

POMORSKI UNIWERSYTET MEDYCZNY W SZCZECINIE



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**ANALIZA MORFOLOGICZNA I FUNKCJONALNA
PARAMETRÓW MIKROKRAŻENIA SIATKÓWKI I NACZYNIÓWKI
W AMD — PRÓBA IDENTYFIKACJI CZYNNIKÓW
WARUNKUJĄCYCH OBRAZ FENOTYPOWY CHOROBY**

Rozprawa doktorska w dziedzinie nauk medycznych i nauk o zdrowiu

Dyscyplina nauki medyczne

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Dziękuję

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1 Wykaz stosowanych skrótów

- AMD — (ang. *age-related macular degeneration*) — zwyrodnienie plamki związane z wiekiem
- ARMS2 gene — (ang. *Age-Related Maculopathy Susceptibility 2 gene*) — gen podatności na AMD
- anty-VEGF — (ang. *Anti-vascular endothelial growth factor*) — przeciwciała przeciw śródbłonkowemu czynnikowi wzrostu
- ATC — (ang. *average thickness center*) — średnia grubość naczyniówki w obszarze pierścienia centralnego
- AV — (ang. *average volume*) — średnia objętość ze wszystkich dziewięciu podpól mapy ETDRS
- AVC — (ang. *average volume center*) — średnia objętość w obszarze pierścienia centralnego
- AVR — (ang. *arteriovenous ratio*) — współczynnik tętniczo-żylny
- bFGF — (ang. *basic fibroblast growth factor*) — podstawowy czynnik wzrostu fibroblastów
- BrM — (ang. *Bruch's membrane*) — błona Brucha
- CC — (ang. *choriocapillaris*) — choriokapilary
- CFH — (ang. *complement factor H*) — czynnik dopełniacza H
- CNV — (ang. *choroidal neovascularization*) — neowaskularyzacja naczyniówkowa
- CRP — (ang. *C-reactive protein*) — białko C-reaktywne
- CSCR — (ang. *central serous chorioretinopathy*) — centralna chorioretinopatia surowicza
- CVI — (ang. *choroidal vascular index*) — współczynnik unaczynienia naczyniówki
- CVDs — (ang. *cardiovascular diseases*) — choroby układu sercowo-naczyniowego
- CRAE — (ang. *central retinal arteriolar equivalent*) — centralny ekwiwalent tętnicy środkowej siatkówki
- CRVE — (ang. *central retinal venular equivalent*) — centralny ekwiwalent żyły środkowej siatkówki
- DVA — (ang. *dynamic vessels analysis*) — dynamiczna analiza naczyń
- EDI-OCT — (ang. *enhanced depth imaging*) — OCT rozszerzonej głębi obrazowania

- FAZ — (ang. *foveal avascular zone*) — strefa beznaczyniowa w obszarze plamki
- HGF — (ang. *hepatocyte growth factor*) — czynnik wzrostu hepatocytów
- GA — (ang. *geographic atrophy*) — zanik geograficzny
- MMPs — (ang. *matrix metalloproteinases*) — metaloproteinazy tkankowe
- MNV — (ang. *macular neovascularization*) — neowaskularyzacja plamkowa
- NIR — (ang. *near infrared reflectance*) — obrazowanie w bliskiej podczerwieni
- OCT — (ang. *optical coherence tomography*) — koherentna tomografia optyczna
- PSD — (ang. *pachychoroid spectrum diseases*) — schorzenia z grupy pachychoroid
- RPE — (ang. *retinal pigment epithelium*) — nabłonek barwnikowy siatkówki
- RVA — (ang. *Retinal Vessel Analyser*) — analizator naczyń siatkówki
- SFCT — (ang. *subfoveal choroidal thickness*) — poddołkowa grubość naczyń siatkówki
- SNP — (ang. *single nucleotide polymorphism*) — polimorfizm pojedynczego nukleotydu
- SVA — (ang. *static vessels analysis*) — statyczna analiza naczyń
- TGF- β — (ang. *transforming growth factor- β*) — transformujący czynnik wzrostu beta
- TIMP — (ang. *tissue inhibitor of metalloproteinases*) — tkankowy inhibitor metaloproteinaz
- WZs — (ang. *watershed zones*) — graniczne strefy spływu
- VEGF — (ang. *vascular endothelial growth factor*) — śródbłonkowy czynnik wzrostu

2 Nota informacyjna

Niniejszą rozprawę doktorską pt. „Analiza morfologiczna i funkcjonalna parametrów mikrokrażenia siatkówki i naczyńówki w AMD — próba identyfikacji czynników warunkujących obraz fenotypowy choroby” stanowi cykl 3 artykułów oryginalnych, których jestem pierwszym autorem. Artykuły opublikowano w międzynarodowych czasopismach naukowych znajdujących się na liście Journal Citation Reports (Thomson Reuters). Łączna wartość współczynnika oddziaływania (Impact Factor — IF) dla cyklu wymienionych prac (według Thomson Reuters Journal Citation Reports 2022) wynosi: 14,885 oraz 420 punktów Ministerstwa Nauki i Szkolnictwa Wyższego. Rozprawę doktorską stanowią następujące publikacje:

- Krytkowska E, Grabowicz A, Mozolewska-Piotrowska K, Ulańczyk Z, Safranow K, Machalińska A. The impact of vascular risk factors on the thickness and volume of the choroid in AMD patients. *Sci Rep*. 2021 Jul 23;11(1):15106. doi: 10.1038/s41598-021-94676-6; IF: 4,996. MNiSW: 140.
- Krytkowska E, Ulańczyk Z, Grabowicz A, Mozolewska-Piotrowska K, Safranow K, Pałucha A, Krawczyk M, Sikora P, Matczyńska E, Stahl A, Machalinski B, Machalinska A. Retinal Vessel Functionality Is Linked With ARMS2 A69S and CFH Y402H Polymorphisms and Choroidal Status in AMD Patients. *Invest Ophthalmol Vis Sci*. 2021 Apr 1;62(4):30. doi: 10.1167/iov.62.4.30; IF: 4,925. MNiSW: 140.
- Krytkowska E, Ulańczyk Z, Grabowicz A, Safranow K, Kawa MP, Pałucha A, Wąsowska A, Matczyńska E, Boguszevska-Chachulska A, Machalińska A. Influence of Clinical and Genetic Factors on the Progression of Age-Related Macular Degeneration: A 3-Year Follow-Up. *J Clin Med*. 2023 Mar 1;12(5):1963. doi: 10.3390/jcm12051963. IF: 4,964. MNiSW: 140.

Zgodnie z Rozporządzeniem Ministra Nauki i Szkolnictwa Wyższego z dnia 30. października 2015 r. (§5.2) do pracy zostały dołączone oświadczenia kandydata oraz pozostałych współautorów publikacji określające indywidualny wkład w powstawanie prac stanowiących rozprawę doktorską.

3 Wprowadzenie

3.1 Epidemiologia zwyrodnienia plamki związanego z wiekiem

Zwyrodnienie plamki związane z wiekiem (ang. *age-related macular degeneration*; AMD) jest przewlekłą, postępującą chorobą centralnej siatkówki dotyczącą głównie osób po 50. roku życia (1). W związku ze starzeniem się społeczeństw jej częstość występowania rośnie i szacuje się, iż do 2040 roku na świecie będzie około 288 milionów chorych. Liczba Europejczyków z rozpoznaniem AMD osiągnie 21,5 miliona, wśród których 4,8 miliony będą stanowiły osoby z późną postacią choroby, prowadzącą do głębokiego i nieodwracalnego upośledzenia widzenia (2). Według danych Polskiego Towarzystwa Okulistycznego w Polsce żyje około 1,5 mln osób z rozpoznaniem AMD.

Wczesne stadium choroby objawia się klinicznie tworzeniem bogatych w lipidy złogów zewnątrzkomórkowych zwanych druzami oraz nieprawidłowościami nabłonka barwnikowego siatkówki (ang. *retinal pigment epithelium*; RPE) widocznymi w badaniu dna oka jako hiper- lub hipopigmentacja. Objawami późnej fazy choroby są: zanik geograficzny (ang. *geographic atrophy*; GA) lub neowaskularyzacja plamki (ang. *macular neovascularization*; MNV), które prowadzą do ciężkiego i trwałego upośledzenia wzroku. W tej chwili całkowite wyleczenie choroby jest niemożliwe. Wprowadzenie terapii doszkliskowej spowolniło tempo utraty wzroku, ale wiąże się z ryzykiem poważnych powikłań związanych z wielokrotnymi nakłuciami gałki ocznej, m.in. odwarstwieniem siatkówki i zapaleniem wnętrza gałki ocznej. Dodatkowo, mimo systematycznie prowadzonego leczenia, u większości pacjentów dochodzi do jedynie krótkotrwałej poprawy ostrości wzroku. Po 7 latach od rozpoznania 37% z nich prezentuje ostrość wzroku rzędu 20/200 lub niższą, co jest jednoznaczne z rozpoznaniem ślepoty prawnej (3). Terapia doszkliskowa jest również ogromnym obciążeniem finansowym i organizacyjnym dla służby zdrowia. W chwili obecnej jedynym postępowaniem o udowodnionej skuteczności w leczeniu i zapobieganiu zanikowi geograficznemu jest prewencja pierwotna obejmująca suplementację antyoksydantów oraz zmianę stylu życia ze szczególnym uwzględnieniem zaprzestania palenia papierosów (4, 5).

3.2 Czynniki ryzyka zwyrodnienia plamki związanego z wiekiem

Aktualnie uważa się, iż AMD powstaje w wyniku wieloczynnikowej interakcji między czynnikami metabolicznymi, genetycznymi i środowiskowymi (6). Należy jednak podkreślić,

że dokładny patomechanizm choroby wciąż nie został jednoznacznie wyjaśniony. Do czynników ryzyka AMD należą: wiek, rasa, oraz styl życia. Wiek jest jednym z najbardziej stałych, niemodyfikowalnych czynników ryzyka: osoby w wieku od 60 do 80 lat są trzykrotnie bardziej narażone na rozwój zaawansowanego AMD w porównaniu z osobami w wieku poniżej 60. roku życia. Z wiekiem dochodzi do rozwoju określonych zmian degeneracyjnych w obszarze plamki, takich jak pogrubienie błony Brucha, akumulacja lipofuscyny w komórkach nabłonka barwnikowego siatkówki oraz tworzenie druzów (7). W oczach osób starszych obserwuje się również nasilenie procesów oksydacyjnych, prowadzących do dysfunkcji mitochondriów. Zmiany te zachodzą zarówno w komórkach nabłonka barwnikowego jak również w choriokapilarach (7).

Dodatni wywiad rodzinny jest uważany za istotny czynnik ryzyka zwyrodnienia plamki żółtej związanego z wiekiem (8). Rodzeństwo pacjenta ma trzykrotnie do sześciokrotnie wyższe ryzyko zachorowania na AMD niż populacja ogólna (9). Badacze prowadzący duże badanie asocjacyjne całego genomu zidentyfikowali 52 warianty kodujące, rozmieszczone w 34 loci, które występowały częściej u pacjentów z AMD (10). Zlokalizowano je w różnych chromosomach, w tym chromosomach 1, 6 i 10. Tan i wsp. przyporządkowali te geny do szlaków, w które są one aktywnie zaangażowane: (a) zapalenie, odpowiedź immunologiczna i aktywacja dopełniacza (C2, C3, C9, CFI, CFH, TLR3, TLR4), (b) metabolizm i transport lipidów (ABCA1, APOE, CETP, LIPC, LPL, VLDLR), (c) przebudowa macierzy zewnątrzkomórkowej i adhezja komórkowa (ACE, ARMS2, ADAMTS9, COL8A1, COL10A1, TIMP3), (d) angiogeneza (CST3, MMP9, TGFBR1, TIMP3, VEGFA) i (e) komórkowa odpowiedź na stres (GSTM1, SOD2, TGFBR1) (11). Nieprawidłowości w funkcjonowaniu powyższych szlaków sygnałowych mogą zaburzać interakcję między fotoreceptorami, nabłonkiem barwnikowym, błoną Brucha i warstwą naczyń włosowatych i tym samym przyczyniać się do rozwoju AMD. Wariantom w obrębie genów CFH i ARMS2/HTRA1 przypisuje się największe znaczenie spośród zidentyfikowanych dotychczas loci związanych z AMD (7). Wykazano, że allele wysokiego ryzyka CFH, ARMS2 i VEGFA były związane z młodszym wiekiem zachorowania, szybszą progresją choroby oraz niewystarczającą odpowiedzią na leczenie doszklistkowe. Medina i wsp. wykazali, iż osoby z wariantami polimorfizmu genu CFH wykazywały opóźnioną i ograniczoną odpowiedź morfologiczną na początkową terapię doszklistkową preparatami anty-VEGF (12). Niektórzy badacze donoszą preferencyjny związek wariantów ryzyka CFH i ARMS2/HTRA1 z różnymi postaciami zaawansowanego AMD. W szczególności warianty ryzyka CFH wydają się

faworyzować progresję w kierunku GA, podczas gdy wariant ryzyka ARMS2 w kierunku MNV (13, 14).

3.3 Patogeneza zwyrodnienia plamki związanego z wiekiem

AMD prowadzi do poważnego upośledzenia wzroku w wyniku uszkodzenia fotoreceptorów plamkowych oraz ich aparatu podporowego: nabłonka barwnikowego, błony Brucha oraz choriokapilarów. Struktury te są ze sobą ściśle powiązane zarówno anatomicznie, jak i czynnościowo, a zaburzenia każdego elementu skutkują degeneracją całego kompleksu, prowadząc ostatecznie do zaburzenia czynności komórek fotoreceptorowych (15).

Nabłonek barwnikowy siatkówki to pojedyncza warstwa komórek sąsiadująca z fotoreceptorami siatkówki. Po stronie zewnętrznej komórki RPE są zakotwiczone przez pódsmosomy w blaszkach podstawnych, które tworzą najbardziej wewnętrzną warstwę błony Brucha. RPE bierze udział w fagocytozie i recyklingu zewnętrznych segmentów fotoreceptorów, a także w pochłanianiu nadmiaru światła, przekształcaniu all-trans retinolu w 11-cis retinal w ramach cyklu wzrokowego, wydzielaniu czynników wzrostu, wymianie składników odżywczych i produktów ubocznych metabolizmu siatkówkowego między komórkami fotoreceptorów a choriokapilarami (16). Wraz z błoną Brucha tworzy zewnętrzną barierę krew-siatkówka. Z wiekiem liczba komórek RPE się zmniejsza, a także kumulują się ziarnistości lipofuscyny oraz toksyczne produkty uboczne cyklu wzrokowego (17). Wykazano, iż zaburzona czynność komórek nabłonka barwnikowego prowadzi do powstawania druzów, a składowe druzów, takie jak białka układu dopełniacza, czy amyloid beta indukują stan zapalny, prowadząc do degeneracji RPE (18). Nabłonek barwnikowy jest również źródłem VEGF, którego podwyższony poziom stymuluje patologiczną angiogenezę.

Błona Brucha to cienka warstwa tkanki łącznej, zbudowana z pięciu (naprzemiennie ułożonych) pasm bogatych w elastynę i kolagen (15). Pełni funkcję podporową dla komórek RPE oraz choriokapilarów, reguluje wzajemną wymianę składników odżywczych, tlenu, płynów i produktów przemiany materii między siatkówką a krążeniem ogólnoustrojowym (19). Ponadto, BrM zapewnia powierzchnię adhezji, wzrostu i rusztowanie dla komórek RPE (15). Błona Brucha ulega ciągłym procesom przebudowy, związanych z aktywnością metaloproteinaz tkankowych (ang. *matrix metalloproteinases*; MMPs) oraz ich inhibitora (ang. *tissue inhibitor of metalloproteinases*; TIMP) a jej prawidłowa funkcja zależy od ich wzajemnej równowagi (19). Z wiekiem dochodzi do pogrubienia BrM, szczególnie w rejonie plamki, a na przestrzeni całego życia jej grubość zwiększa się dwukrotnie. Może być to związane z zaburzoną przemianą cząsteczek macierzy zewnątrzkomórkowej, jak również z akumulacją

lipoprotein. Warstwy kolagenowe z czasem ulegają kalcyfikacji, co nasila kruchość BrM i powoduje jej zwiększoną podatność na pęknięcia. Złogi lipidowe w obrębie błony Brucha z czasem powiększają swoje rozmiary, prowadząc do rozwoju druzów. Tworzą one hydrofobową barierę, która zaburza transport dużych cząstek do komórek nabłonka barwnikowego oraz fotoreceptorów. Dodatkowo, w BrM odkładają się również produkty glikacji i hiperoksydacji, białka układu dopełniacza oraz cząsteczki białka C-reaktywnego (ang. *C-reactive protein*; CRP), które mają toksyczny wpływ na komórki nabłonka barwnikowego, stymulują procesy zapalne oraz angiogenezę (20).

3.3.1 Rola czynników naczyniowych w patogenezie AMD

Siatkówka składa się z dwóch odrębnych czynnościowo warstw: zewnętrznej, czyli RPE oraz siatkówki neurosensorycznej, zawierającej wysoce wyspecjalizowane komórki fotoreceptorowe, posiadające zdolność zamiany bodźca świetlnego w impuls elektryczny (fototransdukcja). Są to komórki o najwyższym zużyciu tlenu w przeliczeniu na gram tkanki w całym organizmie, co powoduje, iż stanowią struktury wysoce wrażliwe na wszelkie zaburzenia w zakresie unaczynienia (21).

Przez wiele lat badania naukowe nad patogenezą i/lub progresją AMD koncentrowały się na zaburzonej funkcji komórek nabłonka barwnikowego, podczas gdy tkanka naczyniówki i siatkówki neurosensorycznej były postrzegane jako biernie poddające się procesom patologicznym wtórnym do degeneracji RPE i BrM (16, 22). W 1997 roku Friedman przedstawił hemodynamiczną teorię AMD, zgodnie z którą pierwotnym zaburzeniem w AMD jest zwiększony opór naczyniowy w łożysku naczyniówki, w wyniku zwiększonej sztywności twardówki, wtórnie do zmian miażdżycowych (23). Autor sugerował, iż postać kliniczna AMD może zależeć od wielkości względnych oporów przepływu w odgałęzieniach tętnicy szyjnej wewnętrznej: tętnic mózgowych oraz tętnicy ocznej. Relatywnie niższy opór naczyniowy w tętnicach mózgowych prowadzi do zmniejszonej perfuzji naczyniówkowej, zaburza przepływ w obrębie choriokapilarów, wpływając niekorzystnie na działanie komórek nabłonka barwnikowego siatkówki i prowadząc do powstania zanikowej postaci AMD. W sytuacji zwiększonego względnego oporu naczyń mózgowych, dochodzi do wzrostu ciśnienia hydrostatycznego w naczyniach naczyniówki, choriokapilarach, zaburzając funkcje RPE oraz błony Brucha i ostatecznie prowadząc do rozwoju wysiękowej postaci AMD. Hipoteza hemodynamiczna nie uzasadniała szerokiego spektrum objawów klinicznych AMD, m.in. współistnienia zaniku geograficznego z neowaskularyzacją plamki (24). Temat niedokrwienia jako czynnika inicjującego AMD nie został więc wyczerpany.

Istotnym ograniczeniem w ocenie naczyniówki jest jej lokalizacja pod nieprzepuszczalnym dla światła nabłonkiem barwnikowym, co czyniło ją przez lata tkanką trudno dostępną dla nieinwazyjnych badań obrazowych. Przełom stanowiło wprowadzenie w 2003 roku do diagnostyki optycznej tomografii koherentnej (SS-OCT), które w sposób szybki, powtarzalny i nieinwazyjny odwzorowuje szczegółową anatomię siatkówki i naczyniówki oraz umożliwia ilościową analizę ich składowych. Wykorzystanie OCT rozszerzonej głębi obrazowania (ang. *enhanced depth imaging*; EDI-OCT) oraz wykorzystanie dłuższej fali świetlnej, umożliwia lepszą penetrację wiązki laserowej i uwidocznienie pełnej grubości tkanki naczyniówki, aż do złącza naczyniówkowo-twardówkowego. Postęp technologiczny zapoczątkował nową erę badań nad zmianami mikrokrażenia naczyniówkowego tak w oczach zdrowych, jak i objętych procesem patologicznym. Gelfand i Ambati w pracy opublikowanej w 2017 roku ponownie postulowali, iż zmiany hemodynamiczne w kapilarach naczyniówkowych stanowią kluczowy czynnik determinujący fenotyp choroby (25). Postulowali, iż zaburzenia hemodynamiczne w choriokapilarach plamkowych mogą być istotnymi czynnikami w patogenezie AMD. Wpływają one na czynność i dalsze ukierunkowanie komórek śródbłonka naczyniowego poprzez uruchomienie szlaku komórkowego prowadzącego do proliferacji komórek śródbłonka choriokapilarów i rozwoju MNV lub poprzez wzmożone procesy apoptozy endotelium do powstania zaniku geograficznego. Dodatkowo, zmiany przepływu w obrębie choriokapilar mogą modulować aktywność procesów immunologicznych oraz angiogenezy, wpływając w ten sposób na obraz kliniczny choroby (25). Według innej teorii, zaburzenie perfuzji naczyniówkowej nie determinuje w sposób bezpośredni rozwoju AMD, ale może istotnie wpływać na obraz kliniczny i przebieg choroby w oczach osób z predyspozycją genetyczną (26).

3.3.1.1 Krążenie naczyniówkowe

Naczyniówka jest jedną z najbardziej unaczynionych tkanek ludzkiego organizmu (27). Jej podstawową funkcją jest zaopatrywanie w tlen i substancje odżywcze zewnętrznych warstw siatkówki, szczególnie w obrębie plamkowej strefy awaskularnej (ang. *foveal avascular zone*; FAZ), gdzie naczyniówka jest wyłącznym źródłem krwi dla siatkówki (27). Dodatkowymi funkcjami naczyniówki są termoregulacja, m.in. naczyniówka pochłania i rozprasza dużą ilość energii cieplnej powstającej w procesie widzenia oraz sekrecja czynników wzrostu takich jak: śródbłonkowy czynnik wzrostu (ang. *vascular endothelial growth factor*; VEGF), podstawowy czynnik wzrostu fibroblastów (ang. *basic fibroblast growth factor*; bFGF), czynnik wzrostu hepatocytów (ang. *hepatocyte growth factor*; HGF)

związane z procesem angiogenezy, transformujący czynnik wzrostu beta (ang. *transforming growth factor-β*; TGF-β) regulujący procesy różnicowania i proliferację komórek, przebudowę macierzy zewnątrzkomórkowej naczyńki oraz procesy immunologiczne (27 – 29).

Naczyniówka jest zbudowana z naczyń krwionośnych o różnej średnicy i budowie, które w rejonie plamki są zorganizowane w trzy warstwy: warstwę choriokapilarów tzn. naczyń włosowatych o porowatych ścianach oraz warstw Sattlera i Hallera, tworzonych przez naczynia o większej średnicy. Krew z naczyniówki jest odprowadzana przez system żył wirowatych do żyły ocznej. Krążenie naczyniówkowe charakteryzuje szybki przepływ oraz niewielka różnica tętniczo-żylna w zakresie zawartości tlenu (27). Implikuje to zwiększone nasilenie procesów oksydacyjnych i predysponuje do powstawania dużej ilości wolnych rodników. Przepływ naczyniówkowy podlega regulacji kontroli neurogennej poprzez włókna układu autonomicznego, podczas gdy autoregulacja przepływu jest słabo wyrażona (30). Warto podkreślić, że naczyniówka w plamce ma odmienną budowę w porównaniu do obszaru okołoplamkowego oraz obwodowego. Naczynia kapilarne w obszarze plamkowym mają krótki przebieg i odchodzą od tętniczek pod kątem prostym, stąd transmisja systemowego ciśnienia tętniczego do choriokapilarów jest bardziej bezpośrednia (31). Według jednej z hipotez, taka anatomia sprawia, iż naczynia mikrokrażenia naczyniówkowego w rejonie plamki są szczególnie podatne na uszkodzenie (32). Naczynia naczyniówki mają organizację płatową, a między poszczególnymi płatami występują graniczne strefy spływu (ang. *watershed zones*; WZs) — obszary gorzej unaczynione i tym samym bardziej podatne na niedokrwienie (33). W rejonie plamki spotykają się liczne WZs, co warunkuje, że naczyniówka w tym obszarze jest wrażliwa na uszkodzenia wynikające z obniżonego przepływu krwi. Badania histopatologiczne oraz obrazowe wykazały, iż w obszarze WZs częściej lokalizują się druzy oraz błona neowaskularna (34). Z wiekiem grubość naczyniówki ulega redukcji, jednak wyniki niektórych badań wykazały, iż w oczach z AMD proces ten jest bardziej nasilony w porównaniu do oczu zdrowych (16). W ostatnich latach wykazano istotną rolę zaburzonego przepływu naczyniówkowego w patofizjologii wielu zagrażających wzrokowi zaburzeń widzenia, w tym jaskry, centralnej chorioretinopatii surowiczej (ang. *central serous chorioretinopathy*; CSCR), czy krótkowzroczności zwyrodnieniowej (35, 36).

Histologicznie naczyniówka składa się z naczyń krwionośnych oraz tkanki pozanaczyniowej złożonej z tkanki łącznej zawierającej liczne komórki barwnikowe, mięśnie gładkie, neurony oraz komórki zapalne (27). Proces utrwalania tkanek podczas obróbki histologicznej powoduje powstawanie artefaktów, w tym jej obkurczenie się tkanki

naczyniowej, co utrudnia ocenę post-mortem zaburzeń wywołanych procesami chorobowymi, zwłaszcza napięcia i struktury naczyń (37).

