnaire outcome on a scale from 0-40	14.7 ± 7.4*	14.8 ± 7.5	0.922
Best-corrected visual acuity (Early Treatment of Diabetic Retinopathy Study letters)	76.9 ± 8.32	75.9 ± 9.22	0.478
Retinal sensitivity on microperimetry (dB)	20.5 ± 4.21	20.3 ± 4.80	0.755
Composite score on vision-related quality of life measured with the NEI-VFQ25	81.2 ± 13.0	83.1 ± 11.5	0.158

Data is n (%), median (range), or mean (SD).

* Data was available for 79 patients.

Abbreviations: HSML: high-density subthreshold micropulse laser treatment; NEI-VFQ25: National Eye Institute Visual Function Questionnaire; PDT: half-dose photodynamic therapy.

SRF compared to the HSML-treated group (67.2% versus 28.8%, respectively; p<0.001), and the relative risk of persistent SRF at the final evaluation visit following HSML treatment was 2.17 (95% confidence interval, 1.49 - 3.16) compared to receiving half-dose PDT (Table 2).

Table 2. Treatment effect on primary and secondary outcome measures

Endpoints	PDT (n)	HSML (n)	P-value
Complete resolution of subretinal fluid			
at first evaluation visit (at 6-8 weeks after treatment)	51.2% (41/80)	13.8% (11/80)	<0.001
at final evaluation visit (at 7-8 months after treatment)	67.2% (45/67)	28.8% (19/66)	<0.001
Change in best-corrected visual acuity (Early Treatment of Diabetic Retinopathy Study letters)*			
at first evaluation visit (at 6-8 weeks after treatment)	+4.60 ± 6.62 (80)	+1.39 ± 8.99 (80)	0.011
at final evaluation visit (at 7-8 months after treatment)	+6.78 ± 8.54 (67)	+4.48 ± 7.29 (66)	0.099
Change in retinal sensitivity on microperimetry (dB)*			
at first evaluation visit (at 6-8 weeks after treatment)	+2.01 ± 3.04 (76)**	+0.92 ± 3.65 (77)**	0.046
at final evaluation visit (at 7-8 months after treatment)	+3.24 ± 3.08 (62)**	+1.38 ± 4.45 (60)**	0.008
Change in composite score on vision-related quality of life using NEI-VFQ25 (on a scale from 0-100)*			
at first evaluation visit (at 6-8 weeks after treatment)	+2.87 ± 8.35 (80)	+2.56 ± 7.36 (79)**	0.800
at final evaluation visit (at 7-8 months after treatment)	+7.03 ± 10.8 (67)	+4.48 ± 9.85 (66)	0.158

Data is n (%), or mean (SD).

*Compared to baseline. **Data was not available for all patients.

Abbreviations: HSML: high-density subthreshold micropulse laser treatment; NEI-VFQ25: National Eye Institute Visual Function Questionnaire; PDT: half-dose photodynamic therapy.

In the half-dose PDT group, the mean PDT laser spot size for the first and second treatments was 4.01 ± 1.57 and 4.04 ± 1.54 mm, respectively. In the HSML treatment group, 187 ± 209 spots with a mean power of 1739 ± 213 mW were applied at the first treatment, and 188 ± 237 spots with a mean power of 1746 ± 184 mW were applied at the second treatment. A total of 99 patients required a second evaluation visit due to undergoing repeat treatment based on the presence of SRF measured on OCT at the first evaluation visit; during the second evaluation visit, 32.4% (11 out of 34 patients) and 15.4% (10 out of 65 patients) of patients in the half-dose PDT group and HSML group, respectively, had a complete resolution of SRF on OCT; however, this difference was not statistically significant (p=0.053).

At the first evaluation visit, the patients in the half-dose PDT group had a significantly higher increase in BCVA compared to the HSML treatment group (4.60 ± 6.62 versus 1.39 ± 8.99 ETDRS letters, respectively; p=0.011). At the final evaluation visit, the increase in BCVA relative to baseline was 6.78 ± 8.54 and 4.48 ± 7.29 ETDRS letters in the PDT and HSML groups, respectively (p=0.099). Retinal sensitivity on microperimetry increased in both treatment groups, and this increase was significantly higher in the PDT group compared to the HSML-treated group at both the first evaluation visit (2.01 ± 3.04 versus 0.92 ± 3.65 dB, respectively; p=0.046) and the final evaluation visit (3.24 ± 3.08 versus 1.38 ± 4.45 dB, respectively; p=0.008)). Finally, the change in vision-related quality of life measured using the NEI-VFQ25 increased in both groups; however, this increase did not differ significantly between the PDT and HSML-treated groups at either the first evaluation visit (2.87 ± 8.35 versus 2.56 ± 7.36, respectively; p=0.800) or the final evaluation visit (7.03 ± 10.8 versus 4.48 ± 9.85, respectively; p=0.158) (Table 2).

During the course of the trial, SRF recurred in 4 patients in the PDT group and 1 patient in the HSML group (corresponding to 5% and 1.3% of patients, respectively (p=0.176)). Twelve AEs were recorded in 10 patients in the PDT group, and 9 AEs were recorded in 7 patients in the HSML group (corresponding to 12.5% and 8.8% of patients, respectively). None of these AEs were treatment-related. A total of 4 patients (2 each in the PDT and HSML groups) reported an SAE that was unrelated to the study treatment, and none of the patients died during the trial. One patient in the HSML group developed a vision-threatening AE in which BCVA decreased by more than 30 ETDRS letters; this decline in visual acuity was considered to have been caused by an increase in SRF, despite treatment. All of the side effects reported in this trial are listed in Table 3.

Table 3. Adverse events

	PDT (80 patients)	**HSML (80 patients)**
Serious adverse events*	2 (2 patients)	2 (2 patients)
Number of adverse events	12 (10 patients)	9 (7 patients)
Viral infectious disease of the upper respiratory tract	0	3
Corneal erosion	2	0
Arthritis	1	0
Allergic response to verteporfin	1	0
Allergic response to dye administered during angiography	1	0
Bladder cancer	1	0
Chalazion	0	1
Finger laceration	1	0
Gingivitis	1	0
Keratoconjunctivitis sicca	0	1
Lateral epicondylitis	1	0
Newly diagnosed arterial hypertension	1	0
Spinal disc herniation	1	0
Squamous cell carcinoma of the skin	1	0
Tenosynovitis	0	1
Uterine fibroids	0	1
Vitamin D deficiency	0	1
YAG laser capsulotomy for posterior capsule opacification	0	1
Vision-threatening adverse events	0	1

*Note that none of the adverse events were related to the study treatment.

Abbreviations: HSML: high-density subthreshold micropulse laser treatment; PDT: half-dose photodynamic therapy.

DISCUSSION

Treatment is usually indicated for patients with cCSC, as persistent SRF leakage in the macula can lead to significant visual disability and decreased vision-related quality of life.[1, 5] To date, however, the best treatment for cCSC has been the subject of controversy due to a lack of prospective randomised controlled trials, leading to a wide variety of recommended treatments.[1, 7, 21] Here, we performed the first large prospective, multicenter, randomised controlled treatment trial in which we compared the 2 most commonly used treatments for cCSC.[1, 7, 11, 12, 14] We found that the efficacy of half-dose PDT with respect to complete resolution of SRF was significantly higher compared to HSML treatment in both short-term and long-term follow-up visits. Moreover, functional outcome was better following half-dose PDT compared to HSML treatment with respect to both increased retinal sensitivity at the first and final evaluation visit and increased BCVA at the first evaluation visit. In contrast, the treatment groups did not differ significantly with respect to BCVA at the final evaluation visit or overall quality of life (measured using the NEI-VFQ-25). Our study was designed primarily to assess a morphological endpoint, namely the complete resolution of SRF, which is a prerequisite for preserving and/or restoring function. In this respect, microperimetry may be a more sensitive and earlier functional endpoint than BCVA, given that the recovery of BCVA can be delayed in cCSC following treatment.[9]

The significantly better anatomical and functional outcome following PDT treatment may be attributed to the fact that PDT targets the choroidal tissue, which appears to be the tissue primarily affected in cCSC. In CSC, the accumulation of SRF – which affects photoreceptor function and thus vision – results from a leaky outer blood-retina barrier at the level of the RPE; however, this impaired barrier function is likely induced by a primary dysfunction of the underlying choroid, given that choroidal abnormalities (imaged using ICGA) often outweigh both the changes in the RPE and leakage visible on fluorescein angiography. The effect of PDT appears to depend on transient choroidal ischemia and subsequent choroidal vascular remodelling;[22] in contrast, the delivery of brief subthreshold micropulses to the RPE during HSML treatment has been suggested to induce the production of intracellular biological factors that stimulate RPE function without causing visible damage to the retina.[23] Given the significant differences in treatment outcome between half-dose PDT and HSML treatment in our study, we suggest that choroidal abnormalities should be targeted in cCSC. Nevertheless, whether HSML treatment has therapeutic effects on the choroid beyond the RPE is currently unknown. None of the patients in either treatment group reported treatment-related AEs. This finding is consistent with previous reports of extremely rare AEs when applying PDT at reduced settings; such rare AEs include choroidal neovascularisation and occur in less than 1% of patients.[11, 14, 24-27] Importantly, choroidal neovascularisation also been described as part of the natural course of cCSC.[28]

In this trial, the observed treatment outcome in both treatment groups was likely the result of the intervention, as we included only patients who had SRF for at least 6 weeks and who presented with typical findings of active cCSC on multimodal imaging; thus, SRF would not be expected to resolve spontaneously in these patients. Moreover, we measured a significant difference in outcome between the half-dose PDT group and the HSML group. In the HSML treatment group, the laser power was in the upper range of subthreshold settings, and adjacent laser spots were treated in order to achieve confluent treatment coverage; in addition, the area to be treated was relatively large and guided by ICGA. Despite these measures to minimise undertreatment with HSML,[20] however, outcome was still more favorable in the group that received half-dose PDT. Importantly, the patients in the PDT group received only half of the dose of verteporfin originally described for treating neovascular age-related macular degeneration;[8] this reduced dose was chosen in order to minimise the occurrence of SAEs following treatment while still providing comparable treatment outcome.[19, 29, 30] However, only in the HSML-treated group a distance of 500 µm from the foveal center was kept in order to prevent possible damage to the fovea, which has been described to be a possible adverse effect of treatment and could have influenced the results of our study. Still, we assume that not including the fovea in the area to be treated could have only minimally affected the study outcome, since the fovea included only a very small part of the total area that required treatment, as treatment was often quite extensive because it was based on ICGA abnormalities while using HSML settings at the upper limit for this treatment technique.

The strengths of the PLACE trial include its prospective design and the relatively large number of patients. In addition, we used the complete resolution of SRF on OCT as the desired treatment effect, as the prolonged presence of SRF can cause irreversible photoreceptor damage.[6] Moreover, our cohort of cCSC patients was well-characterised, and the diagnosis of cCSC was verified in each patient by a central reading center based on typical findings of cCSC on multimodal imaging; in addition, we excluded patients who had previous cCSC treatment, previous or current corticosteroid use, and disease duration longer than 18 months. These criteria resulted in a cohort of patients who ranged from relatively focal cCSC to more extensive cCSC. Finally, with respect to both anatomical and functional outcomes, a follow-up assessment was performed 7 to 8 months after the first treatment; thus, prolonged follow-up studies should be performed in order to assess the long-term efficacy of both treatments, particularly given that SRF recurred in 5 patients during the follow-up period.

