SEMMELWEIS EGYETEM

DOKTORI ISKOLA

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CHOROIDAL THICKNESS CHANGES IN PATIENTS WITH DIABETES MELLITUS

Ph.D. thesis

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List of Abbreviations

AL: axial length

AMD: age related macular degeneration

BCVA: best corrected visual acuity

BM: Bruch's membrane

CC: choriocapillaris

CL: confidence level

CNV: choroidal neovascularization

CRT: central retinal thickness

CSCT: central subfoveal choroidal thickness

CT: choroidal thickness

CVD: choroidal vascularity density

CVI: choroidal vascularity index

CVV: choroidal vascular volume

CXCR4: CXC chemokine 4

DC: diabetic choroidopathy

DM: diabetes mellitus

DME: diabetic macular edema

DNA: deoxyribonucleic acid

DR: diabetic retinopathy

EDI: enhanced depth imaging

F: female

FA: fluorescein angiography

GEE: general estimating equation

HbA1c: glycated hemoglobin A1c

HL: Haller's layer

ICGA: indocyanine green angiography

M: male

NO: nitric oxide

NOS: nitric oxide synthases

NPDR: non-proliferative diabetic retinopathy

OCT: optical coherence tomography

OCTA: optical coherence tomography angiography

PAS: periodic-acid Schiff

PDR: proliferative diabetic retinopathy

PMNs: polymorphonuclear neutrophils

PRP: panretinal photocoagulation

QICC: Corrected Quasi Likelihood under Independence Model Criterion

rANOVA: repeated measures analysis of variance test

RET: retina

RPE: retinal pigment epithelium

SC: suprachoroid

SCL: sclera

SD: standard deviation

SDF-1: stromal cell-derived factor-1

SD-OCT: spectral-domain optical coherence tomography

SL: Sattler's layer

SPSS: Statistical Package for the Social Sciences

SS-OCT: swept-source optical coherence tomography

T1DM: Type 1 diabetes mellitus

T2DM: Type 2 diabetes mellitus

VA: visual acuity

VEGF: vascular endothelial growth factor

1. Introduction

Diabetic retinopathy (DR) and diabetic macular edema (DME) are leading causes of blindness among working age adults worldwide, including Hungary (1). The main retinal insults in diabetes mellitus (DM) are vascular and neural (2). Increased retinal vascular permeability caused by alteration of the blood-retinal barrier as a result of dysfunctional tight junctions, vessel occlusions, leakage and endothelial cell mediated leukostasis lead to retinal edema and ischaemia (2-3).

However, chronic hyperglycemia injures not only the retinal vessels but also the choroidal vessels (4). The choroid provides oxygen and nutrients to the outer retinal layers and it is the only blood supply of avascular fovea (5). In DR several histopathological choroidal abnormalities have been reported, including vascular remodelling with increased tortuosity, obstruction and dilatation of the choriocapillaries, choroidal microaneurysms and choroidal neovascularization (6, 7). Experimental and clinical findings suggested that choroidal vasculopathy may play a vital role in the pathogenesis of DR (8).

Until recently, the clinical evaluation of choroid could only be performed by indocyanine green angiography, laser flowmetry and ultrasonography, but these techniques did not show the three-dimensional anatomy of choroidal layers (7). With the advent of optical coherence tomography (OCT) a non-invasive, high-quality imaging technique for ocular structures became possible (9). Enhanced depth imaging (EDI) of spectral-domain (SD) OCT scans was shown to produce higher resolution with increased depth of penetration, allowing quantitative and cross-sectional imaging of the choroid (10). Swept-source (SS) OCT can achieve even better three-dimensional choroidal images due to its longer wavelength (1050 nm) and faster scanning speed (100,000 A-scan/sec) (11-12). Unlike SD-OCT, SS-OCT machines with using a new software are also capable of creating automatic choroidal volumes and thickness measurements (13).

The choroidal thickness (CT) of diabetic patients has been evaluated in several studies with conflicting results (14-18). In all these studies principally a mixed cohort of treated and non-treated DR eyes was evaluated (8, 19).

There are various metabolic and environmental factors, such as higher glycated hemoglobin A1c (HbA1c) level, a longer duration of diabetes mellitus, hypertension, and dyslipidemia that affect the development and progression of DR (20, 21). In addition, over the past few years, a number of genetic studies have shown that genetic factors may also play an important role in the pathogenesis of DR (22, 23).

Stromal cell-derived factor-1 (SDF-1) is an active chemokine that mobilizes endothelial progenitor cells (24, 25). Accumulating experimental and clinical data suggest that SDF-1 plays a significant role in the micro- and macrovascular complications of DM (26). In terms of ophthalmological complications, SDF-1 plays a crucial role in developing proliferative diabetic retinopathy (PDR) via promoting vascular endothelial growth factor (VEGF)-mediated neoangiogenesis (25, 27, 28) and it is also a key factor of DME by increasing microvascular permeability (29). In addition, the variant of the *SDF-1 gene* is already known to be associated with the increased production of SDF-1 and the development of DR (30, 31, 32). Djuric et al. found that *SDF-1 (c801AA)* homozygosity is associated with the risk of PDR and pointed to a possible role of this allelic variant in the development of PDR (33).

To the best of our knowledge, the molecular mechanism of choroidal changes in diabetes and its relationship to SDF-1 has not been investigated yet.

1.1 Anatomy and physiology of the choroid

1.1.1. Macro- and microscopic anatomy of the human choroid

The choroid is a sponge-like, vascularized and pigmented tissue located between the lamina fusca of the sclera and the retina and making up the posterior part of the uvea (34, 35). It extends from the ora serrata anteriorly to the optic nerve posteriorly (8). Histological

investigations of the choroid demonstrated that it is approximately 200 μm thick at birth and decreases to about 80 μm by the age of 90 (36). Histologically, the majority of the choroid is characterized by its layered vasculature, with stromal tissue accounting for only about 20% of its volume (35, 37).

The choroid is supplied primarily from the long and short ciliary arteries with some contribution from the anterior ciliary arteries (38). The distribution is segmental. Both aforementioned arteries eventually form the choroidal arteries terminating in a lobular pattern and can be considered functionally as ‘end arteries’ (39). Drainage of blood from the choroid is thought to occur exclusively through four to eight vortex veins that ultimately merge with the ophthalmic vein (40).

The following layers are distinguished from the outer retina towards the sclera: Bruch's membrane, the choriocapillaris (CC), Sattler's and Haller's layer, and the suprachoroidal layer (Figure 1) (35). Bruch's membrane is formed by the fusion of the basement membrane of the retinal pigment epithelium (RPE) and the fibrous basement membrane derived from the endothelial cells of the outermost capillaries of the choriocapillaris layer (5). According to Hogan’s classification of the early 1960s, Bruch's membrane consists of five layers, which include the basal lamina of the RPE, the inner collagenous layer, the elastin layer, the outer collagenous layer, and the basal lamina of the choriocapillaris (41). It provides a filtration barrier to regulate exchange between the RPE and choriocapillaris (42). The anatomy of the Bruch’s membrane makes it permeable to ions, metabolic waste, and glucose, and the age-related changes in its anatomy play a significant role in disease incidence (42, 43).

The choriocapillaris layer consists of a network of densely compacted capillaries that serve as the final route of choroidal blood flow (35). These capillaries are organized into a series of hexagonal-shaped lobules, which are smaller at the posterior pole, and become progressively larger towards the periphery (35, 44). The choriocapillaris lobules are separated by many tiny intercapillary spaces (35). These spaces may increase with age and diseases, such as myopia and age-related macular degeneration (AMD), and this enlargement may reflect a reduction in the number or diameter of the choriocapillaris

vessels (35, 45). The capillaries have a distinctly wider lumen than other capillaris bed found in the body, allowing more than one red blood cell to pass through (42). In addition, the choriocapillaris layer has a distinct planar geometry, which allows three to four times more diffusion of molecules compared to the choriocapillaris layer of kidneys (42). The walls of the capillaris vessels are extremely thin and contain multiple fenestrations, which are pore-like structures in the cell membrane (35). These fenestrations are located only on the retinal side of the choriocapillaries (35, 46). The specialized anatomy of the choriocapillaris lamina allows it to provide oxygen, micronutrients, ions, and water to the RPE and regulating outer ocular temperature (5, 42). The high permeability of the fenestrated capillaries also maintains a high oncotic pressure that allows fluid to move out of the retina through the choroid layers and eventually drain out of the sclera (42, 47). The thickness of the choriocapillaris shows regional variability. It is around 10 μm thick at the fovea, the area of highest metabolic activity, and 7 μm thick in the periphery of the retina (35, 48).

Traditionally, the vascular system between the choriocapillaris and the suprachoroid has been subdivided into two different layers based on histological examinations performed by anatomists, such as Sattler and Haller (35). Sattler's layer lies adjacent to the choriocapillaris and is comprised of medium-sized arterioles feeding the choriocapillaris and draining venules, followed by Haller's layer containing larger vessels (35, 49). The stroma (extravascular tissue) contains collagen and elastic fibers, fibroblasts, non-vascular smooth muscle cells and numerous very large melanocytes that are closely apposed to the blood vessels (5). The pigmented appearance of the choroid is due to the presence of melanocytes that in healthy individuals are distributed throughout the stroma with the exception of the choriocapillaris layer (35, 50). Little is currently known about the physiological and pathophysiological characteristics of these two layers (35).

The suprachoroid is a transitional zone between choroid and sclera containing elements of both collagen fibers, fibroblasts and melanocytes (5). The suprachoroid has large endothelial-lined spaces receiving fluid via the uveoscleral route and from the remaining choroid due to an oncotic gradient and emptying into veins (5, 8). The 30-μm-thick outmost

layer of the suprachoroid is the lamina fusca, consisting of several layers of melanocytes and fibroblast-like cells disposed in plates, with bundles of myelinated axons (5, 8).

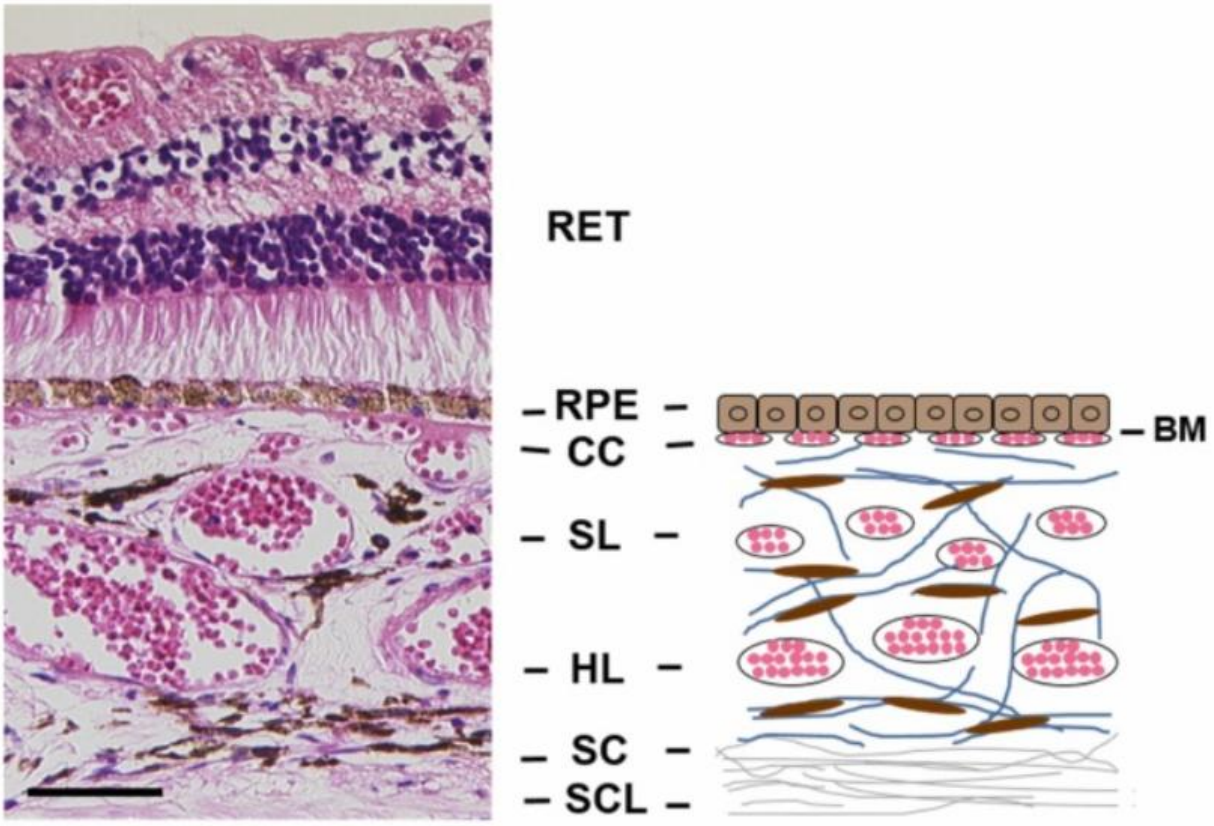

Figure 1: Histological slide (left, standard hematoxylin and eosin) of the human choroid and corresponding sketch (right) as an overview for its general anatomical organization. RET: retina; RPE: retinal pigment epithelium; BM: Bruch's membrane; CC: choriocapillaris; SL: Sattler's layer; HL: Haller's layer; SC: suprachoroid; SCL: sclera. The picture is from the study of Zhang et al. (35).

