LIETUVOS SVEIKATOS MOKSLŲ UNIVERSITETAS
MEDICINOS AKADEMIJA

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HIPOFIZĖS ADENOMOS SĄSAJOS SU REGOS FUNKCIJOMIS IR MOLEKULINIŲ ŽYMENŲ PAIEŠKA

Daktaro disertacija
Biomedicinos mokslai,
medicina (06B)

Kaunas, 2017

Disertacija rengta 2010–2017 metais Lietuvos sveikatos mokslų universitete Medicinos akademijos Akių ligų klinikoje.

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Disertacija bus ginama viešame medicinos mokslo krypties tarybos posėdyje 2017 m. balandžio 13 d. 14 val. Lietuvos sveikatos mokslų universiteto Akių ligų klinikos prof. P. Avižonio auditorijoje.

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LITHUANIAN UNIVERSITY OF HEALTH SCIENCES
MEDICAL ACADEMY

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PITUITARY ADENOMA: ASSOCIATIONS WITH VISUAL FUNCTIONS AND SEARCH FOR MOLECULAR MARKERS

Doctoral Dissertation
Biomedical Sciences,
Medicine (06B)

Kaunas, 2017

Dissertation has been prepared at the Department of Ophthalmology of Medical Academy of Lithuanian University of Health Sciences during the period of 2010–2017.

Scientific Supervisor:

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Dissertation is defended at the Medical Research Council of the Medical Academy of Lithuanian University of Health Sciences.

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Dissertation will be defended at the open session of the Medical Research Council on the 13th of April 2017 at 2 p. m. in the Prof. P. Avižonis Auditorium of Department of Ophthalmology of Lithuanian University of Health Sciences.

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TURINYS

SANTRUMPOS

AP-1 (angl. *activator protein 1*) – aktyvinantis baltymas 1
Bax (angl. *bcl-2-associated X protein)* – bcl-2 susijęs baltymas
DNR – deoksiribonukleininė rūgštis
FKJ – funkcinis kontrastinis jautrumas
F-M 100 – Farnsworth-Munsell 100 atspalvių tyrimas
GKRA – geriausias koreguotas regos aštrumas
FGFR2 (angl. *fibroblast growth factor receptor 2*) – fibroblastų augimo faktoriaus receptorius 2
HA – hipofizės adenoma
IL-17A – interleukinas 17A
KEAF (angl. *vascular endothelial growth factor*) – kraujagyslių endotelio augimo faktorius
MAP (angl. *mitogen-activated protein*) – mitogenais aktyvuojamas baltymas
MIP-2 (angl. *macrophage inflammatory protein 2*) – makrofagų uždegiminis baltymas 2
MMP – matrikso metalo proteinazė
MMP-2 – matrikso metaloproteinazė 2 arba želatinazė A
MMP-9 – matrikso metaloproteinazė 9 arba želatinazė B
NAD – nikotinamido adenino dinukleotidas
NF-κB (angl. *nuclear factor kappaB*) – branduolio κB faktorius
NO – azoto monoksidas
OKT – optinė koherentinė tomografija
PGR – grandininė polimerazės reakcija
PI – proliferacijos indeksas
PI3K (angl. *phosphoinositide-3-kinase*) – fosfatidilinozitolio-3-kinazė
RND – regos nervo diskas
RNK – regos nervų kryžmė
RSKJ – ribinis spalvinis kontrastinis jautrumas
ROR (angl. *retinoic acid receptor-related orphan receptor*) – su retino rūgštimi susiję našlaičiai receptoriai
SIRT1 (angl. *silent mating-type information regulation 2 homologue 1*) – sirtuinas 1
SN – standartinis nuokrypis
STAT3 (angl. *signal transducer and activator of transcription 3*) – signalo perdavėjas ir transkripcijos veiksnys 3
SSN – skirtumas statistiškai nereikšmingas

TGF-β (angl. *transforming growth factor-β*) – transformuojantis augimo faktorius-β
Th (angl. *T helper cells*) – T limfocitai pagalbininkai
TNF (angl. *tumor necrosis factor*) – navikų nekrozės faktorius
TNSS – tinklainės nervinių skaidulų sluoksnis
VS (lot. *versus*) – palyginti

DNR bazių nomenklatūra
A – adeninas
C – citozinas
G – guaninas
T – timinas

ĮVADAS

Hipofizės adenoma (HA) – tai gerybinis intrakranijinis auglys, augantis iš posmegeninės liaukos priekinės dalies – adenohipofizės ląstelių ir pasireiškiantis 16,7 proc. populiacijos [1]. Posmegeninė liauka, arba hipofizė, yra lokalizuota pleištakaulio kūno viršutinės sienos įduboje, kietojo smegenų dangalo maiše, pritvirtintame prie turkiškojo balno diafragmos priekinės dalies, iš šonų apsupta akytųjų veninių ančių [2]. Nustatyta, kad 6–10 proc. HA perauga akytuosius ančius [2, 3], kadangi akytųjų ančių medialinė siena yra plono kietojo smegenų dangalo lateralinė dalis [4, 5], todėl HA gali lengvai plisti lateraliai ir taip pažeisti atitraukiamąjį, akies judinamąjį bei skridininį nervus. Kartais HA gali būti ypač invazinė ir netgi sukelti kaukolės pamato destrukciją [6]. Regos nervų kryžmė (RNK) yra virš posmegeninės liaukos, todėl, jei HA auga supraseliariai, dažnai yra sutrikdomos regos funkcijos [7–14].

HA gali būti tinklainės nervinių skaidulų sluoksnio (TNSS) išplonėjimo priežastis dėl aksonų degeneracijos, sukeltos priekinių regos takų kompresijos. Jis yra objektyviai įvertinamas optinės koherentinės tomografijos (OKT) būdu [15–20]. TNSS sąsajos su regos aštrumo, akipločio pokyčiais yra nagrinėjamos daugelyje mokslinių tyrimų [7–22], tačiau TNSS sąsajos su RNK, HA morfologinėmis charakteristikomis pirmą kartą nagrinėjamos šiame darbe.

Labai svarbu įvertinti ir prognozuoti auglio invazyvumą, kadangi invazinis augimas turi įtakos HA gydymui ir ligos eigos prognozei [23]. Šiuo metu mokslininkai tyrinėja įvairius invazyvumo biologinius žymenis – chromosomines alteracijas ir miRNR, proliferacijos žymenis, onkogenus, navikų supresijos genus, augimo faktorius ir jų receptorius, faktorius susijusius su angiogeneze ar ląstelių adhezija [24–29], tačiau iki šiol nėra nė vieno klinikinėje praktikoje naudojamo žymens, siejamo su HA atsiradimu ar invazyvumu. Matrikso metaloproteinazės 2 (*MMP-2)* rs24386, matrikso metaloproteinazės 9 (*MMP-9)* rs3918242, fibroblastų augimo veiksnio receptoriaus 2 (*FGFR2)* rs2981582, sirtuino 1 (*SIRT1*) rs12778366, signalo perdavėjo ir transkripcijos veiksnio 3 *(STAT3)* rs744166 genotipų dažniai buvo tirti sergantiems įvairiais navikais [30–60], tačiau niekada nebuvo tirti sergantiems HA. Mokslinėje literatūroje paskelbtas tik vienas tyrimas, kuriame nagrinėjamos prouždegiminio citokino IL-17A sąsajos su HA invazyvumu [61, 62]. Daugelis mokslininkų tyrinėja Ki-67 proliferacijos indeksą (Ki-67 PI) kaip galimą HA invazyvumo žymenį, tačiau gaunami duomenys iki šiol išlieka prieštaringi [63–80]. Todėl mes tyrėme bendruose HA patogeneziniuose mechanizmuose dalyvaujančius molekulinius žyme-

nis, kurie padės vertinti HA klinikinę eigą, leis pasirinkti gydymo taktiką, suteiks naujos informacijos apie HA patogenezę.

Darbo aktualumas

HA yra adenohipofizės parenchimos ląstelių gerybinis navikas. HA sudaro apie 10–25 proc. visų intrakranijinių auglių, paplitimas bendrojoje populiacijoje – 16,7 proc. Kliniškai reikšmingos HA pasireiškia 1 iš 1064 gyventojų [1, 81, 82]. Invazinės HA yra agresyvios, nes iš turkiabalnio srities gali plisti į aplinkinius audinius. HA invazyvumas bei naviko sukelti metaboliniai sutrikimai gali nulemti ir letalias baigtis, kadangi beveik kiekvienas žmogaus organas ir audinys yra tiesiogiai ar netiesiogiai veikiamas priekinės ar užpakalinės hipofizės liaukos dalies išskiriamų hormonų. HA atsiradimo mechanizmai iki šiol nėra iki galo ištirti. Manoma, kad tai daugiaveiksnės etiologijos liga, kurios pasireiškimui įtaką daro tiek genetiniai veiksniai, tiek hormoninė būklė, tiek kraujagyslių endotelio augimo faktoriai ir kt. [24–26, 83]. Didžiajai daliai HA sergančių pacientų dėl RNK kompresijos pažeistų regos nervų skaidulų sutrinka regos funkcijos (regos aštrumas, akiplotis, spalvų juslė, kontrastinis jautrumas), atsiranda regos nervų diskų (RND) pažeidimų, todėl oftalmologiniai tyrimo metodai yra svarbūs norint anksti diagnozuoti HA [7–14, 84–88]. Kuo anksčiau diagnozuojamas navikas, tuo labiau tikėtina, kad jį bus galima radikaliai pašalinti ir išsaugoti regos funkcijas. Labai svarbu nustatyti HA navikinio audinio molekulinius ir kraujo imunogenetinius žymenis, padedančius įvertinti HA atsiradimo galimybę, klinikinę šio naviko eigą. Šio tyrimo rezultatai yra svarbūs norint suprasti HA patogenezę, taip pat šios ligos molekulinių mechanizmų tolimesniems tyrimams.

Darbo naujumas

Iki šiol nėra nė vieno klinikinėje praktikoje rutiniškai naudojamo HA žymens, kuris padėtų diagnozuoti HA, leistų pasirinkti gydymo taktiką. Atlikus šį tyrimą inicijuoti naujos krypties tyrimai, t. y. pirmą kartą pasaulyje įvertinti bendruose patogeneziniuose mechanizmuose dalyvaujančių žymenų – *MMP-2* rs243865, *MMP-9* rs3918242, *FGFR2* rs2981582, *SIRT1* rs12778366, *STAT3* rs744166 genotipai sergantiesiems HA. Taip pat pirmą kartą Lietuvoje nustatyta IL-17A koncentracija kraujo serume ir Ki-67 PI sergant HA. Nustatyta 11 apsauginių genotipų derinių ir 2 didinantys HA galimybę.

Darbo tikslas

Nustatyti hipofizės adenomos molekulinius žymenis ir sąsajas su regos funkcijomis.

Darbo uždaviniai:

1. Įvertinti pacientų, kuriems diagnozuota hipofizės adenoma, objektyvių ir subjektyvių oftalmologinių tyrimų duomenis bei sąsajas su hipofizės adenomos klinikiniais ir morfologiniais požymiais.
2. Palyginti Ki-67 PI esant invazinei ir neinvazinei hipofizės adenomai, taip pat palyginti sergančiųjų hipofizės adenoma ir sveikų asmenų IL-17A koncentraciją kraujo serume.
3. Įvertinti *MMP-2* rs243865 ir *MMP-9* rs3918242 genotipų sąsajas su hipofizės adenomos augimo pobūdžiu.
4. Nustatyti galimas *FGFR2* rs2981582, *SIRT1* rs12778366, *STAT3* rs744166 genotipų ir jų derinių sąsajas su hipofizės adenomos atsiradimu.

1. LITERATŪROS APŽVALGA

1.1. Posmegeninės liaukos ir regos nervų kryžmės anatomija

Posmegeninė liauka (hipofizė) yra apie 12 mm pločio ir 8 mm ilgio, 0,5 g svorio endokrininė liauka, sudaryta iš dviejų skirtingos kilmės dalių: priekinės skilties, vadinamos adenohipofize, ir užpakalinės skilties arba neurohipofizės [89]. Adenohipofizės sijose yra liaukinės ląstelės, endokrinocitai, kurie išskiria tropinius hormonus, reguliuojančius atitinkamas periferines liaukas ir savarankiškai sukeliančius endokrininius poveikius (tirotropinį, gonadotropinį, kortikotropinį, laktotropinį, somatotropinį hormonus), neurohipofizėje yra kaupiami hormonai vazopresinas ir oksitocinas. Tarpinė liaukos dalis išskiria hormoną melanotropiną [89, 90]. Liauka yra lokalizuota pleištakaulio kūno viršutinės sienos įduboje, kietojo smegenų dangalo maiše, pritvirtintame prie turkiškojo balno diafragmos priekinės dalies, iš šonų apsupta akytųjų veninių ančių, į kuriuos atsiveria akinės venos [2, 89]. 29 proc. atvejų yra pastebimas fiziologinis hipofizės plėtimasis į akytąjį antį, kadangi akytųjų ančių medialinė siena yra ne tvirta kaulinė struktūra, o plono kietojo smegenų dangalo lateralinė dalis [2, 3]. Tikrasis auglio invazyvumas privalo būti atskirtas nuo lateralinio augimo be invazijos (kai nėra pažeistas kietasis smegenų dangalo maišas) [91], kadangi tai svarbu tolimesnei gydymo taktikai ir prognozei. Akytaisiais ančiais praeina vidinė miego arterija, atitraukiamasis, akies judinamasis, skridininis ir trišakis nervai [92]. Regos nervai pleištakaulio kūno priekyje virš hipofizės sudaro regos nervų kryžmę (chiazmą). Apatinės vidinės tinklainės pusės skaidulos kryžiuojasi žemai ir priekyje, viršutinės vidinės pusės skaidulos kerta kryžmę aukštai ir dorzaliau, geltonosios dėmės skaidulos kryžiuojasi regos nervų kryžmėje. Skaidulos, einančios iš šoninės tinklainės pusės, eina toliau tos pačios pusės regos laidu [89, 93].

1.2. Hipofizės adenomos paplitimas

HA paplitimas bendrojoje populiacijoje yra 16,7 proc. (14,4 proc. autopsijų ir 22,5 proc. radiologinių tyrimų duomenimis) [1]. Kliniškai reikšmingos HA pasireiškia 1 iš 1064 gyventojų [82]. Jungtinių Amerikos Valstijų centrinio smegenų auglių registro CBTRUS (*Central Brain Tumor Registry of the United States*) 2008–2012 m. duomenimis, pirminių piktybinių ir nepiktybinių smegenų ir centrinės nervų sistemos auglių naujų atvejų dažnis JAV – 28,57 atvejai 100 000 gyventojų, iš jų histologiškai patvirtinti gerybiniai hipofizės augliai sudaro 15,5 proc. [81]. HA naujų atvejų dažnis

Belgijoje – 94 atvejai 100 000 gyventojų [82], Švedijos vėžio registro duomenimis (1958–1991 m.), pastebimas HA atvejų skaičiaus augimas nuo 71,3 atvejų 100 000 gyventojų (paplitimas 1959–1979 m.) iki 97,6 atvejų 100 000 gyventojų (paplitimas vertintas po 1980 m.) [94].

Nacionalinio vėžio instituto Vėžio kontrolės ir profilaktikos centro Vėžio registro duomenimis, Lietuvoje 2012 m. 275 asmenys susirgo piktybiniais galvos smegenų navikais ir 118 gerybiniais CNS augliais, tačiau detalių duomenų apie sergamumą HA Lietuvoje nėra [95].

1.3. Sergančiųjų HA regos funkcijų pokyčiai

Regos nervų kryžmė yra lokalizuota virš hipofizės ir, jei navikas auga supraseliariai, dėl RNK kompresijos, dažnai yra matomas regos aštrumo sumažėjimas ir yra dažniausiai temporalinių akipločio defektų [7–14, 85, 86, 74, 96–102]. Yra žinoma, kad regos aštrumo ir akipločio defektai priklauso nuo RNK kompresijos laipsnio [11, 103–105]. Wei-Chen Huang ir Liang-Shong Lee nustatė priklausomybę tarp akipločio defektų ir RNK kompresijos [103]. Apjit Kaur ir bendraautoriai [11] nustatė, kad labiausiai regos aštrumas buvo sumažėjęs pacientams, kuriems nustatytas didesnis nei 2 cm arba obturavęs trečiąjį skilvelį auglys (88,9 proc. nustatytas regos aštrumas nuo 0,1 iki 0), jei HA buvo iki 2 cm arba rasta obturuota trečiojo skilvelio priekinė kišenė, 73,3 proc. atvejų nustatytas regos aštrumas nuo 0,1 iki 0 [11]. Hanbin Wang ir bendraautoriai taip pat nustatė supraseliarinio augimo įtaką regos aštrumui bei neigiamą koreliaciją tarp regos aštrumo ir akipločio ($r = –0,555$; $p = 0,000$) [104]. Kathrin Schmalisch ir kolegos, ištyrę 98 supraseliariai augančias HA, nustatė statistiškai reikšmingas sąsajas tarp regos aštrumo kritimo ir supraseliarinio HA augimo ($p < 0,0001$) [105]. Yra žinoma, kad sergantiesiems HA, dar prieš sutrinkant regos aštrumui ar akipločiui, gali sumažėti kontrastinis jautrumas (KJ) bei spalvų juslė [8, 85, 88, 97, 106–109].

HA gali sukelti peripapilinio tinklainės nervinių skaidulų sluoksnio (TNSS) išplonėjimą, kurį galima įvertinti objektyvaus neinvazinio tyrimo – optinės koherentinės tomografijos (OKT) – būdu [15–20]. Ji buvo sukurta biologinėms sistemoms stebėti matuojant jų optinius atspindžius [110]. TNSS sąsajos su regos aštrumo, akipločio pokyčiais yra nagrinėjamos daugelyje mokslinių tyrimų [7–22], tačiau TNSS kvadrantų storio ryšys su RNK ir HA morfologinėmis savybėmis iki šiol nebuvo tirtas.

1.4. IL-17 A

T limfocitai – pagrindinės specifinio imuninio atsako ląstelės. Limfocitų paviršiuje yra vadinamosios CD molekulės (angl. *cluster of differentiation*). CD4+ yra T limfocitų pagalbininkų skiriamoji molekulė, $CD8^+$ – T limfocitų žudikų. Patekus į organizmą antigenui, naivieji T limfocitai diferencijuojasi į įvairias Th1, Th2 limfocitų pagalbininkų subpopuliacijas, Th17 limfocitus, kurie išskiria specifinius citokinus. Prouždegiminių citokinų IL šeimą sudaro keli citokinai – IL-17A (IL17), IL-17F, IL-17B, IL-17D, IL-17C ir IL-17E (arba IL-25) [111]. IL-17A – tai citokinas, atrastas Rouvier ir kt. 1993 m. (tuo metu pavadintas CTLA-8). Nustatyta, kad IL-17A aminorūgščių seka 57 proc. sutampa su T-limfotropinio Herpes Saimiri viruso amino rūgščių seka [112, 113]. IL-17A koduojantis genas lokalizuotas 6p12 chromosomoje [114]. Nors plačiai žinoma, kad IL17 gamina $CD4^+$ T ląstelės, nustatyta, kad yra ir daugiau ląstelių gaminančių šį citokiną: $CD8^+$ T ląstelės [115, 116], Th17 [117], αβ ir γδTCR $CD4^-CD8^-$ ląstelės [118, 119], į limfoidinio audinio aktyvintojus panašios ląstelės (angl. *lymphoid tissue inducer–like cells*) [120], neutrofilai bei makrofagai [121].

Th diferenciacijoje dalyvauja daug veiksnių. Signalo perdavėjas ir transkripcijos veiksnys 3 (STAT3) yra vienas svarbiausių Th ląstelių diferenciacijoje. Sutrikdžius STAT3 veiklą, sutrikdoma Th diferenciacija iš naiviųjų Th ląstelių, sutrikdant svarbiausią Th17 limfocitų transkripcijos veiksnį – su retino rūgštimi susijusius receptorius našlaičius – RORγt receptorius ar ROR-α. O hiperaktyvi STAT3 forma skatina IL-17A gamybą CD4 ląstelėse [122]. Transformuojantis augimo faktorius-β (TGF-β) ir IL-6 taip pat yra būtini normaliai Th ląstelių diferenciacijai [122, 123].

Nustatyta, kad IL-17A stimuliuoja transkripcijos faktoriaus NF-kappa B aktyvumą ir IL-6 sekreciją fibroblastuose, taip pat yra svarbus T ląstelių proliferacijai [113]. IL-17A yra atsakingas už kraujagyslių endotelio augimo faktoriaus (KEAF) reguliaciją, keratinocitų, makrofagų uždegiminio baltymo 2 (MIP-2), prostaglandinų ir NO gamybą fibroblastuose [124-126]. IL-17A skatina IL-1β ir navikų nekrozės faktoriaus (TNF-α) gamybą makrofaguose ir endotelio ląstelėse [127], be to, aktyvuoja granuliocitų kolonijas stimuliuojantį veiksnį (GCSF) ir CXC chemokinus, kurie stimuliuoja granuliopoezę ir neutrofilų išsiskyrimą į audinius [128–130]. IL-17A taip pat aktyvuoja transkripcijos veiksnius: branduolio veiksnį κB (NF-κB) ir aktyvinantį baltymą 1 (AP-1) [125]. Nustatyta, kad citokinai IL-2 ir IL-15 skatina IL-17 produkciją [131].

Plačiai tyrinėjamas IL-17A poveikis auglių išsivystymui. Duomenys išlieka prieštaringi. Muneo Numasaki ir bendraautoriai nustatė, kad IL-17

in vitro neskatino navikų išsivystymo, tačiau nulėmė greitesnį auglio augimą ir ryškesnę neovaskuliarizaciją nei kontrolinėje pelių grupėje [124]. Eric Tartour [132] savo tyrimu taip pat patvirtino, kad IL-17 neskatino auglio išsivystymo, bet lėmė greitesnį jo augimą. Autoriai taip pat nustatė, kad IL-17A skatina IL-6 ir IL-8 sekreciją gimdos kaklelio vėžinėse ląstelėse. Tartour ir kolegos tirdami gimdos kaklelio vėžines ląsteles, pastebėjo IL-17 tumorogeninį poveikį nesant T ląstelių [132], o naujesnių tyrimų metu nustatyta, kad IL-17A slopina hemopoetinių auglių, mastocitomos ir plazmocitomos vystymąsi dalyvaujant T ląstelėms [133]. Lin Wang ir bendraautoriai [134] patvirtino, kad IL-17 gali lemti naviko augimą, aktyvindamas IL-6, kuris aktyvuoja STAT3. Yra nustatyta, kad padidėjęs IL-17 gaminančių ląstelių skaičius koreliuoja su trumpu pacientų, sergančių hepatoceliuline karcinoma, išgyvenamumu [135]. IL-17 taip pat skatina šlapimo pūslės ir melanomos augimą pelių modeliuose [134]. Jeong-Seok Nam ir bendraautoriai [136] nustatė padidėjusią IL-17A raišką krūties vėžio audinyje, palyginti su sveiku audiniu.

Keliais tyrimais nustatyta, kad IL-17A slopina navikų vystymąsi. Noriyuki Hirahara ir bendraautoriai nustatė, kad IL-17 genas gali slopinti tumorogenezę [137]. Fabrice Benchetrit ir kolegos taip pat nustatė, kad IL-17 gali slopinti naviko augimą [133].

Tik viename tyrime nagrinėta IL-17A koncentracija sergančiųjų HA kraujo serume. Lubin Qiu ir bendraautoriai ištyrė IL-17A koncentraciją kraujo serume 75 asmenims, sergantiems HA, ir nustatė, kad IL-17A koncentracija sergantiesiems invazine HA buvo didesnė nei sergantiesiems neinvazine HA. Invazinių HA grupėje IL-17A koncentracija buvo 95,46 ± 34,09, neinvazinių HA – 56,26 ± 14,03 pg/ml, kontrolinėje grupėje – 23,58 ± 6,55 pg/ml [61, 62]. Lietuvoje tyrimų, kurių metu būtų vertinta IL-17A koncentracija sergantiesiems HA, atlikta nebuvo.

1.5. Ki-67 proliferacijos indeksas

Ki-67 – tai branduolinis antigenas, baltymas, esantis ląstelių branduoliuose. Ki-67 raiška ląstelės ciklo metu yra nevienoda. Ki-67 raiška vyksta G1, S ir G2 fazių metu ir pasiekia maksimumą mitozės pradžioje, bet nevyksta ląstelės ciklo ramybės fazėje G0. Ki-67 proliferacijos indeksas (Ki-67 PI) yra išreiškiamas Ki-67 antigeno teigiamų branduolių procentais tarp visų branduolių [138–141]. Pasaulio sveikatos organizacija 2004 m. endokrininių auglių klasifikacijoje nurodė, kad Ki-67 PI ≥ 3 proc. nulemia HA agresyvų augimo pobūdį ir, esant Ki-67 PI ≥ 3 proc., HA klasifikuojamos kaip atipinės [142].

Vidutinis Ki-67 PI sergant HA, įvairių autorių duomenimis, kinta nuo 0,5 proc. iki 4,7 proc. [65, 66, 70–72, 77, 143]. Daugelis mokslininkų patvirtino Ki-67 PI svarbą vertinant HA invazyvumą ir augimo pobūdį. Luciano Mastronardi ir bendraautoriai [65] nenustatė reikšmingo Ki-67 PI skirtumo tarp intraseliarinių (2,6 ± 3,45 proc.), intrasupraseliarinių (1,91 ± 2,11 proc.), intrasupraparaseliarinių (3,29 ± 5,4 proc.) makroHA ($p = 0,27$), tačiau nustatė statistiškai reikšmingą skirtumą tarp infiltracinio (3,73 ± 5,13 proc.) ir neinfiltracinio augimo HA (2,03 ± 2,41 proc.) ($p = 0,02$), tarp akytąjį antį infiltruojančių (5,61 ± 7,19 proc.) ir neinfiltruojančių (2,09 ± 2,37 proc.) HA ($p = 0,0005$). Kelių tyrimų metu nustatytas statistiškai didesnis Ki-67 PI esant pažeistam kietajam smegenų dangalui (tikrasis invazyvumas) nei neinvazinėse HA [66, 67, 69, 71]. Roger Gejman ir bendraautoriai [144] nustatė neprogresuojančių HA Ki-67 PI 0,41 proc. ± 0,01 proc. (0,08 – 1,2 proc.), progresuojančių HA 1,45 proc. ± 0,09 proc. (0,1–10,6 proc.) ($p = 0,01$). Stefan Wolfsberger ir kolegų [76] atliktame tyrime nustatytas reikšmingai didesnis Ki-67 PI invazinėse navikų grupėse nei neinvazinėse.

Rahul Lath ir kolegos įvertino Ki-67 PI, atsižvelgdami į HA klasifikaciją pagal Hardį. E laipsnio HA nustatytas statistiškai reikšmingai aukštesnis Ki-67 PI (1,44 proc.) nei Hardžio 0 laipsnio navikams (0,36 proc.) [72]. Przemysław Witek ir bendraautoriai įvertino Ki-67 PI sąsajas su HA Knosp laipsniais. Kai Ki-67 PI buvo < 3 proc., tai 45,5 proc. pacientų nustatytos Knosp 3 ar 4 laipsnio HA, kai Ki-67 buvo 3 – 10 proc., tai 33,3 proc. sergančių asmenų nustatytos 3 ar 4 laipsnio HA, kai Ki-67 PI buvo > 10 proc., 100 proc. pacientų nustatytas tikras HA invazyvumas [78].

Jose Alberto Landeiro 35 pacientų ištyrė ryšį tarp Ki-67 PI ir HA pasikartojimo. Jie nustatė, kad Ki-67 PI < 3 proc. pastebėtas 30 HA atvejų, iš jų HA pasikartojimas buvo nustatytas 7,7 proc. (2 pacientams), Ki-67 PI > 3 proc. nustatytas 5 pacientams, 3 iš jų diagnozuotas HA pasikartojimas (60 proc.) [74]. Kyung-Il Paek ir kolegos [71] taip pat nustatė statistiškai didesnį Ki-67 PI pasikartojančių (1,27 proc.) nei nepasikartojančių HA (0,56 proc.) grupėje ($p = 0,027$). Maysam Alimohamadi ir bendraautoriai [80] nustatė didesnį Ki-67 PI pacientams, kuriems buvo nevisiškai pašalintas hipofizės navikas, nei tiems, kuriems buvo pašalintas radikaliai (3,5 *vs.* 1,7 proc.). Laurence Katznelson nustatė, kad Ki-67 PI > 3 proc. dažniau buvo esant makroHA nei mikroHA (44 proc. *vs.* 18 proc.) [79].

Jurgen Honegger ir kolegos nustatė koreliaciją tarp Ki-67 PI ir HA augimo greičio. Esant greitai augančioms HA (> 0,07 proc. augimas per dieną) nustatytas Ki-67 PI > 1,5 proc., o lėtai augančioms (< 0,02 proc. per dieną) – mažesnis nei 1,5 proc. Autoriai nenustatė priklausomybės tarp augimo greičio ir HA invazyvumo [75]. Asen Hadzhiyanev ir bendraautorių

[77] atliktame tyrime nustatyta PI koreliacija su naviko dydžiu ($p = 0,012$), tačiau nerasta priklausomybės nuo invazyvumo, augimo krypties, pasikartojimų. Marco Losa ir bendraautoriai nustatė Ki-67 PI teigiamą koreliaciją su auglio diametru ($r = 0,53$; $p < 0,01$). MakroHA nustatytas didesnis Ki-67 PI ($9,3 \pm 2,7$ proc.) nei mikroHA ($2,8 \pm 0,5$ proc.; $p < 0,002$) [64].