Pierwsze prace dokumentujące obecność ilościowych i jakościowych nieprawidłowości w przepływie krwi w oku we wczesnej i późnej postaci zwyrodnienia plamki żółtej przy użyciu różnych technik obrazowania pochodzą sprzed 30 lat. Pauleikhoff i in. wykazali opóźnione wypełnianie naczyniówki, w obrazach angiografii fluoresceinowej i indocyjaninowej, postulując że opóźnienie to jest skorelowane z pogrubieniem błony Brucha (38). Grunwald w oparciu o badanie laserowej przepływometrii dopplerowskiej wykazał redukcję przepływu w naczyniach siatkówki i naczyniówki w oczach z AMD oraz odwrotną korelację pomiędzy przepływem naczyniówkowym a stadiem AMD (39). Metelitsina i wsp. wykazali, iż zmniejszenie przepływu naczyniówkowego poprzedza rozplem naczyń neowaskularnych w plamce (40). Wyniki badań histopatologicznych również wskazują zredukowany obszar choriokapilarów w oczach pacjentów z AMD w porównaniu do osób zdrowych (41). We wczesnej fazie AMD obserwuje się redukcję fenestracji oraz utratę polaryzacji komórek śródbłonna choriokapilarów, co prowadzi do zaniku tych komórek, powstawania „naczyń-widm” oraz stopniowej redukcji gęstości naczyń już we wczesnej fazie choroby (16, 42). Zmiany te mogą poprzedzać zmiany degeneracyjne komórek RPE (43). W konsekwencji, perfuzja naczyniówkowa ulega redukcji, rozwija się hipoksja nabłonka barwnikowego skutkująca zaburzeniem jego czynności. Zaobserwowano również, iż gęstość choriokapilarów jest odwrotnie skorelowana z gęstością druzów, co przemawia za tym, iż niewystarczające usuwanie produktów przemiany materii w wyniku zaburzonego przepływu jak również wtórnej do hipoksji zaburzonej czynności RPE jest istotnym mechanizmem leżącym u podłoża wczesnych postaci AMD (44).

Aktualnie duże nadzieje pokłada się w obrazowaniu naczyniówki z użyciem OCT. Wykazano, iż poddołkowa grubość naczyniówki (ang. *subfoveal choroidal thickness*; SFCT) jest wiarygodnym i czułym wskaźnikiem zmian towarzyszących wielu stanom fizjologicznym i patologicznym naczyniówki i może być klinicznie wartościowym pośrednim wskaźnikiem stanu poddołkowej perfuzji oka. Wyniki licznych badań wskazują, iż grubość naczyniówki u oczach z AMD jest niższa w porównaniu do oczu zdrowych (45, 46). Zmiany obserwowane są głównie w stadium średniozaawansowanym choroby, chociaż niektórzy autorzy wskazują że mogą one być obecne już we wczesnym jej stadium. Dodatkowo, proces ścieńczenia naczyniówki nasila się z czasem trwania choroby i dominuje u pacjentów z zanikiem geograficznym (47). W postaci wysiękowej wzrost grubości naczyniówki może poprzedzać pojawienie się błony neowaskularnej, dlatego pomiar ten może być więc użytecznym

parametrem do monitorowania aktywności MNV (48). Jednak również w tym zakresie wyniki poszczególnych grup badawczych nie są jednoznaczne (49). Wynika to z faktu, iż na grubość naczyniówki ma wpływ wiele czynników m.in.: długość gałki ocznej, pora dnia, stopień nawodnienia, spożywanie kofeiny, palenie papierosów, ciśnienie tętnicze, czy stosowanie określonych leków. Dodatkowo różnice dokumentowane przez poszczególne zespoły badawcze wynikają również ze stosowania odmiennych aparatów OCT, które wykorzystują różne techniki pozyskiwania i analizy obrazu (50–52). W ostatnim czasie rozwój techniki przetwarzania obrazów OCT w oparciu o binaryzację skanów umożliwił przeprowadzenie analizy różnicowej i oceny ilościowej oddzielnie dla obszarów naczyniowych i zrębu naczyniówki (53). Wskaźnik unaczynienia naczyniówki (ang. *choroidal vascularity index*; CVI) określa stosunek obszaru wewnątrznaczyniowego do całkowitego badanego obszaru naczyniówki (53, 54). Wykazano, iż w porównaniu z pojedynczym pomiarem grubości naczyniówki, CVI jest bardziej obiektywnym i wiarygodnym parametrem odzwierciedlającym zmiany w naczyniówce, co wynika z faktu, że w mniejszym stopniu podlega wpływom czynników zewnętrznych (53). Dowiedziono, że w przeciwieństwie do grubości naczyniówki, wartości CVI były znacząco niższe w oczach w średniozaawansowanym stadium AMD w porównaniu z oczami zdrowej kontroli (55). Doniesienia innych autorów wskazują, że wskaźnik CVI był znacznie niższy u pacjentów z zanikiem geograficznym w porównaniu z grupą zdrowych osób (56). Zaobserwowano również stopniową redukcję wartości tego wskaźnika wraz ze stopniem zaawansowania AMD (57).

Podsumowując, wyniki badań sugerują istotną rolę naczyniówki w patogenezie i progresji AMD. Wyniki poszczególnych grup badawczych nie są jednak spójne, co daje podstawę do dalszych badań w tym obszarze.

3.3.1.2 Krążenie siatkówkowe

Siatkówka otrzymuje krew z tętnicy ocznej, która dopływa do niej dwiema drogami: poprzez krążenie siatkówkowe oraz przez krążenie naczyniówkowe. W przeciwieństwie do krążenia naczyniówkowego, krążenie siatkówkowe charakteryzuje się szybkim przepływem, oraz wysoką różnicą tętniczo-żylną zawartości tlenu oraz wyraźną autoregulacją przepływu (58). Odbywa się to na poziomie naczyń przedwłosowatych, które mogą zmieniać swoją średnicę w zależności od zapotrzebowania metabolicznego. W odróżnieniu do naczyniówki, unerwienie autonomicznie odgrywa tu rolę drugorzędną. Naczynia siatkówki są zorganizowane w dwa główne sploty naczyniowe — powierzchniowy i głęboki. Od wielu lat uważa się, iż stan naczyń mikrokrażenia siatkówkowego w obrazie z oftalmoskopii może odzwierciedlać zmiany

w drobnych naczyniach innych układów i narządów (59). Węższe tętnice na dnie oka korespondują z występowaniem nadciśnienia tętniczego, podczas gdy poszerzone naczynia żyłne z nasilonym stanem zapalnym, hiperglikemią, dyslipidemią, zespołem metabolicznym i otyłością (60). Zmiany w morfologii naczyń siatkówki, takie jak ogniskowe zwężenie tętniczek, ugięcie tętniczo-żyłne oraz uogólnione zwężenie naczyń tętniczych i żylnych siatkówki powiązane z ryzykiem wystąpienia chorób układu sercowo-naczyniowego (ang. *cardiovascular diseases*; CVDs) takich jak udar, czy choroba niedokrwienna serca. Zmiany w naczyniach siatkówki mogą odzwierciedlać uogólnione procesy zachodzące w naczyniach krążenia systemowego w innych narządach i tkankach. Ugięcie tętniczo-żyłne jest związane z dysfunkcją śródbłonna naczyniowego oraz podwyższonymi parametrami zapalnymi, a ogniskowe zwężenie naczyń tętniczych jest uznane za oznakę długotrwałego nadciśnienia tętniczego (61).

Wyniki licznych badań populacyjnych wykazały, że osoby z nadciśnieniem tętniczym i miażdżycą są również bardziej narażone na wystąpienie zwyrodnienia plamki (62 – 64). W 10-letniej obserwacji prowadzonej w ramach populacyjnego obserwacyjnego badania Blue Mountain Eye Study wykazano, iż ogniskowe zwężenie tętniczek oraz ugięcie tętniczo-żyłne, zwiększało ryzyko wystąpienia późnego AMD (65). Udowodniono, iż pacjenci z wczesnym AMD mają wyższe ryzyko udaru mózgu w porównaniu do pacjentów bez cech makulopatii, niezależnie od innych udowodnionych czynników ryzyka (66). W oparciu o powyższe wyniki można wnioskować o istotnej roli naczyniowych czynników ryzyka w patofizjologii AMD.

Aktualnie mamy możliwość wykorzystania obrazowania multimodalnego naczyń siatkówki-zarówno w zakresie ich morfologii, jak i funkcji. Zastosowanie analizatora naczyń siatkówki (ang. *Retinal Vessel Analyser*, RVA) umożliwia ocenę określonych parametrów naczyń siatkówki w sposób obiektywny i powtarzalny (67). W analizie statycznej badania ocenia się takie parametry jak ekwiwalent średnicy tętnicy środkowej siatkówki (CRAE), (ang. *central retinal artery equivalent*; CRAE), ekwiwalent średnicy żyły środkowej siatkówki (ang. *central retinal vein equivalent*; CRVE) i współczynnik tętniczo-żylny (ang. *arterio-venous ratio*; AVR), który reprezentuje stosunek CRAE/CRVE. Faza dynamiczna (ang. *dynamic vessel analysis*; DVA) umożliwia ocenę zmiany średnicy naczyń siatkówki w odpowiedzi na stymulację światłem migoczącym. DVA opiera się na założeniu, iż migotanie światła zwiększa aktywność metaboliczną siatkówki, wywołując rozszerzenie tętnic i żył siatkówki poprzez uwalnianie tlenu azotu, zgodnie z teorią sprzężenia nerwowo-naczyniowego (68). Napięcie naczyniowe wynika z równowagi pomiędzy śródbłonkowymi czynnikami naczynio-rozszerzającymi, takimi jak tlenek azotu, a czynnikami zwężającymi naczynia, jak m.in.

endotelina. Komórki śródbłonna odgrywają więc ważną rolę w modulowaniu napięcia mikronaczyniowego i autoregulacji przepływu (69). Upośledzenie funkcji naczyń może więc odzwierciedlać dysfunkcję śródbłonna, co udowodniono u pacjentów z nadciśnieniem tętniczym, otyłością oraz hipercholesterolemią (70, 71). Związek między występowaniem AMD i chorób układu krążenia sugeruje obecność zaburzeń czynności śródbłonna naczyń siatkówki w oczach z AMD. Yang i wsp. powiązali niski CRAE oraz AVR z wczesną postacią AMD oraz obecnością druzów miękkich (72). W badaniu Toulouie i wsp. wykazano, że obniżony AVR koresponduje ze stopniem zaawansowania AMD (73). Badania czynnościowe naczyń siatkówki wykazały, iż reakcja wazodylatoryjna naczyń tętniczych w oczach z druzami miękkimi oraz podsiatkówkowymi złogami druzoidalnymi jest istotnie zredukowana w porównaniu do oczu zdrowych (74). Wyniki badań w tej materii są jednak niespójne, co wynika z faktu, że literatura opisująca zmiany reaktywności naczyń siatkówki w przebiegu AMD jest nieliczna, a badania prowadzone na stosunkowo niewielkich, niejednorodnych grupach pacjentów, co uniemożliwia jednoznaczne wnioskowanie.

Splot powierzchniowy siatkówki jest zlokalizowany w warstwie włókien nerwowych, a splot głęboki w warstwie spłotowej wewnętrznej. Przepływ w naczyniach spłotu głębokiego można przyżyciowo uwidocznić jedynie techniką angiografii optycznej koherentnej tomografii (ang. *optical coherence tomography angiography*; angio-OCT, OCTA). Jest to pochodna badania optycznej koherentnej tomografii oka, która ocenia różnicę (dekorelację) odbicia fali świetlnej od elementów morfotycznych krwi w naczyniach krwionośnych w kolejnych poprzecznych skanach siatkówki wykonanych w tej samej lokalizacji. W oparciu o analizę skanów OCTA oczu towarzyszących u pacjentów z jednostronną neowaskularną postacią AMD wykazano, iż we wczesnej fazie choroby poza zaburzeniami ukrwienia choriokapilarów, gęstość naczyń obu spłotów naczyniowych siatkówki była zredukowana. Może to sugerować, że nieprawidłowości w przepływie krwi przez naczynia siatkówki są obecne jeszcze przed rozwinięciem błony neowaskularnej (42). W badaniu Trinh i wsp. w oczach ze średnio-zaawansowanym AMD w porównaniu do oczu zdrowych obserwowano zmienioną morfologię oraz redukcję liczby naczyń włosowatych siatkówki obu spłotów siatkówkowych, czemu towarzyszył zanik wewnętrznych warstw siatkówki (75). Toto i wsp. również wykazali, iż zaburzenia w przepływie siatkówkowym pojawiają się dopiero w średniozaawansowanym AMD (76). Nie jest więc ostatecznie ustalone, czy zmiany w przepływie siatkówkowym są istotnym czynnikiem w patogenezie AMD, czy efektem zmniejszonego zapotrzebowania na tlen uszkodzonych w przebiegu AMD komórek fotoreceptorowych.

3.4 Obraz kliniczny choroby

AMD jest chorobą o zróżnicowanym obrazie klinicznym. Najwcześniejszym histopatologicznym objawem choroby są złogi lipidów w błonie Brucha, powstające w wyniku zaburzonej czynności fagocytarnej nabłonka barwnikowego siatkówki (77). Typowe druzy mają postać ogniskowych, białozółtych, uniesionych zmian, zlokalizowanych pomiędzy RPE i BrM, różniących się liczbą, wielkością, kształtem i rozmieszczeniem (78). Twarde druzy występują u 80% populacji w wieku powyżej 30 lat i mają postać okrągłych, dyskretnych żółto-białych plamek o wymiarach poniżej 63 μm . Są uważane za fizjologiczny objaw starzenia. Druzy miękkie są większe, ze słabo zaznaczonymi granicami, mają tendencję do zlewania się, często towarzyszą im zmiany barwnikowe, co jest związane z wyższym ryzykiem rozwoju zaawansowanego AMD (79, 80). Większość systemów klasyfikacji stopnia zaawansowania AMD jest oparta o ocenę rozmiaru druzów oraz obecności zmian barwnikowych (hiperpigmentacja, depigmentacja) w centralnym obszarze plamki w oparciu o kolorowe zdjęcia dna oka (81). Wg opublikowanej w 2019 roku klasyfikacji Ferrisa, obecność średnich ($\geq 63 - < 125 \mu\text{m}$) druzów, bez współistniejących przegrupowań barwnika odpowiada wczesnemu stadium AMD (82). Obecność dużych druzów ($\geq 125 \mu\text{m}$) lub średnich druzów z towarzyszącymi zmianami barwnikowymi wskazuje na średniozaawansowane stadium choroby. Późne stadium AMD może przebiegać pod postacią zmian wysiękowych, kiedy dochodzi do rozwój błony neowaskularnej w plamce (ang. *macular neovascular membrane*; MNV) lub pod postacią zaniku geograficznego (ang. *geographic atrophy*; GA). Rozmiar druzów jest istotnym czynnikiem ryzyka progresji do stadiów późnych AMD. 10-letnie ryzyko progresji dla oczu z małymi druzami jest niskie, podczas gdy pojawienie się na dnie oka zmian barwnikowych wraz z druzami średniej wielkości istotnie zwiększa ryzyko pojawienia się cech późnego AMD w 5-letniej obserwacji (82). Obie postacie późnego AMD związane są ze znaczną utratą ostrości wzroku. W postaci wysiękowej rozplem nieprawidłowych naczyń krwionośnych w rejonie plamki skutkuje pojawieniem się płynu podsiatkówkowego, obrzęku, wysięków oraz wylewów krwi do plamki, prowadząc ostatecznie do uformowania blizny, dezorganizującej całkowicie architekturę plamki. W postaci zanikowej dochodzi do stopniowej atrofii RPE, fotoreceptorów oraz choriokapilarów. Nie jest do końca jasne, w którym momencie dochodzi do określenia kierunku, w którym będzie postępowała choroba.

W ostatnich latach zidentyfikowano różne rodzaje złogów, a także odmienne postacie fenotypowe warunkujące przebieg i rokowanie, jak również odpowiedź na leczenie.

3.4.1 Podsiatkówkowe złogi druzoidalne

Podsiatkówkowe złogi druzoidalne (ang. *subretinal drusenoid deposits*; SDD) zostały po raz opisane przez Mimoun i wsp. w 1990 roku jako „pseudodruzy widoczne w niebieskim świetle” (83). Występują z częstością 9% do 70% w zależności od wieku oraz stadium zaawansowania AMD (84–86). Rozbieżność ta wynika również z odmiennych technik obrazowania, gdyż w ponad 50% przypadków pseudodruzy mogą być niewidoczne w rutynowym badaniu dna oka (87). Najwyższą czułość w wykrywaniu SDD wykazują SD-OCT i obrazowanie w bliskiej podczerwieni (ang. *near infrared reflectance*, NIR) (88). Pseudodruzy, w odróżnieniu od druzów miękkich, częściej występują okołoplamkowo, są zlokalizowane pomiędzy szczytem RPE a siatkówką, mają również inny skład chemiczny. Mogą również warunkować odmienną odpowiedź na leczenie, co wykazano m.in. w badaniu LEAD (*Laser Intervention in Early Stages of Age-Related Macular Degeneration*). Po zastosowaniu podprogowej terapii laserowej badacze zaobserwowali szybszą progresję AMD w oczach z SDD w porównaniu do oczu bez tego podtypu druzów, w których terapia okazała się skuteczna w spowolnieniu postępu choroby (89). Obecność SDD jest niezależnym czynnikiem ryzyka progresji degeneracji plamki do późnych stadiów w 5-letniej obserwacji (90). Obecność SDD bardzo często towarzyszy zanikowi naczyńki, która narasta wraz ze stopniem zaawansowania AMD, a zaburzenia przepływu w choriokapilarach narastają z czasem i korelują ze wzrostem obszaru zajętego przez SDD (91–93, 57). Co ciekawe, zmiany grubości naczyńki nie są ograniczone jedynie do obszaru zajętego przez pseudodruzy, ale dotyczą całego obszaru naczyńki, co może być argumentem przemawiającym za tym, iż SDD są ocznym objawem systemowej choroby naczyniowej (94). Ścieńczenie naczyńki jest związane z redukcją grubości wszystkich warstw naczyńki: Hallera, Sattlera oraz choriokapilarów, przy czym zmniejszaniu ulega zarówno światło tych naczyń, jak i ich gęstość (94, 95). Należy jednak podkreślić fakt, że druzy te mogą występować również w oczach, w których grubość naczyńki jest prawidłowa (96).

Co ciekawe, RPD nie są patognomoniczne dla AMD, występują również innych schorzeniach, jak dystrofia plamkowa Sorsby’ego, pseudoxantoma elasticum, czy w oczach osób z nefropatią IgA (86). Częstość SDD jest niższa u cukrzyków (97). SDD nie występują również w oczach z pachydruzami.

3.4.2 Pachydruzy i pachychoroid

Pachydruzy zostały po raz pierwszy zidentyfikowane w 2018 r. przez Spaide i wsp. jako druzy o odmiennym od wcześniej opisanych fenotypie i ścisłym związku z pogrubiałą

naczyniówką (98). W oftalmoskopii są widoczne jako izolowane lub rozproszone żółto-białe złogi z dobrze zdefiniowanym, ale nieregularnym obrysem, którym, w odróżnieniu od druzów miękkich, rzadko towarzyszy ogniskowa hiperpigmentacja na powierzchni. Złogi te są stosunkowo duże ($\geq 125 \mu\text{m}$ średnicy), lokalizują się pod RPE, najczęściej okołodołeczkowo, wzdłuż łuków naczyniowych oraz okołotarczowo, oszczędzając centrum dołeczka (99 – 100). W badaniu OCT są widoczne w bezpośrednim sąsiedztwie rozdętych naczyń naczyń naczyniówki (98). Pachydrusy stanowią jedną z cech schorzeń z grupy pachychoroidu (ang. *pachychoroid spectrum diseases*, PSD), czyli zaburzeń związanych z pogrubiałą naczyniówką (99). Do schorzeń ze spektrum tej grupy należy m.in. centralna surowicza chorioretinopatia, czy PCV, będąca aktualnie uważana za jeden z podtypów wysiękowego AMD. Ostatnie doniesienia wskazują, iż wśród japońskich pacjentów z wcześniej zdiagnozowanym wysiękowym AMD, u 46% stwierdza się cechy PSD (101). Obecność rozdętych naczyń naczyniówki może prowadzić do ucisku i zaburzeń przepływu a wtórnie do zaniku choriokapilarów (102). W oparciu o obrazy angiografii indocyjaninowej wykazano, iż pachydrusy lokalizują się w obszarze opóźnionego wypełniania choriokapilarów oraz nad rozdętymi naczyniami zewnętrznej naczyniówki, a w późnej fazie badania często są widoczne w obszarze przecieku naczyniowego z naczyniówki (103). Sugeruje się więc, iż pachydrusy są wynikiem zaburzonego przepływu w choriokapilarach w wyniku ich zaniku wtórnie do przewlekłe poszerzonych naczyń warstwy Hallera (104). Wykazano, iż mimo odmiennego fenotypu, pachydrusy, podobnie jak pozostałe złogi, mogą być czynnikiem ryzyka progresji do postaci wysiękowej AMD (105). Odmienne jednak od innych podtypów druzów, pachydrusy zwiększają ryzyko polipoidalnej waskulopatii naczyniówkowej (ang. *polypoidal choroidal vasculopathy*, PCV). Jest to podtyp MNV typu 1 — zlokalizowanej pod nabłonkiem barwnikowym, więc mającej źródło w naczyniówce, której cechą charakterystyczną jest obecność polipów naczyniowych (105). W oczach z PCV pachydrusy są obserwowane w około 50% przypadków, podczas gdy drusy suche i RPD odpowiednio w 12,3% i 6,9% (99). W odróżnieniu od druzów miękkich, które w czasie 5-letniej obserwacji najczęściej wykazywały progresywny wzrost, pachydrusy trzykrotnie częściej wykazywały regresję w czasie, a ich zanikowi nie towarzyszył zanik RPE (106). Co więcej, wykazano, iż obecność pachydruzów w oku towarzyszącym jest związana z możliwością prowadzenia mniej intensywnej terapii AVEGF (107). Łagodniejszy przebieg degeneracji plamki w obecności tych druzów może częściowo tłumaczyć odmienna podatność genetyczna pacjentów z tym typem druzów, czego dowodzi rzadsze występowanie wariantu wysokiego ryzyka ARMS2 A69S w porównaniu do pacjentów prezentujących RPD oraz drusy miękkie (108). Wykazano

również, iż pachydrusy powiększają się znacznie wolniej w porównaniu do druzów miękkich. W oczach z wysiękowym AMD, w których występują oba wyżej wymienione typy druzów, lokalizacja błony neowaskularnej nie pokrywa się z lokalizacją pachydruzów, ale z obszarem występowania druzów miękkich (109). Autorzy postulują, iż pachydrusy mogą być epifenomenem, związanym ze zmienionym przepływem w patologicznie poszerzonych naczyniach naczyniówki. Dane te są jednak sprzeczne z wynikami przedstawionymi przez Shetha i wsp., którzy wykazali powiększanie się pachydruzów, co poprzedzało rozwój MNV zlokalizowanej w obszarze występowania złogów (110).

Podsumowując, w ostatnich latach rozwój technologiczny oraz upowszechnienie nowoczesnych metod obrazowania umożliwiły znaczący postęp w badaniach nad AMD. Liczne zagadnienia wymagają jednak dalszych badań. Otwarte pozostaje pytanie, dlaczego tylko u części pacjentów prezentujących druzy na dnie oka rozwija się choroba, podczas gdy u innych druzy mają postać stałą, nie wykazującą cech progresji do AMD. Podobnie nie jest wyjaśnione, jakie czynniki wpływają na tempo progresji choroby. Kolejną niejasną kwestią jest, dlaczego pacjenci, u których choroba we wczesnych stadiach przedstawia podobny obraz kliniczny, ostatecznie manifestują dwie zupełnie odmienne formy schorzenia: suchą i wysiękową. Zrozumienie patogenezы oraz czynników warunkujących odmienność przebiegu AMD może mieć kluczowe znaczenie dla opracowywania nowych oraz wzmocnienia efektu już istniejących terapii i poprawy jakości życia pacjentów.

4 Główne założenia i cele pracy doktorskiej

Rejon plamki stanowi bardzo aktywny metabolicznie obszar siatkówki, wymagający dynamicznej perfuzji naczyniowej, dzięki czemu dostarczane są substancje odżywcze i tlen, jak również usuwane szkodliwe produkty przemiany materii powstające w procesie fototransdukcji. Badania naukowe wskazują na istotną rolę zaburzeń homeostazy naczyniowej u pacjentów z AMD, aczkolwiek czynniki determinujące obraz fenotypowy oraz progresję choroby nie zostały dotychczas jednoznacznie określone. W niniejszej rozprawie podjęto próbę oceny wybranych parametrów naczyniowych w patogenezie AMD, ze szczególnym uwzględnieniem mikrokrażenia siatkówkowego oraz naczyniówkowego. Postawiono następujące cele pracy:

1. Ocena parametrów naczyniówki w obrazie SD-OCT, takich jak grubość, objętość oraz wskaźnik unaczynienia naczyniówki, celem zobrazowania potencjalnych różnic w morfologii tkanki naczyniowej między oczami z AMD (z uwzględnieniem odmiennych podtypów klinicznych choroby), a grupą kontrolną.
2. Pomiar średnicy oraz reaktywności naczyń siatkówki w oczach pacjentów z AMD w odniesieniu do grupy kontrolnej
3. Analiza korelacji pomiędzy pomiarami opisującymi morfologię naczyniówki, wskaźnikami średnicy naczyń siatkówki, parametrami czynnościowymi mikrokrażenia siatkówkowego a postacią kliniczną, stadium zaawansowania choroby, współistnieniem schorzeń układu sercowo-naczyniowego oraz obecnością określonych polimorfizmów wysokiego ryzyka u pacjentów z AMD.
4. Zidentyfikowanie czynników ryzyka progresji AMD, na przestrzeni 3-letniego okresu obserwacji, z uwzględnieniem udziału czynników naczyniowych w tym procesie.

5 Streszczenia publikacji stanowiących rozprawę doktorską

5.1 The impact of vascular risk factors on the thickness and volume of the choroid in AMD patients

[Wpływ naczyniowych czynników ryzyka na grubość i objętość naczyniówki u pacjentów z AMD]

Celem pracy była ocena parametrów objętości i grubości naczyniówki w obszarze plamki w oczach z AMD oraz analiza wpływu współistniejących ogólnoustrojowych czynników ryzyka naczyniowego na stan naczyniówki.