1.1.2. Physiology of the choroid

The main physiologic function of the choroid is to provide oxygen and nutrients to the highly metabolic outer retinal layers, namely the central avascular fovea and the prelaminar

portion of the optic nerve (8, 51). However, recent studies have revealed its critical role in thermoregulation, intraocular pressure modulation, emmetropization, positional adjustment of the retina, and secreting growth factors (5, 35). Additionally, the choroid functions to drain the aqueous humor from the anterior chamber through the uveoscleral pathway (35, 52).

Despite the conspicuousness of the retinal blood vessels, the major blood supply to the retina is the choroid (5). The photoreceptors are extremely metabolically active cells. They use about 90% of oxygen delivered to the retina, especially in darkness, when the light-gated ion channels are open, and active transport of ions is required to maintain ion homeostasis (5, 8, 53). To achieve this high level of oxygen transport from the choroid, despite the barriers of Bruch's membrane and the RPE, a steep gradient of oxygen tension is required (5). This is maintained by the high blood flow in the choroid, which is probably the highest of any tissue in the body per unit tissue weight, ten-fold higher than in the brain (5, 54, 55). Unlike the retinal circulation (the walls of the capillaries in the retina have no fenestrations, constituting the blood-ocular barrier), the choriocapillaris layer consists of fenestrated blood vessels, allowing prompt delivery of oxygen and nutrients to the outer retina and macula (5, 8, 56). These fenestrations have a high permeability not only to oxygen and glucose but to low molecular weight substances such as albumen and myoglobulin (5).

Although the importance of the choroid, particularly the choriocapillaris layer, has been widely reported in the literature, understanding the structure and function of this vascular bed remains challenging due to the limited availability of appropriate techniques for visualization and evaluation of this layer (8, 42). Significant structural or functional changes in the choriocapillaris have been found in diseases of the posterior pole (42). These pathologies are either directly caused by the changes in the choriocapillaris anatomy or are thought to be significantly influenced by the changes of this layer (42). Abnormal choroidal blood volume and/or compromised flow can result in photoreceptor dysfunction and death (34). In addition, reduced retinal blood flow can modulate vascular endothelial growth factor production and expression, driving changes in the choroidal vasculature (42). It is

essential for researchers and clinicians evaluating posterior segment pathologies (such as AMD, central serous chorioretinopathy and diabetic retinopathy) to understand better this closed-loop feedback relationship between the choroid and retina (42).

1.2. Evaluation of the choroid

1.2.1. Evaluation of the choroid

Until recently, the choroid could be evaluated in vivo by means of indocyanine green angiography (ICGA), laser Doppler flowmetry and ultrasonography (57). ICGA allows the visualization of choroidal vessels and circulation under the retinal pigment epithelium (57). Intravenous administration of exogenous dye (water-soluble tricarbocyanine) is required for this technique (58). Because it is an invasive procedure, it can cause adverse side effects that may be mild (nausea, vomiting, pruritus), moderate (urticaria, thrombophlebitis), or even serious and life-threatening (bronchospasm, laryngeal oedema, myocardial infarction) (59). ICGA has been shown to be better than fluorescein angiography (FA) in visualizing the details of pathophysiological changes involving the choroidal vasculature such as choroidal neovascularization and choroidal polyps (57). In addition, the longer wavelengths used in ICGA allow better visualization of underlying lesions in cases involving blood, exudates and pigment epithelial detachment (57, 60, 61).

Laser Doppler flowmetry is a non-invasive diagnostic method that allows the assessment of hemodynamic parameters of optic nerve head, iris and subfoveal choroidal circulation by determining the average speed and number of erythrocytes moving in a specific volume (57). This technique has been used to show that choroidal circulation is decreased in diseases such as diabetic retinopathy, AMD and retinitis pigmentosa (57, 62, 63).

Ultrasonography has been an important diagnostic tool in the evaluation of the globe and posterior structures in cases of ocular traumas, in opaque media such as corneal leukomas, dense cataracts, and vitreous opacities when the posterior pole cannot be evaluated and in the assessment of advanced diabetic retinopathy or retinal detachments (57, 58). In

particular, it plays a significant role in the diagnosis and follow-up of intraocular tumors (57, 58). However, the low image resolution of ultrasonography makes it difficult to detect small changes in the choroid, and separation of the relatively thin choroid from the retina and sclera may not be possible with this modality (57, 58).

Despite their utility, these methods may not be ideal for visualizing the choriocapillaris as none of them provide in vivo cross-sectional images of the anatomy of the retinal pigment epithelium or choroidal layers, and none provide sufficient data regarding true choroidal thickness and morphology (57, 58). Optical coherence tomography is a relatively new method that provides non-invasive, in vivo images of the posterior pole with a resolution close to histological examination (57, 58). Technically, OCT is a partial coherence interferometer using a super-luminescent diode light source to measure the amplitude of light reflected from tissues of different optical densities to produce high-resolution cross-sectional images of the structure being examined (64). OCT can be thought of as an optical analogue to ultrasound, but by means of light instead of sound (65). One of the main differences between the characteristics of these two methods is that the light has a significantly shorter wavelength, resulting in higher resolution but reduced penetration (66). The image containing depth information from one point in the section of tissue being examined is called A-scan (A mode) in the case of OCT, similar to ultrasound. The cross-sectional B-scan (B mode) image is composed of adjacent A-scan images of the tissue being investigated (66). The depth resolution and tissue penetration of OCT imaging depends on the wavelength of the examination light source, while the lateral resolution depends on the diameter of the light beam (66, 67).

The most common OCT technology available at the moment is the spectral domain OCT (58). This technique examines the target tissue with an exploring beam of light, usually using a light source with a wavelength of 800-870 nm, which provides excellent imaging of the vitreoretinal interface and the retina, but the visualization of the choroid is limited due to depth and density of choroidal tissue and light attenuation by the RPE (58, 68, 69). In 2008, a new imaging technique called enhanced depth imaging OCT was described which can be employed on the SD-OCT machine (70). The zero-delay line is a reference point or

plane defined by a software where imaging is optimal, so that the best resolution can be achieved. In SD-OCT, this point usually corresponds to the vitreoretinal interface, the resolution of deeper layers decreases with distance. The EDI imaging technique moves the zero-delay line back to the level of the outer retina-RPE junction, so that the choroid can be displayed with higher resolution (57). EDI-OCT allows to measure choroidal thickness parameters, but this technique has some limitations (58). In most of the studies, manual or semi-manual methods have been used to quantify choroidal thickness (Figure 2) (71, 72). These methods suffer from large intra- and interobserver variability, and do not have access to the choriocapillaris separately (72). On the other hand, EDI-OCT has some limitations in imaging particularly thick choroids or highly pigmented eyes due to the fact that the choroid itself, especially if it is rich in melanin could absorbs/deflects a great portion of OCT signal (58, 73).

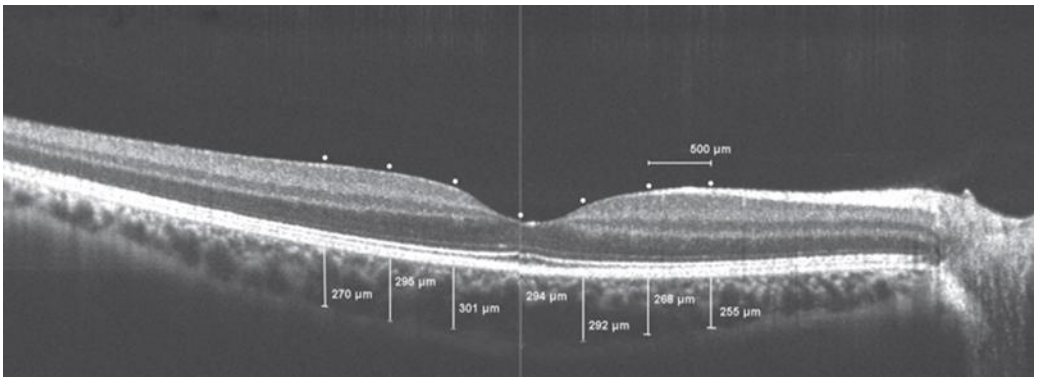

Figure 2: Manual measuring of choroidal thickness at different points on EDI-OCT imaging from the study of Akay et al. (71).

Recently, a new technology called swept source OCT has become available in the clinical setting (58). SS-OCT uses a wavelength-sweeping laser and dual-balanced photodetector, allowing for faster acquisition speeds of 100,000-400,000 A-scans per second (74). SS-OCT and SD-OCT are categorized as Fourier domain optical coherence tomography (75). Unlike SD-OCT, which has a low-coherence super-luminescent diode light source and an

interference spectrum obtained by spectral splitting, SS-OCT generally uses a tunable light source which wavelength varies with time (75, 76). In SD-OCT, broad wavelength light is divided into a spectrum by diffraction and then projected into a spectroscope where light interference is achieved (75). In SS-OCT, a spectroscope is unnecessary, since the light source is already divided into a spectrum through the tunable laser (Figure 3) (75).

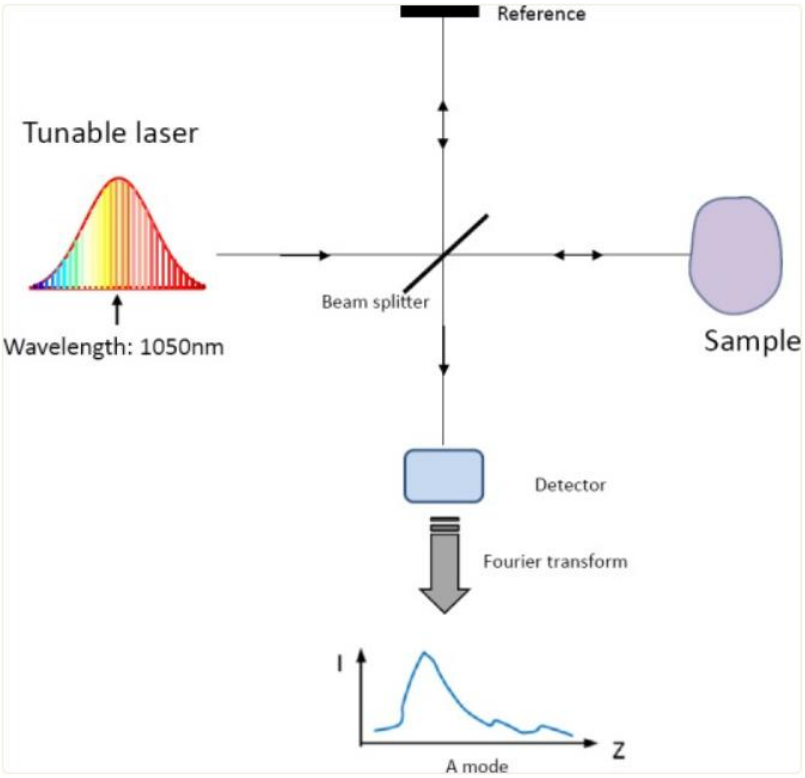

Figure 3. Schematic drawing of the swept source optical coherence tomography device from Kishi S. (75).

This simplified mechanism contributes the high-speed data acquisition in SS-OCT which results in a clearer image compared to SD-OCT (75). The depth of tissue penetration is governed by the wavelength of the light source used (75). In SS-OCT, the median wavelength is 1050 nm compared to the median wavelength of SD-OCT, which is approximately 800-870 nm (75). Consequently, the choroid–sclera boundary has higher

contrast in SS-OCT (76). These features make SS-OCT able to overcome most of the EDI-OCT limitations, allowing a better penetration of the signal and good visualization of both the superficial and the deep structures at the same time in most conditions (58, 77). Commercially available SS-OCT machines can also create automatic choroidal volumes and thickness measurements, which was not possible with most of the spectral-domain machines (13).

OCT angiography is the latest iteration of non-invasive imaging and combines the structural information provided by OCT scans with blood flow detection, without the need to inject any dye (58, 78). This technology is based on the detection of the intrinsic movement of particles in biological tissue to enable the reconstruction of retinal and choroidal vessels (79). When OCTA was firstly introduced in the clinical practice its applications were limited to the study of choroidal circulation for some reasons (58). Mainly, the first OCTA devices were based on SD-OCT technology thus the signal strength was too low to allow good-quality images beyond the RPE (58). On the other hand, OCTA evaluation of the choroidal layers can be complicated by the projection of the superficial vascular network pattern into deeper layers (projection artefacts), which can lead to erroneous results in both qualitative and quantitative assessment of blood flow (80). Over time, software-based correction of projection artefacts has been developed, devices with higher acquisition speed and better resolution have improved the quality of scans, so the first analysis of the choroid with OCTA has been attempted (58). Recently, OCTA devices based on swept-source technology have allowed good visualization of the structure and blood flow in the choriocapillaris layer, but evaluation of the deeper choroidal layers may still be challenging (58, 79).