Sema Yarman ir bendraautoriai nerado Ki-67 PI sąsajų su HA invazyvumu [63]. Severine Dubois ir kolegų [68] tyrimas nepatvirtino Ki-67 PI kaip galimo invazyvumo ar pasikartojimo prognostinio veiksnio. M. Losa ir bendraautoriai [70] taip pat nenustatė Ki-67 PI skirtumų tarp invazinių ir neinvazinių HA, pasikartojančių ir nepasikartojančių HA. Regina Gandour-Edwards ir kolegos [73] nenustatė Ki-67 PI skirtumo tarp sfenoidalinį sinusą pažeidžiančių ir nepažeidžiančių HA.

Nors Ki-67 PI indeksas sergantiesiems HA tyrinėjamas daugelio mokslininkų, tačiau duomenys išlieka prieštaringi. Lietuvoje Ki-67 PI sergantiesiems HA iki šiol nebuvo tirtas.

1.6. *FGFR2* rs2981582, *SIRT1* rs12778366, *STAT3* rs744166 genų polimorfizmų sąsajos su HA

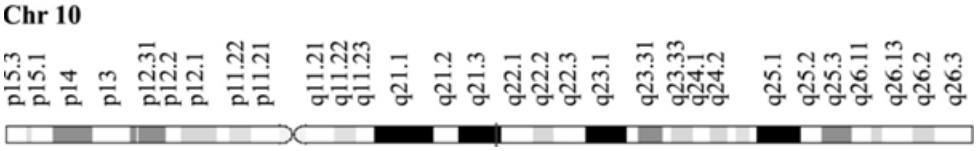
1.6.1. *SIRT1* rs12778366

Sirtuinas 1 (SIRT1) – tai nuo NAD (nikotinamido adenino dinukleotido) priklausoma deacetilazė, priklausanti sirtuinų šeimai [145], kuri yra svarbi balansui tarp ląstelių žūties ir išlikimo per Ku70-Bax kelią [146], p53 [147, 148], FOXO3 [149] ir kitus taikinius palaikyti. SIRT1 dažniausiai yra lokalizuotas ląstelės branduolyje, bet dalis yra ir citozolyje [150]. Vienas svarbiausių SIRT1 taikinių yra p53, kuris yra atsakingas už ląstelės ciklo reguliavimą, apoptozę, DNR pažaidų taisymą. Esant padidėjusiai *SIRT1* raiškai, p53 yra deacetilinamas, dėl to sumažėja p53 aktyvumas, sutrikdoma jo veikla, ląstelės gali išgyventi DNR pažaidas [148]. FOXO baltymai taip pat yra susiję su DNR pažaidų taisymu ir apoptoze, ląstelės ciklo reguliavimu, ląstelių diferenciacija [149, 151–155].

Yra žinoma, kad *SIRT1* raiškos padidėjimas hepatoceliulinės karcinomos audinyje [156], krūties vėžio [157], prostatos vėžio [158], kiaušidžių [159], skrandžio [160], storosios žarnos [161], glioblastomos [162], limfomos [163], ūminės mieloidinės leukemijos [164], odos plokščialąstelinio vėžio [165], skydliaukės vėžio [166] atvejais gali būti susijęs su šių navikų išsivystymu. Tačiau keliuose tyrimuose aprašomi rezultatai, kuriais remiantis *SIRT1* pristatomas kaip genas, slopinantis vėžio vystymąsi [167, 168].

SIRT1 geno rs12778366 polimorfizmas siejamas su krūties vėžiu [30]. Sherine M. Rizk ir bendraautoriai [30] nustatė, kad, esant *SIRT1* rs12778366

T/T genotipui, pastebėtas didesnis kiekis SIRT1 nei esant C/C ar C/T genotipams. T alelio dažnis buvo didesnis pacientams, sergantiems krūties vėžiu, nei sveikiems asmenims [30]. *SIRT1* rs12778366 geno polimorfizmas sergantiems HA, mūsų duomenimis, dar niekada netirtas.



1.6.1.1 pav. *10 chromosoma ir SIRT1 geno lokalizacija (http://www.genecards.org/cgi-bin/carddisp.pl?gene=SIRT1)*

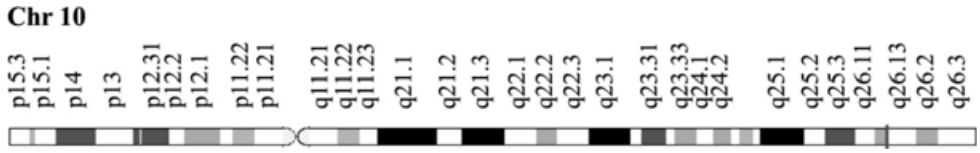
1.6.2. *FGFR2* rs2981582

Fibroblastų augimo faktoriaus receptorius 2 (FGFR2) priklauso FGFR tirozino kinazės receptorių šeimai ir dalyvauja tumorogenezėje aktyvuotamas mitogeninius procesus, sukeldamas invazyvumą ir angiogenezę. Kai vėžinės ląstelės ima išskirti daugiau FGFR su pakitusiu ligando prisijungimo specifiškumu, FGF išskirtas iš šalia esančių ląstelių gali stimuliuoti vėžines ląsteles, sudarydamas parakrininę kilpą [169]. Aktyvuojant FGFRs, yra aktyvuojami įvairūs signalų perdavimo keliai, tokie kaip antiapoptozinis fosfatidilinozitolio-3-kinazės (PI3K), mitogenais aktyvuojamo baltymo (MAP), transkripcijos signalo perdavėjų ir transkripcijos (STAT) keliai, kurie gali paskatinti vėžio progresavimą [170–174].

Padidėjusi *FGFR2* raiška buvo nustatyta sergantiesiems šlapimo pūslės [175] bei plaučių vėžiu [176]. O hepatoceliulinės karcinomos [177], prostatos vėžio [178] atvejais nustatyta sumažėjusi *FGFR2* raiška. *FGFR2* rs2981582 geno polimorfizmas buvo tyrinėjamas sergantiesiems krūtų [31–34, 170, 179–184] ir prostatos vėžiu [185].

FGFR2 rs2981582 polimorfizmas buvo išsamiai tyrinėjamas sergantiesiems krūties vėžiu, tačiau duomenys išlieka prieštaringi. Ying Chen ir bendraautoriai [32] nustatė, kad *FGFR2* rs2981582 G/A ir A/A genotipai yra susiję su mažesne krūties vėžio rizika. Salma Butt ir kolegos [31] nustatė, kad *FGFR2* rs2981582 A/A genotipas statistiškai reikšmingai buvo susijęs su padidėjusia krūties vėžio rizika. Jingxuan Shan ir bendraautoriai [34] taip pat nustatė, kad pacientams, turintiems *FGFR2* rs2981582 A/A genotipą, buvo padidėjusi krūties vėžio rizika. Joanna Ledwoń ir bendraautoriai [33] nustatė rs2981582 polimorfizmo ryšį su sporadiniu ir paveldimu krūties vėžiu.

FGFR2 rs2981582 geno polimorfizmo sąsajos su HA pasaulyje iki šiol dar nebuvo tirtos.



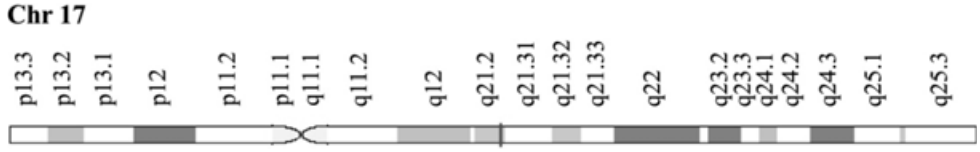
1.6.2.1 pav. *10 chromosoma ir FGFR2 geno lokalizacija (http://www.genecards.org/cgi-bin/carddisp.pl?gene=FGFR2)*

1.6.3. *STAT3* rs744166

Signalo perdavėjas ir transkripcijos veiksnys 3 (STAT3) yra aktyvuojamas vėžinėse ląstelėse ir daugelyje imuninių ląstelių naviko mikroaplinkoje ir yra siejamas su navikinių ląstelių proliferacija, invazija, angiogeneze [186–188]. Šie baltymai yra neaktyvūs citoplazmoje ir yra aktyvuojami fosforilinant tiroziną, dažniausiai per su citokinų receptoriais susijusias kinazes (JAKs) arba augimo veiksnių receptorių tirozinokinazes. Fosforilinta STAT forma patenka į branduolį ir kartu su kitais transkripcijos veiksniais aktyvina transkripciją. Sveikose ląstelėse STAT aktyvintos formos išlieka nuo kelių minučių iki kelių valandų, o vėžinėse ląstelėse STAT išlieka nuolat fosforilintos ar aktyvios formos [189, 190]. Manoma, kad STAT3 dalyvauja navikų vystymesi aktyvinant genus, koduojančius apoptozės inhibitorius (Bcl-xL, Mcl-1), ląstelių ciklo reguliatorius (ciklinai D1/D2, c-Myc) ir angiogenezės aktyvatorius (KEAF) [191].

STAT3 svarba vystantis augliams buvo nagrinėta kolorektalinės karcinomos [192], hepatoceliulinės karcinomos [193], daugybinės mielomos [194], glioblastomos [195], prostatos vėžio [196], galvos ir kaklo vėžio [197] atvejais. Nustatyta padidėjusi *STAT3* raiška augimo hormoną sekretuojančiose HA, palyginti su neaktyviomis HA [198].

STAT3 rs744166 geno polimorfizmas buvo tirtas tik sergantiesiems skrandžio [35, 36], storosios žarnos [37] ir plaučių vėžiu [38], tačiau sąsajos su HA iki šiol nebuvo tirtos.

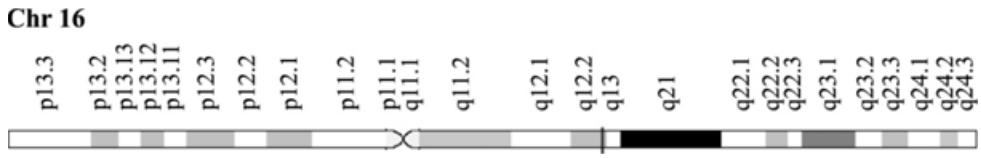


1.6.3.1 pav. *17 chromosoma ir STAT3 geno lokalizacija (http://www.genecards.org/cgi-bin/carddisp.pl?gene=STAT3)*

1.7. Sergančiųjų HA *MMP-2* (-1306 C/T) ir *MMP-9* (-1562 C/T) genų polimorfizmai

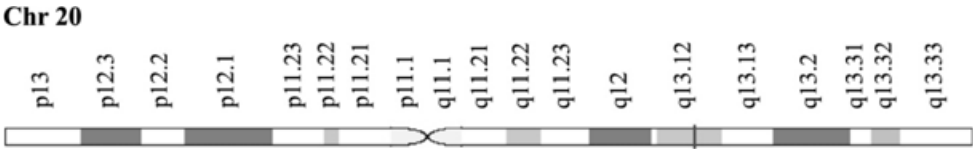
Matrikso metaloproteinazės (MMP) tai yra nuo cinko priklausomos endopeptidazės, dar kitaip vadinamieji matriksinai. MMP dalyvauja užląstelinio matrikso ir bazinės membranos degradacijoje, yra susiję su auglių invazyvumu, metastazavimu bei angiogeneze [12–18]. MMP yra skirstomos į šešias grupes: kolagenazes, želatinazes, stromelizinus, matrilizinus, membraninio tipo MMP ir kitas MMP [199]. MMP gamybą skatina daugelis faktorių: citokinai, augimo faktoriai, stresas, ląstelinis-ekstraląstelinis matriksas, ląstelės–ląstelės tarpusavio sąveika [19]. Manoma, kad yra keturi MMP veikimo keliai: 1) MMP, keičiant ląstelių adhezinį fenotipą į ne adhezinį bei suardant užląstelinę erdvę, veikia ląstelių migraciją, 2) MMP keičia užląstelinės erdvės mikroaplinką ribojant ląstelių proliferaciją bei apoptozę, 3) MMP moduliuoja biologiškai aktyvias molekules, skaldydamos arba išlaisvindamos jas iš užląstelinės erdvės, 4) MMP keičia proteazių aktyvumą, skaldydamos fermentus ar jų slopintojus [19]. Žinomi 24 skirtingi genai, koduojantys MMP proteazių raišką [200]. *MMP* raiškos aktyvumas gali priklausyti nuo įvairių genų polimorfizmo promotoriaus srityje, kai yra pažeidžiama transkripcijos faktorių ar kitų reguliacinių elementų prisijungimo vieta. Yra nustatyta, kad, jei *MMP-2* (-1306 C/T) 1306 bazių poroje citidinas pakeičiamas timino nukleotidu, promotorius praranda 50 proc. aktyvumo [42]. Taip pat žinoma, kad ir *MMP-9* (-1562 C/T) 1562 bazių poroje citidiną pakeitus timino nukleotidu pakinta promotoriaus aktyvumas. C nukleotidą pakeitus į T sutrinka branduolio baltymų komplekso prisijungimas prie DNR grandinės, jei yra T alelis. Nustatyta, kad jei C alelis mutuoja į T, promotoriaus aktyvumas padidėja 1,5 karto [201].

Nustatyta, kad *MMP-2* koduojantis genas yra 16 chromosomos ilgojo peties 16q13-q21 regione (1.7.1 pav.).



1.7.1 pav. *16 chromosoma ir MMP-2 geno lokalizacija (http://www.genecards.org/cgi-bin/carddisp.pl?gene=MMP2)*

MMP-9 koduojantis genas yra 20 chromosomos ilgojo peties 20q11.2-q13.1 regione (1.7.2 pav.).



1.7.2 pav. *20 chromosoma ir MMP-9 geno lokalizacija (http://www.genecards.org/cgi-bin/carddisp.pl?gene=MMP9)*

Daugelio tyrimų duomenimis, nustatyta padidėjusi *MMP-2* raiška įvairių navikų metu, įskaitant krūties [202–207], plaučių [208], kolorektalinį vėžį [209], kasos karcinomą [210], skrandžio ir stemplės vėžį [211–215]. Keliuose tyrimuose nustatyta padidėjusi raiška sergantiesiems glioma [216–218]. Yra tik keli tyrimai, nagrinėjantys *MMP-2* raišką sergantiesiems HA [219–220], ir nėra nė vieno tyrimo, analizuojančio *MMP-2* (-1306 C/T) polimorfizmą sergant HA. Tyrinėtos *MMP-2* (-1306 C/T) polimorfizmo sąsajos su įvairiais kitais navikais (plaučių, stemplės, skrandžio, krūtų, kolorektalinio, šlapimo pūslės vėžiu, glioma), tačiau rezultatai išlieka kontroversiški [48–60].

Keliuose moksliniuose tyrimuose analizuota *MMP-9* raiška HA [220–222] bei prostatos ir skrandžio navikiniame audinyje [39, 40, 223]. Nustatyta statistiškai reikšmingai didesnė *MMP-9* raiška invazinėse HA nei neinvazinėse HA [219, 221, 224–227]. Kai kurie tyrėjai nerado sąsajų tarp naviko invazyvumo ir *MMP-9* raiškos [228]. Keliuose tyrimuose ieškota sąsajų tarp *MMP-9* (–1562 C/T*)* geno polimorfizmo ir prostatos [39], krūties [41, 40], skrandžio [42–44], kolorektalinio [45, 46] ir plaučių vėžio [47]. Diana Schveigert ir kolegos nustatė, kad prostatos vėžio atveju *MMP-9* (-1562 C/T) polimorfizmas susijęs su vėžio diferenciacijos laipsniu [39]. Morteza Sadeghi ir kolegos nustatė, kad C/C ir T/T genotipai lemia krūties vėžio invazyvumą ir metastazavimą [40]. Shuji Matsumura ir bendraautoriai nustatė, kad T alelio buvimas susijęs su skrandžio vėžio progresavimu ir invazyvumu japonų populiacijoje [43]. Frank J.G.M. Kubben ir bendraautoriai nenustatė sąsajų tarp *MMP-9* (-1562 C/T) polimorfizmo ir skrandžio vėžio [44]. Mohammad Jafari ir bendraautoriai nustatė, kad T alelis gali būti susijęs su plaučių vėžio progresavimu [47]. Li-Li Xing savo tyrime nustatė, kad *MMP-9* (-1562 C/T) polimorfizmas susijęs metastazavimu į limfmazgius sergantiesiems kolorektaliniu vėžiu [46].

Rezultatai išlieka kontroversiški. Tyrimų, kuriuose būtų tirta *MMP-9* (–1562 C/T) geno polimorfizmo sąsajos su HA išsivystymu, iki šiol nėra atlikta.

1.8. *FGFR2*, *SIRT1*, *STAT3*, IL-17A ir *MMP* sąsajos

Nustatytas ryšys tarp padidėjusios FGFR raiškos ir STAT3 fosforilinimo vėžinėse ląstelėse. FGF sukeltas STAT3 aktyvavimas, fosforilinant tiroziną, reikalauja aukštos FGFR raiškos ir priklauso nuo su citokinų receptoriais susijusių kinazių (JAKs) arba Src onkogenų (baltymų tirozinkinazių) [174, 229]. STAT3 yra transkripcijos faktorius, ir kai tirozinas yra fosforilinamas, translokuojasi į branduolį, kur reguliuoja genų taikinių transkripciją. FGFR2 aktyvuoja STAT3, taip skatinama c-fos, c-myc ir JunB baltymus koduojančių genų (ankstyvo atsako transkripcijos faktoriai) raiška ir ląstelių proliferacija [174, 230]. Hiperaktyvi STAT3 forma skatina IL-17A gamybą CD4 ląstelėse [122]. STAT3 aktyvavimas padidina MMP, ciklooksigenazės-2 (COX-2) ir angiopoetino-2 (Ang-2), nuo kurių priklauso navikinių ląstelių migracija, angiogenezė ir metastazavimas, raišką [231]. Nustatyta, kad SIRT1 dalyvauja STAT3 acetilinime, slopina STAT3 fosforilinimą bei transkripcinį aktyvumą [232–234]. Taip pat žinoma, kad, esant padidėjusiam SIRT1 aktyvumui, slopinama MMP raiška [235].

Iki šiol nėra nė vieno klinikinėje praktikoje rutiniškai naudojamo molekulinio žymens, kuris būtų siejamas su HA atsiradimu, invazyvumu, pasikartojimu. Todėl mes nusprendėme ištirti dalyvaujančius bendruose patogeneziniuose mechanizmuose ir turinčius sąsajas su įvairiais kitais navikais žymenis (*MMP-2* rs243865, *MMP-9* rs3918242, *FGFR2* rs2981582, *SIRT1* rs12778366, *STAT3* rs744166, Il-17A) bei Ki-67 PI sergantiesiems HA. Tikimės, kad mūsų tyrimo rezultatai padės suprasti HA patogenezę, o ateityje diagnozuoti ir gydyti HA.

2. TYRIMO METODIKA

Moksliniam medicininiam tyrimui atlikti gauti Kauno regioninio biomedicininių tyrimų etikos komiteto (Nr. P2-9/2003) ir Valstybinės duomenų apsaugos inspekcijos (Nr. 2R-581) leidimai.

2.1. Tirtasis kontingentas

Į tyrimą įtraukti asmenys, gydyti LSMU Akių ligų, Neurochirurgijos ir Endokrinologijos klinikose, kuriems diagnozuota HA, pasirašę informuoto asmens sutikimo formą. Šis mokslinis tyrimas yra Lietuvos mokslų tarybos Nacionalinės mokslo programos „Mokslininkų grupės“ finansuojamo projekto „Hipofizės adenomos naujų molekulinių prognostinių žymenų paieška ir sąsajos su regos funkcijomis“ (MIP-008/2014) dalis.

Įtraukimo kriterijai: pacientams HA diagnozė patvirtinta magnetinio rezonanso tomografijos (MRT) tyrimu; sutikimas dalyvauti tyrime; amžius ≥ 18 metų; neserga kitu galvos smegenų ar kitos lokalizacijos augliu; nenustatyta kitų akių ligų (glaukoma, didelio laipsnio refrakcijos ydos, intensyvios optinių terpių drumstys, regos nervo ligos) detalaus oftalmologinio patikrinimo metu.

Tyrimo metu suformuotos tiriamųjų grupės:

I grupė – oftalmologiškai ištirti asmenys, sergantys HA (n=77) (154 akys). Tiriamųjų amžius buvo nuo 24 iki 83 metų. Tiriamuosius sudarė 34 (44,2 proc.) vyrai ir 43 (55,8 proc.) moterys. Šioje grupėje IL-17A ištirtas 60 asmenų (77,9 proc.). Ki-67 PI nustatytas 69 asmenims (89,6 proc.). Sergantieji HA pagal Ki-67 PI suskirstyti į tris grupes:

1. Ki-67 PI < 1 proc.
2. Ki-67 PI = 1 proc.
3. Ki-67 PI > 1 proc.

Kontrolinę grupę OKT rodmenims palyginti sudarė 77 sveiki asmenys (154 akys), į Munsell-Farnsworth 100 atspalvių atrinkimo ir kompiuterinio ribinio spalvinio kontrastinio jautrumo tyrimo kontrolinę grupę atrinkti 99 asmenys (198 akys), į funkcinio kontrastinio jautrumo tyrimo – 198 asmenys (396 akys). IL-17A kontrolinę grupę sudarė 64 asmenys.

Kontrolinės grupės sudarytos atsižvelgiant į amžiaus ir lyties pasiskirstymą sergančiųjų HA grupėje.

II grupė – pacientai, sergantys HA, kuriems ištirtas *MMP-2* rs243865 geno polimorfizmas (n=84).

Kontrolinę *MMP-2* rs243865 geno polimorfizmo grupę sudarė 318 sveiki asmenys, atsižvelgiant į amžiaus ir lyties pasiskirstymą sergančiųjų HA grupėje.

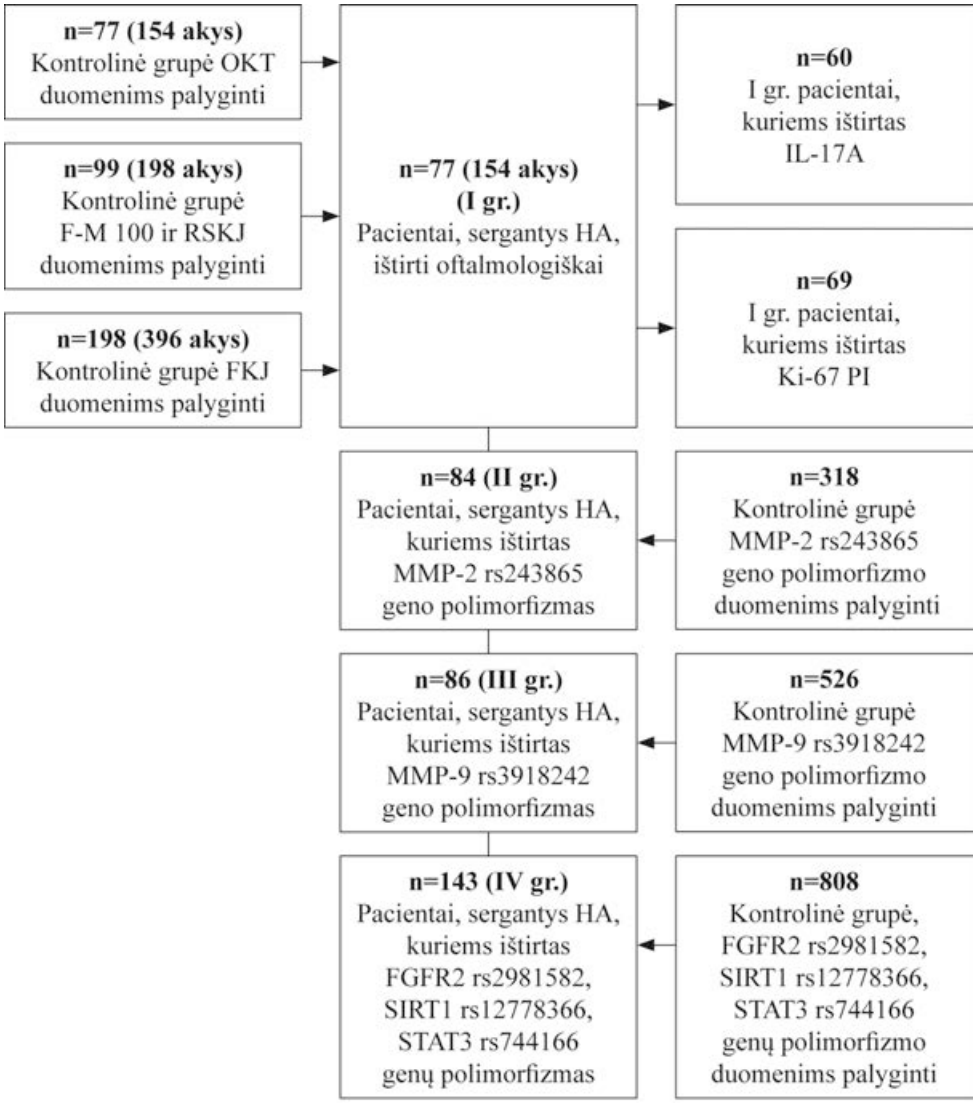
III grupė – pacientai, sergantys HA, kuriems ištirtas *MMP-9* rs3918242 geno polimorfizmas (n=86).

Kontrolinę *MMP-9* rs3918242 geno polimorfizmo grupę sudarė 526 sveiki asmenys, atsižvelgiant į amžiaus ir lyties pasiskirstymą sergančiųjų HA grupėje.

IV grupė – pacientai, sergantys HA, kuriems ištirtas *FGFR2* rs2981582, *SIRT1* rs12778366 bei *STAT3* rs744166 genų polimorfizmas (n=143).

Kontrolinę *FGFR2* rs2981582, *SIRT1* rs12778366 ir *STAT3* rs744166 genų polimorfizmo grupę sudarė 808 sveiki asmenys, atrinkti atsižvelgiant į amžiaus ir lyties pasiskirstymą sergančiųjų HA grupėje.

MMP-2 rs243865 ir *MMP-9* rs3918242 genų polimorfizmo kontrolinė grupė buvo sudaryta iš atsitiktinės Kauno miesto gyventojų imties, kuri buvo surinkta LSMU Kardiologijos instituto Populiacinių tyrimų laboratorijai vykdant tarptautinį HAPIEE (*Health, Alcohol and Psychosocial Factors In Eastern Europe*) projektą (Paesey, ir kt., 2006), CINDI (*Countrywide Integrated Non-communicable Disease Intervention*) projektą (Grabauskas, ir kt., 2003) bei projektą „Sveikas senėjimas“.



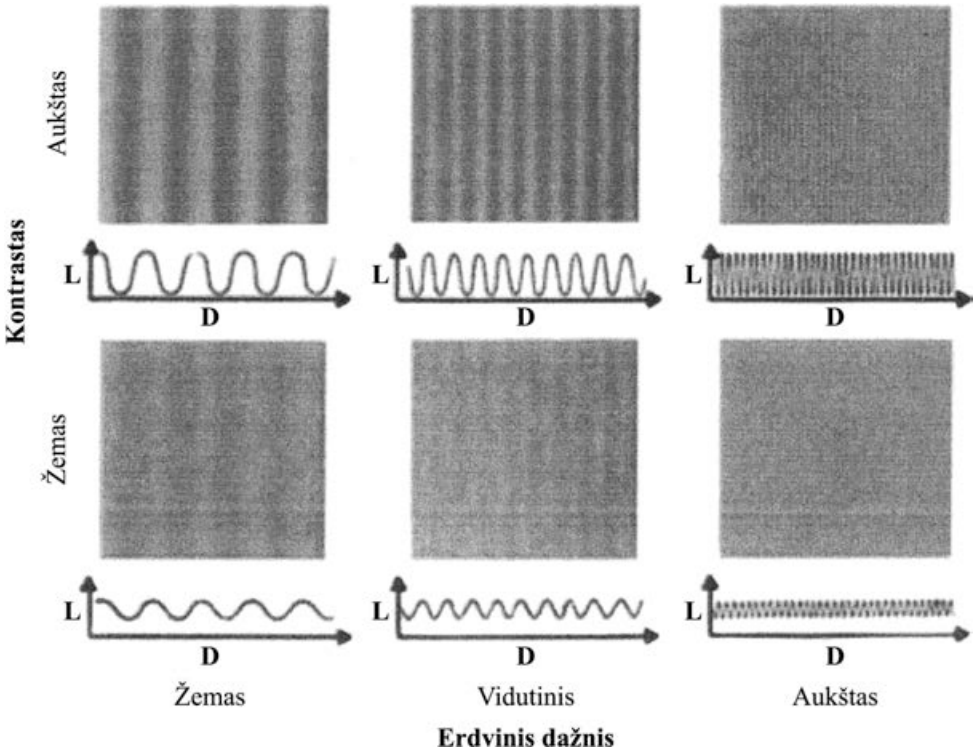
2.1.1 pav. *Tirtieji pacientai*

2.2. Oftalmologinis ištyrimas

Oftalmologiniai tyrimai atlikti LSMU MA Akių ligų klinikoje. Visiems pacientams įvertintas geriausias koreguotas regos aštrumas į tolį, naudojant Landolto žiedus (C optotipus) Sneleno principu (tirta 5 metrų atstumu) dešimtaine sistema. Biomikroskopu įvertinta vokų, junginių, ragenos, rainelės, lęšiuko, stiklakūnio būklė. Tiriamųjų vyzdžiams išplėsti vartotas 1 proc. tropikamido tirpalas. Akių dugnas tirtas naudojant monokulinį tiesioginį oftalmoskopą ir plyšinę lempą, naudojant dvigubai asferinį + 90 D lęšį (*Volk*, JAV). Atliktas statinės perimetrijos pilno regėjimo lauko tyrimas (135 taškai, 87 laipsniai temporaliai) Humphrey Field Analyser, Model 745i aparatu (*Carl Zeiss Meditec Inc.* Dublin, CA, USA), taip pat funkcinis kontrastinio jautrumo tyrimas, pagal dr. Arthur P. Ginsburg metodiką OPTEC 6500 aparatu, kompiuteriniai ribinio spalvinio kontrastinio jautrumo ir Munsell-Farnsworth 100 atspalvių atrinkimo tyrimai, optinė koherentinė tomografija RS-3000 Advance aparatu (*Nidec Co.*, Japan).

