Dissertation

The role of endogenous thrombin potential, fibrinolytic markers and their functional gene polymorphisms in the pathogenesis of central serous chorioretinopathy

submitted by

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Declaration

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the "*Standards of Good Scientific Practice and Ombuds Committee at the Medical University of Graz*".

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Disclosure

Parts of this thesis were published in:

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All co-authors have explicitly agreed to the use of their data in this thesis. Permission for reproduce the content (figures and tables) in this doctoral thesis from the respective copyright holders Taylor & Francis have been obtained.

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Index

Abbreviations

A

A	- Adenine
ACTH	- Adrenocorticotropic hormone
ADP	- Adenosine diphosphate
AF	- Autofluorescence
AMD	- Age-related macular degeneration
AP	- Alternative complement pathway
aPTT	- Activated partial thromboplastin time
AT	- Antithrombin
AUC	- Area under the curve

B

BCVA	- Best corrected visual acuity

C

C	- Cytosine
Ca	- Calcium
C4	- Complement component 4
CCS	- Chorioretinopathia centralis serosa
CFH	- Complement factor H
CI	- Confidence interval
C max	- Peak height
CRP	- C reactive protein
CSC	- Central serous chorioretinopathy
CV	- Coefficient of variation

D

DCP	- Deep capillary plexus
DNA	- Deoxyribonucleic acid

E

ECM - Extracellular matrix

EDTA - Ethylenediaminetetraacetic acid

ETP - Endogenous thrombin potential

EZ - Ellipsoid zone

F

F I - Fibrinogen

F Ia - Fibrin

F II - Prothrombin

F IIa - Thrombin

FA - Fluorescein angiography

FAM - 6-carboxyfluorescein

FLA - Fluoreszenzangiographie

FRET - Förster resonance energy transfer

G

G - Guanine

GCL - Ganglion cell layer

GOT - Glutamic oxaloacetic transaminase

GP - Glycoprotein

GPT - Glutamate-pyruvate transaminase

GR - Glucocorticoid receptor

H

HbA1c - Glycated hemoglobin (hemoglobin A1c)

HPA - Hypothalamic- pituitary axis

I

ICG - Indocyanine green angiography

ICP - Intermediate capillary plexus

INL - Inner nuclear layer

IPL - Inner plexiform layer

IR- AF - Near infrared autofluorescence

L

LDL - Low-density lipoprotein

Lp(a) - Lipoprotein A

M

MMP - Matrix metalloproteinase

MR - Mineralocorticoid receptor

MZ - Myoid zone

N

n - Number

NFL - Nerve fiber layer

O

OCT - Optical coherence tomography

ONL - Outer nuclear layer

OPL - Outer plexiform layer

OR - Odds ratio

P

PAF - Platelet activator factor

PAI-1 - Plasminogen activator inhibitor 1

PED - Pigment epithelial detachment

PLAT - Gene of tissue plasminogen activator

PC - Protein C

PCR - Polymerase chain reaction

PS - Protein S

PT - Prothrombin

R

RPCP - Radial peripapillary capillary plexus

RPE - Retinal pigment epithelium

S

SCP - Superficial capillary plexus

SD - Standard deviation

SD-OCT - Spectral domain optical coherence tomography

SERPINE 1 - Gene of plasminogen activator inhibitor 1

SNP - Single nucleotide polymorphism

SVP - Superficial vascular plexus

SW- AF - Short wavelength autofluorescence

T

T - Thymine

TAFI - Thrombin activatable fibrinolysis inhibitor

TF - Tissue factor

TFPI - Tissue factor pathway inhibitor

TGF- β - Transforming growth factor beta

TIMP - Tissue inhibitors of matrix metalloproteinases

T max - Time to peak

t-PA - Tissue-type plasminogen activator

TXA2 - Thromboxane A2

U

U-PA - Urokinase- type plasminogen activator

V

VEGF - Vascular endothelial growth factor

VIC -Fluorescent dye with an absorbance maximum of 538 nm and an emission maximum of 554 nm

vWF - Von Willebrand factor

Figures

Tables

Abstract

Background:

Central serous chorioretinopathy (CSC) is a common chorioretinal disease, characterized by choroidal hyperpermeability leading to neurosensory and/or retinal pigment epithelial detachments. Hypofibrinolysis due to higher plasma concentrations of plasminogen activator type 1 (PAI-1) or lower activity of tissue-type plasminogen activator (t-PA) has been implicated in the pathogenesis of CSC. Clotting factors, fibrinolysis markers and functional polymorphisms in the PAI-1 (SERPINE 1) and t-PA (PLAT) gene are thus potential risk factors for CSC.
The aim of the present thesis was therefore to investigate a hypothesized association between endogenous thrombin potential (ETP), a global coagulation marker, t-PA antigen plasma levels and the PAI-1 4G/5G and the t-PA -7351C>T gene variants and the presence of CSC.

Methods:

The present dissertation includes data from two different studies.
First, ETP and t-PA antigen plasma levels were investigated in 59/ 63 patients and 58 control subjects. ETP was determined with INNOVANCE® ETP (BCS® XP Coagulation System, Siemens Healthineers, Vienna, Austria), whereas t-PA antigen levels were determined with Quantikine® ELISA (R&D Systems, Inc., Minneapolis, USA).
In a second step, we collected blood samples of 172 CSC patients and 313 control subjects for genotyping of the PAI-1 4G/5G and the t-PA -7351C>T polymorphisms.
Genotypes of the aforementioned polymorphisms were determined by TaqMan™ fluorogenic 5´-exonuclease assays (Applied Biosystems, Vienna, Austria).

Results:

Mean ETP levels (375.0 +- 57.2 mE vs. 367.1 +- 51.3 mE, p=0.43) did not significantly differ between patients and controls. Mean plasma t-PA antigen concentrations were significantly higher in CSC patients compared to controls (3673.1 +- 1281.6 pg/ml vs. 3228.0 +- 1079.8 pg/ml, p=0.04). Allelic frequencies or genotype distributions of neither the PAI-1 4G/5G nor

the t-PA -7531C>T polymorphisms were significantly different between patients with CSC and control subjects (PAI-1 4G/4G: 24.4% vs. 20.4, p=0.36; t-PA -7351CC: 42.4% vs. 46.0%, p=0.50). After adjusting for age and gender presence of the PAI-1 4G/4G genotype was associated with a non-significant odds ratio of 1.21 (95% confidence interval [95% CI]: 0.77 - 1.92, p=0.41), while homozygosity for the t-PA -7351C allele yielded a non-significant odds ratio of 0.91 (95% CI: 0.62 – 1.33, p=0.62) for CSC.

Conclusion:

The present study indicates that increased t-PA antigen plasma concentrations are associated with a higher risk for CSC. In contrast, the present data suggest that neither ETP levels nor the - t-PA -7351C>T and the PAI-1 4G/5G gene polymorphisms are major risk factors for CSC.

Abstract in German

Hintergrund:

Die Chorioretinopathia centralis serosa (CCS) ist eine häufige, chorioretinale Erkrankung, die durch choroidale Hyperpermeabilität zu einer neurosensorischen Abhebung und/ oder retinalen Pigmentepithelabhebung führen kann. Hypofibrinolyse, gekennzeichnet durch erhöhte Plasmakonzentrationen des antifibrinolytischen Parameters Plasminogen Aktivator Inhibitor 1 (PAI 1) und reduzierte Aktivität des profibrinolytischen Gewebsplasminogen-Aktivators (t-PA) wurden in der Literatur bereits im Zusammenhang mit der Entstehung der Chorioretinopathia centralis serosa postuliert. So wurden in dieser Arbeit Gerinnungsfaktoren (endogenes Thrombinpotenzial und t-PA) sowie assoziierte funktionelle Genpolymorphismen (PAI-1 4G/5G, t-PA-7351C>T) als Risikofaktoren in der Pathogenese der Chorioretinopathia centralis serosa untersucht.

Methoden:

Im ersten Teil der Dissertation wurden Plasmaspiegel von ETP und t-PA Antigen von 59/63 PatientInnen und 58 Kontrollen untersucht. ETP wurde mittels INNOVANCE® ETP (BCS® XP System, Siemens Healthineers, Vienna, Austria) gemessen, während t-PA Antigenwerte mittels Quantikine® ELISA (R&D Systems, Inc., Minneapolis, USA) analysiert wurden.
In einem zweiten Teil wurden bei 172 PatientInnen und 313 KontrollprobandInnen die Verteilungen der Genpolymorphismen PAI-1 4G/5G sowie t-PA -7351C>T untersucht. Die Genotypen beider Polymorphismen wurden mittels TaqMan^{TM} fluorogenic 5´-exonuclease assays (Applied Biosystems, Vienna, Austria) bestimmt.

Ergebnisse:

Im Rahmen der Studie konnten wir keinen Unterschied von ETP (375,0+- 57,2 mE vs. 367,1 +- 51,3 mE, p=0,43) zwischen PatientInnen mit CCS und der Kontrollgruppe finden. Die mittleren Plasmakonzentrationen von t-PA Antigen waren signifikant höher in der Patientengruppe verglichen mit der Kontrollgruppe (3673,1 +- 1281,6 pg/ml vs. 3228,0 +- 1079,8 pg/ml, p=0,04).
Die Allelhäufigkeiten und Genotypverteilungen unterschieden sich weder im Fall von PAI-1 4G/5G noch beim t-PA -7351C>T Genpolymorphismus signifikant zwischen PatientInnen

und Kontrollen (PAI-1 4G/4G: 24,4% vs. 20,4; p=0,36; t-PA -7351CC: 42,4% vs. 46,0%; p=0,50). Nach Adjustierung für Alter und Geschlecht waren die errechneten Odds Ratio (ORs) weder für den PAI-1 4G/4G Genotyp (OR 1,21; [95% Konfidenzintervall (KI)]: 0,77 – 1,92; p=0,41) noch den t-PA -7351 CC Genotyp (OR 0,91; [95% KI]: 0,62 – 1,33; p=0,62) signifikant erhöht.

Schlussfolgerung:

Die Daten der vorliegenden Arbeit weisen darauf hin, dass erhöhte Plasmakonzentrationen von t-PA Antigen mit einem erhöhten Risiko für eine CCS assoziiert sind. Hingegen konnte für die beiden untersuchten Genvarianten bzw. dem endogenen Thrombinpotential kein Nachweis als bedeutender Risikofaktor für die CCS erbracht werden.

Introduction

Background

Central serous chorioretinopathy (CSC), a chorioretinal disorder, was first described by von Graefe in 1866 erroneously as „relapsing central luetic retinitis " [1]. It is considered to be the fourth most common nonsurgical retinal disease after age-related macular degeneration (AMD), diabetic retinopathy and branch retinal vein occlusion [2].
Over the last century, CSC was classified according to descriptive terms connected to its pathogenesis, clinical manifestation and characteristic findings in fluorescein- and indocyanine green angiography. Terms such as central serous retinopathy [3], idiopathic central serous choroidopathy [4] and diffuse retinal pigment epitheliopathy were put forward over the last decades. In 1967, Gass characterized the currently favoured term of central serous chorioretinopathy [4].

Abnormal hyperpermeability of choroidal vessels resulting in increased hydrostatic tissue pressure and disturbed ion transport across the retinal pigment epithelium (RPE) have been implicated in the pathogenesis of CSC. This may lead to pigment epithelium detachment (PED) and disruption of the overlying RPE, which causes fluid accumulation below the neurosensory retina. In recent years, new imaging modalities such as optical coherenence tomography (OCT) confirmed the suspected pathogenesis of CSC by providing evidence of an increase in choroidal thickness in patients with acute or chronic CSC. Consequently, CSC counts to the spectrum of pachychoroid diseases. Furthermore, indocyanine green (ICG)-angiography findings, which demonstrated choroidal vacular abnormalities such as choroidal venous dilation, filling defects and hyperpermeability leading to focal choroidal hyperfluorescence, highlight the role of an abnormal choroidal circulation as a potential cause for the disease [5].

Epidemiology

The best documented incidence of the disease is provided by a population- based retrospective cohort and case- control study performed in Olmstedt County, Minnesota, between 1980 to 2002. In this study, a mean annual age-adjusted incidence for men of 9.9 per 100 000, and 1.7 for women was observed. In the literature, the reported incidences of CSC vary between both genders, with men showing a 2.6 to ten times higher incidence than women. Consequently, in several studies male patients constitute 72% to 88% of the study population [5, 2, 6, 7, 8].

There are two age peaks with regard to the prevalence of CSC. The first peak is between the third and fourth decade [2, 9], whereas the second peak is at an older age (with a mean age of 51 years) and no upper age limit [6]. Interestingly, the peak prevalence in women and in patients with chronic CSC seems to be at higher age [2, 10].

In young patients, CSC typically occurs unilaterally with focal leaks. The patients may observe distorted and decreased vision, micropsia, metamorphopsia, reduced contrast sensitivity and mild dyschromatopsia [11]. Patients present most commonly with the acute form of the disease.

Patients older than 50 years at initial presentation are more likely to have both eyes affected and to show diffuse RPE loss and secondary choroidal neovascularisation [6], suggesting an unnoticed earlier onset of the disease.

Ethnicity

The demographic data of CSC seems to vary between different ethnicities. In Asian populations, for example, CSC is more prevalent than among Caucasians [12]. The rates of multifocal and bilateral involvement were also reported to be higher in Asian patients with CSC [13].

CSC occurs less frequently in African American populations, but seems to have a more aggressive course in this ethnicity [14, 15], though this also could be at least partly be explained by a limited access to medical infrastructure.

Desai and coauthors found a lower mean visual acuity at first presentation of African American patients compared with CSC patients of Caucasian descent. Also during follow-up (20/58 vs. 20/32; p=0.04) and at the final visit (20/28 vs. 20/22; p=0.04) after a mean follow-up interval of 21 months, the mean visual acuity was significantly lower among African

American patients with CSC [15].

Clinical presentation

Depending on its duration, CSC can be divided into an acute and a chronic type.

The acute type most commonly occurs in younger patients, and, if a neurosensory detachment is present at the fovea, symptoms such as blurred vision, metamorphopsia, dyschromatopsia, relative central scotoma, reduced contrast sensitivity, micropsia and hypermetropia are reported [2, 11]. Initially, some patients are asymptomatic and are often only diagnosed, when the disease becomes more advanced and the foveal area is affected.
The acute type presents characteristically with mono- and sometimes with multifocal retinal neurosensory detachments as well as a small pigment epithelial detachment. In most cases, the subretinal fluid resolves spontaneously without sequelae within a period of 3-4 months, but the neurosensory detachments may also be recurrent. As a long-term consequence, defects of color discrimination and/or reduced contrast sensitivity may persist. In the acute type, the subretinal fluid is usually clear, but can also appear fibrinous or hazy. Recurrences occur in more than 50% of patients with CSC [16].

In the literature, the term chronic CSC is used, when subretinal fluid persists between 4 and 6 months. When spontaneous absorption of the subretinal fluid is not expected anymore at this interval, therapeutic interventions may be indicated.
Some authors [17, 18, 19] use a cut-off of more than 3 months of duration of symptoms and/or persistance of subretinal fluid for at least 6 months [20, 21, 22, 23].
In the chronic type, often widespread RPE- damage appears as a result of persistent subretinal detachment [24]. Persistent subretinal fluid may also lead to permanent visual loss due to damage of photoreceptor cells, and choroidal neovascularisation may also occur among patients with chronic CSC. Furthermore, also decreased light sensitivity may also persist [25].
The term „diffuse retinal pigment epitheliopathy“ is also used in chronic disease [26], which affects mostly elderly patients at the time of diagnosis. Subretinal lipid appears more common in patients with the chronic type of CSC [2].

Visual acuity ranges from 20/30 to 20/60, and sometimes a weak plus lens can increase visual acuity by correcting the mild hypermetropia caused by the neurosensory detachment [27].

Anatomy and physiology of the retina

Neurosensory retina

The macula or macula lutea, defined as the central area of the posterior pole, is centered between the temporal vascular arcades and the optic disc. Its yellow color is due to the accumulation of lutein and zeaxanthin. The central 1.5 mm of the macula are defined as fovea centralis, which is essential for high spatial acuity and color vision [28].
Due to novel imaging modalities, nomenclature of the single retinal layers changed in the last years. Classified by optical coherence tomography (OCT) according to histological nomenclature [29, 28, 30], the retina can be divided in the following layers according to the IN*OCT consensus study group [31]- the retinal vascular plexus are marked with red (Classification after Campbell and coauthors [32]):

- Internal limiting membrane (consisting of endfeet plates of Mueller cells and astrocytes)
- Nerve fiber layer (axons of ganglion cells, leaving the eye at the posterior pole as optic nerve, glial cells and retinal vessels - radial peripapillary capillary plexus)
- Ganglion cell layer (nuclei of ganglion cells, glial cells, superficial retinal capillary plexus)
- Inner plexiform layer (interneuronal synapsis of bipolar, amacrine and ganglion cells)
- Inner nuclear layer (nuclei of amacrine, horizontal, bipolar and Mueller cells; intermediate retinal capillary plexus above and deep retinal capillary plexus below INL)
- Middle limiting membrane (formed by the attachments of synaptic bodies of photoreceptor cells)
- Outer plexiform layer (synapses between photoreceptors and dendrites of horizontal and bipolar cells)

- Outer nuclear layer (nuclei of photoreceptors) and Henle´s fiber layer (axons of photoreceptors)

- External limiting membrane (junctional complexes of photoreceptors and Mueller cells)

- Myoid zone (myoid portion of inner segments of photoreceptors)

- Ellipsoid zone: interface between inner and outer segments of photoreceptor segments [31]

- Interdigitation zone (tight junction complexes between RPE cells corresponding to Verhoeff´s membrane)

- Retinal pigment epithelium

The main task of the retina is the conversion of light into an electrical signal – so called phototransduction.
Photons are converted in neuronal activity through chemical processes and the neuronal information is sent from the photoreceptors via bipolar cells to ganglion cells and therefore to the optic nerve and brain. Horizontal and amacrine cells connect bipolar cells, the first neurons of visual pathway. In the inner plexiform layer, bipolar cells synapse with ganglion cells, the second neurons, which transform responses from amacrine and bipolar cells into action potentials, transferred to nucleus geniculatum dorsolaterale and the third neuron [28].