1.2.2. Choroidal thickness and its influencing factors

As one of the objective biomarkers for the evaluation of choroid, choroidal thickness may depend on physiological factors of the body and the chosen measurement method (9). The normal CT differs among different measuring instruments. The majority of the studies

reported that the CT measured by SS-OCT (DRI-OCT Triton Plus, Topcon, Tokyo, Japan) was slightly higher than that measured by SD-OCT (Spectralis, Heidelberg Engineering Co.), which could be attributed to the ability of SS-OCT to identify the choroid–scleral interface more precisely (12, 81, 82). With using SS-OCT, the choroidal thickness is measured as the height from the lower boundary of the hyperreflective RPE to the choroid–scleral interface, determined by an automated built-in calibration software (Figure 4) (12).

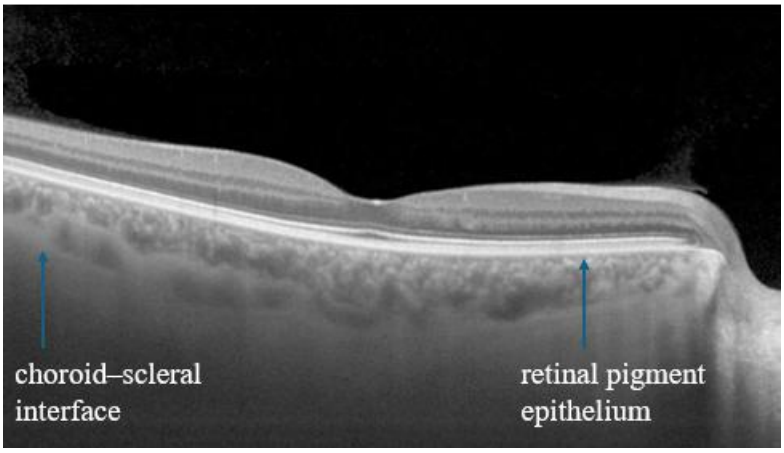

Figure 4: Boundaries of the choroid of a healthy subject examined by SS-OCT (DRI OCT Triton, Topcon Co., Tokyo, Japan). The choroidal thickness values were calculated automatically. Image of our working group.

In several OCT studies, the choroid was found to be thicker in the subfoveal area and then CT decreased from the subfoveal area to the nasal and temporal choroid, with the nasal CT usually being the thinner (11, 15, 83). With using SS-OCT, the mean CT was reported to be 278.5±110.5 μm by Zafar et al. (13), 296±9.7 μm by Philip et al. (84), 299.10 ± 131.2 μm by Bhayana et al. (81) and 300.87 ± 72.256 μm by Moussa et al. (85). Mori et al. recently examined choroidal thickness in healthy eyes using EDI-OCT and showed associations with age, axial length (AL), sex and spherical equivalent (86). With using SS-OCT, Hirano et al. found that the CT was negatively associated with advanced age ($p<0.05$) and AL ($p = 0.042$) (87). Bhayana et al. described a negative correlation between subfoveal

choroidal thickness and age (r=-0.0961, p=0.1392) and axial length (r=-0.3166, p<0.001) (81). Autopsy studies have reported a 1.1 μm decrease of CT per year (88), previous SD-OCT studies have suggested 1.56–1.95 μm for each additional year of age (83, 89), and early SS-OCT studies reported that the mean decrease is about 14 μm for each decade of life (90). It is well known from cross-sectional studies that choroidal thickness decreases with increasing axial length (87). Michalewski et al. examined the CT in patients with wide spectrum of refractive errors (from −22 to +6 diopter) using SS-OCT, and they revealed that choroidal thickness decreases 18.7 μm with every decrease of 1 diopter (R^2 = 0.2) (91). Bhayana et al. found that longer eyes had thinner subfoveal choroid while shorter eyes had thicker choroid and the relationship of subfoveal choroidal thickness with axial length (r=−0.3166, p<0.001) as well as spherical equivalent (r=0.2393, p=0.0002) was statistically significant (81). In previous SD-OCT studies, it has been suggested that CT is thicker in men than in women (92, 93). On the other hand, Ruiz-Medrano et al. (11) and Akhtar et al. (94) found no difference in CT among genders with using SS-OCT. The thickness of the choroid shows a natural diurnal variation (20-60 μm). It thickens during the day and is thickest during the evening and night hours, with the turn being measured around midnight (95, 96). In the future, further studies may be needed to determine what other physiological factors could be responsible for intra-individual variations in choroidal thickness (91). It may vary with race or other genetic predispositions or other unknown factors (91).

1.3. Diabetic choroidopathy (DC) and choroidal thickness changes in diabetes

Diabetes mellitus affects all small and large blood vessels in the body (7). Diabetic microangiopathy is a major contributor to the multi-organ complications of diabetes, including nephropathy, cardiovascular disease and neuropathy (97). In the eye, the focus has always been on the retinal changes that lead to diabetic retinopathy (7). However, it seems logical that the choroidal vasculature would also be affected in DM and the choroid itself would degenerate, leading to diabetic choroidopathy (7).

The first evidence of choroidal involvement in DR came from histopathology, where diabetic eyes showed increased arteriosclerosis and periodic-acid Schiff (PAS)-positive material within the arterial and capillary walls (97, 98). However, the term “diabetic choroidopathy” was introduced in 1985 by Hidayat and Fine, who described the histopathological findings of a small cohort of end-stage, blind and painful diabetic eyes (97, 99). In this study, they described choriocapillaris dropout, luminal narrowing and basement membrane thickening with arteriosclerotic changes in some arteries (7, 99). They reported that a prominent PAS-positive basement membrane material tends to narrow and obliterate the capillary lumens throughout the choroid, with or without fibrosis (97, 99). Histopathological studies of eyes with Type 2 diabetes reported decreased alkaline phosphatase activity in the choriocapillaris, loss of viable endothelial cells, narrowing or even obstruction of the choroidal vasculature, choroidal aneurysms, degenerative changes in Bruch's membrane, and choroidal neovascularization (19, 100). Lutty et al. demonstrated that the decrease in the alkaline phosphatase enzyme activity is related to choriocapillaris loss in DC (101). They described capillary dropout at any stage of DR, even in eyes without retinopathy or mild signs (101). Combining their findings with the existing literature, Cao et al. hypothesized that the unexplained loss of visual acuity in diabetic patients, regardless of the absence of retinopathy, might be due to DC (6).

These histopathological alterations appeared to have an inflammatory basis, with an increased expression of adhesion molecules and polymorphonuclear neutrophils (PMNs) (7, 97). In the study of Lutty et al., PMN numbers were significantly increased in diabetic choroid compared to aged controls and they were often associated with areas of pathology like capillary loss and choroidal neovascularization (102). The increased expression of adhesion molecules and delayed blood flow lead to leukostasis and subsequent mechanical occlusion of the capillary lumen, causing complete obliteration and vascular dropout (97, 102, 103). Another possible hypothesis underlying the pathogenesis of diabetic choroidopathy involved nitric oxide (NO) and NO synthases (NOS) (97, 104). NO is a signaling molecule produced by the vascular endothelium and involved in several physiological functions, including vasodilatation and vascular permeability (97, 104). NOS

are a family of enzymes directly responsible for NO production. The nNOS and eNOS isoforms are directly involved in choroidal blood flow regulation (97, 104, 105). A possible role of nNOS in diabetic choroidopathy was speculated by Sakurai et al., who found evidence of reduced choroidal nNOS expression 6 weeks after the onset of diabetes in their animal study (106). Choroidal nNOS is mainly expressed in the parasympathetic perivascular nerve fibers surrounding the choroidal arteries and veins, suggesting that diabetic choroidal microangiopathy may result from this imbalance (106).

The histopathological findings correlate with the structural changes found in vivo examinations of the choroid. Weinberger et al. described lobular spotty hyperfluorescent and hypofluorescent areas ("salt and pepper" appearance) in patients with non-proliferative diabetic retinopathy (NPDR) using indocyanine green angiography (107). Shiragami et al. saw similar irregularities in diabetic subjects using ICG and the hypofluorescent spots were significantly associated with the severity of DR, while hyperfluorescent spots were significantly associated with elevated HbA1c level (108). Bischoff et al. found an irregular and delayed choroidal filling on ICG in most patients with PDR and about half of the patients with mild non-proliferative diabetic retinopathy (109). Hua et al. considered the following parameters as potential ICG markers for the clinical definition of diabetic choroidopathy: early hypofluorescent spots, late-phase choroidal non-perfusion, inverted inflow phenomena, higher subfoveal choroidal thickness, and larger choroidal areas of nonperfusion (110). With using laser Doppler flowmetry, Schocket et al. described significantly reduced choroidal blood volume and blood flow in PDR (111). Another laser Doppler flowmetry study demonstrated a significant decrease in subfoveal choroidal blood flow in NPDR compared with control (112). The limitation on these studies is that CC flow can only be assessed in subfovea where there is no retinal vascular interference (7).

Recent imaging studies using different types of OCT devices have focused on choroidal thickness changes in diabetes, but the results are contradictory (7, 8, 97).

1.3.1. CT in DM without DR

When comparing CT between diabetic eyes without DR and controls, the results are controversial. With using EDI-OCT, some studies reported that there is no difference in CT between diabetic eyes without retinopathy and controls (Vujosevic et al., 17; Adhi et al., 113), while others reported either a significant increase (Tavares Ferreira et al., 114) or, more commonly, a significant decrease (Kim et al., 14; Esmaeelpour et al., 18; Querques et al., 16). With using SS-OCT, Abadia et al. described the CT significantly thinner is patients with Type 2 DM compared to healthy controls (115).

1.3.2. CT during DR progression and in diabetic macular edema

A decrease in CT in diabetic eyes unrelated to the DR stage was reported in some studies with using EDI-OCT (Esmaeelpour et al., 18; Querques et al., 16) correlating diabetes with a smaller CT. In the EDI-OCT study by Regatieri et al., no difference in CT was found in eyes with non-proliferative diabetic retinopathy compared to healthy controls, but a decrease in CT was described in advanced stages of the disease, such as diabetic macular edema or proliferative diabetic retinopathy (15). Most authors related DME with decreased CT or decreased choroidal circulation using EDI-OCT (Regatieri et al., 15; Querques et al., 16). However, this finding has not been confirmed in the studies of some other authors, who did not report an independent association between DME and CT (Vujosevic et al., 17; Esmaeelpour et al., 18).

With using SS-OCT, Abadia et al. found that the CT in patients with moderate non-proliferative diabetic retinopathy and DME was significantly thinner than in healthy subjects (115). Wang et al. reported an increased CT in the early stage of DR and a further decrease with progression of DR. On the other hand, DME was not significantly associated with CT in their SS-OCT study (116). SS-OCT demonstrated a significant reduction of CT in PDR compared with controls in the study of Lains et al., and the choroid appeared to be thinner in DR eyes than in diabetic eyes without retinopathy (117). Seeing the results of the most recent studies, Campos et al. concluded in their review article, that the evidence seems to indicate that the choroid thins in diabetic eyes, but the correlation with the grade of DR or the presence of DME is still elusive (8).

1.3.3. The influence of treatment of DR on CT

Panretinal photocoagulation (PRP) alters choroidal blood flow in patients with PDR and in most studies using EDI- or SS-OCT, it was associated with a decrease in CT long after the treatment (more than 3 months) (Kim et al., 14; Regatieri et al., 15; Eleiwa et al., 118). Nevertheless, several previous studies using Doppler flowmetry and EDI-OCT have reported significant increases in macular CT shortly after the laser treatment (1 week - 1 month) (119, 120). It was hypothesized that this may be due to a local choroidal inflammatory reaction/obstruction induced by the laser treatment, with vasodilation and consequent increased choroidal blood flow being seen in the untreated macular areas shortly after treatment (119, 120).

There seems to be plenty of agreement that the use of anti-VEGF agents to treat DME decreases CT (Kim et al., 14; Regatieri et al., 15; Lains et al., 121). In some studies, CT has been considered as a prognostic factor for the response to treatment, but the results are contradictory (8). Rayess et al. found that eyes with a thicker baseline CT had better short-term anatomic and functional responses to intravitreal anti-VEGF pharmacotherapy (122). On the other hand, in the study of Savur et al., better visual gain was achieved in DME patients with thinner CT (123).

In conclusion, there is a large body of evidence to suggest that the choroid may be affected by the treatment of DR (8). In their review article, Campos et al. concluded that the strength of a study evaluating CT could be in dealing with treatment naive eyes (8).