2.2.1. Funkcinis kontrastinio jautrumo tyrimas

Funkcinis kontrastinio jautrumo tyrimas (FKJT), naudojant skirtingo kontrasto ir erdvinio dažnio sinuso bangos groteles, atliktas pagal dr. Arthur P. Ginsburg metodiką OPTEC 6500 aparatu. Kontrastinis jautrumas vertintas penkiais standartiniais erdviniais dažniais (1,5; 3,0; 6,0; 12,0; 18,0 ciklai / laipsnis) pagal devynias kontrastingumo pakopas. Ciklų skaičius viename laipsnyje – tai besikeičiančių juodų ir baltų linijų (erdvinių dažnių) skaičius vieno laipsnio kampo regos lauke. Tyrimas atliktas esant geriausiam regos aštrumui nakties (3 cd/m²) ir dienos (85 cd/m²) sąlygomis, be (su) akinančios šviesos. Tyrimo metu pacientas turi apibūdinti paskutinę matomą liniją kiekvienoje eilėje (A, B, C, D ir E) ir pasakyti linijos kryptį: dešinė, vertikali, kairė. Paskutinė tikroji matoma linija kiekvienam erdviniam dažniui pažymima kontrastinio jautrumo kreivėje. Kontrastinis jautrumas vertintas naudojant logaritminę sistemą.



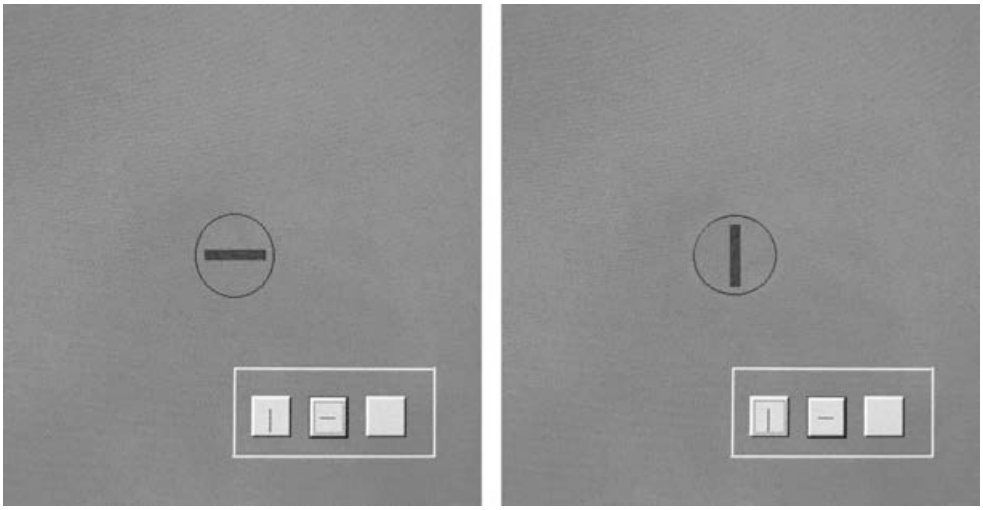
2.2.1.1 pav. *Funkcinis kontrastinio jautrumo tyrimas, naudojant skirtingo kontrasto ir erdvinio dažnio sinuso bangos groteles*

2.2.2. Spalvų juslės tyrimai

Farnsworth-Munsell 100 (F-M 100) atspalvių tyrimo metu reikia sudėlioti 88 vienodo šviesumo ir sotumo visą atspalvių spektrą apimančius spalvinius pavyzdžius pagal atspalvį. Spalviniai pavyzdžiai atsitiktine tvarka rodomi kompiuterio ekrane. Rezultatas vertinamas suma skirtumų tarp tiriamojo nustatyto ir turinčio būti toje vietoje spalvinio pavyzdėlio numerio. Vertinamas spalvų skyrimo laipsnis: labai geras – klaidų skaičius iki 20, normalus vidutinis – klaidų iki 100, sutrikusi spalvų juslė – klaidų daugiau nei 100.

2.2.2.1 pav. *Farnsworth-Munsell 100 spalvų juslės tyrimas*

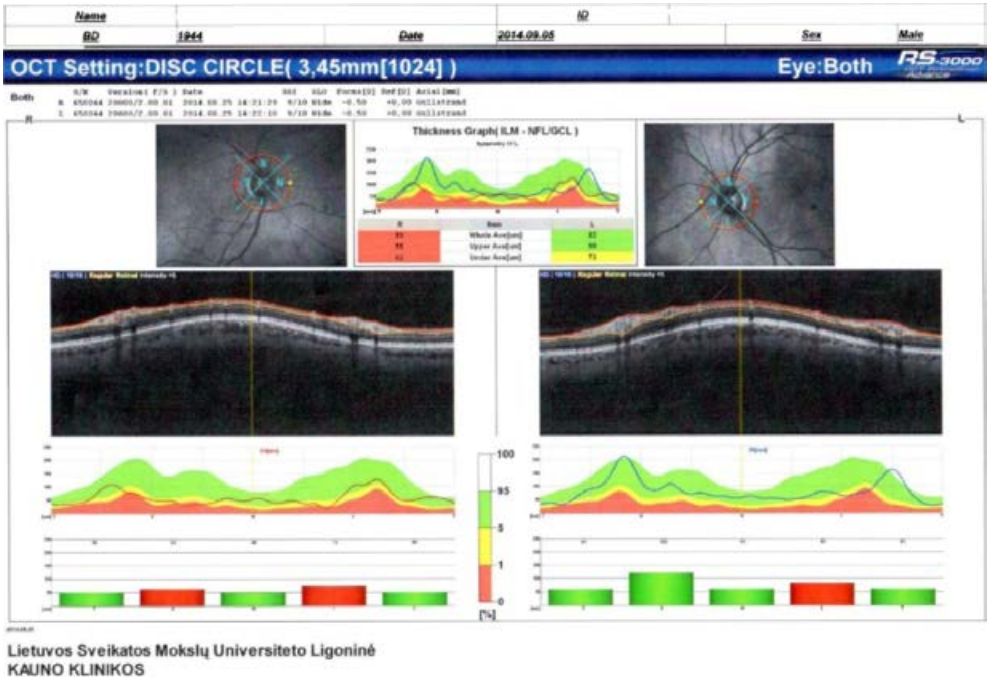
Ribinio spalvinio kontrastinio jautrumo (RSKJ) testo metu pilko fono skritulyje rodomas horizontalus arba vertikalus brūkšnys. Tyrimo metu reikia nurodyti brūkšnio kryptį. Jei spalva nurodoma teisingai, mažinamas spalvos sodris ir keičiamas fono šviesis. Jei kryptis nurodoma klaidingai, padidinamas brūkšnio spalvos sodris. Spalvų juslės tam tikrai spalvai slenkstis nustatomas pagal pirmą teisingą atsakymą po neteisingų atsakymų serijos arba pirmą neteisingą atsakymą po teisingų atsakymų serijos. Paciento spalvų juslės slenkstis – tai vidutinis spalvų juslės slenkstis visoms bandytoms spalvoms.



2.2.2.2 pav. *Ribinio spalvinio kontrastinio jautrumo tyrimas*

2.2.3. Optinė koherentinė tomografija

Tinklainės nervinių skaidulų sluoksnis buvo vertinamas spektrinės optinės koherentinės tomografijos (*RS-3000 Advance Nidec Co.*, Japan) būdu, išplėtus vyzdžius 1 proc. tropikamido tirpalu. Akių dugno vaizdai buvo gauti konfokaliniu skenuojančiu lazeriu, naudojant 785 nm ilgio artimus infraraudoniesiems spindulius. Tinklainės skersinių pjūvių vaizdai gauti optiniu interferometru naudojant infraraudonųjų spindulių, kurių bangos ilgis 880 nm, šaltinį. Naudota „disk circle mode“ programa. Jos metu akių dugnas yra skenuojamas cirkuliariai apie regos nervo diską tokia tvarka – temporalinė dalis, viršutinė, nazalinė ir tada apatinė dalis. Nustatomas peripapiliarinio TNSS vidutinis storis ir atskirai kiekvieno kvadranto storis. Skenavimo krypties A-skenavimų kiekis: 1024.

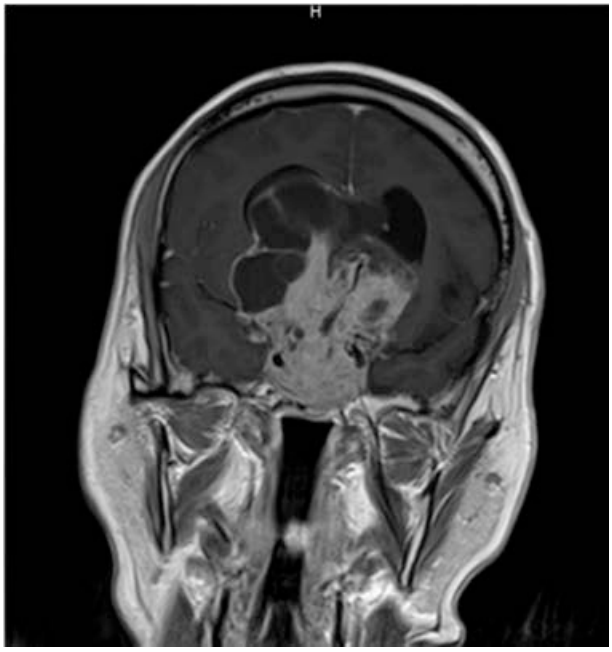


2.2.3.1 pav. *Tinklainės nervinių skaidulų sluoksnio storio pokyčių sergant HA pavyzdys*

2.3. Radiologinė duomenų analizė

HA magnetinio rezonanso tomografijos tyrimai atlikti 1,5 T MR skeneriais (*Siemens MAGNETOM Avanto, Philips ACHIEVA*), naudojant galvos ritę. MRT tyrimui atlikti taikytas standartinis hipofizės skenavimo protokolas, prieš intraveninį kontrastavimą atliekant T1W seką šoninėje ir vainikinėje plokštumose, taip pat T2W/TSE seką vainikinėje plokštumoje. Po intraveninio kontrastinės medžiagos sušvirkštimo atlikta T1W seka vainikinėje ir šoninėje plokštumose. Intraveniniam kontrastavimui naudota kontrastinė medžiaga gadodiamidas (*Omniscan* 0,5 mmol/ml), kontrastinės medžiagos kiekį apskaičiuojant pagal paciento kūno masę.

T2W/TSE seka vainikinėje plokštumoje pasirinkta kaip optimali RNK matuoti. Iš gautų T2W/TSE sekos vainikinės plokštumos vaizdų išrinktas pjūvis su optimaliai diferencijuojama RNK. RNK storis matuotas ties RNK viduriu bei lateralinėse kryžmės dalyse. Įvertintos ir užfiksuotos RNK deformacijos ir signalo pakitimai. Taip pat išmatuotas mažiausias atstumas tarp hipofizės naviko viršutinės ribos ir RNK. MRT duomenų analizė atlikta retrospektyviai gydytojos radiologės S. Jakštienės.



2.3.1 pav. *Invazinės hipofizės adenomos pavyzdys*

HA augimo ir invazijos pobūdžiui įvertinti naudojome Hardžio klasifikaciją, modifikuotą Vilsono [23], bei Knosp klasifikaciją [236].

Hipofizės adenomų supra- bei paraseliarinio augimo Hardžio klasifikacija, modifikuota Vilsono:

Supraseliarinis augimas

A – plėtimasis (augimas) į supraseliarinę cisterną,

B – obturuota trečio skilvelio priekinė kišenė,

C – ryški trečio skilvelio dugno dislokacija.

Paraseliarinis augimas

D – intrakranijinis (intraduralinis):

1. į priekinę daubą,
2. į vidurinę daubą,
3. į užpakalinę daubą.

E – augimas į akytąjį antį ar po akytuoju ančiu (ekstraduralinis).

Turkiabalnio dugno pažeidimas buvo klasifikuojamas į I–IV laipsnius:

I – turkiabalnis normalus ar lokaliai deformuotas; auglys <=10 mm.

II – turkiabalnis padidėjęs; auglys >=10 mm.

III – lokali turkiabalnio dugno perforacija.

IV – difuzinė turkiabalnio dugno destrukcija.

I, II laipsnis – turkiabalnio dugnas nepažeistas ir tuomet HA vertinome kaip neinvazinę.

III, IV laipsnis – turkiabalnio dugnas pažeistas ir HA vertinta kaip invazinė.

HA invazijos į akytąjį antį klasifikacija pagal Knosp:

0 – nėra augimo link akytojo ančio.

1 – navikas stumia medialinę akytojo ančio sienelę, tačiau neperauga hipotetinės linijos tarp dviejų vidinės miego arterijos segmentų centrų.

2 – navikas perauga hipotetinę liniją tarp dviejų vidinės miego arterijos centrų, tačiau neperauga hipotetinės linijos išvestos per lateralinę miego arterijos sienelę.

3 – navikas auga lateraliau vidinės miego arterijos.

4 – navikas visiškai apsupa vidinės miego arterijos akytojo ančio segmentą.

Pagal Knosp klasifikaciją, tik 3 ir 4 laipsnio HA vertintos kaip invazinės.

2.4. Deoksiribonukleininės rūgšties išskyrimas ir genų polimorfizmų tyrimas

Visiems pacientams iš venos buvo imamas periferinis kraujas genetiniams tyrimams atlikti. Deoksiribonukleininės rūgšties (DNR) išskyrimas, hipofizės navikinio audinio molekulinių ir kraujo imunogenetinių žymenų tyrimai buvo atliekami LSMU Neuromokslų instituto Oftalmologijos bei Neuroonkologijos ir genetikos laboratorijose, LSMU Laboratorinės medicinos klinikoje ir LSMU Patologinės anatomijos klinikoje. Imunogenetinius tyrimus atliko genetikė A.Vilkevičiūtė. DNR bankas papildytas LSMU Neuromokslų instituto Neuroonkologijos ir genetikos laboratorijoje sukauptais DNR mėginiais.

DNR išskirti kraujas buvo surinktas į vakuuminius mėgintuvėlius su EDTA (etilendiamintetraacetatu). Tyrimo metu naudota genominė DNR buvo išskirta iš periferinio kraujo leukocitų, naudojant *Thermo Scientific GeneJET Genomic DNA Purification Kit* ir *Thermo Scientific MagJET Genomic DNA Kit* DNR skyrimo rinkinius. Genominės DNR skyrimas atliktas remiantis gamintojo rekomendacijomis.

DNR koncentracija išmatuota spektrofotometru „*Agilent Technologies, Cary 60 UV – Vis*“. DNR, RNR, oligonukleotidų ir mononukleotidų kiekis nustatytas vandeniniuose tirpaluose, matuojant tirpalo absorbciją (optinį tankį) ultravioletinių bangų ilgyje. Nukleino rūgštys nustatytos panaudojant

260 nm bangos ilgio spindulius. Baltyminės tirpalo priemaišos įvertintos matuojant santykį bangos ilgių prie 260 nm ir 280 nm.

Genų polimorfizmo tyrimas (*MMP-2* rs243865, *MMP-9* rs3918242, *SIRT1* rs12778366, *FGFR2* rs2981582 ir *STAT3* rs744166) buvo atliktas naudojant tikro laiko grandininės polimerazės reakcijos (TL-PGR) metodą.

Genotipavimas buvo atliekamas TL-PGR gausintuvu „*Rotor – Gene Q*“. *MMP-2* rs243865, *MMP-9* rs3918242, *SIRT1* rs12778366, *FGFR2* rs2981582 ir *STAT3* rs744166 genotipuoti skirti rinkiniai, pradmenys ir molekuliniai žibukai buvo sukurti kompanijos „*Applied Biosystems*“. DNR mėginiai, laikyti –20º C temperatūroje, buvo atšildyti ir nucentrifuguoti prieš atliekant TL-PGR. Kiekvienai reakcijai buvo naudojama 1 µl matricinės bandinių DNR (~10 ng) ir 9 µl PGR reakcijos mišinio, kurio sudėtis kiekvienam tiriamam polimorfizmui nurodoma 2.4.1 lentelėje. PGR vykdyta pagal 2.4.2 lentelėje aprašytas sąlygas.

2.4.1 lentelė. *TL-PGR mišinio paruošimas MMP-2 rs243865, SIRT1 rs12778366, FGFR2 rs2981582, STAT3 rs744166 ir MMP-9 rs3918242 genų polimorfizmams nustatyti*

Reagentai	**Pradinė koncent-racija**	**Galutinė koncentracija (tūris 10 µl)**	***MMP-2* (rs243865) 1 pvz., µl**	***SIRT1* (rs12778366) 1 pvz., µl**	***FGFR2* (rs2981582) 1 pvz., µl**	***STAT3* (rs744166) 1 pvz., µl**	***MMP-9* (rs3918242) 1 pvz., µl**
TaqMan Universal Master Mix II, no UNG („Applied Biosystems“, Lietuva)	2X	1X	5	5	5	5	5
Sterilus H_2O („Thermo Fisher Scientific“, Lietuva)	–	–	3,5	3,5	3,5	3,5	2,8
„Applied Biosystems“ genotipavimo rinkinys (C_ 3225943_10 (*MMP*-2 rs243865)	20X	1X	0,5	–	–	–	–
„Applied Biosystem“ genotipavimo rinkinys C_ 1340370_10 (*SIRT1* rs12778366)	–	–	–	0,5	–	–	–
„Applied Biosystem“ genotipavimo rinkinys C_ 2917302_10 (*FGFR2* rs2981582)	–	–	–	–	0,5	–	–
„Applied Biosystem“ genotipavimo rinkinys C_ 3140282_10 (*STAT3* rs744166)	–	–	–	–	–	0,5	–
Pirmaujančios sekos pradmuo, „Applied Biosystem“, UK	10 pmol/µl	0,16 pmol/µl	–	–	–	–	0,4
Atsiliekančios sekos pradmuo, „Applied Biosystem“, UK	10 pmol/µl	0,16 pmol/µl	–	–	–	–	0,4
VIC žymėtas molekulinis žibukas „Applied Biosystem“, UK	20 pmol/µl	0,4 pmol/µl	–	–	–	–	0,2
6 FAM žymėtas molekulinis žibukas, „Applied Biosystem“, UK	20 pmol/µl	0,4 pmol/µl	–	–	–	–	0,2

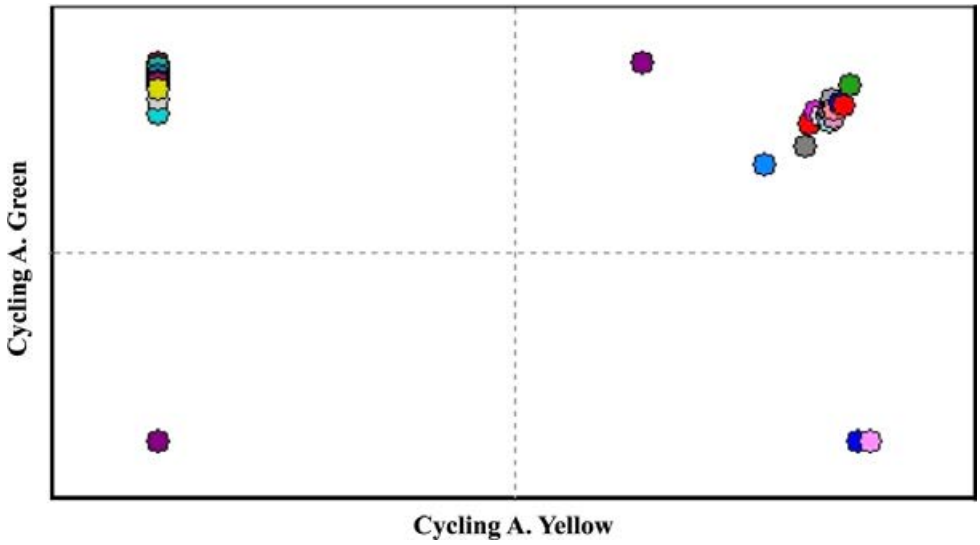
2.4.2 lentelė. *Optimalios genotipavimo reakcijos sąlygos, nustatant MMP-2 rs243865, MMP-9 rs3918242, SIRT1 rs12778366, FGFR2 rs2981582 ir STAT3 rs744166 genotipus tikro laiko PGR gausintuvu „Rotor Gene-Q“*

	Pradmenų sekos ir molekuliniai žibukai	**PGR sąlygų protokolai**	
MMP-2 rs243865	„Applied Biosystem“ patentas.	95ºC 10 min.	40 ciklų: 92 ºC 15 sek. 60 ºC 60 sek.
SIRT1 rs12778366	„Applied Biosystem“ patentas.	95ºC 10 min.	40 ciklų: 92 ºC 15 sek. 60 ºC 60 sek.
FGFR2 rs2981582	„Applied Biosystem“ patentas.	95ºC 10 min.	40 ciklų: 92 ºC 15 sek. 60 ºC 60 sek.
STAT3 rs744166	„Applied Biosystem“ patentas.	95ºC 10 min.	40 ciklų: 92 ºC 15 sek. 60 ºC 60 sek.
MMP-9 rs3918242	F:5’-CAGATCACTTGAGTCAGAA-3’ („Applied Biosystem“, UK) R: 5’-GGTGTAGTATCACTCTGTCA- 3’ („Applied Biosystem“, UK) 5’FAM- TGGCGCACGCCTATAATACCA-MGBNFQ 3’ („Applied Biosystem“, UK) 5’VIC-TGGCGCATGCCTATAATACCAGC-MGBNFQ 3’ („Applied Biosystem“, UK)	95ºC 10 min.	40 ciklų: 95 ºC 15 sek. 60 ºC 60 sek.

F – pirmaujanti seka, R – atsiliekanti seka.

Metodas:

1. PGR mišinys paruošiamas 72 mėginiams.
2. Mišinys išpilstomas po 9 µl į 0,1 ml tūrio mėgintuvėlius, kurie yra sujungti juostelėmis po 4.
3. Į 71 mėgintuvėlį įpilama po 1 µl DNR, o paskutiniajame mėgintuvėlyje, kaip neigiama kontrolė, įpilama sterilaus vandens.
4. Mėgintuvėliai sudedami į specialų 72 mėgintuvėliams skirtą diską.
5. Nustatoma programa vienam iš polimorfizmų identifikuoti. Genotipuojant naudota Alelių nustatymo programa (angl. *Allelic Discrimination*).
6. Pagal skirtingų detektorių fluorescencijos intensyvumo santykį programa nustato individų genotipus. Tam tikri kanalai atpažįsta skirtingus fluorescencinius dažus: „*Yellow*“ kanalas atpažįsta VIC fluorescenciniais dažais žymėtus molekulinius žibukus, o „*Green*“ – FAM fluorescenciniais dažais žymėtus molekulinius žibukus (2.4.4.1 pav.). Programai baigus darbą, gauti rezultatai naudojami statistiniams skaičiavimams atlikti.



2.4.4.1 pav. *Alelių nustatymo programos rezultatų diagrama*

2.5. IL-17A koncentracijos kraujo serume nustatymas

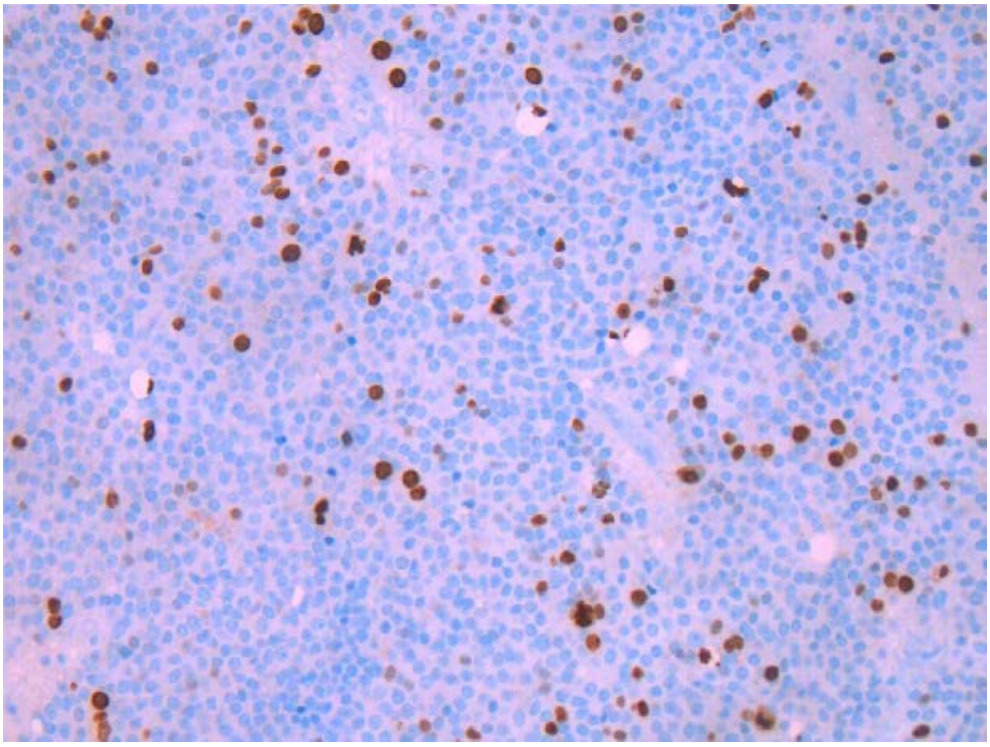
Asmenų, sergančių HA, ir kontrolinės grupės asmenų IL-17A koncentracijai kraujo serume nustatyti buvo atlikta imunofermentinė analizė, naudojant komercinį ELISA (angl. *enzyme linked immunosorbent assay*) rinkinį pagal gamintojo pateiktą protokolą („*ThermoFisher Scientific Human IL17A ELISA Kit*“). Tirpalai, skirti tyrimui atlikti, paruošti pagal gamintojų protokolą. Kalibracinei kreivei nustatyti buvo paruošti žinomų IL-17A koncentracijų etaloniniai tirpalai: 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62,5 pg/ml, 31,25 pg/ml ir 0 pg/ml. Į sterilias 96 šulinėlių ELISA plokšteles (į kiekvieną šulinėlį) buvo įpilta po 50 uL biotinilintų antikūnų. Etaloniniai tirpalai ir tiriamieji mėginiai buvo išpilstyti į šulinėlius po 50 uL po du kartus. Uždengta plokštelė inkubuota 2 valandas kambario temperatūroje ir 3 kartus plauta plovimo buferiu. Į kiekvieną šulinėlį įpilta po 100 uL streptavidino konjugato su krienų peroksidaze ir uždengus inkubuota 1 valandą. Kiekvienas šulinėlis ir vėl 3 kartus plautas plovimo buferiniu tirpalu. Į kiekvieną šulinėlį, pridėjus substrato tirpalo, inkubuota 30 min. kambario temperatūroje, reakcija vykdyta tamsoje. Į kiekvieną šulinėlį pridėjus po 100 uL reakcijos sustabdymo reagento, mėlyna spalva pakito į geltoną. Absorbcija buvo matuota mikroplokštelių skaitytuvu esant 450 nm ir 550 nm bangų ilgiams, o koncentracijai skaičiuoti buvo naudojamas skirtumas tarp optinių tankių, esant šiems bangos ilgiams. Mėginių koncentracija buvo nustatoma naudojant etaloninių junginių kreivę.

2.6. Ki-67 proliferacijos indekso nustatymas

Ki-67 PI nustatymas atliktas LSMU Patologinės anatomijos klinikoje gydytojos patologės I. Gudinavičienės. Darbe tirtų biologinių žymenų baltymų raiška parafininiuose pjūviuose analizuota IHC tyrimo metodu naudojant Ventana BenchMark XT dažymo automatą (*Ventana Medical Systems, Tucson,* Arizona, JAV). Parafininiai pjūviai deparafinizuoti naudojant *Ventana* deparafinizavimo reagentą. Antigenų epitopams atkurti naudotas *Ventana* ląstelių kondicionavimo tirpalas (pH 8,4) – 100^{o} C 60 min. Vėliau pjūviai inkubuoti monokloniniu antikūnu – 37° C 32 min. ir identifikuoti, naudojant *Ventana Iview DAB* detektavimo sistemą. IHC reakcijos pabaigoje pjūviai kontrastuoti Gillo hematoksilinu, po to melsvinti vandeninio buferinio ličio karbonato tirpalu ir uždengti dengiamaisiais stikleliais.

Antikūnas	Gamintojas	Klonas	Skiedimo santykis
Ki-67	*Spring*	SP6	1:200

Ki-67 PI yra teigiamai nusidažiusių ir visų branduolių santykis, išreikštas procentais (2.6.1 pav.).



2.6.1 pav. *Ki-67 teigiami branduoliai*

2.7. Statistinė analizė

Statistinė duomenų analizė atlikta naudojant statistinės programos paketą „*Statistical Package for the Social Sciences, version 23,0 for Windows*“ (*SPSS for Windows*, 23,0 versija, JAV). Hipotezė apie matuojamų požymių reikšmių normalųjį skirtinį tikrinta taikant Kolmogorovo-Smirnovo ir Šapiro-Vilko testus. Tiriamiesiems požymiams, turintiems normalųjį skirstinį, buvo taikomos šios aprašomosios statistikos charakteristikos: vidurkis (standartinis nuokrypis) arba vidurkis (± standartinė vidurkio paklaida). Tiriamiesiems požymiams, kurie netenkino normaliojo skirstinio kriterijų, buvo taikomos šios aprašomosios statistikos charakteristikos: mediana, minimali bei maksimali reikšmės, vidutinis rangas.