The blood supply of the inner retina is provided by the central retinal artery, which divides into 4 branches supplying each quadrant of the retina. In about 20- 32% [33, 34], a cilioretinal artery is additionally present and supplies the region of the papillomacular bundle. The arterial intraretinal branches supply 4 capillary layers, one known as radial peripapillary capillary plexus located in the peripapillary nerve fiber layer, one superficial capillary plexus in the ganglion cell layer, one intermediate and one deep capillary plexus located above and below the inner nuclear layer [32], whereas the outer retina is supplied by the choriocapillaries,

as outlined below.

In total, approximately 5% of the oxygen used in the fundus is thought to be provided through the retinal vascular system, whereas the choroid supplies the residual part [28].

The blood retinal barrier consists of tight junctions of endothelial cells in the retinal vasculature (inner blood-retinal barrier) as well as at the level of retinal pigment epithelium (outer blood-retinal barrier).

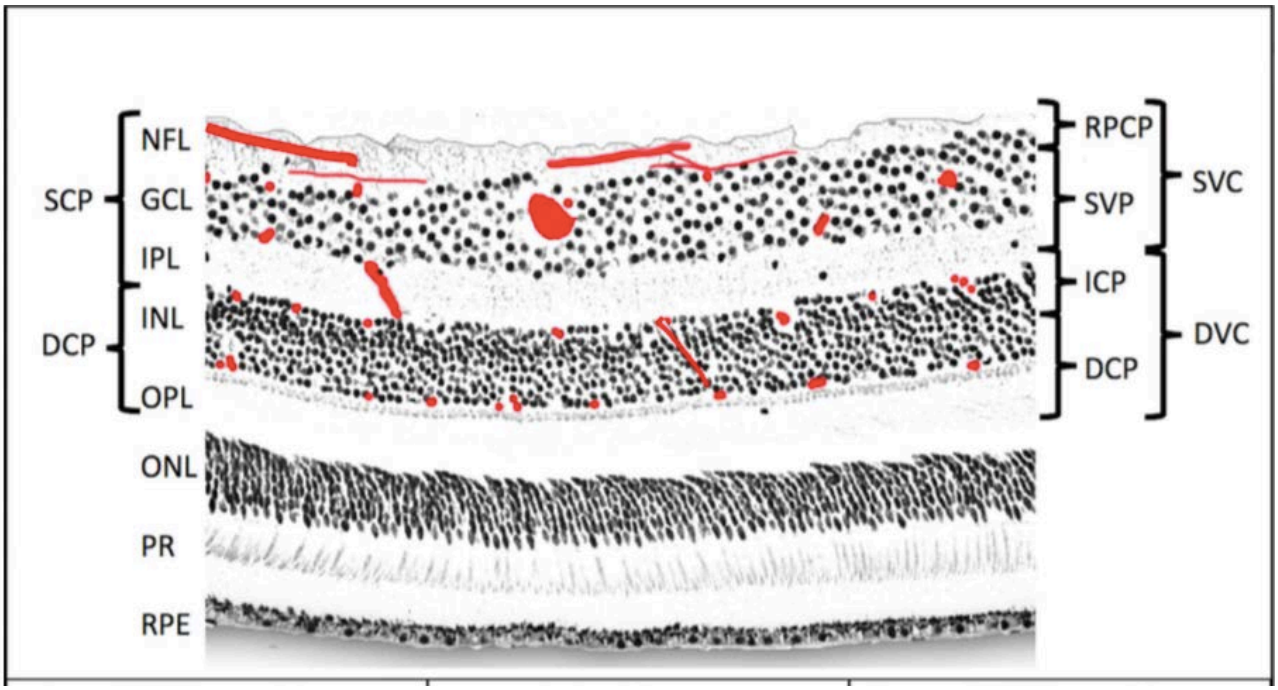


Figure 1 Overview of retinal layers and the retinal vascular plexus (reproduced from Campbell [32] , open access article distributed under the terms of the Creative Commons CC BY license)

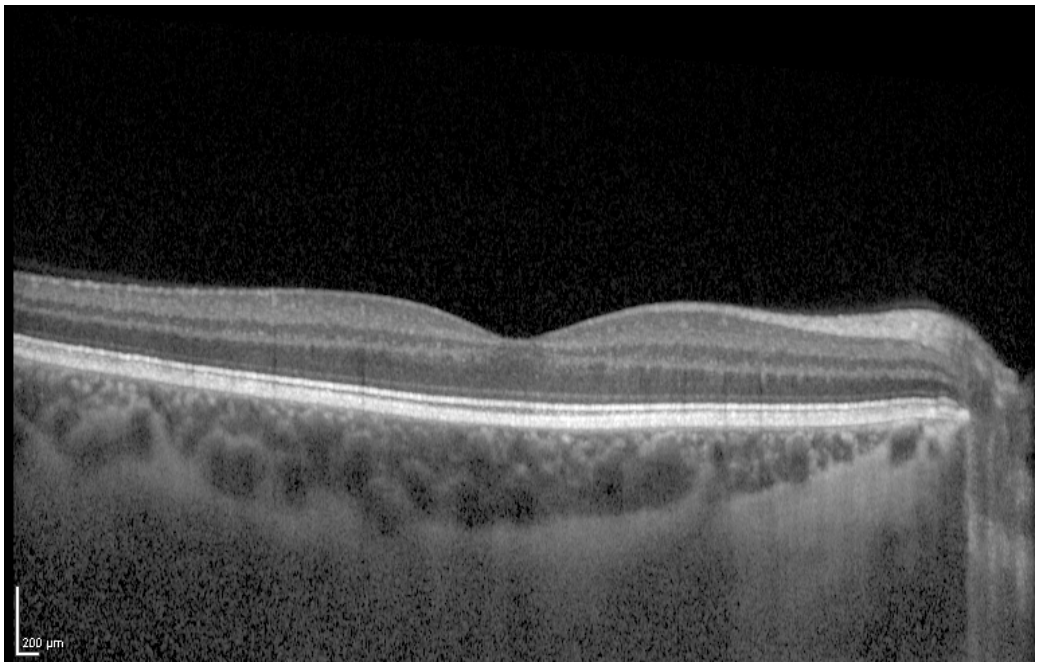
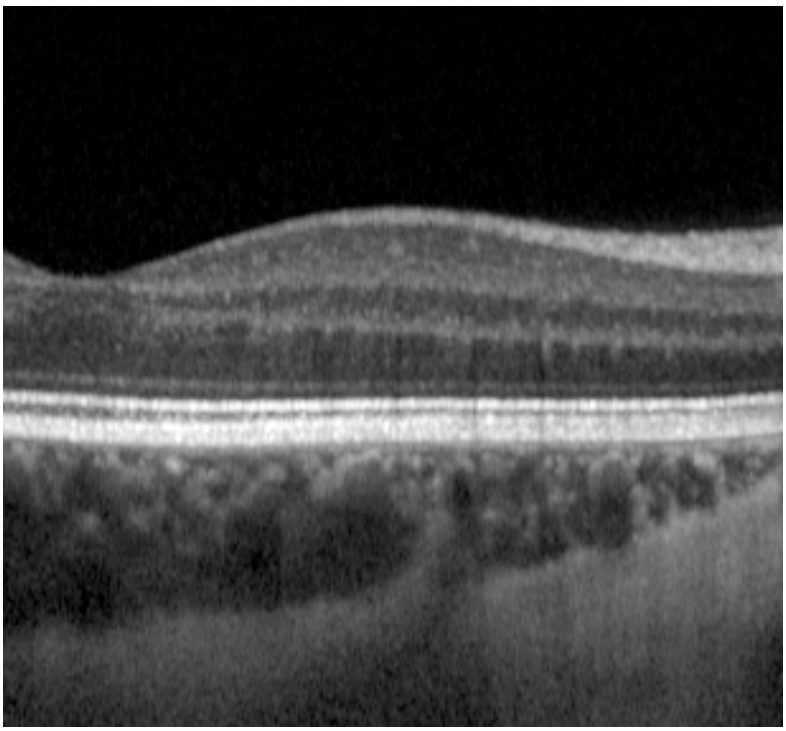


Figure 2 Retinal layers, scanned by Optical coherence tomography



NFL
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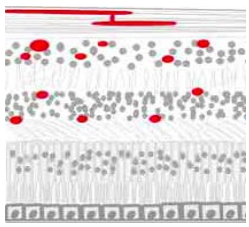

Figure 3 Comparison of retinal layers in OCT vs. schematic histology (schematic histology reproduced from Spaide [35] with permission of publisher American Medical Association)

Retinal pigment epithelium (RPE)

The RPE consists of a monolayer of pigmented, hexagonal, cuboid cells. The size and number of the cells varies depending on the localization. Among the lateral sides of the RPE cells, there are interconnections of tight junctions, forming the outer bood- retinal barrier. The RPE supports several functions in the visual process via absorbing light, phagocytosis of the outer segments of photoreceptors and maintaining the subretinal space [28].

Lysosomes within the RPE digest the phagocyted material. Normally, fluid of the subretinal space is dehydrated by RPE cells, resulting in special bonding properties between the neurosensory retina and RPE.

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Histology of the choroid

Bruch´s membrane

The Bruch`s membrane separates the RPE and the choriocapillaris. It consists of elastin- rich and collagen- rich extracellular material. Supporting the migration, differentiation and cell adhesion of the RPE, the Bruch`s membrane restricts retinal and choroidal cell migration [35].

The five acellular layers of Bruch`s membrane are [36]:

1) the basement membrane of the RPE
2) the inner collagenous layer
3) the porous elastic layer
4) the outer collagenous layer
5) the basement membrane of the endothelium of the choriocapillaris

With a general thickness between 2 and 4 μm [37], the Bruch´s membrane functions as regulator of passive diffusion of serum components, molecules and other transporting systems between choriocapillaris and RPE.
Environmental and genetic factors are important for the structure of Bruch`s membrane. Furthermore, also physiological and pathological changes of Bruch`s membrane and adjacent structures including choriocapillaris, RPE, outer retinal segments can influence its functional integrity. During normal aging, changes in RPE and Bruch´s membrane like irregular basement membrane thickening, RPE basolateral infoldings, deposits in basal lamina, intra-Bruch and sub- RPE deposits and choroidal neovascularisation in Bruch`s membrane are observed [38, 39, 40].

Choriocapillaris and choroidal vasculature

The choriocapillaris consists of a dense one-layer meshwork of capillaries with slightly increased lumen. The fenestrated endothelial cells in a distinct layer play an important role in the filtration and secretion of various factors like expression of vascular endothelial growth factor receptor -1 and -2 at the retinal side of the layer. Therefore, the function of choriocapillaris and its endothelial integrity diminishes with decreasing VEGF levels. Intracellular adhesion molecule -1, which is in charge of adherence of macrophages and neutrophils to endothelial cells, is also expressed by the choriocapillaris.
One of the most important functions of the choriocapillaris is to supply the RPE and outer retina, especially in the foveal avascular zone and the the prelaminar portion of the optice nerve head with oxygen and metabolites [41, 42] In normal aging changes like atrophy of the choriocapillaris and damage of endothelial cells are found. Ramrattan and coauthors [37] observed a decrease of density of the choriocapillaris during lifetime, other studies suggested a transformation of a fenestrated sinusoidal system in a tubular vascular system with loss of fenestrations with age [43, 44].

The architecture of the choroid is controversially discussed: there is a model of anatomical and functional lobules. Anatomical lobules can be found in electronic microscopy, but are not compatible with the functional lobules in angiography and vice versa. First, the anatomical lobule is supplied by peripheral feeding arterioles and a central collecting venule. In the functional lobule, a central feeding arteriole and peripheral draining venoules are responsible for the blood supply. Draining venules and feeding arterioles of the posterior pole join the capillary plexus in a rectangle form. At the posterior pole towards equatorial and peripheral choroid, the architecture of the choroid is splitted in segments, and each lobule of choriocapillaris is an independent unit with no anastomosis with the adjoining lobules. Findings in fluorescein angiography showed a precise pattern of the arrangement of the singular lobules, but they can vary in size and shape, depending of the area of the eye.
At the posterior pole, the choriocapillaris is arranged more compact compared to the periphery [45].

Histologically, the choroid consists of two main layers beside the choriocapillaris: the Sattler layer, characterized by medium- sized choroidal vessels, and the Haller layer, which contains large- sized choroidal vessels.

Three orbital branches of the ophthalmic artery, the anterior ciliary arteries, the short posterior ciliary arteries and the long posterior ciliary arteries supply the arterial blood of the choroidal circulation.
The blood of the choroid is drained by the four vortex veins into the superior and inferior orbital veins.
The vortex veins drainage includes beside the corresponding choroid quadrant also the same quadrant of the ciliary body and the iris. Normally, there are no free anastomoses between the single vortex veins. Because of these observations, Hayreh suggested that blood flow in the choriocapillaris, choroidal arterial- and venous system is strictly segmental and there are no anastomoses between the adjacent segments at any level of the choroid, opposite to the retina, where adjacent arterial segments overlap via veins. This indicates every choroidal artery as an end- artery [45].

In animals, there was a multiple higher choroidal blood flow than in the retina [46], whereas the blood flow rate of the choriocapillaris was four times slower than in the inner retinal capillaries, as shown by measurement of red blood cell velocity in rats. Bhutto and Lutty suggested that slow red blood cell velocity was due to the lobular architecture of the choriocapillaris, rectangle choroidal microvasculature to capillaries and oval shape of the lumen of capillaries [47].

Pathophysiology

The pathogenesis of CSC has still not yet been fully elucidated.
One hypothesis suggests that choroidal vascular hyperpermeability as found in ICG angiography causes increased tissue hydrostatic pressure beneath the RPE leading to secondary dysfunction of the overlying RPE [6]. This may lead to pigment epithelial detachment (PED) and disruption of the overlying RPE, which causes fluid accumulation under the neurosensory retina in areas with RPE atrophy and pigment mottling. In recent years, new imaging modalities like OCT confirmed the presumed pathogenesis by showing an increase of choroidal thickness among CSC patients - CSC counts to the spectrum of pachychoroid diseases. In ICG- angiography, filling delays of the choriocapillaris and choroidal arteries and venous dilatation, further focal hyperfluorescence of the choroid can be observed, which highlightes the role of abnormal choroidal circulation as possible cause of the disease [5].

Gass hypothesized a focal increase in the permeability of the choriocapillaris as a primary cause for RPE dysfunction leading to serous retinal detachment, pigment epithelial detachment and rarely to serofibrinous subretinal fluid [4]. Another study group observed diffuse hyperpermeability around active leakage sites only in indocyanine green angiography, but not in fluorescein angiography (FA), hypothesizing that hyperpermeability primarily occured in the choroid subsequently leading to RPE damage [48].
Choroidal ischemia could also be caused by alterations in choroidal circulation.
Hayashi found areas of choroidal ischemia and leakage of indocyanine green from the choriocapillaris. In FA and ICG- angiography, a delay in arterial filling and dilated capillaries and dilated draining venules in one or more choroidal lobules provide evidence for a role of impaired choroidal circulation in the pathogenesis of CSC [49].

Other authors suggested impaired autoregulation of the choroidal blood flow as a potential cause of choroidal vascular hyperpermeability. Tittl and coauthors hypothesized a persistent abnormally regulatory response to be involved in the pathogenesis of chronic CSC [50].
They found impaired subfoveal choroidal blood flow regulation in patients with even inactive chronic CSC.

Another hypothesis suggests RPE- dysfunction as a major cause for CSC, which leads to leakage of fluid into the subretinal space resulting in a neurosensory retinal detachment. Spitznas and coauthors hypothesized that a change of the direction of ion secretion due to focal RPE damage may result in increased fluid movement towards the retina instead into the direction of the choroid [8].

This hypothesis is supported by findings of an animal model, which showed small nonrhegmatogenous retinal detachments over rabbit RPEs damaged by laser photocoagulation [51]. Marmor published additional results implying that serous retinal detachments may be caused either by a diffuse metabolic dysfunction of the RPE or focal RPE damage [52].

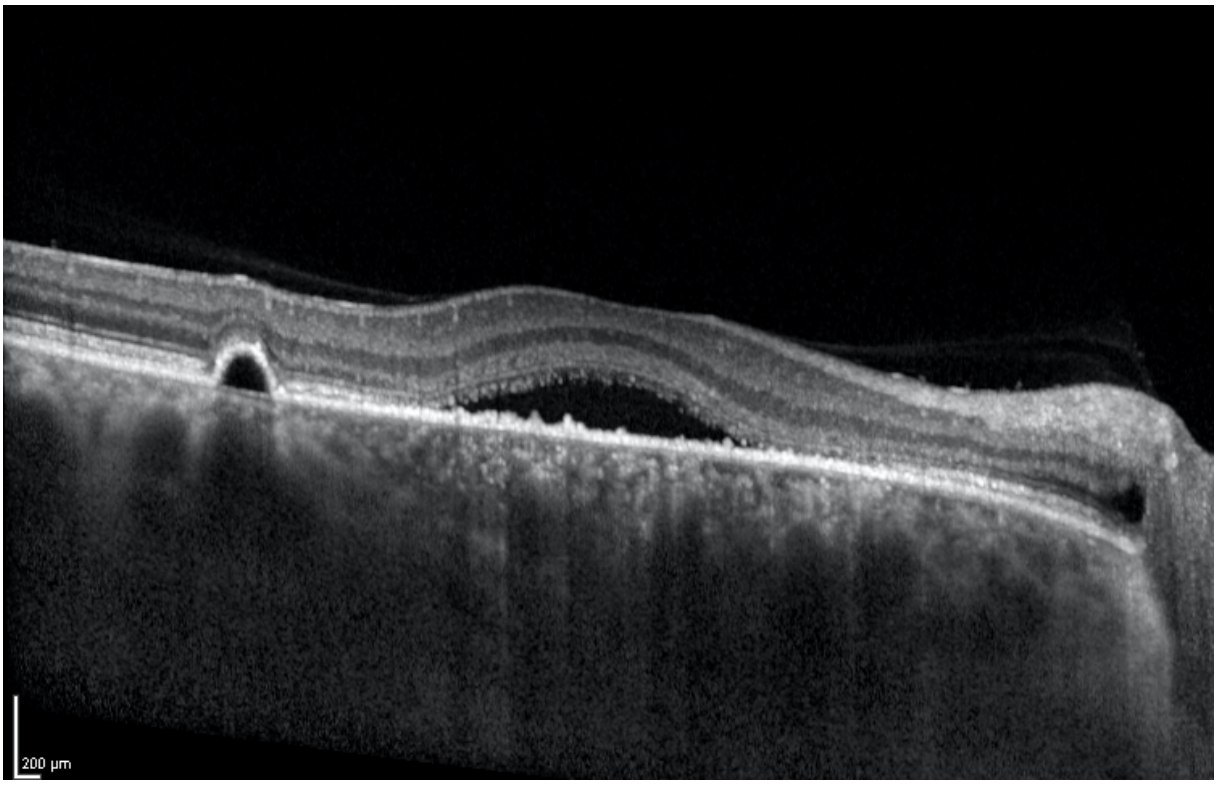


Figure 4: PED and neurosensory detachment in a patient with CSC

Ryan suggested that both impaired RPE function and increased fluid leakage from the choriocapillaris play an essential role in the pathogenesis of CSC [53].