In conclusion, the results of our large prospective, multicenter, randomised controlled treatment trial show that half-dose PDT provides superior outcome compared to HSML treatment in patients with cCSC. This finding provides key insight into developing best-practice guidelines for treating CSC. In addition, the results of this trial may be used to justify changing the existing reimbursement restrictions in several countries regarding the off-label use of verteporfin in PDT for treating cCSC.

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6.2

PHOTODYNAMIC THERAPY IN CHRONIC CENTRAL SEROUS CHORIORETINOPATHY WITH SUBRETINAL FLUID OUTSIDE THE FOVEA

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ABSTRACT

Purpose: To assess the efficacy of photodynamic therapy (PDT) in patients with chronic central serous chorioretinopathy (cCSC), in whom subretinal fluid (SRF) was solely present outside the foveal area.

Methods: In this retrospective study, 16 eyes of 15 cCSC patients who received half-dose PDT because of notable subjective visual complaints due to the presence of extrafoveal SRF, were included. An ophthalmic examination was performed before treatment, including Early Treatment of Diabetic Retinopathy Study best-corrected visual acuity measurement, applanation tonometry, slit-lamp examination, and indirect ophthalmoscopy, followed by multimodal imaging, including fundus photography, fundus autofluorescence, spectral-domain optical coherence tomography (OCT), enhanced-depth imaging OCT of the choroid, fluorescein angiography, and indocyanine green angiography.

Results: In 7 treated patients (47%) PDT led to a decrease in visual complaints at the first evaluation visit. At this visit, extrafoveal SRF on OCT had resolved in 14 eyes (88%), whereas a complete resolution of extrafoveal SRF had occurred in all eyes at final follow-up visit. At baseline, posterior cystoid retinal degeneration was also present in 5 eyes (31%) and this remained present at all evaluation visits in these patients. Choroidal thickness decreased statistically significantly in the treated eyes, both foveally and at the location of the maximum height of extrafoveal SRF. No complications of PDT were observed.

Conclusions: Half-dose PDT treatment of cCSC patients with visual complaints due to extrafoveal SRF accumulation is a safe procedure leading to complete SRF resolution, a decrease in choroidal thickness, and a reduction in visual symptoms.

INTRODUCTION

Central serous chorioretinopathy (CSC) is a chorioretinal disease that can eventually lead to vision loss as a result of irreversible retinal damage, mainly affecting middle-aged men. Despite the fact that the disease has already been described by Von Graefe in 1866, the exact pathogenetic mechanism of CSC is still unknown.[1] The disease is characterised by an accumulation of serous subretinal fluid (SRF). This leakage results from dysfunction of the retinal pigment epithelium (RPE) outer blood-retinal barrier, most probably caused by choroidal congestion, thickening, and hyperpermeability.[2-5] Compared to a control group, a significant increase in choroidal thickness (CT) in both affected eyes and non-affected fellow eyes has been described, supporting the hypothesis that CSC is a bilateral disorder which can present unilaterally.[3, 6, 7]

In chronic CSC (cCSC), treatment is generally initiated in case of the presence of vision loss due to SRF accumulation under the fovea.[4, 8] Based on the currently available literature, photodynamic therapy (PDT) and micropulse laser treatment appear to be the most appropriate treatment modalities for the disease.[8] In up to 73% of cCSC patients complete resolution of SRF can be achieved after micropulse laser treatment, whereas this occurs in up to 100% of cases after PDT.[9-11] However, no results of large randomised controlled treatment trials have been published yet.[2]

PDT is thought to induce choroidal changes due to a temporary decrease in the perfusion of the choriocapillary layer and due to choroidal vascular remodelling, resulting in a reduction in fluid leakage from the choroid to the subretinal space.[12] However, some patients develop a temporary worsening of visual complaints in the first 2 weeks after PDT, and very rare complications such as choroidal ischemia, choroidal neovascularisation, and RPE atrophy have been described after PDT in cCSC.[13-16] Because of these possible complications and because of the fact that in cCSC patients extrafoveal SRF less often causes visual symptoms compared to foveal SRF, PDT is usually only performed in the cCSC patient group with foveal SRF. However, some patients without foveal SRF do have significant visual symptoms that require treatment. No studies on the use of PDT in cCSC patients with only extrafoveal SRF have been conducted thus far. In this retrospective study, we assessed the safety and efficacy of half-dose PDT on both visual complaints and SRF on optical coherence tomography (OCT) in cCSC patients with extrafoveal SRF.

METHODS

Patients

Sixteen eyes of 15 patients could be included in this study. In these patients solely extrafoveal SRF could be detected on OCT prior to treatment. However, subjectively disabling visual complaints such as metamorphopsia, impaired color vision, and blurred peripheral vision led to the decision to schedule a treatment. PDT treatment had been performed between November 2014 and January 2017. Diagnosis of cCSC was established by fundoscopy, digital color fundus photography (Topcon Corp., Tokyo, Japan), fundus autofluorescence (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany), spectral-domain OCT (Spectralis HRA+OCT) and enhanced-depth imaging (EDI-)OCT of the choroid (Spectralis HRA+OCT), fluorescein angiography (FA; Spectralis HRA+OCT), and indocyanine green angiography (ICGA; Spectralis HRA+OCT). All of the following had to be present to set the cCSC diagnosis: disease duration of more than 4 months, serous SRF on OCT, ≥ 1 area of a hot spot of leakage or diffuse leakage in combination with irregular RPE window defects on FA, and corresponding hyperfluorescence on ICGA. Patients in whom evidence of other diagnoses than cCSC were present, or cases with evidence of complications such as polypoidal choroidal vasculopathy and/or choroidal neovascularisation, were excluded.

Local ethics committee and institutional review board approval was obtained. The study followed the tenets of the Declaration of Helsinki.

Photodynamic therapy treatment

Half-dose intravenous (3 mg/m^2) verteporfin (Visudyne®; Novartis Europharm Ltd., Horsham, West Sussex, UK) was administrated over a period of 10 minutes. At exactly 15 minutes after the start of the verteporfin infusion, a contact glass (Volk® PDT lens) was positioned on the affected eye, and the laser beam was projected on the area to be treated. The zone to be treated was chosen based on hyperfluorescent areas on mid-phase (10') ICGA, corresponding to SRF on OCT and hyperfluorescent hot spots of leakage on mid-phase (3') FA. For the PDT treatment, a fluency of 50 J/cm^2, treatment duration of 83 seconds, and a laser wavelength of 689 nm (Carl Zeiss Meditec, Dublin, CA, USA) were used.

Ophthalmological examinations

Ocular complaints were recorded and Early Treatment of Diabetic Retinopathy Study (ETDRS) best-corrected visual acuity (BCVA) was measured at the last visit before PDT and at least at 1 evaluation visit after PDT. When ETDRS BCVA was not available, a previously described conversion method was used.[17] The effect of treatment on SRF was assessed with spectral-domain OCT imaging. Moreover, the effect on intraretinal cystoid spaces without intraretinal leakage (posterior cystoid retinal degeneration) was also studied on these OCT images.[18, 19]

For the treated eyes, the following findings were measured manually on EDI-OCT with use of the caliper tool in Heidelberg Eye Explorer (version 1.9.10.0; Heidelberg Engineering) at the last visit before PDT and at least at 1 evaluation visit after PDT: foveal CT (distance from the outer part of the hyperreflective RPE layer to the hyperreflective line of the inner surface of the sclera, on EDI-OCT) and CT at the location of the maximum height of extrafoveal SRF (distance from the outer part of the external limiting membrane to the outer part of the RPE layer). Complete resolution of SRF on OCT was considered to be the desired anatomical treatment effect. For comparison with CT in the treated eyes, subfoveal CT was also measured on EDI-OCT in untreated fellow eyes.

Statistical analysis

For statistical analyses, a dependent t-test was used in SPSS Statistics (version 23; IBM Corp., Armonk, NY, USA) to compare both ETDRS and CT at evaluation visits with ETDRS and CT before PDT. The level of statistical significance was set at $p<0.05$.

RESULTS

The 15 cCSC patients (16 eyes; 14 male patients, 1 female patient) who were included in this study had a mean age of 52 ± 13 years (range, 35 - 80). In the treated eyes, cCSC had been diagnosed for the first time at 21 ± 21 months (range, 3 - 83) before PDT. Prior to PDT treatment, ETDRS BCVA in the affected eyes was 78 (Snellen equivalent: 20/29) ± 18 letters (range, 21 - 95). Bilateral signs of cCSC were present in 10 patients (67%). Posterior cystoid retinal degeneration was present in 5 eyes (31%) at the last visit before PDT. Nine included eyes (56%) had received previous CSC treatment because of foveal SRF, including micropulse laser treatment (7 eyes) and half-dose PDT (2 eyes). All patients had received this previous treatment within a year before the half-dose PDT performed within this study. The mean PDT spot size in the treated eyes was 5.0 ± 1.7 mm (range, 2.2 - 7.2), and the verteporfin dosage was 2.9 ± 0.4 ml (range, 2.0 - 3.3). Two patients were treated with 2 PDT spots in 1 session. Out of 12 treated eyes of 12 patients, for which this information was available, the fovea was included in the treatment spot in 10 eyes (84%). Patient characteristics are summarised in Table 1.

At the first evaluation visit after a mean of 63 days (range, 7 - 161) after half-dose PDT, a reduction in visual symptoms had occurred in 7 patients (47%). At that visit, ETDRS BCVA was 80 (Snellen equivalent: 20/25) ± 16 letters (range, 35 - 96), which was not statistically significantly different from the last visit before PDT ($p=0.074$). At the last evaluation visit after a mean of 325 days (range, 189 - 351) after PDT, ETDRS BCVA was 78 (Snellen equivalent: 20/29) ± 20 letters (range, 20 - 93) as compared to the last visit before PDT ($p=0.836$).

Table 1. Clinical characteristics of patients with central serous chorioretinopathy who received photodynamic therapy for extrafoveal subretinal fluid

Patient	Age	Gender	Baseline ETDRS BCVA	Duration of CSC (days)	Bilateral CSC	Recurrent CSC	Previous CSC treatment affected eye
1	50	M	85	352	Y	N	N
2	51	F	86	488	N	N	micropulse laser treatment (2*)
3	70	M	77	421	Y	Y	N
4	37	M	59	1299	Y	Y	N
5	80	M	21	129	Y	N	half-dose photo-dynamic therapy (foveal SRF)
6	72	M	89	725	Y	N	N
7	35	M	72	474	Y	Y	micropulse laser treatment (1*)
8	51	M	90	547	N	Y	micropulse laser treatment (2*)
9	47	M	92	1518	Y	N	half-dose photo-dynamic therapy (foveal SRF)
10	49	M	84	333	N	N	micropulse laser treatment (2*)
11	44	M	90	349	N	N	micropulse laser treatment (2*)
12	41	M	67	315	Y	N	micropulse laser treatment (2*)
13	50	M	76	671	N	N	micropulse laser treatment (2*)
14	47	M	95	211	Y	N	N
15	57	M	81	83	Y	N	N
	57	M	75	2523	NA	Y	N

Abbreviations: BCVA: best-corrected visual acuity; CSC: central serous chorioretinopathy; ETDRS: Early Treatment of Diabetic Retinopathy Study; NA: not applicable; SRF: subretinal fluid.