MMP-2 rs243865, *MMP-9* rs3918242, *SIRT1* rs12778366, *FGFR2* rs2981582 ir *STAT3* rs744166 genų polimorfizmo pasiskirstymas sergantiesiems HA ir kontrolinės grupės asmenims vertintas pagal Hardžio-Vainber-

go pusiausvyros modelį (http://www.oege.org/software/hwe-mr-calc.shtml). Sergančiųjų HA ir kontrolinės grupės *MMP-2* rs243865, *MMP-9* rs3918242, *SIRT1* rs12778366, *FGFR2* rs2981582 ir *STAT3* rs744166 genų polimorfizmo pasiskirstymo homogeniškumui palyginti taikytas χ^2 ir Fisher Exact testas, naudojant vienpuses ir dvipuses alternatyvas. Atlikus dvinarę logistinę regresinę analizę, įvertintas galimybių santykis (GS) HA pasireikšti, atsižvelgiant į paveldėjimo modelius bei genotipų derinius. Ši analizė atlikta ir atskiroms HA grupėms pagal HA invazyvumą bei pasikartojimą, nurodant GS su 95 proc. patikimumo intervalu (PI). Pasirenkant geriausią paveldėjimo modelį buvo vertinamas Akaike informacinis kriterijus (AIC), kurio mažiausia vertė nurodo tinkamiausią modelį. IL-17A koncentracijoms palyginti skirtingose genotipų grupėse, kai duomenų pasiskirstymas nėra normalus, taikyti neparametriniai Kruskalo-Voliso ir Mano-Vitnio U testai. Statistinėms hipotezėms tikrinti pasirinkome kriterijaus reikšmingumo lygmenį 0,05. Statistiškai reikšmingas skirtumas buvo nustatomas tada, kai p reikšmė $< 0,05$.

3. REZULTATAI

3.1. HA charakteristikos

Ištirti 77 pacientai – 43 moterys (55,8 proc.) ir 34 vyrai (44,2 proc.). Keturiasdešimt šešiems pacientams (59,7 proc.) buvo diagnozuota invazinė HA ir 31 pacientui (40,3 proc.) neinvazinė HA. Atsinaujinusi HA nustatyta 12 pacientų (15,6 proc.).

Supraseliarinis augimo pobūdis diagnozuotas 55 pacientams (71,4 proc.). HA supraseliarinio augimo laipsniai pagal Hardžio klasifikaciją, modifikuotą Vilsono, pateikti 3.1.1 lentelėje.

3.1.1 lentelė. *Hipofizės adenomos supraseliarinio augimo laipsniai pagal Hardžio klasifikaciją, modifikuotą Vilsono, pacientų, sergančiųjų hipofizės adenoma, grupėje*

Supraseliarinio augimo laipsniai	Pacientų skaičius, n (proc.)
0	22 (28,6)
A	25 (32,5)
B	21 (27,3)
C	9 (11,7)

Paraintrakranijinis augimas diagnozuotas tik 10 asmenų (13,0 proc.). Parakaverninis augimas diagnozuotas 56 pacientams (73,7 proc.), tikroji akytojo ančio invazija (3–4 laipsnis pagal Knosp klasifikaciją) nustatyta 26 pacientams (33,8 proc.), sergantiems HA (3.1.2 lentelė).

3.1.2 lentelė. *Parakaverninio augimo laipsniai pagal Knosp klasifikaciją, pacientų, sergančių hipofizės adenoma, grupėje*

Parakaverninio augimo laipsniai	Pacientų skaičius, n (proc.)
0	21 (27,3)
1	16 (20,8)
2	14 (18,2)
3	15 (19,5)
4	11 (14,3)

Turkiabalnio dugno destrukcija (III–IV laipsnis) buvo diagnozuota 40 pacientų, sergančių HA (51,9 proc.) (3.1.3 lentelė).

3.1.3 lentelė. *Pacientų, sergančių hipofizės adenoma, turkiabalnio dugno pažeidimas (sfenoidalinis augimas) pagal Hardžio klasifikaciją, modifikuotą Vilsono*

Turkiabalnio dugno pažeidimo laipsniai	Pacientų skaičius, n (proc.)
0	3 (3,9)
I	10 (13)
II	24 (31,2)
III	25 (32,5)
IV	15 (19,5)

Keturiasdešimt septyniems pacientams (61 proc.) nustatyta RNK deformacija MRT tyrimo būdu. RNK signalo pokyčiai rasti 13 pacientų, sergančių HA (16,9 proc.).

3.2. Regos nervo kryžmės charakteristikos

RNK ir jos santykis su HA buvo nustatomi įvertinant 73 sergančiųjų HA MRT nuotraukas. Keturių nuotraukų nebuvo galima įvertinti dėl blogos MRT kokybės.

Nustatyta dešinės pusės RNK aukščio mediana 2,0 mm (min – 0,1; maks – 3,7), kairės pusės 2,0 mm (min – 0; maks – 4,0), vidurinės dalies 1,7 mm (min – 0,1; maks – 3,7). Atstumo tarp HA ir RNK mediana 0 mm (min – 0; maks – 7,6). RNK storio skirstiniai statistiškai reikšmingai skyrėsi pacientams, kuriems nustatytas supraseliarinis HA augimas, ir tų, kuriems nebuvo supraseliarinio HA augimo, grupėse ($p < 0,001$) (3.2.1 lentelė).

3.2.1 lentelė. *Regos nervo kryžmės aukštis pacientų, sergančių hipofizės adenoma su ir be supraseliarinio augimo, grupėse*

Regos nervo kryžmė	HA su supraseliariniu augimu: mediana (min; maks); vidutinis rangas	HA be supraseliarinio augimo: mediana (min; maks), vidutinis rangas	p reikšmė*
Dešinė pusė	1,7 (0,1;3,6); 60,81	2,6 (1,3;3,7); 112,28	<0,001
Vidurinė dalis	1,6 (0,1;3,4); 64,25	2,2 (1,1;3,7); 101,78	<0,001
Kairė pusė	1,7 (0;3,6); 60,63	2,8 (1,8;3,7); 112,83	<0,001

*Mano-Vitnio U testas.

3.3. Sergančiųjų HA regos aštrumo ir akipločio pokyčiai

Nustatytas geriausio koreguoto regos aštrumo (GKRA) vidurkis sergant HA 0,76 ± 0,33; mediana 0,95 (min 0; maks 1,0). GKRA skirstiniai, sergantiems HA su supraseliariniu plitimu (vidutinis rangas 71,40) ir be supraseliarinio plitimo (vidutinis rangas 92,76) skyrėsi statistiškai reikšmingai (p = 0,004). Buvo nustatyta silpna teigiama koreliacija tarp RNK dešinės pusės, vidurio bei kairės pusės aukščio ir GKRA (r = 0,349; 0,276; 0,307; p < 0,001).

Akiplotis be pakitimų buvo nustatytas 82 akyse (53,2 proc.), 13 akių (18,4 proc.) diagnozuota dalinė temporalinė hemianopsija (akiplotis susiaurėjęs < 87° iš lateralinės pusės, defektas nesiekia vertikalaus meridiano), 51 akyje (33,1 proc.) – pilna temporalinė hemianopsija (akiplotis susiaurėjęs 87° iš lateralinės pusės, defektas siekia vertikalųjį meridianą) ir 6 akyse (3,9 proc.) nustatytas koncentriškai susiaurėjęs akiplotis. Dvi akys neištirtos dėl potrauminio aklumo.

3.4. Sergančiųjų HA TNSS pokyčiai

Kontrolinę grupę OKT rodmenų palyginimui sudarė 77 asmenys (154 akys). Demografinės pacientų, sergančių HA, ir sveikų asmenų charakteristikos pateiktos 3.4.1 lentelėje.

3.4.1 lentelė. *Pacientų, sergančių hipofizės adenoma, ir oftalmologiškai sveikų asmenų demografinės charakteristikos*

Veiksniai	Grupė		p reikšmė
	HA (n=77)	Kontrolinė grupė (n=77)	
Vyrai, n (proc.)	34 (44,2)	24 (31,2)	SSN
Moterys, n (proc.)	43 (55,8)	53 (68,8)	SSN
Amžius, mediana	50,0	49,5	SSN

SSN – skirtumas statistiškai nereikšmingas.

Nustatytas statistiškai reikšmingai suplonėjęs priešoperacinis TNSS sluoksnis apie RND visuose keturiuose kvadrantuose pacientams, sergantiems HA, palyginti su kontroline grupe (3.4.2 lentelė).

3.4.2 lentelė. *TNSS pokyčiai sveikų asmenų ir pacientų, sergančių hipofizės adenoma, grupėse*

OKT	Kontrolinė grupė: mediana (min; maks); vidutinis rangas	Pacientai, sergantys HA: mediana (min; maks); vidutinis rangas	p reikšmė*
Temporalinis kvadrantas	70 (50; 91); 184,74	60 (16; 109); 120,63	<0,001
Viršutinis kvadrantas	130 (102; 197); 179,41	119 (47; 178); 126,07	<0,001
Nazalinis kvadrantas	84 (56; 111); 176,68	71 (25; 132); 128,85	<0,001
Apatinis kvadrantas	132 (100; 169); 181,50	118 (44; 159); 123,93	<0,001

*Mano-Vitnio U testas.

Nustatėme statistiškai reikšmingai suplonėjusį visų kvadrantų TNSS pacientams, turintiems akipločio defektų (pilną, dalinę hemianopsiją ar koncentriškai susiaurėjusį akiplotį), palyginti su pacientais, kuriems nebuvo akipločio defektų ($p < 0,001$). TNSS storio skirstiniai apie RND pasikartojančių HA ir nepasikartojančių HA grupėse statistiškai reikšmingai skyrėsi tik temporaliniuose ir nazaliniuose kvadrantuose (3.4.3 lentelė).

3.4.3 lentelė. *Tinklainės nervinių skaidulų sluoksnio storio pokyčiai pacientų, sergančių pasikartojančia ir nepasikartojančia hipofizės adenoma, grupėse*

TNSS storis, µm	Pasikartojanti HA: mediana (min; maks); vidutinis rangas	Nepasikartojanti HA; mediana (min; maks); vidutinis rangas	p reikšmė*
Temporalinis kvadrantas	47 (16;87); 54,67	61 (61;29); 80,03	0,009
Viršutinis kvadrantas	111 (70;158); 64,33	122 (47;178); 78,20	SSN
Nazalinis kvadrantas	62,5 (29;126); 59,00	74 (25;132); 79,21	0,038
Apatinis kvadrantas	113 (71;149); 60,00	120 (44;159); 79,02	SSN

*Mano-Vitnio U testas. SSN – skirtumas statistiškai nereikšmingas.

TNSS storio skirstiniai statistiškai reikšmingai skyrėsi tik temporaliniame kvadrante pacientams, kuriems pasireiškė supraseliarinis HA augimas ir kuriems nebuvo supraseliarinio HA augimo (3.4.4 lentelė).

3.4.4 lentelė. *Tinklainės nervinių skaidulų sluoksnio storis pacientų, kuriems pasireiškė supraseliarinis hipofizės adenomos augimas ir kuriems nebuvo supraseliarinio augimo, grupėse*

TNSS storis, µm	HA su supraseliariniu augimu	HA be supraseliarinio augimo	p reikšmė
Viršutinis kvadrantas	117,45 (24,340) Vidurkis, (standartinis nuokrypis)	118,67 (21,644) Vidurkis, (standartinis nuokrypis)	SSN*
Temporalinis kvadrantas	68,63; 54 (16; 109) Vidutinis rangas; mediana (min; maks)	94,50; 68 (22; 94) Vidutinis rangas; mediana (min; maks)	0,001**
Apatinis kvadrantas	94,50; 117 (67; 159) (vidutinis rangas, mediana; min; maks)	82,57; 120 (44; 155) (vidutinis rangas; mediana, min; maks)	SSN**
Nazalinis kvadrantas	70,10 (20,666) Vidurkis (standartinis nuokrypis)	74,28 (23,080) Vidurkis (standartinis nuokrypis)	SSN*

*Stjudento t testas. **Mano-Vitnio U testas. SSN – skirtumas statistiškai nereikšmingas.

TNSS storio skirstinių analizė, atlikta atsižvelgiant į supraseliarinio augimo laipsnį, parodė temporalinio TNSS kvadranto suplonėjimą lyginant 0 ir B grupes ($p = 0,009$), 0 ir C ($p < 0,001$), A ir C ($p < 0,001$), B ir C ($p < 0,001$) (3.4.5 lentelė).

3.4.5 lentelė. *Pacientų, sergančių hipofizės adenoma, tinklainės nervinių skaidulų sluoksnio kvadrantų storis, priklausomai nuo supraseliarinio augimo laipsnių*

Supraseliarinis augimas (Hardžio klasifikacija, modifikuota Vilsono)	TNSS temporalinis kvadrantas Mediana (min; maks)	TNSS viršutinis kvadrantas Mediana (min; maks)	TNSS nazalinis kvadrantas Mediana (min; maks)	TNSS apatinis kvadrantas Mediana (min; maks)
0	68 (22; 94)	124 (60; 164)	74 (25; 132)	120 (44; 155)
A	63 (16; 109)	122 (70; 178)	73 (29; 126)	119 (67; 159)
B	59 (19; 91)	118 (47; 167)	67,5 (33; 109)	117 (71; 156)
C	45 (21; 54)	110 (61; 162)	64 (35; 101)	115 (84; 149)

Nustatyta stipriausia temporalinio kvadranto TNSS storio koreliacija su atstumu tarp RNK ir HA ($r = 0,401$, $p < 0,001$), koreliacija su viršutiniu, nazaliniu, apatiniu TNSS kvadrantais buvo silpna ($r = 0,079; 0,074; 0,113$) ir statistiškai nepatikima ($p = 0,351; 0,380; 0,180$).

RNK aukštis statistiškai patikimai koreliavo su TNSS visų kvadrantų storiu ($p < 0,05$) (3.4.6 lentelė).

3.4.6 lentelė. *Regos nervo kryžmės aukščio ir tinklainės nervinių skaidulų sluoksnio storio sąsajos*

TNSS		**RNKA dešinės pusės**	**RNKA vidurinės dalies**	**RNKA kairės pusės**
Temporalinis kvadrantas	Koreliacijos koeficientas	0,540	0,393	0,530
	p reikšmė	<0,001	<0,001	<0,001
Viršutinis kvadrantas	Koreliacijos koeficientas	0,346	0,320	0,341
	p reikšmė	<0,001	<0,001	<0,001
Nazalinis kvadrantas	Koreliacijos koeficientas	0,243	0,187	0,240
	p reikšmė	0,003	0,025	0,004
Apatinis kvadrantas	Koreliacijos koeficientas	0,321	0,256	0,343
	p reikšmė	<0,001	0,002	<0,001

3.5. Sergančiųjų HA Munsell-Farnsworth 100 atspalvių atrinkimo ir ribinio spalvinio kontrastinio jautrumo tyrimo rezultatai

F-M 100 ir RSKJ tyrimai atlikti 67 asmenims. 10 asmenų tyrimai neatlikti, kadangi nesuprato tyrimų, atsisakė tirtis ar pavargo. Į kontrolinę grupę atrinkti 99 sveiki asmenys (198 akys).

Nustatėme vidutinį klaidų skaičių F-M 100 kontrolinėje grupėje – 87,39 (24,106), sergančiųjų HA – 201,95 (106,071). F-M 100 tyrimo rezultatai pasiskirstė nuo 28 iki 148 klaidų sveikų tiriamųjų grupėje, o pacientų, sergančių HA, nuo 6 iki 608 klaidų. RSKJ tyrime vidutinis klaidų skaičius sveikų asmenų grupėje – 1,33 (0,649), sergančių HA – 3,806 (3,425). RSKJ tyrimų rezultatai sveikų tiriamųjų grupėje pasiskirstė nuo 0,26 iki 3,5, o pacientų, sergančių HA, nuo 0,66 iki 18,36. Tyrimų rodmenys buvo statistiškai reikšmingai geresni oftalmologiškai sveikų pacientų nei sergančių HA ($p < 0,001$) (3.5.1 lentelė).

3.5.1 lentelė. *Farnsworth-Munsell 100 atspalvių ir ribinio spalvinio kontrastinio jautrumo tyrimų duomenys oftalmologiškai sveikų asmenų ir pacientų, sergančių hipofizės adenoma, grupėse*

Tyrimas	Klaidų skaičius sergančiųjų HA grupėje (n=67, 134 akys) Vidutinis rangas, mediana (min; maks)	Klaidų skaičius kontrolinėje grupėje (n=100, 198 akys) Vidutinis rangas, mediana (min; maks)	p vertė*
F-M 100	242,94; 186 (6; 608)	113,87; 88,00 (28;148)	<0,001
RSKJ	235,24; 2,735 (0,66; 18,36)	116,21; 1,230 (0,26; 3,5)	<0,001

*Mano-Vitnio U testas.

Nenustatyta statistiškai reikšmingų SKJ pokyčių palyginus pacientus, sergančius invazine ir neinvazine HA (p = 0,49), tačiau nustatytas statistiškai reikšmingai didesnis klaidų skaičius atliekant F-M 100 tyrimą (p = 0,041) (3.5.2 lentelė).

3.5.2 lentelė. *Farnsworth-Munsell 100 ir ribinio spalvinio kontrastinio jautrumo tyrimų duomenys pacientų, sergančių invazine ir neinvazine hipofizės adenoma, grupėse*

Tyrimas	Klaidų skaičius sergančiųjų invazyvine HA grupėje Vidutinis rangas, mediana (min; maks)	Klaidų skaičius sergančiųjų neinvazine HA grupėje Vidutinis rangas, mediana (min; maks)	p reikšmė*
F-M 100	72,26; 216 (68;608)	58,44; 160 (6;388)	0,041
RSKJ	65,41; 3,05 (76;18,36)	60,87; 3,31 (1,36;17,5)	SSN

*Mano-Vitnio U testas. SSN – skirtumas statistiškai nereikšmingas.

Nustatytas statistiškai reikšmingas klaidų skaičiaus skirtumas tarp sergančiųjų supraseliarinio augimo HA ir HA be supraseliarinio augimo RSKJ tyrimo metu (p < 0,001), o F-M 100 atveju skirtumas nebuvo statistiškai reikšmingas (p = 0,126) (3.5.3 lentelė).

3.5.3 lentelė. *Farnsworth-Munsell 100 ir ribinio spalvinio kontrastinio jautrumo tyrimų duomenys pacientų, sergančių hipofizės adenoma su supraseliariniu hipofizės adenomos plitimu ir be supraseliarinio plitimo, grupėse*

Tyrimas	**Klaidų skaičius, nustatytas sergantiems supraseliarinio plitimo HA Vidutinis rangas, mediana (min; maks)**	**Klaidų skaičius, nustatytas sergantiems HA be supraseliarinio plitimo Vidutinis rangas, mediana (min; maks)**	**p reikšmė***
F-M 100	69,86; 194 (6; 608)	58,78; 148 (40; 432)	SSN
RSKJ	73,95; 3,085 (0,76; 18,36)	39,29; 1,835 (0,66; 11,87)	<0,001

*Mano-Vitnio U testas. SSN – skirtumas statistiškai nereikšmingas.

Nenustatyta statistiškai reikšmingo klaidų skaičiaus skirtumo tarp pasikartojančios HA grupės ir nepasikartojančios HA RSKJ tyrimo metu (p = 0,72), o F-M 100 atveju nustatytas statistiškai reikšmingas skirtumas (p = 0,03) (3.5.4 lentelė).

3.5.4 lentelė. *Farnsworth-Munsell 100 ir ribinio spalvinio kontrastinio jautrumo tyrimų duomenys pacientų, sergančių pasikartojančia ir nepasikartojančia hipofizės adenoma, grupėse*

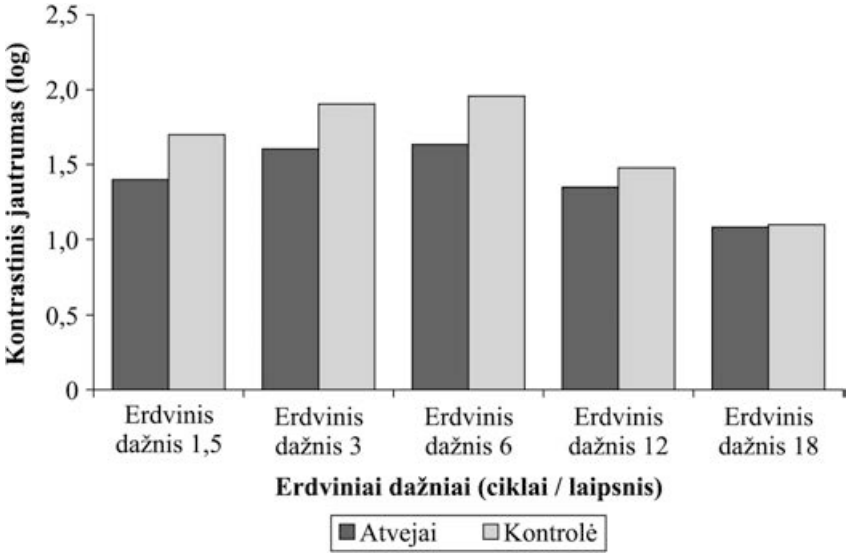
Tyrimas	**Klaidų skaičius, nustatytas sergantiems pasikartojančia HA Vidutinis rangas; mediana (min; maks)**	**Klaidų skaičius, nustatytas sergantiems nepasikartojančia HA Vidutinis rangas; mediana (min; maks)**	**p reikšmė***
F-M 100	84,72; 216 (112; 440)	63,62; 162 (6; 608)	0,03
RSKJ	66,33; 3,18 (1,35; 8,52)	63,03; 2,695 (0,66; 18,36)	SSN

*Mano-Vitnio U testas. SSN – skirtumas statistiškai nereikšmingas.

3.6. Sergančiųjų HA funkcinio kontrastinio jautrumo pokyčiai

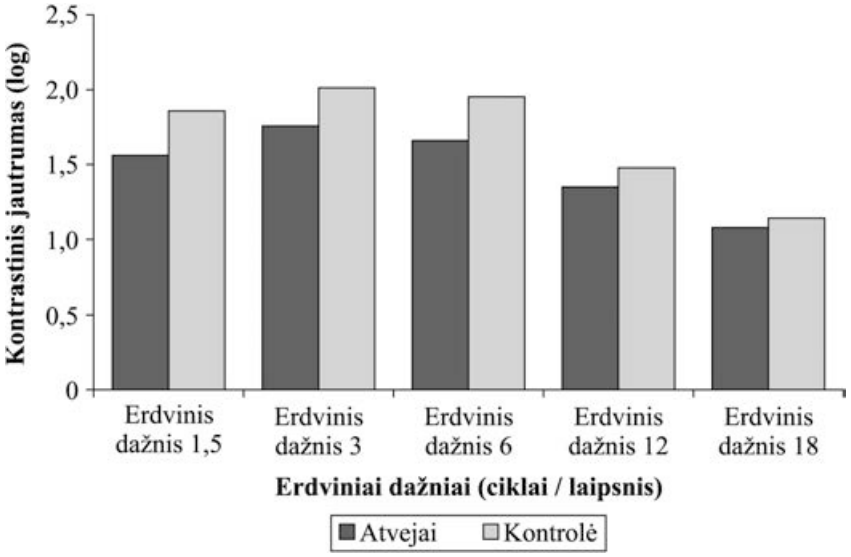
FKJ tyrimas atliktas 77 asmenims (154 akys), sergantiems HA, ir palygintas su 396 sveikų asmenų akių FKJ duomenimis. Šiame tyrime rodmenys perskaičiuoti į logaritmus.

Tyrimo rezultatai pateikiami 3.6.1–3.6.4 pav.



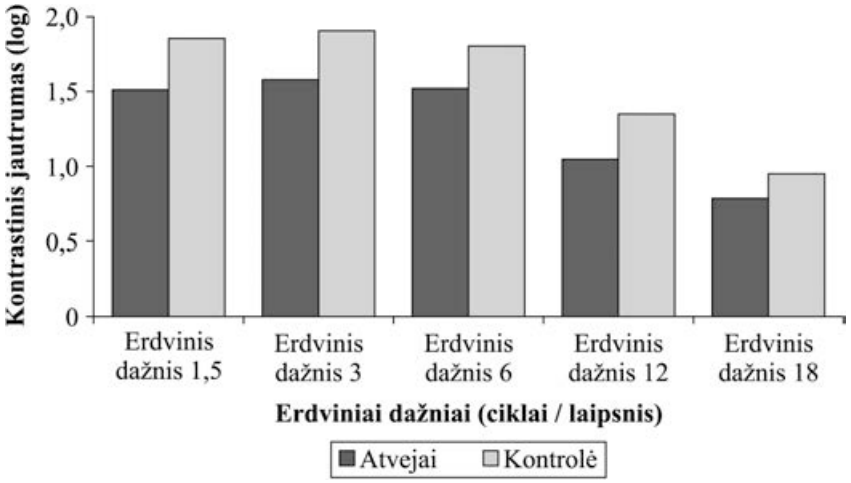
3.6.1 pav. *Funkcinio kontrastinio jautrumo tyrimų, atliktų dienos sąlygomis be akinančios šviesos, rodmenys pacientams, sergantiems hipofizės adenoma, ir kontrolinės grupės asmenims*

Nustatytas statistiškai reikšmingas FKJ sumažėjimas 1,5, 3, 6, 12, 18 erdviniuose dažniuose (ciklai / laipsnis) ($p < 0,001$) sergantiesiems HA dienos sąlygomis be akinančios šviesos.



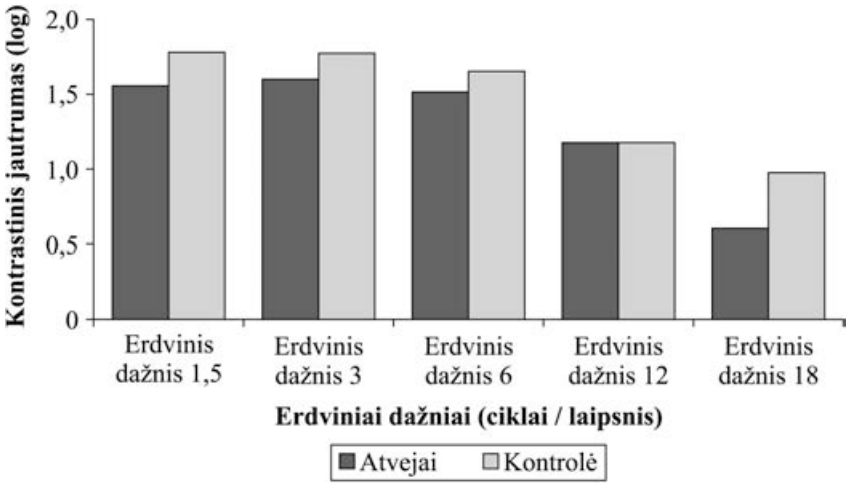
3.6.2 pav. *Funkcinio kontrastinio jautrumo tyrimų, atliktų dienos sąlygomis su akinančia šviesa, rodmenys pacientams, sergantiems hipofizės adenoma, ir kontrolinės grupės asmenims*

Sergantiesiems HA nustatytas statistiškai reikšmingas FKJ sumažėjimas 1,5, 3, 6, 12, 18 erdviniuose dažniuose (ciklai / laipsnis) ($p < 0,001$) dienos sąlygomis su akinančia šviesa.



3.6.3 pav. *Funkcinio kontrastinio jautrumo tyrimų, atliktų nakties sąlygomis be akinančios šviesos, rodmenys pacientams, sergantiems hipofizės adenoma, ir kontrolinės grupės asmenims*

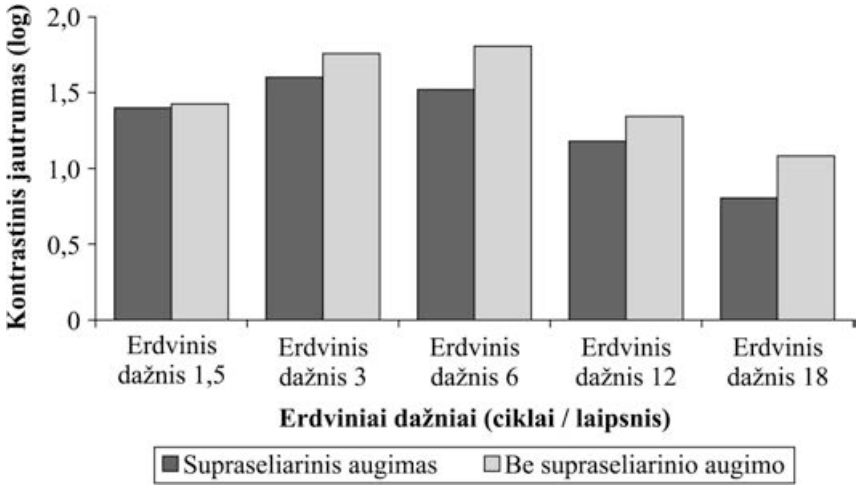
Sergantiesiems HA nustatytas statistiškai reikšmingas FKJ sumažėjimas 1,5, 3, 6 ir 12 erdviniuose dažniuose (ciklai / laipsnis) ($p < 0,001$) nakties sąlygomis be akinančios šviesos.



3.6.4 pav. *Funkcinio kontrastinio jautrumo tyrimų, atliktų nakties sąlygomis su akinančia šviesa, rodmenys pacientams, sergantiems hipofizės adenoma, ir kontrolinės grupės asmenims*

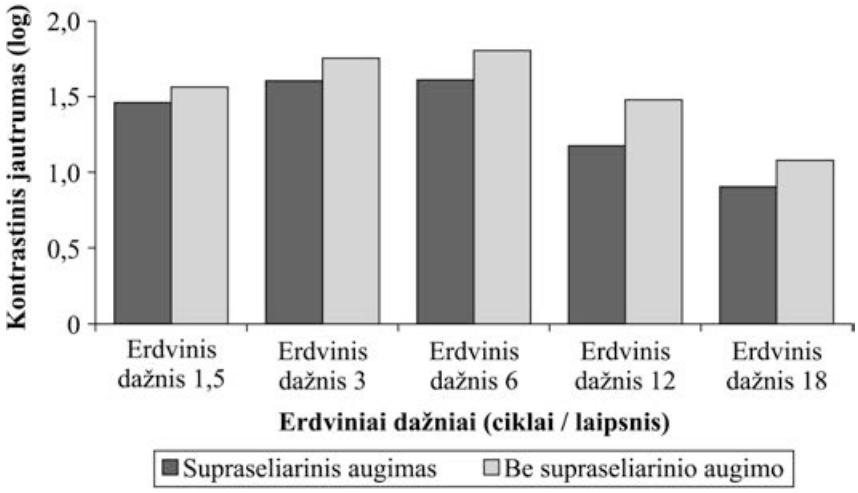
Sergantiesiems HA nustatytas statistiškai reikšmingas FKJ sumažėjimas 1,5, 3, 6 ir 18 erdviniuose dažniuose (ciklai / laipsnis) ($p < 0,05$) nakties sąlygomis su akinančia šviesa.