A pigment epithelial detachment seems to develop as a result of choroidal dysfunction and interruption of the junctions of the basement membrane of the RPE and the inner collagenous layer of Bruch´s membrane, followed by an „in- flow“ of serous fluid out of the

choriocapillaris. These findings are confirmed by ICG- angiography, which show a leakage at the level of the choroid, which cannot be observed by fluorescein angiography.
PEDs are angiographically verified only in 10% of CSC cases [54, 55], whereas PEDs can be detected by OCT in 33-63% of patients with CSC [56, 57].

Increased platelet aggregation, hypercoagulability, increased blood viscosity and microthrombus formation leading to choroidal ischemia have also been implicated in the pathogenesis of CSC [58, 59].

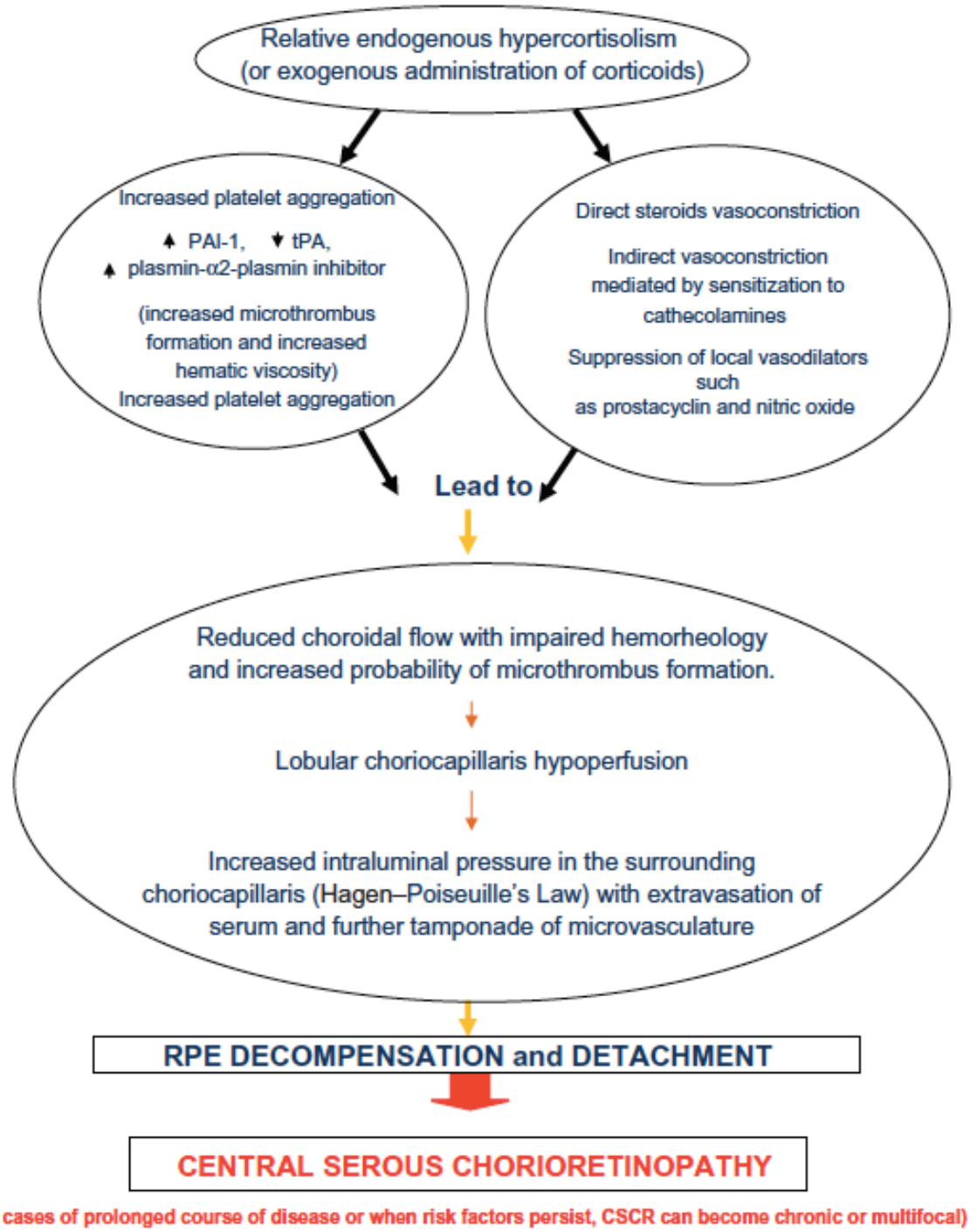


Figure 5 Overview of a pathogenetic model, reproduced from Caccavalle and coauthors [60] with permission of publisher Dove Medical Press Limited

Imaging in CSC

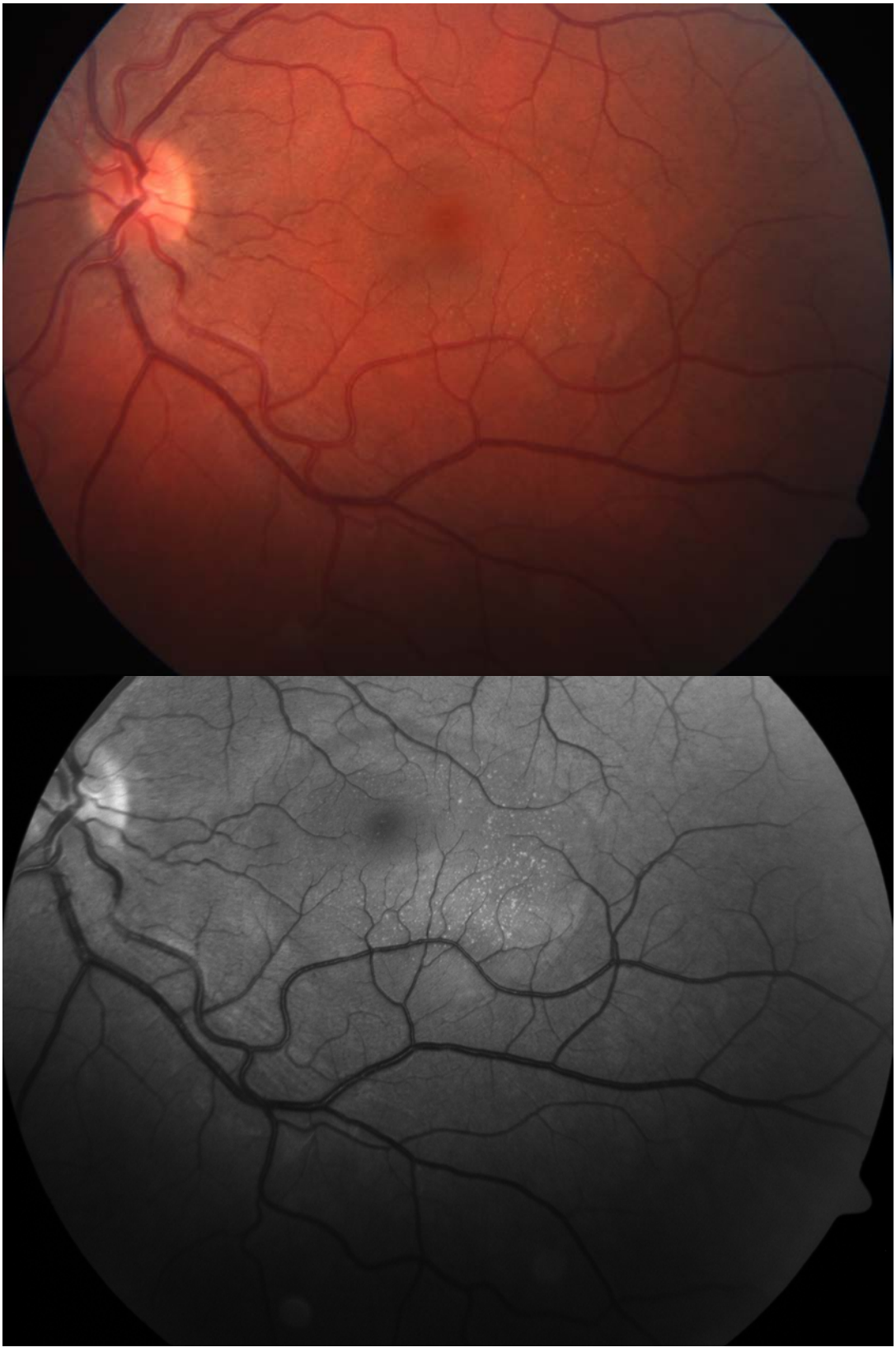


Figure 6 A fundus of a patient with CSC with neurosensory detachment and „yellow dots“, as shown by the red-free image below.

Optical coherence tomography (OCT) in CSC

In the last decade, multimodal imaging of chorioretinal diseases has become more and more important and provided a more detailed insight into the manifestations and pathogenesis of numerous diseases. Optical coherence tomography (OCT), a non- invasive tool provides information on retinal pathologies in real time and in situ with high resolutions, comparable to excisional biopsy and histopathology [61]. Images are generated cross- sectional or three-dimensional by measurement of magnitude of backreflected or backscattered light and echo time delay.

Spectral- Domain (SD)- OCT non- invasively depicts the different retinal and choroidal layers. Because of these advantages, the OCT also plays an important role in the diagnosis and follow-up of CSC. OCT technology allows the measurement of retinal and choroidal layer thickness, detection and quantification of subretinal fluid and PEDs and to provide additional information concerning the integrity of RPE and photoreceptors. Furthermore, granulation of photoreceptor outer segments at the detached retina, which are seen as hyperreflective foci (equal or higher reflectivity than RPE, hard exudates excluded), long-persistent neurosensory detachment and long- lasting disruption of the ellipsoid zone at the fovea are associated with a poor visual outcome [25, 62, 63] and therefore may be used as prognostic biomarkers. Lee and coauthors found neurosensory retinal detachments in 100% of study population, photoreceptor granulations at the macula in 21%, PEDs in 33.9% and irregular changes of the RPE, RPE bumps in around 8% and in less than 5% RPE defects [64].

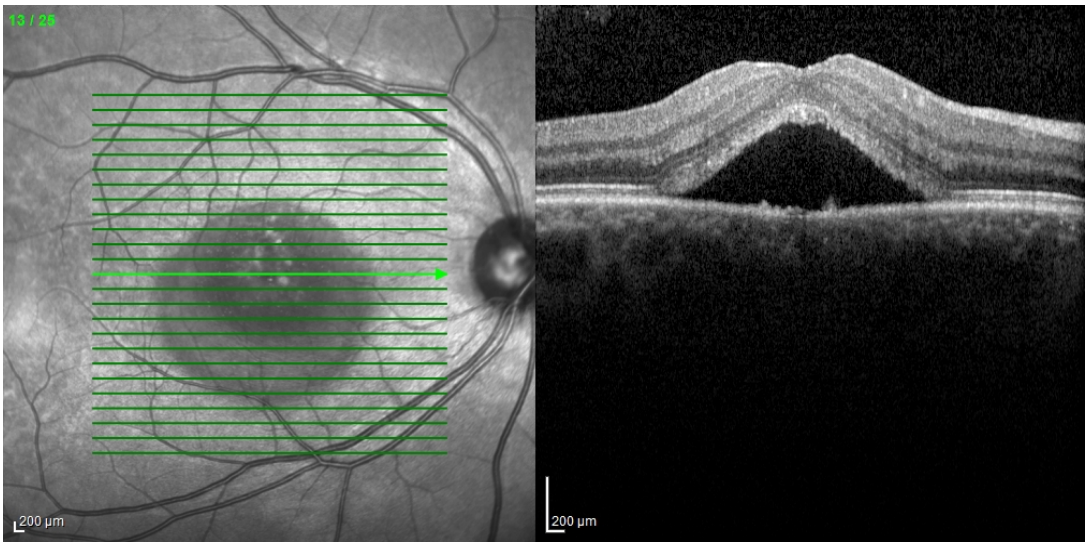


Figure 7 OCT of a right eye with acute central serous chorioretinopathy. Typically, the elevation of the neurosensory retina from the retinal pigment epithelium (RPE) is reflected by the dark triangle shaped area in the center of the scan, corresponding to the dark area in the infrared image to the left. On the bottom, there are accumulated shed photoreceptors discs presenting as granular hyperreflective material in OCT

Hyperreflective foci, as described above, are mainly observed at the outer retina of the macular region and at the leakage site identified by fluorescein angiography and above the PED on the outer retinal layer [65, 64].

The importance of choroidal dysfunction in the pathogenesis of CSC is further supported by findings in OCT, which showed that the thickness of the choroid is increased by up to 50% - 80% in patients with CSC compared to the choroid of healthy age- matched controls [66, 24, 67]. Additionally, in patients with unilateral CSC, an increased choroidal thickness was also found in the unaffected eye when compared to healthy controls [68].
Furthermore, Gupta and coworkers reported that three- dimensional single layer RPE- map analysis showed RPE- irregularities in asymptomatic eyes, which suggested preclinical stages of RPE dysfunction, resulting in the formation of RPE bumps [69].

Fluorescein angiography

Fluorescein angiography (FA), an examination based on the intravenous injection of sodium fluorescein, allows mainly the visualization of the retinal blood flow. Only 20% of the fluorescein are circulating freely in the retinal and choroidal vasculature, the other 80% are mainly albumin- bound [28].

Through activation of a molecule by light of a certain wavelength and therefore release of a photon, fluorescence can be detected. Depending on the wavelength of fluorescence, special filters are required. In the case of fluorescein, white light passes through a blue excitatory filter. Blue light (465-490nm) excites the free fluorescein molecules, resulting in a stimulation of yellow- green light (520-530nm), which is detected by the camera system, blocking the reflected blue light by a barrier filter (yellow-green). So only the yellow- green fluorescence of the fluorescein molecules is recorded. Normally, fluorescein can not exit retinal vessels because of the inner blood- retinal barrier, and fluorescein particles out of the choriocapillaries can not pass through the outer blood retinal barrier, which is formed by the tight junctions between RPE cells, into the subretinal space [28].

In acute CSC a single and sometimes multiple leakage points can be identified by FA in the area or close to subretinal detachment [5, 70]. There are 2 classical types of diffusion- the "inkblot"- pattern, present in up to 71% and the "mushroom/smokestack"-pattern, which can be found in up to 14-24% [5, 71].

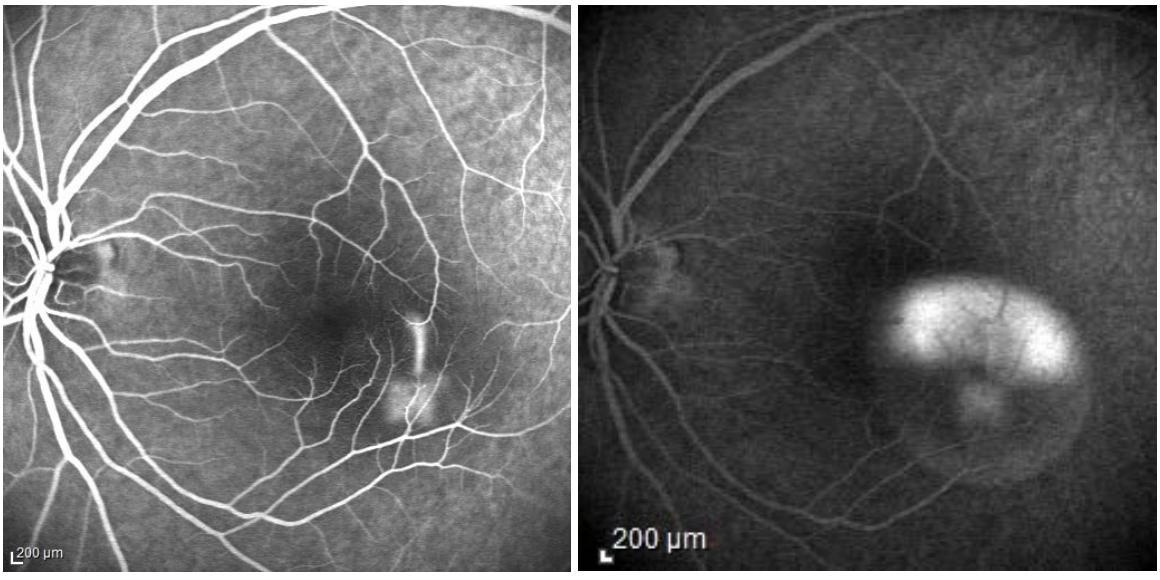


Figure 8: A smokestack- pattern in FA, early and late phase

The inkblot- pattern shows an increasing circular hyperfluorescence originating from a central pinpoint. In the smokestack- pattern, the hyperfluorescence is arising with lateral diffusion, producing the appearance of a mushroom- like image. This pattern is typically for the primary acute episode of the disease [71]. In the mid and late phase, diffuse circular hyperfluorescence as dye pooling in the subretinal detachment can be observed. In cases with only scant fluorescein exudation, there is often no exudation detectable in ICG angiography [72]. Focal choroidal hypoperfusion is better visible in ICG angiography compared to FA. If chronic, multifocal hyperfluorescent leakage through diffuse RPE defects is typically observed [73] . Piccolino and coauthors found a fluorescein leakage in areas of hypoperfusion of the choriocapillaris [74].