1.4. Stromal cell-derived factor-1 and its role in the pathogenesis of DR

Diabetic retinopathy and diabetic choroidopathy are specific microvascular complications of diabetes mellitus (2, 7). In addition to a variety of metabolic, environmental and other factors, several candidate genes have been suggested to be associated with the pathophysiology of DR (20, 21, 124).

Stromal cell-derived factor-1 is a highly active small chemokine that was originally isolated from murine bone marrow stromal cells (20, 125). The signaling pathway of SDF-1 and its receptor CXC chemokine 4 (CXCR4) has been implicated in angiogenesis, tumor growth, embryogenesis, and wound healing (20, 126). A growing body of experimental and clinical data suggest that SDF-1 plays an important role in the micro- and macrovascular complications of DM (26). In the recent CATIS trial, serum SDF-1 levels were independently associated with a higher cardiac autonomic neuropathy and cardiovascular risk score in diabetic patients, suggesting a causative interaction between plasma SDF-1 levels and the development of cardiovascular events, including stroke, and decreased all-cause mortality in diabetic patients (26).

It was recently demonstrated, that SDF-1 could play a crucial role in the expression of vascular endothelial growth factor, which is the main effector of ocular neovascularization, and the key inducer of vascular permeability associated macular edema (29, 127). In the study of Chen et al., the SDF-1 level in vitreous of PDR patients was significantly higher than that of patients operated on idiopathic macular hole (28). Butler et al. (25) and Brooks et al. (29) reported that the vitreous concentration of SDF-1 increased with various stages of macular edema with or without PDR. In the study of Meleth et al., SDF-1 levels measured in human serum were higher in patients with at least severe non-proliferative diabetic retinopathy than in patients with less severe diabetic retinopathy (128).

There is emerging evidence indicating that variation in the human *SDF-1 gene* can have an influence on SDF-1 levels (129). SDF-1 has two transcriptional splice variants: *sdf-1α* and *sdf-1β*. The nucleotide transition G to A of the *SDF-1-3′* is located at nucleotide position 801 of the *sdf-1β* transcript (130, 131). *SDF-1 (c801AA)* homozygosity is associated with higher SDF-1 production (130, 131). Djuric et al. reported that homozygous carriers of the *SDF-1 (c801AA)* genotype are more frequent in diabetic patients with proliferative retinopathy (33). Peng et al. found that the *SDF-1 (c801AA)* genotype was ten times more common in Taiwanese patients with NPDR or PDR than in patients without DR (20). They concluded, that genetic analysis of the SDF-1 polymorphism may be suggested for DR individuals (20).

Previously, the choroid has been largely ignored as a potential source of growth factors (5). In recent years, several studies demonstrated the choroidal synthesis of growth factors involved in angiogenesis, such as vascular endothelial growth factor, basic fibroblast growth factor and hepatocyte growth factor (132, 133). In the study of Bhutto et al., SDF-1 appeared to be constitutively produced by RPE and was also present in the choroidal stroma of all subjects. They hypothesized that an increase in circulating CXCR4 cells during inflammatory processes leads to an accumulation of these cells in RPE / Bruch's membrane / choriocapillaris complex and then, these ocular infiltrated inflammatory cells may produce angiogenic factors that play a role in the formation of CNV in age-related macular degeneration (134). Regarding the molecular mechanism of choroidal neovascularization in diabetes and its relationship to SDF-1, we only have animal studies (135, 136). Gao et al. found that hyperglycemia promotes the vasculogenesis in CNV, which might be triggered by VEGF and SDF-1 production (135).

To the best of our knowledge, the relationship between choroidal microvascular changes in diabetes and SDF-1 has not been investigated yet.

2. Objectives

Our research aimed to measure choroidal thickness in diabetic patients using the latest generation SS-OCT and to correlate it to the presence, the severity and the therapy of DR and to the metabolic status. We also investigated the association between the SDF-1-3′ (c801G > A) gene variant with choroidal thickness parameters.

2.1. Choroidal thickness changes in non-treated eyes of patients with diabetes mellitus

In this study we investigated the CT of diabetic subjects, by observing CT changes in different stages of the natural course of the disease. We investigated the association of CT and diabetes or the severity of DR in treatment naive eyes. We also evaluated the influence of systemic risk factors (type and duration of DM, HbA1c level, hypertension) on choroidal thickness values.

2.2. Choroidal thickness changes in patients with treated diabetic retinopathy

To measure CT in diabetic eyes and to correlate it with the therapy (panretinal photocoagulation) of diabetic retinopathy.

2.3. Association of SDF-1-3' gene variant with diabetic retinopathy

This study investigated the *SDF-1-3'* variant in diabetic patients to assess its relevance in the development of DM-associated PDR and DME. Furthermore, the association between *SDF-1-3'* variant and retinal and choroidal thickness parameters was also evaluated after adjustment for known (metabolic or environmental) risk factors.

3. Methods

The study was conducted in accordance with the tenets of the Declaration of Helsinki and the applicable national and local ethics committee and institutional review board requirements. Ethical approval was obtained from the Institutional Review Board (Semmelweis University Regional and Institutional Committee of Sciences and Research Ethics, TUKEB 839/PI/010). Written informed consent was obtained before the examination from each patients.

Key materials and methods information based on the researches of our study group published earlier (137, 138, 139).

3.1. Study participants

Patients were recruited from the Ophthalmological Department of Semmelweis University Budapest, from June 2015 to June 2017.

3.1.1. Choroidal thickness changes in non-treated eyes of patients with diabetes

Inclusion criteria: All patients with confirmed diabetes were enrolled regardless of the presence or absence of any signs of DR. The diagnosis of Type 1 and Type 2 diabetes was based on the criteria from the American Diabetes Association (140).

The control group included patients without diabetes who had no other systemic diseases.

Exclusion criteria: Patients with corneal or vitreal opacity, uveitis, degenerative, vasoocclusive retinal disease or dystrophy were excluded. A history of refractive error equal to or greater than ± 3 diopters and evidence of glaucoma were also exclusion criteria. Eyes with a history of any type of intraocular surgery (except cataract surgery), any previous pharmacologic intravitreal or subtenon treatment and any type of retinal laser therapy (panretinal or macular grid photocoagulation) were also rejected, because of the

modifying effect of these treatment modalities on retinal and choroidal thickness values (118 – 123).

3.1.2. Choroidal thickness changes in patients with treated diabetic retinopathy

Inclusion criteria: All patients with confirmed diabetes were enrolled regardless of the presence or absence of any signs of DR. The diagnosis of Type 1 and Type 2 diabetes was based on the criteria from the American Diabetes Association (140).

The control group included patients without diabetes who had no other systemic diseases.

Exclusion criteria: Patients with corneal or vitreal opacity, uveitis, degenerative, vasoocclusive retinal disease or dystrophy were excluded. A history of refractive error equal to or greater than ± 3 diopters and evidence of glaucoma were also exclusion criteria. Eyes with a history of any type of intraocular surgery (except cataract surgery) and any previous pharmacologic intravitreal or subtenon treatment were also rejected. In this study, we divided our patients with proliferative diabetic retinopathy into two subgroups - eyes that had not yet received any treatment and eyes that had been treated with panretinal photocoagulation for proliferation - to study the long-term effect of PRP on choroidal thickness. In all treated eyes included in the study, the laser treatment preceded the OCT examination by at least 3 months, due to the transient choroidal thickening directly associated with photocoagulation, as reported in the literature earlier (119, 120).

3.1.3. Association of SDF-1-3' gene variant with diabetic retinopathy

Inclusion criteria: All patients with any signs of diabetic retinal microvascular complications were included in the study.

Exclusion criteria: Patients with a history of previous intraocular surgery, ocular trauma, or any other retinal or neurological disease, intraocular inflammation, or tumor were excluded from the study. Patients with prior anti-VEGF or laser treatment for DME or PDR were also

excluded. The control group consisted of randomly selected sex- and age-matched unrelated volunteers who were referred for spectacle prescription or general routine eye care. All cases and controls were Caucasian and Hungarian. The inclusion and exclusion criteria were the same for the DM and sex- and age-matched control groups, except that patients in the control group had no evidence of DR.

3.2. Clinical history and laboratory parameters

Clinical data, anthropometric data, together with patient's and family history, duration of diabetes were recorded. Blood hemoglobin A1c level was measured at the beginning of the study. Additionally, antihypertensive treatment was recorded and patients were considered to suffer from hypertension if they were treated for the disease.

3.3. Ophthalmological examination

After autorefractometry examination, best corrected visual acuity was measured with a Snellen chart. Each subject underwent a slit-lamp examination, a Goldmann applanation tonometry and a dilated fundus examination with a 90D lens biomicroscopy. Diagnostic and staging criteria of DR were applied according to the guidelines of Early Treatment Diabetic Retinopathy Study (ETDRS) (141). We divided our diabetic patients into three groups - no DR, non-proliferative retinopathy and proliferative retinopathy - and recorded the presence of diabetic macular edema. DME was determined by the presence of any retinal thickening (confirmed on SS-OCT) or hard exudates in the posterior pole on biomicroscopy (141, 142). PDR was identified if any signs of neovascularization were detectable on fundus biomicroscopy with or without vitreous hemorrhage (141). Fluorescein angiography was performed in ambiguous cases (143).

3.3.1. Choroidal thickness measurement using SS-OCT

All enrolled eyes were examined with swept-source OCT (DRI OCT Triton, Topcon Co., Tokyo, Japan) using a light source of a wavelength-sweeping laser centered at 1050 nm with a 1000-Hz repetition rate and producing images of tissue with an axial resolution of 5.3 μm (144, 145). Most of the examinations were performed by the author. After pupil dilatation with 0.5% tropicamide and 0.5% phenylephrine, a three-dimensional (3D) macular volumetric raster scan protocol (consisting of 512 A-scans and 256 B-scans) was performed to acquire retinal and choroidal thickness map in the macular region (12 × 9 mm) with a central fixation. CT was defined as the distance between the outer border of the retinal pigment epithelium and the chorio-scleral interface, determined by an automated built-in calibration software (3D Macular Volumetric Raster Scan Protocol, Triton software version 3) (146). In each image of the 3D data set, lines of both RPE and the chorio-scleral border were reviewed manually by two trained observers to avoid automated segmentation errors. After the retinal and choroidal thickness map was obtained, a grid (6 × 6 mm) used by the ETDRS study was applied to the map to automatically calculate the CT values for each sectors (141, 144). The grid was subdivided into nine sectors: with the inner ring being 1–3 mm and the outer ring being 3–6 mm from the foveal center. The rings were segmented into four quadrants (superior, inferior, nasal and temporal). The central sector was defined as being within 1000 μm of the center of the fovea and registered as central subfoveal choroidal thickness (CSCT) (Figure 5) (144, 146). Mean whole macular choroidal thickness was calculated by averaging the CT values in all the nine ETDRS sectors. To exclude the diurnal variation of CT, the OCT examinations were always performed in the morning around 10 a.m. (96).

At the same time, central retinal thickness (CRT) was also measured in all participants.

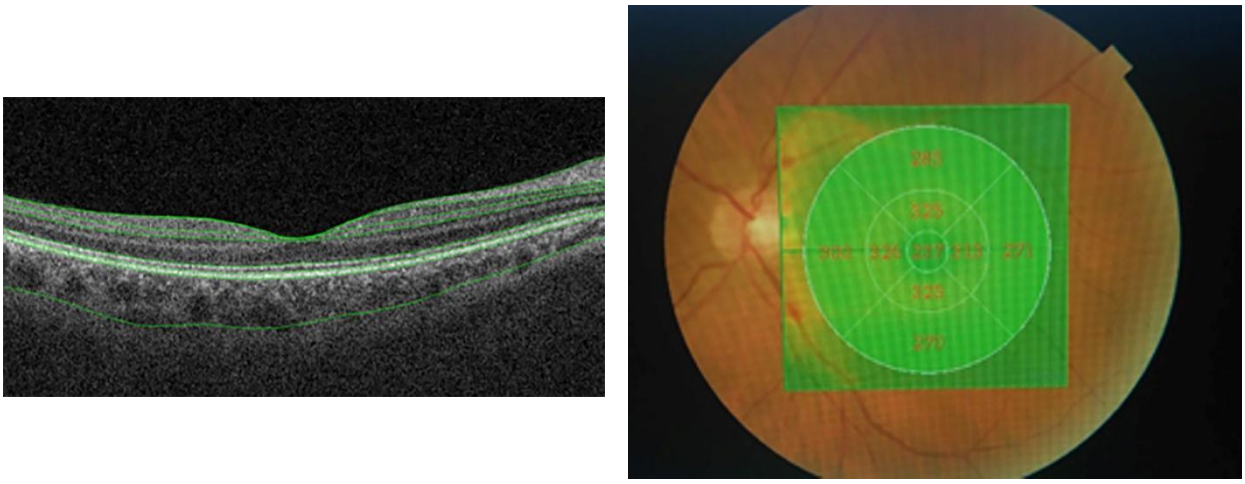

Figure 5: Image of the retina and the choroid (a) and a normal choroidal thickness map (b) by swept-source optical coherence tomography (DRI OCT Triton, Topcon Co., Tokyo, Japan). Own image, published in (137).