A complete resolution of extrafoveal SRF had occurred in 14 eyes (88%) at the first evaluation visit after PDT, whereas posterior cystoid retinal degeneration had not disappeared in any of the eyes. In the 9 patients for whom EDI-OCT images of sufficient quality were available both before PDT and at the first evaluation, the subfoveal CT was 408 ± 93 µm (range, 200 - 509) at this first visit after PDT, which was a significant decrease as compared to before PDT (452 ± 95 µm (range, 238 - 554); p=0.043). CT at the location of the maximum height of extrafoveal SRF was 407 ± 99 µm (range, 213 - 544) before PDT, which was statistically significantly higher compared to CT at the first evaluation after PDT (351 ± 102 µm (range, 172 - 489); p=0.015). Before PDT, subfoveal CT in the untreated fellow eye was 415 ± 91 µm (range, 244 - 489), which did not differ from CT at the first evaluation visit (411 ± 96 µm (range, 224 - 502); p=0.711). At the

final follow-up visit of 6 patients for whom EDI-OCT images of sufficient quality were available, the subfoveal CT was 397 ± 50 µm (range, 341 - 468) and the extrafoveal CT was 371 ± 84 µm (range, 245 - 475), which was significantly lower than the CT before PDT (p=0.001 and p=0.003). At that moment, the CT of the untreated fellow eyes was 422 ± 78 µm (range, 303 - 496), which did not differ from CT before PDT (p=0.953). Multimodal imaging of a patient before and after half-dose PDT is depicted in Figure 1.

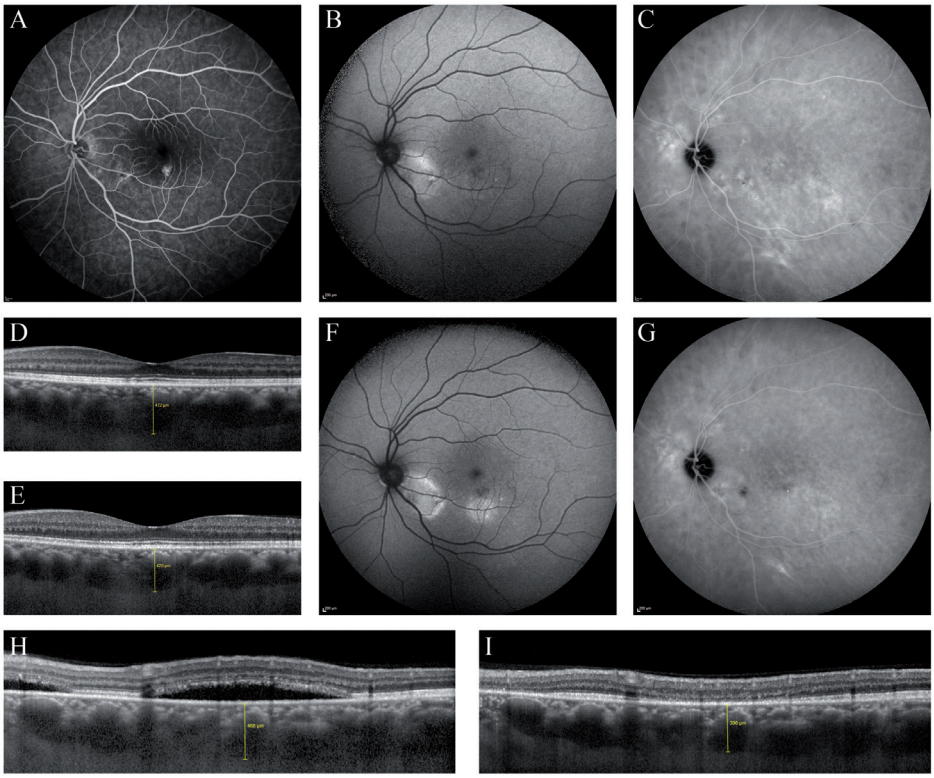

Figure 1. Characteristics on multimodal imaging of a patient with chronic central serous chorioretinopathy with only extrafoveal subretinal fluid, who was treated with half-dose photodynamic therapy.
A-D. Fluorescein angiography (FA; A), fundus autofluorescence (FAF) imaging (B), indocyanine green angiography (ICGA; C), and a foveal optical coherence tomography (OCT) scan (D) of a 47-year-old male patient with chronic central serous chorioretinopathy before half-dose photodynamic therapy (PDT). FA showed hyperfluorescent areas, with a hot spot of leakage inferiorly of the fovea. On FAF, both hyper- and hypoautofluorescent abnormalities were present. ICGA revealed the presence of areas of hyperfluorescence, larger compared to the extent of the abnormalities on FA. On the foveal OCT scan, no subretinal fluid (SRF) was present. Some subtle retinal pigment epithelium abnormalities could be detected, and the choroidal thickness (CT) was 472 µm.

E-G. At the evaluation visit at 6 weeks after half-dose PDT, the subfoveal CT on OCT (E) had decreased to 420 µm. FAF imaging (F) revealed a slight increase in the extent of hyperautofluorescent abnormalities, but the extent of hyperfluorescent abnormalities on ICGA (G) had clearly decreased.
H-I. On the OCT scan (H), inferiorly of the fovea, obtained before half-dose PDT, on which the maximum height of extrafoveal SRF could be detected, the CT was 468 µm. At 6 weeks after treatment, a complete resolution of SRF on OCT (I) had occurred and the CT had decreased to 396 µm.
The complaints of the patient, which mainly consisted of metamorphopsia, had not changed at the evaluation visit after treatment.

At final evaluation visit, extrafoveal SRF had disappeared in all patients. The 2 patients in whom extrafoveal SRF had not resolved at the first evaluation visit received an additional half-dose PDT treatment, after which a complete resolution of SRF occurred. Both patients had previously received treatment for cCSC, including micropulse laser treatment (1 eye) and half-dose PDT (1 eye). Characteristics on OCT before and at the evaluation visits after half-dose PDT are summarised in Table 2.

Table 2. Characteristics on optical coherence tomography in chronic central serous chorioretinopathy patients who received half-dose photodynamic therapy for extrafoveal subretinal fluid

Patient	Reduction of complaints after PDT	Pre-PDT SCT (µm)	Post1-PDT SCT (µm)	Post2-PDT SCT (µm)	Pre-PDT extrafoveal CT (µm)	Post1-PDT extrafoveal CT (µm)	Post2-PDT extrafoveal CT (µm)	Pre-PDT SCT fellow eye (µm)	Post1-PDT SCT fellow eye (µm)	Post2-PDT SCT fellow eye (µm)
1	Y	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	Y	549	420	430	482	385	365	478	447	496
3	N	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	N	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	N	NA	NA	NA	NA	NA	NA	NA	NA	NA
6	N	NA	NA	NA	NA	NA	NA	NA	NA	NA
7	Y	NA	NA	NA	NA	NA	NA	NA	NA	NA
8	N	NA	NA	NA	NA	NA	NA	NA	NA	NA
9	N	413	427	NA	334	348	NA	489	416	NA
10	N	238	200	NA	344	268	NA	244	224	NA
11	Y	485	372	341	441	294	245	472	475	451
12	Y	416	361	344	382	324	306	348	368	354
13	Y	447	482	NA	213	172	NA	465	469	NA
14	N	492	486	385	454	485	410	458	496	492
15	Y	554	509	468	544	489	475	475	502	437
	Y	472	420	413	468	396	423	306	299	303

Abbreviations: NA: not available; PDT: photodynamic therapy; POST1: first evaluation visit after PDT; POST2: last evaluation visit after PDT; SCT: subfoveal choroidal thickness.

DISCUSSION

To the best of our knowledge, this is the first study describing the outcome of PDT treatment in cCSC patients in whom only extrafoveal SRF was present on OCT. A complete resolution of SRF occurred in 88% of patients at first evaluation visit, and in all patients at final follow-up visit. Also, CT in the treated eyes decreased significantly at the evaluation visits both at the location of the maximum height of extrafoveal SRF and in the fovea. A decrease in visual complaints was reported for 47% of treated patients.

The percentage of patients in whom SRF disappeared after half-dose PDT is in line with the outcome of other studies that included patients with foveal SRF, for whom it has been described that treatment could prevent the occurrence of permanent photoreceptor damage.[9, 20, 21] This complete resolution occurred despite the fact that the majority of eyes in our study had previously received treatment for cCSC. In addition to a resolution of SRF, treatment resulted in a significant reduction in CT, both extrafoveally and foveally. A comparable subfoveal CT reduction independently from including the fovea in the PDT treated area has been previously described.[11, 22] Such an effect that is distant from the area that was actually treated with the PDT spot may be explained by choroidal remodelling after PDT treatment.[12] Apart from the finding that SRF and visual symptoms resolved in a noteworthy number of cCSC patients with extrafoveal SRF included in this study, these treatment effects in these cCSC patients may also decrease the likelihood of either recurrence of SRF or progression to foveal SRF leakage at a later date, which could lead to irreversible damage.[23]

Based on the available, mostly retrospective evidence on the safety and efficacy of PDT using reduced treatment settings in cCSC,[8, 13] our first-line choice for the treatment of cCSC is PDT, and this treatment resulted in a complete resolution of SRF in all the included patients with extrafoveal SRF. However, the optimal treatment and timing of treatment for cCSC is subject to controversy, due to the lack of large prospective randomised controlled trials. We are currently performing a large prospective randomised controlled multicenter treatment trial, the PLACE trial, comparing half-dose PDT with high-density subthreshold micropulse laser treatment for cCSC.[24] In this trial both anatomical and functional parameters are taken into account, for a prolonged follow-up period.[24] However, for patients included in this trial and in other treatment trials on cCSC generally the presence of SRF in the fovea is mandatory to be eligible for inclusion. Limitations of the current study include the small number of included patients, its retrospective nature, and the relatively short follow-up period. Since PDT treatment can lead to both a temporary increase in visual complaints and to serious complications, it should be performed with its associated risks in mind especially in patients without SRF in the fovea.[13-16]

In conclusion, half-dose PDT treatment of cCSC patients with notable visual complaints due to extrafoveal SRF accumulation induces complete SRF resolution and leads to a decrease in CT and a reduction in visual symptoms.