Palyginome FKJ tyrimo rezultatus pacientų, sergančių HA su supraseliariniu plitimu ir be supraseliarinio plitimo. Tyrimo rezultatai pateikiami 3.6.5–3.6.8 pav.



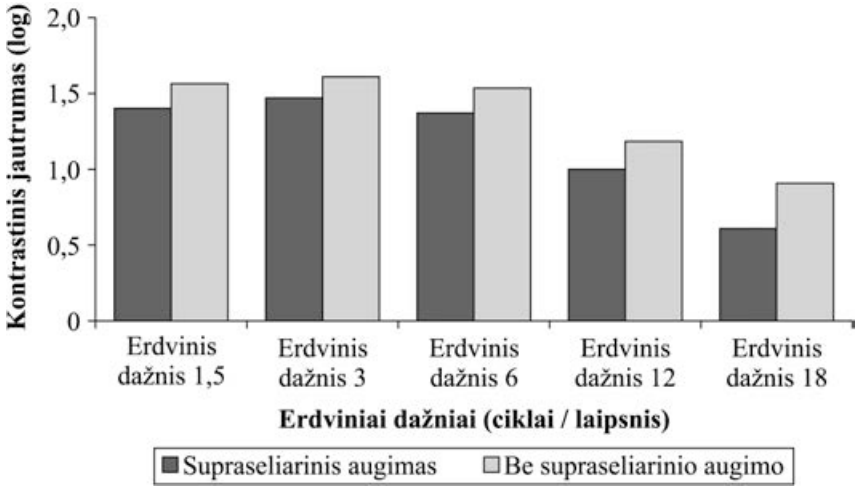
3.6.5 pav. *Funkcinio kontrastinio jautrumo tyrimų atliktų dienos sąlygomis be akinančios šviesos, rodmenys pacientams, sergantiems hipofizės adenoma su supraseliariniu augimu ir be supraseliarinio augimo*

Nustatytas statistiškai reikšmingas FKJ sumažėjimas 1,5, 3, 6, 12 ir 18 erdviniuose dažniuose (ciklai / laipsnis) ($p < 0,05$) sergantiesiems supraseliarinio augimo HA dienos sąlygomis be akinančios šviesos.



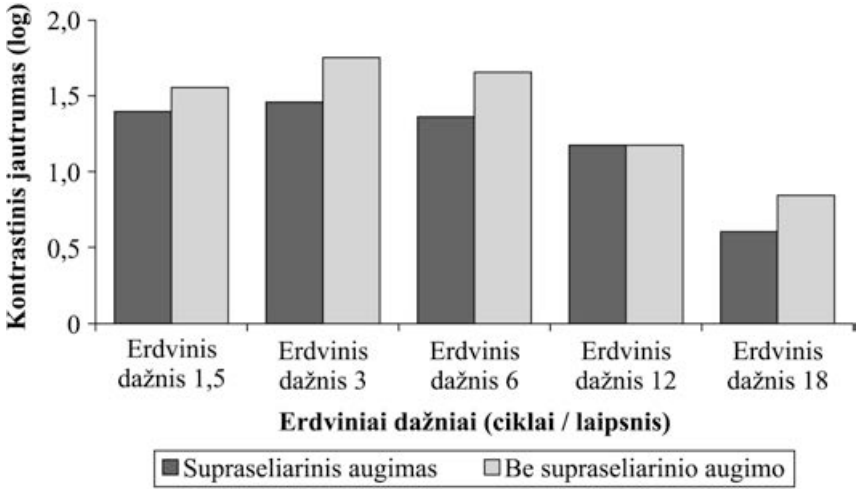
3.6.6 pav. *Funkcinio kontrastinio jautrumo tyrimų, atliktų dienos sąlygomis su akinančia šviesa, rodmenys pacientams, sergantiems hipofizės adenoma su ir be supraseliarinio augimo*

Sergantiesiems supraseliarinio augimo HA statistiškai reikšmingai sumažėjo FKJ tyrimo, atlikto dienos sąlygomis su akinančia šviesa, rezultatai 1,5, 3, 6, 12 ir 18 erdviniuose dažniuose (ciklai / laipsnis) ($p < 0,05$).



3.6.7 pav. *Funkcinio kontrastinio jautrumo tyrimų, atliktų nakties sąlygomis be akinančios šviesos, rodmenys pacientams, sergantiems hipofizės adenoma su ir be supraseliariniu augimo*

FKJ tyrimo rezultatai statistiškai reikšmingai sumažėjo 1,5, 3 ir 6 erdviniuose dažniuose (ciklai / laipsnis) ($p < 0,05$) sergantiesiems supraseliarinio augimo HA nakties sąlygomis be akinančios šviesos.



3.6.8 pav. *Funkcinio kontrastinio jautrumo tyrimų, atliktų nakties sąlygomis su akinančia šviesa, rodmenys pacientams, sergantiems hipofizės adenoma su ir be supraseliariniu augimo*

FKJ tyrimo rezultatai statistiškai reikšmingai sumažėjo 1,5, 3, 6, 12 ir 18 erdviniuose dažniuose (ciklai / laipsnis) ($p < 0,001$) sergantiesiems supraseliarinio augimo HA nakties sąlygomis su akinančia šviesa.

3.7. Sergančiųjų HA KI-67 proliferacijos indeksas

Ištirti 69 hipofizės adenomos audinio mėginiai. Dalies pacientų navikinio audinio nepavyko ištirti, kadangi buvo per mažas audinio kiekis, nustatyta navikinio audinio nekrozė, nepavyko paimti audinio operacijos metu. Ki-67 PI ištirtas 37 moterims (53,6 proc.) ir 32 vyrams (46,4 proc.). Nenustatyta statistiškai reikšmingų Ki-67 PI skirtumų tarp vyrų ir moterų ($p = 0,092$).

Ki-67 PI < 1 proc. nustatytas 34 pacientams (49,3 proc.), sergantiems HA, Ki-67 PI 1 proc. – 17 asmenų (24,6 proc.), Ki-67 PI > 1 proc. – 18 pacientų (26,1 proc.). Nustatyta, kad Ki-67 PI statistiškai reikšmingai priklauso nuo HA invazyvumo ($p = 0,039$), tačiau nenustatyta Ki-67 PI sąsajų su pasikartojimu ($p = 0,6$) ir HA augimo kryptimi ($p > 0,05$) (3.7.1 lentelė).

3.7.1 lentelė. *Ki-67 proliferacijos indeksas HA navikiniame audinyje, atsižvelgiant į hipofizės adenomos augimo pobūdį*

Augimo pobūdis		**Ki-67 PI < 1 proc. (n)**	**Ki-67 PI ≥ 1 proc. (n)**	**p vertė***
Supraseliarinis augimas	Taip	24	26	SSN
	Ne	10	9	
Parakavernozinis augimas	Taip	10	15	SSN
	Ne	24	20	
Sfenoidalinis augimas	Taip	16	21	SSN
	Ne	18	14	

**Stjudento t testas. SSN – skirtumas statistiškai nereikšmingas.

3.8. Sergančiųjų HA IL-17A koncentracija kraujo serume

Tiriant IL-17A koncentracijos skirtumus HA (n=60) ir kontrolinėje grupėje (n=64) bei atskirose HA skirtingo augimo pobūdžio grupėse, nustatyta statistiškai reikšmingai didesnė citokino IL-17A koncentracija pacientų, sergančių HA, kraujo serume palyginti su sveikų asmenų grupe (minimali reikšmė / mediana / maksimali reikšmė: 1,95/42,12/76,80 pg/ml *vs.* 0,39/8,19/74,57, $p < 0,001$) (3.8.1 lentelė), tačiau, lyginant atskiras HA grupes, skirtumų nenustatyta ($p > 0,05$) (3.8.2 lentelė).

3.8.1 lentelė. *IL-17A koncentracijos kraujo serume pacientų, sergančių hipofizės adenoma, ir sveikų asmenų grupėje, atsižvelgiant į hipofizės adenomos augimo pobūdį*

HA augimo pobūdis	**Grupė (n)**	**IL-17A koncentracija (pg/ml) min/mediana/maks**	**p vertė***
Invazyvumas	Invazinė (29)	1,95/42,12/76,80	SSN
	Neinvazinė (25)	3,10/42,12/76,80	
Pasikartojimas	Pasikartojanti HA (5)	1,95/42,12/76,80	SSN
	Nepasikartojanti HA (49)	3,10/42,12/76,80	
Supraseliarinis augimas	Yra (38)	1,95/42,12/76,80	SSN
	Nėra (16)	4,29/39,95/55,12	
Parakavernozinis augimas	Yra (18)	1,95/44,29 /76,80	SSN
	Nėra (36)	3,10/41,04/76,80	
Parasfenoidalinis augimas	Yra (24)	1,95/42,12/76,80	SSN
	Nėra (30)	3,10/42,12/76,80	

*Mano-Vitnio U testas. SSN – skirtumas statistiškai nereikšmingas.

3.9. Sergančiųjų HA genų polimorfizmų pokyčiai

3.9.1. Sergančiųjų HA *MMP-2* (-1306 C/T) geno polimorfizmo pokyčiai

Išanalizavus 87 pacientų, sirgusių HA, *MMP-2* (-1306 C/T) rs243865 geno polimorfizmą, ir palyginus su 318 sveikų asmenų polimorfizmu, nenustatyta statistiškai reikšmingų skirtumų pagal genotipų (C/C, C/T ir T/T) pasiskirstymą (atitinkamai 50 proc., 44 proc., 6 proc. *vs.* 59,75 proc., 33,96 proc., ir 6,29 proc.). *MMP-2* (-1306 C/T) C/T genotipas statistiškai reikšmingai dažniau buvo nustatytas moterims, sergančioms HA, nei sveikoms moterims: 33,66 proc. *vs.* 49,1 proc.; p = 0,041. Atlikus dvinarę logistinę regresinę analizę, nustatyta, kad genotipai nebuvo statistiškai reikšmingi prognozuojant galimybę susirgti HA, lyginant pacientus, sergančius HA, ir sveikus asmenis.

Nustatėme, kad *MMP-2* (-1306 C/T) C/T genotipas, palyginti su C/C genotipu, didino neinvazyvios hipofizės adenomos galimybę 3 kartus, palyginti su C/C + T/T genotipais – 2,8 karto, o *MMP-2* (-1306 C/T) C/T + T/T genotipai, palyginti su C/C genotipu, šią galimybę didino 2,7 karto.

Kiti *MMP-2* (-1306 C/T) geno polimorfizmo analizės rezultatai aprašyti mūsų straipsnyje *Role of MMP-2 (–1306 C/T) Polymorphism in Pituitary Adenoma. Scientifica. 2016; 2016: 2839697.*

3.9.2. Sergančiųjų HA *MMP-9* (-1562 C/T) geno polimorfizmo pokyčiai

Ištyrėme 86 pacientų, sergančių HA, *MMP-9* (-1562 C/T) rs3918242 geno polimorfizmą ir palyginome su 526 sveikų asmenų geno polimorfizmu.

MMP-9 genotipų ir alelių dažniai sveikų asmenų grupėse atitiko Hardžio-Vainbergo dėsnį (p > 0,05), o pacientų, sergančių HA, neatitiko (p < 0,05). Atlikus *MMP-9* (-1562 C/T) geno polimorfizmo analizę, nustatytas statistiškai reikšmingas skirtumas pagal genotipų pasiskirstymą, tarp pacientų, sergančių HA, ir sveikų asmenų (p = 0,003). C/C genotipas statistiškai reikšmingai dažniau buvo nustatytas pacientams, sergantiems HA, nei sveikiems asmenims (81,4 proc. *vs.* 64,6 proc., p = 0,002), o C/T genotipas rečiau nustatytas sergantiems HA nei sveikiems asmenims (14,0 proc. *vs.* 32,1 proc., p < 0,001) (3.9.2.1 lentelė).

3.9.2.1 lentelė. *MMP-9 (-1562 C/T) genotipų dažniai pacientų, sergančių hipofizės adenoma, ir kontrolinėje grupėse*

Genas	Genotipas/ alelis	Dažnis (proc.)				
		Kontrolinė grupė n (proc.) (n=526)	**p HVE**	**HA grupė n (proc.) (n=86)**	**p HVE**	**p reikšmė**
MMP-9 (-1562 C/T) rs3918242	*Genotipas*					
	C/C	**340 (64,6)[1]**	0,469	**70 (81,4)[1]**	**0,0029**	
	C/T	**169 (32,1)[2]**		**12 (14,0)[2]**		**0,003**
	T/T	17 (3,2)		4 (4,7)		
	Iš viso	526 (100)		86 (100)		
	Alelis					
	C	849 (80,70)		152 (88,37)		
	T	203 (19,30)		20 (11,63)		

MMP – matrikso metaloproteinazė, p reikšmė – reikšmingumo lygmuo (alfa=0,05), p vertė HVE – reikšmingumo lygmuo pagal Hardžio-Vainbergo dėsnį, skirtumai laikomi statistiškai reikšmingais, kai p<0,05.[1]**p=0,002**, [2]**p<0,001**.

Išanalizavus *MMP-9* (-1562 C/T) genotipus, nenustatyta statistiškai reikšmingų skirtumų pagal genotipų (C/C, C/T ir T/T) pasiskirstymą vyrų ir moterų, sergančių HA, grupėse (atitinkamai 81,5 proc., 14,8 proc., 3,7 proc. *vs.* 81,2 proc., 12,5 proc., 6,2 proc.) (3.9.2.2 lentelė). Nustatyti statistiškai reikšmingi skirtumai lyginant moteris, sergančias HA, ir sveikas moteris. *MMP-9* (–1562 C/T) C/C genotipas dažniau buvo nustatomas HA sergančioms moterims nei sveikoms kontrolinės grupės moterims (81,5 proc. *vs.* 64,6 proc.; p = 0,018*)*. C/T genotipas buvo rečiau nustatomas HA sergančioms moterims nei sveikoms (14,8 proc. *vs.* 32,3 proc.; p = 0,01). *MMP-9* (-1562 C/T) T/T genotipų dažnis tarp moterų grupių nesiskyrė. Vyrų grupėje tik *MMP-9* (-1562 C/T) C/T genotipo dažnis statistiškai reikšmingai skyrėsi tarp vyrų, sergančių HA, ir sveikų vyrų grupės (12,5 proc. *vs.* 31,9 proc.; p = 0,023).

3.9.2.2 lentelė. *MMP-9 (-1562 C/T) genotipų dažniai pacientų, sergančių hipofizės adenoma, ir kontrolinėje grupėse, atsižvelgiant į lytį*

Genas	**Genotipas/ alelis**	**Dažnis (proc.)**							
		Kontrolinė grupė n (proc.)		**p HVE**	**p reikšmė**	**HA grupė n (proc.)**		**p HVE**	**p reikšmė**
		Moterys n=316	**Vyrai n=210**			**Moterys n=54**	**Vyrai n=32**		
	Genotipas								
	C/C	**204 (64,6)[1]**	136 (64,8)	0,991	0,868	**44 (81,5)[1]**	26 (81,2)	0,836	1,00
MMP-9	C/T	**102 (32,3)[2]**	**67 (31,9)[3]**		0,928	**8 (14,8)[2]**	**4 (12,5)[3]**		1,00
(-1562 C/T)	T/T	10 (3,2)	7 (3,3)		0,915	2 (3,7)	2 (6,2)		0,626
rs3918242	Iš viso	316 (100)	210 (100)			54 (100)	32 (100)		
	Alelis								
	C	510 (80,7)	339 (80,71)			96 (88,89)	56 (87,5)		
	T	122 (19,3)	81 (19,29)			12 (11,11)	8 (12,5)		

MMP – matrikso metaloproteinazė, p reikšmė – reikšmingumo lygmuo (alfa=0,05), p vertė HVE – reikšmingumo lygmuo pagal Hardžio-Vainbergo dėsnį, skirtumai laikomi statistiškai reikšmingais, kai p<0,05.**[1]p=0,018, [2]p=0,01, [3]p=0,023.**

Atlikus dvinarę logistinę regresinę analizę, nustatyta, kad, HA galimybę mažino C/T genotipas, palyginti su C/C (GS = 0,345, 95 proc. PI: 0,182–0,654; p = 0,01), C/T + T/T genotipai, palyginti su C/C genotipu (GS = 0,418, 95 proc PI: 0,236–0,740; p = 0,003), C/T genotipas, palyginti su C/C + T/T genotipais (GS = 0,343, 95 proc. PI: 0,181–0,648; p = 0,001), T alelis, palyginti su C aleliu, taip pat mažino HA galimybę (GS = 0,551, 95 proc. PI: 0,337–0,901; p = 0,018) (3.9.2.3 lentelė).

3.9.2.3 lentelė. *MMP-9 (-1562 C/T) geno polimorfizmo ir hipofizės adenomos sąsajos (dvinarė logistinė regresinė analizė)*

Modelis	Genotipas	GS (95 proc. PI)	p reikšmė	AIC
Kodominantinis	C/C C/T T/T	1 0,345 (0,182–0,654) 1,143 (0,373–3,5)	 **0,01** 0,815	489,539
Dominantinis	C/C C/T+T/T	1 0,418 (0,236–0,740)	 **0,003**	490,616
Recesyvinis	C/C+C/T T/T	1 1,461 (0,479–4,449)	 0,505	50,423
Overdominantinis	C/C+T/T C/T	1 0,343 (0,181–0,648)	 **0,001**	478,593
Adityvinis	---	0,551 (0,337–0,901)	**0,018**	494,457

GS – galimybių santykis, AIC – akaike informacinis kriterijus, p – reikšmingumo lygmuo, statistiškai reikšmingas skirtumas buvo nustatomas tada, kai p reikšmė <0,05.

Dvinarė logistinė regresinė analizė atlikta pacientams, sergantiems HA, ir sveikiems kontrolinės grupės asmenims, atsižvelgiant į lytį (3.9.2.4 lentelė). Vyrų grupėje HA galimybę mažino C/T genotipas, palyginti su C/C genotipu (GS = 0,312, 95 proc. PI: 0,105–0,931; p = 0,037) ir C/T genotipas, palyginti su C/C + T/T genotipais (GS = 0,305, 95 proc. PI: 0,103–0,904; p = 0,032). Analizė parodė, kad moterų grupėje HA galimybę mažino C/T genotipas, palyginti su C/C genotipu (GS = 0,364, 95 proc. PI: 0,165–0,801; p = 0,012), C/T, palyginti su C/T + T/T genotipais (GS = 0,365, 95 proc. PI: 0,166–0,802; p = 0,012), bei C/T + T/T, palyginti su C/C genotipu (GS = 0,414, 95 proc. PI: 0,201–0,854; p = 0,017), bei T alelis palyginti su C aleliu (GS = 0,520, 95 proc. PI: 0,275–0,983; p = 0,044).

3.9.2.4 lentelė. *MMP-9 (-1562 C/T) geno polimorfizmo ir hipofizės adenomos sąsajos, atsižvelgiant į lytį (dvinarė logistinė regresinė analizė)*

Modelis	Genotipas	GS (95 proc. PI)	p reikšmė	AIC
Vyrai				
Kodominantinis	C/C C/T T/T	1 0,312 (0,105–0,931) 1,495 (0,294–7,601)	 **0,037** 0,628	189,034
Dominantinis	C/C C/T+T/T	1 0,424 (0,167–1,077)	 0,071	189,340
Recesyvinis	C/C+C/T T/T	1 1,933 (0,384–9,745)	 0,424	190,485
Overdominantinis	C/C+T/T C/T	1 0,305 (0,103–0,904)	 **0,032**	187,253
Adityvinis	---	0,603 (0,278–1,310)	0,201	191,239
Moterys				
Kodominantinis	C/C C/T T/T	1 0,364 (0,165–0,801) 0,927 (0,196–4,381)	 **0,012** 0,924	306,013
Dominantinis	C/C C/T+T/T	1 0,414 (0,201–0,854)	 **0,017**	305,045
Recesyvinis	C/C+C/T T/T	1 1,177 (0,251–5,525)	 0,836	311,512
Overdominantinis	C/C+T/T C/T	1 0,365 (0,166–0,802)	 **0,012**	304,022
Adityvinis	---	0,520 (0,275–0,983)	**0,044**	306,912

GS – galimybių santykis, AIC – akaike informacinis kriterijus, p – reikšmingumo lygmuo, statistiškai reikšmingas skirtumas buvo nustatomas tada, kai p reikšmė <0,05.

MMP-9 (–1562 C/T) C/C genotipas dažniau nustatytas neinvazinių, invazinių ir nepasikartojančių HA grupėse palyginti su kontroline grupe (atitinkamai 81,8 proc. *vs.* 64,6 proc; p = 0,021; 81,0 proc. *vs.* 64,6; p = 0,041; 81,8 proc. *vs.* 64,6 proc.; p = 0,005). Nenustatyta skirtumo tarp neinvazinių ir invazinių, pasikartojančių ir nepasikartojančių HA.

Tolimesnė analizė parodė, kad *MMP-9* (-1562 C/T) C/T genotipas rečiau pasitaikė invazinėse, nepasikartojančiose ir pasikartojančiose HA palyginti su kontroline grupe (atitinkamai 9,5 proc. *vs.* 32,1 proc; p = 0,001; 15,2 proc. *vs.* 32,1 proc; p = 0,004; 10,0 proc. *vs.* 32,1; p = 0,047) (3.9.2.5 ir 3.9.2.6 lentelės).

3.9.2.5 lentelė. *MMP-9 (-1562 C/T) genotipų dažniai tiriamųjų, sergančių hipofizės adenoma, ir kontrolinėje grupėje, atsižvelgiant į invazyvumą*

Genas	**Genotipas/ alelis**	**Dažnis (proc.)**					
		Kontrolinė grupė n (proc.) (n=526)	**p HVE**	**Neinvazinė HA n (proc.) (n=44)**	**p HVE**	**Invazinė HA n (proc.) (n=42)**	**p HVE**
	Genotipas						
	C/C	**340 (64,6)[1,2]**	0,469	**36 (81,8)[1]**	0,507	**34 (81,0)[2]**	**<0,001**
MMP-9	C/T	**169 (32,1)[3]**		8 (18,2)		**4 (9,5)[3]**	
(-1562 C/T)	T/T	17 (3,2)		0 (0)		4 (9,5)	
rs3918242	Iš viso	526 (100)		44 (100)		42 (100)	
	Alelis						
	C	849 (80,70)		80 (90,91)		72 (85,71)	
	T	203 (19,30)		8 (9,09)		12 (14,29)	

MMP – matrikso metaloproteinazė, p reikšmė – reikšmingumo lygmuo (alfa=0,05), p vertė HVE – reikšmingumo lygmuo pagal Hardžio-Vainbergo dėsnį, skirtumai laikomi statistiškai reikšmingais, kai p<0,05.[1] **p=0,021,**[2] **p=0,041,** [3] **p=0,001**.

3.9.2.6 lentelė. *MMP-9 (-1562 C/T) genotipų dažniai tiriamųjų, sergančių hipofizės adenoma, ir sveikų asmenų grupėse, atsižvelgiant į pasikartojimą*

Genas	**Genotipas/ alelis**	**Dažnis (proc.)**					
		Kontrolinė grupė n (proc.) (n=526)	**p HVE**	**Nepasikartojanti HA n (proc.) (n=66)**	**p HVE**	**Pasikartojanti HA n (proc.) (n=20)**	**p HVE**
	Genotipas						
	C/C	**340 (64,6)[1]**	0.469	**54 (81,8)[1]**	0,103	16 (80,0)	0,007
MMP-9	C/T	**169 (32,1)[2,3]**		**10 (15,2)[2]**		**2 (10,0)[3]**	
(-1562C/T)	T/T	17 (3,2)		2 (3,0)		2 (10,0)	
rs3918242	Iš viso	526 (100)		66 (100)		20 (100)	
	Alelis						
	C	849 (80,70)		118 (89,39)		34 (85,0)	
	T	203 (19,30)		14 (10,61)		6 (15,0)	

MMP – matrikso metaloproteinazė, p reikšmė – reikšmingumo lygmuo (alfa=0,05), p vertė HVE – reikšmingumo lygmuo pagal Hardžio-Vainbergo dėsnį, skirtumai laikomi statistiškai reikšmingais, kai p<0,05.[1] **p=0,005,** [2] **p=0,004,** [3] **p=0,047.**

Atlikta dvinarė logistinė regresinė analizė neinvazinių HA ir kontrolinėje grupėse (3.9.2.7 lentelė). Neinvazinės HA galimybę mažino C/T genotipas, palyginti su C/C genotipu (GS = 0,447, 95 proc. PI: 0,203–0,983; p = 0,045), C/T + T/T genotipai, palyginti su C/C genotipu (GS = 0,406, 95 proc. PI: 0,185–0,892; p = 0,025), T alelis, palyginti su C aleliu (GS = 0,409, 95 proc. PI: 0,193–0,866; p = 0,021). Invazinės HA galimybę mažino C/T genotipas, palyginti su C/C genotipu (GS = 0,237, 95 proc. PI: 0,083–0,678; p = 0,007), C/T + T/T genotipai, palyginti su C/C genotipu (GS = 0,430, 95 proc. PI: 0,195–0,948; p = 0,036), invazinės HA galimybę didino T/T genotipas, palyginti su C/C + C/T genotipais (GS = 3,152, 95 proc. PI: 1,010–9,835; p = 0,048), invazinės HA galimybę mažino C/T genotipas, palyginti su C/C + T/T genotipais (GS = 0,222, 95 proc. PI: 0,078–0,633; p = 0,005).

3.9.2.7 lentelė. *MMP-9 (-1562 C/T) geno polimorfizmo ir invazinės bei neinvazinės hipofizės adenomos sąsajos (dvinarė logistinė regresinė analizė)*

Modelis	**Genotipas**	**GS (95 proc. PI)**	**p reikšmė**	**AIC**
Neinvazinė HA				
Kodominantinis	C/C C/T T/T	1 0,447 (0,203–0,983) 0 (–)	 **0,045** 0,998	308,535
Dominantinis	C/C C/T+T/T	1 0,406 (0,185–0,892)	 **0,025**	308,035
Recesyvinis	C/C+C/T T/T	1 0 (–)	 0,998	311,145
Overdominantinis	C/C+T/T C/T	1 0,469 (0,214–1,032)	 0,060	309,878
Adityvinis	---	0,409 (0,193–0,866)	**0,020**	307,187
Invazinė HA				
Kodominantinis	C/C C/T T/T	1 0,237 (0,083–0,678) 2,353 (0,749–7,393)	 **0,007** 0,143	302,361
Dominantinis	C/C C/T+T/T	1 0,430 (0,195–0,948)	 **0,036**	298,548
Recesyvinis	C/C+C/T T/T	1 3,152 (1,010–9,835)	 **0,048**	300,429
Overdominantinis	C/C+T/T C/T	1 0,222 (0,078–0,633)	 **0,005**	302,203
Adityvinis	---	0,698 (0,372–1,310)	0,263	302,240

GS – galimybių santykis, AIC – akaike informacinis kriterijus, p – reikšmingumo lygmuo, statistiškai reikšmingas skirtumas buvo nustatomas tada, kai p reikšmė <0,05.

Atlikus dvinarę logistinę regresinę analizę nepasikartojančių HA ir kontrolinėje grupėse, buvo nustatyta, kad nepasikartojančios HA galimybę mažino C/T genotipas, palyginti su C/C genotipu, (GS = 0,373, 95 proc. PI: 0,185–0,750; p = 0,006), C/T + T/T genotipai, palyginti su C/C genotipu (GS=0,406, 95 proc. PI: 0,212–0,779; p = 0,007), C/T genotipas, palyginti su C/C + T/T genotipais (GS = 0,377, 95 proc. PI: 0,188–0,758; p = 0,006) bei T alelis, palyginti su C aleliu, (GS = 0,492, 95 proc. PI: 0,276–0,878; p = 0,016) (3.9.2.8 lentelė).

Atlikus dvinarę logistinę regresinę analizę lyginant pacientus, sergančius pasikartojančiomis HA, ir sveikus asmenis, genotipai nebuvo statistiškai reikšmingi vertinant polimorfizmo sąsajas su HA.

3.9.2.8 lentelė. *MMP-9 (-1562 C/T) geno polimorfizmo ir nepasikartojančios bei pasikartojančios hipofizės adenomos sąsajos (dvinarė logistinė regresinė analizė)*

Modelis	Genotipas	GS (95 proc. PI)	p reikšmė	AIC
Nepasikartojanti HA				
Kodominantinis	C/C C/T T/T	1 0,373 (0,185–0,750) 0,741 (0,166–3,297)	 **0,006** 0,694	410,785
Dominantinis	C/C C/T+T/T	1 0,406 (0,212–0,779)	 **0,007**	409,409
Recesyvinis	C/C+C/T T/T	1 0,936 (0,211–4,143)	 0,930	417,933
Overdominantinis	C/C+T/T C/T	1 0,377 (0,188–0,758)	 **0,006**	408,952
Adityvinis	---	0,492 (0,276–0,878)	**0,016**	411,211
Pasikartojanti HA				
Kodominantinis	C/C C/T T/T	1 0,251 (0,057–1,106) 2,500 (0,531–11,762)	 0,068 0,246	171,102
Dominantinis	C/C C/T+T/T	1 0.457 (0,151–1,387)	 0,167	175,346
Recesyvinis	C/C+C/T T/T	1 3,327 (0,714–15,498)	 0,126	173,731
Overdominantinis	C/C+T/T C/T	1 0,235 (0,054–1,023)	 0,054	170,208
Adityvinis	---	0,736 (0,303–1,784)	0,497	175,041

GS – galimybių santykis, AIC – akaike informacinis kriterijus, p – reikšmingumo lygmuo, statistiškai reikšmingas skirtumas buvo nustatomas tada, kai p reikšmė buvo <0,05.