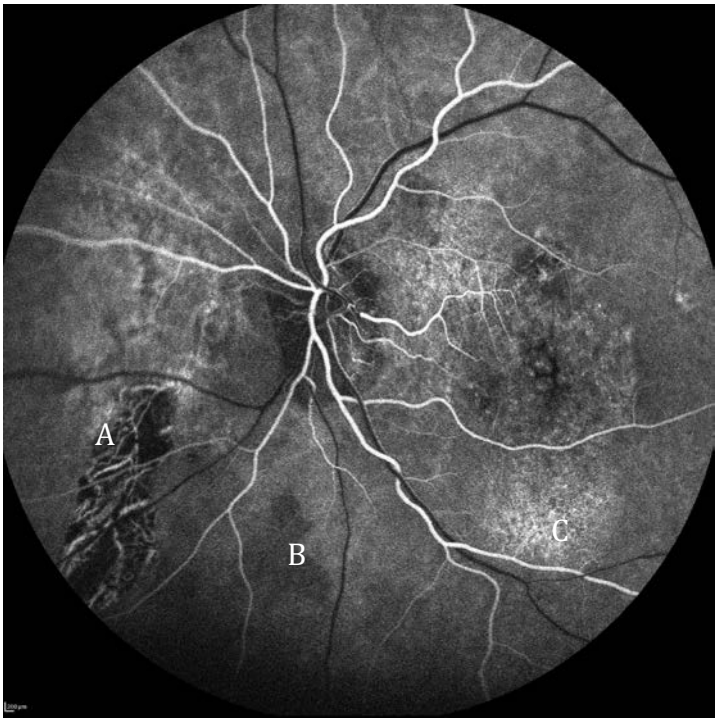


Figure 9 RPE atrophy (A) and focal hypoperfusion (B), further hyperfluorescence (C) in early phase FA- angiography

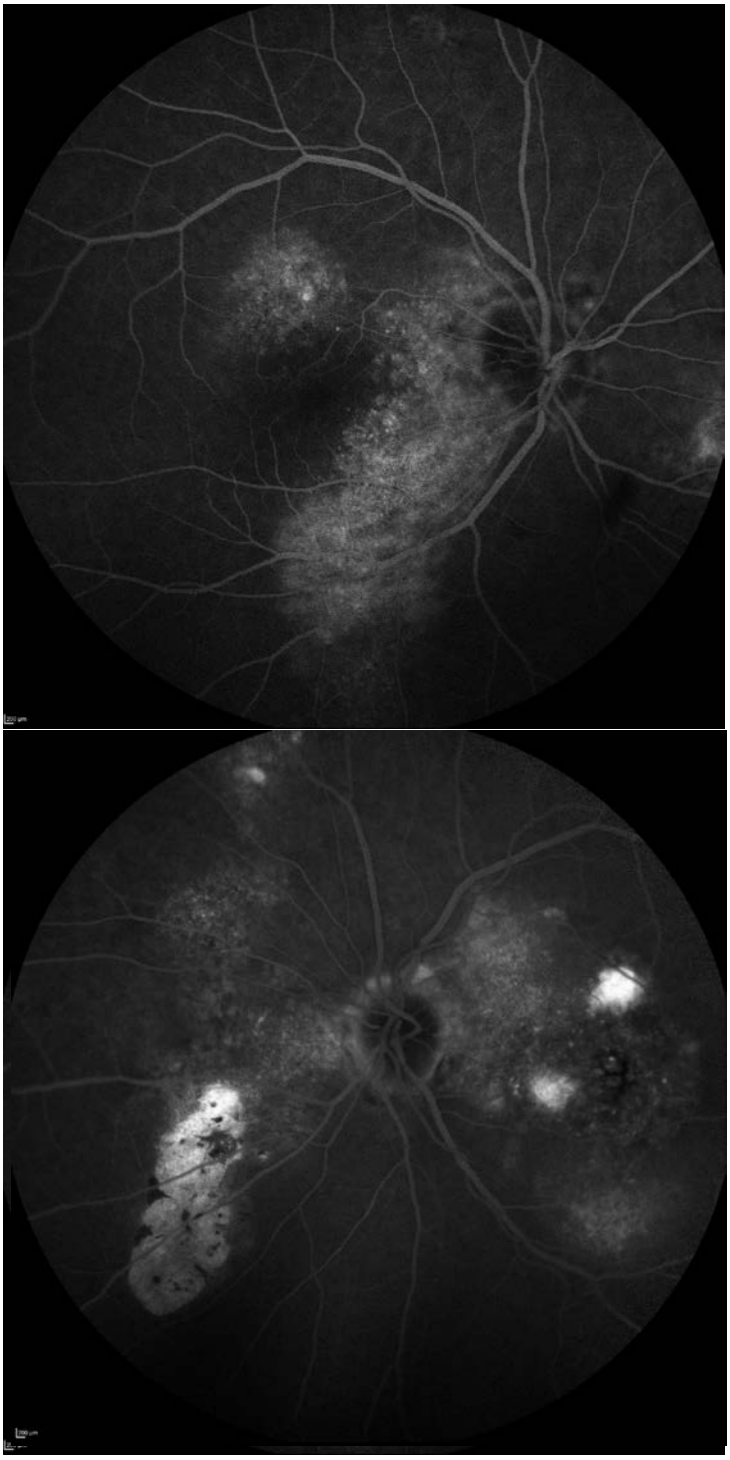


Figure 10: Typical, bilateral angiographic changes in a chronic CSC patient in FA

Indocyanine green (ICG)- angiography

Indocyanine green is a tricarbocyanine water- soluble dye, which after intravenous injection is rapidly bound to proteins in 98%. Diffusion through the choriocapillaries is therefore limited. Because of these characteristics, ICG is predisposed for imaging of the choroid. The maximum emission of ICG is around 835 nm wavelength. The recording of ICG-fluorescence works through a diode laser illumination (790-805 nm) and barrier filters with 500 and 810 nm [28].

In CSC, several studies showed changes in the choroidal circulation, areas with capillary and venous hyperfluorescence around leakage sites and elsewhere, indicating hyperpermeability of choroidal vessels [48 49]. Additionally, some research groups found filling delays of the choroicapillaris and choroidal arteries, followed by capillary and venous congestion, and sometimes focal areas of choroidal ischemia [49 75 76]. Only in cases with capillary and venous congestion, leakage at the level of RPE in FA was observed by Prünte and Flammer, speculating that delay of arterial filling is caused by capillary and venous congestion [75]. This hypothesis was supported by Kitaya and coworkers, who also found FA leakage points at the RPE only in areas of delayed arterial filling as observed by ICG- angiography, hypothesizing occlusion of the choriocapillaris. Chronic choroidal hyperpermeability may affect the RPE metabolically and/or mechanically, thus leading to changes of the RPE function [75, 76].

Kitaya and coworkers also observed a significantly lower choroidal blood flow in eyes with CSC compared to the fellow eyes, which again points to a role of impaired choroidal perfusion in the pathogenesis of CSC.

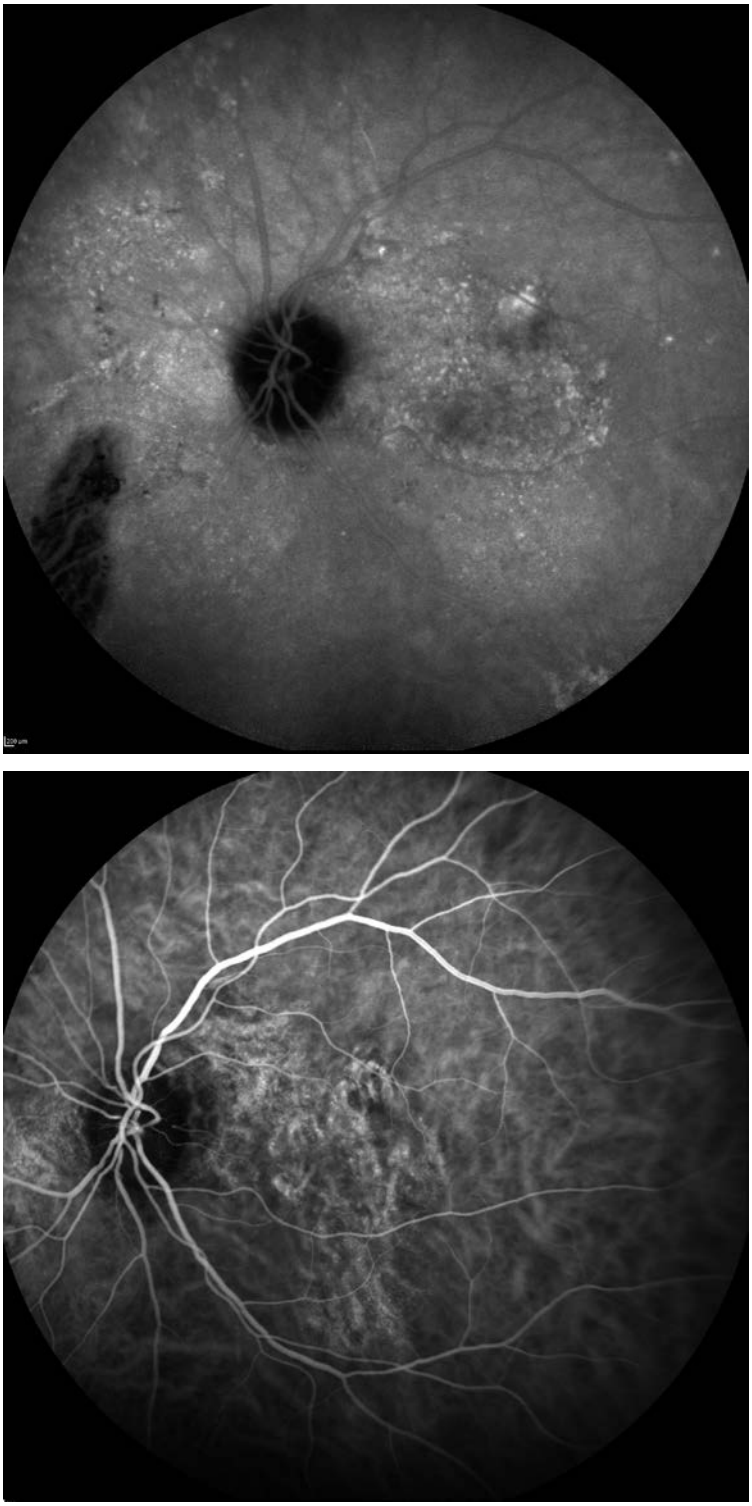


Figure 11: ICG- angiography image of chronic CSC

Among different studies choroidal hyperfluorescence was found in 37% to 100% [48, 72, 75, 59]. Interestingly, changes similar to those observed in the CSC eyes were also found in unaffected fellow eyes [59]. This strengthens the hypothesis of the choroid as the primary localization for the development of CSC.

Fundus Autofluorescence (FAF)

Autofluorescence photography is a rapid method to record functional images via detection of the stimulated emission of light after excitation energy of naturally occurring fluorophores. In the human RPE cells, the most important fluorophore is lipofuscin. Lipofuscin originates as a by- product of phagocytosis of photoreceptor outer segments by RPE cells. Furthermore, RPE cells contain melanin, which also has fluorescent properties.
Therefore, autofluorescence can detect pathologies and loss of RPE cells. A loss of RPE cells is accompanied with a loss of autofluorescence [28].
The fundus autofluorescence includes 2 types, short wavelength (SW-AF) exciting light, produced by lipofuscin, and near infrared AF (IR-AF) exciting light, originating from melanin.

IR-AF is more sensitive for detection of RPE- abnormalities in CSC than SW- AF.
Zhang and coauthors found RPE detachments to show hypo IR-AF, hypothesizing that the serous accumulation blocked autofluorescence of choroidal melanin [77].
In CSC, hyper SW- AF seems to originate from subretinal deposits and RPE cell aggregation, contributing to retinal degeneration [77], whereas hypoautofluorescence is observed in the area of leakage in most of the patients, indicating RPE detachment or defect of RPE at this site [78].
Sekiryu et al found that the BCVA decreased in eyes with granular hypo IR-AF after retinal reattachment, possibly due to the damage of RPE cells [79].

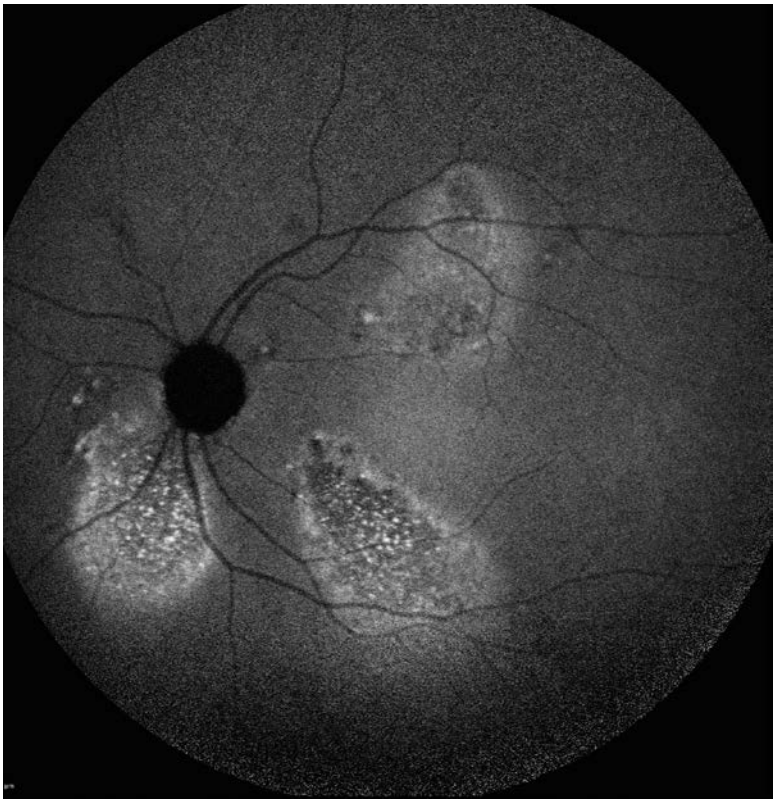
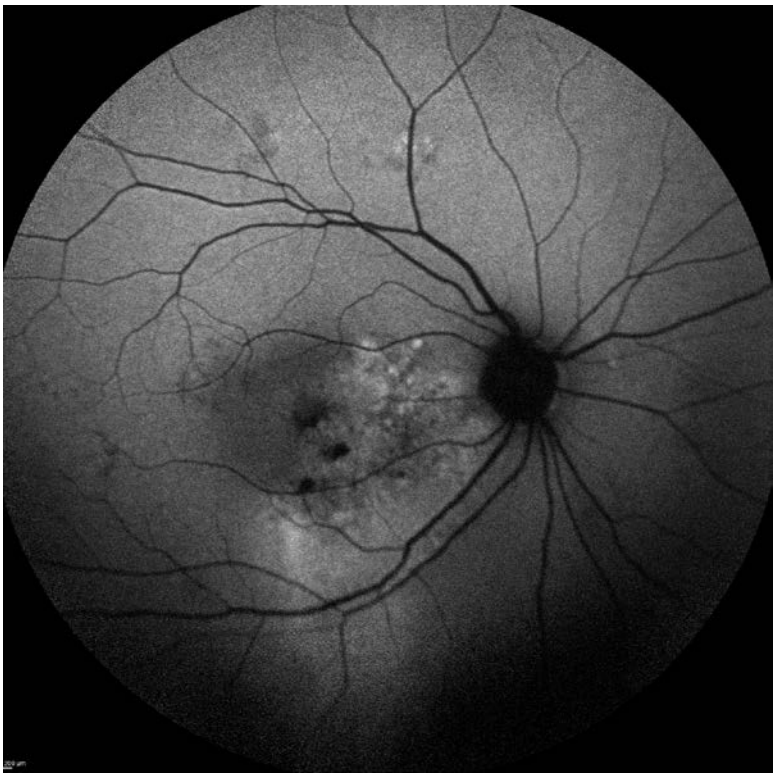


Figure 12: Autofluorescence in bilateral chronic CSC. Typically, areas of atrophy are hypoautofluorescent.



Risk factors

Overview

There are several established risk factors for CSC, including corticosteroid exposure due to endogenous overproduction [80] or therapeutic administration of steroids [81, 82, 83], psychological stress [84], male gender [6], type A personality [3, 7] , pregnancy [85, 86] and endocrine disorders in the adrenocorticotropic axis. [80] Patients with autoimmunologic diseases like systemic lupus erythematosus [87] or a history of organ transplantation [88] also have been reported to develop CSC usually in the setting of current corticosteroid intake.
Additional associations with coronary heart disease, arterial hypertension, peptic ulcer diseases, anithistamines and psychopharmalogical medications have also previously been reported [89].
Recently, new risk factors have been identified in the development of the disease, including genetic variants encoding complement factor H [90] and cadherin 5 [91], as well as shift work [92], which leads to disturbance of the circadian biological rhythm.

Shift work

Shift work is defined as a working period out of the regular „daylight hours“ from 7 AM to 6/ 7 PM, including working in the morning, evening, night and all other kinds of irregularities. Due to circadian misalignment and sleep disturbances, shift work may lead to an increased risk of cardiovascular disease, depression, obesity, diabetes mellitus and cancer [93]. Furthermore, increased activities of the hypothalamic- pituitary adrenal axis and the autonomic sympathoadrenal system caused by sleep disturbances possibly result in altered secretion of cortisol and catecholamine hormones. Shift work is known to affect levels of stress hormones because of their circadian regulation, wake/sleep cycle, activity and daylight exposure. Because of elevated urine and plasma cortisol levels [94] and increased levels of plasma catecholamines in patients with CSC [80], Bousquet and coauthors hypothesized an association-between shift work and the development of CSC, which was subsequently confirmed by a significant increased prevalence of shift work and sleep disturbances in patients with CSC compared to healthy control subjects [92]. Setrouk and coworkers also found chronic CSC patients to be more likely exposed to irregular working hours [95].

Psychosomatic factors

Psychosocial stress factors like critical illness, divorce or job loss, often in combination with poor coping mechanisms have been found to be associated onset of CSC. Furthermore, personality traits and psychic disturbances were implicated as contributing or predisposing factors in the development of CSC. Type A behavior pattern, which is known to be an established risk factor for coronary heart disease, was found more frequently among patients with CSC [3, 96].