3.4. Deoxyribonucleic acid (DNA) extraction and genotyping

Blood samples remaining after the routine examination were used for genotyping. Genomic DNA was extracted from whole blood using the QIAamp Blood Mini kit (Qiagen, Hilden, Germany). Genotyping of the *SDF-1-3' (c801G > A)* variant was performed as follows: DNA fragments were amplified in a reaction mix containing 10% buffer, 2 MgCl2, dNTP, sdf-1 specific primers (F: CAGTCAACCTGGGCAAAGCC, R: CCTGAGAGTCCTTTTGCGGG) and recombinant Taq polymerase (Invitrogen, Budapest, Hungary), using the following PCR conditions: initial denaturation at 94 ◦C for 5 minutes, followed by 35 cycles of denaturation at 94 ◦C for 1 minute, annealing at 55 ◦C for 1 minute and extension at 72 ◦C for 1 minute, ending with a final extension at 72 ◦C for 5 minutes and cooling to 4 ◦C in a Perkin-Elmer Thermo Cycler (PE, Model 2400; Norwalk, CT, USA). The PCR products were digested with Msp1 (Sigma Chemical Co., Budapest, Hungary) at 37 ◦C for 4 hours. The cleavage products were electrophoresed on a 3% agarose gel and stained with GelRed™ (Biotium, CA, USA).

3.5. Statistical analysis

3.5.1. Choroidal thickness changes in non-treated eyes of patients with diabetes

Statistical analysis was performed with SPSS (Statistical Package for the Social Sciences) software (version 23.0, IBM Inc., Chicago, IL, USA). The repeated measures analysis of variance test (rANOVA) was used to analyze the differences between the study groups. The effect of predisposing factors on CT and DR stage was assessed by univariable and multivariable regression analysis using general estimating equation (GEE). Both rANOVA and GEE allow adjustment for within-subject correlation of parameters (right eye vs. left eye) by taking into account between-eye correlations. In addition, the inclusion of the most relevant systemic risk factors as covariates into GEE models permits one to simultaneously control for their effect on the dependent variables simultaneously. Model fit was assessed by the QICC (Corrected Quasi Likelihood under Independence Model Criterion) value. For the predictive models, we divided the available data into separate partitions randomly and developed our models on one of these partitions and used the other for predictive model assessment. We used a random partition to generate a training set (40% of eyes) and a test set (60% of eyes), the latter of which was used for calculating predicted probability of developing DR as a function of age. Data are expressed as mean ± SD (standard deviation); the significance level was set to $p < 0.05$.

3.5.2. Choroidal thickness changes in patients with treated diabetic retinopathy

Statistical analysis was performed with SPSS software (version 23.0, IBM Inc., Chicago, IL, USA). The repeated measures analysis of variance test (rANOVA) followed by Tukey's post hoc test was used to analyze the differences between the study groups, as the repeated measures tests allow us to take into account for the correlation between the data from the two eyes of the same individual. Data are expressed as mean ± SD (standard deviation); the significance level was set to $p < 0.05$.

3.5.3. Association of SDF-1-3' gene variant with diabetic retinopathy

Statistical analysis was performed using SPSS software (version 21.0, IBM Inc., Chicago, IL, USA). The Shapiro-Wilk test was used for normal distribution, and parametric or non-parametric statistical tests were used to compare data from the two study groups. We calculated allele and genotype frequencies in patients and healthy controls through direct counting. The Hardy-Weinberg equilibrium was assessed for both variants in diabetic and control subjects by comparing the observed and expected frequencies of the genotypes using chi-squared analysis. To determine the effect of the *SDF-1-3'* gene variant among multiple predictors on the development of DME or PDR, multivariable regression analyses were performed using binomial logistic regression models, adding duration and type of DM, hypertension and HbA1c level as covariates to adjust for their effect on the development of DME or PDR. Kaplan-Meier life table analysis was used to construct curves for the time to onset of diabetic macular edema, and the log-rank test was used to compare their distribution. The significance level was set at $p < 0.05$ for all statistical analyses.

4. Results

Key results information based on the researches of our study group published earlier (137, 138, 139).

4.1. Choroidal thickness changes in non-treated eyes of patients with diabetes

4.1.1. Baseline characteristics

A total of 96 eyes from 48 patients with diabetes and 46 eyes from 23 healthy controls were included in the study. Eighteen patients (38%) had Type 1 DM (T1DM) and 30 patients (62%) had Type 2 DM (T2DM). Baseline demographic and clinical characteristics of enrolled patients are summarized in Table 1.

Table 1: Baseline characteristics of enrolled patients (DM: diabetes mellitus; M: male; F: female; HbA1c: hemoglobin A1c; BCVA: best corrected visual acuity, VA: visual acuity), published in (137). Note: data are mean ± standard deviation.

	Control patients (n = 23)	**Patients with diabetes** (n = 48)	**p value**
Gender (M/F)	8/15	22/26	> 0.05
Age (years)	59.8 ± 16.78	58.75 ± 13.74	> 0.05
Duration of DM (years)	-	17.73 ± 9.61	-
HbA1c (%)	-	7.64 ± 1.11	-
HbA1c (mmol/mol)	-	61 ± 12	-
Hypertension, yes/no (%)	9/14 (40%)	37/11 (78%)	**< 0.001**
BCVA (Snellen VA ratio)	0.78 ± 0.32	0.51 ± 0.37	**< 0.001**

4.1.2. Comparison of choroidal thickness in patients with diabetes and controls

The mean CT in the central subfoveal region was 244.45 ± 64.79 µm in patients with diabetes and 278.32 ± 82.67 µm in controls. In the whole analyzed area, mean CT was 225.09 ± 60.32 µm in diabetic and 258.89 ± 73.75 µm in control eyes. Table 2 shows mean CT in the different ETDRS subfields. A significantly thinner CT was measured in patients with diabetes in the central subfoveal region ($p = 0.009$) as well as in the whole macular area ($p = 0.005$).

Next, when comparing between-eye asymmetry in the two study groups by calculating the difference in CT values from eyes of the same subject, we found no difference between the diabetic and the control group (30.63 ± 33.69 vs. 27.76 ± 30.91 µm).

Table 2: Mean choroidal thickness in the different Early Treatment Diabetic Retinopathy Study subfields and mean whole macular choroidal thickness (ETDRS: Early Treatment Diabetic Retinopathy Study), published in (137). Note: data are mean ± standard deviation.

Subfield / ETDRS grid sector	**Control eyes** (n = 46)	**Diabetic eyes** (n = 96)	**p value**
Central subfoveal (µm)	278.32 ± 82.67	244.45 ± 64.79	**0.009**
Inner ring (µm)	264.44 ± 70.59	229.11 ± 64.18	**0.004**
Outer ring (µm)	233.92 ± 73.04	201.71 ± 58.59	**0.006**
Mean whole macular choroid (µm)	258.89 ± 73.75	225.09 ± 60.32	**0.005**

4.1.3. Systemic factors and choroidal thickness in the diabetic group

In univariable regression models, age, gender, hypertension and the duration of diabetes were significantly correlated with CT (Table 3).

Table 3: The effect of systemic factors on choroidal thickness in univariable regression models (CT: choroidal thickness; F: female; M: male; DM: diabetes mellitus; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; HbA1c: hemoglobin A1c; CL: confidence level), published in (137).

	Beta (ß)	**Lower CL 95** (%)	**Upper CL 95** (%)	**p value**
Central subfoveal CT				
Age (years)	-0.54	-0.69	-0.38	**< 0.001**
Gender (F vs. M)	0.26	0.08	0.44	**0.005**
Duration of DM (years)	-0.22	-0.39	-0.04	**0.02**
Hypertension (yes vs. no)	-0.29	-0.47	-0.12	**0.001**
Type of DM (T1DM vs. T2DM)	0.15	-0.05	0.36	> 0.05
HbA1c (%; mmol/mol)	-0.11	-0.29	0.07	> 0.05
Mean whole macular CT				
Age (years)	-0.56	-0.71	-0.41	**< 0.001**
Gender (F vs. M)	0.25	0.07	0.43	**0.007**
Duration of DM (years)	-0.21	-0.39	-0.03	**0.02**
Hypertension (yes vs. no)	-0.32	-0.49	-0.14	**0.003**
Type of DM (T1DM vs. T2DM)	0.17	-0.03	0.37	> 0.05
HbA1c (%; mmol/mol)	-0.04	-0.23	0.14	> 0.05

Aging showed significant negative correlation with CT in the central subfoveal region ($p < 0.001$) as well as in the whole analyzed area ($p < 0.001$, Table 3). On average, the mean decrease in CT was 26 μm/10 years in the central subfoveal region, and 25 μm/10 years in the whole macular area. Male subjects proved to have a significantly thinner choroid than females had (Table 3): the mean difference was 34.6 μm (95% CI 10.76–58.46 μm) in the central subfoveal region and 30.7 μm in the whole analyzed area (95% CI 8.56–52.79 μm).

Hypertension was also found to associate significantly with a decreased CT in patients with diabetes in the whole analyzed area (Table 3). In the central subfoveal region mean CT was 232.75 ± 59.4 μm in patients with HT, and 268.96 ± 69.63 μm in patients without HT ($p = 0.001$). The mean CT in the whole macular area was 205.2 ± 56.85 μm in patients with HT and 248.71 ± 61.36 μm in patients without hypertension ($p < 0.001$).

Duration of diabetes was significantly correlated with a reduction of choroidal thickness in the whole analyzed area (Table 3). The mean decrease in CT was 15 μm/10 duration years in the central subfoveal region and 13 μm/decade in the whole macular area.

There was no significant difference in central subfoveal and mean overall CT between patients with Type 1 and Type 2 DM ($p > 0.05$). The mean CT in the central subfoveal region was 255.91 ± 73.52 μm in patients with T1DM and 235.53 ± 56.2 μm in patients with T2DM. In the whole macular area, mean CT was 236.7 ± 76.94 μm in patients with T1DM and 216.06 ± 50.68 μm in patients with T2DM. The type of DM did not correlated significantly with CT ($p > 0.05$) (Table 3).

The level of HbA1c proved not to be associated significantly with CT (Table 3).

During simultaneous analysis of the effect of systemic factors on CT in multivariable regression models, the duration of diabetes was significantly negatively associated with a decreased CT both in the central subfoveal region (β -0.16, $p = 0.03$) and in the whole analyzed area (β -0.19, $p = 0.01$), after adjusting for the effects of age, gender, hypertension, type of DM and HbA1c level.

4.1.4. Choroidal thickness and severity of diabetic retinopathy

As detailed above, in patients with diabetes eyes were grouped according to DR stage: no DR (n = 17), NPDR (n = 18), NPDR + DME (n = 45) and PDR (n = 16). Patients with DME only graded NPDR in this study.

According to analysis of variance, there was a significant difference among means of choroidal thickness in different stages of DR ($p = 0.002$), with thinner CT in cases with more advanced DR (Table 4). However, post hoc analysis using the Newman–Keuls test identified significantly different means of CT only in eyes without retinopathy vs. eyes with proliferative retinopathy ($p < 0.001$, Table 4). There was no significant difference in CT values between eyes with NPDR + DME compared to the other groups without DME with post hoc analysis ($p > 0.05$, Table 4).

Table 4. Choroidal thickness in the ETDRS subfields in different stages of DR (ETDRS: Early Treatment Diabetic Retinopathy Study, DR: diabetic retinopathy, NPDR: nonproliferative diabetic retinopathy, DME: diabetic macular edema, PDR: proliferative diabetic retinopathy), published in (137). Note: data are mean ± standard deviation; p values were calculated using analysis of variance (ANOVA) test; *: $p < 0.001$, compared to eyes with no DR using post-hoc analysis with Newman-Keuls test.

Subfield / ETDRS grid sector	**Eyes with no DR (n = 17)**	**Eyes with NPDR without DME (n = 18)**	**Eyes with NPDR with DME (n = 45)**	**Eyes with PDR (n = 16)**	**p value**
Central subfoveal, (µm)	294.48 ± 66.6	257.22 ± 74.31	236.78 ± 52.73	218.67 ± 58.88*	**0.002**
Inner ring, (µm)	282.08 ± 60.07	251.34 ± 74.87	225.59 ± 54.15	187.2 ± 42.95*	**< 0.001**
Outer ring, (µm)	255.89 ± 53.09	219.68 ± 62.99	197.54 ± 48.53	166.51 ± 48.81*	**< 0.001**

In predictive multivariable logistic regression analysis, CT < 225 µm was a significant predictor of the presence of DR in the central subfoveal region (odds ratio (OR) = 5.27; p = 0.02) and likewise in the whole analyzed area (OR 4.97; p = 0.04), after adjusting for the effects of age, gender, disease duration and hypertension. Figure 6 shows the predicted probability plot of the presence of DR in patients with a CT < 225 µm compared to those with a CT > 225 µm, after adjusting the effects of the aforementioned risk factors.