3.9.3. SIRT1 rs12778366, *FGFR2* rs2981582 ir *STAT3* rs744166 genų polimorfizmų pokyčiai

SIRT1 rs12778366, *FGFR2* rs2981582 ir *STAT3* rs744166 genotipų dažniai įvertintas pacientams, sergantiems HA, ir sveikiems asmenims (3.9.3.1 lentelė).

SIRT1 genotipų ir alelių dažniai pacientų, sergančių HA, ir sveikų asmenų grupėse neatitiko Hardžio-Vainbergo dėsnio ($p < 0,05$). Atlikus *SIRT1* rs12778366 polimorfizmo analizę, nustatytas statistiškai reikšmingas skirtumas pagal genotipų pasiskirstymą tarp pacientų, sergančių HA, ir kontrolinės grupės asmenų ($p < 0,001$). T/C genotipas HA grupėje nustatytas rečiau nei sveikų asmenų grupėje (0 proc. *vs.* 17,5 proc., $p < 0,001$), o C/C genotipas buvo dažnesnis HA grupėje nei sveikų asmenų grupėje (18,9 proc. *vs*. 2,5 proc., $p < 0,001$) (3.9.3.1 lentelė).

FGFR2 genotipų ir alelių dažniai pacientų, sergančių HA, ir sveikų asmenų grupėse neatitiko Hardžio-Vainbergo dėsnio ($p < 0,05$). Atlikus statistinę analizę, nenustatyta statistiškai reikšmingų skirtumų pagal genotipų (G/G, G/A ir A/A) pasiskirstymą, lyginant kontrolinę grupę su pacientais, sergančiais HA (atitinkamai 41,6 proc. *vs*. 39,2; 53,1 *vs.* 58,7; 5,3 *vs.* 2,1; $p = 0,174$) (3.9.3.1 lentelė).

STAT3 rs744166 genotipų ir alelių dažniai sveikų asmenų grupėje neatitiko Hardžio-Vainbergo dėsnio ($p < 0,05$), bet atitiko pacientų, sergančių HA grupėje ($p > 0,05$). Atlikus *STAT3* rs744166 polimorfizmo analizę, nustatyti statistiškai reikšmingi genotipų pasiskirstymo skirtumai tarp pacientų, sergančių HA, ir kontrolinės grupės ($p = 0,012$). G/G genotipas rečiau pasitaikė asmenims, sergantiems HA, nei sveikų asmenų grupėje (9,1 proc. *vs*. 19,1 proc., $p = 0,003$) (3.9.3.1 lentelė).

3.9.3.1 lentelė. *SIRT1 rs12778366, FGFR2 rs2981582 ir STAT3 rs744166 genotipų dažniai pacientų, sergančių hipofizės adenoma, ir sveikų asmenų grupėse*

Genas	Genotipas/ alelis	Dažnis (proc.)				
		Kontrolinė grupė n (proc.) (n=808)	**p HVE**	**HA grupė n (proc.) (n=143)**	**p HVE**	**p reikšmė**
SIRT1 rs12778366	*Genotipas*					
	T/T	647 (80,1)	<0,01	116 (81,1)	<0,001	
	T/C	141 (17,5)[1]		0 (0)[1]		
	C/C	20 (2,5)[2]		27 (18,9)[2]		**p<0,001**
	Iš viso	808 (100)		143 (100)		
	Alelis					
	T	1435 (88,8)		232 (81,1)		
	C	181 (11,2)		54 (18,9)		
FGFR2 rs2981582	*Genotipas*					
	G/G	336 (41,6)	<0,001	56 (39,2)	<0,001	
	G/A	429 (53,1)		84 (58,7)		
	A/A	43 (5,3)		3 (2,1)		p=0,174
	Iš viso	808 (100)		143 (100)		
	Alelis					
	G	1101 (68,13)		196 (68,53)		
	A	515 (31,87)		90 (31,47)		
STAT3 rs744166	*Genotipas*					
	G/G	**154 (19,1)3**	<0,001	**13 (9,1)3**	0,354	
	G/A	363 (44,9)		68 (47,55)		
	A/A	291 (36,0)		62 (43,35)		**p=0,012**
	Iš viso	808 (100)		143 (100)		
	Alelis					
	G	671 (41,5)		94 (32,87)		
	A	945 (58,5)		192 (67,13)		

SIRT1 – sirtuinas 1, *FGFR2* – fibroblastų augimo faktoriaus receptorius 2, *STAT3* – signalo perdavėjas ir transkripcijos veiksnys 3, p reikšmė – reikšmingumo lygmuo (alfa=0,05), p vertė HVE – reikšmingumo lygmuo pagal Hardžio-Vainbergo dėsnį, skirtumai laikomi statistiškai reikšmingais, kai p<0,05. **[1]p<0,001; [2]p<0,001; [3]p=0,003.**

Visi trys genotipai išanalizuoti atsižvelgiant į pacientų, sergančių HA, ir kontrolinės grupės asmenų lytį (3.9.3.2 lentelė). Išanalizavus *SIRT1* rs12778366 polimorfizmą (T/T, T/C ir C/C) pagal tiriamųjų lytį, nenustatyta statistiškai reikšmingų rodmenų skirtumų tarp moterų ir vyrų, sergančių HA (atitinkamai 80,7 proc., 0 proc., 19,3 proc. *vs.* 81,8 proc., 0 proc., 18,2 proc.) (3.9.3.2 lentelė). Palyginus *SIRT1* rs12778366 genotipų dažnį tarp moterų, sergančių HA, ir sveikų moterų, rasti statistiškai reikšmingi skirtumai. T/C genotipas rečiau nustatytas moterims, sergančioms HA, nei sveikoms moterims (0 proc. *vs.* 2,7 proc., $p < 0,001$) ir C/C genotipas buvo dažniau nustatytas HA sergančioms moterims nei kontrolinės grupės moterims (19,3 proc. *vs.* 2,7 proc., $p < 0,001$). Nustatant T/T genotipą, statistiškai reikšmingo skirtumo nebuvo. Nustatyta, kad C/T genotipas statistiškai reikšmingai retesnis vyrams, sergantiems HA, nei kontrolinės grupės vyrams (0 proc. *vs.* 17,4 proc.; $p < 0,001$), o C/C genotipas dažniau nustatytas sergantiesiems HA nei sveikiems vyrams (18,2 proc. vs. 2,0 proc., $p < 0,001$) (3.9.3.2 lentelė).

Atlikus *FGFR2* rs2981582 polimorfizmo analizę atsižvelgiant į tiriamųjų lytį, statistiškai reikšmingų skirtumų nenustatyta (3.9.3.2 lentelė).

Atlikus *STAT3* rs744166 polimorfizmo genotipų (G/G, G/A ir A/A) analizę, taip pat nenustatyta statistiškai reikšmingų skirtumų tarp vyrų ir moterų, sergančių HA (atitinkamai 8,0 proc., 48,9 proc., 43,2 proc. *vs.* 10,9 proc., 45,5 proc., 43,6 proc.) (3.9.3.2 lentelė). Palyginus *STAT3* rs744166 genotipų dažnį tarp sveikų moterų ir moterų, sergančių HA, nustatyta, kad *STAT3* rs744166 G/G genotipas rečiau pasitaikė moterims, sergančioms HA, nei sveikoms moterims: 8,0 proc. *vs.* 20,4 proc., $p = 0,004$. *STAT3* rs744166 G/A ir A/A genotipų pasiskirstymas tarp sveikų ir sergančių HA moterų nesiskyrė. *STAT3* rs744166 genotipai nesiskyrė sveikų ir sergančių vyrų grupėse (3.9.3.2 lentelė).

3.9.3.2 lentelė. *SIRT1 rs12778366, FGFR2 rs2981582 ir STAT3 rs744166 genotipų dažniai pacientų, sergančių hipofizės adenoma, grupėje ir sveikų asmenų grupėje, atsižvelgiant į lytį*

Genas	**Genotipas/ alelis**	**Dažnis (proc.)**							
		Kontrolinė grupė n (proc.)		**p HVE**	**p reikšmė**	**HA grupė n (proc.)**		**p HVE**	**p reikšmė**
		Moterys (n=510)	**Vyrai (n=298)**			**Moterys (n=88)**	**Vyrai (n=55)**		
SIRT1 rs12778366	*Genotipas*								
	T/T	407 (79,8)	240 (80,5)	0,811	0,801	71 (80,7)	45 (81,8)	0,866	0,866
	T/C	**89 (17,5)[1]**	**52 (17,4)[3]**		1,000	**0 (0)[1]**	**0 (0)[3]**		1,00
	C/C	**14 (2,7)[2]**	**6 (2,0)[4]**		0,518	**17 (19,3)[2]**	**10 (18,2)4**		0,866
	Iš viso	510 (100)	298 (100)			88 (100)	55 (100)		
	Alelis								
	T	903 (88,5)	532 (89,3)			142 (80,7)	90 (81,8)		
	C	117 (11,5)	64 (10,7)			34 (19,3)	20 (18,2)		
FGFR2 rs2981582	*Genotipas*								
	G/G	218 (42,7)	118 (39,6)	0,675	0,381	39 (44,3)	17 (30,9)	0,197	0,117
	G/A	265 (52,0)	164 (55,0)		0,398	48 (54,5)	36 (65,5)		0,224
	A/A	27 (5,3)	16 (5,4)		1,0	1 (1,1)	2 (3,6)		0,559
	Iš viso	510 (100)	298 (100)			88 (100)	55 (100)		
	Alelis								
	G	701 (68,73)	400 (67,11)			126 (71,59)	70 (63,64)		
	A	319 (31,27)	196 (32,89)			50 (28,41)	40 (36,36)		

3.9.3.2 lentelės tęsinys

Genas	Genotipas/ alelis	Dažnis (proc.)							
		Kontrolinė grupė n (proc.)		p HVE	p reikšmė	HA grupė n (proc.)		p HVE	p reikšmė
		Moterys (n=510)	Vyrai (n=298)			Moterys (n=88)	Vyrai (n=55)		
STAT3	*Genotipas*								
rs744166	G/G	**104 (20,4)[5]**	50 (16,75)	0,378	0,207	**7 (8,0)[5]**	6 (10,9)	0,815	0,563
	G/A	229 (44,9)	134 (45,0)		0,986	43 (48,9)	25 (45,5)		0,733
	A/A	177 (34,77)	114 (38,25)		0,312	38 (43,2)	24 (43,6)		1,00
	Iš viso	510 (100)	298 (100)			88 (100)	55 (100)		
	Alelis								
	G	437 (42,84)	234 (39,26)			57 (32,39)	37 (33,64)		
	A	583 (57,16)	362 (60,74)			119 (67,61)	73 (66,36)		

SIRT1 – sirtuinas 1, *FGFR2* – fibroblastų augimo faktoriaus receptorius 2, *STAT3* – signalo perdavėjas ir transkripcijos veiksnys 3, p reikšmė – reikšmingumo lygmuo (alfa=0,05), p vertė HVE – reikšmingumo lygmuo pagal Hardžio-Vainbergo dėsnį, skirtumai laikomi statistiškai reikšmingais, kai p<0,05. **[1] p<0,001; [2] p<0,001; [3] p<0,001; [4] p<0,001; [5] p=0,004.**

Atlikus *SIRT1* rs12778366 analizę, nustatyta, kad, HA galimybę didino C/C genotipas, palyginti su T/T genotipu (GS = 7,530, 95 proc. PI: 4,087–13,873; (p < 0,001), C/C genotipas, palyginti su T/T + T/C genotipais (GS = 9,171, 95 proc. PI: 4,982–16,881; p < 0,001), ir C alelis, palyginti su T aleliu (GS = 1,584, 95 proc. PI: 1,187–2,115; p = 0,002).

Atlikus dvinarę logistinę regresinę analizę, nenustatyta statistiškai reikšmingų *FGFR2* rs2981582 genotipo sąsajų su HA, lyginant HA sergančius pacientus ir sveikus asmenis.

STAT3 rs744166 analizė parodė, kad HA galimybę mažina G/G genotipas, palyginti su A/A genotipu (GS = 0,396, 95 proc. PI: 0,211–0,743; p = 0,004), G/G genotipas, palyginti su A/A + G/A genotipais (GS = 0,425, 95 proc. PI: 0,234–0,71; p = 0,005), bei G alelis, palyginti su A aleliu (GS = 0,702, 95 proc. PI: 0,541–0,911; p = 0,008) (3.9.3.3 lentelė).

3.9.3.3 lentelė. *SIRT1 rs12778366 ir STAT3 rs744166 genų polimorfizmų ir hipofizės adenomos sąsajos (dvinarė logistinė regresinė analizė)*

Genas	Modelis	Genotipas	GS (95 proc. PI)	p reikšmė	AIC
SIRT1 rs12778366	Kodominantinis	T/T T/C C/C	1 0 (0) 7,530 (4,087–13,873)	 0,995 **<0,001**	720,516
	Recesyvinis	T/T+T/C C/C	1 9,171 (4,982–16,881)	 **<0,001**	780,895
	Adityvinis	–	1,584 (1,187–2,115)	**0,002**	780,214
STAT3 rs744166	Kodominantinis	A/A G/A G/G	1 0,879 (0,603–1,282) 0,396 (0,211–0,743)	 0,504 **0,004**	801,223
	Recesyvinis	A/A+G/A G/G	1 0,425 (0,234–0,71)	 **0,005**	799,670
	Adityvinis	–	0,702 (0,541–0,911)	**0,008**	801,881

GS – galimybių santykis, AIC – akaike informacinis kriterijus, p – reikšmingumo lygmuo, skirtumai laikomi statistiškai reikšmingais, kai p<0,05.

Atlikta dvinarė logistinė regresinė analizė HA sergančių pacientų ir sveikų asmenų grupėse, atsižvelgiant į lytį (3.9.3.4 lentelė).

Atlikus *SIRT1* rs12778366 analizę vyrų imtyje, nustatyta, kad HA didino galimybę C/C genotipas, palyginti su T/T genotipu (GS = 8,889, 95 proc. PI: 3,076–25,683; p < 0,001) ir C/C genotipas, palyginti su T/T + T/C genotipais (GS = 10,815, 3,748–31,205; p < 0,001). Moterims HA galimybę didino C/C genotipas, palyginti su T/T genotipu (GS = 6,961, 95 proc. PI: 3,285–14,750; p < 0,001), C/C genotipas, palyginti su T/T + T/C genotipais (GS = 8,483, 95 proc. PI: 4,008–17,955; p < 0,001) bei C alelis, palyginti su T aleliu (GS = 1,580, 95 proc. PI: 1,100–2,271; p = 0,013).

Atlikus *FGFR2* rs2981582 dvinarę logistinę regresinę analizę atsižvelgiant į lytį, nenustatyta statistiškai reikšmingų *FGFR2* rs2981582 genotipo sąsajų su HA.

STAT3 rs744166 dvinarė logistinė regresinė analizė parodė, kad moterų grupėse, HA galimybę mažino G/G genotipas, palyginti su A/A genotipu (GS = 0,314, 95 proc. PI: 0,135–0,727; p = 0,007), G/G genotipas, palyginti su A/A + G/A genotipais (GS = 0,337, 95 proc. PI: 0,151–0,752; p = 0,008) bei G alelis, palyginti su A aleliu (GS = 0,654; 95 proc. PI: 0,469–0,912; p = 0,012), o vyrų imtyje HA genotipai nebuvo statistiškai reikšmingi vertinant HA galimybę.

3.9.3.4 lentelė. *SIRT1 rs12778366 ir STAT3 rs744166 genų polimorfizmų ir hipofizės adenomos sąsajos, atsižvelgiant į tiriamųjų lytį (dvinarė logistinė regresinė analizė)*

Genas	Lytis	Modelis	Genotipas	GS (95 proc. PI)	p reikšmė	AIC
SIRT1 rs12778366	Vyrai	Kodominantinis	T/T T/C C/C	1 0 (0) 8,889 (3,076–25,683)	 0,997 **<0,001**	275,783
		Recesyvinis	T/T+T/C C/C	1 10,815 (3,748–31,205)	 **<0,001**	290,082
	Moterys	Kodominantinis	T/T T/C C/C	1 0 (0) 6,961 (3,285–14,750)	 0,996 **<0,001**	450,358
		Recesyvinis	T/T+T/C C/C	1 8,483 (4,008–17,955)	 **<0,001**	474,428
		Adityvinis	---	1,580 (1,100–2,271)	**0,013**	497,979
STAT3 rs744166	Moterys	Kodominantinis	A/A G/A G/G	1 0,875 (0,542–1,411) 0,314 (0,135–0,727)	 0,583 **0,007**	496,249
		Recesyvinis	A/A+G/A G/G	1 0,337 (0,151–0,752)	 **0,008**	494,549
		Adityvinis	---	0,654 (0,469–0,912)	**0,012**	497,077

GS – galimybių santykis, AIC – akaike informacinis kriterijus, p – reikšmingumo lygmuo, skirtumai laikomi statistiškai reikšmingais, kai $p<0,05$.

Atlikta *SIRT1* rs1277836, *FGFR2* rs2981582 ir *STAT3* rs744166 polimorfizmų analizė atsižvelgiant į HA invazyvumą ir pasikartojimą (3.9.3.5 ir 3.9.3.6 lentelės).

SIRT1 rs12778366 T/C genotipas buvo rečiau nustatomas sergantiesiems neinvazinėmis ir nepasikartojančiomis HA, palyginti su kontroline grupe (atitinkamai 0 proc. *vs.* 17,5 proc.; p = 0,021, 0 proc. *vs.* 17,5 proc.; p < 0,001; p < 0,001). Nenustatyta statistiškai reikšmingo skirtumo tarp neinvazinių ir invazinių, pasikartojančių ir nepasikartojančių HA grupių (3.9.3.5 ir 3.9.3.6 lentelės).

Taip pat nustatėme, kad C/C genotipas dažniau pasitaikė invazinių ir pasikartojančių HA grupėse, palyginti su kontroline grupe (atitinkamai 18,8 proc. *vs.* 2,5 proc.; p = 0,041, 19,4 proc. *vs.* 2.5 proc.; p = 0,047) (3.9.3.5 ir 3.9.3.6 lentelės).

FGFR2 rs2981582 G/G genotipas rečiau buvo nustatomas sergantiesiems neinvazinėmis HA, palyginti su kontroline grupe: 27,6 proc. *vs.* 41,6 proc.; p = 0,038, bet G/A genotipas buvo nustatomas dažniau sergantiesiems neinvazinėmis HA nei kontrolinėje grupėje (72,4 proc. *vs.* 53,1 proc.; p = 0,004) ir sergantiesiems invazinėmis HA (72,4 proc. *vs.* 49,4 proc., p = 0,009) (3.9.3.5 lentelė).

Nenustatyta statistiškai reikšmingų *FGFR2* rs2981582 genotipo sąsajų su HA pasikartojimu (3.9.3.6 lentelė).

STAT3 rs744166 G/G genotipas buvo rečiau nustatomas sergantiesiems invazinėmis ir nepasikartojančiomis HA, palyginti su kontroline grupe (atitinkamai 4,7 proc. *vs.* 19,1 proc.; p < 0,001, 6,2 proc. *vs.* 19,1 proc.; p < 0,001). *STAT3* rs744166 G/G genotipas buvo dažniau nustatomas sergantiesiems neinvazinėmis HA, palyginti su sergančiaisiais invazinėmis HA (15,5 proc. *vs.* 4,7 proc.; p = 0,038), ir sergantiesiems pasikartojančiomis HA, palyginti su sergančiaisiais nepasikartojančiomis HA (19,4 proc. *vs.* 6,2 proc.; p = 0,036) (3.9.3.5 ir 3.9.3.6 lentelės).

3.9.3.5 lentelė. *SIRT1 rs1277836, FGFR2 rs2981582 ir STAT3 rs744166 genotipų dažniai pacientų, sergančių hipofizės adenoma, grupėje ir sveikų asmenų grupėje, atsižvelgiant į invazyvumą*

Genas	**Genotipas/ alelis**	**Dažnis (proc.)**					
		Kontrolinė grupė n (proc.) (n=808)	**p HVE**	**Neinvazinė HA n (proc.) (n=58)**	**p HVE**	**Invazinė HA n (proc.) (n=85)**	**p HVE**
SIRT1 rs12778366	*Genotipas*						
	T/T	647 (80,1)	<0,001	47 (81,0)	<0,001	69 (81,2)	<0,001
	T/C	**141 (17,5)[1,3]**		**0 (0)[1]**		**0 (0)[3]**	
	C/C	**20 (2,5)[2,4]**		**11 (19,0)[2]**		**16 (18,8)[4]**	
	Iš viso	808 (100)		58 (100)		85 (100)	
	Alelis						
	T	1435 (88,8)		94 (81,0)		138 (81,2)	
	C	181 (11,2)		22 (19,0)		32 (18,8)	
FGFR2 rs2981582	*Genotipas*						
	G/G	**336 (41,6)[5]**	<0,001	**16 (27,6)[5,7]**	<0,001	40 (47,1)[7]	0,043
	G/A	**429 (53,1)[6]**		**42 (72,4)[6,8]**		**42 (49,4)[8]**	
	A/A	43 (5,3)		0 (0)		3 (3,5)	
	Iš viso	808 (100)		58 (100)		85 (100)	
	Alelis						
	G	1101 (68,13)		74 (63,79)		122 (71,76)	
	A	515 (31,87)		42 (36,21)		48 (28,24)	

3.9.3.5 lentelės tęsinys

Genas	Genotipas/ alelis	Dažnis (proc.)					
		Kontrolinė grupė n (proc.) (n=808)	**p HVE**	**Neinvazinė HA n (proc.) (n=58)**	**p HVE**	**Invazinė HA n (proc.) (n=85)**	**p HVE**
STAT3	*Genotipas*						
rs744166	G/G	**154 (19,1)[9]**	<0,001	**9 (15,5)[2]**	0,313	**4 (4,7)[1,2]**	0,031
	G/A	363 (44,9)		23 (39,7)		45 (52,9)	
	A/A	291 (36,0)		26 (44,8)		36 (42,4)	
	Iš viso	808 (100)		58 (100)		85 (100)	
	Alelis						
	G	671 (41,5)		41 (35,34)		53 (31,18)	
	A	945 (58,5)		75 (64,66)		117 (68,82)	

SIRT1 – sirtuinas 1, *FGFR2* – fibroblastų augimo faktoriaus receptorius 2, *STAT3* – signalo perdavėjas ir transkripcijos veiksnys 3, p reikšmė – reikšmingumo lygmuo (alfa=0,05), p vertė HVE – reikšmingumo lygmuo pagal Hardžio-Vainbergo dėsnį, skirtumai laikomi statistiškai reikšmingais, kai p<0,05. **[1] p=0,021; [2] p=0,041; [3] p<0,001; [4] p<0,001; [5] p=0,038; [6] p=0,004;[7] p=0,024; [8] p=0,009; [9] p<0,001; [10] p=0,038.**

3.9.3.6 lentelė. *SIRT1 rs1277836, FGFR2 rs2981582 ir STAT3 rs744166 genotipų dažniai pacientų, sergančių hipofizės adenoma, grupėje ir sveikų asmenų grupėje, atsižvelgiant į pasikartojimą*

Genas	**Genotipas/ alelis**	**Dažnis (proc.)**					
		Kontrolinė grupė n (proc.) (n=808)	**p HVE**	**Nepasikartojanti HA n (proc.) (n=112)**	**p HVE**	**Pasikartojanti HA n (proc.) (n=31)**	**p HVE**
SIRT1 rs12778366	*Genotipas*						
	T/T	647 (80,1)	<0,001	91 (81,2)	<0,001	25 (80,6)	<0,001
	T/C	**141 (17,5)[1,3]**		**0 (0)[1]**		**0 (0)[3]**	
	C/C	**20 (2,5)[2,4]**		**21 (18,8)[2]**		**6 (19,4)[4]**	
	Iš viso	808 (100)		112 (100)		31 (100)	
	Alelis						
	T	1435 (88,8)		182 (81,25)		50 (80,6)	
	C	181 (11,2)		42 (18,75)		12 (19,4)	
FGFR2 rs2981582	*Genotipas*						
	G/G	336 (41,6)	<0,001	44 (39,3)	<0,001	12 (38,7)	0,067
	G/A	429 (53,1)		66 (58,9)		18 (58,1)	
	A/A	43 (5,3)		2 (1,8)		1 (3,2)	
	Iš viso	808 (100)		112 (100)		31 (100)	
	Alelis						
	G	1101 (68,13)		154 (68,75)		42 (67,74)	
	A	515 (31,87)		70 (31,25)		20 (32,26)	

3.9.3.6 lentelės tęsinys

Genas	Genotipas/ alelis	Dažnis (proc.)					
		Kontrolinė grupė n (proc.) (n=808)	**p HVE**	**Nepasikartojanti HA n (proc.) (n=112)**	**p HVE**	**Pasikartojanti HA n (proc.) (n=31)**	**p HVE**
STAT3	*Genotipas*						
rs744166	G/G	**154 (19,1)[5]**	<0,001	**7 (6,2)[5,6]**	0,083	**6 (19,4)[6]**	0,305
	G/A	363 (44,9)		56 (50,0)		12 (38,7)	
	A/A	291 (36,0)		49 (43,8)		13 (41,9)	
	Iš viso	808 (100)		112 (100)		31 (100)	
	Alelis						
	G	671 (41,5)		70 (31,25)		24 (38,71)	
	A	945 (58,5)		154 (68,75)		38 (61,29)	

SIRT1 – sirtuinas 1, *FGFR2* – fibroblastų augimo faktoriaus receptorius 2, *STAT3* – signalo perdavėjas ir transkripcijos veiksnys 3, p reikšmė – reikšmingumo lygmuo (alfa=0,05), p vertė HVE – reikšmingumo lygmuo pagal Hardžio-Vainbergo dėsnį, skirtumai laikomi statistiškai reikšmingais, kai p<0,05. **[1] p<0,001; [2] p<0,001; [3] p=0,005; [4] p=0,047; [5] p<0,001; [6] p=0,036.**

Atlikta dvinarė logistinė regresinė analizė neinvazinių, invazinių HA ir kontrolinėje grupėse (3.9.3.7 lentelė).

Išanalizavus *SIRT1* polimorfizmą neinvazinės HA galimybę didino C/C genotipas, palyginti su T/T genotipu (GS = 7,571, 95 proc. PI: 3,426–16,734; p < 0,001), C/C genotipas, palyginti su T/T + T/C genotipais (GS = 9,221, 95 proc. PI: 4,175–20,367; p < 0,001), bei C alelis, palyginti su T aleliu (GS = 1,649, 95 proc. PI: 1,065–2,554; p = 0,025). Atlikus invazinių HA ir kontrolinės grupės analizę, nustatyta, kad galimybę susirgti invazine HA didina C/C genotipas, palyginti su T/T genotipu (GS = 7,501, 95 proc. PI: 3,715–15,147; p < 0,001), C/C genotipas, palyginti su T/T + T/C genotipais (GS = 9,136, 95 proc. PI: 4,528–18,434; p < 0,001), bei C alelis, palyginti su T aleliu (GS = 1,616, 95 proc. PI: 1,120–2,330; p = 0,010).

FGFR2 rs2981582 dvinarė regresinė logistinė analizė parodė, kad neinvazinės HA galimybę didina G/A genotipas, palyginti su G/G genotipu (GS = 2,056, 95 proc. PI: 1,136–3,721; p = 0,017), G/A + A/A genotipai, palyginti su G/G genotipu (GS = 1,869, 95 proc. PI: 1,033–3,380; p = 0,039), ir G/A genotipas, palyginti su G/G + A/A genotipais (GS = 2,319, 95 proc. PI: 1,283–4,193; p = 0,005).

STAT3 rs744166 dvinarė logistinė regresinė analizė parodė, kad invazinės HA galimybę mažina G/G genotipas, palyginti su A/A genotipu (GS = 0,210, 95 proc. PI: 0,073–0,601; p = 0,004), G/G genotipas, palyginti su A/A + G/A genotipais (GS = 0,210, 95 proc. PI: 0,076–0,581; p = 0,003), G alelis, palyginti su A aleliu (GS = 0,651, 95 proc. PI: 0,467–0,908; p = 0,011).

3.9.3.7 lentelė. *SIRT1 rs12778366, FGFR2 rs2981582 ir STAT3 rs744166 genų polimorfizmų ir neinvazinės ir invazinės hipofizės adenomos sąsajos, atsižvelgiant į tiriamųjų lytį (dvinarė logistinė regresinė analizė)*

Genas	HA tipas	Modelis	Genotipas	GS (95 proc. PI)	p reikšmė	AIC
SIRT1 rs12778366	Neinvazinė	Kodominantinis	T/T T/C C/C	1 0 (0) 7,571 (3,426–16,734)	 0,996 **<0,001**	390,145
		Recesyvinis	T/T+T/C C/C	1 9,221 (4,175–20,367)	 **<0,001**	406,092
		Adityvinis	---	1,649 (1,065–2,554)	**0,025**	425,148
	Invazinė	Kodominantinis	T/T T/C C/C	1 0 (0) 7,501 (3,715–15,147)	 0,996 **<0,001**	509,448
		Recesyvinis	T/T+T/C C/C	1 9,136 (4,528–18,434)	 **<0,001**	533,418
		Adityvinis	---	1,616 (1,120–2,330)	**0,010**	459,515
FGFR2 rs2981582	Neinvazinė	Kodominantinis	G/G G/A A/A	1 2,056 (1,136–3,721) 0 (0)	 **0,017** 0,998	419,170
		Dominantinis	G/G G/A+A/A	1 1,869 (1,033–3,380)	 **0,039**	425,028
		Overdominantinis	G/G+A/A G/A	1 2,319 (1,283–4,193)	 **0,005**	420,961
STAT3 rs744166	Invazinė	Kodominantinis	A/A G/A G/G	1 1,002 (0,630–1,595) 0,210 (0,073–0,601)	 0,993 **0,004**	553,313
		Recesyvinis	A/A+G/A G/G	1 0,210 (0,076–0,581)	 **0,003**	551,313
		Adityvinis	---	0,651 (0,467–0,908)	**0,011**	558,771

GS – galimybių santykis, AIC – akaike informacinis kriterijus, p – reikšmingumo lygmuo, skirtumai laikomi statistiškai reikšmingais, kai p<0,05.