Typically, type A behavior is characterized by an agressive nature and competitive driving, sense of urgency and hostile personality.

A possible explantation for the increased prevalence of CSC in patients with type A personality might be the stimulation of the adrenomedullary- sympathetic system [3] , with consequently increased plasma levels of catecholamines in patients with CSC [97]. Moreover, Williams and coauthors observed also higher cortisol levels in persons with a type A personality [98].

Genetics in CSC

In the literature, only some cases of familial clustering of CSC were reported. Interestingly, Weenink and coauthors reported that 42% of the relatives with chronic CSC also showed evidence of CSC or RPE atrophy [99].

Based on the reports on familial clustering of CSC, Miki and coworkers investigated five gene polymorphisms of the complement factor H (CFH) as potential risk factors for CSC in a Japanese population [90]. Four of the five CFH gene polymorphisms were risk-conferring (CFH rs800292, CFH rs 3753394, CFH rs1329428, CFH rs2284664) and one polymorphism was protective against CSC (CFH rs1065489). The mechanism by which CFH affects CSC risk is so far not fully elucidated. Yet, CFH binds to adrenomedullin, which itself has been shown to induce vasodilation in the choroid and to increase choroidal blood flow [100, 101].
In a large study including 292 Dutch patients with chronic CSC, the risk-conferring effect of CFH rs1329428 and CFH rs800292 was confirmed [102]. The protective effect of CFH rs1065488, which has also been observed by Miki and coworkers, was also found in the Dutch study population.
Furthermore, the ARMS2 Ala69Ser (rs10490924) gene polymorphism was found to exert a protective effect against CSC.
In a small study conducted in a Greek population, Moschos and coworkers reported an increased risk for CSC among homozygous carriers of the CFH rs3759394, CFH rs1329428 or CFH rs1065489 polymorphisms [103]. In contrast to the studies by Miki et al and de Jong et al, the CFH rs1065489 polymorphism was significantly associated with an increased risk for CSC among Greek patients.
When interpreting these data, it is important to keep in mind that the Greek study included only 41 patients with CSC and 78 control subjects.

In 2015 Breukink and coworkers reported that the copy numbers of the complement C4B gene were associated with risk of chronic CSC [104].
Studying 197 patients with chronic CSC and 303 healthy control subjects, absence of C4B was significantly associated with increased risk for CSC, whereas three copies of C4B significantly decreased the risk for CSC.

A possible mechanism by which genomic C4B copy number affects CSC risk is the observation that that the genomic copy number of C4B is associated with cortisol release after adrenocorticoptropic hormone stimulation [105].

Glucocorticoid hormones act by binding to glucocorticoid as well as mineralocorticoid receptors. In 2017 van Dijk and coworkers studied the role of functional gene polymorphisms of the glucocorticoid receptor (NR3C1) and the mineralocorticoid receptor (NR3C2) genes as potential risk factors for CSC [106]. Analyzing 336 patients with chronic CSC and 1314 control subjects, they found a significantly increased risk for CSC among carriers of the rs2070951 polymorphism of the NR3C2 gene.

Finally, Schubert and coworkers reported that two genetic variants of cadherin 5, rs7499886A>G and rs1073584C>T, were significantly associated with CSC risk [91]. Cadherin 5 is the major cell-cell adhesion molecule in the vascular endothelium, and was downregulated by corticosteroids, thus potentially leading to an increased permeability of the choroidal vessels.

Corticosteroids

Glucocorticoids and adrenergic hormones have previously been shown to play an important role in the development of CSC. Oral [87], inhalational or intranasal [86], intraarticular, epidural [82] or intravenous [107] administration of corticosteroids were shown to result in the development of CSC-related acute exudative changes.

Furthermore, also risk factors such as type A personality, psychological stress, male gender and Cushing´s disease are associated with abnormal steroid regulation. Glucocorticoids have been implicated to sensitize epinephrine receptors to circulating adrenergic hormones. Garg and coauthors found significantly elevated plasma cortisol and 24-hour urine cortisol levels in patients with CSC [94]. Haimovici reported elevated 24-hour urine cortisol levels in up to 50% of CSC patients. In a study by Tittl et al, individuals with CSC were more likely to use corticosteroid and psychopharmacological medication compared to gender- and age-matched controls [7].

In a prospective case control study 56% of patients had a history of either endogenous or exogenous exposure to steroids [83]. In another case- control study, Haimovici and coworkers found systemic steroid use (OR 37.1; 95% CI 6.2-221.8) to be a strongly associated with increased risk for CSC. Pregnancy was also found to be significantly associated with CSC risk (OR 7.1; 95% CI 1.0-50.7) [86].

Coagulation

Platelet activation- primary hemostasis

After endothelium damage, circulating platelets bind to the collagen in the endothelial cell through collagen specific glycoprotein Ia/IIa surface receptors.
Von Willebrand factor (vWF), which is released by the endothelial cells and platelets, supports this adhesion. It further strenghtens the linkage between platelets, collagen fibrils and GP Ib. Therefore, platelets get activated and release serotonin, platelet activating factor (PAF), plasma adenosin diphosphat (ADP), vWF, platelet factor 4 (PF4) and thromboxane A2 (TXA2). This process activates additional platelets. Fibrinogen cross- links with GP IIb/IIIa, which is necessary for the aggregation of platelets.

The coagulation cascade- secondary hemostasis

In coagulation, fibrin clots are formed by blood. The two pathways - the extrinsic and the intrinsic pathway play an important role in fibrin formation.

After tissue factor (TF) expression- also known as tissue thromboplastin- caused by vascular injury, the extrinsic pathway starts. TF acts as a cofactor with the active form of factor VII, which supports the activation of factor X to factor Xa. The TF- Factor VII complex can also activate factor IX to factor IXa, which can activate factor X, too.

In the intrinsic pathway, the activation of factor XII takes place in the presence of kininogen and kallicrein. Through factor XIIa, factor IX is converted to its active form factor IXa.

The intrinsic and extrinsic pathyway converge at the level of factor X. The factor Xa- Va complex activates prothrombin (factor II) in its active form thrombin (factor IIa). Thrombin primarily converts fibrinogen (factor I) into fibrin (factor Ia).

Stabilization of the clot, formed by fibrin monomers, takes place by factor XIIIa. Thrombin is involved in the activation of factors V, VIII and XIII.
Inhibiting factors of fibrin formation are antithrombin (AT), TF pathway inhibitor (TFPI), protein C (PC) and protein S (PS) [108, 109, 110].

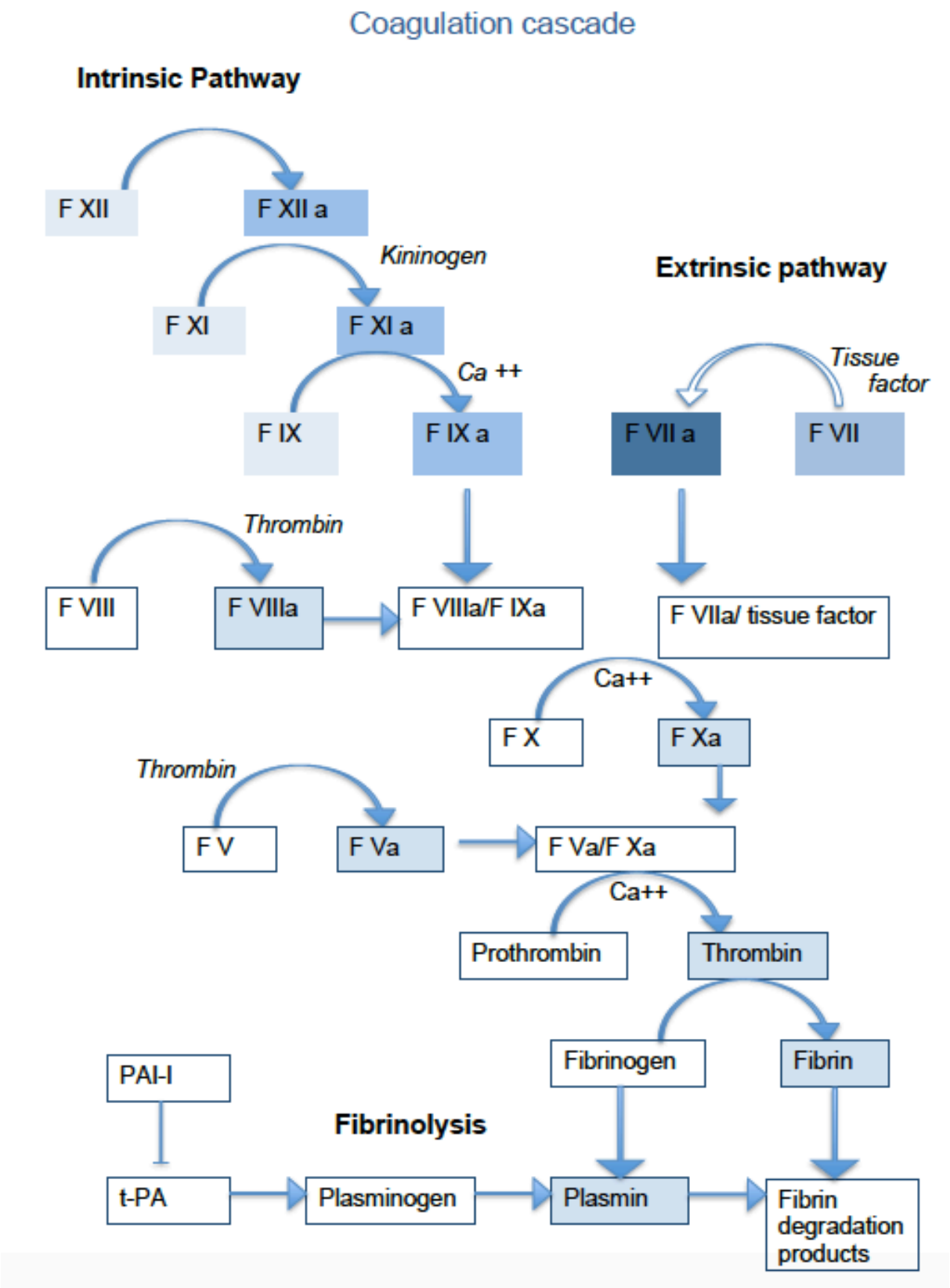


Figure 13: Overview of the coagulation cascade

The fibrinolytic system

When the injured blood vessel recovers, the residual fibrin clot becomes dissoluted via the fibrinolytic system. The key proenzyme plasminogen is mainly converted through tissue plasminogen activator (t-PA) and also through urokinase-type plasminogen activator (u-PA) into its active form plasmin, the central protease in fibrinolysis. Both, u-PA and t-PA, form complexes with a low-density lipoprotein (LDL)- receptor like protein and are cleared by the liver. Plasmin and also plasminogen possess specific lysine binding sites, which enable the specific binding to fibrin and the interaction of plasmin with alpha 2- antiplasmin [111]. Plasmin cleaves fibrin through proteolysis in soluble fibrin degradation products, some of which act as chemotactic and immunomodulatory agents [112], and activates matrix metalloproteinases (MMPs). MMPs degrade extracellular matrix (ECM).

The complex of fibrin, plasminogen and t-PA also promotes the local interaction between t-PA and plasminogen and therefore increases the conversion of plasminogen into plasmin [113]. Further enhancement of fibrinolysis takes place through the partial degradation of fibrin via plasmin, resulting in additionally disposable binding sites of plasminogen and t-PA with lysin on fibrin fragments. A possible inhibition in the fibrinolytic process can occur through inhibition of the plasminogen activators (t-PA and u-PA) by plasminogen activator inhibitors (PAI) or at the level of plasmin by mainly alpha 2- antiplasmin [111]. A third level of the regulation of plasmin involves the “Thrombin activatable Fibrinolysis inhibitor (TAFI)“, a carboxypeptidase responsible for clot stabilisation. It indirectly inhibits the fibrin breakdown through reduction of the capacity of plasminogen and t-PA binding to the fibrin surface. TAFI is activated through thrombin [114].

Tissue Plasminogen Activator (t-PA)

Tissue plasminogen activator (t-PA) is an active serine protease involved in vascular remodeling, fibinolysis, synaptic plasticity and neurodegeneration in the brain following trauma. Freely circulating t-PA is synthesized and secreted by fibroblasts, melanoma cells, vascular endothelial cells and neural cells [115, 116]. It consists of 530 amino acids. The serine protease inhibitors Serpin I1 and Serpin E1 (PAI 1) sequester freely circulating t-PA. The main function of the fibrin selectively t-PA is the conversion of plasminogen into plasmin. Reduced expression of t-PA is associated with thrombosis and formation of emboli, while increased activity of t-PA induces excessive bleeding. The universal role of t-PA arises also due to changes in t-PA expression in different organs, resulting in hypoxia, stroke and other injuries in the brain. Through activation of matrix metalloproteinases (MMPs) it is also implicated in blood-brain barrier breakdown, vascular remodeling, neurogenesis, angiogenesis and axonal regeneration.

The human t-PA gene is localized on chromosome 8. A polymorphism, characterized by a C to T transposition at the position -7351 of the t-PA gene, has been associated with vascular t-PA release. Carriers of the homozygous t-PA -7351CC genotype were found to have significantly higher t-PA release than those carrying either the t-PA -7351CT or -7351TT genotype [117].

t-PA antigen plasma levels are also determined by environmental factors such as plasminogen activator-inhibitor-1 activity, hypertriglyceridemia, hypercholesterolemia, chronic low-dose aspirin therapy and the intake of statins [116, 118].

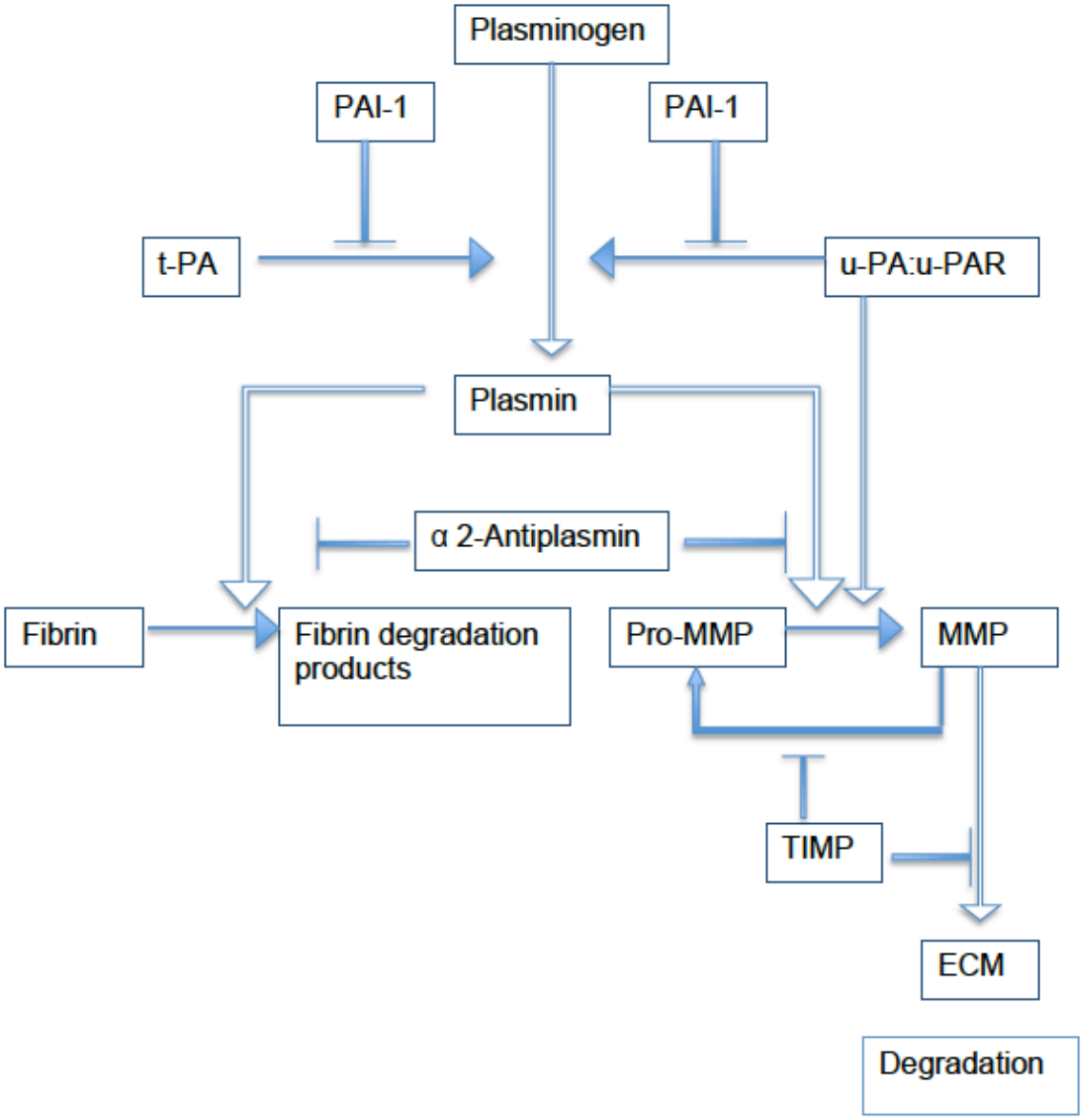


Figure 14: Schematic representation of the fibrinolytic system (after Collen[119] with permission of publisher Georg Thieme Verlag KG)

Plasminogen activator-inhibitor (PAI) 1

PAI-1 or Serpin E1 is a single- chain glycoprotein consisting of 379 amino acids and belongs to the familiy of serine proteinase inhibitors. In healthy individuals, high variability of PAI-1 plasma levels has been observed. Its main function is the regulation of the activity of the fibrinolytic system by inhibiting t-PA and u-PA.

Increased plasma levels of PAI-1, inducing reduced fibrinolysis, are frequently found among patients with deep vein thrombosis, stroke, myocardial infarction or coronary artery diseas [120].

The PAI-1 gene is located on chromosome 7, and after adjustment for age and sex the heritability of plasma PAI-1 levels has been reported to be 26% [121].

A common functional variant in the promoter region of the PAI-1 gene, which is characterized by the insertion or deletion of guanosine (PAI-1 4G/5G), has been shown to affect PAI-1 plasma concentrations [122]. Homozygotes for the PAI-1 4G allele were found to have higher plasma PAI-1 concentrations than heterozygotes or homozygotes for the PAI-1 5G allele.