Do badania włączono 354 pacjentów z AMD: 175 z postacią suchą, 179 z postacią wysiękową AMD oraz 121 zdrowych osób stanowiących grupę kontrolną. Wszyscy uczestnicy zostali poddani obszernemu badaniu okulistycznemu obejmującemu pomiar ostrości wzroku, ciśnienia wewnątrzgałkowego oraz szczegółową ocenę dna oka. Wykonano również skany optycznej koherentnej tomografii z modulem rozszerzonego obrazowania głębi (EDI-OCT; Heidelberg Engineering, Heidelberg, Germany) celem oceny grubości, objętości oraz wskaźnika unaczynienia naczyniówki (*choroidal vascularity index*, CVI). Pomiar grubości i objętości naczyniówki uśredniono w oparciu o mapę ETDRS (*Early Treatment Diabetic Retinopathy Study*) i oznaczono jako: ATC (*average thickness center*; średnia grubość w obszarze pierścienia centralnego), AVC (*average volume center*; średnia objętość w obszarze pierścienia centralnego) oraz AV (*average volume*; średnia objętość ze wszystkich dziewięciu podpól mapy ETDRS). Do oszacowania CVI wykorzystano półautomatyczną metodę binaryzacji opisaną wcześniej przez Sonodę z późniejszymi modyfikacjami Agrawala za pomocą oprogramowania ImageJ (wer. 1.53e). Stadium zaawansowania choroby ustalono w oparciu o klasyfikację Ferrisa. Zebrano również obszerny wywiad medyczny ze szczególnym uwzględnieniem historii chorób układu krążenia, jak również dane dotyczące aktualnie zażywanych leków, palenia tytoniu oraz aktywności fizycznej.

Analiza wieloczynnikowa z uwzględnieniem wieku i płci oraz palenia papierosów jako zmiennych niezależnych wykazała, iż wysiękowa postać AMD była niezależnym czynnikiem związanym z wyższymi wartościami ATC, AVC oraz niższymi wartościami CVI w porównaniu z grupą kontrolną (odpowiednio: $\beta = +0,18$, $p = 0,0007$, $\beta = +0,18$, $p = 0,0008$) oraz z suchym AMD ($\beta = +0,17$, $p = 0,000003$ dla ATC i AVC oraz $\beta = -0,30$ $p < 0,0001$ dla

CVI). W grupie pacjentów z suchą postacią AMD zaobserwowano ujemne korelacje między stopniem zaawansowania choroby a AV ($R_s = -0,18$, $p = 0,0004$), AVC ($R_s = -0,19$, $p = 0,0002$), ATC ($R_s = -0,19$, $p = 0,0003$) oraz CVI ($R_s = -0,16$; $p = 0,003$), co może wskazywać, iż zanik naczyń naczyniówki jest większy w późniejszych fazach choroby. Dodatkowo obniżenie parametrów AV, ATC, AVC oraz CVI było związane z niższą ostrością widzenia (odpowiednio: $R_s = +0,14$, $p < 0,001$; $R_s = +0,12$, $p = 0,002$; $R_s = +0,12$; $p = 0,002$; $R_s = +0,19$, $p < 0,001$). Może to świadczyć o tym, że postępująca atrofia naczyniówki w oczach z AMD wiąże się z większym stopniem upośledzenia czynnościowego.

U pacjentów z AMD ze współistniejącym nadciśnieniem i chorobą niedokrwienną serca wszystkie parametry opisujące naczyniówkę, tzn. ATC, AVC i AV były istotnie niższe w porównaniu do osób bez schorzeń towarzyszących (odpowiednio: $p = 0,001$, $p = 0,002$, $p = 0,003$ dla pacjentów z nadciśnieniem; $p = 0,003$, $p = 0,01$, $p = 0,0006$ dla pacjentów z chorobą niedokrwienną serca). Czas trwania nadciśnienia był odwrotnie skorelowany z ATC, AVC i AV (odpowiednio: $R_s = -0,13$, $p < 0,05$; $R_s = -0,12$; $p < 0,05$, $R_s = -0,12$; $p < 0,05$), natomiast czas trwania IHD ujemnie skorelowany z AV ($R_s = -0,15$, $p < 0,05$). W grupie AMD pacjenci z zawałem mięśnia sercowego w wywiadzie (*eng. myocardial infarction*; MI) mieli istotnie niższe wartości AVC i ATC niż pacjenci bez MI (odpowiednio: $p = 0,04$ i $p = 0,05$). W grupie kontrolnej nie zaobserwowano takich zależności.

Wyniki niniejszego badania wskazują na, iż krążenie naczyniówkowe w oczach z AMD jest znacznie bardziej podatne na uszkodzenia w obecności ogólnoustrojowej choroby naczyniowej.

5.2 Retinal Vessel Functionality Is Linked With ARMS2 A69S and CFH Y402H Polymorphisms and Choroidal Status in AMD Patients

[Funkcjonalność naczyń siatkówki jest powiązana z polimorfizmami ARMS2 A69S i CFH Y402H oraz statusem naczyniówki u pacjentów z AMD]

Celem pracy było porównanie średnicy oraz reaktywności naczyń siatkówki na migoczący bodziec świetlny u pacjentów ze zwyrodnieniem plamki związanym z wiekiem oraz u zdrowych uczestników. Dodatkowo, analizie poddano wpływ określonych wariantów genetycznych na parametry naczyń siatkówki.

Do badania zrekrutowano 354 pacjentów ze zwyrodnieniem plamki związanym z wiekiem oraz 121 osób z grupy kontrolnej. Wszyscy uczestnicy zostali poddani obszernemu badaniu okulistycznemu i szczegółowemu badaniu dna oka i siatkówki w oparciu o analizę

obrazów optycznej koherentnej tomografii (Heidelberg Engineering, Heidelberg, Niemcy). Przeprowadzono również statyczną i dynamiczną analizę naczyń siatkówki (ang. *retinal vessel analysis*, RVA) z kamery dna oka FF450 plus (Zeiss AG, Jena, Niemcy) oraz oprogramowania VISUALIS i VesselMap (IMEDOS Systems, Ltd., Jena). Ocenie poddano: centralny ekwiwalent tętniczy siatkówki (CRAE), który odnosi się do średnicy tętnicy środkowej siatkówki; centralny ekwiwalent żylny siatkówki (CRVE), który odnosi się do średnicy żyły środkowej siatkówki; i stosunek tętniczo-żylny (AVR), który reprezentuje stosunek CRAE/CRVE. W badaniu dynamicznym ocenie poddano odpowiedź na bodziec migoczący naczyń tętniczych (ang. *dynamic analysis of arteries*; DAA) oraz naczyń żylnych (ang. *dynamic analysis of veins*; DAV), które opisywały zmianę średnicy naczynia po zadziałaniu bodźca świetlnego w stosunku do średnicy wyjściowej. Analiza genetyczna polimorfizmów CFH Y402H i ARMS2 A69S została przeprowadzona za pomocą systemu Illumina NextSeq500 (Illumina, San Diego, CA, USA).

Nie stwierdzono istotnych statystycznie różnic między grupą oczu z AMD a grupą kontrolną w zakresie badanych parametrów statycznej analizy naczyń siatkówki, w tym: CRAE (odpowiednio: 181,4 IU, 181,8 IU, $p = 0,55$), CRVE (odpowiednio: 215,2 IU, 213,6 IU, $p = 0,88$) oraz współczynnika tętniczo-żylnego (0,85 w obu grupach badanych, $p = 0,31$). Reaktywność naczyń tętniczych i żylnych na bodziec migoczący również nie różniła się istotnie między obiema badanymi grupami (DAA: 1,6 w grupie AMD, 1,8 w grupie kontrolnej, $p = 0,31$; DAV: 3,0 w grupie AMD, 3,3 w grupie kontrolnej, $p = 0,22$). Współczynnik tętniczo-żylny (AVR) był istotnie wyższy w oczach z wczesnym AMD ($0,87 \pm 0,08$) w porównaniu do oczu z zaawansowaną postacią choroby ($0,85 \pm 0,09$, $p = 0,03$). W grupie oczu z AMD reaktywność naczyń tętniczych po stymulacji światłem korelowała dodatnio zarówno z grubością naczyniówki ($R_s = 0,14$, $p = <0,000961$), jak i objętością naczyniówki ($R_s = 0,23$, $p < 0,0001$). Nie stwierdzono takich zależności w grupie kontrolnej. Homozygoty TT dla polimorfizmu ARMS2A69S wykazywały istotnie niższą reaktywność naczyń tętniczych na światło ($1,47 \pm 1,50$) w porównaniu z homozygotami GG ($2,38 \pm 1,79$; $p = 0,008$) oraz pacjentami z wariantem GG + GT ($2,28 \pm 1,84$; $p = 0,01$). Dodatkowo pacjenci z wariantem TC + CC w polimorfizmie CFH Y402H prezentowali mniejszą reaktywność naczyń żylnych siatkówki na bodziec świetlny ($3,24 \pm 1,71$) w porównaniu z homozygotami TT ($3,83 \pm 1,68$; $p = 0,03$).

Wyniki badania sugerują, iż stan czynnościowy mikrokążenia siatkówkowego u pacjentów z AMD zależy od współistnienia genetycznych wariantów ryzyka oraz jest ściśle skorelowany z zaawansowaniem choroby oraz grubością i objętością naczyniówki.

5.3 Influence of Clinical and Genetic Factors on the Progression of Age-Related Macular Degeneration: A 3-Year Follow-Up

[Wpływ czynników klinicznych i genetycznych na progresję zwyrodnienia plamki żółtej związanego z wiekiem w 3-letnim okresie obserwacji]

Celem badania była analiza wpływu wybranych czynników klinicznych, demograficznych i środowiskowych oraz genetycznych na progresję zwyrodnienia plamki żółtej związanego z wiekiem (AMD).

Do badania włączono 94 uczestników z wcześniej zdiagnozowaną wczesną lub średniozaawansowaną postacią AMD w co najmniej jednym oku. Przeprowadzono szczegółowy wywiad medyczny z uwzględnieniem chorób naczyniowych oraz ich czynników ryzyka. U każdego z uczestników dwukrotnie, w odstępie 3 lat wykonano szczegółowe badanie okulistyczne wraz z multimodalnym obrazowaniem siatkówki i naczyniówki (Heidelberg Engineering, Heidelberg, Niemcy). Stopień zaawansowania AMD określono w oparciu o klasyfikację Ferrisa.

Po 3 latach 48 pacjentów wykazało cechy progresji AMD, podczas gdy u 46 choroba pozostała stabilna. W wieloczynnikowej analizie regresji skorygowanej o płeć i wiek, progresja choroby była istotnie związana z gorszą początkową ostrością wzroku (OR = 6,74, 95% CI = 1,24 – 36,79, $p = 0,03$) oraz obecnością wysiękowego podtypu AMD w oku towarzyszącym (OR = 3,79, 95% CI = 0,94 – 15,2, $p = 0,05$). Ponadto, większe ryzyko progresji wystąpiło u pacjentów suplementujących tyroksynę (OR = 4,77, CI = 1,25 – 18,25, $p = 0,002$). Wariant CC CFH Y402H był związany z wyższym ryzykiem progresji choroby w porównaniu z fenotypem TC+TT (OR = 2,76, 95% CI: 0,98 – 7,79, $p = 0,05$). Grubość naczyniówki w dołku nie była istotnie związana z postępem choroby (OR = 0,1, 95% CI = 0,99 – 1,0, $p = 0,12$). Nie wykazano również związku między progresją choroby a obecnością podsiatkówkowych złogów druzoidalnych ($p = 0,12$), pachydruzów ($p = 1,0$), pachyvessels ($p = 0,83$), jak również cech pachychoroid ($p = 0,36$).

Wyniki badania sugerują, iż morfologia naczyniówki nie jest niezależnym czynnikiem wpływającym na przebieg AMD w 3-letniej obserwacji, zwłaszcza w oczach z zaawansowaną postacią AMD w oku towarzyszącym.

6 Publikacje stanowiące rozprawę doktorską

6.1 The impact of vascular risk factors on the thickness and volume of the choroid in AMD patients

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OPEN The impact of vascular risk factors on the thickness and volume of the choroid in AMD patients

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Disturbances in choroidal microcirculation may lead to the onset and progression of age-related macular degeneration (AMD). We aimed to assess changes in the choroidal volume and thickness in the macular region in AMD eyes and to investigate whether coexisting vascular risk factors alter choroidal status. We enrolled 354 AMD patients (175 dry, 179 wet AMD) and 121 healthy controls. All participants underwent a complete ophthalmologic examination and assessment of choroidal thickness and volume. A multivariate analysis adjusted for age, sex, and smoking status revealed that wet AMD was an independent factor associated with higher average thickness of the central ring area (ATC) and average volume of the central ring area (AVC) and lower choroidal vascularity index (CVI) compared to controls ($\beta = +0.18$, $p = 0.0007$, $\beta = +0.18$, $p = 0.0008$, respectively) and to dry AMD ($\beta = +0.17$, $p = 0.00003$ for both ATC and AVC and $\beta = -0.30$, $p < 0.0001$ for CVI). ATC, AVC and average volume (AV) were lower in AMD patients with hypertension and ischaemic heart disease (IHD). The duration of hypertension was inversely correlated with ATC, AVC and AV ($R_s = -0.13$, $p < 0.05$; $R_s = -0.12$; $p < 0.05$, $R_s = -0.12$; $p < 0.05$, respectively) while IHD duration negatively correlated with AV ($R_s = -0.15$, $p < 0.05$). No such associations were observed in the control group. Our findings show that the choroidal vascular system in eyes with AMD is much more susceptible to damage in the presence than in the absence of systemic vascular disease.

Age-related macular degeneration (AMD) is a progressive disease leading to a severe decrease in visual acuity¹. Clinically relevant disease is most prevalent in the elderly population, but early signs of AMD can also be found in individuals under 50 years of age². Despite extensive research, the pathomechanism of this disease has not been clarified³.

Recently, the role of the choroid in AMD development has been investigated^{4,5}. This research was made possible by the introduction of the new technique OCT and enhanced depth imaging (EDI-OCT), which provide better penetration and visualization of the entire choroid up to the uveal-scleral junction⁶. OCT allows noninvasive quantitative assessment of retinal and choroidal structures in vivo. Technological advancements have initiated a new age of research on the role of choroidal microcirculation in healthy, aged, and diseased eyes^{7,8}. The choroid is one of the most vascularized tissues in the body. Its main function is supplying oxygen and nutrients to the outer retina, particularly the foveal avascular zone (FAZ), where the choroid is the exclusive blood source for the retina. Recently, the choroid was proven to play a role in the pathophysiology of many vision-threatening ocular disorders, including central serous chorioretinopathy (CSR) and degenerative myopia^{9–11}. Choroid thinning is a natural process associated with ageing, but some researchers have shown that in eyes with AMD, this process is much more pronounced^{4,12,13}. It is likely that the vascular structure of the choroid undergoes the same pathological changes associated with ageing and the development of atherosclerosis as vessels in other regions^{12,13}.

Previous studies have evaluated changes upon CT in both dry and wet AMD; however, the results have been inconsistent¹⁴. In a study by Jonas et al. that evaluated patients with unilateral AMD, no statistically significant difference between the affected eyes and contralateral unaffected eyes was observed¹⁵. However, Razavi et al. found CT values to be significantly higher in eyes with neovascular AMD than in unaffected eyes¹⁶. Notwithstanding, most studies have focused on neovascular or atrophic forms of AMD and have shown that the thinning of the choroid may be a consequence of late-stage disease rather than a cause. A few studies suggested that the

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thinning of the choroid may originate in the early stages of AMD, although this finding is inconsistent with the results presented in other studies^{17,18}. This discrepancy between studies may be partly due to imperfections in the subfoveal CT measurement technique. A single manual CT scan in the subfoveal region can lead to biased results. An analysis of volume based on central 6-mm perfoveal B-scans of the choroid could provide more precise information about choroidal status, as demonstrated in our previous research¹⁹. An additional valuable parameter recently used in the analysis of choroidal vascularity is the choroidal vascularity index (CVI) obtained by binarization of OCT images^{20,21}. The results of the research indicate that CVI is a parameter with lower variability and is influenced by fewer physiological factors compared to the thickness of the choroid, therefore it can be considered a relatively stable parameter for the assessment of changes in choroidal microcirculation²⁰. CVI values in patients with age related macular degeneration appeared to be significantly lower in patients with AMD compared to healthy controls^{22,23}. In addition, CVI has been shown to decrease with disease duration²⁴. Interestingly, either in the study of Koh et al. or Giannaccare et al. subfoveal choroidal thickness did not differ between AMD and healthy eyes, while CVI was significantly lower^{22,24}.

Thus, the aim of the study was to assess choroidal volume, thickness and choroid vascular index changes for a detailed analysis of choroidal microcirculation in eyes with dry and exudative AMD. We also investigated whether coexisting vascular risk factors alter the choroidal microvasculature in AMD eyes⁷.

Methods

Subjects and initial management. Patients with a diagnosis of AMD were enrolled in this study. A detailed medical and ophthalmological assessment was provided by the First Department of Ophthalmology of Pomeranian Medical University in Szczecin. We collected data on medical history, current drug use, smoking status and physical activity status from the enrolled subjects, with a particular focus on their history of clinical cardiovascular diseases, including physician-diagnosed heart and vascular diseases. Before the ophthalmic examination, arterial blood pressure was measured (three measurements taken at 5-min intervals gave the mean result) for every participant using a noninvasive blood pressure system with a manual aneroid manometer. Then, the systemic mean arterial pressure (MAP) was calculated: $\text{MAP} = \text{diastolic BP} + 1/3 (\text{systolic BP} - \text{diastolic BP})$ mmHg. We also assessed waist circumference [cm], waist/hip ratio (WHR), and body mass index (BMI) [weight (kg)/height (m)²] of all enrolled subjects. To calculate cumulative pack-years, the reported average number of cigarettes smoked per day and the number of years the patient smoked were collected. Finally, with the help of a member of the research team, each participant completed the International Physical Activity Questionnaire (IPAQ), comprising 7 questions regarding all types of physical activity (lasting 10 min or longer) in the previous week. Physical activity scores are presented as MET-min per week and were calculated as described previously²⁵. In brief, each activity-specific factor was multiplied by the number of days spent performing the activity and the time in minutes spent performing the activity daily. Weekly activity was measured by adding the score for each of the activities performed.

In accordance with the tenets of the Declaration of Helsinki, a consent form was signed by all patients before trial enrolment.

Ophthalmologic examination. The patients underwent a complete ophthalmologic examination (biomicroscopic examination of the eye anterior and posterior segments, determination of visual acuity, colour fundus imaging, IOP measurement with a Goldmann applanation tonometer, and axial length and anterior chamber depth calculation). Only eyes with an axial length within the normal range, i.e. 22–25 mm were included in the study²⁶. The above mentioned tests were performed to eliminate known factors that interfere with reliable OCT image analysis. If the presence of a neovascular membrane could not be clearly excluded or confirmed, a fluorescence angiography test was performed. Additionally, fundus autofluorescence examination was performed for more accurate drusen visualization to allow correct determination of the AMD stage.

Optical coherence tomography. Enhanced-depth imaging was performed on both eyes from each subject using the Heidelberg Spectralis SD OCT (870 nm) device (Heidelberg Engineering, Heidelberg, Germany). The A-scan rate was 70,000 scans/s, a light source centred on 870 nm was used, and the axial and transverse tissues resolutions were 3.9 and 6 μm , respectively. To exclude confounding factors, measurements were performed by an experienced technician after 30 min of rest at the same time of the day after pupil dilation with 1% tropicamide solution. In addition, the patients were instructed not to smoke for 6 h or drink any fluids for 1 h before the examination. During scanning, the OCT Spectralis device collected two images using a continuous double laser scan, including an infrared image from a scanning laser ophthalmoscope (SLO) and an OCT scan. The SLO images were used as references for the OCT scans. In addition, a system for actively tracking and correcting eye movements was used. To obtain SD-OCT images of the macular region, a $25^\circ \times 25^\circ$ volume acquisition protocol was used to obtain 49 cross-sectional B-scans. Choroidal segmentation was performed manually after the automated retinal layer segmentation software was disabled. An experienced retina specialist moved the reference lines of the built-in automated segmentation from the retinal boundaries to the choroidal boundaries. This method allowed the use of the automatic retinal thickness and volume map features of the built-in software. The automated software was used to calculate choroidal volume in a similar manner as that used for retinal volume analysis. The details of obtaining OCT images have been described previously¹⁹.

Choroidal volume measurements were made at all points within the 9 Early Treatment Diabetic Retinopathy Study (ETDRS) subfields, which were automatically provided by Heidelberg Engineering software, were averaged, and are presented as the AV (average volume). Choroidal thickness and volume measurements from the central area according to the ETDRS map were calculated as the ATC (average thickness in central ring area) and AVC (average volume in central ring area) (Fig. 1). For a more precise assessment of choroidal vasculature, we also

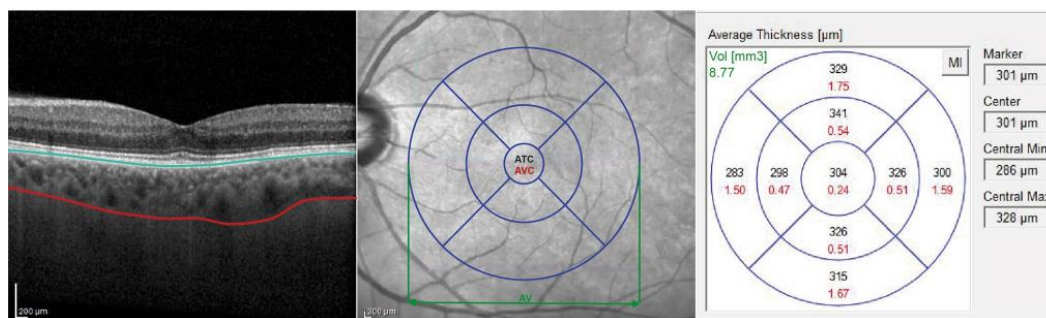


Figure 1. Left: enhanced depth imaging-optical coherence tomography (EDI-OCT) scan of macular region with marked retinal pigment epithelium (blue line) and scleral-veal junction (red line). Middle: fundus photography with the Early Treatment Diabetic Retinopathy Study (ETDRS) grid applied. Averaged measurements from the central ETDRS region are presented as the averaged thickness center (ATC) and to the average volume center (AVC). Averaged volumetric measurements from the entire area of the ETDRS grid are presented as average volume (AV). Right: the averaged measurements of choroidal thickness (μm) (marked in black) and volume (mm³) (marked in red) presented for 9 ETDRS subfields. Values of AV displayed in green.

calculated the choroidal vascularity index (CVI). We used the semi-automated method described previously by Sonoda et al.²⁷ with later modifications of Agrawal et al.^{28,29}. In brief, EDI OCT scan passing through the subfoveal region was selected and used for analysis. Images with a poorly demarcated choroidal-scleral interface (CSI) were excluded from the analysis. Binarization of the choroidal area in that scan was performed using the ImageJ software (ver. 1.53e; Java 1.8.0_172 (64-bit); <https://imagej.nih.gov/ij>). The polygon tool was used to select the region of interest (ROI). Next, the selected region was plotted across the entire length of the EDI OCT scan to standardize the area of the ROI among all patients. The upper boundary of the ROI was manually traced along the choroid-RPE junction and the lower boundary along the CSI. Application of auto-threshold was performed after conversion to eight-bit images. Brightness was reduced to allow clear visualization of the choroidal vessels and minimize noise. Niblack's auto local threshold tool was then applied, to allow demarcation of the luminal or vascular area and the stromal area. The image was then converted back to an RGB (red, green, blue) image, and the luminal area was determined using the colour threshold tool. Across all three different thresholding steps (auto-threshold, Niblack's auto local threshold and colour threshold), we used the default standard settings in the ImageJ plugin that allow us to standardize the thresholding values for all the scans. Choroidal vascularity index was defined as the proportion of luminal area to the total choroidal area and computed for all images. Choroidal thickness, volume and CVI measurements were all performed with one grader. Each measurement was performed three times and averaged. OCT scans in which it was not possible to clearly visualize the choroidal-scleral interface were excluded from the study.

To identify correlations between the stage of the disease and specific systemic and ocular parameters, patients in the study group were assigned to one of three subgroups depending on the status of the disease. Grading of AMD based on evaluation of colour fundus images and OCT using the Ferris classification system was performed by the examining clinician²¹. Group 1 included patients with medium drusen (63–125 μm) but without pigmentary abnormalities, who were considered to have early AMD. Group 2 patients, who had large drusen or pigmentary abnormalities associated with at least medium drusen, were considered to have intermediate AMD. Group 3 included patients with a diagnosis of late-stage AMD including both forms: advanced geographic atrophy and macular neovascularization of any type. Patients with no visible drusen or pigmentary abnormalities and those with small drusen (< 63 μm) were included in the control group because these features are considered signs of the physiological ageing process.

Patients were diagnosed with the dry subform of AMD when there were drusen, pigmentary abnormalities and/or atrophic changes, including geographic atrophy found on colour fundus images and OCT. The wet subform of AMD was diagnosed when the existence of the macular neovascular membrane was confirmed by the presence of intra- and sub-retinal haemorrhages, exudations and/or vascularized PED on colour fundus images, OCT or angiography³. All wet AMD patients enrolled in the study were anti-VEGF treatment naïve.

Statistical analysis. The Mann-Whitney U test was used to compare quantitative and rank variables between groups. The strength of associations between quantitative and rank variables was assessed by Spearman rank correlation coefficients (Rs). Fisher's exact test was used to compare qualitative variables between groups. A multivariate general linear regression model (GLM) adjusted for age, gender and smoking status was used to determine independent factors associated with the thickness and volume of the choroid in AMD patients, transformed logarithmically to normalize their distributions. Standardized β coefficients were calculated to show the direction and strength of the associations. A *p* value < 0.05 was considered statistically significant.

Parameter	AMD group	Control group	p value
Number of subjects	354	121	X
Sex (male/female)	135/219	32/89	0.02
Age [years] (mean $\pm$ SD)	73.4 $\pm$ 8.0	73.1 $\pm$ 6.0	0.41
Outdoor/indoor working conditions	40.1/59.9%	33.1/67.0%	0.19
Current smokers [%]	13.6%	6.3%	0.0503
Former smokers [%]	51.4%	30.9%	<0.001
Smoking pack-years [mean $\pm$ SD]	13.6 $\pm$ 18.9	6.0 $\pm$ 13.1	<0.001
Period without smoking [years] (mean $\pm$ SD)	6.8 $\pm$ 10.9	5.3 $\pm$ 10.2	0.055
BMI [kg/m ²] (mean $\pm$ SD)	26.9 $\pm$ 4.2	26.6 $\pm$ 3.7	0.43
WHR [arbitrary units] (mean $\pm$ SD)	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	0.13
MAP [mmHg] (mean $\pm$ SD)	98.3 $\pm$ 11.1	98.7 $\pm$ 9.7	0.86
Hypertension [%]	64.7%	71.1%	0.27
Duration of hypertension [years] (mean $\pm$ SD)	1.2 $\pm$ 4.2	9.2 $\pm$ 9.9	0.27
History of myocardial infarction [%]	6.2%	6.2%	1
History of ischemic heart disease [%]	16.2%	11.3%	0.33
Duration of ischemic heart disease [years] (mean $\pm$ SD)	1.2 $\pm$ 4.2	0.8 $\pm$ 3.3	0.26
AL [mm]	22.82 $\pm$ 1.52	23.13 $\pm$ 0.89	0.21

Table 1. Characteristics of the patients in the study groups. Mann–Whitney U test/Fisher's exact test. Bolded values indicate significance at $p < 0.05$.