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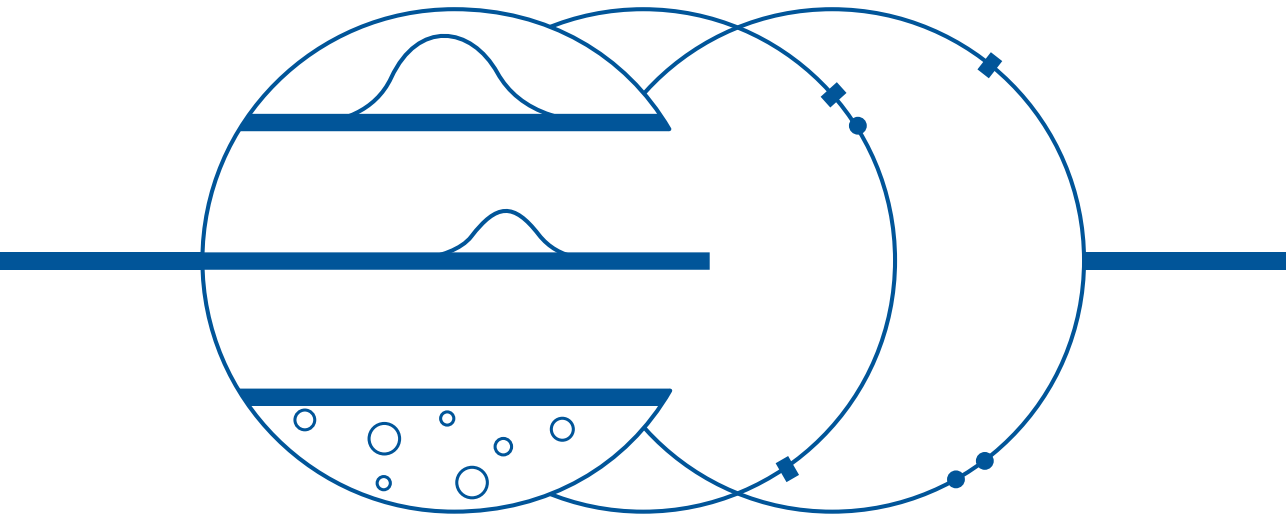
7

SUMMARY AND GENERAL DISCUSSION



The aim of this thesis was to increase the understanding of central serous chorioretinopathy (CSC), by performing research that covers the spectrum from pathogenesis to treatment. In this chapter, the most important findings and new insights from the studies described in this thesis will be integrated, and clinical implications and future perspectives will be discussed.

PATHOGENESIS OF CSC

Since the German ophthalmologist Albrecht von Graefe was the first to describe CSC as 'recurrent central retinitis' in 1866,[1] attention has been paid to this mysterious eye disease. Choroidal involvement in CSC was suggested by the American ophthalmologist Alfred Edward Maumenee, based on the outcome of angiographic examinations.[2] In 1967, the current term 'central serous chorioretinopathy' was eventually introduced by Donald Gass.[3] However, the exact pathogenesis of CSC still has to be unraveled.

Several associations with CSC have previously been identified in clinical practice, out of which corticosteroid use, male gender, and a relatively young age are the most pronounced risk factors.[4-7] [8, 9] Moreover, type A individuals (described as outgoing, ambitious, organised, sensitive, impatient, anxious, and proactive) have been described to be at risk to develop CSC.[10, 11] Familial CSC has also been reported, which underlines a genetic basis for the disease.[12-15]

In this thesis, a number of new insights have added to the understanding of the pathogenesis of CSC.

Association of chronic CSC (cCSC) with corticosteroids and the hypothalamus-pituitary-adrenal (HPA) axis

Corticosteroid use has been found to be the most important risk factor for CSC, with odds ratios of up to 37, whereas previous corticosteroid intake has been described in up to 52% of CSC patients.[16-19] Exogenous hypercortisolism has been associated with an increased risk to develop CSC, independent of the dosage and route of administration.[16, 17, 20-22] Endogenous hypercortisolism (Cushing's syndrome) has also been linked to CSC: a previous study found that 5% of 60 patients with active Cushing's disease proved to have a history of CSC before the endocrinological diagnosis had been established.[23] Moreover, increased cortisol levels in urine and plasma of CSC patients have been described.[24-26] However, the precise underlying mechanism of this relationship is currently unclear.

Association of cCSC with single nucleotide polymorphisms in steroid genes

Since corticosteroids are the most pronounced risk factor for CSC, the glucocorticoid-binding glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR) have been suggested

to be involved in CSC pathogenesis. In a study in rats, choroidal abnormalities resembling findings in CSC patients were observed after intravitreal injection of aldosterone and corticosterone (the rodent equivalent to cortisol). Neutralization of this effect was seen after administration of the MR antagonist canrenoate. Vessel dilatation via the upregulation of the endothelial vasodilatory calcium-dependent potassium channel has been described to be the underlying mechanism for this effect of MR activation.[8, 27] Moreover, oral administration of the MR antagonists spironolactone and eplerenone has been found to lead to a therapeutic effect in a numerous percentage of patients, but still there is a lack of prospective randomised controlled trials on this medication for CSC patients.[27-31]

Therefore, our identification of an increased risk of CSC in patients with the variant rs2070951 and the GA haplotype in the MR gene (*NR3C2*) is important (Chapter 2.2). Moreover, we describe a decreased risk of CSC for the CA haplotype, and no association with CSC for 3 variants in the GR gene (*NR3C1*) and 1 other variant in the MR gene, that have been described to alter its protein activity.[32-39] Our findings are a genetic confirmation of the possible role of MR in CSC pathogenesis. Remarkably, the detected *NR3C2* haplotypes and its associated risks for CSC have been previously associated with differences in perceived chronic stress, providing a possible link between genetic and clinical CSC risk factors.[10, 39-42] Stratification of patients into different groups based on variants and haplotypes in the MR would be an interesting option to further investigate the clinical course and optimal treatment for CSC. Moreover, further studies on the role of GR and MR in CSC, in the perspective of clinical and genetic risk factors, can shed a light on the functional and pathophysiological consequences of these associations in CSC.

To further assess the role of corticosteroids in CSC, we are currently studying the effect of the administration of different types of these substances to primary human choroidal endothelial cells (CECs). We have developed a unique and robust culturing protocol for CECs, with which CECs can be isolated from cadaveric human donors. Magnetic-activated cell sorting with anti-human CD31 is performed for CEC isolation, and characteristics of endothelial cells are confirmed using different techniques. The role of MR and GR in the pathogenesis of CSC, and in other diseases in which CECs and/or corticosteroids are involved, can thereafter be further studied and clarified. Since knowledge on the individual vulnerability in the pathogenesis of CSC can be obtained in the most reliable way by studying newly generated CECs from CSC patients, we have also planned to develop the generation of vascular endothelium from blood cell-derived induced pluripotent stem cells. We will compare cells from patients that did and did not develop CSC after treatment with corticosteroids: those responses to cortisol that are unique to patient-derived CECs are strong candidates for CSC-associated pathways.

Clinical findings

In this thesis, the detection of abnormalities within the CSC spectrum in 54% of 13 patients with primary hyperaldosteronism, most often caused by adrenal overproduction of aldosterone, on extensive ophthalmological phenotyping supports the role for MR (Chapter 4.1), which has been hypothesised previously based on earlier basic research.[8, 27, 28, 31]

MR stimulation by aldosterone may lead to choroidal effects through induction of either oxidative stress, inflammation, and/or fibrosis.[43, 44] These choroidal effects could be observed during ophthalmological phenotyping, but the exact underlying mechanisms of action still have to be clarified. Overactivation of aldosterone-preferring MRs or occupancy of cortisol-preferring MRs has been suggested, which is in contrast with the physiological situation, in which MR mainly functions as a cortisol-binding receptor.[45]

Because of the fact that the corticosteroid-binding GR is even more widely expressed in human body than MR,[46] and because CSC has also been described to develop as a result of administration of GR-selective glucocorticoids in some patients,[16, 19] further studies on this receptor are also of interest.

To gain knowledge on the relationship between exogenous corticosteroid use and both CSC and other ophthalmological diseases, we performed extensive ophthalmological phenotyping in asymptomatic renal transplant patients who had used a high cumulative dose of oral corticosteroids (Chapter 4.2). The susceptibility to develop retinal abnormalities apparently also depends on other unknown risk factors, because although we found these changes in 59% of 37 patients, findings characteristic for the spectrum of CSC were present in 10 patients. However, active CSC was found in only 2 patients (5%). Vice versa, previous corticosteroid intake has been described in up to 52% of CSC patients, and local administration of corticosteroids may even be enough as a trigger to develop CSC in persons who may be especially suspectible.[16-19] Interindividual genetic and local anatomical differences may explain the limited occurrence of CSC in chronic corticosteroid users while others appear hypersensitive to CSC development as a response to steroid administration.

However, it is still uncertain if a straightforward causal relationship between corticosteroid use and CSC exists in a subset of patients. To date, it is unexplained why some patients develop CSC after prolonged use of relatively high (cumulative) doses of corticosteroids, while other appear to develop CSC only after sporadic and/or local low-dose use. Strikingly, only few patients seem to develop active CSC, despite the fact that corticosteroid use is very common in our society, and up to half of the CSC patients do not report previous use of corticosteroids. There is a wide interindividual variation in normal cortisol levels and in cortisol receptor activation thresholds, possibly leading to different thresholds for the development of CSC and other cortisol-related

symptoms and pathology. It is unclear if steroids are either mandatory for the development of CSC or if they comprise an additional trigger to an already existing disease state. In patients who have never used exogenous corticosteroids, an effect could also have been induced by endogenous steroids like cortisol.

We here provide further support for the link between corticosteroids and CSC, as we were the first to report several patients in whom cCSC was the principal manifestation of relatively mildly symptomatic endogenous hypercortisolism (Cushing's syndrome), that had not been recognised until the referral from our ophthalmology department (Chapter 4.3), despite the known relationship between endogenous hypercortisolism and CSC.[17, 22] This underlines the importance of considering possible hypercortisolism and the associated non-ophthalmological complaints in CSC patients.

Signs of Cushing's syndrome include facial rounding ('moon face'), weight gain, muscle weakness, flushing of the face (plethora), and reddish-purple skin striae, but these can be subtle. Patients can also experience amenorrhoea, bruising after a minor trauma, glucose intolerance/type 2 diabetes mellitus, libido loss, and metabolic syndrome (a combination of abdominal obesity, elevated fasting plasma glucose, hypertension, high serum triglycerides, and low high-density lipoprotein). In all 4 patients that were included in our case series, complete resolution of subretinal fluid (SRF) occurred after Cushing's syndrome surgery, and during follow-up no reactivation of disease was observed. This is in line with available literature, in which discontinuation of corticosteroid use (if possible) is the first step in treatment of CSC.[47] As cCSC can be the first and principal manifestation of subclinical Cushing's syndrome, we advocate referring CSC patients with potential signs indicative of Cushing's syndrome for endocrinological analysis.

As a part of this thesis, we performed a cross-sectional study, in which we assessed whether Cushing's syndrome prevalence is increased among well-phenotyped cCSC patients and whether detailed endocrinological phenotyping indicates an increased HPA axis activity in cCSC patients that do not have symptoms that could be characteristic for Cushing's syndrome (Chapter 3.1). In none of the included 86 cCSC patients biochemical criteria indicative of Cushing's syndrome were found. However, compared to healthy controls, HPA axis activity was increased in cCSC patients. Patients had higher 24 hours urinary free cortisol, and accompanying higher waist circumference and diastolic blood pressure, whereas diurnal cortisol rhythmicity and feedback were not different. Based on these findings, we advise against biochemical screening for hypercortisolism in CSC patients, unless additional clinical features that could be typical for Cushing's syndrome are present. However, our results do indicate that CSC is associated with

increased activity of the HPA axis, which could be important for research on pathophysiology and disease management of CSC.