Previous studies have shown that both genetic and environmental factors influence PAI-1 plasma levels [123, 124].

Among environmental factors, alcohol drinking, smoking, increased body mass-index, insulin resistance and physical activity are well known to affect PAI-1 plasma concentrations. As for lipid parameters, PAI-1 is positively associated with triglycerides and LDL-cholesterol, and negatively with HDL cholesterol.

Males are known to have higher plasma PAI-1 concentrations than females, and postmenopausal females show higher plasma PAI-1 levels than premenopausal females [124].

Endogenous thrombin potential (ETP)

Thrombin generation, a complex enzymatic mechanism, plays an essential role in the coagulation cascade. The more thrombin is generated, the more likely is the development of thrombosis.

Endogenous thrombin potential (ETP) respresents the potential thrombin-generating capacity of the coagulation cascade. In case of hypercoagulability ETP is increased, while in case of hypocoagulability ETP decreases [125, 126].

In 2007 van Hylckama Vlieg and coworkers reported that persons with an increased ETP had a significantly higher risk of a first deep vein thrombosis [127]. High ETP, however, was not associated with a significantly increased risk for recurrent venous thrombosis.

Wexels and coworkers also reported significantly higher ETP values in patients with venous thromboembolism [128]. In contrast to the findings by van Hylckama Vlieg and coworkers, Eichinger et al found high ETP to be associated with increased risk for recurrent venous thrombosis [129].

As for retinal vein occlusions, Cellai and coworkers evaluated ETP in 81 patients with retinal vein occlusions and 78 control subjects [130]. They found significantly higher ETP values in patients with retinal vein occlusions compared to control subjects.

Purpose of the dissertation

Based on previous indocyanine green angiography findings, showing choroidal vascular abnormalities including choroidal venous dilation, hyperpermeability and a choroidal filling defect, two Japanese research groups investigated the potential role of hypofibrinolysis in CSC. Activity of tissue-type plasminogen activator (t-PA), which is the principal activator of physiologic fibrinolysis, was found to be significantly lower in CSC patients compared to the control group. Furthermore, plasma concentrations of plasminogen activator type 1 (PAI-1), a serine protease that directly blocks the action of t-PA, were found to be significantly higher among CSC patients [131, 132]. The latter finding has recently been confirmed in a Turkish population, thus suggesting that hypofibrinolysis contributes to the pathogenesis of CSC [133].

First, we wanted to investigate ETP and t-PA antigen plasma levels in CSC patients and control subjects. In a second step we investigated two functional gene polymorphisms – PAI-1 4G/5G and tPA -7351C>T as potential risk factors for CSC in a second cohort [134].

Methods

Part 1- ETP and t-PA antigen plasma levels

Enrolment of study participants

In this prospective case-control study 67 patients with the diagnosis of CSC were included at the Department of Ophthalmology, Medical University of Graz. Patients were enrolled from February 2013 to December 2015. The study was approved by the local Ethics committee (25-070 ex 12/13).
All patients underwent complete ophthalmologic examination including best-corrected visual acuity (BCVA), slit lamp exmination, dilated indirect fundoscopy and optical coherence tomography. If necessary, fluorescein and indocyanine green angiography were performed. All patients signed an informed consent form after explanation of the study protocol by a physician and had a chance to discuss all open questions. The patients were informed that a blood sample of 23 ml was collected exclusively for the study. The control group consisted of 60 subjects, who were seen at the outpatient department of the Department of Ophthalmology.

Inclusion criteria

Inclusion criteria for patients with CSC comprised age over 18 years and diagnosis of active acute or chronic CSC (presence of neurosensory detachment and/ or pigment epithelial detachment on OCT). The control group consisted of patients, who were seen at the outpatient department of the Department of Ophthalmology, Medical University for reasons other than CSC including corneal foreign bodies, corneal erosion, posterior vitreous detachment, conjunctivitis, posterior capsule opacity or dry eye syndrome, and did not show evidence of CSC on OCT imaging.

Exclusion criteria

For CSC patients the exclusion criteria were as follows:

- age under 18 years
- denied consent or withdrawal of consent
- inactive acute or chronic CSC
- age-related macular degeneration
- choroidal polypoidal vasculopathy
- diabetic macular edema
- retinal vascular occlusions
- intake of corticosteroids within the last 6 months
- intake of any platelet aggregation inhibitors, anticoagulants or fibrinolytic drugs

For controls the exclusion criteria were as follows:

- age under 18 years
- denied consent or withdrawal of consent
- any history of chorioretinal disease and presence of ophthalmological findings consistent with the diagnosis of CSC as shown by OCT imaging
- intake of corticosteroids within the last 6 months
- intake of any platelet aggregation inhibitors, anticoagulants or fibrinolytic drugs

Patients with diabetes mellitus, defined as HbA1c level over 6.5%, or use of antidiabetic drugs, or any pathologies of the hepatic metabolism were excluded were excluded (routinely measurement of glutamate oxalacetate transaminase-GOT and glutamate-pyruvate transaminase- GPT). Furthermore, patients with intake of any platelet aggregation inhibitors, anticoagulants or fibrinolytic drugs were not eligible for study participation.

Four CSC patients were excluded because of partially missing laboratory parameters in tPA antigen, eight CSC patients were excluded for partially missing laboratory parameters in ETP, 2 control patients had to be excluded because of incomplete data. Therefore, a total of 63 patients and 58 controls underwent the study relevant measurements of t-PA antigen. ETP levels (AUC and Cmax) were measured in 59 patients and 58 controls.

Sample collection

Blood samples were drawn from the antecubital vein. Of each patient and control subject 3 tubes were collected after ophthalmologic examination, one tube containing serum separator clot activator (VACUETTE®, GreinerBioOne, Kremsmünster, Austria, Tube 6 ml Z Serum Separator Clot Activator) and Lithium Heparin (VACUETTE®, GreinerBioOne, Kremsmünster, Austria, Tube 8 ml LH Lithium Heparin).
Samples for determination of fibrinolytic parameters were collected in tubes with 3.2% sodium citrate (VACUETTE®, GreinerBioOne, Kremsmünster, Austria, Tube 9 ml 9NC Coagulation sodium citrate 3.2%).

After blood samples were collected, they were transferred to the laboratory at the Department of Ophthalmology, where plasma was isolated within 30 min by centrifugation at 4000 U for 20 min.

From each plasma sample, 3 plasma aliquots a 500 µl were transferred to Nunc Cryo tubes, using a standard pipette with unfiltered pipette tips. The samples were labeled with a specific participant identification number according to the privacy protection guidelines and were frozen and stored at -80°C until analysis was performed. The position of each tube in storage boxes was documented in Excel files, with participant´s identification number and date of sample collection, in order to easily relocate any specific sample.

Biochemical analysis

Following laboratory parameters were routinely measured:
To exclude any abnormalities in the glucose metabolism, each patient had to have HbA1c levels lower than 6.5% (DCA Systems HbA1c Reagent Kit, Siemens).
Furthermore, serum concentrations of cholesterol and triglycerides, GOT, GPT, C- reactive protein (CRP) and Lipoprotein A (LpA) were determined by standard methods at the Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Graz.

Plasma concentrations of ETP were determined using INNOVANCE® ETP (BCS® XP System, Siemens, Austria). The intra-assay coefficient of variation (CV) of ETP was 1.8%, the inter-assay coefficient of variation (CV) was 1.3%.
This ETP Kit works as a global hemostasis function test system to assess endogenous thrombin potential. The area under the curve (AUC) and peak height (Cmax) have been shown to be of diagnostic relevance:
Increased AUC was found to correlate with an increased risk for venous thrombosis after discontinuation of anticoagulation, increased AUC and additionally increased
C max levels have been demonstrated in patients with prothrombin mutation G 20210.
Further, AUC and Cmax are known to be influenced by treatment with vitamin K antagonists and AUC and C max [135] have been reduced under therapy with direct inhibitors (for instance Argatroban).
The Kit functions through the initiation of the coagulation processes through incubation of plasma with phospholipids, activator and calcium ions. This can eventually result in the generation of thrombin. The conversion of a specific slow reacting chromogenic substrate of a short wavelength (405 nm) over time demonstrates the generation and subsequent inactivation of thrombin. The substrate conversion curve is corrected through a mathematic algorithm, to substract the activity of bound thrombin, which is not active, from the substrate conversion curve. Therefore, the result indicates the activity of free thrombin and corresponds to the "thrombin generation curve". The area under the curve is referred to ETP.

Plasma concentrations of the t-PA antigen were analysed by the Quantikine® Human t-Plasminogen Activator/t-PA Immunoassay (R&D Systems, Inc., Minneapolis, USA), based on the quantitative sandwich enzyme immunoassay technique.
Therefore, a monoclonal antibody specific for human t-PA has been pre-coated onto a microplate, where standards and samples are pipetted into the wells. If t-PA was present, it was bound by the immobilized antibody.
After removing all unbound residues, an enzyme-linked polyclonal antibody specific for human t-PA was added to the wells. Again, any unbound antibody enzyme reagent was washed out. Furthermore, a substrate solution was added to the wells and color developed in proportion to the amount of t-PA bound in the initial step.
The color development was stopped and the intensity of the color measured. Mean intraassay coefficients of variation (CV) varied between 3.1 and 7.2, the corresponding mean interassay CVs varied between 4.0 and 6.6% [135].

Overview of the single procedure steps (according to the company´s website):

1) Bring all reagents and samples to room temperature, prepare all reagents, standard dilutions, and samples as directed in the product insert.
2) Addition of 50 µL of Assay Diluent to each well.
3) Addition of 50 µL of Standard, control or sample to each well.
4) Coverage with a plate sealer, and incubation at room temperature for 2 hours on a horizontal orbital microplate shaker.
5) Aspiration of each well and washing, repeating the process 3 times for a total of 4 washes.
6) Addition of 200 µL of Conjugate to each well. Coverage with a new plate sealer, and incubation at room temperature for 2 hours on the shaker.
7) Aspiration and washing 4 times.
8) Addition of 200 µL Substrate Solution to each well. Incubation at room temperature for 30 minutes on the benchtop. Protect from light.
9) Addition of 50 µL of Stop Solution to each well. Reading at 450 nm within 30 minutes with a wavelength correction to 540 nm or 570 nm.

Part 2- Gene polymorphisms affecting the fibrinolytic system in CSC [134]

Enrolment of study participants

In this single center case-control study 172 patients with CSC and 313 control subjects were recruited at the Department of Ophthalmology, Medical University of Graz.
All patients underwent an ophthalmologic examination including best-corrected visual acuity (BCVA), slit lamp examination, dilated indirect fundoscopy and optical coherence tomography (Spectralis, Heidelberg Engineering, Heidelberg, Germany). In every patient, fluorescein- and/or indocyanine green angiography were performed to confirm the diagnosis of CSC (Heidelberg Engineering, Heidelberg, Germany). All patients signed an informed consent form after explanation of the study protocol by a physician and had a chance to ask any remaining questions. The study was approved by the local Ethics Committee (26-495 ex 13/14).
The patients were informed that a blood sample of 3 ml was collected exclusively for the study. Control subjects were recruited among patients attending the outpatient department of the local department of Ophthalmology for other reasons than CSC. At the time of enrolment each of the control subjects underwent OCT imaging to exclude any ophthalmologic abnormalities of the posterior pole consistent with CSC.

Inclusion criteria

For inclusion into the study group the following criteria had to be fulfilled:
Age over 18 years with an acute or chronic type of CSC or proven previous episode of CSC in the past (fluorescein and/or indocyanine green angiography findings consistent with the diagnosis of CSC had to be present).
Patients with a history of CSC were contacted for enrolment into the study.

To be eligible for study enrolement, a fluorescein and/or indocyanine green angiography had to be present for all patients with CSC.

Control subjects were seen at the outpatient department of the Department of Ophthalmology, Medical University Graz, for reasons other than CSC, such as corneal foreign bodies, posterior vitreous detachment, conjunctivitis, posterior capsule opacity, dry eye syndrome or corneal erosion, and acquaintances were asked for participation in the study. In all subjects OCT imaging without findings consistent with the diagnosis of CSC was necessary for enrolment.

Exclusion criteria

For CSC patients the exclusion criteria were as follows:

- age under 18 years
- denied consent or withdrawal of consent
- age-related macular degeneration
- choroidal polypoidal vasculopathy
- diabetic macular edema
- retinal vascular occlusion

For controls the exclusion criteria were as follows:

- age under 18 years
- denied consent or withdrawal of consent
- any history of chorioretinal diseases, presence of pathologies of the posterior pole consistent with CSC on OCT imaging

Sample collection

Blood samples were drawn from the antecubital vein. Of each patient and control subject, one tube was collected after ophthalmologic examination, containing 3 ml EDTA blood (VACUETTE®, GreinerBioOne, Kremsmünster, Austria, 3 ml K3E K3EDTA).
After blood sampling, the probes were immediately frozen at -80°C until further analysis.

Genotyping

After transport to the Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Graz, the samples were treated as follows: DNA was isolated via MagNA pure LC DNA Isolation Kit (Roche Diagnostics, Vienna, Austria) through lysis of the cell membranes and degradation of proteins. Therefore, glass particles with integrated magnetic nanoparticles connect with DNA, so the DNA can be separated from residues through magnetism. After several circles of cleaning, the DNA is released by elution at 70°C.
The TaqMan SNP Genotyping Assay (Applied Biosystems, Vienna, Austria) utilizes the 5´Exonuclease activity of AmpliTaq Gold DNA Polymerase. This assay is based on the principle that the polymorphic region is amplified by PCR in the presence of two probes, complementary to one of the SNP alleles, labeled with two different fluorescent dyes. It contains two locus specific PCR primers, flanking the SNP to detect, and two allele- specific oligonucleotide TaqMan probes with a fluorescent reporter dye at the 5´end and a non-fluorescent quencher at the 3´end. The intact probe excites only minimal fluorescence due to fluorescent resonance energy transfer effect from the 5´fluorophore to the 3´quencher, because fluorescence of the fluorophore is quenched by the fluorescent resonance energy transfer effect (FRET). Due to cleavage by the 5´exonuclease activity of AmpliTaq Gold DNA Polymerase during each PCR cycle, where the intact probe is hybridized to the target allele, the fluorescent signal is generated. PCR primers amplify a specific locus on the genomic DNA template, and each fluorescent dye- labeled hybridization probe reports the presence of its associated allele in the DNA sample[136].

For each fluorogenic 5´exonuclease assay, probes and primers were designed through Applera´s Assays-by Design custom service. Probes, which were complementary to common

alleles, were labeled with VIC at the 5´ nucleotide, whereas rare alleles were investigated through their complementary probes, labeled with FAM (6- carboxy- fluorescein) [137].
For analysis of the PLAT -7351C>T polymorphism, the two TaqMan probes only differ in one base of the SNP- one TaqMan probe contains a cystosin, the other thymidine. Dependent on the particular SNP, the correct TaqMan probe binds on the SNP- if there is a cytosine on the SNP, a thymidine- TaqMan probe attaches more likely. In the case of PAI 4G/5G (SERPINE1**),** the gene polymorphism is characterized by the inseration/deletion of guanosine.

For purposes of quality control of genotyping, analysis of 96 samples was repeated and results from both analyses showed no discrepancies.

PLAT -7351C>T: [136] AGGAGCC[C/T]GCCCCAGACACAGCCATGGCCTGGGACTCTGGGGTCACCCTG GGGTCAGAAG FP: 5 ` -AGTGATCTCATTGCCGAGGTG-3` RP: 5 ` -CCCAGAGTCCCAGGCCA-3` C-probe: 5-AAAGGAGCCCGCCCCAGACA-3` T-probe: 5-CCAAAGGAGCCTGCCCCAGAC-3`
SERPINE1 (PAI 1 -675 4G/45G): [137] GGGCACAGAGAGAGTCTGGACACGT[-/G]GGGGAGTCAGCCGTGTATCATCGG FP:5´- TCTTTCCCTCATCCCTGCC.3´ RP:5`-CCAACCTCAGCCAGACAAGG-3 4C-probe: 5´-(VIC)ACACGGCTGACTCCCCACGT-3´ 5C- probe:5`-(FAM)ACGGCTGACTCCCCCACGT-3´

Figure 15: Overview of the single sequences with forward primer, reverse primer and different TaqMan probes.

Statistics

Statistical analysis of part 1 of the dissertation (ETP and t-PA antigen plasma levels) was performed at the Institute for Medical Informatics, Statistics and Documentation of the Medical University of Graz by "SAS 9.4 for Windows" software. Metric values were described as mean ± standard deviation, non-parametric values as median (range). Evaluation for normal distribution was done visually by QQ-plots. In case of a normal distribution, differences between the groups were evaluated by Student's T-Test. Group differences in non-parametric parameters were analyzed by Mann-Whitney-U Test. Difference in sex was evaluated by Fisher´s exact test. A p-value of less than 0.05 was considered to be statistically significant.

Statistical analysis of part 2 of the dissertation (genetic analysis) was done using SPSS 22.0 for Windows. Statistical analysis of the differences in sex and age between both groups were analysed with with fisher´s exact test in sex and Student´s T-test in age, respectively. Metric values were analyzed by Student´s t-test and presented as mean ± standard deviation.
Odds ratios (ORs) and 95% confidence intervals (95% CIs) were determined by logistic regression analysis, additionally adjusted for age and sex.
The criterion for statistical significance was $p < 0.05$ [134].

Results

Part 1 – ETP and t-PA antigen plasma levels

Patients characteristics

Study participants

Inclusion (0=yes, 1=no)	Group		
	CCS patients	controls	Sum
0	63	58	121
1	4	2	6
sum	67	60	127

Table 1: Inclusion of study participants

Four patients and two controls were excluded from analysis because of incomplete data (inclusion=1). Consequently, the final study group comprised 63 (52.1%) patients with CSC and 58 controls (47.9%).

Normal distribution was visualized using QQ plots, and was present in the parameters ETP_AUC and t-PA antigen. Only the parameter of ETP Cmax (mE/min) showed a deviation from normal distribution. Therefore, the Mann-Whitney-U-Test was used for correct interpretation of these parameters.