Parameter	Dry AMD group	Wet AMD group	p value
Number of subjects	175	179	X
Sex (male/female)	52/123	83/96	0.001
Age [years] (mean $\pm$ SD)	72.7 $\pm$ 8.0	74.1 $\pm$ 7.9	0.07
Outdoor/indoor working conditions [%]	38.3/61.7%	41.9/58.1%	0.52
Current smokers [%]	10.6%	16.6%	0.14
Former smokers [%]	45%	57.7%	0.03
Smoking pack-years (mean $\pm$ SD)	10.1 $\pm$ 16.1	17.0 $\pm$ 20.8	0.002
Period without smoking [years] (mean $\pm$ SD)	6.3 $\pm$ 10.8	7.3 $\pm$ 11.0	0.24
BMI [kg/m ²] (mean $\pm$ SD)	26.9 $\pm$ 4.3	26.9 $\pm$ 4.2	0.99
WHR [arbitrary units] (mean $\pm$ SD)	0.88 $\pm$ 0.1	0.91 $\pm$ 0.1	0.002
MAP [mmHg] (mean $\pm$ SD)	97.1 $\pm$ 10.4	99.5 $\pm$ 11.7	0.1
Hypertension [%]	65%	64.4%	1.0
Duration of hypertension [years] (mean $\pm$ SD)	8.4 $\pm$ 9.6	7.9 $\pm$ 9.4	0.64
History of myocardial infarction [%]	7.6%	4.9%	0.36
History of ischemic heart disease [%]	15.1%	17.2%	0.65
Duration of ischemic heart disease [years] (mean $\pm$ SD)	1.4 $\pm$ 4.8	1.1 $\pm$ 3.5	0.59
AL [mm]	23.75 $\pm$ 0.97	23.12 $\pm$ 0.86	0.38

Table 2. Characteristics of the patients in the study groups. Mann–Whitney U test/Fisher's exact test. Bolded values indicate significance at $p < 0.05$.

Ethics approval. The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Pomeranian Medical University in Szczecin, Poland (ethical code is KB-0012/141/13).

Consent to participate. Informed consent was obtained from all subjects involved in the study.

Consent for publication. The manuscript has been read and approved by all named authors.

Results

Study groups characteristics. We enrolled a total of 354 patients with AMD: 175 with dry and 179 with wet forms of the disease. As a control group, we enrolled 121 healthy subjects. Tables 1 and 2 summarize the clinical characteristics of the AMD patients (divided into dry and wet subgroups) and the controls. Our study groups did not differ in age or the occurrence of well-known atherosclerotic risk factors: hypertension, a history

	Control		Dry AMD		Wet AMD		<i>p</i> value		
	Median	IQR	Median	IQR	Median	IQR	(Dry vs. control)	(Wet vs. control)	(Dry vs. wet)
AV [mm ³]	6.96	2.82	6.77	3.55	6.71	2.81	0.641	0.841	0.957
ATC [μm]	273.5	117	269	146	286	161.5	0.907	0.022	0.017
AVC [mm ³]	0.21	0.09	0.21	0.11	0.22	0.13	0.937	0.022	0.015
CVI [%]	0.66	0.03	0.65	0.04	0.64	0.05	0.01	<0.001	<0.001

Table 3. Differences between choroidal parameters in the dry and wet AMD and control groups. *IQR* interquartile range. Statistical significance was established at $p < 0.05$ (bolded).

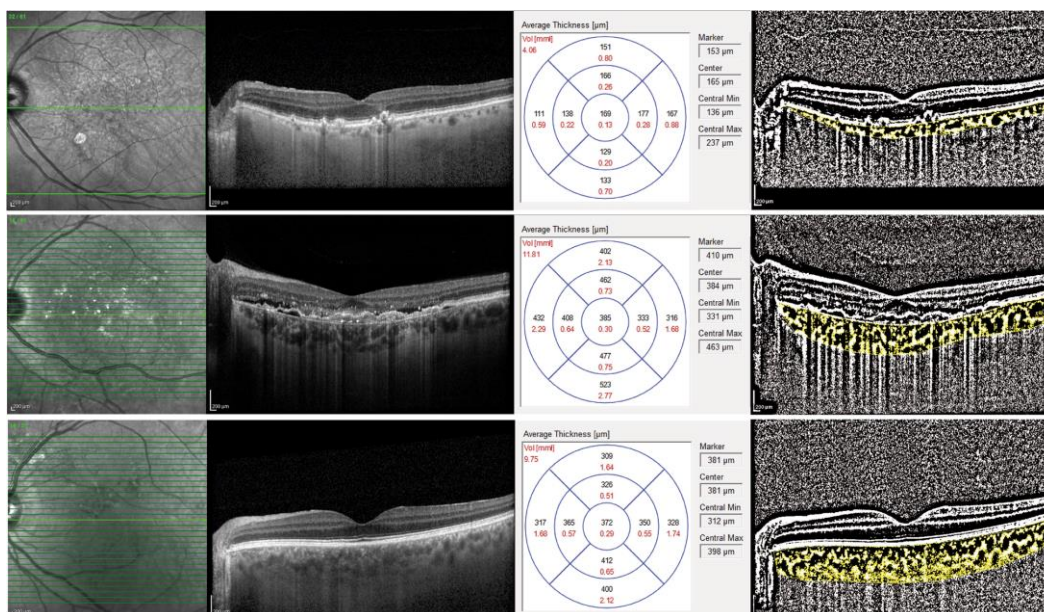


Figure 2. Representative EDI-OCT and CVI scans and averaged measurements of the choroidal thickness and volume in eyes with dry AMD (top line), wet AMD (middle line) and in healthy eyes (bottom line).

of ischaemic heart disease or myocardial infarction. However, the AMD group had a higher proportion of past smokers and a longer duration of smoking in pack-years than the controls ($p = 0.0004$ and $p < 0.0001$, respectively). We also did not find any correlation between AL and choroidal parameters including AV, ATC, AVC and CVI in wet ($R_s = -0.21$, $p = 0.65$; $R_s = -0.11$, $p = 0.82$; $R_s = -0.07$, $p = 0.88$; $R_s = -0.03$, $p = 0.93$; respectively), dry ($R_s = -0.39$, $p = 0.13$; $R_s = -0.36$, $p = 0.16$; $R_s = -0.36$, $p = 0.16$; $R_s = -0.02$, $p = 0.95$; respectively) and control ($R_s = -0.55$, $p = 0.1$; $R_s = -0.59$, $p = 0.07$; $R_s = -0.6$, $p = 0.07$; $R_s = -0.16$, $p = 0.68$; respectively) study groups.

The proportion of past smokers and the duration of smoking in pack-years were significantly higher in the wet AMD group than in the dry AMD group ($p = 0.03$ and $p = 0.002$, respectively). Furthermore, participants with dry AMD presented lower WHR values than those with wet AMD, which may have been partially related to the difference in gender distribution between study groups.

Choroid status in AMD and healthy control eyes. The main outcome of the study was to assess the differences in choroidal parameters between the dry and wet AMD subgroups and the control group. Table 3 provides the choroidal thickness and eye volume in the three study groups. We found that both the average thickness and volume in the central ETDRS ring area were significantly higher in wet AMD eyes than in dry AMD eyes ($p = 0.017$, $p = 0.015$, respectively) and control eyes ($p = 0.022$ and $p = 0.022$, respectively). Accordingly, CVI values were significantly lower in wet AMD compared to dry subtype of disease, and values recorded in both AMD subtypes were found to be significantly lower compared to healthy controls.

Representative choroidal OCT and CVI scans of the patients with exudative AMD and dry AMD are shown in Fig. 2.

A multivariate analysis of patients and controls adjusted for age, sex, and smoking status (pack-years) revealed that wet AMD was an independent factor associated with higher ATC, AVC and lower CVI when compared to controls ($\beta = +0.18$, $p = 0.0007$, $\beta = +0.18$, $p = 0.0008$, $\beta = -0.42$ $p < 0.0001$; respectively) and to dry AMD ($\beta = +0.17$, $p = 0.00003$ for both ATC and AVC and $\beta = -0.30$ $p < 0.0001$ for CVI). The covariate most strongly associated with ATC and AVC in these analyses was age ($\beta = -0.32$ for wet AMD vs controls, $\beta = -0.43$ for wet vs dry AMD, $p < 0.000001$ for both). The wet and dry groups did not differ in terms of average choroidal volume either between themselves from the control group ($p = 0.96$, $p = 0.64$, and $p = 0.84$, respectively).

In the dry AMD group, choroidal parameters strongly depend on macular morphology and function. Decreases in total choroidal volume, thickness and volume in the central ETDRS area were all associated with lower visual acuity ($R_s = +0.14$, $p < 0.001$; $R_s = +0.12$, $p = 0.002$; and $R_s = +0.12$, $p = 0.002$, respectively). Similarly, CVI correlated with visual acuity in AMD group ($R_s = +0.19$, $p < 0.001$). This may indicate that ongoing atrophy of the choroid in eyes with AMD is associated with a greater degree of functional impairment in the context of AMD. No such correlations were observed in the control group. Accordingly, in dry AMD group we observed negative correlations between the stage of the disease and the AV, AVC and ATC ($R_s = -0.18$, $p = 0.0004$; $R_s = -0.19$, $p = 0.0002$, $R_s = -0.19$, $p = 0.0003$; respectively). Similar association was observed with respect to CVI ($R = -0.16$; $p = 0.003$). It may indicate that choroidal vascular atrophy appears to be more pronounced in the later phases of the disease.

Correlations between choroidal parameters and the clinical characteristics of the patients. In the AMD group, the age of the participants was strongly negatively correlated with both the choroidal thickness and volume in the central ETDRS area ($R_s = -0.4$, $p < 0.001$, $R_s = -0.4$, $p < 0.001$, respectively) and with the AV ($R_s = -0.49$, $p < 0.001$). A similar relationship was observed in the control group: the age of participants was strongly negatively correlated with the ATC ($R_s = -0.22$, $p < 0.001$), the AVC ($R_s = -0.22$, $p < 0.001$) and the AV ($R_s = -0.45$, $p < 0.001$). In the AMD group, age negatively correlated with CVI ($R = -0.11$, $p = 0.01$), while in the control group, no statistically significant correlation was observed ($R = -0.006$, $p = 0.93$). The above observations confirm the fact that choroidal thinning occurs with age and that the process is more severe in patients with AMD.

Since there is a causative association between vascular diseases and dyslipidemia, we wanted to see if the concentration of blood lipids in specific fractions affects choroid morphology. In the AMD group, the total cholesterol level was positively correlated with the average thickness and volume in the central area and with the total choroidal volume ($R_s = 0.14$, $p = 0.02$; $R_s = 0.14$, $p = 0.02$; $R_s = +0.16$, $p = 0.007$; respectively). A similar relationship was shown between all aforementioned choroidal parameters and HDL concentrations ($R_s = 0.15$, $p = 0.01$; $R_s = 0.15$, $p = 0.01$; $R_s = +0.15$, $p = 0.01$; respectively). The results suggest that patients with hypercholesterolemia and/or a high HDL concentration have thicker choroids. No such correlations were found in the control group. There was no correlation between choroidal parameters and blood triglyceride concentration in any of the studied groups. Interestingly, choroidal parameters did not statistically differ between statin users and non treated patients.

Because smoking is one of the proven risk factors for the development of vascular diseases, we also evaluated the effect of smoking on parameters of choroid. We considered the duration of smoking, the time since smoking cessation for former smokers, the impact of active smoking and the number of smoked cigarettes expressed in pack-years. Surprisingly, we observed no effect of smoking on measured choroidal parameters in either patients with AMD or in healthy controls.

Since there is evidence that habitual physical activity significantly decreases the risk of vascular diseases, we investigated the association between choroidal parameters and regular physical activity. In the AMD group, physical activity (calculated in MET) appeared to be positively correlated with choroidal thickness and volume in the central ETDRS subfield ($R_s = +0.13$, $p = 0.04$ and $R_s = +0.12$, $p = 0.04$, respectively) and with the average volume ($R_s = +0.15$, $p = 0.009$). There were no such relationships in the control group. We also checked whether obesity affects choroidal parameters and found a negative correlation between CVI and WHR in the AMD group ($R_s = -0.13$, $p = 0.008$).

Relationship between choroid parameters and systemic vascular diseases. Since available data suggest that choroidal parameters are dependent on underlying systemic vascular disease, we investigated the association between choroidal parameters and several systemic risk factors and conditions. We noted that AMD patients with concurrent vascular diseases had significant reductions in choroidal thickness and volume (Table 4). In the AMD group, patients with concomitant hypertension had a significantly smaller average choroidal thickness and volume in the central ring area and a significantly smaller total choroidal volume than those without hypertension ($p = 0.001$, $p = 0.002$, and $p = 0.003$, respectively). Importantly, the duration of hypertension inversely correlated with both the ATC and AVC ($R_s = -0.13$, $p = 0.003$; $R_s = -0.12$; $p = 0.04$, respectively) and with the AV ($R_s = -0.12$; $p = 0.04$) which indicates that AMD patients with longer HA durations had thinner choroids. No such associations were observed in the control group. Subsequently, we assessed the effect of concurrent IHD on choroidal parameters in AMD patients and found that those with IHD presented a significantly smaller central average choroidal thickness, volume and total choroidal volume than patients without IHD ($p = 0.003$, $p = 0.01$, and $p = 0.0006$, respectively). Accordingly, in the control group, patients with ischaemic heart disease had significantly lower average choroidal volume parameters than patients without IHD ($p = 0.05$ for AVC and $p = 0.01$ for AV). Importantly, negative correlations between IHD duration and the AV were identified ($R_s = -0.15$, $p = 0.01$), corroborating the observed relationship. Analogous associations regarding a history of myocardial infarction were observed. In the AMD group, patients with MI had significantly lower AVCs and ATCs than patients without MI ($p = 0.04$ and $p = 0.05$, respectively). No such associations were observed in the

	AMD group			Control group		
	H+	H–		H+	H–	
Hypertension (H)	Median (IQR)	Median (IQR)	<i>p</i> value	Median (IQR)	Median (IQR)	<i>p</i> value
ATC [μm]	256.5 (119.5)	315 (163)	0.001	264 (98.25)	296.7 (109.75)	0.21
AVC [mm^3]	0.2 (0.1)	0.25 (0.13)	0.002	0.21 (0.08)	0.24 (0.09)	0.20
AV [mm^3]	6.37 (2.85)	6.98 (3.76)	0.003	6.56 (2.84)	7.29 (3.65)	0.15
Ischemic heart disease (IHD)	IHD+	IHD–		IHD+	IHD–	
	Median (IQR)	Median (IQR)	<i>p</i> value	Median (IQR)	Median (IQR)	<i>p</i> value
ATC [μm]	243.25 (89.25)	286 (143)	0.011	238.5 (198.5)	280.5 (93.5)	0.057
AVC [mm^3]	0.19 (0.07)	0.23 (0.11)	0.009	0.19 (0.16)	0.22 (0.08)	0.052
AV [mm^3]	5.97 (1.63)	6.79 (3.33)	0.003	5.47 (3.99)	6.98 (2.51)	0.010
Myocardial infarction (MI)	MI+	MI–		MI+	MI–	
	Median (IQR)	Median (IQR)	<i>p</i> value	Median (IQR)	Median (IQR)	<i>p</i> value
ATC [μm]	225 (96.5)	281.75 (141)	0.045	248 (79.5)	278 (101.5)	0.49
AVC [mm^3]	0.18 (0.08)	0.22 (0.11)	0.042	0.19 (0.07)	0.22 (0.08)	0.14
AV [mm^3]	5.81 (1.94)	6.67 (3.16)	0.228	5.86 (2.61)	6.92 (2.91)	0.10
Ischaemic stroke (IS)	IS+	IS–		IS+	IS–	
	Median (IQR)	Median (IQR)	<i>p</i> value	Median (IQR)	Median (IQR)	<i>p</i> value
ATC [μm]	251.75 (51.5)	280.5 (143.75)	0.435	280.5 (174.5)	270.5 (101.5)	0.48
AVC [mm^3]	0.2 (0.04)	0.22 (0.11)	0.434	0.22 (0.14)	0.21 (0.08)	0.50
AV [mm^3]	5.74 (1.01)	7.14 (3.13)	0.275	6.9 (4.56)	6.86 (2.53)	0.41
Limb ischaemia (LI)	LI+	LI–		LI+	LI–	
	Median (IQR)	Median (IQR)	<i>p</i> value	Median (IQR)	Median (IQR)	<i>p</i> value
ATC [μm]	240.5 (110)	280 (143)	0.587	301.75 (72)	269.5 (121)	0.34
AVC [mm^3]	0.19 (0.09)	0.22 (0.11)	0.58	0.24 (0.06)	0.21 (0.1)	0.34
AV [mm^3]	5.82 (2.78)	6.64 (3.09)	0.459	6.63 (1.46)	6.92 (2.74)	0.68

Table 4. Differences in choroidal parameters between the AMD group and the control group depending on the presence of concomitant vascular diseases and vascular risk factors. *IQR* interquartile range. Statistically significant differences with $p < 0.05$ are in bold.

control group. The results show that vascular disorders affect choroidal flow, as indicated by decreased choroidal thickness and volume, and this phenomenon is much more pronounced in patients with AMD.

Additionally, we checked whether systemic therapy for vascular diseases influences the choroidal structure. In the AMD group, use of antihypertensive treatment was associated with a lower ATC (hypotensive drug use: median = 250.75 μm , no hypotensive drug use: median = 320.75 μm , $p < 0.001$), AVC (hypotensive drug use: median = 0.2 mm^3 , no hypotensive drug use: median = 0.25 mm^3 , $p < 0.001$) and AV (hypotensive drug use: median = 6.23 mm^3 , no hypotensive drug use: median = 7.05 mm^3 , $p < 0.001$). A similar relationship was identified in the eyes of AMD patients using nonsteroidal anti-inflammatory drugs. Both ATC (NSAID use: median = 244 μm , no NSAID use: median = 287 μm , $p = 0.004$), AVC (NSAID use: median = 0.19 mm^3 , no NSAID use: median = 0.24 mm^3 , $p = 0.003$) and AV (NSAID use: median = 5.99 mm^3 , no NSAID use: median = 6.88 mm^3 , $p < 0.001$) were reduced in patients taking nonsteroidal anti-inflammatory drugs compared to individuals not receiving such therapy. In contrast, no such differences were found in the control group.

Discussion

Choroidal structure is of particular interest in AMD because abnormalities in choroidal circulation have been hypothesized to contribute to or even precede the development of AMD^{5,14}. Some studies suggested that AMD might have a vascular origin, with inadequate choroidal perfusion leading to ischaemia of the retinal pigment epithelium and subsequent production of VEGF, which can result in the formation of a choroidal neovascular membrane^{5,30}. This is in line with the results of the current study, which showed that choroidal atrophy increased with age in AMD patients. The reduction in blood flow in the choroidal vessels observed in AMD eyes may be the result of accelerated ageing processes. This leads to narrowing of the choriocapillaris lumen, loss of tissue cellularity, and thinning of the choroid, especially the choriocapillaris layer^{13,31}. In the present study, the average values of choroidal thickness and volume in the central ring area of the ETDRS grid in wet AMD eyes were significantly higher than those in both control eyes and dry AMD eyes. Additionally, dry AMD eyes did not exhibit differences in these parameters compared to healthy control eyes. This finding is in agreement with a study by Noori et al., who showed that both the mean CT and CV of the subfoveal area in wet AMD eyes were significantly greater than those in dry AMD and healthy eyes^{32,33}. Similarly, Razavi et al. showed thicker choroids in wet AMD eyes than in healthy control eyes in the central ring and in all 9 subfields of the ETDRS grid. This result may be attributed to the presence of oedema, exudation and haemorrhages secondary to the active choroidal neovascular membrane. According to Invernizzi et al., increasing CT in active³⁴ neovascularization was secondary to an enlargement of choroidal vessel diameters with or without a corresponding increase in vessel density rather

than from tissue thickening secondary to oedema³⁵. This might be the result of elevated levels of intraocular VEGF, which leads to choroidal vessel dilation, an increase in blood flow and proinflammatory and proedematous effects³⁶. However, in the present study, CVI was significantly lower in both AMD study groups compared to the control group that confirms the presence of vascular atrophy in AMD. Interestingly, unlike choroidal thickness and volume, the CVI in the wet form of AMD was lower compared to the control group as well as compared to the dry form of the disease. Since the choroidal vascularity index is derived from the ratio of luminal area to total choroid area, a decrease in its value may result from either advanced vascular atrophy or an increase in the volume of all tissue. In the latter case, it may result from the development of the fibrovascular membrane and inflammatory infiltrates in the extravascular tissue of the choroid in response to the increased concentration of proinflammatory and proangiogenic mediators, including VEGF. Other studies assessing choroidal thickness in AMD eyes using OCT imaging have shown mixed results. Chung et al. showed a decrease in choroidal thickness at four points located 1500 µm nasally, temporally, above and below the foveal centre in both dry and exudative subtypes of AMD compared to healthy controls³⁷. Similar results were documented by Jonas et al., who showed that CT at a horizontal distance of 1000 µm from the centre of the fovea was not significantly associated with any AMD subtype¹⁵. It is worth noting that in both studies, point choroidal thickness measurements taken at certain distances from the centre of the fovea were analysed separately (not averaged). CT measurements limited to one or even several points provide a very limited indication of choroidal status. Central choroidal thickness is not, in general, a good predictor of the wider distribution of choroidal thickness on this scale. In contrast, we assessed the averaged thickness parameter obtained from the central ring of the ETDRS grid, which allows the evaluation of the area most frequently affected by the disease in the course of AMD. Moreover, we analysed the average choroidal volume, which allows avoidance of a possible error of a single measurement and errors resulting from the physiological difference in the thickness of the choroid in different quadrants, as the lower nasal sector always presents the lowest value due to the neural tube closure process, while the upper sector, especially the upper temporal sector, physiologically presents the greatest thickness³⁶.

The current study showed a negative correlation between average choroidal thickness, volume, choroidal vascularity index and stage of the disease. This indicates a progressive loss of tissue thickness as the disease progresses. Accordingly, in the current study, there was a strong correlation between choroidal thickness, volume as well as CVI and visual acuity. Thus, a smaller choroidal thickness was associated with poorer visual function. Interestingly, Kang et al. found a significant correlation positive between choroidal thickness and visual outcomes in the eyes of patients with nAMD treated with an intravitreal injection of ranibizumab³⁸. They concluded that thicker choroids have more potential for recovery due to a relatively preserved choriocapillaris. Similarly, Metelitsina et al. showed that visual loss was associated with decreased choroidal circulation³⁹. We would like to emphasize that this is the first study using averaged choroidal thickness and volume measurements to describe the state of the choroid in eyes with different AMD subtypes and at subsequent stages of the disease. This approach provides detailed and reliable information regarding the choroidal parameters of the eyes of AMD patients.

In the present study, we confirmed that concomitant hypertension affected the choroidal microcirculation in AMD patients, leading to the loss of thickness and volume. This leads to the conclusion that the choroidal tissue of AMD patients is more prone to injury due to hypertension than those of healthy eyes. Indeed, the thickness and volume of the choroid in AMD patients in our study was negatively correlated with the duration of hypertension. The results of a study by Ellakwa et al. demonstrated that choroidal thickness was decreased in patients with systemic arterial hypertension. The authors attributed this finding to the process of arteriolar sclerosis and vascular contraction caused by high intravascular pressure in the choroid⁴⁰. Thus, we conclude that prolonged elevated systemic blood pressure impairs compensatory mechanisms and leads to decreased choroidal blood flow, which is followed by ischaemia.

The current evidence suggests that there are multiple similarities between pathogenic mechanisms of AMD and cardiovascular disease^{41,42}. However, epidemiological studies have not found a consistent association between AMD and CVD^{43–46}. In the current study, patients with AMD and coexisting ischaemic heart disease had significantly lower thickness and volume values than those who did not present CAD. Based on these results, we conclude that the presence of CVD more strongly affects the choroidal microcirculation in patients with AMD than in those without macular degeneration. However, the term CVD generally refers to atherosclerotic disease of medium and large vessels, while the diameter of the choroid is more closely related to the microvessels than to large-diameter coronary vessels⁴⁷. Most likely, the results of our study do not show a simple connection between the two diseases but rather reflect the state of systemic processes associated with biological ageing and other mechanisms underlying the development of cardiovascular disease and AMD. We found that the choroidal thickness and volume were all significantly lower in the eyes of AMD patients receiving antihypertensive and antiplatelet drugs as well as anticoagulants than in the eyes of patients who did not receive such pharmacotherapy. This may suggest that improvements in blood rheological parameters (platelet function) and a decrease in the level of systemic blood pressure or refinement of heart function do not reduce the rate of choroid atrophy in patients with AMD. It is possible that changes in these parameters do not affect the real cause of AMD, which has not been fully established.

There have been a few studies on changes in choroidal parameters directly after exercises in healthy participants, but no studies have analysed the influence of habitual physical activity on choroidal parameters in AMD patients^{48,49}. We showed a significant positive correlation between choroidal thickness and volume and physical activity in AMD patients, as patients with AMD who reported more intensive physical activity had a thicker choroid than those with a less active lifestyle. Indeed, an active lifestyle was found to be associated with a lower risk of AMD in several previous studies, probably due to increased antioxidant enzyme activity and increased resistance to oxidative stress, which is thought to be one of the key processes in the pathogenesis of AMD^{50–54}.

Because lipids represent at least 40% of the volume of drusen, it can be presumed that lipid metabolism is involved in the pathogenic mechanism of age-related macular degeneration⁵⁵. In this study, plasma levels of

total cholesterol and HDL were positively correlated with choroidal thickness and volume. Wong et al. linked the occurrence of a thicker choroid in patients with hypercholesterolaemia and the accumulation of lipids in the suprachoroid region and hypertrophy of endothelial cells and vascular smooth muscle, which was previously observed in animal models⁵⁶. Interestingly, the use of statins did not appear to adversely affect choroidal parameters in our study. This may be due to the pleiotropic effects of statins, which at least partially regulate certain disturbances that contribute to the pathogenesis of AMD.