Atlikome *SIRT1* rs12778366 polimorfizmo dvinarę logistinę regresinę analizę, vertindami nepasikartojančias, pasikartojančias HA ir kontrolinę grupes. Palyginus nepasikartojančių HA ir kontrolinę grupę, nustatyta, kad nepasikartojančios HA galimybę didino C/C genotipas, palyginti su T/T genotipu (GS = 7,465; 95 proc. PI: 3,896–14,307; p < 0,001), C/C genotipas, palyginti su T/T + T/C genotipais (GS = 9,092; 95 proc. PI: 4,748–17,411; p < 0,001), ir C alelis, palyginti su T aleliu (GS = 1,592; 95 proc. PI: 1,153–2,199; p = 0,005) (3.9.3.8 lentelė). Pasikartojančios HA galimybę didino *SIRT1* rs12778366 C/C genotipas, palyginti su T/T (GS = 7,764; 95 proc. PI: 2,868–21,019; p < 0,001), ir C/C genotipas, palyginti su T/T + T/C genotipais (GS = 9,456, 95 proc. PI: 3,495–25,586; p < 0,001).

Nenustatyta statistiškai reikšmingų *FGFR2* rs2981582 genotipo sąsajų su HA atsiradimu.

STAT3 rs744166 dvinarės logistinės regresinės analizės metu nustatėme, kad nepasikartojančios HA galimybę mažino G/G genotipas, palyginti su A/A genotipu (GS = 0,270; 95 proc. PI: 0,119–0,610; p = 0,002), G/G genotipas, palyginti su A/A + G/A genotipais (GS = 0,283, 95 proc. PI: 0,129–0,621; p = 0,002), bei G alelis, palyginti su A aleliu (GS = 0,653; 95 proc. PI: 0,487–0,876; p = 0,005) (3.9.3.8 lentelė). Nenustatyta statistiškai reikšmingų genotipo sąsajų su HA pasikartojimu.

3.9.3.8 lentelė. *SIRT1 rs12778366 ir STAT3 rs744166 genų polimorfizmų ir nepasikartojančios bei pasikartojančios hipofizės adenomos sąsajos (dvinarė logistinė regresinė analizė)*

Genas	HA tipas	Modelis	Genotipas	GS (95 proc. PI)	p reikšmė	AIC
SIRT1 rs12778366	Nepasikartojanti	Kodominantinis	T/T T/C C/C	1 0 (0) 7,465 (3,896–14,307)	 0,995 **<0,001**	614,042
		Recesyvinis	T/T+T/C C/C	1 9,092 (4,748–17,411)	 **<0,001**	645,812
		Adityvinis	---	1,592 (1,153–2,199)	**0,005**	678,223
	Pasikartojanti	Kodominantinis	T/T T/C C/C	1 0 (0) 7,764 (2,868–21,019)	 0,996 **<0,001**	247,718
		Recesyvinis	T/T+T/C C/C	1 9,456 (3,495–25,586)	 **<0,001**	255,407
STAT3 rs744166	Nepasikartojanti	Kodominantinis	A/A G/A G/G	1 0,916 (0,606–1,385) 0,270 (0,119–0,610)	 0,678 **0,002**	673,559
		Recesyvinis	A/A+G/A G/G	1 0,283 (0,129–0,621)	 **0,002**	671,731
		Adityvinis	---	0,653 (0,487–0,876)	**0,005**	677,047

GS – galimybių santykis, AIC – akaike, p – reikšmingumo lygmuo, skirtumai laikomi statistiškai reikšmingais, kai p<0,05.

3.9.4. SIRT1, *FGFR2* ir *STAT3* genotipų polimorfizmų derinių sąsajos su HA atsiradimu

Atlikome *SIRT1* rs12778366, *FGFR2* rs2981882 ir *STAT3* rs744166 genotipų derinių sąsajų tyrimus. Gauti genotipų derinių duomenys buvo palyginti tarp pacientų, kuriems nustatyta HA, bei sveikų asmenų grupės. Statistiškai reikšmingi genotipų deriniai pateikiami 3.9.4.1 lentelėje.

3.9.4.1 lentelė. *SIRT1, FGFR2 ir STAT3 genotipų deriniai, didinantys ar mažinantys hipofizės adenomos galimybę*

Genotipų deriniai	GS (95 proc. PI)	p vertė
SIRT1 T/T + *FGFR2* G/G + *STAT3* G/G	3,475 (1,073–11,258)	0,038
SIRT1 T/T + *FGFR2* G/A + *STAT3* A/A	0,627 (0,407–0,967)	0,035
SIRT1 C/C + *FGFR2* G/G + *STAT3* G/A	0,170 (0,059–0,492)	0,001
SIRT1 C/C + *FGFR2* G/G + *STAT3* A/A	0,058 (0,006–0,560)	0,014
SIRT1 C/C + *FGFR2* G/A + *STAT3* G/A	0,130 (0,029–0,585)	0,008
SIRT1 C/C + *FGFR2* G/A + *STAT3* A/A	0,042 (0,009–0,199)	<0,001
SIRT1 C/C + *FGFR2* G/G	0,151 (0,065–0,349)	<0,001
SIRT1 C/C + *FGFR2* G/A	0,092 (0,038–0,224)	<0,001
SIRT1 T/T + *STAT3* G/G	2,699 (1,338–5,444)	0,006
SIRT1 C/C + *STAT3* G/G	0,260 (0,072–0,933)	0,039
SIRT1 C/C + *STAT3* G/A	0,151 (0,065–0,349)	<0,001
SIRT1 C/C + *STAT3* A/A	0,045 (0,012–0,162)	<0,001
FGFR2 G/A + *STAT3* A/A	0,595 (0,398–0,890)	0,011

SIRT1 – sirtuinas 1, *FGFR2* – fibroblastų augimo faktoriaus receptorius 2, *STAT3* – signalo perdavėjas ir transkripcijos veiksnys 3, GS – galimybių santykis, PI – pasikliautinasis intervalas, p – reikšmingumo lygmuo, skirtumai laikomi statistiškai reikšmingais, kai p<0,05.

Atlikus logistinę regresinę analizę buvo nustatyta 11 apsauginių ir 2 genotipų deriniai didinantys HA galimybę. *SIRT1* T/T + *FGFR2* G/G + *STAT3* G/G genų variantas didino HA galimybę 3,475 karto (GS = 3,475; 95 proc. PI: 1,073–11,258, p = 0,038), o *SIRT1* T/T + *STAT3* G/G genų variantas HA didino galimybę 2,699 karto (GS = 2,699; 95 proc. PI: 1,338–5,444, p = 0,006).

Genų variantai, mažinantys HA galimybę:

SIRT1 T/T + *FGFR2* G/A + *STAT3* A/A (GS = 0,627; 95 proc. PI: 0,407–0,967, p = 0,035).

SIRT1 C/C + *FGFR2* G/G + *STAT3* G/A (GS = 0,170; 95 proc. PI: 0,059–0,492, p = 0,001).

SIRT1 C/C + *FGFR2* G/G + *STAT3* A/A (GS = 0,058; 95 proc. PI: 0,006–0,560, p = 0,014).

SIRT1 C/C + *FGFR2* G/A + *STAT3* G/A (GS = 0,130; 95 proc. PI: 0,029–0,585, p = 0,0080.

SIRT1 C/C + *FGFR2* G/A + *STAT3* A/A (GS = 0,042; 95 proc. PI: 0,009–0,199, p < 0,001).

SIRT1 C/C + *FGFR2* G/G (GS = 0,151; 95 proc. PI: 0,065–0,349, p < 0,001).

SIRT1 C/C + *FGFR2* G/A (GS = 0,092; 95 proc. PI: 0,038–0,224, p < 0,001).

SIRT1 C/C + *STAT3* G/G (GS = 0,260; 95 proc. PI: 0,072–0,933, p = 0,039).

SIRT1 C/C + *STAT3* G/A (GS = 0,151; 95 proc. PI: 0,065–0,349, p < 0,001).

SIRT1 C/C + *STAT3* A/A (GS = 0,045; 95 proc. PI: 0,012–0,162, p < 0,001).

FGFR2 G/A + *STAT3* A/A (GS = 0,595; 95 proc. PI: 0,398–0,890, p = 0,011).

4. REZULTATŲ APTARIMAS

4.1. Sergančiųjų HA regos funkcijų pokyčiai

RNK yra lokalizuota virš hipofizės ir, jei navikas auga supraseliariai, dėl kryžmės kompresijos, dažnai būna regos aštrumo bei akipločio pakitimų. Daugelis mokslininkų nustatė sutrikusias regos funkcijas sergantiesiems HA [7-14, 74, 84, 85, 97–102, 237]. Mūsų tyrime taip pat nustatytas sumažėjęs GKRA ir akipločio defektai (dalinė temporalinė hemianopsija, pilna temporalinė hemianopsija, koncentriškai susiaurėjęs akiplotis) sergantiesiems HA. Tik viename tyrime Siddharth Ogra ir bendraautoriai 7 atvejais (13 proc.) iš 103 sergančiųjų rado homoniminių akipločio defektų [238]. Mūsų tyrime nė vienam pacientui homoniminių akipločio defektų nenustatyta. Paprastai homoniminė hemianopsija rodo ne kryžmės pažeidimą, o pažeidimą už RNK priešingoje pusėje nei nustatytas akipločio defektas.

HA gali sukelti peripapilinio TNSS išplonėjimą, kurį galime objektyviai įvertinti objektyvaus neinvazinio tyrimo – optinės koherentinės tomografijos – būdu. Šiuo metu OKT plačiai naudojama oftalmologijoje nervinių skaidulų būklei įvertinti. Mūsų atliktame tyrime nustatytas statistiškai reikšmingas TNSS išplonėjimas visuose keturiuose kvadrantuose HA sergantiems pacientams. Tai patvirtina mūsų ankstesnio tyrimo rezultatus, kai, ištyrę 20 HA sergančių pacientų ir palyginę su 20 kontrolinės grupės asmenų, nustatėme TNSS išplonėjimą visuose keturiuose kvadrantuose apie RND [239]. Chan Hee Moon ir bendraautoriai taip pat nustatė statistiškai reikšmingai išplonėjusį TNSS HA sergantiems pacientams (n=18), palyginti su kontroline grupe (n=20) [15]. Charlotta Johansson ir Bertil Lindblom [240] ištyrė 16 pacientų, sergančių HA, kuriems visiems pasireiškė bitemporalinė hemianopsija, tačiau ne visose akyse nustatytas išplonėjęs TNSS. Manome, kad tokie rezultatai galėjo būti gauti dėl mažo tiriamųjų skaičiaus. TNSS sąsajos su RNK, HA morfologinėmis charakteristikomis iki šiol nebuvo tirtos.

Mūsų tyrimo išskirtinė dalis – išsami radiologinė HA ir RNK analizė. Nustatėme, kad sergančiųjų HA vidurinės dalies RNK mediana 1,7 mm (min 0,1; maks 3,7), kai normalus RNK aukštis turėtų būti maždaug 3,5 mm [241]. RNK storis statistiškai patikimai skyrėsi tarp HA su ir be supraseliarinio plitimo. Yra svarbu įvertinti HA augimo kryptį, kadangi supraseliarinis HA augimas gali sukelti regos funkcijų sutrikimus. Nustatėme, kad TNSS storis temporaliniame kvadrante buvo statistiškai patikimai mažesnis pacientams, sergantiems HA su supraseliariniu plitimu nei be supraseliarinio plitimo. Taip pat nustatėme statistiškai patikimai blogesnį GKRA

pacientų, kuriems nustatytas supraseliarinis HA augimas, grupėje nei tiems, kuriems nebuvo supraseliarinio HA augimo. Mūsų rezultatai patvirtina Schmalisch ir kolegų atlikto tyrimo rezultatus, kai nustatyta statistiškai reikšminga priklausomybė tarp regos aštrumo mažėjimo ir supraseliarinio HA augimo [105].

Nustatėme kontrastinio jautrumo ir spalvų juslės sutrikimus sergantiesiems HA. Mūsų tyrimo rezultatai sutampa su kitų mokslininkų duomenimis [85, 88, 97, 109], kurie taip pat nustatė sutrikusias šias regos funkcijas sergantiesiems HA. Moksliniame tyrime, kurį atliko dr. Kęstutis Šinkūnas [109], ištyrus 40 HA sergančių asmenų ir 80 sveikų asmenų, taip pat nustatyta, kad sergančiųjų HA spalvų juslės slenkstis yra statistiškai reikšmingai aukštesnis nei sveikų asmenų. Šinkūnas savo disertacijoje nagrinėjo ir sergančiųjų HA spalvų juslės pokyčius. F-M 100 testo metu nustatyta, kad asmenys, sergantys HA, padarė 3 kartus daugiau klaidų nei sveikieji. Mūsų atliktame tyrime spalvų juslės bei KJ rodmenys taip pat buvo statistiškai reikšmingai geresni sveikų pacientų nei sergančių HA. Tyrimo metu mes papildomai įvertinome sąsajas tarp F-M 100 ir RSKJ tyrimo rezultatų ir HA augimo pobūdžio ir nustatėme, kad tyrimų rodmenų skirtumai nebuvo statistiškai reikšmingi tarp pacientų, sergančių invazine ir neinvazine HA, RSKJ tyrimo atveju, o F-M 100 tyrimo metu pastebėtas statistiškai reikšmingas skirtumas. Nustatytas statistiškai reikšmingas RSKJ skirtumas tarp supraseliarinio augimo HA ir HA be supraseliarinio augimo grupėse, o F-M 100 tyrimo atveju skirtumas nebuvo statistiškai reikšmingas.

Taigi, įtarus turkiabalnio srities patologiją, reikėtų atlikti ne tik įprastinius oftalmologinius tyrimus, bet ir detaliai ištirti spalvų juslę bei kontrastinį jautrumą.

4.2. Sergančiųjų HA IL-17A pokyčiai

Tik viename tyrime mokslininkai nagrinėjo IL-17A koncentraciją sergančiųjų HA kraujo serume. Qiu ir bendraautoriai [61, 62] prieš operaciją ištyrė IL-17 koncentraciją kraujo serume 75 HA sergantiems asmenims ir nustatė, kad IL-17 koncentracija sergantiesiems invazine HA buvo didesnė nei sergantiesiems neinvazine HA. Invazinių HA grupėje IL-17 koncentracija buvo 95,46 ± 34,09, neinvazinių HA 56,26 ± 14,03 pg/ml, kontrolinėje grupėje 23,58 ± 6,55 pg/ml. Mes taip pat nustatėme statistiškai reikšmingai didesnę citokino IL-17A koncentraciją pacientų, sergančių HA, kraujo serume, palyginti su kontroline sveikų asmenų grupe, tačiau nenustatyta statistiškai reikšmingų skirtumų tarp skirtingo augimo pobūdžio HA.

Mes galime kelti hipotezę, kad citokinas IL-17A gali būti svarbus HA atsiradimui, tačiau neturi įtakos HA augimo pobūdžiui.

4.3. Sergančiųjų HA Ki-67 proliferacijos indekso pokyčiai

Ki-67 proliferacijos indeksas intensyviai tyrinėjamas, kaip galimas HA invazyvumo žymuo, tačiau gaunami duomenys iki šiol išlieka prieštaringi. Tai vienintelis HA žymuo, kuris PSO endokrininių auglių klasifikacijoje yra įvardintas kaip agresyvaus HA augimo rodiklis [142].

Mūsų tyrimas patvirtina kitų mokslininkų gautus rezultatus, kad Ki-67 PI yra statistiškai reikšmingai didesnis esant pažeistam kietajam smegenų dangalui (tikrasis invazyvumas) nei neinvazinėse HA [65–67, 69, 71, 72, 76, 78], tačiau keliuose moksliniuose tyrimuose nenustatyta Ki-67 PI sąsajų su HA invazyvumu [63, 64, 68, 73]. Mes nenustatėme Ki-67 PI priklausomybės nuo pasikartojimo, tačiau Paek ir kolegos [71] nustatė statistiškai didesnį Ki-67 PI (1,27 proc.) pasikartojančių HA nei nepasikartojančių HA atvejais (0,56 proc.) ($p = 0,027$). Mūsų tyrimo metu nustatėme invazinėse HA statistiškai reikšmingai didesnį Ki-67 PI nei neinvazinėse HA, tačiau nenustatyta Ki-67 PI sąsajų su pasikartojimu ir HA augimo kryptimi.

Ki-67 PI tyrimų rezultatai išlieka prieštaringi, todėl reikia išsamesnių tyrimų su didesne tiriamųjų imtimi.

4.4. Sergančiųjų HA *MMP-2* (-1306 C/T) geno polimorfizmo analizė

Pasaulyje daugiau nėra atlikta tyrimų, nagrinėjančių *MMP-2* (-1306 C/T) geno polimorfizmo sąsajas su HA išsivystymu ir augimo pobūdžiu. Daugelis mokslininkų nagrinėjo *MMP-2* (-1306 C/T) geno polimorfizmo svarbą vystantis įvairiems navikams [48–60]. Taip pat keliuose tyrimuose nustatyta padidėjusi *MMP-2* raiška hipofizės navikiniame audinyje [219–220]. Remdamiesi šiais tyrimais, mes iškėlėme hipotezę, kad *MMP-2* geno polimorfizmas gali turėti įtakos HA atsiradimui, taip pat invaziniam HA augimo pobūdžiui. Išanalizavus 84 pacientų, sergančių HA, *MMP2* (-1306 C/T) polimorfizmą ir palyginus su 318 kontrolinės grupės asmenų, nustatėme, kad *MMP-2* (-1306 C/T) C/T genotipas, palyginti su C/C ir C/C + T/T genotipais, didino neinvazyvios HA galimybę 3 ir 2,8 kartų, o C/T + T/T genotipai, palyginti su C/C genotipu, šią galimybę didino 2,7 karto. Nenustatyta statistiškai reikšmingų skirtumų pagal genotipų pasiskirstymą, tik C/T genotipas buvo dažniau nustatytas moterims, sergančioms HA, nei sveikoms moterims. Galime daryti išvadą, kad *MMP-2* (-1306 C/T) C/T genotipas gali sąlygoti HA atsiradimą moterims.

4.5. Sergančiųjų HA *MMP-9* (-1562 C/T) geno polimorfizmo analizė

Mūsų duomenimis, daugiau nėra atlikta pasaulyje tyrimų, nagrinėjančių *MMP-9* (-1562 C/T) geno polimorfizmo sąsajas su HA. Ankstesniuose tyrimuose, nagrinėjančiuose HA atsiradimą, invazyvumą, didesnis dėmesys buvo skiriamas *MMP-9* raiškai HA audiniuose [219–222, 224–227], bet ne geno polimorfizmui. Nustatėme, kad *MMP-9* (-1562 C/T) T/T genotipas, palyginti su C/C + C/T genotipais, didino invazinės HA galimybę 3,2 karto.

Taip pat nustatėme, kad *MMP-9* (-1562 C/T) C/C genotipas, dažniau pasireiškė pacientų, sergančių HA, grupėje nei sveikų asmenų grupėje. *MMP-9* (-1562 C/T) C/C genotipas buvo pastebimas dažniau sergantiems neinvazine, invazine ir nepasikartojančia HA, palyginti su sveikais asmenimis. Todėl mes galime kelti hipotezę, kad *MMP-9* (-1562 C/T) C/C geno polimorfizmas gali būti svarbus HA atsiradimui, tačiau nėra reikšmingas HA pasikartojimui.

4.6. Sergančiųjų HA *SIRT1* rs12778366*, FGFR2* rs2981582 ir *STAT3* rs744166 genų polimorfizmų analizė

SIRT1, FGFR2, STAT3 genų polimorfizmų įtaka įvairių navikų išsivystymui, analizuota daugelyje tyrimų [30–38, 170, 179–185], tačiau mūsų duomenimis, nėra tyrimų, kurių metu būtų įvertintos sąsajos su HA.

Mes pirmą kartą pasaulyje ištyrėme *SIRT1* rs12778366 genotipą sergantiesiems HA. C/C genotipas dažniau nustatytas HA sergantiems asmenims nei sveikiems (18,9 *vs*. 2,5 proc., $p < 0,001$). Todėl *SIRT1* rs12778366 C alelis ir C/C genotipas gali būti siejamas su didesne galimybe susirgti HA. Taip pat nustatyta, kad HA galimybę 7,5 karto didino C/C genotipas, palyginti su T/T genotipu, C/C genotipas, palyginti su T/T + T/C genotipais šią galimybę didino 9 kartus. Tyrimų, nagrinėjančių *SIRT1* rs12778366 geno polimorfizmo sąsajas su HA atsiradimu, daugiau pasaulyje nėra atlikta.

Ištyrę *FGFR2* rs2981582 geno polimorfizmą, mes nenustatėme statistiškai reikšmingų skirtumų tarp genotipų pasiskirstymo, lygindami kontrolinę ir HA grupes. Taip pat nenustatėme skirtumų analizuodami HA invazyvumą ar pasikartojimą. Tyrimų, nagrinėjančių *FGFR2* rs2981582 geno polimorfizmo sąsajas su HA, daugiau pasaulyje nėra atlikta.

Keliuose tyrimuose nagrinėta *STAT3* rs744166 polimorfizmo svarba įvairių auglių išsivystymui, tačiau nėra nė vieno tyrimo, analizuojančio sąsajas su HA išsivystymu, invazyvumu, pasikartojimu. Mes pirmieji nustatėme, kad *STAT3* rs744166 G/G genotipas rečiau pasitaikė pacientams, sergantiems HA, nei sveikiems asmenims (9,1 *vs*. 19,1 proc., $p = 0,012$). G/G genotipas dažniau nustatytas sergantiems neinvazinėmis HA, palyginti

su invazinėmis (15,5 *vs.* 4,7 proc., p = 0,038), ir sergantiesiems pasikartojančiomis HA palyginti su sergančiaisiais nepasikartojančiomis HA (19,4 *vs.* 6,2 proc., p = 0,036). Galime daryti išvadą, kad *STAT3* rs744166 geno polimorfizmas gali būti siejamas su HA invazyvumu ir pasikartojimu.

Tyrimo metu metu nustatėme 2 genotipų derinių variantus didinančius HA galimybę. *SIRT1* T/T + *FGFR2* G/G + *STAT3* G/G genotipų derinių variantas didino HA galimybę 3,5 karto, o *SIRT1* T/T + *STAT3* G/G genotipų derinių variantas didino HA galimybę 2,7 karto. Taigi, svarbu ne tik įvertinti atskirų genų polimorfizmų sąsajas su HA išsivystymu, bet ir genotipų derinius, kurie gali mažinti ar didinti HA galimybę.

IŠVADOS

1. Atlikus pacientų oftalmologinį ištyrimą, nustatyta:
   - Sumažėjęs funkcinis kontrastinis jautrumas hipofizės adenoma sergantiems pacientams.
   - Sutrikusi spalvų juslė pacientams, sergantiems invazine ir pasikartojančia hipofizės adenoma, bei sumažėjęs spalvinis kontrastinis jautrumas pacientams, sergantiems supraseliarinio augimo hipofizės adenoma.
   - Suplonėjęs priešoperacinis tinklainės nervinių skaidulų sluoksnio storis visuose keturiuose regos nervo disko kvadrantuose pacientams, sergantiems hipofizės adenoma. Tinklainės nervinių skaidulų sluoksnio storis pacientams, kuriems nustatytas supraseliarinis hipofizės adenomos augimas, palyginti su pacientais, kuriems nenustatytas supraseliarinis augimas, buvo sumažėjęs tik temporaliniame kvadrante.

2. Invazinėse hipofizės adenomose nustatytas didesnis Ki-67 proliferacijos indeksas nei neinvazinėse hipofizės adenomose.
   Nustatyta didesnė citokino IL-17A koncentracija pacientų, sergančių hipofizės adenoma, kraujo serume, palyginti su sveikais asmenimis.

3. *MMP-2* (-1306 C/T) C/T genotipas, palyginti su C/C genotipu, didino neinvazyvios hipofizės adenomos galimybę 3 kartus, o palyginti su C/C + T/T genotipais – 2,8 karto. *MMP-2* (-1306 C/T) C/T + T/T genotipai, palyginti su C/C genotipu, šią galimybę didino 2,7 karto.
   MMP-9 (-1562 C/T) T/T genotipas, palyginti su C/C + C/T genotipais, didino invazinės hipofizės adenomos galimybę 3,2 karto.

4. *SIRT1* C/C genotipas, palyginti su T/T genotipu, hipofizės adenomos galimybę didino 7,5 karto, o palyginti su T/T + T/C genotipais – 9 kartus. *SIRT1* T/T + *FGFR2* G/G + *STAT3* G/G genotipų derinių variantas hipofizės adenomos galimybę didino 3,5 karto, o *SIRT1* T/T + *STAT3* G/G genotipų derinių variantas – 2,7 karto.

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PUBLIKACIJOS DISERTACIJOS TEMA

Leidiniuose, referuojamuose Mokslinės informacijos instituto (*Thomson Reuters*) duomenų bazėje „ISI Web of Science“ ir turinčiuose citavimo rodiklį:

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Changes of Visual Functions in Patients With Pituitary Adenoma

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***Key Words**: pituitary adenoma; visual functions; Farnsworth-Munsell 100 hue test.*

***Summary.** Background and Objective. The aim of this study was to evaluate associations between visual functions (visual acuity, perimetry, optic nerve disc condition, and color contrast sensitivity) and pituitary adenoma (PA) diameter.*

Material and Methods. In the study, 20 patients with PA, which was confirmed by computed tomography or magnetic resonance imaging scans, were examined. The patients were divided into 2 groups: those with a PA diameter of ⩽1 cm (14 eyes) and with a PA diameter of >1 cm (26 eyes). The control group comprised 40 healthy age- and gender-matched persons (80 eyes). The diameter of PA, visual acuity, and perimetry were analyzed; the F-M 100 hue test for color discrimination was used in patients with PA.

Results. Visual acuity was better in the control group as compared with both groups of patients (1.0 vs. 0.90 [SD, 0.50] and 0.64 [SD, 0.21]; P=0.01; respectively). The results of the Farnsworth-Munsell 100 hue test were also better in the control group compared with the patients with PA of ⩽1 cm and >1 cm (error score of 80.1 [SD, 53.0] vs. 131.8 [SD, 30.6] and 244.68 [SD, 51. 6], respectively; P=0.011). There was a very strong positive correlation between the error score of the F-M 100 hue test and PA diameter (r=0.905), but the correlation between the error score and visual acuity (r=−0.32), perimetry (r=0.21), and eye fundus changes (r=0.36) and PA diameter was weak.

Conclusions. Our results showed that PA can cause the impairments of visual acuity, perimetry, and color contrast sensitivity. The computerized F-M 100 hue test can be one of the methods for an early diagnosis of chiasm damage in patients with PA.

Introduction

The pituitary gland is a small endocrine gland, weighing about 0.5 g. It is slightly larger in the brain of women than men. Pituitary adenoma (PA) is the most common pathological process occurring in the sella turcica. PAs account for 12%–15% of all brain tumors (1). Ezzat et al. (2) reported the estimated prevalence of pituitary adenomas to be 14.4% and 22.5% in pooled autopsy and radiological series, respectively. Davis et al. (3) reported that pituitary adenomas occurred with a prevalence rate of 190–280 cases per 1 000 000. It is a benign tumor originating in adenohypophysial cells of the anterior lobe of the pituitary gland (4). The classification of PAs is based on the secretion of hormones. It can be a secreting (functional) or a non-secreting (nonfunctioning) pituitary adenoma. According to the size, adenomas are classified into microadenomas (⩽10 mm) and macroadenomas (>10 mm) (5). Women have a 2-fold increased risk of developing PA in comparison with men (4). Most commonly, PA is a nonmalignant tumor; however, it tends to renew/recur itself (6). Usually this tumor is soft and has no capsule, which could isolate it from the surrounding mass of microglia. That is the reason why it can grow and infiltrate the surrounding structures. Adenomas may cause symptoms in 2 ways: 1) due to tumor-related hypersecretion or hyposecretion of hormones. In this case, the tumor causes compression to a normally functioning hypophysis; or 2) due to compression of PA to the surrounding structures (5).

PA can often cause injury to the optic chiasm. Hypophysis is in the sella turcica, 8–13 mm lower than the optic chiasm. Therefore, when it increases, it can easily compress the optic nerve fibers in the chiasm. Microadenomas can have a negligible effect on the visual system or on the function of other glands, whereas macroadenomas can cause visual function impairment (7–9). Visual function impairment depends on the diameter of PA and its contact with optic pathways. If PA is small, it cannot reach the optic chiasm, and visual function impairment may not be observed (10). When PA compresses the frontal part of the optic nerve, impairments in visual field, visual acuity, and color contrast sensitivity are possible. Visual impairments can also be triggered by a microadenoma when it grows directly to the optic pathways and causes swelling of the pituitary gland (5).

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The long-lasting compression of the chiasm induces primary optic nerve atrophy, which directly impairs the visual function. Functionally active PAs usually appear with specific clinical symptoms because of hormone hypersecretion. Functionally active PAs cause less damage to the visual function than the nonfunctioning gland, because functioning PAs become symptomatic due to hormone secretion. Nonfunctioning PAs can grow slowly, compress the optic chiasm, which is directly above the pituitary gland, and cause progressive visual loss (11). Among patients with intracranial tumors, the PA prevalence is high, and diagnosing the tumor, an overall visual system examination is required.