To further assess HPA axis activity and the potential stress association in cCSC in our systematic evaluation, patients also completed questionnaires regarding stress-related problems. Based on these questionnaires, no evidence for a higher prevalence of type A personality characteristics in cCSC patients was found (Chapter 3.2), which is in contrast with available literature.[10, 19] Moreover, at the moment of endocrinological screening no difference in stress levels of cCSC patients in whom SRF was present, indicating active CSC, and patients without SRF was found. No association between 24 hours urinary free cortisol and questionnaire outcomes was present, which further demonstrates that cCSC disease activity may not be as strongly associated with psychosocial stress as is traditionally thought. This led us to conclude that there might not be a rationale for stress reduction therapies in cCSC as advised by some ophthalmologists. However, since the questionnaires were not filled in shortly after the intial development of cCSC, no conclusions can be drawn on (the absence of) an association between stress and the initial development of cCSC.

In this thesis, we found no differences in the outcome of extensive endocrinological screening when comparing male and female cCSC patients. Thus, this has not provided clues on the background of the striking male preponderance in cCSC. However, variation in the susceptibility to develop CSC between genders could still be due to subtle differences in hormonal and/or other endocrinological abnormalities, which could even be only local in the ocular environment, warranting further research.

We conclude that both in males and in females a mild increase in cortisol levels may already lead to the development of cCSC in susceptible individuals, since the absolute difference in urinary free cortisol levels between our patients and healthy controls was minor. Both exogenous and endogenous hypercortisolism could induce either abnormal activation or altered consequences of the activation of cytoplasmic steroid receptors, which normally enter the cell nucleus and stimulate transcription of several genes. Changed metabolic and anti-inflammatory effects of corticosteroids may have a damaging effect on the choroid and/or the retinal pigment epithelium (RPE), which can eventually lead to either CSC or other diseases that are part of the pachychoroid clinical spectrum in a subset of patients.

It is currently unclear how the RPE becomes dysfunctional in CSC, through primary RPE effects and/or as a result of the underlying choroidal dysfunction, with subsequent leakage of SRF. Damage to the RPE via the effect of steroids on fibroblasts and the extracellular matrix has been described.[48, 49] Steroids have also been found to modify electrophysiological parameters of the RPE, as measured with electro-oculography, which could reflect a decreased fluid

absorption capacity of these cells, as can be seen in CSC.[50] Moreover, choriocapillary fragility and permeability,[51, 52] an increase in transcription of adrenergic receptors,[53] and prothrombotic effects have been associated with steroid use, which could be of importance in CSC.[54]

Association of cCSC with the complement system

The complement system, an essential part of our innate immune system consisting of a number of proteins that are usually inactive, can be activated in response to pathogen exposition. Pathology can result from underactivation or overactivation of this system: either decreased protection or damage to own cells can occur. Three main pathways are involved in activation of the complement system: the classical pathway, the alternative pathway, and the mannose-binding lectin pathway. Several factors that provide information on complement activity (complement factors 3, 4, B, D, P, the activation product complement 3d, and the complement 3d/complement factor 3 ratio) and on complement inhibition (complement factors H and I) can be assessed in serum. Activation products complement component 5a and soluble complement components 5b-9 can be measured in plasma samples.[55-58] All complement genes that are associated with the complement activation pathways are expressed locally in the human RPE-choroidal complex.[59]

Association of cCSC with single nucleotide polymorphisms in complement genes

We have previously found single nucleotide polymorphisms in the *age-related maculopathy susceptibility 2* (*ARMS2*), *complement factor H* (*CFH*), and copy number variations in the *complement component 4B* (*C4B*) gene have been associated with CSC in Caucasians,[60-62] and the association with *CFH* single nucleotide polymorphisms was also identified in an Asian CSC cohort.[63] Both genetic risk and protective factors have been observed in these studies.[60, 61, 63] Most intriguingly, variants in *ARMS2* and *CFH* that are protective for CSC confer risk of age-related macular degeneration (AMD), and vice versa, which has indicated the existence of a genetic and pathophysiological overlap between these diseases.[60]

The *ARMS2* gene has been found to interact with the extracellular matrix, which biochemically and structurally supports surrounding cells. Changes in function of the extracellular matrix could lead to an increase in either rigidity or elasticity of cells nearby, which could subsequently cause cellular detachments of the RPE and/or the choroid.[64] Moreover, this could induce either an increase in choroidal vascular resistance or a decrease in the blood flow support through vessels, which has been described to be involved in CSC pathogenesis. The protein product of the *CFH* gene, factor H, is an inhibitor of the alternative complement pathway. After linking with adrenomedullin, factor H may lead to choroidal vasodilation characteristic for CSC by mechanisms that have not been clarified yet.[65] For the *ARMS2* and *CFH* gene, differences between subgroups of CSC patients were observed: the most typical CSC patients showed a different genetic profile compared to patients with atypical CSC. Atypical CSC patients had

a phenotype that considerably overlapped with other retinal diseases, such as AMD, which illustrates the need for correct clinical classification.[60] The number of *C4B* copies, possibly present to increase immunological diversity,[66] also has an effect on CSC risk: we have found that having no *C4B* copies appears to lead to an increased risk of CSC, whereas the presence of 3 *C4B* copies has a protective effect. *C4B* copy numbers have been described to influence the reactivity of the HPA axis, which can be in line with CSC pathogenesis.[67]

In this thesis, we also describe the first unbiased genome-wide association study (GWAS) on cCSC (Chapter 2.3). Interestingly, in this GWAS we identified the locus in the *CFH* gene, that we also found previously in the aforementioned targeted studies.[60, 63] Moreover, other risk-conferring and protective *CFH* haplotypes were found. Additional evidence for altered regulation of the complement system in cCSC and novel candidate genes and pathways implicated in this disease were detected. We identified predicted differences in expression of the *CFH, CFHR1*, *CFHR4,* and *KCNT2* genes which are located in the *CFH* locus on chromosome 1 around the lead variant of the GWAS. As the *CFH, CFHR1, CFHR4*, and *CD46* genes are important regulators of the complement cascade, taken together, the genetic studies described in this thesis therefore point to a potentially important role of the complement system in cCSC pathogenesis.

We also found that the *CD46* (chromosome 1) and *TNFRSF10A* (chromosome 8) genes were predicted to differ in expression, but the exact role of both the *TNFRSF10A* and *KCNT2* gene in the eye is unclear. *TNFRSF10A* encodes a member of the tumor necrosis factor-receptor superfamily, which is involved in regulation of cell apoptosis,[68] whereas the fact that the *KCNT2* gene encodes a potassium channel could imply a role in the ion- and water balance in the eye, which could be of importance in CSC.[69, 70]

Clinical findings

To decipher the functional effect of the genetic link between the complement system and CSC, we then tested possible signs of complement activation and inhibition in serum and plasma of cCSC patients (Chapter 3.3). After all, several studies have shown abnormal systemic complement activation in AMD, and we hypothesised that certain abnormalities could also be found in cCSC. However, we did not detect either systemic activation or inhibition of the complement system in cCSC patients, compared to a group of matched healthy controls, after correction for multiple testing and taking covariates into account.

This outcome may have been caused by the relatively small effect sizes for the known genetic associations of *CFH* in CSC, as compared to AMD. In AMD, systemic complement activation was previously found, next to several genetic associations.[57, 58, 60] For some of the complement variables the number of included patients and controls could have led to underpowered results in our study, although it was sufficiently powered to detect differences in the important

complement 3d/complement factor 3 ratio which were previously found in AMD. Moreover, CD46, CFHR1, and CFHR4 levels, that were found to be associated with cCSC in our GWAS (Chapter 2.3), have not been measured in peripheral blood until now.

The functional translation of the outcome of genetic studies in CSC should thus be further focused on during future research. Local complement activation and inhibition could be assessed in aqueous humor. However, for obtaining aqueous humor in CSC patients an extra, invasive procedure should be undertaken, since cCSC is usually not treated with intravitreal injections with anti-vascular endothelial growth factor,[8, 71] during which aqueous humor sampling can be performed. Local complement activation and inhibition can also be tested in CECs and RPE cells, that could be obtained out of blood cell-derived induced pluripotent stem cells of CSC patients and healthy controls. It would be even of more interest to study the interplay between these different types of cells in a model, since local intraocular complement activation has been previously observed in a small cohort of AMD patients, but together with an absence of differences in complement activity in plasma in that specific study.[72]

Association of cCSC with antiretinal antibodies

The role of antiretinal antibodies (ARAs) in CSC pathogenesis has also been assessed within this thesis (Chapter 3.4). Such antibodies have been previously found in AMD patients and uveitis patients.[73, 74] We detected ARAs in 54% of CSC patients, which was significantly higher compared to controls. Moreover, staining of the photoreceptors occurred significantly more often in CSC patients. The clinical significance of ARAs in CSC patients is currently unknown. Damage to the outer blood-retina barrier may lead to antibody formation against photoreceptors and RPE, which could induce aggravation of the chorioretinal disease. However, ARAs may also play a major primary part in CSC pathogenesis.

A relatively frequent occurrence of CSC in patients with immune-mediated diseases such as systemic lupus erythematosus and membranoproliferative glomerulonephritis has been described.[75, 76] This immune predisposition could lead to CSC by the inflammatory disease itself. Moreover, mimicry between microbial proteins and ocular antigens has been hypothesised to be involved in CSC pathogenesis. As such, (subtle) infections may be involved in the pathogenesis of CSC. The effect of the presence of ARAs in CSC patients and the involvement of infections in the occurrence of CSC can be assessed in future larger-scale studies.

CLINICAL CHARACTERISTICS OF CSC

A combination of findings on multimodal imaging leads to the CSC diagnosis. Within this thesis, the distinction of familial CSC from non-familial CSC has been studied, and a definition

of severe CSC has been proposed, in order to be able to divide CSC patients into subgroups. Moreover, knowledge on other diseases that can resemble CSC and should be borne in mind during ophthalmological examination, has been obtained.

Spectrum of phenotypes in CSC and familial CSC

Familial CSC has been found to occur sporadically.[12-15] Within this thesis, findings on multimodal ophthalmological imaging have been described in a unique cohort of families with multiple members affected by CSC (Chapter 2.1), to gain knowledge on CSC etiology and to determine if familial CSC is a separate entity within the CSC spectrum. During imaging of 103 subjects from 23 families, SRF on optical coherence tomography and/or hot spots of leakage on fluorescein angiography indicative of CSC were detected in 45 subjects (44%). Findings characteristic for CSC, but without the presence of SRF on optical coherence tomography and/or hot spots of leakage on fluorescein angiography, were observed in an additional 27 family members (26%), which led to a remarkably high percentage of affected family members.