Conclusions

AMD is a disease with an unexplained pathomechanism; therefore, effective therapeutic and prevention strategies are still lacking. The results of the current study indicate a relationship between vascular risk factors and choroidal thinning, which suggests an involvement of the choroid in the pathogenesis of age-related macular degeneration. Additionally, our findings show that the choroidal vascular system in eyes with AMD is much more susceptible to damage in the context of coexisting systemic vascular disease. This indicates a double-impact mechanism in which choroids that are altered due to genetic predisposition and/or as the result of certain pathological processes become more susceptible than the choroids of non-AMD eyes to progressive damage resulting from ageing and/or blood circulation disorders.

Data availability

The data that support the findings of this study are available on request from the corresponding author, A.M.

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Author contributions

Conceptualization, A.M.; ophthalmologic examination, E.K., K.M.-P.; database management, A.G.; statistical analysis, K.S.; writing—original draft preparation, E.K., Z.U.; writing—review and editing, A.M.; supervision, A.M.

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Competing interests

The authors declare no competing interests.

Additional information

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6.2 Retinal Vessel Functionality Is Linked With *ARMS2* A69S and *CFH* Y402H Polymorphisms and Choroidal Status in AMD Patients

Retina

Retinal Vessel Functionality Is Linked With *ARMS2* A69S and *CFH* Y402H Polymorphisms and Choroidal Status in AMD Patients

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PURPOSE. We aimed to investigate the reactivity of retinal vessels to a flickering stimulus in patients with age-related macular degeneration (AMD) and healthy participants. We also assessed whether the parameters of retinal vessels are dependent on genetic predisposition.

METHODS. A total of 354 patients with AMD and 121 controls were recruited for the study. All participants underwent thorough ophthalmologic examination and static and dynamic retinal vessel analysis. AMD risk polymorphisms were genotyped in the *CFH* and *ARMS2* genes.

RESULTS. We found no differences between the AMD group and controls in central retinal arteriolar equivalent (CRAE), central retinal venular equivalent (CRVE), arteriovenous ratio (AVR), dynamic analysis of arteries (DAAs), or dynamic analysis of veins (DAVs). Eyes with early AMD presented with significantly higher AVR values than eyes with late AMD. In the AMD group, DAA correlated positively with both choroidal thickness ($R_s = 0.14$, $P = 0.00096$) and choroidal volume ($R_s = 0.23$, $P < 0.0001$), and no such associations were observed in the controls. We found significantly lower DAA (1.47 ± 1.50) in TT homozygotes for the *ARMS2* A69S polymorphism in comparison with GG homozygotes (2.38 ± 1.79) and patients with GG + GT genotypes (2.28 ± 1.84). We also observed less prominent DAV (3.24 ± 1.71) in patients with TC + CC genotypes in the *CFH* Y402H polymorphism compared with TT homozygotes (3.83 ± 1.68).

CONCLUSIONS. Our findings suggest that retinal microcirculation appears to be associated with the genetic background, choroidal parameters, and clinical features of the patients with AMD.

Keywords: age-related macular degeneration (AMD), *CFH*, *ARMS2*, retinal vasculature, choroid

Age-related macular degeneration (AMD) is a disease that affects the macular region of the retina, causing progressive decrease in central vision. Clinically relevant disease is most prevalent in the elderly population, but early signs of AMD can also be found in individuals below 50 years of age.¹ Early clinical manifestations of the disease are the formation of lipid-rich extracellular deposits called drusen and abnormalities of the retinal pigment epithelium (RPE) seen on funduscopy as hyper- or hypopigmentation.² Symptoms of the late phase of disease are geographic atrophy (GA) or choroidal neovascularization (CNV), both leading to severe and permanent visual impairment and legal blindness that affect quality of life and functional independence.³

AMD is considered a disease with a complex pathogenesis, and metabolic, functional, genetic, and environmental factors seem to contribute to it.⁴ Age is one of the most constant unmodifiable factors; 60 to 80-year-old people are at a threefold greater risk of developing advanced AMD than persons younger than 60 years.^{5,6} A positive family history is also considered an important risk factor for AMD because siblings of patients have a three to sixfold higher risk than the general population.⁷ To date, using genomewide screening approaches, 34 genetic loci, including 52 gene variants that have been linked to AMD, have been identified.⁸ All these genes can be clustered into five physiologically relevant pathways: immune

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AMD: Retinal Vessels Function and CFH, ARMS2, Choroidal Status

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response and complement (*CFH*, *C9*, *C2/CFB*, *CFI*, and *C3*), extracellular matrix remodeling (*COL8A1*, *COL10A1*, *TGFBR1*, *TIMP3*, *ADAMTS9*, *HTRA1*, and *B3GALT1*), lipid transport (*APOE*, *LIPC*, *CETP*, *BAIAP2*, and *L2*), angiogenesis (*VEGFA*, *TGFBR1*, and *ADAMTS9*), and cell survival (*ARMS2*, *RAD51B*, and *TNFRSF10*).^{9,10} The significance of genetic predisposition extends beyond the high susceptibility of a person to develop AMD and may also affect the response to treatment.¹¹ Some researchers have reported a preferential association of *CFH* and *ARMS2/HTRA1* risk variants with different forms of advanced AMD.^{12,13} Specifically, *CFH* and *ARMS2* risk variants have been associated with progression toward GA and CNV, respectively.^{13,14} Even though this may imply a different influence of the two genes, their variants substantially increase the likelihood of both types of late AMD, indicating their contribution to biological processes before the onset of advanced disease.

Furthermore, cardiovascular risk factors, including smoking, hypertension, and obesity, have been suggested to intensify microvascular aging processes.^{15,16} It has been suggested that vascular factors play an important role in AMD pathogenesis,¹⁷ and that choroidal and retinal blood flows are disturbed in AMD, which may result in the initiation or progression of AMD.^{18,19} As we have shown in our previous study, the state of the retinal microvasculature may also reflect generalized disturbances in cardiovascular disorders (CVDs), such as stroke, coronary heart disease, and hypertension.²⁰ Retinal vascular caliber changes, assessed quantitatively on fundus photographs, have been shown to predict CVD independently of traditional risk factors, suggesting that, compared to other risk measures of CVD, retinal vascular changes may carry additional prognostic information.²¹

It has been suggested by McClintic et al. that functional assessments of the retinal microcirculation may be more useful in CVD risk prediction than static analysis alone.²² Thus, a dynamic vessel analyzer (DVA) estimates retinal vessel functionality at baseline and after flicker stimulation. It is based on the premise that in accordance with the neurovascular coupling theory, flicker light increases retinal neural activity, provoking retinal arterial and venous dilation via the release of nitric oxide (NO).²³ Endothelial cells play an important role in modulating microvascular tone and autoregulation because NO is an endothelial-derived vasodilator, whereas endothelin is a potent vasoconstrictor.²⁴ Importantly, diminished arterial and venous dilative responses have been linked to different vascular diseases.²⁵ Because AMD has been previously linked to cardiovascular diseases, such a relationship may be confirmed with altered vessel reactivity in the DVA test. Indeed, dynamic vessel analysis in eyes with drusen and RPD showed diminished retinal arterial dilation in response to flicker light stimulation.²⁶ However, the literature describing the changes in DVA in the course of AMD is scarce,^{27,28} and studies have been conducted on relatively small groups of patients, which makes inference impossible.

The aim of the present study was to compare the differences in the reactivity of the retinal vessels to a flickering stimulus in patients with AMD and healthy participants and to assess whether the status of retinal vessels is dependent on concomitant vascular diseases. Subsequently, we searched for the correlation between flicker-induced vasodilation and morphological status of the retina and choroid on the basis of spectral-domain optical coherence tomography (SD-OCT) images. We also assessed the relation-

ship between high-risk AMD polymorphisms, including *CFH* Y402H and *ARMS2* A69S, and both static and dynamic retinal vascular function. We hypothesize that static and dynamic retinal vascular function is associated with high-risk AMD polymorphisms, choroidal status, and concomitant vascular diseases.

MATERIALS AND METHODS

Subjects and Initial Management

We recruited 354 patients with AMD and 121 age-matched controls with no symptoms or signs of macular degeneration from the outpatient population of the First Department of Ophthalmology of Pomeranian Medical University in Szczecin, Poland. All patients with AMD were enrolled in the study at the moment of diagnosis, prior to the introduction of anti-VEGF treatment. The exclusion criteria were significant chronic systemic conditions (diabetes mellitus, renal failure, neoplastic disease, hepatic dysfunction, etc.) or ongoing retinal disease except AMD (in the AMD group). All patients enrolled in the study signed a consent form before enrollment, in accordance with the tenets of the Declaration of Helsinki.

Data regarding medical history and current drug use were collected with a particular focus on the history of cardiovascular diseases, including physician-diagnosed heart and vascular diseases. Prior to ophthalmic examination, the actual arterial blood pressure (BP) was directly measured in all subjects using a noninvasive BP system with a manual aneroid manometer. The mean result from 3 measurements obtained with 5-minute resting intervals was calculated. The systemic mean arterial pressure (MAP) was calculated as follows: $\text{MAP} = \text{diastolic BP} + 1/3 (\text{systolic BP} - \text{diastolic BP})$ mm Hg. The following medical parameters were also assessed: waist circumference (cm), waist/hip ratio (WHR), and body mass index (BMI; weight [kg]/height [m]²). Using the reported average number of cigarettes smoked per day and the number of years of smoking, we calculated cumulative pack-years.

Ophthalmologic Examination

All participants underwent complete ophthalmologic examination, including best corrected distance visual acuity with Snellen charts, slit lamp biomicroscopy, and detailed fundus examination after pupil mydriasis with 1% tropicamide solution, to determine the ocular health status of the fundus. OCT images were obtained using a Spectralis OCTA (Heidelberg Engineering, Heidelberg, Germany). IOP measurement with the Goldmann applanation tonometer, axial length, and anterior chamber depth calculations were performed to eliminate known factors that interfere with reliable OCT image analysis. If the presence of a neovascular membrane could not be clearly excluded or confirmed, a fluorescence angiography test was performed. The results were analyzed for the presence of factors that met the exclusion criteria, which included any posterior eye disease that could potentially affect the choroidal or retinal vasculature, including but not limited to glaucoma, retinal vessel occlusion, retinopathy of any type, high myopia (≥ 6 D), uveitis, and ocular tumor. Eyes were excluded if they had cataract surgery in the previous 3 months, and any previous laser therapy or vitreoretinal surgery. Additionally, fundus autofluorescence examination was performed for more accurate drusen classification.

The severity of AMD was classified according to Ferris et al.²⁹: (1) early AMD: medium drusen (63–125 µm) and no pigmentary abnormalities, (2) intermediate AMD: large drusen or pigmentary abnormalities associated with at least medium drusen, and (3) late AMD: lesions associated with neovascular AMD or geographic atrophy. The examinations were carried out in a blinded manner.

Retinal Vessel Analysis

For static retinal vessel analysis (SVA), the FF450 plus fundus camera (Zeiss AG, Jena, Germany) was used, and 30 degrees retinal photographs of each subject were collected and analyzed using VISUALIS and VesselMap Software (IMEDOS Systems, Ltd., Jena, Germany). Examination was conducted in a half-lit room. The participant focused on the fixation bar within the retinal camera, whereas the fundus was examined under green light; thus, preserved central vision is crucial for precise test conduction. The standard parameters for this evaluation were as follows: central retinal arteriolar equivalent (CRAE), which relates to the diameter of the central retinal artery; central retinal venular equivalent (CRVE), which relates to the diameter of the central retinal vein; and arteriovenous ratio (AVR), which represents the CRAE/CRVE ratio. After 20 minutes, DVA was conducted. The major temporal arterial and venous segments approximately 1.5 mm long were evaluated in each eye. The measurements were located 1- to 2-disc diameters from the optic disc. The selection criteria for the arterial and venous segment locations were as follows: no crossing or bifurcation in the measured segment, a curvature not exceeding 30 degrees, a distance from the neighboring vessels of at least one vessel diameter, and sufficient contrast with the surrounding fundus. The measurement of the baseline vessel diameter for 50 seconds was followed by 3 cycles of 20-second flicker provocation and 80-second steady illumination, during which the vessel diameter returned to baseline. The total duration of the measurement, including baseline and observation between flicker provocations, was 352 seconds. The response was measured as the difference between the mean vascular diameter for the last 10 seconds of flicker stimulation and the mean vascular diameter for the 30 seconds immediately preceding this flicker stimulation, divided by the latter value. The response was expressed as the mean of the calculations for the three flicker cycles. Only one artery and one vein were measured in each eye.

Genotyping

Venous blood samples (approximately 7.5 mL) collected in EDTA tubes were centrifuged (2000 rpm, 4°C, 10 minutes). Then, red blood cells were lysed using ammonium chloride-based lysing buffer (BD Biosciences, Franklin Lakes, NJ, USA). Nucleated cells were then counted, and DNA isolation was subsequently performed with a total DNA isolation kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol. AMD risk polymorphisms were genotyped as previously described.³⁰ In *CFH*, rs1061170, encoding a Y402H interchange, was genotyped by restriction analysis with *EagI*, *HhaI*, and *Hsp92II* enzymes. In *ARMS2*, LOC387715 rs10490924, encoding an A69S interchange, was determined by direct DNA sequence analysis using an Applied Biosystems 3130 XL instrument for DNA sequencing. Molecular analysis was performed by

an outward cooperating laboratory according to generally approved standards.

Statistical Analysis

Because most of the analyzed retinal vessel parameters showed distributions significantly different from normal distribution (Shapiro-Wilk test, $P < 0.05$), nonparametric tests were applied. The Kruskal-Wallis and Mann-Whitney U tests were used to compare quantitative and rank variables between groups. The strength of associations between quantitative and rank variables was measured with Spearman rank correlation coefficients (R_s). Fisher's exact test was used to compare qualitative variables between groups. We considered any P values < 0.05 as statistically significant. Because simultaneous testing of multiple comparisons within a family of hypotheses carries the risk of increasing rejection error, the conservative Bonferroni's correction was calculated to decrease the risk of indication of a significant difference when it resulted only from random variation. Consequently, the nominal significance level of each test was lowered by a specific correction for multiple comparisons. To take into account the issue of multiple testing, we corrected the P value threshold by dividing the standard 0.05 level byproduct of the number of retinal vessel parameters (5), the number of ophthalmological, clinical and genetic parameters correlated with retinal vessel parameters (30), and the number of study groups (2). Thus, associations of retinal vessel parameters with the other variables at $P < 0.05/300 = 0.00016$ was considered statistically significant after Bonferroni correction for multiple testing.

RESULTS

Clinical Characteristics of the Study Groups

In this study, we enrolled 354 patients with AMD (175 dry AMD and 179 wet AMD) and 121 healthy controls. Table 1 lists the clinical features of the patients and controls. In the study groups, we examined vascular-related risk factors because epidemiological evidence collected so far suggests that atherosclerosis is involved in AMD. However, no significant differences in age or well-known atherosclerotic risk factors, including hypertension, history of ischemic heart disease, or cardiac infarction, were detected between the AMD and control groups. The proportion of past smokers and the number of smoking pack-years were significantly higher in the AMD group than in the controls ($P = 0.0004$ and $P < 0.0001$, respectively).

Retinal Vessel Parameters

Next, we analyzed retinal vessel parameters in the study groups (Table 2). The eyes of the patients with AMD and controls did not differ significantly in parameters evaluated by SVA and DVA. Both CRAE, relating to the diameter of the central retinal artery, and CRVE, relating to the diameter of the central retinal vein, did not show statistically significant differences between AMD and control eyes ($P = 0.55$ and $P = 0.88$, respectively). Accordingly, we observed no differences between the groups in AVRs ($P = 0.31$). Regarding the dynamic retinal vessel analysis, no significant differences in either arterial or venous responses to flicker stimulation were identified between eyes from patients with AMD and controls ($P = 0.31$ and $P = 0.22$, respectively).

TABLE 1. Characteristics of the Study Groups

Parameter	AMD Group	Control Group	P Value*
Number of subjects	354	121	—
Sex M/F	135/219	32/89	0.02
Patient's age, y, mean $\pm$ SD	73.4 $\pm$ 8.0	73.1 $\pm$ 6.0	0.41
BMI, kg/m ² , mean $\pm$ SD	26.9 $\pm$ 4.2	26.6 $\pm$ 3.7	0.43
WHR, arbitrary units, mean $\pm$ SD	0.90 $\pm$ 0.09	0.88 $\pm$ 0.09	0.13
Waist circumference, cm, mean $\pm$ SD	103.3 $\pm$ 9.1	102.1 $\pm$ 7.3	0.33
MAP, mm Hg, mean $\pm$ SD	98.30 $\pm$ 11.10	98.72 $\pm$ 9.66	0.86
Current smokers, N	44 (14%)	6 (6%)	0.0503
Former smokers, N	166 (51%)	30 (31%)	0.0004
Smoking pack-years, mean $\pm$ SD	13.6 $\pm$ 18.9	6.0 $\pm$ 13.1	0.00007
Period without smoking, y, mean $\pm$ SD	6.8 $\pm$ 10.9	5.3 $\pm$ 10.2	0.055
Hypertension, N	209 (65%)	69 (71%)	0.27
Duration of hypertension, y, mean $\pm$ SD	8.2 $\pm$ 9.5	9.2 $\pm$ 9.9	0.27
History of ischemic heart disease, N	52 (16%)	11 (11%)	0.33
Duration of ischemic heart disease, y, mean $\pm$ SD	1.2 $\pm$ 4.2	0.8 $\pm$ 3.3	0.26
History of myocardial infarction, N	20 (6%)	6 (6%)	1.00
History of cerebral stroke, N	9 (3%)	3 (3%)	1.00
History of peripheral artery disease, N	16 (5%)	6 (6%)	0.61
History of aortic aneurysm, N	5 (2%)	0	0.59
Hypotensive drugs/vasodilators, N	210 (65%)	68 (70%)	0.39
Hormonal drugs, N	55 (17%)	20 (21%)	0.45
Thyroxine, N	44 (14%)	20 (21%)	0.11
Steroids, N	6 (2%)	1 (1%)	1.00
Other hormonal drugs, N	4 (1%)	0	0.58
Statins, N	86 (27%)	35 (36%)	0.07
NSAIDs, N	65 (20%)	19 (20%)	1.00
Cardiac medications/anti-arrhythmic drugs, N	45 (14%)	14 (14%)	0.87
Anti-asthmatic drugs, N	24 (7%)	3 (3%)	0.16
Antidepressants, N	15 (5%)	5 (5%)	0.79

N, number of subjects.

* Mann-Whitney test/Fisher's exact test. In bold, a P value < 0.05 was considered statistically significant.

TABLE 2. Retinal Vessel Parameters in the Study Groups

Parameter	AMD Group Median (IQR)	Control Group Median (IQR)	P Value*
AVR	0.85 (0.09)	0.85 (0.09)	0.31
CRAE	181.4 (23.1)	182.8 (21.18)	0.55
CRVE	215.2 (27.1)	213.6 (26.43)	0.88
DAA (%)	1.6 (2.9)	1.8 (2.9)	0.31
DAV (%)	3.0 (2.5)	3.3 (2.4)	0.22

IQR, interquartile range; AVR, arteriovenous ratio; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; DAA, dynamic analysis of arteries; DAV, dynamic analysis of veins.

* Mann-Whitney U test.

Interestingly, when we analyzed the same parameters in groups of patients with different stages of AMD (Table 3), we observed significantly higher AVR values in eyes with early AMD than in eyes with late AMD (median = 0.87 and median = 0.85, respectively, $P = 0.03$). This may indicate that AMD progression is linked with structural changes in the retinal vasculature.

Representative static vessel analyses of the patients with early and late stage of AMD are shown in the Figure.

Correlations Between Retinal Vessel Parameters and Retinal and Choroidal Characteristics

Next, we investigated possible correlations between retinal vessel parameters and ophthalmological characteristics of

our patients with AMD and controls. In both patients with AMD and controls, choroidal thickness correlated positively with CRAE ($R_s = 0.21$, $P < 0.0001$ in AMD eyes and $R_s = 0.20$, $P = 0.007$ in controls, respectively) and CRVE ($R_s = 0.14$, $P = 0.0007$ in AMD eyes and $R_s = 0.28$, $P = 0.0002$ in controls, respectively). Similarly, choroidal volume correlated positively with CRAE ($R_s = 0.24$, $P < 0.0001$ in AMD eyes and $R_s = 0.30$, $P < 0.0001$ in controls, respectively) and CRVE ($R_s = 0.25$, $P < 0.0001$ in AMD eyes and $R_s = 0.38$, $P < 0.0001$ in controls, respectively). This finding indicates that the thicker the choroid is, the larger the diameter of both the central retinal vein and central retinal artery. Interestingly, visual acuity correlated positively with CRAE in AMD eyes ($R_s = 0.14$, $P = 0.001$), whereas no such association was observed for controls. Associations of choroidal thickness with CRAE in the AMD group and associations of choroidal volume with CRAE and CRVE for both patients with AMD and controls remained significant after correction for multiple tests ($P < 0.00016$).

Then, we investigated whether flicker-induced vasodilation is associated with morphological changes in the retina and choroid obtained from OCT images. In the AMD group, arterial response to flicker stimulation correlated positively with both choroidal thickness ($R_s = 0.14$, $P = 0.00096$) and choroidal volume ($R_s = 0.23$, $P < 0.0001$), and no such associations were observed in the controls. This indicates that the function of the vascular endothelium in the arterial vessels of the retina is linked with parameters of choroidal vessels: the higher the flicker-induced arterial vasodilatation values are, the thicker and larger the volume of the choroid.

TABLE 3. Retinal Vessel Parameters in Patients With Different Stages of AMD

Parameter	<i>P</i> Value*	Early Median (IQR)	Intermediate Median (IQR)	Late Median (IQR)	<i>P</i> Value† E vs. I	<i>P</i> Value† I vs. L	<i>P</i> Value† E vs. L
AVR	0.05	0.87 (0.08)	0.85 (0.09)	0.85 (0.08)	0.33	0.11	0.03
CRAE	0.21	184.50 (21.0)	185.2 (16.85)	180.18 (21.25)	0.68	0.08	0.43
CRVE	0.31	210.55 (25.7)	216.5 (25.4)	214.18 (24.8)	0.17	0.94	0.14
DAA (%)	0.74	1.65 (2.5)	1.98 (2.65)	1.5 (2.65)	0.49	0.55	0.78
DAV (%)	0.95	3.35 (1.95)	3.03 (1.65)	3.1 (2.2)	0.70	0.94	0.87

* Kruskal–Wallis test.

† Mann–Whitney *U* test.

E, early; I, intermediate; L, late; AVR, arteriovenous ratio; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; DAA, dynamic analysis of arteries; DAV, dynamic analysis of veins.

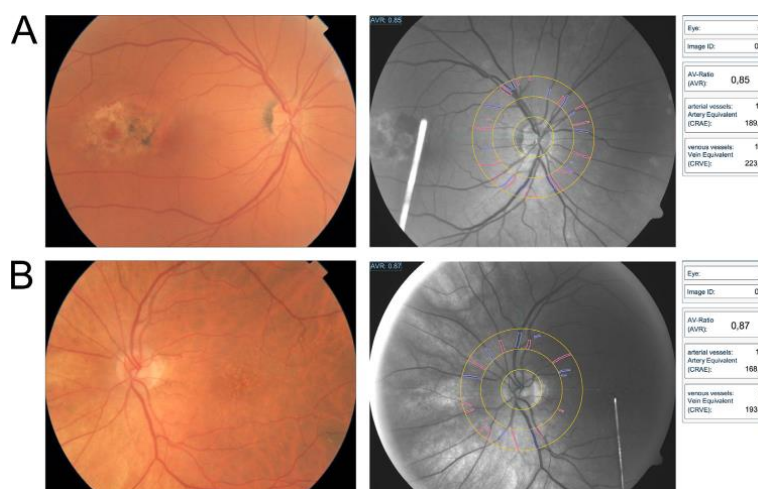
In bold, a *P* value < 0.05 was considered statistically significant.

FIGURE. Static vessel analysis of a patient with late (A) and early (B) AMD, with AVR, CRAE, and CRVE values presented.

Interestingly, in the AMD group, we found a positive correlation between arterial response to flicker stimulation and total retinal volume ($R_s = 0.16$, $P = 0.0003$). This indicates that better vascular endothelial function implies less advanced retinal atrophy. Moreover, we also found strong positive correlations between arterial and venous responses to flicker stimulation in both patients with AMD ($R_s = 0.28$, $P < 0.0001$) and controls ($R_s = 0.33$, $P < 0.0001$). Associations of arterial response to flicker stimulation with choroidal volume in AMD group and associations of arterial with venous responses to flicker stimulation for both patients with AMD and controls remained significant after correction for multiple tests ($P < 0.00016$).

Correlations Between Retinal Vessel Parameters and Clinical Characteristics of the Patients

Because narrowing of vessels in the course of atherosclerosis is tightly linked with the development and progression of AMD, we focused on associations between analyzed retinal vessel parameters and general characteristics of our patients with AMD and controls, including medical history and current drug use. We observed a negative correlation

between flicker-induced arterial vasodilation and age in both the AMD and control groups ($R_s = -0.16$, $P = 0.00018$ and $R_s = -0.21$, $P = 0.005$, respectively). In the AMD group, we also observed a negative correlation between WHR values and AVRs ($R_s = -0.12$, $P = 0.003$). This indicates that overweight and obesity are associated with a prominent narrowing of the ocular microvasculature. Accordingly, higher MAP values were accompanied by lower AVRs ($R_s = -0.19$, $P < 0.0001$), further supporting the notion of narrowing the arteries in the eyes of patients who present with increased BP. Association of MAP with AVR remained significant after correction for multiple tests ($P < 0.00016$).