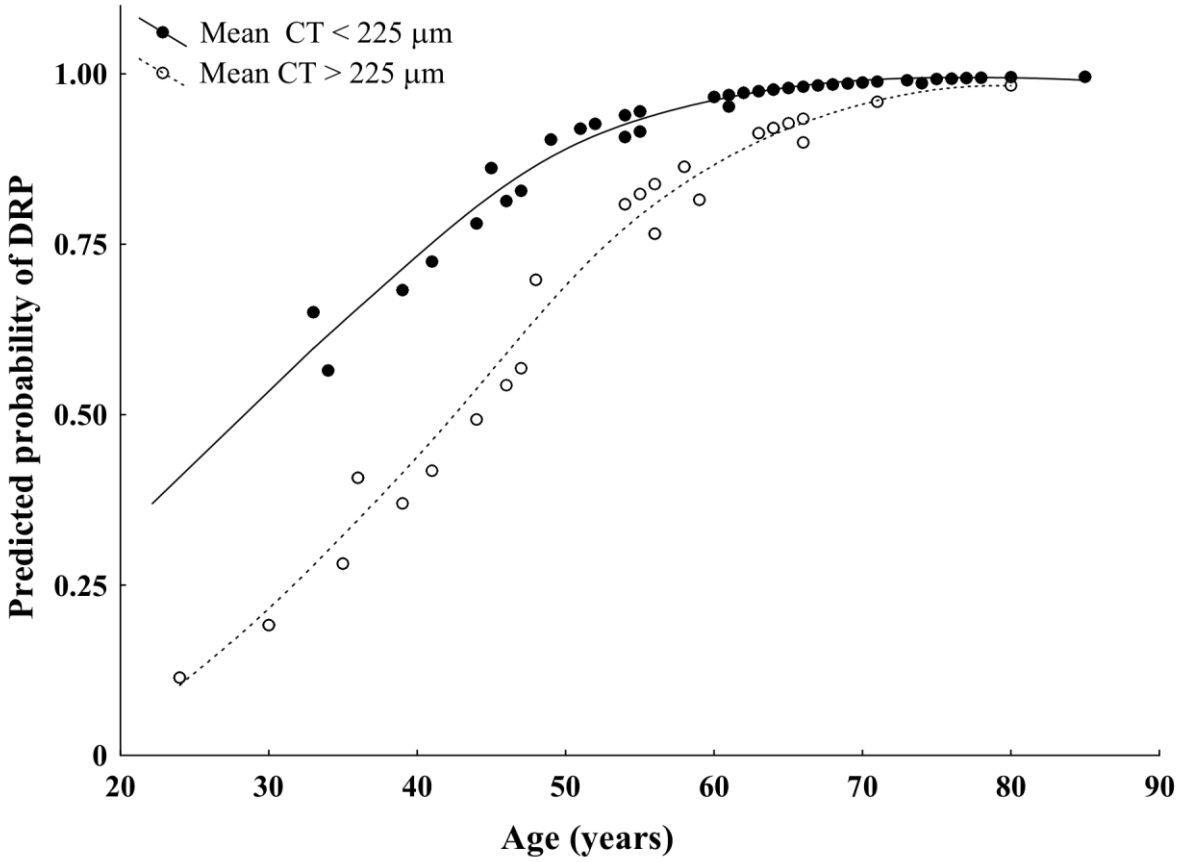

Figure 6: Probability curves for the development of diabetic retinopathy as a function of age in eyes with choroidal thickness less than 225 µm (n = 36) vs. CT more than 225 µm (n = 23) from the test set (37 eyes), after adjusting for the effects of age, gender, disease duration and hypertension (DRP: diabetic retinopathy, CT: choroidal thickness). Lines represent logarithmic fit. Own representation, published in (137).

4.2. Choroidal thickness changes in patients with treated diabetic retinopathy

4.2.1. Baseline characteristics

A total of 117 eyes of 60 diabetic patients and 45 eyes of 24 healthy controls were analyzed. 3 eyes from the diabetic and 3 eyes from the control group were excluded from the study due to the compliance with exclusion criteria. Data from 42 males (31 diabetics and 11 controls) and 42 females (29 diabetics and 13 controls) were included in the study; 24 (40%) of our diabetic patients had Type 1 DM, while 36 (60%) had Type 2 DM. Baseline demographic and clinical characteristics of enrolled patients are summarized in Table 5.

Table 5. Baseline characteristics of enrolled patients (DM: diabetes mellitus; M: male; F: female; HbA1c: hemoglobin A1c; BCVA: best corrected visual acuity, VA: visual acuity), published in (138). Note: data are mean ± standard deviation.

	Eyes with diabetes (n = 117)	**Control eyes** (n = 45)	**p value**
Gender (M/F)	58/59	24/21	> 0.05
Age (years)	59.26 ± 13.86	59.8 ± 16.78	> 0.05
Duration of DM (years)	18.43 ± 12.13		-
HbA1c (%)	8.21 ± 1.01	5.42 ± 0.37	**< 0.0001**
Hypertension, yes/no (%)	83/34 (71)	18/27 (40)	**< 0.05**
BCVA (Snellen VA ratio)	0.44 ± 0.22	0.78 ± 0.32	**< 0.0001**

4.2.2. Choroidal thickness association with the severity and the therapy of diabetic retinopathy

As detailed above, in patients with diabetes eyes were grouped according to DR stage: no DR (n = 18), NPDR (n = 62) and PDR (n = 37). Of the 37 eyes with proliferative retinopathy included in the study, 16 had not yet received any treatment and 21 had been treated with panretinal photocoagulation at least 3 months prior to the study. Central subfoveal and mean whole macular CT were found to be significantly lower in patients with PDR and in those who had been treated with PRP compared those diabetic patients who had no signs of neovascularization (no DR + NPDR group) (Table 6). DME was detected in 60 eyes (51%). There was no significant difference in CT values between eyes with DME compared to the other patients without DME ($p > 0.05$, Table 6).

Table 6. Central subfoveal and mean whole macular choroidal thickness based on the presence of proliferative retinopathy and diabetic macular edema (CT: choroidal thickness, PRP: panretinal photocoagulation), published in (138). Note: data are mean ± standard deviation.

		Central subfoveal CT, μm	**p value**	**Mean whole macular CT**, μm	**p value**
Diabetic macula edema	**No** (n = 57)	260.15 ± 92.38	-	243.3 ± 19.57	-
	Yes (n = 60)	240.37 ± 73.23	> 0.05	222.48 ± 20.35	> 0.05
Proliferative diabetic retinopathy	**No** (n = 80)	264.54 ± 60.54	-	249.39 ± 23.32	-
	Yes (n = 37)	229.94 ± 81.02	**0.01**	213.15 ± 20.18	**< 0.001**
	PRP-treated (n = 21)	221.13 ± 68	**0.007**	208.18 ± 21.12	**< 0.001**

4.3. Association of SDF-1-3′ gene variant with diabetic retinopathy

4.3.1. Patient characteristics

The baseline characteristics and the central subfoveal retinal and choroidal thickness values of the various diabetic and control groups are presented in Table 7.

Table 7. Laboratory parameters and retinal and choroidal thickness values in the central subfoveal area of the control and the diabetic groups (M: male, F: female, PDR: proliferative diabetic retinopathy, DME: diabetic macular edema, DM: diabetes mellitus, HbA1c: hemoglobin A1c, CRT: central retinal thickness, CSCT: central subfoveal choroidal thickness, T1DM: Type 1 diabetes mellitus, T2DM: Type 2 diabetes mellitus, NA: not applicable), published in (139). Note: data are mean ± standard deviation. The Student t-test is for continuous variables, and the Chi-square test is for categorical variables.

Variables	**Controls** (n = 31)	**T1DM** (n = 28)	**T2DM** (n = 75)	**p value** (T1 vs. T2DM)	**p value** (DM vs. control)
Age (years)	61.0 ± 15.0	52.7 ± 18.0	64.6 ± 9.76	**0.001**	0.63
Gender (M/F)	13/24	13/15	37/38	0.698	0.06
PDR, yes/no (eyes)	NA	19/28	40/75	**0.015**	NA
DME, yes/no (eyes)	NA	14/28	30/75	0.295	NA
DM duration (years)	NA	25.3 ± 12.3	16.6 ± 8.12	**0.011**	NA

Variables	**Controls** (n = 31)	**T1DM** (n = 28)	**T2DM** (n = 75)	**p value** (T1 vs. T2DM2)	**p value** (DM vs. control)
HbA1C (mmol/L)	<6.5	8.07 ± 1.51	7.59 ± 1.73	0.307	NA
Hypertension, yes/no	11/31	20/28	75/75	**0.007**	**<0.001**
CRT (μm)	270 ± 49.8	277 ± 95.9	362 ± 188	**0.006**	**0.02**
CSCT (μm)	249 ± 109	245 ± 67.2	225 ± 80.9	0.23	0.09

4.3.2. Genotype distribution in diabetic and control patients

Hardy-Weinberg's criteria were fulfilled in all groups. Table 8 summarizes the genotype frequencies of *SDF-1-3' (c801G > A)* in the diabetic and control groups. The distribution of heterozygous and homozygous *SDF-1-3' (c801G > A)* genotypes was similar in diabetic and control subjects.

Table 8. SDF-1-3' (c801G > A) genotype frequencies in control and diabetic patients (DM: diabetes mellitus), published in the study of our research group (139). Note: p-values were calculated using the Chi-squared test.

Genotypes	**AA**	**AG + GG**	**p - values**
Control patients (n = 31)	3 (9.7%)	28 (90.3%)	0.99
DM patients (n = 103)	10 (9.7%)	93 (90.3%)	
Genotypes	**AA + AG**	**GG**	
Control patients (n = 31)	11 (35.5%)	20 (64.5%)	0.38
DM patients (n = 103)	48 (46.6%)	55 (54.4%)	

4.3.3. Association of retinal and choroidal thickness with SDF-1 (c801A) allele

As shown in Figure 7, the central retinal thickness was significantly higher ($p < 0.006$), and central subfoveal choroidal thickness was lower in DM patients with the presence of the SDF-1 (c801A) allele ($p < 0.08$).

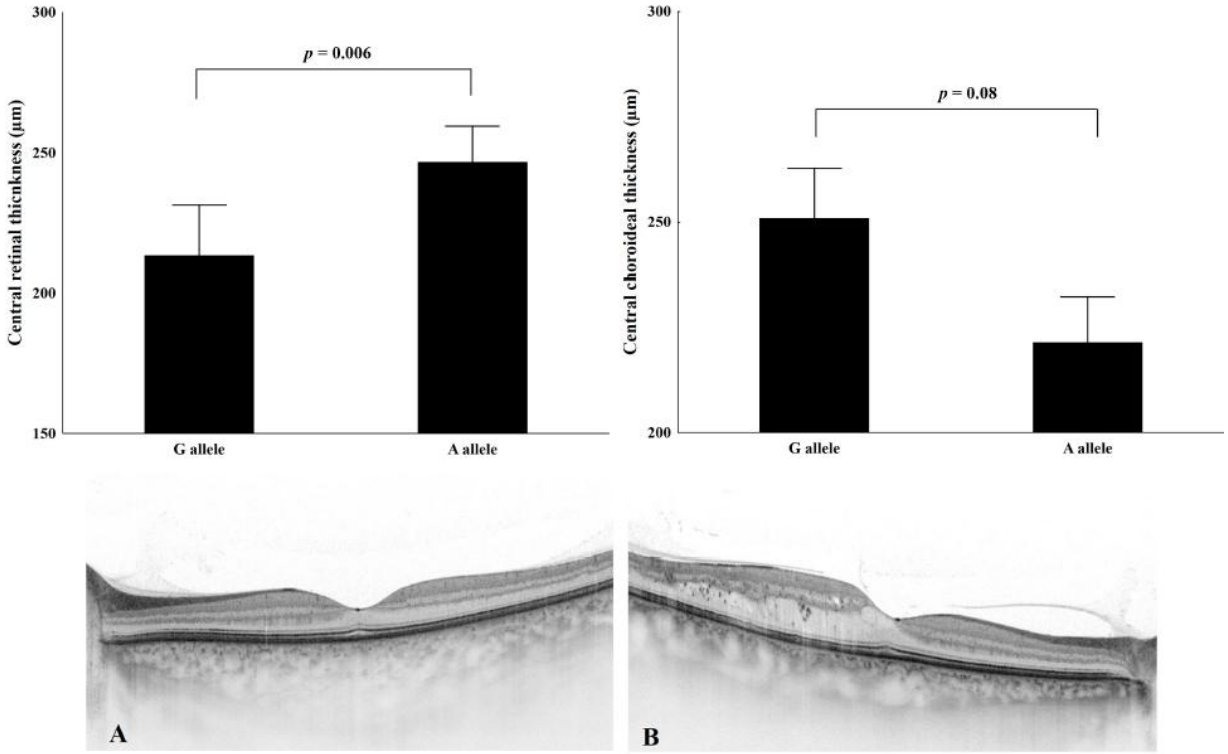

Figure 7. Association of SDF-1-3′ A and G alleles with central retinal thickness and central subfoveal choroidal thickness. Representative images of two patients of our study cohort: image and layers of the retina and the choroid by swept-source optical coherence tomography (DRI OCT Triton, Topcon Co., Tokyo, Japan), representation and image of our study group, published in (138). Panel (A): A 55-year-old women with Type-1 diabetes mellitus without any sign of diabetic retinopathy or diabetic macula edema and standard choroidal thickness (CSCT = 301 µm); SDF-1 (c801G) genotype. Panel (B): A 64-year-old man with Type-1 diabetes mellitus, slight diabetic macular edema with thinner choroid (CSCT = 186 µm); SDF-1 (c801A) genotype. Note: p-values were calculated using the Kruskal–Wallis test.