Age at CSC diagnosis, gender, corticosteroid use, response to different CSC treatments, medical history, and presence of choroidal neovascularisation did not differ when comparing the familial CSC cohort to non-familial CSC patient cohorts, which leads to a challenge in the recognition of familial CSC.[8, 22, 77-81] However, a relatively high percentage of probands was diagnosed with recurrent and/or bilateral CSC, compared to sporadic CSC in literature.[82] The presence of these signs of relatively severe CSC may be indicative of a higher risk of family members to develop CSC, but this warrants further study. The inheritance pattern in most of these families appeared to be autosomal dominant, which has its implications for clinical practice. We recommend regular ophthalmological examination for asymptomatic subclinically affected family members, since abnormalities can increase over time.

New insights into the differential diagnosis of CSC

A serous retinopathy resembling CSC has been previously found to occur as a side effect of treatment with several mitogen-activated protein kinase kinase (MEK) inhibitors. This targeted treatment option, prescribed to an increasing number of patients with metastatic uveal melanoma and metastatic cutaneous melanoma, has an effect on intracellular signal transduction, and induces gene transcription for multiple cellular processes.[83, 84] We found that the time-dependent, reversible retinopathy caused by the MEK inhibitor binimetinib usually does not lead to ophthalmological complaints and a best-corrected visual acuity (BCVA) decrease, and we described this clinical picture as *MEK-associated serous retinopathy* (Chapter 5.1). The occurrence of SRF on optical coherence tomography is usually bilateral and fairly symmetrical, and is detected after a median of 14 days after the start of binimetinib. No other findings typical for CSC are found on multimodal imaging: on optical coherence tomography no RPE changes and no pachychoroid is present, while fluorescein angiography does not show hot

spots of leakage. However, various antiretinal and anti-RPE antibodies can be detected in the peripheral blood of these patients (Chapter 5.1). In MEK-associated serous retinopathy, direct toxicity to the RPE may also be an important pathogenetic factor, apart from a possible role for anti-RPE and antiretinal antibodies.

In this thesis, the molecular mechanism by which the MEK inhibitor binimetinib leads to serous retinopathy has also been analysed (Chapter 5.2). We describe a correlation between inhibition of the mitogen-activated protein kinase (MAPK) signaling pathway, in which MEK is involved, and the serous retinopathy: in retinal cells (both the neuroretina and RPE) the MAPK pathway is inhibited by binimetinib and reactivation occurs after discontinuation of administration of this medication. This mirrors the reversibility of MEK-associated serous retinopathy.

We also describe a similar serous retinopathy during prescription of the MEK inhibitor pimasertib, which is developed for the treatment of metastatic cutaneous melanoma (Chapter 5.3). This serous retinopathy was found in all patients that were included, and like binimetinib did not lead to any visual complaints. The observed abnormalities were assumed to be indicative of more extensive dysfunction of the retina and/or RPE, since lesions occurred both foveally and extrafoveally, which was similar to the serous retinopathy after binimetinib use. Moreover, a retinal vein occlusion occurred in 1 of the patients, which caused unilateral vision loss and necessitated subsequent intravitreal bevacizumab treatment. An effect of MEK inhibition on retinal vasculature has been described to be the underlying cause of retinal vein occlusions, which have been previously found to occur after administrating other MEK inhibitors.[85, 86]

We have also described that a striking pachychoroid and resultant pachychoroid pigment epitheliopathy as well as CSC can be present in myopic patients,[87] despite the fact that myopia has been described to be protective for CSC and that choroidal thickness in myopic patients is generally relatively low.[88-92] Our findings have implications for the therapeutic approach of patients with similar refractive errors and thus a lower risk to develop CSC, since treatment for CSC differs from other diseases that are part of the differential diagnosis of this disease.

As another differential diagnosis of cCSC in patients above the age of 50, we have published on a clinical spectrum of neovascular AMD without drusen in the fellow eye.[93] We have shown that the presence of drusen is not a prerequisite to develop neovascular AMD, despite the fact that drusen have traditionally been considered a *condition sine qua non* for the diagnosis of AMD. Interestingly, the pathophysiological background and therapeutic response in patients with unilateral AMD without drusen in the fellow eye may differ from typical drusen-associated AMD patients. Further classification of patients with neovascular AMD will contribute to the most appropriate treatment for this patient group. Neovascular AMD and neovascular CSC have been suggested to have overlapping phenotypes.[94] After excluding cases of neovascularisation

in patients in whom imaging suggested a CSC background (or any other possibly explaining pre-existent retinal disease), some patients with shallow type 1 neovascularisation/branching vascular network and/or polypoidal choroidal vasculopathy may still be easily mistaken for cCSC. Additional clinical and genetic studies on this phenotype of neovascular AMD without drusen, which may be a heterogeneous spectrum, are carried out to further delineate this intriguing clinical subgroup from diseases such as drusenoid AMD and CSC.

PHOTODYNAMIC THERAPY: THE FIRST-LINE TREATMENT OF CHOICE IN CCSC

Numerous suggested treatment options have emerged in an attempt to accelerate resolution of SRF accumulation in cCSC patients (Table 1), due to the previous lack of large prospective randomised controlled trials.[8] Thus far, photodynamic therapy (PDT) with reduced settings, high-density subthreshold micropulse laser (HSML) treatment, and oral administration of MR antagonists (either eplerenone or spironolactone) have been described as the most promising treatments.[8, 93] Out of these options, half-dose PDT and HSML treatment are most often used. However, in several countries reimbursement restrictions regarding cCSC treatment exist, since the optimal treatment for cCSC has only been studied retrospectively and included patient groups differ among these studies, which further stresses the need for randomised controlled trials to facilitate reimbursement for viable treatments.

The PLACE trial: the first large prospective multicenter randomised controlled treatment trial for cCSC

In this thesis, we present the results of the PLACE trial, the much-anticipated prospective randomised controlled trial comparing the two most-used laser treatment options for cCSC (Chapter 6.1). In PLACE (**P**hotodynamic therapy versus micropulse **la**ser treatment in chronic **ce**ntral serous chorioretinopathy; clinicaltrials.gov identifier: NCT01797861; EudraCT number: 2012-004555-36), conducted in 5 centers in 4 European countries, the effect of half-dose PDT and HSML treatment in cCSC has been studied, in terms of complete resolution of SRF on optical coherence tomography (primary outcome measure) and outcome on BCVA, microperimetry, and a questionnaire on visual functioning (secondary outcome measures).

We found that the efficacy of half-dose PDT with respect to complete resolution of SRF was significantly higher compared to HSML treatment in both short-term and long-term follow-up visits. Moreover, functional outcome was better following half-dose PDT compared to HSML treatment with respect to both increased retinal sensitivity on microperimetry at the first and final evaluation visit and increased BCVA at the first evaluation visit. In contrast, the treatment groups did not differ significantly with respect to BCVA at the final evaluation visit or overall quality of life (measured using a questionnaire on visual functioning). The better outcome of PDT treatment

may be attributed to the fact that PDT targets the choroidal tissue, which appears to be the tissue primarily affected in cCSC. This is in contrast with HSML treatment, during which delivery of brief subthreshold micropulses to the RPE has been suggested to induce the production of intracellular biological factors that stimulate RPE function. This does not cause visible damage to the retina, and most probably does not have an effect on choroidal dysfunction.[94]

Table 1. Therapeutic interventions in central serous chorioretinopathy

Conventional thermal laser treatment	Argon laser
Photodynamic therapy (PDT)	PDT with standard settings Half-dose PDT Half-fluence PDT Half-time PDT
High-density subthreshold micropulse laser treatment	577 nm wavelength 810 nm wavelength
Anti-vascular endothelial growth factor treatment	Aflibercept (Eylea ®) Bevacizumab (Avastin ®) Ranibizumab (Lucentis ®)
Systemic treatment	5α reductase inhibitor *finasteride* Antiplatelet treatment *aspirin* Antimetabolite treatment m*ethotrexate* Beta blocker *nadolol* Carbonic anhydrase inhibitor *acetazolamide* Glucocorticoid receptor antagonist *mifepristone* Helicobacter pylori infection treatment *amoxicillin and metronidazole* *amoxicillin, clarithromycin, and omeprazole* Mineralocorticoid receptor antagonist *eplerenone* *spironolactone*

In the HSML treatment group, the laser power was in the upper range of subthreshold settings, and adjacent laser spots were applied in order to achieve confluent treatment coverage; in addition, the area to be treated was relatively large and guided by indocyanine green angiography. Despite these measures in order to prevent possible undertreatment with HSML,[95] treatment outcome was still significantly more favorable in the group that received half-dose PDT. The pivotal findings in the PLACE trial in favor of half-dose PDT may alleviate reimbursement restrictions for the off-label use of verteporfin in PDT for the treatment of cCSC that currently exist in numerous countries, also because of the fact that no treatment-related adverse events have been observed during the trial.

PDT in patients with severe CSC

Between cCSC cases, the extent of retinal abnormalities may vary strongly. A significant subgroup of cCSC patients displays more widespread RPE atrophy, diffuse leakage on angiography imaging, posterior cystoid retinal degeneration (a combination of photoreceptor atrophy and intraretinal fluid), subretinal fibrin, and choroidal neovascularisation.[98-102] We have studied the clinical spectrum and therapeutic outcome of patients with characteristics that are presumed to be typical for more severe CSC. Most importantly, these patients with severe cCSC were found to respond to reduced-settings PDT, in terms of BCVA increase and resolution of SRF, in a way that was comparable to patients with clinical cCSC that would not qualify as severe. Therefore, it seems that reduced-settings/half-dose PDT is also the treatment of choice in more severe cCSC cases that partially do not comply with the PLACE trial inclusion criteria, for instance because of the presence of intraretinal fluid without CNV or the presence of diffuse atrophic RPE alterations.(paper by Mohabati et al., under review)

PDT in CSC patients who use corticosteroids

As corticosteroid use is the most evident risk factor for CSC, with described odds ratios of up to 37,[19] cessation of the use of this medication is the first step in CSC treatment, despite the fact that it is unknown in what percentage of patients this discontinuation could lead to SRF resolution.[47] Since corticosteroid treatment cannot be ceased in all patients, due to the severity of some medical conditions for which this type of medication is prescribed, the outcome of PDT in this patient group has been studied by our group.[103] Moreover, we assessed if patients with steroid-related CSC respond in a similar way to PDT in comparison with CSC patients who have never used steroids. Based on the results of our study, we concluded that PDT is equally effective in both patient groups and we found that continuation of corticosteroid use did not adversely influence treatment outcome. However, in CSC patients it is still required to discuss a possible stoppage of corticosteroid use in an early stage, as this can lead to complete SRF resolution.

PDT in CSC patients with SRF outside the fovea

Historically, only cCSC patients with visual complaints due to foveal SRF have been considered eligible for treatment. Despite the risk of complications after PDT,[104-106] and the fact that extrafoveal SRF relatively seldom leads to visual symptoms, significant complaints can also occur in these patients. In this thesis, we describe a resolution of extrafoveal SRF in all PDT-treated patients, together with a decrease in complaints in 47% of 15 patients (Chapter 6.2). We hypothesise that treating this patient group led to preventing permanent photoreceptor damage. In addition, a significant decrease in choroidal thickness after PDT treatment was shown both foveally and at the location of the most pronounced SRF, which may also reduce the risk of future disease recurrence, although larger studies with longer follow-up are needed to address this topic.