Our next aim was to further assess whether static and dynamic retinal vessel characteristics differ in the eyes of patients with known atherosclerotic risk factors and a history of cardiovascular disease. We observed significantly lower DAA values in patients with AMD with ischemic heart disease and aortic aneurysm than in individuals without such diseases (ischemic heart disease: median = 0.85, no ischemic heart disease: median = 2.25, $P = 0.002$; aortic aneurysm: median = 0.6, no aortic aneurysm: median = 1.95, $P = 0.04$). No such differences were observed in controls. In patients with AMD with hypertension, we also observed significantly lower CRAE (hypertension: median

Table 4. Retinal Vessel Parameters in Patients With Different High Risk AMD Genotype^a

Parameter	CFH Y402H Genotype Groups									
	TT (N = 61)		TC + CC (N = 234)		TT + TC (N = 194)		CC (N = 101)		P Value ^b	
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	P Value ^b	P Value ^b (TT vs. CC)
AVR	0.84 ± 0.06	0.84 (0.09)	0.85 ± 0.06	0.85 (0.08)	0.85 ± 0.07	0.85 (0.09)	0.85 ± 0.06	0.86 (0.07)	0.33	0.98
CRAE	183.74 ± 14.66	183.74 (23.36)	181.44 ± 15.12	180.75 (19.55)	181.96 ± 15.06	182.73 (20.01)	181.91 ± 15.0	181.5 (20.6)	0.26	1.0
CRVE	185.75 ± 19.59	185.75 (29.79)	180.75 ± 18.10	180.75 (27.8)	182.73 ± 19.91	182.73 (27.8)	181.5 ± 17.88	181.5 (27.88)	0.08	0.76
DAA, %	2.50 ± 2.09	2.0 (3.5)	2.06 ± 1.73	1.63 (2.5)	2.08 ± 1.7	1.8 (2.7)	2.08 ± 2.0	1.5 (2.7)	0.29	0.62
DAV, %	3.83 ± 1.68	3.4 (2.6)	3.24 ± 1.71	3.0 (1.9)	3.36 ± 1.71	3.05 (2.0)	3.38 ± 1.7	3.1 (2.1)	0.03	0.82
ARMS A69S Genotype Groups										
Parameter	GG (N = 95)		GT + TT (N = 201)		GG + GT (N = 51)		TT (N = 45)		P Value ^b	
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	P Value ^b	P Value ^b (GG vs. TT)
AVR	0.86 ± 0.07	0.86 (0.07)	0.84 ± 0.06	0.85 (0.09)	0.85 ± 0.06	0.85 (0.08)	0.86 ± 0.07	0.87 (0.09)	0.12	0.27
CRAE	183.52 ± 15.62	183.52 (21.35)	181.15 ± 14.63	180.75 (19.8)	181.92 ± 14.98	182.0 ± 15.22	182.0 ± 15.22	183.0 (24.18)	0.43	0.74
CRVE	182.95 ± 19.22	182.95 (27.3)	180.75 ± 18.10	180.75 (27.3)	182.73 ± 19.91	182.73 (27.8)	181.5 ± 17.88	181.5 (27.88)	0.85	0.32
DAA, %	2.38 ± 1.79	1.95 (2.6)	2.03 ± 1.82	1.5 (2.5)	2.28 ± 1.8	1.95 (2.5)	2.08 ± 2.0	1.47 ± 1.50	0.11	0.01
DAV, %	3.44 ± 1.51	3.2 (1.8)	3.31 ± 1.81	3.05 (2.2)	3.42 ± 1.68	3.2 (2.1)	3.01 ± 1.89	2.5 (1.4)	0.4	0.14

^a Kruskal-Wallis test for comparison among three genotypes of indicated polymorphism.^b Mann-Whitney U test.

AVR, arteriovenous ratio; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; DAA, dynamic analysis of arteries; DAV, dynamic analysis of veins; N, number of subjects.

In bold, a P value < 0.05 was considered statistically significant.

= 179.93, no hypertension: median = 186.88, $P = 0.001$) and CRVE (hypertension: median = 212.45, no hypertension: median = 218.4, $P = 0.006$).

Finally, we investigated whether specific drugs could also affect retinal vessel parameters. In the AMD group, taking hypotensive medication was associated with lower values of the same parameters as those observed for hypertensive subjects: CRVE (hypotensive drug use: median = 212.88, no hypotensive drug use: median = 217.7, $P = 0.02$) and CRAE (hypotensive drug use: median = 180.55, no hypotensive drug use: median = 186.6, $P = 0.002$). Accordingly, lower CRAEs were observed in the eyes of the patients taking anticoagulants (anticoagulant users: median = 178.7, no anticoagulant users: median = 183.4, $P = 0.04$). No such differences were found in the controls. Interestingly, DAV was reduced in patients with AMD taking nonsteroidal anti-inflammatory drugs (NSAID use: median = 2.6, no NSAID use: median = 3.25, $P = 0.04$) and taking cardiac medications (cardiac drug use: median = 1.0, no cardiac drug use: median = 1.95, $P = 0.03$).

Associations Between *CFH* Y402H and *ARMS2* A69S Polymorphisms and Retinal Vessel Parameters

In order to explore whether SNPs associated with an increased risk of AMD (*CFH* Y402H and *ARMS2* A69S) influence retinal vessel characteristics in our patients, we correlated these parameters with different genotypes in these two SNPs (Table 4). We found that the TT genotype in *CFH* Y402H was associated with an evidently higher venous response to flicker stimulation than the TC + CC genotypes (median = 3.4, median = 3.0, respectively, $P = 0.03$). However, we should be rather cautious in interpreting the results of the Mann–Whitney test because the Kruskal–Wallis test showed only borderline differences between the TT and TC + CC genotype groups ($P = 0.08$). Importantly, in the case of *ARMS2* A69S, we observed significant differences in arterial vasodilatation values between the tested genotypes ($P = 0.04$, Kruskal–Wallis test). In detail, GG homozygotes presented the highest, whereas TT homozygotes presented the lowest DAA responses. Moreover, patients with GG + GT genotypes presented with a significantly higher arterial response to flicker stimulation than TT homozygotes ($P = 0.01$).

DISCUSSION

AMD remains the leading cause of blindness in the industrialized world. However, the exact pathogenesis of this disease remains largely unknown. Recently, disturbances in choroidal and retinal blood flows leading to chronic hypoxia have been implicated in the initiation or progression of AMD. Because the state of the retinal microvasculature may also reflect generalized processes occurring in the course of various CVDs and a number of CVD risk factors (age, obesity, cigarette smoking, hypertension, and high cholesterol) have also been associated with an increased risk of AMD,³¹ the link between CVDs and AMD is receiving more attention. Thus, in this study, we aimed to assess the reactivity of retinal vessels to flickering stimuli in patients with AMD and to investigate whether retinal vascular function is associated with disease grading, choroidal and retinal characteris-

tics, genetic background, and clinical and demographic data, with a particular focus on vascular-related risk factors.

First, we evaluated static and dynamic retinal vessel parameters in our study groups. We did not find any differences in retinal vessel diameters between AMD and control eyes at baseline or after flicker light application. Functional assessments of the retinal microcirculation in patients with AMD have not been extensively investigated. In 2019, Rabiolo et al. found no significant difference between eyes with drusen, reticular pseudodrusen and controls for CRAE, CRVE, and AVR, which is in agreement with our results.²⁶ In the same study, DVA revealed significantly reduced arterial dilation in eyes with drusen compared with controls, contradicting our results, as we observed no significant alterations in this parameter. However, it is worth noting that the study by Rabiolo et al. was conducted on a very small group of 23 patients with drusen. In another study by Lanzl et al., no significant differences in mean arterial dilation were found in patients with AMD in comparison with controls,²⁷ further supporting our results. The 10 patients with exudative AMD enrolled in this study were evaluated pre- and post-treatment with a single intravitreal application of bevacizumab, and, interestingly, arterial constriction after stimulation occurred more slowly in the post-treatment group than in the control group. However, the scarce literature on retinal vessel analysis in AMD and the small sample size in those studies makes it difficult to draw valuable conclusions.

Our results are in concordance with several studies on large cohorts, including the Blue Mountains Eye Study, Beijing Eye Study, and Beaver Dam and Rotterdam Eye Study (BDES), in which the authors found no association between the diameter of retinal vessels and AMD risk.^{32–34} Accordingly, we found no statistically significant differences between the AMD and control groups in terms of AVR. However, when we investigated the particular AMD stages, we observed lower AVR values in eyes with late AMD. This may support the hypothesis that the structural changes in the retinal vasculature might be accompanied by progression of AMD. In fact, retinal changes depending on the AMD stage have previously been described by Yang et al., as dilated retinal arteriolar caliber was shown to be associated with early AMD.³⁵ Similarly, Jeganathan et al. identified wider venular caliber to be associated independently with early AMD, whereas there was no significant association between retinal arteriolar caliber and early AMD or between arteriolar or venular caliber and late AMD.³⁶ The authors attributed this association to some cardiovascular risk factors, such as inflammatory processes, oxidative stress, or nitric oxide (NO) production dysregulation. Interestingly, a smaller AVR was also reported in the context of increased carotid artery stiffness, which is considered an early marker of atherosclerosis.³⁷

Next, we focused on the correlations between retinal vessel parameters and choroidal characteristics of the patients. Several previous studies noted that abnormal choroidal circulation may be involved in the development of AMD.^{38–40} Choriocapillaris dropout has been observed from early stages of AMD, and vascular dropout appears to be linearly related to the progression of AMD based on histopathological studies.^{18,41,42} Several previous studies have proposed that AMD affects both the quantity and morphology of inner retinal and choroidal vasculature.^{17,43,44} In early patients with AMD with reticular pseudodrusen, retinal thinning was accompanied by choroidal and retinal vascular loss, which further suggests a possible link

between retinal atrophy and choroidal vasculature alterations.⁴⁵ The results of our study are in agreement with these previously published works and show that with the loss of choroidal tissue, a reduction in the diameter of retinal arterioles and venules occurs. It is probable that chronic choroidal ischemia may contribute to retinal atrophy and decreased metabolic demands that may induce a secondary decrease in retinal vascular flow.⁴⁵ The positive correlation between the arterial response to flicker stimulation and choroidal thickness and volume may in part support the hemodynamic theory of AMD proposed by Friedman.⁴⁶ According to this theory, lipids accumulate in Bruch's membrane and sclera, similar to atherosclerotic plaques in the arteries, resulting in the higher stiffness of the tissues. This leads to increased choroidal vascular resistance, which further leads to either an increase in choriocapillaris hydrostatic pressure or a decrease in choroidal perfusion, depending on the relative resistances of the ophthalmic artery and the cerebral artery.⁴⁷ This further supports the notion that AMD is the eye localization of the systemic disease of atherosclerosis.

In fact, several studies have linked atherosclerotic changes and a markedly increased risk of CVD with the incidence of AMD. Taniguchi et al. reported that neovascular AMD is associated with atherosclerosis, whereas Klein et al. similarly reported the presence of carotid plaques and carotid artery intima-media thickness to be weakly associated with the incidence of late AMD.^{48,49} The Age-Related Eye Disease Study (AREDS) found advanced AMD to be associated with increased cardiovascular mortality,⁵⁰ whereas the Atherosclerosis Risk in Communities Study (ARCS) observed a significant association between late AMD and incident coronary heart disease (CHD) in a population at high risk for CHD.⁵¹ Therefore, to better understand possible associations between AMD and atherosclerosis risk factors, we investigated the relationship between analyzed retinal vessel parameters and general characteristics of our patients with AMD and controls, including medical history and current drug use. We found significant associations between both static and dynamic retinal vessel parameters and ischemic heart disease, aortic aneurysm, and hypertension. Our results confirm the previously described relationship between arterial hypertension and the reduced diameter of the retinal vessels, where it might take the form of generalized or focal arteriolar narrowing as a result of local autoregulatory mechanisms in response to elevated intraluminal pressure.^{52,53} Either remodeling or rarefaction of retinal/choroidal vessels may contribute to an increase in vascular resistance, resulting in a decrease in blood flow and ischemia, and these effects may be reflected by changes in retinal vessel static and dynamic tests. Indeed, the history of ischemic heart disease, aortic aneurysm, or hypertension in our patients with AMD was associated with a significantly decreased reactivity of arterial vessels to flicker stimulation. All these diseases are characterized by complex etiopathogenesis related to both environmental and genetic factors, but atherosclerosis remains the common point of these entities,⁵⁴ once again suggesting its vital role in AMD pathogenesis.

In patients with AMD, both antihypertensive and anticoagulant therapies were associated with significantly reduced CRAE and CRVE parameters. According to our previous study, conducting effective BP-lowering therapy does not go hand in hand with the normalization of the functions of the vascular endothelium.²⁰ This may indicate sources of microcirculation disturbances other than elevated BP in patients

with hypertension. In contrast, it was postulated that statin use might potentially contribute to reduced progression of AMD due to lipid-lowering, anti-inflammatory, and anti-angiogenic effects.⁵⁵ We also observed a reduced venous response to flicker stimulation in patients with AMD using NSAIDs or cardiac medications. Rubio-Ruiz et al. proposed that in chronic, low-grade inflammatory conditions, such as aging, COX-2 contributes greatly to vasoconstriction; thus, NSAIDs directly affect vascular responses.⁵⁶ On the other hand, Modjtahedi et al. recently described a lower risk of exudative AMD in longer-term NSAID users, suggesting its protective effect, although the group concluded a generally low impact of NSAIDs on AMD incidence.⁵⁷

Finally, we found a significantly lower arterial response to flicker stimulation in TT homozygotes for the *ARMS* A69S polymorphism and a less prominent venous response to flicker stimulation in patients with TC + CC genotypes in the *CFH* Y402H polymorphism. Carriers of at least one high-risk C allele in the Y402H variant are two to three times more likely to develop AMD than homozygous individuals with T alleles,⁵⁸ whereas individuals harboring TT alleles in the *ARMS* A69S polymorphism have up to a 10-fold increase in the risk of late AMD. Our results suggest that high-risk alleles in these polymorphisms are associated with decreased arterial or venous responses to flicker stimulation, however this does not necessarily mean a causal relationship. In population-based trials, the pathways surrounding venous retinal microcirculation maladaptation have demonstrated strong correlations with indicators of systemic inflammation,^{59,60} which corresponds to the function of *CFH* and *ARMS2*, as *CFH* is a crucial regulator of the complement system, whereas *ARMS2* might also play a role in the pro-inflammatory pathway.^{61,62} Although there have been several studies conducted in murine models on the role of *CFH* in retinal development and function,^{63,64} there is less evidence of the influence of *CFH* on retinal vessel parameters in humans. Only one study investigated retinal vessel dilation in healthy young carriers of the C risk allele at the Y402H polymorphism and found no abnormal flicker-induced retinal vessel dilation²⁸; however, the study included only 18 homozygous C carriers. We cannot exclude the possibility that the vascular response could alter over the lifespan of these individuals and be poorer when they develop AMD. To the best of our knowledge, our study provides the first evidence that high-risk alleles in the *CFH* Y402H and *ARMS2* A69S polymorphisms are associated with decreased arterial or venous responses to flicker stimulation in a population of patients with AMD.

CONCLUSIONS

Overall, we analyzed static and dynamic retinal vessel parameters in patients with AMD with a particular focus on morphological changes in the choroid, underlying vascular disorders, and high-risk AMD polymorphisms. Our findings suggest that retinal microcirculation appears to be associated with the genetic background, choroidal parameters, and clinical features of the patients with AMD.

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6.3 Influence of Clinical and Genetic Factors on the Progression of Age-Related Macular Degeneration: A 3-Year Follow-Up

Article

Influence of Clinical and Genetic Factors on the Progression of Age-Related Macular Degeneration: A 3-Year Follow-Up

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Abstract: The aim of the present study was to analyze the relationship of age-related macular degeneration (AMD) progression with clinical characteristics, demographic, and environmental risk factors that would affect disease development. In addition, the influence of three genetic AMD polymorphisms (CFH Y402H, ARMS2 A69S, and PRPH2 c.582-67T>A) on AMD progression was investigated. In total, 94 participants with previously diagnosed early or intermediate AMD in at least one eye were recalled for an updated re-evaluation after 3 years. The initial visual outcomes, medical history, retinal imaging data, and choroidal imaging data were collected to characterize the AMD disease status. Among the AMD patients, 48 demonstrated AMD progression, and 46 showed no disease worsening at 3 years. Disease progression was significantly associated with worse initial visual acuity (OR = 6.74, 95% CI = 1.24–36.79, $p = 0.03$) and the presence of the wet AMD subtype in fellow eyes (OR = 3.79, 95% CI = 0.94–15.2, $p = 0.05$). In addition, a higher risk of AMD progression appeared in the patients with active thyroxine supplementation (OR = 4.77, CI = 1.25–18.25, $p = 0.002$). The CC variant of CFH Y402H was associated with AMD advancement compared to the TC+TT phenotype (OR = 2.76, 95% CI: 0.98–7.79, $p = 0.05$). Identifying risk factors of AMD progression may lead to earlier intervention and better outcomes, preventing the expansion of the late stage of the disease.

Keywords: age-related macular degeneration (AMD); progression; CFH Y402H; ARMS2 A69S; PRPH2; choroid; thyroid hormones

1. Introduction

Macular degeneration is a known major cause of vision loss in people over 60 years of age, and its frequency increases with age [1]. The number of people living with age-related macular degeneration (AMD) was expected to reach 196 million worldwide in 2020 and is estimated to increase to 288 million by 2040 [2]. In developed countries, approximately 10% of the population over the age of 65 years and 25% over the age of 75 years have been diagnosed with different forms of AMD. It is further estimated that 85% of all AMD patients have early and intermediate AMD, while 15% of all AMD patients are affected by the advanced stages of the disease, including central geographic atrophy, active choroidal neovascularization, or inactive choroidal neovascularization [3]. Importantly, no treatment options are available for the early and intermediate stages of AMD. Intravitreal anti-VEGF therapy for wet AMD is also unsatisfactory because 25% of eyes achieve visual acuity of 0.1 or less within 5 years despite active treatment [4]. The lack of effective treatment is due, in part, to the complexity of the disease as not only multiple genetic loci

but also environmental risk factors have been shown to be involved in AMD pathology. During the last several decades, different large observational cohort- and population-based research studies have documented strong evidence supporting associations between AMD development and diverse risk factors, such as sociodemographic features, medical status, and selected biochemical variables [5,6]. However, there are inconsistencies among the literature data regarding the factors that may affect AMD progression. Therefore, we performed an analysis of demographic data, lifestyle data, medical history, and existing comorbidities combined with genetic risk factors for AMD and evaluated the relevance of such features for AMD progression over 3 years. All subjects who participated in this follow-up were submitted to multimodal retinal imaging and underwent a general medical questionnaire. The aim of the present study was to examine the relationship of AMD progression with clinical characteristics, demographic, and environmental risk factors that would affect disease development over a period of time. Furthermore, in 2018, our group were the first to report the relationship between the presence of the peripherin gene (PPH2) c.582-67T>A variant and the occurrence of AMD [7]. The peripherin gene (PRPH2) encodes specific proteins that are crucial for the proper formation and function of the outer segments of rods and cones [8]. Thus, we investigated the influence of three genetic AMD polymorphisms (CFH Y402H, ARMS2 A69S, and PRPH2 c.582-67T>A) and their effect on AMD deterioration.

2. Materials and Methods

2.1. Subjects and Initial Management

Initially, 244 patients with clinically diagnosed early or intermediate AMD in at least one eye were randomly selected from among the patients of the outpatient clinic of the 1st Ophthalmology Department in 2016–2017. The follow-up examination was planned for 2019–2020. Due to the COVID-19 pandemic and the massive disruption to healthcare, 150 patients did not turn up for a follow-up checkup within 3 years or refused to participate in the project altogether. Finally, 94 people were followed up, which was 38.5% of the originally recruited participants. Data on medical history, current drug use, smoking status, and physical activity from the enrolled subjects, with a particular focus on their history of clinical cardiovascular diseases, including physician-diagnosed heart and vascular diseases, were collected. We also assessed waist circumference [cm], waist/hip ratio (WHR), and body mass index (BMI) [weight (kg)/height (m)²] of all enrolled subjects. The reported average number of cigarettes smoked per day and the number of years the patient smoked were collected to calculate cumulative pack-years. Finally, with the help of a member of the research team, each participant completed the International Physical Activity Questionnaire (IPAQ), comprising seven questions regarding all types of physical activity (lasting 10 min or longer) in the previous week. Physical activity scores are presented as MET-min per week and were calculated as described previously [9]. Medical history was taken personally by a medical worker (doctor or trained nurse), on the basis of the data provided by patients and their medical records, including a statement from a primary care physician. In accordance with the tenets of the Declaration of Helsinki, a consent form was signed by all patients before trial enrolment.

2.2. Ophthalmologic Examination

The patients underwent a detailed ophthalmologic examination as follows: measurement of visual acuity using the logarithm of the minimum angle of resolution (LogMAR) charts; slit-lamp examination of the anterior and posterior eye segments; color fundus photography (CFP); and Goldmann applanation tonometry. Axial length and anterior chamber depth were assessed to eliminate known factors that interfere with reliable OCT image analysis. Additionally, fundus autofluorescence (FAF) and near-infrared reflectance (NIR) imaging examinations were performed for more accurate drusen visualization to allow correct determination of the AMD stage. In eyes where the presence of CNV could not be unequivocally excluded, fluorescence angiography was performed. Patients presenting

with ocular media opacity due to corneal, lens, or vitreous changes potentially influencing OCT scan quality were excluded. The exclusion criteria also encompassed ophthalmic and systemic conditions that may affect the choroidal thickness, such as high refractive errors (over ± 4 diopters of spherical equivalent), glaucoma, choroiditis, retinopathy (any type), vitreomacular changes (such as an epiretinal membrane), a history of retinal detachment, or serious ocular trauma. Eyes were excluded if they had cataract surgery in the previous 6 months, as well as any previous laser therapy or vitreoretinal surgery.

To obtain EDI-OCT images of the macular region, a $30^\circ \times 25^\circ$ volume acquisition protocol was used, achieving 61 cross-sectional B-scans (Spectralis, Heidelberg Engineering, Germany). The measurements were performed by an experienced technician after pupil dilation with a 1% tropicamide solution after 30 min of rest and at the same time of the day (between 9.00 and 11.00 a.m.). FAF images were obtained using an excitation blue light of 488 nm and a barrier filter beginning at 500 nm. High-contrast digital NIR images were acquired using the 815 nm diode laser of the Spectralis System. The images were captured at the time of acquisition of the macular OCT scans.

Grading of AMD based on the evaluation of color fundus and OCT images using the Ferris classification system was performed by the examining clinician [10]. The early AMD group included patients with medium drusen (63–125 μm) but without pigmentary abnormalities. The intermediate AMD group comprised patients who had large drusen or pigmentary abnormalities associated with at least medium drusen. Patients with lesions associated with neovascular AMD or geographic atrophy were classified as having late AMD. Geographic atrophy was defined by the presence of sharply demarcated atrophic lesions of the outer retina, resulting from loss of photoreceptors, RPE, and underlying choriocapillaris layer based on CFP, FAF, and NIR imaging [11]. Wet form AMD was diagnosed in eyes with features of pathological vessels that were confirmed by ophthalmoscopy, coherence tomography, or fluorescein angiography.

We analyzed the influence of the size and type of drusen on the risk of AMD progression. The largest drusen in the circle area with a diameter of 6 mm centered in the fovea was manually evaluated. The size of the drusen was estimated as the product of their height (the distance between Bruch's membrane and the RPE at the highest point of the druse) and their width at the base along the BrM. The type of drusen was determined based on its appearance in color fundus photography, OCT, and FAF images. Hard drusen were described as punctate, small-medium sized ($<125 \mu\text{m}$), and yellow-white lesions with discrete borders observed in CFP, OCT, and IR images. Soft drusen were identified as yellow-white mound-like elevations with blurred boundaries and gradually reduced density from the center to the periphery observed in CFP images, which corresponded to the moderately reflective dome-shaped sub-RPE deposits present on OCT scans [12,13]. Pachydrusen were identified when isolated or scattered yellow-white deposits, which had a better-defined and more complex outer border compared to regular round soft drusen, were observed in CFP images [14]. On the OCT scans, pachydrusen were located adjacent to the pachyvessels [15]. Subretinal drusenoid deposits (SDD) were recognized when discrete white-yellowish interlacing network deposits were present in the color photography, and they corresponded with a round or triangular, well-defined, hyper-reflective subretinal deposit accumulation of material that forms sharp peaks and may lead to abruption of the inner and outer photoreceptor segment layer on the OCT scans. On FAF and IR images, SDD were observed as an area of hyperautofluorescence/reflectance on the center of each reticular pseudodrusen surrounded by a hyporeflective halo, forming the characteristic appearance of the target [16,17].

The diagnosis of pachychoroid was based on the presence of subfoveal choroidal thickness $>350 \mu\text{m}$ or an extrafoveal focus that exceeded fovea choroidal thickness by at least $50 \mu\text{m}$ as well as the presence of pachyvessels identified on EDI-OCT b-scans [18–20]. Pachyvessels were defined as dilated outer choroidal vessels compressing the overlying choriocapillaris and Sattler's layers observed on cross-section EDI-OCT images [20–22]. Only good quality scans with well-defined choroid-sclera junction were further analyzed.

To estimate disease progression, all abovementioned ophthalmologic tests were repeated after 36 months. To identify the impact of specific systemic and ocular parameters on AMD deterioration, patients were assigned to one of two subgroups depending on the status of disease progression. AMD progression was defined as the change in disease stage from early to intermediate AMD as well as the presence of late-stage disease features in the eyes previously classified as early or intermediate in at least one eye. No AMD progression was established when none of the signs of a more advanced stage of disease were observed.

2.3. Genotyping

Venous blood samples (approximately 7.5 mL) collected in EDTA tubes were centrifuged (2000 rpm, 4 °C, 10 min), and red blood cells were lysed using ammonium chloride-based lysing buffer (BD Biosciences, Franklin Lakes, NJ, USA). Nucleated cells were then counted, and DNA isolation was subsequently performed with a total DNA isolation kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol. AMD-risk polymorphisms were genotyped as previously described [7]. In CFH, rs1061170, encoding a Y402H interchange, was genotyped by restriction analysis with EagI, HhaI, and Hsp92II enzymes. In ARMS2, LOC387715 rs10490924, encoding an A69S interchange, was determined by direct DNA sequence analysis using an Applied Biosystems 3130 XL instrument for DNA sequencing. The genetic analysis of the PRPH2 gene was performed by exon capturing with Molecular Inversion Probes (MIPs) and subsequent sequencing of amplified libraries with the Illumina NextSeq 500 system [7]. Molecular analysis was performed by the Genomed SA bioinformatic team as well as an outwards cooperating laboratory according to generally approved standards.