Age

	N	Mean value	SD	Median	Min	Max
CCS	63	46.0	10.6	45.0	29.0	76.0
controls	58	45.9	9.5	45.5	25.0	64.0

Table 2: Age distribution of patiens and controls, study 1

There was no statistically significant difference in age between patients and controls (p=0.98)

Sex

The CSC group comprised 57 male and 6 female individuals, with a mean age of 46.0 years (SD= 10.6) at time of sample testing. In the control group 58 individuals, 56 males and 2 females with a mean age of 45.9 years (SD= 9.5) were enrolled. There was no significant difference between groups in gender distribution using fisher´s exact test (p=0.28).

Sex	group		
	CSC	control subjects	Total
male	57	56	113
	90.48	96.55	
female	6	2	8
	9.52	3.45	
Total	63	58	121
	52.07	47.93	100

Table 3 Sex distribution of CSC patients and controls

Laboratory parameter

Endogenous Thrombin potential

Endogenous Thrombin potential (AUC)

Parameter	Group	N	Mean Value	SD	p-value (T-Test)
ETP_AUC [mE]	CSC	59	375	57.2	0.43
	Controls	58	367.1	51.3	

Table 4: Overview of results of ETP_AUC

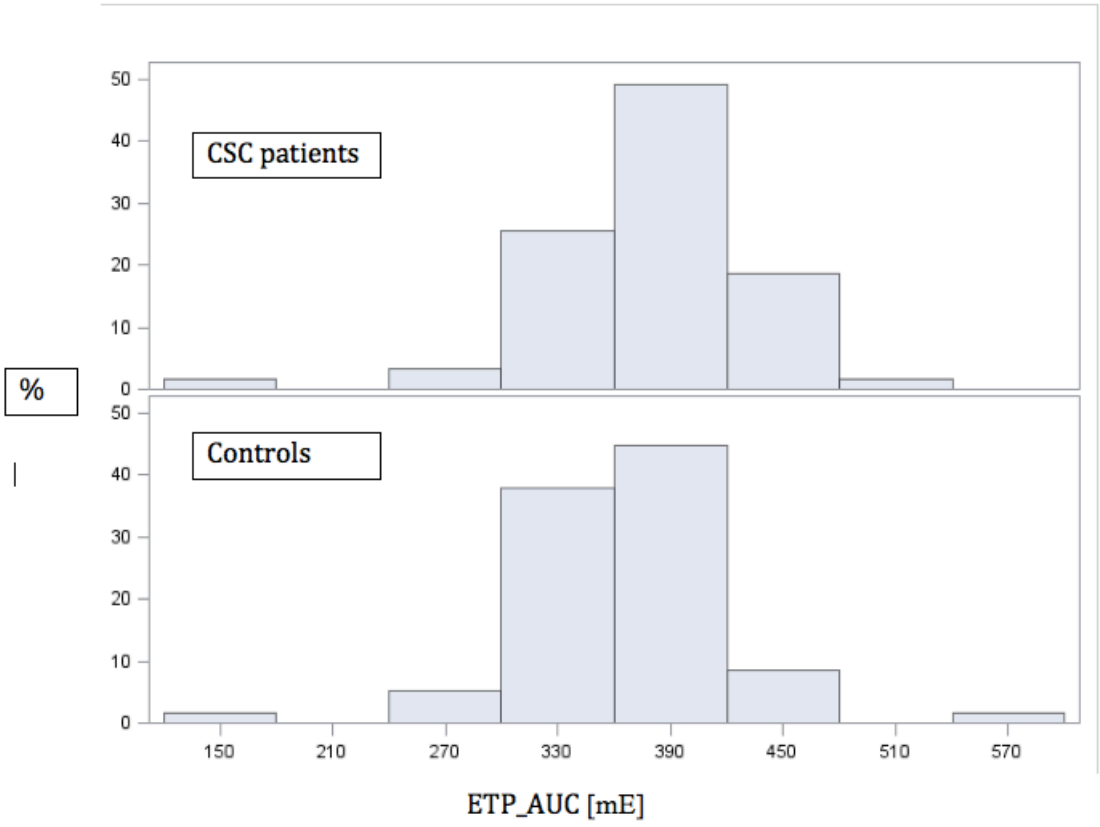


Figure 16 Comparison of ETP_AUC levels (%) between CSC patients and controls

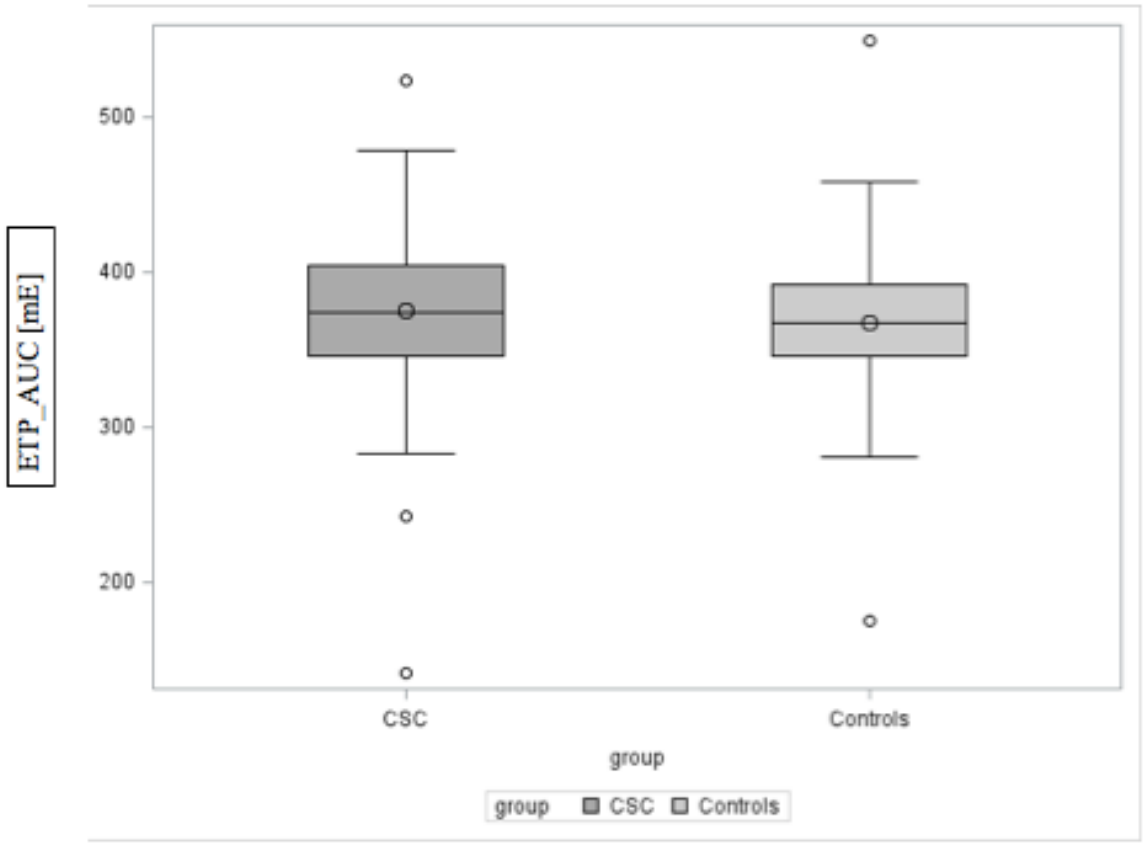


Figure 17 ETP_AUC. Box plot diagram for CSC patients and controls

Summary of ETP_AUC results

The present study comprised 59 patients with CSC and 58 control subjects for ETP. The mean age of patients was 46.0 ± 10.6 years (range: 29 –76 years) and 45.9 ± 9.5 years (range: 25 – 64 years) among controls (p=0.98).

No significant differences in ETP_AUC were found between patients with CSC and control subjects (p=0.43).

ETP (C max)

Parameter	Group	N	Median	Min	Max	p- value (MWU)
ETP_Cmax [mE/min]	CSC	59	106.7	83.2	330.3	0.07
	Controls	58	104.2	28.8	150.8	

Table 5: Results of ETP (Cmax)

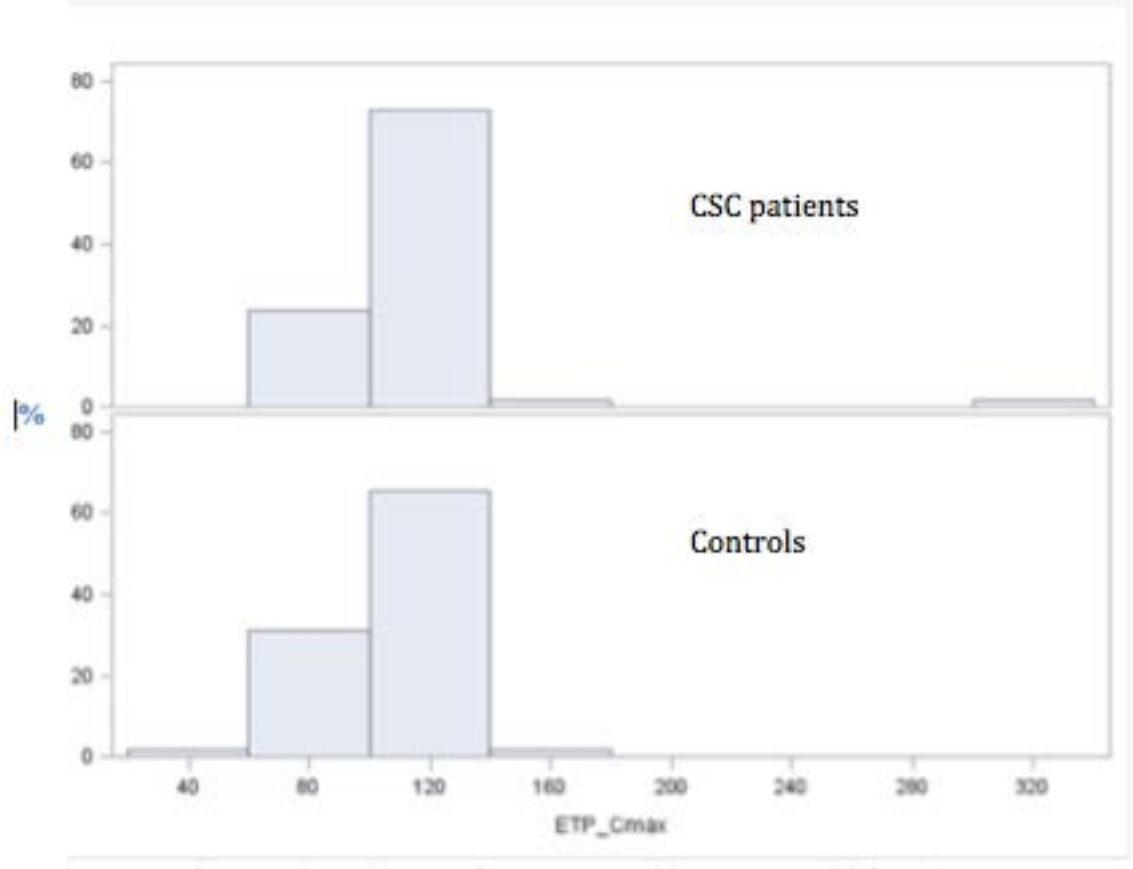


Figure 18 Comparison of ETP Cmax levels (%) between CSC patients and controls

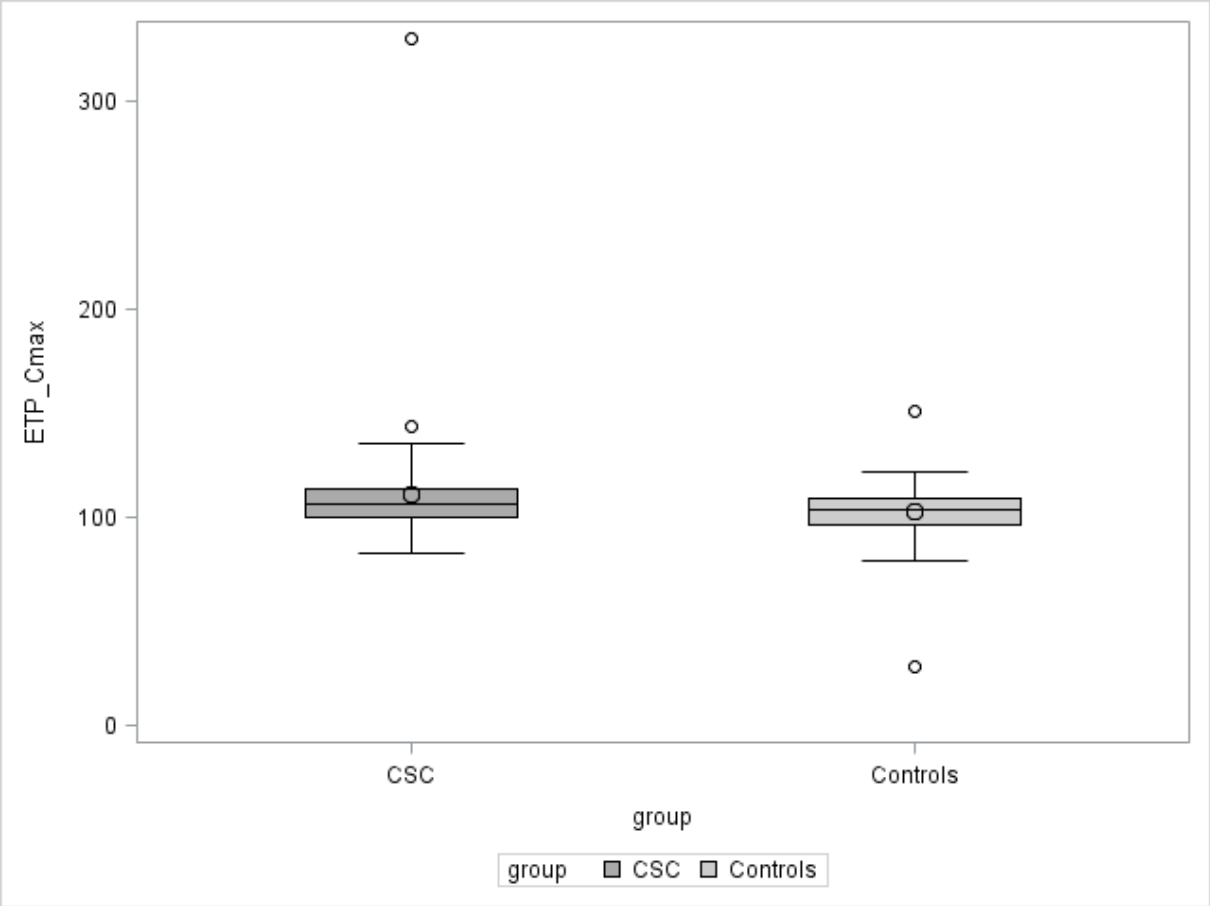


Figure 19 ETP _C max. Box plot diagram for CSC patients and controls

Summary of ETP_C max results

No significant differences in ETP_ C max were found between both groups (p=0.07).

t-PA antigen

Parameter	Group	N	Mean Value	SD	p-value (T-Test)
t-PA antigen [pg/ml]	CSC	63	3673.1	1281.6	0.04
	Controls	58	3228	1079.8	

Table 6: Results of plasma t-PA antigen levels

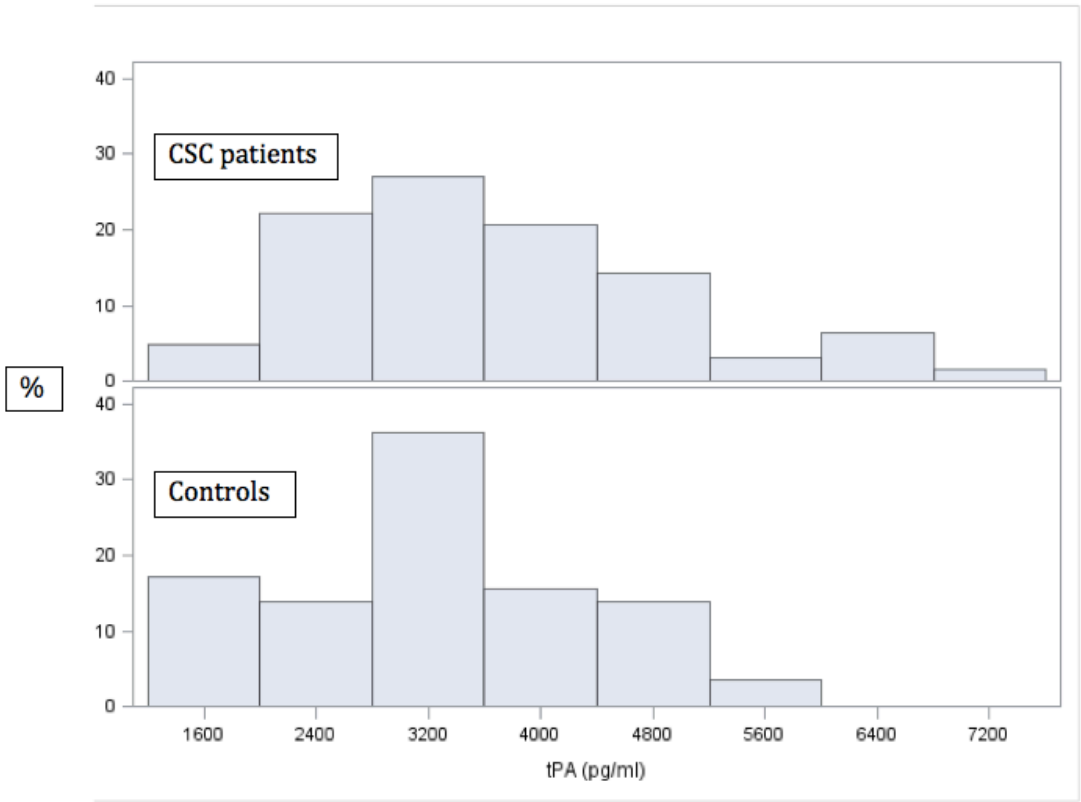


Figure 20 Distribution of t-PA plasma antigen levels (%) in CSC patients and controls