Based on the findings in this thesis, we conclude that half-dose PDT should be the first cCSC treatment option both for patients with foveal SRF and for patients with extrafoveal SRF and marked visual complaints. Moreover, when a patient currently uses corticosteroids that cannot be discontinued due to a severe medical condition, PDT with reduced settings is the first-line treatment of choice for cCSC. Currently existing reimbursement restrictions for the off-label use of verteporfin in treating cCSC should thus be reconsidered.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The studies presented in this thesis have provided important new insights from pathogenesis to treatment of CSC, and show that combining interdisciplinary clinical, genetic, and fundamental research is essential to understand complex diseases such as CSC.

In this thesis, we presented information on the genetic background of CSC patients, and further highlighted a role of the complement system, for which the functional translation should be focused on during future research. Antiretinal antibodies were also found to be relevant in CSC, but its exact role still has to be discovered. Moreover, the association we found between several haplotypes of the MR gene (*NR3C2*) and CSC is a genetic confirmation of the possible role of MR in CSC pathogenesis, which is targeted therapeutically by MR antagonists such as eplerenone and spironolactone. Because CSC has also been described to develop as a result of administration of GR-selective glucocorticoids in many patients, the role of MR and GR in the pathogenesis of CSC can be clarified by research that we are currently conducting: the effect of administration of cortisol (which binds to both MR and GR), aldosterone (which binds to MR), and dexamethasone (which binds to GR) on gene expression of CECs, in which the origin of CSC can most probably be found, is being assessed by performing RNA sequencing. Additionally,

exome sequencing data of patients with familial CSC, which we are interpreting at present, will contribute to gain further genetic and pathophysiological insight into CSC pathogenesis.

To further study the consequences of hypercortisolism, which has been described to be the most pronounced risk factor for CSC, we performed extensive ophthalmological phenotyping and detected retinal abnormalities in 59% of asymptomatic renal transplant patients who had used a high cumulative dose of oral corticosteroids, including findings characteristic for (the spectrum) of CSC in 45% of these. This confirms the importance of corticosteroids as a risk factor for CSC, but also leads us to conclude that the susceptibility to develop these changes depends on other (yet unknown) risk factors. For example, interindividual genetic and local anatomical differences could explain our findings. However, it is still questionable if a straightforward causal relationship between this exogenous hypercortisolism and findings characteristic for CSC exists in a subset of patients.

Based on the first large endocrinological screening of CSC patients, we conclude that biochemical screening for hypercortisolism in CSC patients is not required, unless additional clinical features that could be typical for endogenous hypercortisolism (Cushing's syndrome) are present. However, our results indicate that CSC is associated with increased activity of the HPA axis, which could therefore be important for research on pathophysiology and disease management of CSC. In contrast with available literature, we have shown that cCSC disease activity is not associated with psychosocial stress, concluding that stress reduction may not be of important benefit to improve CSC. We also did not find evidence for a higher prevalence of type A personality characteristics in cCSC patients.

A spontaneous resolution of or decrease in SRF is not uncommon in CSC, illustrating the difficulty using retrospective, non-randomised, non-controlled studies in assessing whether a treatment in cCSC is successful. On the basis of several large studies on the treatment of cCSC that have been included in this thesis, mandatory steps have been taken towards an evidence-based treatment guideline that is currently lacking for cCSC. We performed the PLACE trial, the first-ever large multicenter prospective randomised controlled treatment trial for cCSC, which led us to conclude that half-dose PDT is superior to HSML. Our additional treatment studies lend further support to the conclusion that PDT with reduced settings should be the first-line treatment of choice for cCSC. To be able to perform this treatment in such patients, currently existing reimbursement restrictions for the off-label use of verteporfin in treating cCSC may have to change in many countries. Still, in a noteworthy percentage of cCSC patients a complete resolution of SRF cannot be achieved after PDT with reduced settings, which necessitates additional research for optimal treatments.

Recently, oral medication with MR antagonists has been introduced for treatment of cCSC, and its worldwide use to treat cCSC has increased notably. However, the efficacy of this type of medication is under debate. Both spironolactone and eplerenone can be administered to induce antagonism of MR, but eplerenone treatment has been found to induce less hormonal side effects compared to spironolactone. Again, until recently large prospective randomised controlled trials were lacking for this treatment of cCSC. Several hospitals in Ireland and the United Kingdom then started to include patients in the VICI trial, in which participants are randomly allocated to be treated with either eplerenone or a placebo capsule containing no active ingredients. To compare the effect of eplerenone to a standard half-dose PDT, our group commenced the SPECTRA trial (clinicaltrials.gov identifier: NCT03079141; EudraCT number: 2016-004119-11). The SPECTRA trial is therefore expected to provide important additional comparative insights into the treatment of cCSC in addition to the PLACE and VICI trials. Its results will enable to establish an evidence-based treatment guideline for cCSC that incorporates the 3 current most-used treatments for cCSC: PDT, eplerenone, and HSML treatment. A best-practice treatment guideline will improve visual outcome and quality of life of this relatively frequently occurring retinal disease.

We have prepared to study the interactions between the most prominent risk factors for CSC in a superior human-based model using both post-mortem choroid and pluripotent induced stem cells out of the blood of patients and healthy controls. These will contribute to the identification of tissue-specific disease mechanisms in CSC, and may identify new treatment targets. We will perform the administration of its most pronounced risk factor (corticosteroids) to the cell type in which the origin of CSC can most probably be found, with which we have already acquired unique experience during the last few years. Because corticosteroids affect primarily expression of genes (leading to differences in protein and function), we will undertake genome-wide analysis of gene expression, and this open approach maximises the chance to identify the actual mechanism of disease. Since we will also take gender into account during the analysis of the effect of corticosteroids on human stem cell-based CECs, we will combine the 3 most important risk factors for CSC.

The outcome of clinical, genetic, and fundamental research thus has to be combined to further identify mechanisms that could be involved in the origin of CSC, and to provide further guidance in translating insights from bench to bedside and detecting optimal and – preferably – personalised CSC treatment.

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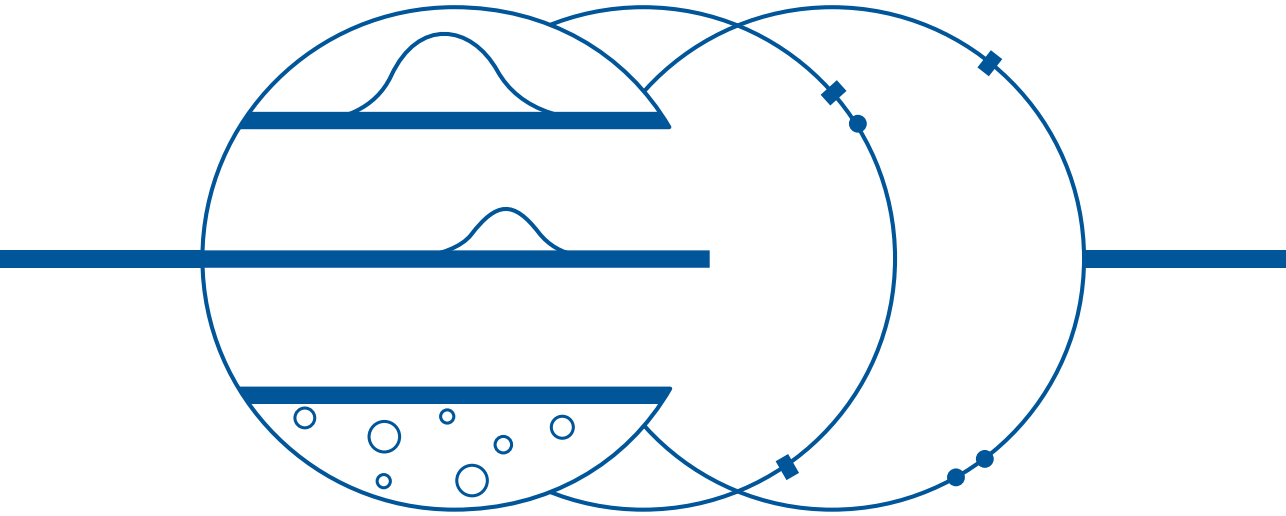
8

DUTCH SUMMARY
ACKNOWLEDGEMENTS
ABOUT THE AUTHOR
LIST OF PUBLICATIONS



DUTCH SUMMARY

Chronische centrale sereuze chorioretinopathie (CSC) is een oogziekte waarbij er een vochtophoping onder het centrale deel van het netvlies (de *retina*) ontstaat. Dit vocht ontstaat hoogstwaarschijnlijk onder invloed van afwijkingen in het vaatvlies (het *choroïd*), dat bij CSC patiënten vaak verdikt en hyperpermeabel is. Deze choroïdale abnormaliteiten leiden tot schade aan het retinaal pigment epitheel, waardoor vocht naar onder het netvlies lekt. Patiënten met CSC kunnen een breed scala aan visusklachten ontwikkelen; dit treedt met name op wanneer het vocht het centrale deel van de retina (de *fovea*) bereikt.
De ziekte wordt vooral gezien bij mannen die een leeftijd van tussen de 30 en 60 jaar hebben. Daarnaast worden exogeen hypercortisolisme (gebruik van corticosteroïd-houdende medicatie) en endogeen hypercortisolisme (syndroom van Cushing) beschouwd als de belangrijkste risicofactoren voor CSC, terwijl een associatie met stress en met de type A persoonlijkheid eveneens beschreven is. Echter, ondanks dat CSC de op drie na meest voorkomende 'natte maculopathie' is, is over het ziektemechanisme, de klinische kenmerken en de optimale behandeling nog veel onbekend.
Met dit proefschrift wordt beoogd inzicht te krijgen in de genetische en klinische aspecten van CSC, en hiermee meer te weten te komen over ziektemechanismen die een rol spelen. Bovendien is het doel kennis te verwerven over de optimale behandeling van deze chorioretinale ziekte; de uitkomsten van de eerste grote gerandomiseerde gecontroleerde behandelstudie voor chronische CSC (de PLACE trial) zijn hiervoor van evident belang. Door de onderzoeken die deel uitmaken van dit proefschrift zal men kennis verkrijgen over de ziekte zelf en haar optimale behandeling, waardoor afname van zowel visus als visus-gerelateerde kwaliteit van leven voorkomen kan worden.

Hoofdstuk 1 is de algemene introductie van dit proefschrift. In dit hoofdstuk wordt de anatomie van het oog, en in het bijzonder van retina en choroïd, beschreven. Bovendien worden technieken voor de evaluatie van deze structuren besproken. Het tweede deel van de algemene introductie behelst een inleiding over CSC, waarbij specifiek wordt ingegaan op de pathogenese, behandelopties en differentiaal diagnose van deze veelvoorkomende chorioretinale ziekte.