As age-dependent thinning is a crucial factor in determining the role of the choroid in retinal pathologies (86 – 90), we also performed regression analysis. We found a significant correlation between age and central subfoveal choroidal thickness in diabetic and control subjects (Figure 8, left panel, $p < 0.001$, $r = -0.57$). Hypertension was also a significant predictor of central choroidal thinning in our DM patients (Figure 8, right panel, $p < 0.05$).

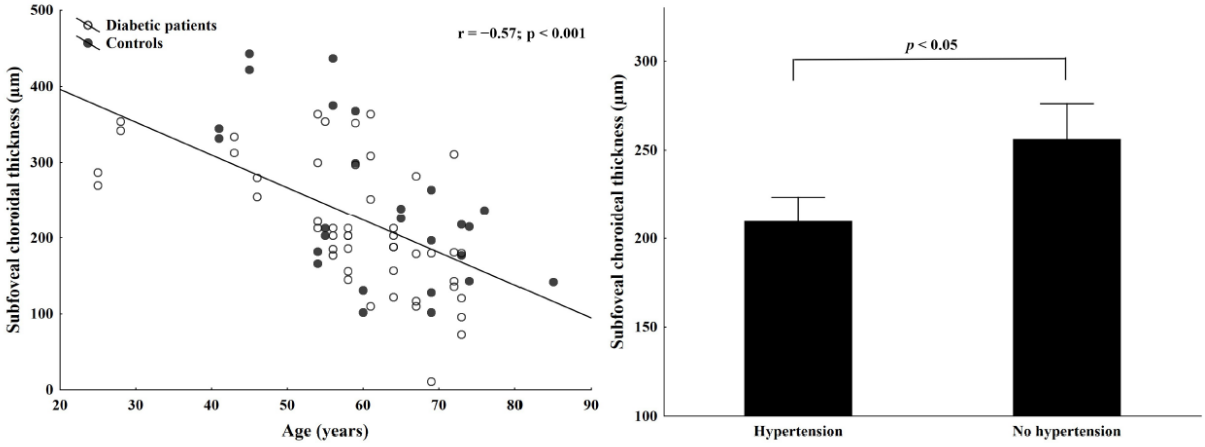

Figure 8. Regression analysis between age and central subfoveal choroidal thickness in the diabetic and control study groups (left) and the effect of hypertension on choroidal thickness in the whole cohort (right), representation of our study group, published in (138).

4.3.4. Genetic association with sight-threatening retinal complications

Table 9 summarizes the *SDF-1-3' (c801G > A)* genotypes in different severity groups of diabetic retinopathy. The *SDF-1-3' (c801G > A)* variant was a significant predictor of DME in the multivariable regression model (OR: 2.48, 95% CL: 1.21–5.08; $p = 0.01$) after adjusting for the effect of risk factors for DME, such as the duration of DM, HbA1C level and hypertension (141, 142). In addition, the *SDF-1-3' (c801G > A)* variant did not predict PDR in our cohort.

Table 9. SDF-1-3' (c801G > A) genotype frequencies in the eyes of diabetic patients with/without DME or with/without PDR (DM: diabetes mellitus, PDR: proliferative diabetic retinopathy, DME: diabetic macular edema), published in the study of our research group (139). Note: p-values were calculated using the Chi-squared test.

Genotypes	**AA + AG**	**GG**	**p - values**
DM eyes without PDR (n = 117)	60 (51.3%)	57 (48.7%)	0.16
DM eyes with PDR (n = 89)	36 (40.4%)	53 (59.5%)	
DM eyes without DME (n = 112)	43 (38.4%)	69 (61.6%)	**0.01**
DM eyes with DME (n = 94)	53 (56.38%)	41 (43.61%)	

Kaplan-Meier analysis showed that DME occurs significantly earlier in patients carrying the SDF-1 (c801A) allele (log-rank test, p = 0.02).

5. Discussion

In our prospective cross-sectional studies, we investigated the CT of diabetic subjects with SS-OCT, by observing CT changes in different stages of the disease. Furthermore, the association between *SDF-1-3'* variant and choroidal thickness parameters, in addition its relevance in the development of DM-associated PDR and DME was also evaluated after adjustment for known (metabolic or environmental) risk factors (5, 8). Our results revealed that in treatment naive patients, DM itself and the severity of DR affects CT significantly. CT even proved to be an independent predictor of the presence of DR. To the best of our knowledge, our research was the first SS-OCT study describing this type of association between diabetic choroidopathy and DR (137). On the other hand, when we examined the association of *SDF-1-3'* gene variant with diabetic retinopathy, our data suggested that the *SDF-1 (c801A)* variant is a relevant contributor to the development of DME. The binary logistic regression analysis, including the most studied influencing clinical parameters, suggested that this polymorphism remains a strong, independent, and predictive factor for the development of the disease. Finally, OCT measurements supported the association between genetic variants and macular retinal and choroidal pathologies (139).

Previous studies have already described that physiological factors could be responsible for inter-individual variations in choroidal thickness, such as age, gender and axial length (81, 86 – 90). In several studies, CT was also associated with systemic factors, such as hypertension, HbA1c level and duration of diabetes (147 – 151). The role of these factors (especially hypertonia and hyperglycaemia) in the pathogenesis of DR has also been studied extensively over the past 30 years (108). In healthy population, reduction of CT was reported significantly with aging (81, 87, 90). Hypertension was found to be the other most prominent factor affecting the choroid (147, 148). Yilmaz et al. (147) and Papathanasiou et al. (148) described that choroidal thickness decreased in patients with systemic arterial hypertension compared to normotensive controls in their studies. Akay et al. hypothesized that this may be caused by arteriolar sclerosis and vascular contraction caused by high intravascular pressure in the choroid (71). In our diabetic study group, we also found

significant correlation between age, systemic arterial hypertension and a decreased CT using univariable regression models. The mean reduction in CT was 26 μm/10 years in the central subfoveal region and 25 μm/10 years in the whole macular area (137). The association of CT and disease duration has been evaluated by Abadia et al. (115), who found a weak negative correlation between Type 2 DM duration and CT in their total study group. In our patients with diabetes, the disease duration was significantly associated with a decreased CT (137). At the same time, in contrast to the publications of Ünsal et al. (149) and Kim et al. (14) - who found a negative correlation between the central CT and HbA1c levels - in our study, there was no significant correlation between HbA1c level and choroidal thickness. However, we used only one HbA1c level, measured at the time of the ophthalmological examination. Conflicting results concerning inter-sex differences in CT have been reported earlier (92 – 94). In our study, male subjects proved to have a significantly thinner choroid than females. When we considered the main acknowledged physiological and systemic risk factors influencing DR progression (age, disease duration, gender, HbA1c level and hypertension) (152), the duration of diabetes remained significantly negatively associated with a decreased CT (137).

When investigating the association of CT and diabetes or the severity of DR in treatment-naive patients, we found a significant reduction of CT with both the presence and the severity of the disease, even after adjusting for the effects of the confounding factors (137, 152). CT changes with DR progression have been reported previously in several studies using different types of OCT devices, showing both choroidal thinning and thickening, as cited above (14 – 18, 113 – 117).

One reason for these conflicting results could be the use of different types of measurement devices. SS-OCT allows increased precision in the visualization of the choroid–sclera boundary (notably in DME patients with thickened retinas) (145, 153). Moreover, with the use of automated measuring software, a more precise and reproducible CT mapping can be produced compared to EDI-OCT (12, 13). It is in light of this that we chose this innovative new device for our study.

Choroidal thickness can vary between individuals according to age, gender, and axial length, as cited above (81, 86-90). On the other hand, some systemic factors, such as hypertension, HbA1c level and duration of diabetes could also affect the CT parameters (147 – 151). At the intra-individual level, it is important to take into consideration, that choroidal thickness can fluctuate during the day (95, 96). Several studies have not accounted for the effect of these intra- and inter-individual confounders, which may be another reason for conflicting results in the evaluation of diabetic choroidopathy (8, 15, 18, 97, 110, 122). Comparing to previous SS-OCT publications, one of the strengths of our study is the parallel evaluation of these influencing physiological and systemic factors. Furthermore, owing the effect of axial length to CT, patients with refractive error equal to or greater than ± 3 diopters were excluded from our study. Due to the diurnal variation of CT, the OCT examinations were always performed at the same period of the day in our research.

Another explanation for the conflicting results could be the mixed cohorts of treated and non-treated eyes. Seeing the influence of panretinal photocoagulation, most authors described a decrease of CT more than 3 months after the treatment (14, 15, 118). Concerning the use of anti-VEGF agents to treat DME, choroidal thinning was found (14, 15, 121). In the studies investigating the choroidal thickness in non-treated eyes of patients with diabetes and the association of SDF-1-3′ gene variant with diabetic retinopathy, we examined only treatment naive eyes, to avoid the effect of PRP or anti-VEGF treatment on CT values.

In our study examining CT in non-treated eyes of patients with diabetes, a significantly thinner choroid was measured in patients with diabetes compared to healthy controls. Between-eye asymmetry was similar in the two study groups. There was no significant difference in CT values between patients with Type 1 and Type 2 DM. In addition, in the diabetic group, we found a significant difference among means of CT in different stages of DR, with significantly thinner CT in cases with proliferative retinopathy similar to earlier SD- and SS-OCT studies (16, 18, 115, 117). At the same time, CT proved to be an independent significant predictor of the presence of DR, even after controlling for the

effects of systemic risk factors. When we classified our patients with diabetes in two groups according to CT values in the whole macular region (CT < 225 μm and CT > 225 μm, as 225 μm was found to be the mean whole macular CT in diabetic eyes), we found that patients with CT < 225 μm had a five times increased risk of developing DR than those with CT > 225 μm, independently of their age. Our results lead us to consider whether CT could be an important biomarker to identify the high-risk group of patients with diabetes with more rapid disease progression (137).

It can be hypothesized that the underlying cause of our observations is a progressive reduction of choroidal blood flow and volume, coupled with progressive damage to the choriocapillaris layer; as was reported in diabetic eyes earlier in histopathological, angiographic and Doppler flowmetry studies (99 – 101, 107 – 112). Schocket et al. and Nagaoka et al. suggested that choroidal hypoperfusion might trigger the development of DR due to retinal tissue hypoxia and overexpression of VEGF (111, 112). In some histological and clinical publications, choriocapillaris degeneration or dropout was found to be associated with unexplained loss of visual acuity without any signs of clinical retinopathy (6, 101), but these layers are too small to be detected with cross-sectional OCT devices. The introduction of OCT angiography may allow a better understanding of the deeper retinal vascular network and the choriocapillaris layer (58, 79). Findings from various OCTA studies indicate a significant decrease in choriocapillaris perfusion parameters in diabetic patients without diabetic retinopathy compared to healthy individuals (154). Dai et al. reported a significant increase in the percentage and average size of choriocapillaris flow deficits in diabetic patients without DR (155). Zlatanović et al. demonstrated a significant reduction in choriocapillaris flow area and vascular density in diabetic patients without DR compared to healthy subjects ($p < 0.001$ for both) (156). However, some studies have claimed that diabetic patients without DR do not exhibit significant variations in choroidal vascular indices examining with OCTA (157, 158). Therefore, while OCTA can aid in the early diagnosis of choroidal microangiopathy, it is essential to emphasize that OCTA may have some limitations for detecting the choroidal vasculature including the reduced ability to visualize intervascular spaces within the

choriocapillaries, which could make it difficult to distinguish individual choriocapillaries (154, 159). Furthermore, with using en-face SS-OCT, loss of vessels in the Sattler's layer, focal narrowing and vascular stumps in the Haller's layer were also identified in diabetic patients (160, 161). Due to the limitations of current OCTA technologies in visualizing deeper choroidal layers, some authors have suggested that OCTA may not be the most suitable tool for detecting choroidal changes in patients with diabetes (155, 158). In our study, we were able to evaluate the whole macular choroidal structure using SS-OCT.