2.4. Statistical Analysis

Qualitative variables were compared between groups with Chi-squared or Fisher's exact tests. Because the distributions of most quantitative variables were significantly different from a normal distribution, the nonparametric Mann–Whitney test was used for their analysis. A logistic regression model adjusted for age and sex was used for multivariate analysis of potential predictors of AMD progression. Odds ratio (OR) and 95% confidence interval (95% CI) values were calculated for comparison of the AMD progression group to the no progression group, which was considered a reference. OR values for quantitative parameters reflected odds of AMD progression associated with an increase in a parameter by one unit (continuous variables) or class (rank variables). $p < 0.05$ was considered statistically significant.

3. Results

The follow-up data (36 months after the initial evaluation) were collected from 94 patients. Among the study subjects, 48 patients presented with AMD progression, and 46 patients showed no disease worsening.

3.1. Systemic Factors Associated with AMD Progression

We aimed to assess the effects of systemic factors on AMD progression. The data regarding demographic factors, medical history of the patients, and medication use are presented in Table 1. Patient age, sex, iris color, and AMD family history were not associated with disease advancement. There was also no association between the presence of selected, well-defined systemic diseases (such as hypertension, ischemic heart disease, cardiac infarction, peripheral artery disease, or limb ischemia) and AMD progression. Similarly, there was no association between medication use, except for thyroxine use, and disease development. Thyroxine use was a significant predictor of disease deterioration (OR = 3.75, 95% CI = 1.08–12.97, $p = 0.05$), and this association remained significant in multivariable logistic regression analysis adjusted for age, sex, and presence of ischemic heart disease (OR = 4.77; 95% CI = 1.25–18.25, $p = 0.002$).

Table 1. Characteristics of the study subjects. Disease progression with regard to demographic data and medical history. The data are presented as the mean $\pm$ SD or %.

Parameter	AMD Progression	No AMD Progression	OR (95% CI)	p-Value *
Number of subjects	48	46	—	—
Sex (male/female)	16/32	16/30	0.93 (0.39–2.22)	1.00
Patient's age [years] (min–max)	72.7 (56–84)	70.4 (54–85)	1.04 (0.98–1.11)	0.07
Iris colour (dark/light)	11/37	18/28	0.46 (0.19–1.15)	0.12
Education	Basic (%)	50%	1.03 (0.66–1.60)	0.99
	Vocational (%)	50%		
	Secondary (%)	52.5%		
	Higher (%)	51.7%		
AMD family history	46.7%	53.3%	0.81 (0.26–2.49)	0.78
Currently smoking	36.4%	52.6%	0.51 (0.14–1.94)	0.35
Formerly smoking	41.9%	58.7%	0.51 (0.21–1.19)	0.14
BMI (kg/m ²)	26.8 $\pm$ 4.2	27.0 $\pm$ 4.9		0.68
Physical activity (MET)	1624.5 $\pm$ 2079.3	1890.4 $\pm$ 2501.8		0.24
Medical history				
Hypertension	50%	50%	0.93 (0.38–2.30)	1.00
History of ischemic heart disease	55.6%	50.6%	1.22 (0.30–4.98)	1.00
History of myocardial infarction	60%	50.6%	1.46 (0.23–9.47)	1.00
History of peripheral artery disease	60%	50.6%	1.46 (0.23–9.47)	1.00
History of limb ischemia	60%	50.6%	1.46 (0.23–9.47)	1.00
Medications use				
Hypotensive drugs/vasodilators	50.8%	50%	1.03 (0.41–2.57)	1.00
Thyroxine	75%	44.4%	3.75 (1.08–12.97)	0.05
Steroids	50%	50%	1.00 (0.06–17.18)	1.00
Statins	48%	52%	0.87 (0.34–2.22)	0.82
NSAIDs	55.6%	49.3%	1.28 (0.45–3.69)	0.79
Cardiac medications/antiarrhythmic drugs	50%	50.6%	0.45 (0.10–1.97)	0.31
Antiasthmatic drugs	75%	49.4%	3.07 (0.30–31.74)	0.62
Antidepressants	75%	49.4%	3.07 (0.30–31.74)	0.62
Vitamins and antioxidants	50%	51.6%	0.94 (0.39–2.27)	1.00
Xanthines (lutein, zeaxanthin)	53.3%	44.8%	1.41 (0.57–3.47)	0.50
Omega-3 rich oils	51.9%	48.7%	1.14 (0.48–2.68)	0.83
Resveratrol	54.8%	46.81%	1.37 (0.59–3.21)	0.53

* Mann–Whitney/Chi-squared or Fisher's exact test. The statistically significant result is marked in bold.

3.2. Clinical Parameters Associated with AMD Progression

We next discriminated the initial clinical features of the disease that were linked with AMD advancement, e.g., visual acuity, choroidal thickness, retinal thickness, drusen type, drusen size, AMD stage, and wet AMD subtype in one eye. Patients with worse initial visual acuity scores were more likely to develop AMD progression (OR = 3.56, 95% CI = 0.96–13.1, $p = 0.04$) after 3 years of observation. This association remained significant in multivariate logistic regression analysis adjusted for age and sex (OR = 6.74, 95% CI = 1.24–36.79, $p = 0.03$).

Interestingly, in patients who initially presented with thinner choroids, AMD advancement was more likely in the follow-up (OR = 0.99, 95% CI = 0.99–1.00, $p = 0.049$). However, in a multivariate regression analysis by sex and age, this factor was not significantly associated with AMD progression (OR = 0.1, 95% CI = 0.99–1.0, $p = 0.12$). Neither the presence of pachychoroid features nor the coexistence of pachyvessels in patients with AMD were significantly associated with disease progression.

Among the clinical parameters, the wet AMD subtype in one eye was a particularly important predictor of disease deterioration in the other eye (OR = 4.17, 95% CI = 1.06–16.46, $p = 0.04$), and this association remained significant in multivariate analysis adjusted for age and sex (OR = 3.79, 95% CI = 0.94–15.2, $p = 0.05$). There was no association of disease progression with initial retinal thickness, drusen size, and type or AMD stage. Detailed results are presented in Tables 2 and 3.

Table 2. State of disease progression with regard to the baseline clinical characteristics of the study subjects. The data are presented as the mean $\pm$ SD.

Clinical Parameter	AMD Progression	No AMD Progression	OR (95% CI)	<i>p</i> -Value *
Visual acuity (logMAR)	0.49 $\pm$ 0.35	0.35 $\pm$ 0.31	3.56 (0.96–13.10)	0.04
Choroidal thickness in the foveal region (μ m)	207.5 $\pm$ 84.3	245.4 $\pm$ 96.6	0.995 (0.990–1.00)	0.0497
Pachychoroid (Y/N)	4/44	1/44	4.00 (0.42–38.37)	0.36
Pachyvessels (Y/N)	19/29	19/26	0.90 (0.38–2.07)	0.83
Retinal thickness in the central ETDRS area (μ m)	317.4 $\pm$ 80.8	300.6 $\pm$ 55.0	1.00 (1.00–1.00)	0.52
Drusen size	2.43 $\pm$ 0.65	2.43 $\pm$ 0.73	0.99 (0.54–1.81)	0.80
Soft drusen	65%	69%	0.82 (0.34–1.98)	0.83
Hard drusen	25%	22%	1.17 (0.44–3.08)	0.81
Subretinal drusenoid deposits (SDD)	42%	24%	2.21 (0.90–5.44)	0.12
Pachydrusen	10%	11%	0.93 (0.25–3.52)	1.00
AMD stage	Early	8.7%	1.03 (0.56–1.90)	0.18
	Intermediate	56.5%		
	Late	34.8%		
Wet AMD in one eye (yes [%])	37.5%	32.6%	4.17 (1.06–16.46)	0.04

* Mann–Whitney/Fisher’s exact test. Statistically significant results are marked in bold.

Table 3. Multivariate logistic regression analysis of patients considering the presence of AMD progression as a dependent variable, adjusted for age and sex.

Independent Variables	OR	−95% CI	+95% CI	<i>p</i> -Value
Thyroxine supplementation	6.42	1.6	25.79	0.008
Visual acuity (logMAR)	5.1	1.05	24.69	0.039
Choroidal thickness in the foveal region (μ m)	0.1	0.99	1.0	0.12
Wet AMD in one eye (yes [%])	0.99	4.49	20.3	0.048
CFH	2.76	0.98	7.79	0.05

Statistically significant results are marked in bold.

Next, to test whether patients with AMD who progressed to late stage differ from those who progressed to intermediate stage, we performed a comparative analysis of the two subgroups. The subgroup that progressed to the late stage consisted of 35 patients, while the subgroup that progressed to the intermediate stage consisted of 13 participants. There were no significant differences between the groups in terms of age, gender distribution,

and the coexistence of systemic vascular diseases and their risk factors. Accordingly, the analysis showed no significant differences in thyroxine supplementation ($p = 0.73$), initial visual acuity ($p = 0.34$), and central choroidal thickness ($p = 0.11$) between the groups. Detailed results are available in Tables S2–S4 in Supplementary Materials.

3.3. Genetic Factors Influencing AMD Progression

Finally, we analyzed whether the genetic background of the patients influences AMD progression (Table 3). We selected three single nucleotide polymorphisms (SNPs) for this analysis, namely, CFH Y402H, ARMS2 A69S, and PRPH2 c.582-67T>A, which were strong AMD risk factors in our previous study [7].

In multivariable logistic regression analysis adjusted for age, sex, and initial visual acuity, the CC variant of CFH Y402H was significantly associated with AMD advancement compared to the TC + TT phenotype (OR = 2.76, 95% CI: 0.97–7.79, $p = 0.05$). The tested variants of the ARMS2 and PRPH2 genes did not show a significant relationship with the progression of AMD. Detailed results are presented in Tables 3 and 4.

Table 4. State of disease progression with regard to the genetic background of patients.

Tested SNP	Genotype	% of Patients with AMD Progression	% of Patients without AMD Progression	<i>p</i> -Value *	Genotypes or Alleles	OR (95% CI)	<i>p</i> -Value *
CFH Y402H	TT	41.2%	58.8%	0.19	CC + TC vs. TT	1.47 (0.50–4.36)	0.48
					CC vs. TC + TT	2.40 (0.92–6.21)	0.069
	TC	41.7%	58.3%		CC vs. TT	2.43 (0.70–8.41)	0.16
					C vs. T allele	1.69 (0.90–3.18)	0.10
	CC	63.0%	37.0%		TC vs. TT	1.02 (0.32–3.29)	0.97
					CC vs. TC	2.38 (0.85–6.63)	0.09
ARMS2 A69S	GG	46.9%	53.1%	0.69	TT + GT vs. GG	1.09 (0.44–2.65)	0.85
					TT vs. GT + GG	1.91 (0.42–8.60)	0.39
	GT	46.3%	53.7%		TT vs. GG	1.89 (0.38–9.27)	0.43
					T vs. G allele	1.18 (0.62–2.26)	0.61
	TT	62.5%	37.5%		GT vs. GG	0.98 (0.39–2.47)	0.96
					TT vs. GT	1.93 (0.41–9.16)	0.40
PRPH2 c.582-67T>A (rs3818086)	TT	42.1%	57.9%	0.13	AA + TA vs. TT	1.37 (0.49–3.88)	0.55
					AA vs. TA + TT	0.40 (0.14–1.20)	0.098
	TA	58.1%	41.9%		AA vs. TT	0.63 (0.17–2.40)	0.50
					A vs. T allele	0.82 (0.44–1.52)	0.53
	AA	31.6%	68.4%		TA vs. TT	1.91 (0.64–5.70)	0.24
					AA vs. TA	0.33 (0.11–1.04)	0.054

* Chi-squared test.

4. Discussion

In our cohort, the multivariate logistic regression analyses failed to identify any significant differences in the current and past medical status or demographic characteristics between those who presented with disease progression and those who showed no AMD worsening. Similarly, we found no association between medication use, except for thyroxine use, and disease advancement. Thyroxine use was a significant predictor of AMD progression. Thyroid dysfunction (both hypo- and hyperthyroidism) has been previously analyzed in the context of an increased risk of AMD [23]. Interestingly, a positive associ-

ation between levels of fT4 and macular degeneration was observed in the prospective, population-based Rotterdam study performed on approximately 15,000 subjects recruited at different ages and observed over more than two decades [24]. Importantly, even in restricting analyses to euthyroid individuals with a normal range of fT4, higher fT4 values were still associated with an increased risk of AMD development in the study. Furthermore, Farvadin et al. showed a relationship between serum free T4 levels and wet AMD. [25] Gopinath et al., in multivariate regression analysis adjusted for age, sex, current smoking, fish consumption, and presence of high risk CFH and ARMS2 variants, demonstrated that overt hyperthyroidism (low TSH and high free T4 levels) in older patients is independently associated with a 3-fold increase in the risk of AMD development; however, they did not verify any significant positive association between serum free T4 levels and incident AMD in their study. Researchers have also found an association between the development of AMD and thyroxine supplementation in both current and past users [26]. The Beaver Dam Eye study also reported an association between therapeutic thyroid hormone use and early AMD, while a case-control study of the Age-Related Eye Disease Study Research Group reported an association between thyroxine supplementation and a higher risk of geographic atrophy [27,28]. However, there is no consensus among researchers regarding the association between thyroid medications and the risk of AMD. While the meta-analysis by Xu et al. published in 2021 confirmed that thyroid disorders may increase the risk of AMD, no link to the use of thyroid medications was found [29]. Thyroid hormone signaling regulates retinal development and maintenance via regulating cone maturation and expression of cone opsins [30]. Hyperthyroidism has been observed to accelerate the basal metabolic rate and oxidative metabolism by inducing mitochondrial enzymes, which may induce local cellular oxidative stress [31]. Recent molecular and in vivo studies have implicated thyroid hormone signaling in cone photoreceptor viability [32]. It has been discovered that inhibition of thyroid hormone signaling protects photoreceptors from oxidative stress-induced cell death in a mouse model of age-related macular degeneration [33]. However, there is evidence that thyroid hormones may negatively influence retinal pigment epithelium (RPE) cells, resulting in further degeneration of photoreceptors [34]. Furthermore, it has been observed that deficiency of type 2 iodothyronine deiodinase, which is the crucial enzyme for fT4 cellular metabolism, reduces cellular necroptosis and the activity of oxidative stress responses in retinas in a mouse model of Leber congenital amaurosis [35]. These findings may serve as molecular evidence for the causal inference between macular/retinal degeneration and increased concentrations of thyroid hormones due to natural or pharmacological reasons, implicating the specific pathophysiologic role of these hormones in the progression of AMD. Importantly, thyroid dysfunction is related to vascular function, atherosclerosis, and cardiovascular disease [36]. Both oxidative stress and vascular dysfunction make choroid and retinal tissue more susceptible to the development of degenerative changes. Taken together, these findings warrant the need for further research to investigate the biological relationship between AMD progression and thyroid hormone imbalance, especially because it is a modifiable risk factor. We did not find the AMD progression to be related with baseline clinical characteristics such as retinal and choroidal thickness, drusen size, and drusen type. However, we observed that worse initial visual acuity and wet AMD subtype in one eye significantly correlated with AMD advancement. Progression from early to late AMD is associated with severe and irreversible vision loss [37]. Therefore, deteriorating vision and its decreased acuity are important outcome measures for defining AMD progression in prospective clinical trials [38]. In a group of more than 40,000 patients from Great Britain with a diagnosis of early/intermediate AMD, Chakravarthy et al. observed that better VA was significantly associated with a decreased rate of progression to advanced AMD [39]. Studies of wet AMD (wAMD) eyes treated with AVEGF have shown that patients with good initial visual acuity are more likely to maintain good vision during 2 years of follow-up [40,41]. Some researchers have emphasized the relationship between VA and choroidal thickness in both healthy and AMD eyes. [42–44] Baseline CT is also regarded as a predictive factor of VA outcome in patients with wAMD. In a case series study, greater

initial subfoveal choroidal thickness was found to be associated with a better anatomic and functional clinical outcome in eyes with wAMD after intravitreal aflibercept therapy [45]. Eyes with an initially thinner choroid thickness may have a lower “choroid reserve.” In the age-related decrease in choroidal flow, impaired nutrition of the RPE, as a result of insufficient blood supply to the choroid, leading to progressive loss of photoreceptors and deterioration of visual acuity, may appear earlier or be more pronounced compared to eyes with higher CT. However, some studies have reported no correlation between choroidal thinning and worsening retinal function in AMD eyes [46]. In the current study, subfoveal choroidal thickness was not risk a factor of AMD progression in multivariate regression analysis. Several studies have investigated whether decreased choroidal thickness is a risk factor for progression to advanced AMD. Lee et al. reported that a reduced subfoveal CT correlates with a higher progression rate of nonexudative AMD. [43] In another study, researchers have identified that a thinner choroid is a risk factor for the development of new onset atrophy in dry AMD eyes with drusenoid lesions. [47] Similarly, Fan et al. reported that AMD patients with decreased subfoveal CT at baseline are more likely to develop advanced macular atrophy at the 18-month follow-up [48]. Another prospective study has reported that irregular choroidal vessels are predictive of earlier development of both late disease forms, namely, GA and neovascular AMD [49]. However, not all studies clearly indicate a relationship between AMD progression and the presence of a thinner choroid. In the study, Manjunath et al. showed that patients with AMD have a wide range of choroidal thicknesses in different stages and subtypes of the disease [50]. Similarly, Keenan et al. showed that in AMD, the choroid undergoes bidirectional changes as it becomes thicker as drusen develops in the early stages of the disease and thinner in the later stages [51]. It seems that the most valuable studies on the role of the choroid in AMD will be those based on long-term observation of the patient in all phases of the disease and analyses, taking into account local and systemic confounding factors.

Interestingly, Sakurada et al. reported that the unilateral neovascular AMD subtype is a particularly important predictor of disease deterioration in the other eye during the mean follow-up period of 36 months [52]. Gangnon et al. stated that severe AMD in the study eye is associated with an increased incidence of AMD and accelerated progression in its fellow eye [53]. Similarly, the prospective, longitudinal study by Silva et al. reported a 3-year progression from early to late exudative AMD in fellow eyes of patients with wet AMD in the other eye [54]. Moreover, patients with early/intermediate AMD and a diagnosis of CNV in the fellow eye have the fastest rate of disease progression. [45,55] These findings strongly support the results obtained from the present study.

It has been confirmed that AMD has a strong genetic basis. To date, more than half of the genomic heritability of AMD can be explained by genetic variants in 34 known AMD-associated loci, which are mostly involved in the regulation of complement system activation [55]. Common SNPs within CFH, including rs1061170 that encodes a tyrosine to histidine substitution at position 402 (Y402H), were first identified as major susceptibility variants for AMD [56]. Multiple prospective studies have confirmed that the minor allele of this variant is a risk allele for the incidence of early AMD (HR 1.2–2.3) as well as for the progression to both late forms of AMD, such as wet AMD (HR 1.48–2.5) and geographic atrophy (HR 1.38–3.65) [57–59]. In the present study, the multivariable logistic regression analysis adjusted for age, sex, and visual acuity revealed that among the tested AMD-associated loci, the CC variant of CFH Y402H was associated with AMD advancement over a 3-year period with borderline significance. This finding confirmed data from the newest meta-analyses based on pooled studies, including 17,174 individuals 45 years of age or older participating in 6 population-based cohort studies, 2 clinic-based studies and 1 case-control study, demonstrating that CFH risk variants are the leading drivers of late forms of AMD [60,61]. In contrast, the PRPH2 variant c.582-67T>A (rs3818086) showed no association with AMD progression. This may indicate that this gene plays a role in the pathomechanism associated with the onset of the disease, but not in its progression, which requires further research in a larger group of patients.

5. Conclusions

In conclusion, the 3-year follow-up of AMD patients recruited to a single-center, prospective study confirmed that worse visual acuity as well as the presence of unilateral CNV are a strong risk factor for AMD progression. The presence of this feature implies particular vigilance in patient management and in planning follow-up visits. Identifying modifiable risk factors for disease progression, such as thyroid hormone imbalance, is particularly valuable as a potential target of prevention and requires further study in a larger and more homogeneous group of patients.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/jcm12051963/s1>, Table S1: Clinical characteristics of the follow up participants and lost in follow-up subjects. The data are presented as the mean $\pm$ SD or %. Statistically significant results are marked in bold.; Table S2: Clinical characteristics of the subjects according to progression to intermediate or late AMD stage. The data are presented as the mean $\pm$ SD or %. Statistically significant results are marked in bold.; Table S3: Differences in clinical parameters between the eyes that progressed to intermediate and to late stages of AMD. The data are presented as the mean $\pm$ SD. Statistically significant results are marked in bold.; Table S4: Differences in genetic factors between the eyes that progressed to intermediate and to late stages of AMD; Table S5: Clinical characteristics of the AMD subjects. Late-stage baseline eyes were excluded from the analysis.; Table S6: Differences in clinical features between eyes with and without progression of AMD. Eyes with baseline late AMD were excluded from analysis.; Table S7: State of disease progression with regard to the genetic background of patients. Eyes with baseline late AMD excluded from analysis.

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SUPPLEMENTARY MATERIALS

Table S1. Clinical characteristics of the follow up participants and lost in follow-up subjects. The data are presented as the mean $\pm$ SD or %. Statistically significant results are marked in bold.

Parameter	Follow up group	Lost in follow up group	p-value*
Number of subjects	94	150	—
Sex (male / female)	32/62	52/98	1.0
Patient's age [years] (min-max)	71.56 (54-85)	73.3 (49-91)	0.08
Iris colour (dark/light)	29/65	35/113	0.23
Education	Basic (%)	10.75	0.59
	Vocational (%)	15.05	
	Secondary (%)	43.01	
	Higher (%)	31.18	
AMD family history	15.96	12.84	0.57
Currently smoking	12.36	14.07	0.84
Formerly smoking	48.31	44.44	0.59
BMI (kg/m ²)	26.88 (4.53)	27.05 (3.99)	0.62
Physical activity (MET)	1585.09 (2243.62)	1755.99(2288.65)	0.26
Hypertension	67.42	62.96	0.57
History of ischemic heart disease	10.23	15.56	0.32
History of peripheral artery disease	5.68	6.67	1.0
History of limb ischemia	5.68	3.7	0.52
Hypotensive drugs/vasodilators	68.54	64.44	0.57
Thyroxine	18.18	11.85	0.24
Steroids	2.27	2.22	1.0
Statins	28.09	25.93	0.76
NSAIDs	20.22	20.0	1.0
Cardiac medications/antiarrhythmic drugs	8.99	14.81	0.22
Antiasthmatic drugs	4.49	8.15	0.41
Antidepressants	4.49	4.48	1.0
Vitamins and antioxidants	65.17	42.22	0.001
Xanthines (lutein, zeaxanthin)	67.42	54.81	0.07
Omega-3 rich oils	58.43	41.04	0.013
Resveratrol	47.19	34.81	0.07

* Mann–Whitney/Chi-squared or Fisher's exact test.

Table S2. Clinical characteristics of the subjects according to progression to intermediate or late AMD stage. The data are presented as the mean $\pm$ SD or %. Statistically significant results are marked in bold.

Parameter	Progression to intermediate stadium	Progression to late stadium	p-value*
Number of subjects	13	35	
Patient's age [years] (min-max)	70.7 (56-83)	74 (58-84)	0.31
Iris color (dark/light)[%]	30.8/69.2	20/80	0.46
Education	Basic (%)	5.7	0.08
	Vocational (%)	14.3	
	Secondary (%)	42.9	
	Higher (%)	37.1	
AMD family history (%)	7.7	17.1	0.46
Currently smoking	7.7	9.4	1.0
Formerly smoking	23.1	46.9	0.19
BMI (kg/m ²)	27.63 (5.15)	26.41 (3.82)	0.56
Physical activity (MET)	2534.42(3079)	1254(1406.6)	0.1
Medical history			
Hypertension	69.2	65.6	1.0
History of ischemic heart disease	15.4	9.4	0.62
History of peripheral artery disease	7.7	6.25	1.0
History of limb ischemia	7.7	6.25	1.0
Medications use			
Hypotensive drugs/vasodilators	69.2	68.8	1.0
Thyroxine	30.8	25.8	0.73
Steroids	0	3.1	1.0
Statins	30.8	25	0.72
NSAIDs	23.1	22.9	1.0
Cardiac medications/antiarrhythmic drugs	23.1	3.1	0.07
Antiasthmatic drugs	0	9.4	0.55
Antidepressants	0	9.4	0.55
Vitamins and antioxidants	53.9	68.8	0.5
Xanthines (lutein, zeaxanthin)	38.5	84.4	0.004
Omega-3 rich oils	30.8	71.9	0.02
Resveratrol	23.1	62.5	0.02

* Mann–Whitney/Chi-squared or Fisher's exact test.

Table S3. Differences in clinical parameters between the eyes that progressed to intermediate and to late stages of AMD. The data are presented as the mean $\pm$ SD. Statistically significant results are marked in bold.

Clinical parameter	Progression to intermediate stadium	Progression to late stadium	p-value*
Visual acuity (logMAR)	0.42 (0.39)	0.51 (0.34)	0.34
Choroidal thickness in the foveal region (μ m)	238.23 (86.5)	196.07 (81.8)	0.11
Pachychoroid (Y/N)%	7.69/92.31	8.57/91.43	1.0
Pachyvessels (Y/N) %	38.5/61.54	40/60	1.0
Retinal thickness in the central ETDRS area (μ m)	332.04 (96.6)	311.95 (75)	0.52
Soft drusen	61.54/38.46	65.71/ 34.29	1.0
Hard drusen	38.46/61.54	20/80	0.26
Subretinal drusenoid deposits (SDD)	8.33/ 91.67	54.29/45.7	0.007
Pachydrusen	23.08/76.92	5.71/94.29	0.12

* Mann–Whitney/Fisher's exact test.

Table S4. Differences in genetic factors between the eyes that progressed to intermediate and to late stages of AMD.