Hoofdstuk 2 bevat studies waarin we informatie hebben verzameld over de genetische oorzaak van CSC. In **hoofdstuk 2.1** voerden wij uitgebreide oogheelkundige fenotypering uit in families waarin meerdere leden CSC hadden. Dit leidde tot de detectie van (eerder) actieve CSC, zich presenterend door ten minste de aanwezigheid van subretinaal vocht op de optische coherentie tomografie scan of lekkage op de fluorescentie angiogram, in 44% van de 103 onderzochte personen. In nog eens 26% van de onderzochte personen werden afwijkingen gevonden die karakteristiek waren voor CSC, maar bij deze personen werd geen actieve ziekte gezien. Een relatief groot percentage van de probands in onze studie had ernstige CSC, en het overervingspatroon van de ziekte was autosomaal dominant in de meeste families. **Hoofdstuk 2.2** beschrijft de uitkomst van een studie waarin de associatie van varianten in

de glucocorticoïd receptor (GR) en de mineralocorticoïd receptor (MR) met chronische CSC bekeken is. Aangezien het gebruik van corticosteroïden een belangrijke risicofactor voor CSC is, en deze hormonen aan de GR en de MR binden, is betrokkenheid van beide receptoren voor CSC eerder gesuggereerd. Wij vonden een veranderd risico op CSC in patiënten met bepaalde varianten in het *NR3C2* gen, wat de rol van het MR gen in CSC benadrukt. Voor het *NR3C1* gen, dat codeert voor de GR, vonden we dit niet. In **hoofdstuk 2.3** is de uitkomst van de eerste genoombrede associatiestudie ('genome-wide association study') opgenomen. Naast de al bekende varianten in het *complement factor H* gen werden in deze studie in dit gen andere varianten gevonden, die leiden tot een veranderd CSC-risico. Bovendien werden bij het voorspellen van expressie van enkele andere genen die betrokken zijn bij de regulering van het complement systeem – onderdeel van het aangeboren immuunsysteem – verschillen gezien tussen patiënten en controlepersonen, wat het belang van het complement systeem in de pathogenese van CSC benadrukt.

Hoofdstuk 3 beschrijft de uitkomsten van studies waarin systemische abnormaliteiten in CSC patiënten bekeken zijn. De uitkomst van complete endocrinologische fenotypering van chronische CSC patiënten is terug te lezen in **hoofdstuk 3.1** en **hoofdstuk 3.2**. Ondanks dat in de literatuur een verband tussen endogeen hypercortisolisme (syndroom van Cushing) en CSC beschreven is, vonden wij in geen van de 86 onderzochte patiënten biochemische criteria die zouden kunnen wijzen op aanwezigheid van het syndroom van Cushing. Echter, tekenen van verhoogde activiteit van de hypothalamus-hypofyse-bijnieras in de geanalyseerde patiënten waren wél aanwezig, wanneer deze vergeleken werden met gezonde controlepersonen. Zodoende is endocrinologische screening van CSC patiënten slechts nodig wanneer zij zich presenteren met klinische kenmerken die bij het syndroom van Cushing zouden kunnen passen. In onze systematische cross-sectionele studie vonden wij bovendien geen aanwijzingen dat stress geassocieerd is met ziekteactiviteit in CSC, wat om die reden tegen het adviseren van stressreductie als behandeling van CSC pleit. Eveneens vonden we niet dat CSC patiënten vaker een type A persoonlijkheid hebben dan gezonde controlepersonen, wat de 'mythe' rondom persoonlijkheidstype en CSC weerlegt.
In **hoofdstuk 3.3** is nader onderzoek verricht naar de betrokkenheid van het complement systeem bij chronische CSC. In vergelijking met gezonde controlepersonen werden in het bloed van patiënten geen aanwijzingen gevonden voor activatie of inhibitie van het complement systeem. Zodoende is het functionele effect van de varianten in genen die betrokken zijn bij het complement systeem nog onbekend, en zal dit in toekomstig onderzoek bekeken moeten worden. Het functionele effect van antiretinale antistoffen, beschreven in **hoofdstuk 3.4**, moet eveneens nog bepaald worden; deze antistoffen, die in patiënten met chronische CSC significant vaker voorkomen dan in controlepersonen, kunnen een belangrijke primaire rol spelen in CSC of juist secundair aan chronische schade van het netvlies ontstaan.

Hoofdstuk 4 gaat verder in op de rol van steroïden in CSC. **Hoofdstuk 4.1** beschrijft de uitkomsten van uitgebreide oogheelkundige fenotypering in patiënten met primair hyperaldosteronisme (syndroom van Conn). Abnormaliteiten die passen bij (het spectrum van) CSC werden gevonden in 54% van de gescreende patiënten. Dit onderzoek bevestigt de belangrijke rol voor de MR in de pathogenese van CSC, zoals beschreven in hoofdstuk 2.2, aangezien het steroïdhormoon aldosteron enkel bindt aan die receptor. Voor het steroïdhormoon cortisol is dit anders, aangezien het aan zowel de MR als de GR bindt. In **hoofdstuk 4.2** is de uitkomst van oogheelkundige fenotypering van 37 patiënten, die minimaal 2 jaar lage doses corticosteroïden gebruikten na een transplantatie, terug te lezen. In 59% van deze patiënten, die geen oogklachten hadden, werden retinale abnormaliteiten gevonden, waarvan 45% karakteristiek was voor (het spectrum van) CSC. Wij concluderen hieruit dat CSC slechts ontstaat in patiënten met een bepaalde susceptibiliteit voor (cortico)steroïden, en dat andere risicofactoren eveneens van belang zijn. Echter, het verband tussen hypercortisolisme en CSC is evident: in **hoofdstuk 4.3** beschrijven wij in een retrospectief onderzoek dat chronische CSC in 4 van onze patiënten een eerste manifestatie van het syndroom van Cushing bleek, zonder dat daar andere symptomen van mogelijk hypercortisolisme bij gevonden werden.

Hoofdstuk 5 voegt kennis toe aan de diffentiaal diagnose van CSC. In **hoofdstukken 5.1, 5.2 en 5.3** wordt de *MEK-geassocieerde sereuze retinopathie* bestudeerd. Deze tijdsafhankelijke, reversibele sereuze retinopathie, die over het algemeen niet leidt tot visusklachten, ontstaat als bijwerking bij het gebruik van mitogeen-geactiveerde proteïne kinase kinase (MEK) remmers, die frequent voorgeschreven worden aan patiënten met gemetastaseerd uveamelanoom en gemetastaseerd huidmelanoom. In **hoofdstuk 5.1 en 5.2** beschrijven wij het ontstane beeld na toediening van de MEK remmer binimetinib, terwijl **hoofdstuk 5.3** ingaat op oogheelkundige veranderingen die kunnen worden geïnduceerd door de MEK remmer pimasertib.

In **hoofdstuk 6** wordt ingegaan op de optimale behandeling van CSC. In **hoofdstuk 6.1** presenteren we de uitkomsten van de eerste grote gerandomiseerde gecontroleerde behandelstudie voor chronische CSC (de PLACE trial). Op basis van deze door onderzoekers geïnitieerde studie, die uitgevoerd werd in 5 ziekenhuizen in 4 Europese landen, concluderen wij dat fotodynamische therapie met halve dosis verteporfine superieur is aan behandeling met high-density subthreshold micropuls laser. Dit geldt zowel voor de primaire uitkomstmaat van de studie (compleet verdwijnen van subretinaal vocht op de optische coherentie tomografie scan) als voor enkele secundaire uitkomstmaten (visus, retinale sensitiviteit bij microperimetrie, visus-gerelateerde kwaliteit van leven op basis van beantwoording van een vragenlijst). Zodoende zou fotodynamische therapie beschouwd moeten worden als de behandeling van voorkeur voor chronische CSC, en lijkt de weg vrijgemaakt voor het veranderen van bestaande restricties in het vergoeden van fotodynamische therapie voor de behandeling van chronische CSC. Dit wordt bovendien kracht bijgezet door **hoofdstuk 6.2**. Hierin wordt het resultaat van fotodynamische

therapie bestudeerd in een patiëntengroep die visusklachten had, en subretinaal vocht dat zich enkel buiten de fovea bevond. In alle patiënten verdween het subretinale vocht na behandeling, en genoemde visusklachten namen af bij 47% van hen.
In beide studies werden complicaties en klachten na fotodynamische therapie met halve dosis verteporfine zelden tot nooit gezien.

Hoofdstuk 7 is de algemene discussie van dit proefschrift, waarin de belangrijkste bevindingen worden samengevat en in een breder perspectief worden geplaatst. Het eerste deel van de discussie gaat in op de pathogenese van CSC: associaties van CSC met achtereenvolgens corticosteroïden en de hypothalamus-hypofyse-bijnieras, het complement systeem en antiretinale antistoffen worden bediscussieerd. Het belang van bekende genetische associaties en van genetische associaties die wij gevonden hebben wordt besproken, tezamen met de uitkomst van onze uitgebreide oogheelkundige fenotypering van patiënten die mogelijk 'at risk' zouden zijn voor het ontwikkelen van CSC. Bovendien wordt de noodzaak tot endocrinologische screening van CSC patiënten en bepaling van aanwezigheid van antiretinale antistoffen en de activiteit van het complement systeem in deze patiëntengroep bediscussieerd. Eveneens wordt ingegaan op klinische karakteristieken van CSC: familiale CSC is in kaart gebracht, en de differentiaal diagnose van CSC is uitgebreid. Hierna wordt de optimale therapie van CSC besproken, waarin een prominente rol weggelegd is voor fotodynamische therapie. De discussie wordt afgesloten met het bespreken van toekomstige studies, die van belang zullen zijn voor het verkrijgen van nog meer inzicht in de ziektemechanismen en optimale behandeling van CSC.

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ABOUT THE AUTHOR

Elon Hendrik Cornelis van Dijk was born on the 14th of August, 1990, in Rotterdam, The Netherlands. After his graduation with honors from the Marnix Gymnasium in Rotterdam in 2007, he started to study Medicine at the Erasmus University Medical Center Rotterdam. As a part of his medical school, he spent time in Barcelona (Spain), Cotonou (Benin (Africa)), and Paramaribo (Surinam (South America)). He developed a special interest in ophthalmology, which led to doing his senior clinical and research internship at the Rotterdam Eye Hospital/ Rotterdam Ophthalmic Institute, under supervision of Prof. dr. Jan C. van Meurs. This further increased his enthusiasm for research in ophthalmology, and led to completing his clinical rotations and obtaining his medical degree with honors.

In April 2014, Elon started working on the PhD project "Central serous chorioretinopathy – from pathogenesis to treatment", which was supervised by Prof. dr. Camiel J.F. Boon and Prof. dr. Gré P.M. Luyten, at the Department of Ophthalmology at Leiden University Medical Center in The Netherlands. The research results are presented in this thesis.

During his PhD, Elon received the Dutch Association for Research in Vision and Ophthalmology (ARVO) Travel Grant in 2016 and the Young Retina Specialists 'Science Slam' award during the EURETINA congress in 2017. He was co-organiser of the 'Dutch Ophthalmology PhD Students' (DOPS) congress, and currently is a board member of the Foundation for Extracurricular Activities for Dutch Residents in Ophthalmology. Moreover, he finished the Ride New York (cycling 660 kilometers in 4 days), in order to collect money for the Foundation 'Niet Blind', and ran the Rotterdam Marathon. He has presented his scientific work at several national and international conferences, and was co-applicant for several subsidies that have been awarded to the group of Prof. dr. Camiel J.F. Boon.

In November 2017, Elon commenced his residency in ophthalmology at the Leiden University Medical Center.

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