Among the therapeutic options for diabetic retinopathy, we examined the effect of panretinal laser treatment on choroidal thickness in the second part of our study. Laser photocoagulation is an intervention that is commonly used to treat diabetic retinopathy, in which light energy is applied to the retina with the aim of stopping the growth and development of new blood vessels and thereby preserving vision (162). Panretinal photocoagulation alters choroidal blood flow in patients with PDR and in most studies using EDI-OCT, it was associated with a decrease in CT long after the treatment (more than 3 months) (14, 15). During our literally search, we came across only one study using SS-OCT: Eleiwa et al. found the decrease of average SFCT in severe NPDR and PDR 3 months after PRP treatment (118). Zhang et al. formulated three hypotheses to explain the CT reduction long after PRP (163). First, during photocoagulation, heat dissipates from the RPE and subsequent thermal damage spreads to the adjacent outer retina and choroid. This results in choroidal damage, which leads to failure of the choroidal vasculature to reperfuse or reorganise, manifesting as a reduction in choroidal thickness (163). The second hypothesis was that in addition to its damaging effect on the retinal pigment epithelium, PRP reduces vascular endothelial growth factor release. It is well known that VEGF has a crucial role in the function of human vascular tissues (including the choroid), maintaining the normal permeability of vascular tissues in adults (164). A decrease in secretion of VEGF after PRP may lead to a reduction in the dilation and permeability of the choroidal blood vessels (163). The third hypothesis was that after widespread destruction of the outer retina by the laser, the hypoxic inner retina may be closer to the choriocapillaris with its highly saturated choroidal circulation. This could lead to autoregulation and reduce

choroidal circulation (163). The results of our study, which showed a significant reduction in CT at least three months after laser treatment, are consistent with these hypotheses. However, there was no significant difference between patients with PDR and the PRP-treated group in our study. Due to this outcome, we cannot rule out the influence of PDR on our results.

When we investigated the association of *SDF-1-3'* gene variant with diabetic retinopathy, we found that the *SDF-1 (c801AA)* genotype increases the risk of DME in diabetic patients. We also demonstrated a significant association between the *SDF-1 (c801AA)* genotype and the development of DME, independent of the duration or type of DM, or other systemic parameters such as HbA1c levels or hypertension, which factors could be relevant to the development of diabetic retinopathy (139, 152). Since changes in retinal thickness are also known biomarkers of DME progression and prognosis (142), we investigated the association of the SDF-1 (c801A) allele with central macular retinal thickness. We showed that the central macular retinal thickness was increased in patients carrying the SDF-1 (c801A) allele (139).

In addition to retinal thickening, central subfoveal choroidal thickness was thinner in our diabetic patients carrying the SDF-1 (c801A) allele; however, age and hypertension seemed to have a more significant predictive value for choroidal thinning (139). Regarding the relationship of choroidal structure and SDF-1, we only have in vitro studies (134, 135, 136). To the best of our knowledge, our study was the first investigating the association between choroidal thickness changes in diabetes and *SDF-1-3'* gene variant (139).

The signaling pathway of SDF-1 and its receptor CXCR4 were demonstrated to have roles in different pathological and physiological mechanisms, including embryogenesis, wound healing, angiogenesis, tumor growth and proliferation (126). When SDF-1 binds to its receptor on the endothelial cells, it induces angiogenesis by releasing growth factors, such as VEGF or transforming growth factor beta (127, 165). In terms of ophthalmological complications - seeing the results of in vitro studies - SDF-1 is a potent stimulator of VEGF-mediated neoangiogenesis, which plays a crucial role in developing AMD or PDR and it could be a key inducer of vascular permeability associated with DME (25, 27-29,

134). In an animal study of Cai et al., the expression of SDF-1 and VEGF in RPE cells increased when cultured under high glucose. They suggested that hyperglycemia increases the expression of SDF-1 and VEGF in RPE cells, which play a role in formation of CNV (136). In the study of Brooks et al., both intravitreal VEGF and SDF-1 were significantly higher in patients with PDR than in patients with NPDR. Levels of SDF-1 were markedly increased in patients with DME compared with those without DME. Moreover, intraocular injection of triamcinolone acetonide resulted in dramatic reductions of VEGF and SDF-1 to nearly undetectable levels, eliminated DME, and caused regression of active neovascularization in their research (29).

In our study, we found a strong correlation between DME and the SDF-1 (c801A) allele. Although there was no association with the development of PDR (139). As this correlation has already been published in T2DM patients in a recent Taiwanese study (20), we assume that the relatively small sample size or the inclusion of T1DM patients may explain the lack of significance and the discrepancy with literature (139). It should also be considered that our study population was white Caucasians of Hungarian ethnic origin. Therefore, the possibility of ethnicity as a confounding factor cannot be excluded (139).

Incorporating our genetic findings into DME clinical practice, *SDF-1-3' (c801G > A)* variant may be an ideal biomarker and also a future therapeutic target for high-risk diabetic retinopathy patients, especially those carrying the SDF-1 (c801A) allele (139). Furthermore, as was published in the CATIS study, elevated plasma SDF-1 was significantly associated with an increased risk of recurrent stroke and cardiovascular events in ischemic stroke patients with diabetes mellitus but not in those without DM (26). SDF-1 antagonists may be helpful in the future, not only for ocular complications but also for the prevention of recurrent stroke, cardiovascular events, and all-cause mortality (26, 139).

Regarding the molecular mechanism of choroidal changes in different diseases and their relationship to SDF-1, we only have in vitro studies (134-136, 165, 166). In postmortem eyes of patients with AMD, the presence of SDF-1 and CXCR4 was most prominent in RPE cells and choroidal stroma by immunolocalization (134). In the study of Jin et al., SDF-1 induced proliferation in the choroid-retinal endothelial cell line under hypoxic

conditions (166). In our study, the central subfoveal choroidal thickness was thinner in diabetic patients carrying the SDF-1 (c801A) allele (139). However, our results suggest an association between *SDF-1-3′ (c801G > A)* genetic variants and choroidal pathology, further investigations are needed to understand the role of the SDF-1 and CXCR4 signaling pathways in choroidal changes in diabetic patients.

The principal strengths of our study - comparing to previous SS-OCT publications - are the investigation of treatment naive eyes, the parallel evaluation of the influence of systemic risk factors and the simultaneous analysis of both eyes of the same subject using marginal effects model, to take into account the correlation between the subject's two eyes.

It is evident that this study is not without limitations, including the small sample size and the inclusion of a mixed cohort of patients with Type 1 and Type 2 diabetes. The nature of the cross-sectional design of our study prevents cause-and-effect inference, meaning that our results cannot establish a clear causal relationship between the examined risk factors and choroidal thickness. In future, whenever possible, transversal matching should be replaced by longitudinal analysis. This requires a longer follow-up period but provides more information about the evolution of CT with DR or DME, while avoiding age-related changes and individual variability (8). Although SS-OCT technology was adopted in our study - which has higher resolution and more accuracy for CT measurement than EDI-OCT (12, 13) -, we used a grid only in 6 × 6 mm size to automatically calculate the CT values. A larger area may have provided more representative information about choroidal changes. On the other hand, the evaluation of choroidal thickness using different types of OCT devices has been proven to be affected by several intra- and inter-individual confounders, resulting in conflicting results in literature evaluating diabetic choroidopathy (14 – 18, 113 – 117). In recent years, alternative indices obtained through post-processing imaging, such as choroidal vascularity index (CVI), choroidal vascularity density (CVD), and choroidal vascular volume (CVV), have been considered for investigating choroidal changes, particularly in the medium vessels of Sattler's and the large vessels of Haller's layer (97). CVI represents the proportion of luminal area, corresponding to the vascular component, over the total choroidal area (97, 167, 168). CVD can be calculated by binarizing the en-

face swept-source OCT to enhance the visualization of the choroidal vasculature (97, 169). In contrast, CVV was calculated by multiplying the average CVD by the macular area and the maximal choroidal thickness (97, 169). Using these indices may improve the understanding of the relationship between the choroid and the pathophysiology of diabetic retinopathy in the future. In our SS-OCT device, using these indices was not yet available.

6. Conclusions

We found that DM itself, in addition to the severity of DR, affects CT significantly in treatment-naive eyes; even after adjusting for the effects of confounding systemic risk factors. CT < 225 μm proved to be an independent predictor of the presence of DR. To the best of our knowledge, our study was the first describing this type of association between diabetic choroidopathy and diabetic retinopathy. Examining the choroidal thickness of diabetic patients may help ophthalmologists to identify the high-risk group of patients with more rapid disease progression.

In the second part of our study, choroidal thinning was found to be correlated with the presence of PDR and the panretinal photocoagulation therapy for diabetic retinopathy.

Evaluating the association of *SDF-1-3'* gene variant with diabetic retinopathy, we found that *SDF-1-3' (c801G > A)* is involved in the development of macular complications in DM independent of critical clinical factors, suggesting that SDF-1 may be a future therapeutic target for high-risk patients, especially those carrying the SDF-1 (c801A) allele. The central subfoveal choroidal thickness was lower in DM patients with the presence of the SDF-1 (c801A) allele in our research. During our literally search, our study was found to be the first investigating the association between *SDF-1-3'* variant and choroidal thickness parameters.

Our results could confirm the importance of CT measurement in patients with diabetes. However, beyond the experimental and in vivo findings, the role of choroidal abnormalities in the pathogenesis of DR is not clearly defined. The majority of literature indicates that as the severity of the disease increases, there is also an observable decline in the choroidal microvasculature. This was also demonstrated in our study. Any change or damage of the choroid may affect the overlying retina. This can lead to hypoxia and the appearance of DR lesions, or the progression of existing retinopathy. However, it is not well known whether DR lesions are associated with reduced CT or whether CT decreases prior to the appearance of DR signs. To better understand the role of choroidal changes in the pathophysiology of

DR, further SS-OCT studies with a larger cohort and longer follow-up duration are warranted. Knowledge of the imaging features of the choroid is essential for creating an optimal model to study the exact pathogenic relationship and potential therapeutic implications.

Summarizing our new results:

1. Evaluating CT in treatment-naive eyes, CT < 225 μm proved to be an independent predictor of the presence of DR (137).
2. When we considered the main acknowledged physiological and systemic risk factors influencing DR progression (age, disease duration, gender, HbA1c level and hypertension), the duration of diabetes remained significantly negatively associated with a decreased CT in treatment-naive eyes (137).
3. When we investigated the association of *SDF-1-3′* gene variant with sight-threatening retinal complications, we found that *SDF-1-3′ (c801G > A)* is involved in the development of macular complications in DM independent of critical clinical factors (139).
4. DME occurred earlier in patients carrying the SDF-1 (c801A) allele in our study (139).
5. In addition to retinal thickening, central subfoveal choroidal thickness was thinner in our diabetic patients carrying the SDF-1 (c801A) allele (139).

7. Summary

The growing number of people with DM worldwide has led to several problems. Diabetic retinopathy, a microvascular complication of DM is the leading cause of visual impairment in working-age adults in industrialized countries. The choroidal layer supplies blood to the outer layers of the retina and may play a key role in the pathophysiologic mechanism of DR. The most consequential changes of the choroid primarily impact the choriocapillaris layer, though they can also affect the larger vessels located in the outer choroidal layers. A better understanding of the morphology and function of this vascular structure could facilitate the management of DR and improve therapeutic decision-making in diabetic patients. Choroidal thickness has been found to be an important index for the quantification of choroidal structure.

The first part of our study, we measured CT in treatment naive eyes of diabetic subjects with SS-OCT, which is a useful non-invasive device to evaluate CT parameters and correlated it to the presence and severity of DR. We found a significant reduction of CT with both the presence and the severity of the disease, even after adjusting for the effects of confounding systemic factors. Disease duration seemed to be associated with a reduction of choroidal thickness. Decreased CT proved to be correlated with the presence of DR.

Our second objective was to correlate CT with the therapy of DR. Our results showed significantly lowered CT in patients with PDR and in patients at least 3 months after PRP treatment.

Ultimately, we investigated the association between the *SDF-1-3' (c801G > A)* variant and the development of DME or PDR. Central retinal and CT parameters were also measured in this part of our study. *SDF-1-3' (c801G > A)* variant was found to be a relevant contributor to the development of DME, especially those carrying the SDF-1 (c801A) allele. Finally, OCT measurements supported the association between genetic variants and macular retinal and choroidal pathologies.

Our findings provide further evidence to suggest that choroidal alterations may be a potential pathway in the pathogenesis of diabetic retinopathy. Further studies are required to clarify the exact mechanism behind these findings.

8. References

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9. Bibliography of the candidate's publications

9.1. Publications related to the PhD thesis

1. **Horváth H**, Kovács I, Sándor GL, Czakó C, Mallár K, Récsán Zs, Somogyi A, Nagy ZZ, Ecsedy M. Choroidal thickness changes in non-treated eyes of patients with diabetes: swept-source optical coherence tomography study. Acta Diabetol. 2018;55(9):927-934. -**IF: 2,996**
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Σ Impact factor: 8,436

9.2. Publications not related to the PhD thesis

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