Tested SNP	Genotype	% of patients with progression to intermediate stage	% of patients with progression to late stage	p-value*	Genotypes or alleles	OR (95% CI)	p-value*
CFH Y402H	TT	48.86	57.14	0.29	CC+TC vs TT	1.91(0.36-10.32)	0.44
					CC vs TC+TT	2.32 (0.71-14.61)	0.12
	TC	40.0	60.0		CC vs TT	3.5(0.5-24.56)	0.2
					C vs T allele	2.18 (0.81-5.82)	0.12
	CC	17.65	82.35		TC vs TT	1.13 (0.62-15.71)	0.9
ARMS2 A69S				0.44	CC vs TC	3.11 (0.62-15.71)	0.16
	GG	40.0	60.0		TT+GT vs GG	2.0 (0.5-7.0)	0.32
					TT vs GT+GG	0.63 (0.09-4.33)	0.63
	GT	21.05	78.95		TT vs GG	1.0 (0.13-7.89)	1.0
					T vs G allele	1.27(0.46-3.49)	0.64
PRPH2 c.582-67T>A (rs3818086)	TT	60.0	60.0	0.32	GT vs GG	2.5 (0.55-11.33)	0.23
					TT vs GT	0.4 (0.05-3.27)	0.38
	TT	12.5	87.5		AA+TA vs TT	0.26 (0.03-2.39)	0.32
					AA vs TA+TT	0.38 (0.06-2.21)	0.27
	TA	32.0	68.0		AA vs TT	0.14 (0.01-2.0)	0.12
					A vs T allele	0.53 (0.2-1.4)	0.2
	AA	50.0	50.0		TA vs TT	0.3 (0.03-2.9)	0.28
					AA vs TA	0.47 (0.08-2.87)	0.41

* Chi-squared test.

Table S5. Clinical characteristics of the AMD subjects. Late-stage baseline eyes were excluded from the analysis. The data are presented as the mean $\pm$ SD or %.

Parameter	AMD progression	No AMD progression	p-value*
Number of subjects	27	24	-
Patient's age [years] (min-max)	70.04 (56-81)	69.78 (54-83)	0.77
Iris colour (dark/light)	20.83/79.17	44.44/55.56	0.14
Education	Basic (%)	11.11	0.75
	Vocational (%)	14.81	
	Secondary (%)	48.15	
	Higher (%)	25.93	
AMD family history	25.0	11.11	0.28
Currently smoking	4.35	15.38	0.35
Formerly smoking	43.48	53.85	0.57
BMI (kg/m ²)	26.84 (4.5)	26.34 (4.6)	0.54
Physical activity (MET)	1609.11 (1504.3)	2079.94 (3104.6)	0.86
Hypertension	61.54	65.22	1.0
History of ischemic heart disease	12.0	8.7	1.0
History of peripheral artery disease	0	8.7	0.22
History of limb ischemia	0	8.7	0.22
Hypotensive drugs/vasodilators	65.22	57.69	0.77
Thyroxine	69.23	30.77	0.1
Steroids	3.85	0	1.0
Statins	30.43	30.77	1.0
NSAIDs	26.09	15.38	0.48
Cardiac medications/ antiarrhythmic drugs	8.7	11.54	1.0
Antiasthmatic drugs	4.35	0	0.5
Antidepressants	4.35	0	0.5
Vitamins and antioxidants	73.91	69.23	0.76
Xanthines (lutein, zeaxanthin)	69.57	57.69	0.55
Omega-3 rich oils	60.87	53.85	0.77
Resveratrol	52.17	38.46	0.4

* Mann–Whitney/Chi-squared or Fisher's exact test.

Table S6. Differences in clinical features between eyes with and without progression of AMD. Eyes with baseline late AMD were excluded from analysis. The data are presented as the mean $\pm$ SD or %.

Clinical parameter	AMD progression	No AMD progression	p-value*
Visual acuity (logMAR)	0.39 (0.31)	0.24 (0.18)	0.24
Choroidal thickness in the foveal region (μ m)	217.6 (83.05)	258 (93.93)	0.08
Pachychoroid (%)	12.50/	3.85	0.34
Pachyvessels (%)	46.15	37.50	0.58
Retinal thickness in the central ETDRS area (μ m)	269.67 (25.62)	272.7 (25.6)	0.82
Soft drusen (%)	58.33	69.23	0.56
Hard drusen (%)	45.83	19.23	0.07
Subretinal drusenoid deposits (SDD) (%)	25.0	14.81	0.49
Pachydrusen (%)	12.5	11.54	1.0
AMD stage	Early (%)	14.81	0.2
	Intermediate (%)	85.19	

* Mann–Whitney/Fisher’s exact test.

Table S7. State of disease progression with regard to the genetic background of patients. Eyes with baseline late AMD excluded from analysis. Statistically significant results are marked in bold.

Tested SNP	Genotype	% of patients with AMD progression	% of patients without AMD progression	p-value*	Genotypes or alleles	OR (95% CI)	p-value*
CFH Y402H	TT	37.50	62.50	0.8	CC+TC vs TT	1.4 (0.29–6.8)	0.67
					CC vs TC+TT	1.5 (0.44–5.09)	0.52
	TC	41.18	58.82		CC vs TT	1.67 (0.3–9.16)	0.56
					C vs T allele	1.37 (0.57–3.32)	0.48
	CC	50.0	50.0		TC vs TT	1.17 (0.21–6.56)	0.86
ARMS2 A69S				0.69	CC vs TC	1.43 (0.38–5.44)	0.6
	GG	40.0	60.0		TT+GT vs GG	1.27 (0.38–4.22)	0.7
					TT vs GT+GG	1.82 (0.24–33.7)	0.4
	GT	42.86	57.14		TT vs GG	3.0 (0.23–38.87)	0.39
					T vs G allele	1.34 (0.54–3.33)	0.53
PRPH2 c.582-67T>A (rs3818086)	TT	66.67	33.33	0.38	GT vs GG	1.13 (0.32–3.9)	0.85
					TT vs GT	2.67 (0.21–34.2)	0.44
	TA	52.17	47.83		AA+TA vs TT	2.51 (0.56–11.16)	0.23
					AA vs TA+TT	0.84 (0.2–3.55)	0.82
	AA	40.0	60.0		AA vs TT	1.78 (0.28–11.12)	0.54
					A vs T allele	1.3 (0.56–3.04)	0.17
					TA vs TT	2.91 (0.61–13.83)	0.17
					AA vs TA	0.61 (0.14–2.76)	0.52

* Chi-square test.

7 Wnioski

1. Parametry opisujące anatomię naczyniówki u pacjentów z AMD pozostają w związku z obrazem klinicznym i stadiem choroby. Może to wskazywać na istotną rolę zaburzeń krążenia naczyniówkowego w patogenezie schorzenia.
2. Współistnienie ogólnoustrojowych schorzeń o podłożu naczyniowym jest czynnikiem związanym ze zmniejszoną grubością i objętością naczyniówki w oczach z AMD. Można zatem wnioskować, iż układ naczyniówkowy w oczach z AMD jest znacznie bardziej podatny na uszkodzenie w obecności ogólnoustrojowych chorób krążenia.
3. Obecność genetycznych wariantów ryzyka determinuje stan czynnościowy mikrokrażenia siatkówkowego w oczach z AMD. Reaktywność naczyń siatkówki koresponduje z grubością i objętością naczyniówki, co wskazuje na funkcjonalną łączność obu łożysk naczyniowych w przebiegu choroby.
4. Schorzenia układu naczyniowego oraz parametry funkcjonalne i morfologiczne siatkówki i naczyniówki nie stanowią niezależnych wykładników progresji choroby. Do bezpośrednich czynników ryzyka postępu choroby należą: wiek, przyjmowanie tyroksyny, obecność wysiękowej postaci AMD w oku towarzyszącym oraz predyspozycja genetyczna.

8 Streszczenie rozprawy doktorskiej w języku polskim

AMD jest postępującą degeneracyjną chorobą centralnej siatkówki, która jest główną przyczyną ślepoty w krajach rozwiniętych. AMD jest uznawane za chorobę o złożonym mechanizmie, u podstaw której leży podatność genetyczna związana z obecnością określonych wariantów genowych. Zgodnie z teorią hemodynamiczną, rozwój AMD jest konsekwencją nieprawidłowego przepływu krwi w rejonie plamki żółtej. Badania naukowe wykazują cechy zaburzonej homeostazy naczyniowej u pacjentów z AMD, aczkolwiek czynnik pierwotnie inicjujący te zmiany, jak również wpływ czynników naczyniowych na rozwój odmiennych fenotypowo postaci klinicznych oraz na dynamikę progresji choroby nie został dotychczas określony. W niniejszych trzech pracach oryginalnych poddano ocenie wybrane parametry naczyniowe w patogenezie AMD, ze szczególnym uwzględnieniem mikrokrażenia siatkówkowego oraz naczyniówkowego. Postawiono następujące cele pracy:

1. Ocena parametrów naczyniówki w obrazie SD-OCT, takich jak grubość, objętość oraz wskaźnik unaczynienia naczyniówki, celem zobrazowania potencjalnych różnic w morfologii tkanki naczyniowej między oczami z AMD (z uwzględnieniem odmiennych podtypów klinicznych choroby), a grupą kontrolną.
2. Pomiar średnicy oraz reaktywności naczyń siatkówki w oczach pacjentów z AMD w odniesieniu do grupy kontrolnej
3. Analiza korelacji pomiędzy pomiarami opisującymi morfologię naczyniówki, wskaźnikami średnicy naczyń siatkówki, parametrami czynnościowymi mikrokrażenia siatkówkowego a postacią kliniczną, stadium zaawansowania choroby, współistnieniem schorzeń układu sercowo-naczyniowego oraz obecnością określonych polimorfizmów wysokiego ryzyka u pacjentów z AMD.
4. Zidentyfikowanie czynników ryzyka progresji AMD, na przestrzeni 3-letniego okresu obserwacji, z uwzględnieniem udziału czynników naczyniowych w tym procesie.

Do badań włączono pacjentów z AMD w różnym stadium i o różnorodnym obrazie klinicznym choroby oraz osoby zdrowe, stanowiące grupę kontrolną. Wszyscy uczestnicy badania zostali poddani obszernemu badaniu okulistycznemu, zebrano od nich również szczegółowy wywiad medyczny. Stosując multimodalne techniki obrazowania siatkówki i naczyniówki przeprowadzono klasyfikację stopnia zaawansowania AMD, scharakteryzowano różne typy druzów (druzy miękkie, podsiatkówkowe złogi druzoidalne, pachydruzy) oraz

oceniono morfologię naczyniówki pod kątem obecności cech pachychoroid. Poddano analizie parametry grubości, objętości oraz wskaźnik unaczynienia naczyniówki, przeprowadzono także statyczną i dynamiczną ocenę naczyń tętniczych i żylnych w oparciu o analizator naczyń siatkówkowych. Uzyskane wyniki zestawiono z grupą kontrolną zdrowych ochotników. Przeprowadzono analizę w kontekście współwystępowania określonych wariantów genetycznych oraz systemowych schorzeń naczyniowych oraz ich czynników ryzyka. W celu określenia czynników determinujących progresję AMD pacjentów poddano 3-letniej obserwacji.

W przedstawionym cyklu prac oryginalnych wykazano, iż grubość, objętość oraz wskaźnik unaczynienia naczyniówki różnią między oczami prezentującymi odmienne postaci kliniczne oraz stopień zaawansowania AMD. Parametry te były bardziej zredukowane w oczach pacjentów ze współistniejącymi schorzeniami naczyniowymi. Średnica i reaktywność naczyń siatkówki nie były istotnie różne w oczach z AMD w porównaniu do oczu zdrowych, obserwowano jednak stopniową redukcję wskaźnika tętniczo-żylnego wraz ze wzrostem stadium choroby. Wykazano również, iż parametry dynamicznej analizy naczyń siatkówki korelują z grubością i objętością naczyniówki, a także zależą od obecności określonych wariantów genetycznych wysokiego ryzyka AMD. Wykazano, iż niezależnymi czynnikami powiązanymi z progresją choroby w 3-letnim okresie obserwacji są: wiek, suplementacja tyroksyny, obecność wysiękowej postaci AMD w oku towarzyszącym oraz predyspozycja genetyczna.

Na podstawie cyklu prac sformułowano następujące wnioski:

1. Parametry opisujące anatomię naczyniówki u pacjentów z AMD pozostają w związku z obrazem klinicznym i stadium choroby. Może to wskazywać na istotną rolę zaburzeń krążenia naczyniówkowego w patogenezie schorzenia.
2. Współistnienie ogólnoustrojowych schorzeń o podłożu naczyniowym jest czynnikiem związanym ze zmniejszoną grubością i objętością naczyniówki w oczach z AMD. Można zatem wnioskować, iż układ naczyniówkowy w oczach z AMD jest znacznie bardziej podatny na uszkodzenie w obecności ogólnoustrojowych chorób krążenia.
3. Obecność genetycznych wariantów ryzyka determinuje stan czynnościowy mikrokrażenia siatkówkowego w oczach z AMD. Reaktywność naczyń siatkówki koresponduje z grubością i objętością naczyniówki, co wskazuje na funkcjonalną łączność obu łożysk naczyniowych w przebiegu choroby.
4. Schorzenia układu naczyniowego oraz parametry funkcjonalne i morfologiczne siatkówki i naczyniówki nie stanowią niezależnych wykładników progresji choroby. Do

bezpośrednich czynników ryzyka postępu choroby należą: wiek, przyjmowanie tyroksyny, obecność wysiękowej postaci AMD w oku towarzyszącym oraz predyspozycja genetyczna.

9 Streszczenie rozprawy doktorskiej w języku angielskim

AMD is a progressive degenerative disease of the central retina that is the leading cause of blindness in developed countries. AMD is considered a disease with a complex mechanism, which is based on genetic susceptibility associated with the presence of specific gene variants. According to hemodynamic theory, the development of AMD is a consequence of abnormal blood flow in the macular region. Numerous studies showed features of impaired vascular homeostasis in patients with AMD, although the primary factor initiating these changes, as well as the impact of vascular factors on the development of phenotypically different clinical forms and on the dynamics of disease progression, has not been determined so far. In these three original papers, selected vascular parameters in the pathogenesis of AMD were assessed, with particular emphasis on the retinal and choroidal microcirculation. The following study objectives were defined:

1. Evaluation of choroidal parameters on SD-OCT, including choroidal thickness, volume and vascularity index, to visualize potential differences in vascular tissue morphology between AMD eyes (in different clinical subtypes of the disease) and controls.
2. Measurement of the diameter and reactivity of the retinal vessels in the eyes of patients with AMD in relation to the control group.
3. Analysis of correlations between measurements describing choroidal morphology, retinal vessel diameter equivalents, functional parameters of retinal microcirculation and the clinical form, stage of the disease, coexistence of cardiovascular diseases and the presence of certain high-risk polymorphisms in patients with AMD.
4. Identification of risk factors for AMD progression over a 3-year observation period, including the contribution of vascular factors in this process.

The study included patients with AMD at various stages and with a diverse clinical picture of the disease, as well as healthy controls. All study participants underwent an extensive ophthalmological examination and a detailed medical history was collected from them. Using multimodal retinal and choroidal imaging techniques, AMD staging was performed, different types of drusen were characterized (soft drusen, subretinal drusenoid deposits, pachydrusen) and choroidal morphology was assessed for the presence of pachychoroid features. Choroidal thickness, volume and the vascularity index were analyzed, and the static and dynamic assessment of arteries and veins was performed using the retinal vessels analyzer. The obtained

results were compared with those obtained in the control group of healthy volunteers. The analysis was performed taking into account the coexistence of specific genetic variants and systemic vascular diseases and their risk factors. A 3-year follow-up was conducted to assess the determinants of AMD progression. In the presented series of original works, it was shown that the thickness, volume and vascularity index of the choroid differ between eyes presenting various clinical forms and stages of AMD. These parameters were more reduced in the eyes of patients with coexisting vascular diseases. The diameter and reactivity of the retinal vessels were not significantly different in the eyes with AMD compared to the healthy eyes, however, a gradual reduction of the arteriovenous index was observed with increasing disease stage. It has also been shown that the parameters of dynamic retinal vessels analysis correlate with the thickness and volume of the choroid, and depend on the presence of specific high-risk AMD genetic variants. It was shown that the independent factors associated with the progression of the disease in the 3-year observation period were: age, thyroxine supplementation, the presence of exudative AMD in the fellow eye and genetic predisposition. Based on the series of original papers, the following conclusions were drawn:

1. The parameters describing the choroidal anatomy in patients with AMD are related to the clinical picture and the stage of the disease. This may indicate an important role of choroidal microcirculation disorders in the pathogenesis of the disease.
2. The presence of systemic vascular disease is a factor associated with reduced choroidal thickness and volume in eyes with AMD. Therefore, it can be concluded that the choroidal system of eyes with AMD is more susceptible to damage in the presence of systemic circulatory diseases.
3. The presence of genetic risk variants determines the functional state of the retinal microcirculation in eyes with AMD. The reactivity of the retinal vessels corresponds to the thickness and volume of the choroid, that indicates a functional connection of both vascular beds in the course of the disease.
4. Diseases of the vascular system as well as functional and morphological parameters of the retina and choroid are not independent factors of disease progression. Direct risk factors for disease progression include age, thyroxine intake, presence of exudative AMD in the fellow eye, and genetic predisposition.

10 Oświadczenia współautorów publikacji

Dr hab. Anna Boguszevska-Chachulska

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OŚWIADCZENIE

Potwierdzam mój wkład pracy w przygotowanie następującej publikacji naukowej:

Influence of Clinical and Genetic Factors on the Progression of Age-Related Macular Degeneration: A 3-Year Follow-Up

Mój wkład pracy polegał na analizie danych i wyniósł 2%.

Anna Boguszevska-Chachulska
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OŚWIADCZENIE

Potwierdzam mój wkład pracy w przygotowanie następujących publikacji naukowych:

The impact of vascular risk factors on the thickness and volume of the choroid in AMD patients

Mój wkład pracy polegał na kompletowaniu bazy danych pacjentów i wyniósł 5%.

Retinal Vessel Functionality Is Linked With *ARMS2* A69S and *CFH* Y402H Polymorphisms and Choroidal Status in AMD Patients

Mój wkład pracy polegał na kompletowaniu bazy danych pacjentów i wyniósł 3%.

Influence of Clinical and Genetic Factors on the Progression of Age-Related Macular Degeneration: A 3-Year Follow-Up

Mój wkład pracy polegał na kompletowaniu bazy danych pacjentów i wyniósł 5%.

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OŚWIADCZENIE

Potwierdzam mój wkład pracy w przygotowanie następującej publikacji naukowej:

Influence of Clinical and Genetic Factors on the Progression of Age-Related Macular Degeneration: A 3-Year Follow-Up

Mój wkład pracy polegał na analizie danych i wyniósł 5%.



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OŚWIADCZENIE

Potwierdzam mój wkład pracy w przygotowanie następujących publikacji naukowych:

Retinal Vessel Functionality Is Linked With *ARMS2* A69S and *CFH* Y402H Polymorphisms and Choroidal Status in AMD Patients

Mój wkład pracy polegał na analizie danych i wyniósł 2%.

Influence of Clinical and Genetic Factors on the Progression of Age-Related Macular Degeneration: A 3-Year Follow-Up

Mój wkład pracy polegał na analizie danych i wyniósł 2%.

KRAWCZYK MARIUSZ

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OŚWIADCZENIE

Potwierdzam mój wkład pracy w przygotowanie następujących publikacji naukowych:

The impact of vascular risk factors on the thickness and volume of the choroid in AMD patients

Mój wkład pracy polegał na przeprowadzaniu badań okulistycznych, analizie uzyskanych wyników oraz przygotowaniu manuskryptu do publikacji i wyniósł 48%.

Retinal Vessel Functionality Is Linked With *ARMS2* A69S and *CFH* Y402H Polymorphisms and Choroidal Status in AMD Patients

Mój wkład pracy polegał na przeprowadzaniu badań okulistycznych, analizie uzyskanych wyników oraz przygotowaniu manuskryptu do publikacji i wyniósł 25%.

Influence of Clinical and Genetic Factors on the Progression of Age-Related Macular Degeneration: A 3-Year Follow-Up

Mój wkład pracy polegał na zaplanowaniu badania, prowadzeniu badań okulistycznych, analizie uzyskanych wyników oraz przygotowaniu manuskryptu do publikacji i wyniósł 40%.

Elżbieta Krytkowska

Prof. dr hab. n. med. Anna Machalińska
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Pomorski Uniwersytet Medyczny w Szczecinie
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OŚWIADCZENIE

Potwierdzam mój wkład pracy w przygotowanie następujących publikacji naukowych:

The impact of vascular risk factors on the thickness and volume of the choroid in AMD patients

Mój wkład pracy polegał na zaplanowaniu badania, nadzorem nad jego realizacją, recenzowaniu i nadzorem redakcyjnym nad manuskrypcem oraz udostępnieniu infrastruktury i wyniósł 30%.

Retinal Vessel Functionality Is Linked With *ARMS2* A69S and *CFH* Y402H Polymorphisms and Choroidal Status in AMD Patients

Mój wkład pracy polegał na zaplanowaniu badania, nadzorem nad jego realizacją, recenzowaniu i nadzorem redakcyjnym nad manuskrypcem oraz udostępnieniu infrastruktury i wyniósł 25%.

Influence of Clinical and Genetic Factors on the Progression of Age-Related Macular Degeneration: A 3-Year Follow-Up

Mój wkład pracy polegał na zaplanowaniu badania, nadzorem nad jego realizacją, nadzorem redakcyjnym nad manuskrypcem oraz udostępnieniu infrastruktury i wyniósł 32%.

Prof. dr hab. n. med. Anna Machalińska
1324762
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OŚWIADCZENIE

Potwierdzam mój wkład pracy w przygotowanie następującej publikacji naukowej:

Retinal Vessel Functionality Is Linked With *ARMS2* A69S and *CFH* Y402H Polymorphisms and Choroidal Status in AMD Patients

Mój wkład pracy polegał na nadzorowaniu przebiegu eksperymentu oraz udostępnieniu infrastruktury do jego przeprowadzenia i wyniósł 5%.

K I E R O W N I K
Zakładu Patologii Ogólnej
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OŚWIADCZENIE

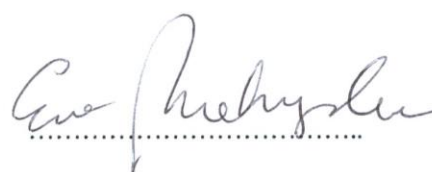
Potwierdzam mój wkład pracy w przygotowanie następującej publikacji naukowej:

Retinal Vessel Functionality Is Linked With *ARMS2* A69S and *CFH* Y402H Polymorphisms and Choroidal Status in AMD Patients

Mój wkład pracy polegał na analizie danych i wyniósł 2%.

Influence of Clinical and Genetic Factors on the Progression of Age-Related Macular Degeneration: A 3-Year Follow-Up

Mój wkład pracy polegał na analizie danych i wyniósł 2%.



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OŚWIADCZENIE

Potwierdzam mój wkład pracy w przygotowanie następujących publikacji naukowych:

The impact of vascular risk factors on the thickness and volume of the choroid in AMD patients

Mój wkład pracy polegał na przeprowadzaniu badań okulistycznych pacjentów i wyniósł 2%.

Retinal Vessel Functionality Is Linked With *ARMS2* A69S and *CFH* Y402H Polymorphisms and Choroidal Status in AMD Patients

Mój wkład pracy polegał na przeprowadzaniu badań okulistycznych pacjentów i wyniósł 2%.

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KATARZYNA MOZOLEWSKA-PIOTROWSKA
specjalista z wydziału okulistyki
51.5.2015

Dr Andrzej Pałucha

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OŚWIADCZENIE

Potwierdzam mój wkład pracy w przygotowanie następujących publikacji naukowych:

Retinal Vessel Functionality Is Linked With *ARMS2* A69S and *CFH* Y402H Polymorphisms and Choroidal Status in AMD Patients

Mój wkład pracy polegał na analizie danych i wyniósł 2%.

Influence of Clinical and Genetic Factors on the Progression of Age-Related Macular Degeneration: A 3-Year Follow-Up

Mój wkład pracy polegał na analizie danych i wyniósł 2%.



dr Andrzej Pałucha
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Prof. dr hab. n. med. Krzysztof Safranow
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OŚWIADCZENIE

Potwierdzam mój wkład pracy w przygotowanie następujących publikacji naukowych:

The impact of vascular risk factors on the thickness and volume of the choroid in AMD patients

Mój wkład pracy polegał na przeprowadzeniu analizy statystycznej uzyskanych wyników i przygotowaniu manuskryptu do publikacji i wyniósł 5%.

Retinal Vessel Functionality Is Linked With *ARMS2* A69S and *CFH* Y402H Polymorphisms and Choroidal Status in AMD Patients

Mój wkład pracy polegał na przeprowadzeniu analizy statystycznej uzyskanych wyników i przygotowaniu manuskryptu do publikacji i wyniósł 5%.

Influence of Clinical and Genetic Factors on the Progression of Age-Related Macular Degeneration: A 3-Year Follow-Up

Mój wkład pracy polegał na przeprowadzeniu analizy statystycznej uzyskanych wyników i przygotowaniu manuskryptu do publikacji i wyniósł 5%.

Krzysztof Safranow

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OŚWIADCZENIE

Potwierdzam mój wkład pracy w przygotowanie następującej publikacji naukowej:

Retinal Vessel Functionality Is Linked With *ARMS2* A69S and *CFH* Y402H Polymorphisms and Choroidal Status in AMD Patients

Mój wkład pracy polegał na analizie danych i wyniósł 2%.



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STATEMENT

I confirm my contribution to the preparation of the following scientific publication:

Retinal Vessel Functionality Is Linked With *ARMS2* A69S and *CFH* Y402H Polymorphisms and Choroidal Status in AMD Patients

My contribution was based on data analysis and amounted to 2%.



.....

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OŚWIADCZENIE

Potwierdzam mój wkład pracy w przygotowanie następujących publikacji naukowych:

The impact of vascular risk factors on the thickness and volume of the choroid in AMD patients

Mój wkład pracy polegał na przygotowaniu maszynopisu do publikacji i wyniósł 10%.

Retinal Vessel Functionality Is Linked With *ARMS2* A69S and *CFH* Y402H Polymorphisms and Choroidal Status in AMD Patients

Mój wkład pracy polegał na przygotowaniu maszynopisu do publikacji i wyniósł 25%.

Influence of Clinical and Genetic Factors on the Progression of Age-Related Macular Degeneration: A 3-Year Follow-Up

Mój wkład pracy polegał na przygotowaniu maszynopisu do publikacji i wyniósł 5%.



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OŚWIADCZENIE

Potwierdzam mój wkład pracy w przygotowanie następującej publikacji naukowej:

Influence of Clinical and Genetic Factors on the Progression of Age-Related Macular Degeneration: A 3-Year Follow-Up

Mój wkład pracy polegał na analizie danych i wyniósł 2%.

Anna Wąsowska
.....

11 Piśmiennictwo

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