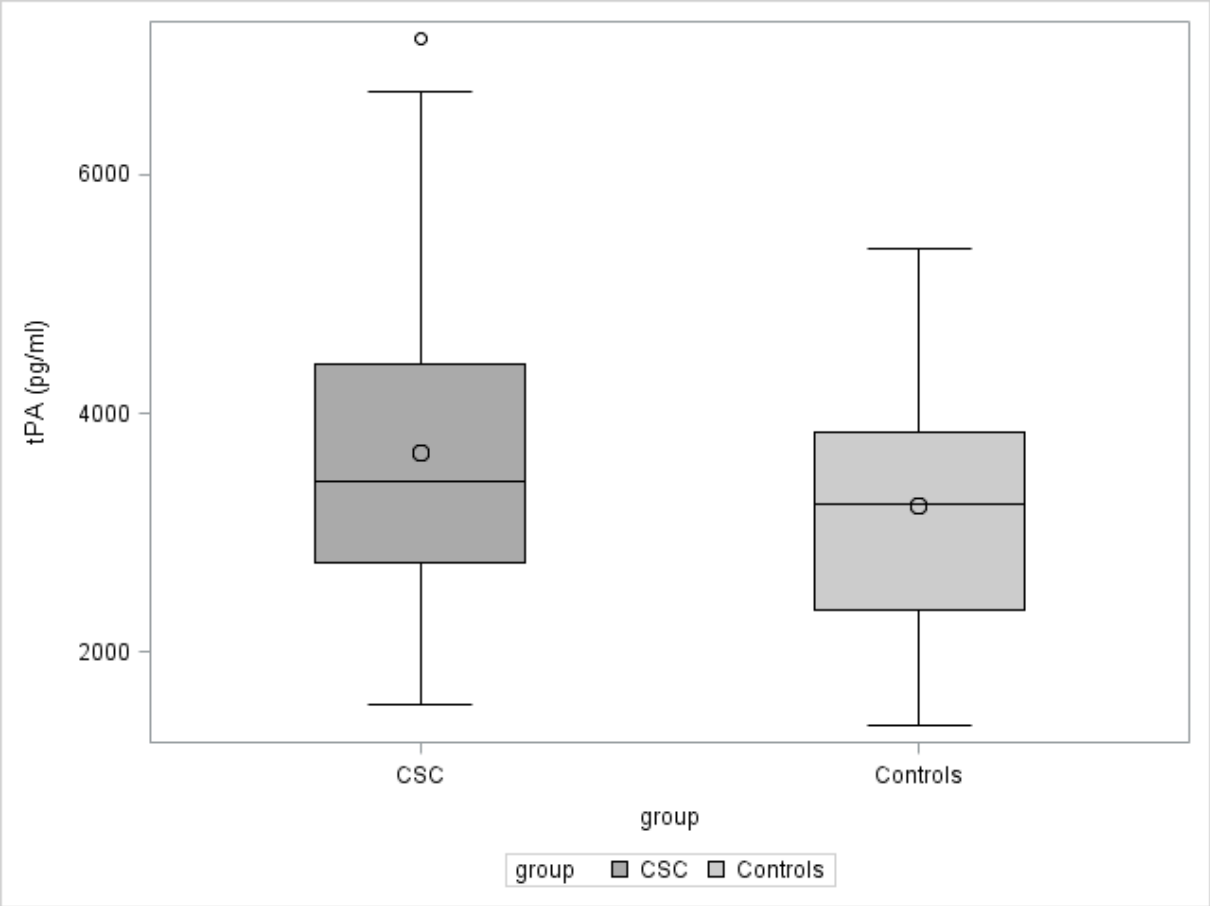


Figure 21 t-PA antigen plasma levels. Box plot diagram for CSC patients and controls

Summary of t-PA antigen plasma concentrations

The present study comprised 63 patients with CSC (57 [90.48%] males) and 58 control subjects (56 [96.55%] males) for the analysis of plasma t-PA antigen concentrations. The mean age of patients was 46.0 ± 10.6 years (range: 29 –76 years) and 45.9 ± 9.5 years (range: 25 –64 years) among controls (p=0.98).

As for t-PA antigen plasma levels, patients with CSC showed significantly higher levels than control subjects (p=0.04).

Results Part 2

PAI-1 4G/5G and t-PA -7351C>T gene polymorphisms in CSC [134]

Genotypes of the PAI-1 4G/5G and t-PA -7351C>T polymorphisms were determined in 172 patients with CSC and 313 control subjects.
No significant differences in either genotype or allele frequencies were found between both groups. Presence of the homozygous PAI-1 4G/4G genotype was associated with a non-significant adjusted OR of 1.21 (95%CI: 0.77-1.92; p=0.41) for CSC.
Homozygosity for the t-PA -7351C-allele was associated with a non-significant OR of 0.91 (95%CI: 0.62-1.33; p=0.62) for CSC.

Genotype distributions were in line with those predicted by the Hardy-Weinberg equilibrium. The present study had a statistical power of 0.85 to detect an OR of 1.8 for carriers of the t-PA -7351CC genotype, and a statistical power of 0.82 to detect an OR of 1.9 of the PAI-1 4G/4G genotype at a significance level of 0.05.

Demographic data

Age distribution

	Mean value	N	SD	Median	Minimum	Maximum
Controls	49.18	313	17.38	49.2	19.4	85.6
Patients	54.02	172	11.86	53.25	31.7	78.6
Total	50.9	485	15.81	50.2	19.4	85.6

Table 7: Age distribution of patients and controls, part 2.

After testing with fisher´s exact test, there was a significant difference in age between both groups (p=0.004)

Gender distribution

The CSC group consisted of 172 patients (142 male and 30 female individuals), with a mean age of 54.02 years (SD 11.86) at the time of sample testing. In the control group 313 individuals (215 males and 98 females) with a mean age of 49.18 years (SD 17.38) were enrolled.

PAI-1 4G/5G gene polymorphism [134]

Table 8: Genotype distributions and allelic frequencies of the PAI- 1 4G/5G polymorphism

	Control subjects	Patients	P
PAI-1 4G/5G:			
4G/4G	64 (20.4)	42 (24.4)	0.48
4G/5G	166 (53.0)	91 (52.9)	
5G/5G	83 (26.5)	39 (22.7)	
5G allele frequency	0.530	0.491	0.48

Table 9: Overview of crude odds ratio and odds ratio adjusted for age and sex for the homozygous PAI-1 4G/4G genotype

	Crude OR	95% CI-Min	95% CI-Max	Adjusted OR	95% CI-Min	95% CI-Max
PAI-1 4G/4G	1.26	0.79	2.0	1.21	0.77	1.92

t-PA -7351C>T polymorphism [134]

	Control subjects	Patients	P
t-PA-7351C>T:			
CC	144 (46.0)	73 (42.4)	0.60
CT	127 (40.6)	78 (45.3)	
TT	42 (13.4)	21 (12.2)	
T allele frequency	0.337	0.349	0.59

Table 10: Genotype distributions and allelic frequencies of the t-PA -7351C>T polymorphism

	Crude OR	95% CI-Min	95% CI-Max	Adjusted OR	95% CI-Min	95% CI-Max
t-PA-7351 CC	0.87	0.58	1.28	0.91	0.62	1.33

Table 11: Overview of crude odds ratio and odds ratio adjusted for age and sex for the homozygous t-PA -7351CC genotype

Discussion

CSC is the fourth most common nonsurgical retinal disease. It commonly affects both eyes and severe visual impairment may occur due to the development of choroidal neovascularisations, cystoid retinal degeneration or chorioretinal atrophy[2].

Numerous risk factor for CSC have previously been identified including increased psychological stress, type A personality pattern, local or systemic administration of corticosteroids, increased endogenous plasma cortisol levels and arterial hypertension [7, 86, 80].

Over recent years, genetic polymorphisms encoding proteins of the complement cascade, cadherin 5 and the mineralocorticoid receptor have been suggested as major risk factors for CSC [90, 102, 103, 104, 91, 106].

Nevertheless, not all cases can be fully explained by the known risk factor alone. Thus, the identification of other as yet unknown risk factors is of paramount importance.

ICG findings in CSC patients showed choroidal venous dilation, choroidal hyperpermeability and choroidal filling defects, pointing to a role of impaired choroidal blood flow in the pathogenesis of the disease [59, 76].

In line with these findings, two Japanese and one Turkish study group each reported significantly increased plasma concentraions of PAI-1 in patients with CSC compared to control subjects [138, 132, 133]. As pointed out above, PAI-1 is the major inhibitor of t-PA and increased plasma concentrations lead to a hypofibrinolytic state. Interestingly, Yamada and coworkers reported a significantly lower t-PA activity in CSC patients than in the control group.

Both increased PAI-1 plasma levels and a low activity of t-PA, the principal activator of the physiologic fibrinolysis, contribute to a hypofibrinolytic state, which may at least in part explain the ICG findings observed among CSC patients.

In contrast to the fibrinolytic system, the role of a procoagulant imbalance as a potential risk factor for CSC has so far not been investigated. Hypercoagulability can be caused by numerous distinct alterations in the coagulation cascade. ETP reflects the balance between pro- and anticoagulant factors and thus can be used to investigate hyper- and hypocoagulability [139, 126].
It has also been suggested to provide a method for quantifying the composite effect of multiple risk factors.

In the present study we hypothesized that a hypercoagulable state might also contribute to the presence of CSC. Yet, ETP did not significantly differ between CSC patients and control subjects, suggesting that a procoagulant state is unlikely a major risk factor for CSC.
Mean plasma t-PA antigen levels, which were also determined in this cohort of study participants, were found to be significantly higher in CSC patients than in control subjects. This is in line with the findings of Yamada et al, who also found higher plasma t-PA antigen concentrations in Japanese patients with CSC than among controls [132].

Previous studies have established a significant correlation between increased plasma PAI-1 levels and increased plasma t-PA antigen concentrations [140, 124, 141].
As is also true for the assay used in our study, the plasma t-PA antigen concentration measured consists of free active t-PA and of t-PA bound to substrates such as PAI-1.
Thus, increased plasma t-PA antigen concentrations - as measured in the present analysis- might be due to increased plasma PAI-1 levels. Nevertheless, as a limitation of the present analysis, plasma PAI-1 concentrations and t-PA activity were not additionally determined in this cohort of study participants because too little plasma was available for analysis.

Due to these results we analysed in the second part of this thesis two functional polymorphisms of the PAI-1 and t-PA gene (PAI-1 4G/5G and t-PA -7351C>T) as potential genetic risk factors for CSC.

Genetics

The functional PAI-1 4G/5G and t-PA -7351C>T polymorphisms were analyzed in a second, larger cohort of CSC patients of Caucasian descent [134]. As mentioned above, increased plasma PAI-1 concentrations and lower t-PA activity have previously been found among patients with CSC [138, 132, 133].

Besides environmental factors, genetic polymorphisms have also been shown to affect the expression of these proteins [140, 124, 118, 123].

As for PAI-1, a common polymorphism in the promoter region of the PAI-1 gene, which is characterized by the insertion or deletion of guanosine (PAI-1 4G/5G), has been shown to affect plasma concentraions of PAI-1 [122]. Homozygotes for the PAI-1 4G allele were shown to have higher plasma PAI-1 concentrations than heterozygotes or homozygotes of the PAI-1 5G allele. Genotypes of the PAI-1 4G/5G polymorphism were determined in 172 patients with CSC and 313 control subjects.

Neither genotype nor allele frequencies of the PAI-1 4G/5G polymorphism differed significantly between patients and control subjects [134]. This is in line with a previous study in a Turkish population, which analyzed 60 patients with CSC and 50 control subjects and did not find either a significant diference in genotype distributions of this polymorphism between both groups [133]. However, their study was limited by the very small number of participants being analyzed.

Importantly, our analysis had a statistical power of 0.82 to detect an odds ratio of 1.9 for carriers of the homozygous PAI-1 4G/4G genotype at a significance level of 0.05.

The present result thus suggests that the PAI-1 4G/5G gene polymorphism is unlikely a major risk factor for CSC. Further, it extends our knowledge of this polymorphism as a potential risk factor for CSC to a Central European population of Caucasian descent [134].

In 2002 Freeman and coworkers studying 537 adults from 89 randomly ascertained healthy families found that after adjustment for age and sex the heritability of PAI-1 was 28% and that the at the time identified gene variants, which included the PAI-1 4G/5G gene polymorphism, accounted for only a small fraction of this heritability [121].

Moreover, Margaglione and coworkers studied the influence of environmental and genetic determinants on PAI-1 plasma concentrations in a general population without clinical evidence of atherosclerosis [123]. In a univariate analysis, they found plasma PAI-1

concentrations to be significantly higher in males, alcohol drinkers, cigarette smokers and homozygotes for the PAI-1 4G allele.
In a multivariate analysis, PAI-1 plasma levels were predicted by body mass index, triglycerides, male sex, cigarette smoking, the PAI-1 4G/5G polymorphism and the ACE I/D polymorphism.
The authors demonstrated that the contribution of the PAI-1 4G/5G polymorphism to plasma PAI-1 concentrations was small compared to environmental determinants. Other studies have suggested that this polymorphism does not exert a great effect on basal PAI-1 concentrations, but influences the PAI-1 gene expression in response to very low density lipoproteins or cytokines such as interleukin 1 [124, 142, 143].
Taking the fact into account that environmental factors strongly determine PAI-1 plasma concentrations, our finding that the PAI-1 4G/5G polymorphism is not associated with the presence of CSC does not imply that PAI-1 itself does not play an essential role in the pathogenesis of CSC. Unfortunately, plasma samples for determining PAI-1 concentrations were not available, which is a limitation of this analysis.

In the present study a t-PA polymorphism, which is characterized by C to T transition at position -7351 of the t-PA gene (t-PA -7351C>T), was also investigated as a potential risk factor for CSC. [117] Subjects with the homozygous t-PA -7351CC genotype had been found to have twice as high t-PA release rate than heterozygotes or homozygotes for the t-PA -7351T allele. Data with regard to this functional t-PA polymorphism as a risk factor for CSC had not been reported by other studies. The novel finding of this analysis is thus that no significant differences in either genotype distributions or allelic frequencies of the t-PA -7351C>T polymorphism could be found between patients and control subjects [134].
Homozygosity for the t-PA -7351C allele was associated with a non-significant odds ratio of 0.87 for CSC. Importantly, the present study had a statistical power of 0.85 to detect an odds ratio of 1.8 for carriers of the t-PA -7351CC genotype, thus suggesting that this polymorphism is unlikely a major risk factor for CSC at least in a Central European population of Cauacasian descent.
In the study by Freeman and coworkers, which determined the genetic contribution of hemostatic factors in healthy families, the heritability for t-PA was reported to be 23% [121]. Geppert and coworkers analyzed baseline t-PA antigen concentrations in 366 patients with angiographic evidence of coronary artery sclerosis [118]. In a multivariate analysis, t-PA antigen concentrations were predicted by PAI-1 activity, hypertriglyceridemia, low dose aspirin

intake, type of angina, hypercholesterolemia and multivessel disease. Furthermore, gene transcription of t-PA is well known to be modulated by the intake of statins or by growth factors such as vascular endothelial growth factor (VEGF) or transforming growth factor beta (TGF-ß) and hormones like strogen and dexamethasone [116].
Thus again, our finding that the t-PA -7351C>T polymorphism is not associated with CSC risk does not imply that t-PA itself does not play a major role in the pathogenesis of CSC.

This has already been suggested by Yamada et al demonstrating increased plasma t-PA antigen concentrations and lower plasma t-PA activity among Japanese patients with CSC [132].
In the first part of this thesis, a significantly higher plasma t-PA antigen concentration was found in patients with CSC compared to the control group too.
As we pointed out in the publication of the genetic results in the journal *Ophthalmic Genetics*, recent technological advances made the simultaneous analysis of numerous gene polymorphisms in a single study possible [134].
Yet, together with the use of a significance threshold of 0.05 a high rate of false positive results will be observed. One possible approach to this problem is focusing on a few plausible hypotheses and potential risk factors with a high probability of positive association.

Therefore, in this analysis only the two PAI-1 and t-PA gene variants with the highest probability for a positive association with CSC were investigated.
In the meantime, three genome-wide association studies in European and Asian populations have been performed. None of these studies detected a PAI-1 or a t-PA gene polymorphism as a significant risk factor for CSC [144, 145, 146].
Interestingly, a genome-wide association study on choroidal thickness, which itself is an important feature in the pathogenesis of CSC, identified two susceptibility polymorphisms - CFH rs800292 and VIPR2 rs3793217 [147]. Both polymorphisms were subsequently analysed in a large case-control study demonstrating a significant association with CSC in a Japanese population.
In contrast to gene variants of factors involved in hemostasis, gene polymorphisms encoding proteins of the complement cascade such as CFH have been repeatedly found to be associated with CSC risk and are therefore thought to play an important role in the pathogenesis of this disease [90, 102, 103].

Conclusion

In this thesis we investigated whether ETP, a parameter reflecting hyper- or hypocoagulability, would be significantly increased among patients with CSC, thus suggesting that not only a hypofibrinolytic state, but also hypercoagulability would play a role in the pathogenesis of CSC. Nevertheless, we did not find a significant difference in ETP levels between patients with CSC and control subjects.

In line with a previous study by Yamada and coworkers, plasma concentrations of the t-PA antigen were significantly higher in CSC patients than among control subjects. The plasma t-PA antigen concentration measured reflects the sum of free active t-PA and t-PA bound to PAI-1. This again points to a role of hypofibrinolyis in the pathogenesis of CSC. Other fibrinolytic parameters such as t-PA activity or PAI-1 plasma concentrations were not measured because too little plasma was available for analysis.

In a further step, the t-PA -7351C>T and the PAI-1 4G/5G gene polymorphisms were investigated in a second, larger cohort of CSC patients of Caucasian origin.

As a novel finding, neither genotype distributions nor allele frequencies of the t-PA -7351C>T polymorphism differed significantly between both groups.

In line with the findings of Sogutlu Sari and coworkers in a Turkish population, a significant difference to the PAI-1 4G/5G polymorphism was not found. This extends our knowledge of the role of this polymorphism in CSC to a Central European population of Caucasian origin.

Our findings that both the PAI-1 4G/5G and the t-PA -7351C>T polymorphisms were not associated with increased risk for CSC, does not imply that plasma PAI-1 concentrations and t-PA activity themselves do not play a major role in the pathogenesis of CSC as both factors are strongly determined by environmental factors.

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