The aim of this study was to evaluate associations between visual functions (visual acuity, perimetry, optic nerve disc condition, and color contrast sensitivity) and PA diameter.

Material and Methods

A total of 20 patients diagnosed with PA (40 eyes, study group) and 40 healthy patients (80 eyes, control group) were enrolled into the study. PA was confirmed by computed tomography (CT) or magnetic resonance imaging (MRI) scans. The patients in the study group were divided into 2 groups according to the PA diameter: patients with the PA diameter of 1 cm and less (14 eyes) and patients with the PA diameter of more than 1 cm (26 eyes).

The inclusion criteria were as follows: 1) PA identified and confirmed by CT/MRI; 2) good patient's general condition; 3) patient's consent to take part in the study.

The exclusion criteria were as follows: 1) infectious eye diseases (history of keratitis, acute or chronic uveitis), glaucoma, optic nerve diseases, degeneration or dystrophy of the central part of the retina, high-degree refraction defects, lens opacities because of obscurity or poor photography quality of eye fundus; 2) systemic disease (diabetes, malignant diseases, systemic connective tissue disease, chronic infectious diseases, tissue or organ transplant surgery; 3) brain tumors of other localization; and 4) patient's refusal to participate in the study.

In this study, visual acuity as well as the transparency of the cornea and lens and the fundus were investigated in patients. Biomicroscopy was performed in order to assess the corneal and lenticular transparency. Noncorrected and best-corrected visual acuity (measured in decimals from 0.1 to 1.0) was evaluated using Landolt's rings (C optotypes) by Snellen test types at a 5-m distance from the chart.

The lenses were evaluated by biomicroscopy. The lenses were examined using a slit lamp, positioning the illumination source at a 45-degree angle and the light beam split to a 2-mm width.

To reach the best-corrected visual acuity, refraction was performed during each examination. The intraocular pressure was measured in order to exclude the patients with glaucoma. Moreover, the pupils of the subjects were dilated with 1% tropicamide. After dilation of the pupils, funduscopy was performed with an ophthalmoscope of the direct monocular type and the slit-lamp using a double aspheric lens of +78 diopters.

The Farnsworth-Munsell 100 hue test (F-M 100 hue test), which is a computer test of color sensitivity, was applied to all the patients (Fig. 1). The test was carried out under artificial daylight illumination; care was taken to use the same instructions during all the testing sessions.

The F-M 100 hue test required the arrangement of color samples by tone. The majority of samples were of the same brightness and intensity in color. Four boxes containing 85 plastic color samples were provided. Two color samples in each box were repeated and used as supportive colors, while other color samples were arranged so that a consistent transition of tones between the two supportive colors were achieved. The color samples were in such a manner as to cover the entire range of tones. The samples differed in tone, but their colors were approximately of the same brightness and intensity. Two minutes were given for each box series, though the speed of accomplishment of the test was not highly accentuated. A sequence number was assigned to each color sample. The result was evaluated by the total number of differences between the number of the color sample chosen by a subject and the number of the color sample actually belonging to the position. The degree of color distinction was assessed. The sensitivity of colors might be very high (when the number of mistakes is up to 20), normal (up to 100), or impaired (more than 100).

Stimuli were generated and presented on a color monitor for calibration. The chromaticity of the monitor phosphors was calibrated using a spectrometer (VIS-LIGA of STEAG microParts GmbH). The computerized test was compared with the original test using a Bland and Altman plot (12).

Statistical analysis was performed using the computer program SPSS/W 13.0 (Statistical Package for the Social Sciences for Windows, Inc., Chicago, Illinois, USA). The data were expressed as absolute numbers (percentage) or means and standard deviation (SD). The Mann-Whitney *U* and Kruskal-Wallis tests were used for the comparison of 2 or 3 groups, respectively. The correlation between the diameter of PA and Farnsworth-Munsell 100 hue test, visual acuity, perimetry, and fundus changes was evaluated by using the Spearman's correlation coefficient. Differences were considered statistically significant if $P<0.05$.

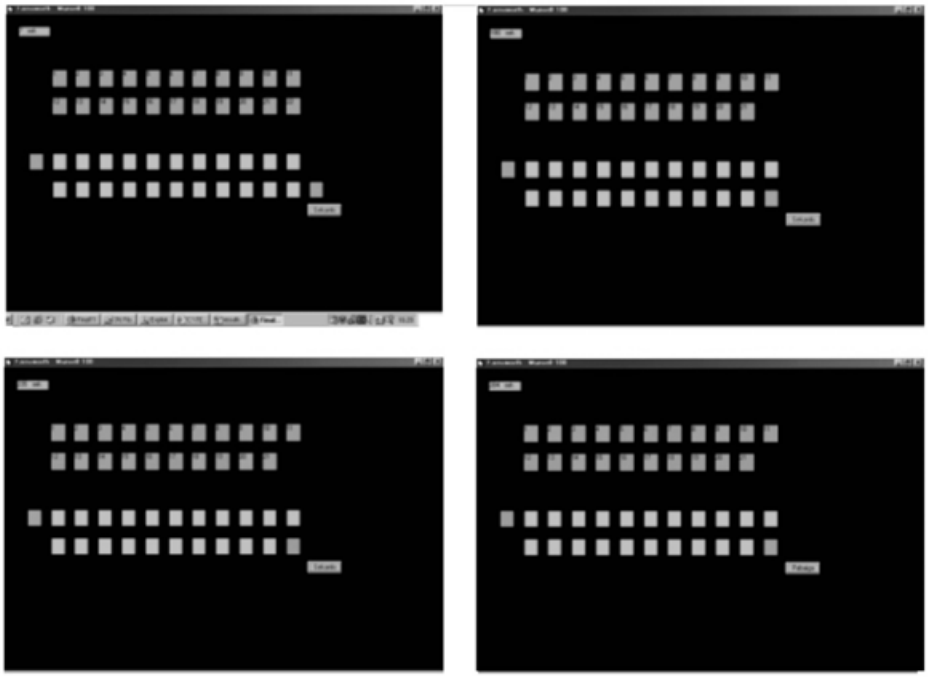

Fig. 1. Farnsworth-Munsell 100 hue test

Results

Twenty patients (40 eyes) diagnosed with pituitary adenoma by CT/MRI were examined. There were 16 women (80%) and 4 men (20%). The mean age of the patients was 51 years (SD, 13.07). Of the 40 patients with a mean age of 50 years (SD, 10.3) in the control group (80 eyes), 30 were women (75%) and 10 were men (25%). The control and study groups were matched for age and gender.

In the control group, visual acuity was 1.0. Visual acuity was worse in the group of patients with PA of >1 cm as compared with the patients with PA of ⩽1 cm (0.64 [SD, 0.21] vs. 0.9 [SD, 0.50], $P<0.05$). A weak negative correlation was found between visual acuity and PA diameter ($r=-0.32$, $P<0.05$).

The results of the Farnsworth-Munsell 100 hue test were also better in the control group compared with the patients with PA of ⩽1 cm and >1 cm (error score of 80.1 [SD, 53.0] vs. 131.8 [SD, 30.6] and 244.68 [SD, 51.6], respectively; $P=0.011$) (Fig. 2). There was a very strong positive correlation between the error score of the F-M 100 hue test and the PA diameter ($r=0.905$, $P=0.008$).

Patients who were diagnosed with PA had visual field impairment. Visual field impairment was determined in 71% of the patients in the group with PAs of ⩽1 cm. Concentric visual field was found in 8 (57%) of eyes, 2 eyes (14%) had bitemporal hemianopsia, and 4 eyes (29%) had the intact visual field (Fig. 3). In the group with PAs of >1 cm, visual field impairment was determined in 86% of the patients; concentric constriction and bitemporal hemianopia was documented in 16 (63%) and 6 patients (23%), respectively. Visual acuity was intact in 4 patients (14%). Visual field impairment was more common in the patients with PA >1 cm, but the difference

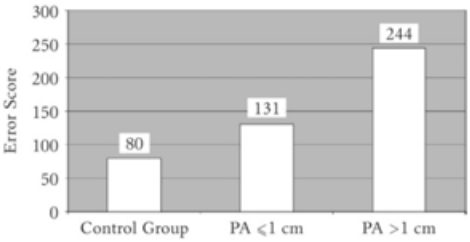

Fig. 2. Error scores of the Farnsworth-Munsell 100 hue test in 3 groups of the patients

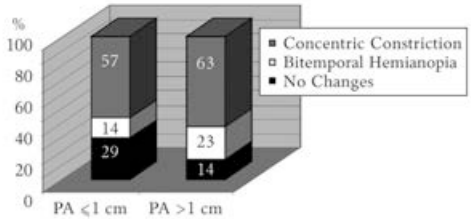

Fig. 3. Visual field impairments by pituitary adenoma diameter

was not significant. There was a weak positive correlation between visual field impairment and PA diameter (r=0.21, P<0.05).

The patients diagnosed with PA showed optic nerve disc changes in the fundus of the eye (Table). Optic nerve disc changes were significantly more common in the patients with PA than the healthy controls (20% vs. 50%, P=0.009), and there was a weak positive correlation between eye fundus changes and PA diameter (r=0.36, P<0.05).

Table. Optic nerve disc changes in patients with a pituitary adenoma (PA) diameter of ⩽1 cm and >1 cm

Change	Eyes, n (%)		*P* value
	PA ⩽1 cm	PA >1 cm	
Unchanged optic nerve disc	6 (15)	6 (15)	1.0
Optic nerve disc pallor	6 (15)	14 (35)	0.69
Optic nerve disc atrophy	2 (5)	6 (15)	0.26

Discussion

Visual functions in patients with PA were analyzed in this study. Our analysis revealed that PA can cause the impairments of visual acuity, perimetry, eye fundus, and color contrast sensitivity in patients with PA, and the computerized F-M 100 hue test can be one of the methods for an early diagnosis of chiasm damage in patients with PA.

Molecular genetic research has identified a number of genetic defects that might be involved in pituitary tumorigenesis. Few genes have been reported to be associated with inherited forms of pituitary tumors. Approximately 5% of all pituitary tumors arise in a familial setting (11). The etiology of pituitary adenomas has not been clearly understood until now. A relationship between adenomas and multivarious environmental factors, such as smoking, mobile phone use, etc., was studied. However, no scientifically confirmed environmental risk factors associated with the development of pituitary adenomas have been identified so far (13, 14).

PA affects people of all ages. It can occur at any age, but its prevalence increases with age (31). A study by Thomas et al. showed that the mean age of PA patients was 43 years (range, 16–69 years); the ratio of women to men was 1:2 (27). Other study by Elgamal et al. reported the mean patients' age of 42 years (range, 14–83) and a female-to-male ratio of 1.4:1 (16). In our study, the mean age of PA patients was slightly greater, i.e., 51 years (range, 25–70), and the ratio of women to men was 3.8:1.

The impairment of visual functions may be found in patients with PA. The present investigation showed that visual acuity impairment can be found even when the diameter of PA less than 1 cm (visual acuity, 0.9 [SD, 0.50]). The level of damage directly depended on the of diameter adenoma and the location of compression on optic pathways (15). Other researchers also determined visual acuity impairment in 54.8% of the patients (n=37) who were diagnosed with PA (16).

In literature, information about the impairment of color contrast sensitivity can only be found when visual acuity is altered, i.e. in advanced PA stages. Researchers have also determined a great impairment in color contrast sensitivity in patients diagnosed with PA (17). In Grochowski's opinion, the examination of contrast sensitivity is a susceptible method for the determination of visual pathway compression. However, it should not be used separately from other examination methods of the visual system (18). Gutowski et al. evaluated 11 patients who had tumors in the sella turcica and had no visual acuity and visual field impairment; however, color contrast sensitivity impairment was found (19).

Our study showed that the results of the F-M 100 hue test were better in the control group than in the groups with PA ⩽1 cm and >1 cm (80.1 [SD, 53.0] vs. 131.8 [SD, 30.6] and 244.7 [SD, 51.6], P=0.011; respectively). Dain et al. reported that the results of the F-M 100 hue test depended on the human race, but the difference was insignificant if the examination was performed in the same group of age and with the same pupil diameter. However, the mentioned authors noted that the difference was significant between Asians and brown-eyed Europeans (20). With reference to the study by Kinnear et al., the results of the F-M 100 hue test can vary depending on age. The authors reported the number of mistakes ranging from 43 to 364 (21). The researchers examined 10 patients (20 eyes) of the same age and ophthalmologically healthy patients similar to our group (50–59 years old); and the number of mistakes was 90, but in the group of 60–69-year-old persons, the number of mistakes increased to 120 (21). Based on the results of the F-M 100 hue test, Kessel et al. reported that the Danish healthy human population made 83 mistakes (SD, 79) (22). Our study of healthy human population showed very similar results, i.e., 80.1 (SD, 53).

The evaluation of visual field defects is very important in diagnosing PA, especially in primary stages of the development. Patients with PA have unique visual field defects – usually the visual field begins to constrict from the upper temporal sector (23). The prevalence of visual field defects in PA, with reference to various studies, varies from 37% to 96% (15, 24–26). Another very characteristic and unique symptom of PA is a constriction of the visual field from temporal sides to a complete bilateral temporal hemianopia. Thomas et al. reported that theoretically optic chiasm compression caused by PAs led to bitemporal defects. According to their results, bitemporal hemianopsia was the second most common presentation (27). Their study showed that visual field defects were determined in 94.6% of the patients. The unique visual field constricted from the temporal side for PA was determined in 69 of the 93 patients (74.2%). Bitemporal hemianopia was diagnosed in 19 patients (20.4%). In 24 patients (25.8%), 3 sectors of the visual field were lost. Nonspecific visual field defects for PA were determined in 19 patients (20.4%) (27). The results of our study are in line with the findings of these studies. Our study showed that in the group with a PA diameter of ⩽1 cm, field defects were determined in 71% of patients. Of these patients, 57% had the concentric constricted vision field and 14% bitemporal hemianopia. In the group with a PA diameter of >1 cm, visual field defects were determined in 85% of patients. Of these patients, 63% had the concentric constricted field of vision and 23% bitemporal hemianopia. In the group with a PA diameter of >1 cm, visual field defects were twice as common in comparison with the group with a PA diameter of <1 cm. However, bitemporal hemianopia was more common in other scientific studies: the visual field was damaged in all the patients, and 50% of the patients were diagnosed with bitemporal hemianopia (28). Other nonspecific defects in the visual field were found in 55 eyes of 29 patients (44%), and 19 of them had bitemporal hemianopia (69%) (13). A study by Elegemal et al. also reported that 37 patients had visual field defects (65.5%) (16).

In a retrospective study by Kerrison et al., 62 patients with a mean age of 54 years (SD, 15 years; range, 22 to 83) were examined (33 men and 29 women). Visual field defects were analyzed before and after surgical treatment of pituitary adenoma and abnormal static threshold perimetry (29). The authors reported that the early fast phase (1 week after surgery) of improvement might lead to normalization of visual fields in some individuals. The early slow phase (1 month to 4 months) was the period of the most notable improvement. The late phase (6 months to 3 years) of mild improvement did not appear significant overall but may be marked in some individuals. Each of these phases might have one or more mechanisms underlying the observed improvement (29).

With reference to Monteiro et al., 30 patients (60 eyes) with pituitary adenoma were examined. All patients underwent neuro-ophthalmic examination and MRI before and after optic chiasm decompression. The study showed the tumor size to be the best predictive factor for visual loss, and the factors associated with visual recovery were the degree of optic atrophy, the severity of VF defect, and the tumor size. The authors reported that diagnosing pituitary adenomas before optic atrophy becomes severe might be related to a better prognosis in such patients (30).

The earliest defect of visual function is the impairment of color contrast sensitivity. The progressive impairment of color contrast sensitivity could warn about the development of the disease and progression; therefore, the examination of color contrast sensitivity is an informative and useful test to investigate visual functions.

Conclusions

Our results showed that PA can cause the impairments of visual acuity, perimetry, and color contrast sensitivity. Computerized F-M 100 hue test can be one of the methods for an early diagnosis of chiasm damage in patients with PA.

Statement of Conflict of Interest

The authors state no conflict of interest.

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Received 11 March 2012, accepted 30 March 2013

KJO
pISSN: 1011-8942 eISSN: 2092-9382

Korean J Ophthalmol 2016;30(4):295-301
http://dx.doi.org/10.3341/kjo.2016.30.4.295

Original Article

The Application of a New Maximum Color Contrast Sensitivity Test to the Early Prediction of Chiasma Damage in Cases of Pituitary Adenoma: The Pilot Study

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Purpose: Our objective was to estimate the maximum color contrast sensitivity (MCCS) thresholds in individuals with chiasma opticum damage.

Methods: The pilot study tested 41 people with pituitary adenoma (PA) and 100 age- and gender-matched controls. Patients were divided into two groups according to PA size, PA ≤1 cm or PA >1 cm. A new MCCS test program was used for color discrimination.

Results: The mean total error score (TES) of MCCS was 1.8 in the PA ≤1 cm group (standard deviation [SD], 0.38), 3.5 in the PA >1 cm group (SD, 0.96), and 1.4 in the control group (SD, 0.31; $p < 0.001$). There was a positive correlation between tumor size and MCCS result ($r = 0.648$, $p < 0.01$). In the group that had PA-producing hormones, the TES was 2.5 (SD, 1.09), compared to 4.2 value in the non-functioning PA group of patients that did not have clinically significant hormone excess (SD, 3.16; $p < 0.01$). In patients with normal visual acuity (VA) or visual field MCCS, the TES was 3.3 (SD, 1.8), while that in patients with VA <0.00 was 4.6 (SD, 2.9).

Conclusions: Results of the MCCS test TES were 1.9 times better in patients with PA ≤1 cm compared to patients with PA >1 cm ($p < 0.01$). In PA patients with normal VA, the TES was 2.35 times worse than that of healthy persons ($p < 0.01$).

Key Words: Macroadenoma, Maximum color contrast sensitivity, Micoradenoma, Pituitary, Visual function impairment

Pituitary adenoma (PA) is a common benign monoclonal neoplasm that accounts for approximately 15% to 20% of primary intracranial tumors [1]. Compared with men, women have a two-fold increased risk of developing PA [2]. This condition develops as a non-malignant tumor that grows from the frontal lobe adenohypophysis cells of the hypophysis [2]. It is the most common pathological process in sella turcica, but no scientifically-based environmental risk factors have been identified [2-4].

Pituitary tumors can be subdivided into non-secretory

Received: May 19, 2015 *Accepted*: October 19, 2015

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(non-functioning) and secretory (functioning) types. Secretory PAs become symptomatic because they secrete hormones. Non-secretory pituitary tumors might grow slowly, compress the optic chiasm, which is directly above the pituitary gland, and cause progressive visual loss [2]. Adenomas are classified according to size into microadenomas (smaller than 1 cm) and macroadenomas (larger than 1 cm). Microadenomas have less of an effect on the visual system or on the function of other glands, whereas macroadenomas can cause visual impairment [5-7]. Visual function impairment depends on the PA diameter and its contact with optic pathways. If the PA is small, it cannot reach the optic nerve chiasm, and visual function impairment might not occur [8].

Early diagnosis of PA is essential because long-standing chiasmal compression indicates primary optic atrophy and a poor prognosis for visual recovery following surgical decompression [2]. Detailed visual examination is very important for early PA diagnosis. Various visual functions are tested, including the general health of the visual system, central pathways of vision, and visual system cognitive perception. Studies have shown that visual acuity (VA) assessment using the typical Snellen chart and Landolt rings (Coptotypes) alone is insufficient for visual function testing because it provides limited information about central vision; accurate diagnosis requires both VA determination and contrast sensitivity [9]. The purpose of the present study was to assess visual function (VA, visual field, and color contrast sensitivity) in order to achieve early prediction of chiasma damage in patients with PA. The maximum color contrast sensitivity (MCCS) test is especially useful for follow-up of PA patients after PA surgery. Impairment revealed by the MCCS test could indicate recurrence, which can be confirmed though a brain imaging study.

Materials and Methods

The study group comprised patients (41 patients, 82 eyes) who were referred to the Clinic of Neurosurgery, Hospital of Lithuanian University of Health Sciences, for a consultation with a neuroophthalmologist due to worsened visual function (VA and visual field). All these patients underwent color contrast sensitivity testing, and the diagnosis of PA was confirmed by nuclear magnetic resonance imaging (MRI) of the brain.

The control group consisted of patients who had no ophthalmologic pathology on examination and who agreed to undergo color contrast sensitivity testing. Of the 300 patients who underwent color contrast sensitivity testing, 100 (198 eyes) were included into the control group matched for age and gender to the study group. The patients in the study group were divided into two groups according to PA size. Based on PA diameter, the first group comprised patients with a PA diameter of 1 cm or less, i.e., microadenoma; the second group of patients had a PA diameter larger than 1 cm, i.e., macroadenoma.

The inclusion criteria were as follows: (1) PA determined and confirmed using MRI, (2) generally healthy, (3) consented to take part in the study, (4) age between 18 and 65 years. The exclusion criteria were as follows: (1) contagious eye diseases, high degree of refraction error (patients with high degree of myopia ≥6.0 diopters and a high degree of hyperopia ≥5.0 diopters), or lens opacities (nuclear, cortical, and posterior subcapsular cataract) except minor opacities, keratitis, acute or chronic uveitis in anamnesis, glaucoma, optic nerve disease, retina central part degenerations or dystrophy; (2) systemic disease (diabetes, oncological disease, systemic connective tissue disease, and chronic infectious disease, state after the tissue or organ transplant); (3) other brain tumors; (4) congenital color vision deficiency; and (5) patient refusal to participate in the study.

Healthy patients with no ophthalmological eye disorder based on detailed ophthalmological evaluation were included if they did not have any eye disorders (patients with any refractive error were not included in the study) or if they were taking epileptic or sedative drugs.

In this research, non-corrected and best-corrected VA were evaluated using the logarithm of the minimum angle of resolution chart at a standard 4-meter distance from the chart. Refraction was determined during each examination in order to obtain the best-corrected VA. Biomicroscopy was performed to assess corneal and lenticular transparency. The subjects' pupils were dilated with 1% tropicamide. After dilation, funduscopy was performed with a direct monocular ophthalmoscope and a slit-lamp using a double aspheric lens of +78 diopters.

The MCCS test was carried out under artificial daylight illumination; care was taken to use the same instructions in all testing sessions. The light was directed at an angle of

approximately 90° from the patient's side, the viewing angle was about 60° positioned at about 45° to the plate surface without a glare from the monitor.

In the MCCS computer test [10], the subject's task was to determine the correct direction of a bar in a circle, indicated by pressing a button. If the direction was unclear, a blank button was pressed. Each time the button was pressed, a blank screen appeared; 1 second later, another circle with a randomly chosen bar direction was presented. If the direction of the bar in the circle was chosen incorrectly, its color was automatically highlighted. After the direction of the bar was selected correctly, the intensity of its color was automatically dulled; due to the change in intensity of the bar, the brightness of the background of the circle appeared to change. The first correct answer after a series of incorrect answers or the first incorrect answer after a series of correct answers was accepted as the subject's maximum sensitivity to the target color of a bar. When this maximum sensitivity was determined, the color of the bar was changed, and the test was started again. The bar was presented in a total of six colors: red, green, blue, greenish blue, violet, and yellow. Once a subject's sensitivity to all these colors had been assessed, all findings were recorded in a database, and the results of the test were presented in a result window. The grey background luminance of the monitor was 350 cd/m^2 [10]. The luminance of the surrounding area was 400 cd/m^2 [10].

Statistical analyses were performed to assess the VA of each eye; the visual field and MCCS test results were estimated separately for each eye. Statistical analysis was performed using the SPSS ver. 13.0 (SPSS Inc., Chicago, IL, USA). The data are expressed as absolute number, percentage, or mean and standard deviation (SD). The Mann-Whitney *U*-test and Kruskal-Wallis test were used to compare the groups. The correlations between PA diameter and MCCS test results, VA, perimetry, and fundus changes were evaluated using Spearman's correlation coefficient. Differences were considered statistically significant at $p < 0.05$.

Results

A total of 141 participants were enrolled in the study. There were 34 men (34%) and 66 women (66%) in the control group and 8 men (19.5%) and 33 women (80.5%) in the study group. The mean age of the patients with a diagnosis of PA was 51.7 years (SD, 10.4), while that of the control group and 51.4 years (SD, 10.8).

The VA was 0.00 in the control group and -0.04 in the group with PA ≤1 cm (distributed from 0.3 to 0.00). In the group with PA >1 cm, the VA was -0.20 (distributed from 2 to 0.00). The VA was significantly worse in patients with PA diameter >1 cm compared to that of those with PA diameter ≤1 cm ($p = 0.0281$). VA deficit was found in 50 of the patients (61%) with PA: 34 patients (66%) with PA >1 cm and 16 patients (57%) with PA <1 cm. A weak negative correlation was found (r = 0.341, $p = 0.002$) between tumor size and VA. VA decreased in 10% of persons with PA diameter ≤1 cm and in 40% of patients with PA >1 cm. There were no blind patients in the PA patients groups.

The analysis indicated that 77% of patients with PA ≤1 cm had an affected visual field (concentric narrowing was detected in 62% of patients, bitemporal hemianopia was found in 15% of patients). The visual field was affected in 86% of patients with PA >1 cm (concentric narrowing was detected in 57% of patients, bitemporal hemianopsia was found in 29% of patients) (Table 1). There was no correlation (r = 0.053, $p = 0.634$) between tumor size and visual field damage.

This was the first study to analyze changes to the optic nerve disc (OND) in patients with PA ≤1 cm and >1 cm and in those with hormone-producing PA. The OND was more frequently affected in patients with PA with a diameter greater than 1 cm and in patients with non-functioning PA. The analysis indicated that 16.66% of patients with OND atrophy had a PA ≤1 cm, while 22.22% of patients with PA >1 cm experienced OND atrophy. However, there was no statistically significant difference between these groups (Table 2).

Our research showed that MCCS was affected in 73.17% of individuals with PA. Color contrast sensitivity was

Table 1. Visual field damage in patients with PA

Parameter	Concentric narrowing	Bitemporal hemianopsia	Normal visual field
PA ≤1 cm	15 (62)	4 (15)	5 (23)
PA >1 cm	31 (57)	16 (29)	7 (14)
p-value	0.8053	0.2726	0.4976

Values are presented as number (%).
PA = pituitary adenoma.

Table 2. OND changes in patients with PA ≤1 cm and >1 cm

Change	PA ≤1 cm	PA >1 cm	*p*-value
Normal OND	10 (41.67)	12 (22.22)	0.1034
OND pallor	10 (41.67)	30 (55.56)	0.8061
OND atrophy	4 (16.66)	12 (22.22)	0.7634

Values are presented as number (%).
OND = optic nerve disc; PA = pituitary adenoma.

affected in 13.3% of patients with PA ≤1 cm and was affected in 81.1% of patients with PA >1 cm. The total error score (TES) of MCCS was 1.8 (SD, 0.38) in the group with PA ≤1 cm, 3.5 in the group with PA >1 cm (SD, 0.96), and 1.4 in the control group (SD, 0.31). The difference between the TES values of MCCS in individuals with PA and the controls was significant ($p < 0.001$). There was a positive correlation between tumor size and MCCS results ($r = 0.648$, $p < 0.001$).

When VA and visual field were normal, the TES MCCS test results were 3.3 (SD, 1.8), compared to 4.6 in those with a VA less than 0.00 (SD, 2.9). Even for PA patients with normal VA, the TES was 2.35 times worse than that of healthy individuals ($p < 0.01$).

The average diameter of the PA was 2.4 cm in patients with a VA of 0.00 (SD, 1.01), 3.6 cm in patients with a VA in the range of 0.00 to 0.04 (SD, 3.2) ($p < 0.01$), and 4.83 cm in those with a VA <0.04 (SD, 2.1). The average diameter of the PA was 3.1 cm (SD, 0.95) when the VA was less than 0.00. When the VA was normal, the diameter of the PA was half the size of that of patients with VA <0.04. We grouped all patients in order to calculate the specificity and sensitivity of the MCCS test. According to our data, the sensitivity of this test was 71.95% and the specificity was 75%.

Discussion

PAs are classified based on size into microadenoma (≤1 cm) and macroadenoma (>1 cm). The size of the adenoma corresponds with the compromising effects on the optic chiasm, cranial nerves, and cavernous sinuses, but tumor size does not reflect clinical importance [11]. In addition, macroadenomas can cause local symptoms such as visual disturbances when the optic chiasm is compressed [12]. This classification is supplemented by immunochemistry and functional status. Pituitary tumors are classified as functioning or non-functioning on the basis of ability to produce and secrete mature hormones [13,14]. Patients might experience headaches, visual disorders, and cranial nerve dysfunction from compressive effects, while changes in hormone expression are either due to pituitary stalk disruption or pituitary failure due to compression of normal pituitary tissue [15]. Since MRI techniques have improved and are used more widely in the general population, PAs are more frequently incidentally diagnosed; however, pituitary tumors such as non-functioning PA might not be identified for many years.

In cases of PA, one of the most important aspects of diagnosis is to thoroughly investigate visual function. The complexity of the examination includes VA, perimetry, and fundus examination of both eyes. Although these examination methods are very useful, they do not completely reflect the condition of the visual system. We used the new MCCS test to more accurately examine visual functions in our research. This test is extremely sensitive to the earliest stages of visual function alterations. The qualitative estimation of color contrast sensitivity is very important for diagnosing PA and can provide valuable information for diagnosing the disease and determining progression [16]. These alterations depend on changes in the entire visual tract from the cones in the retina to the cerebral extrastriatal regions [17].

In our research, VA decreases in accordance with adenoma diameter. VA was particularly affected in patients with PA >1 cm. VA deficit was found in 34 patients (66%) with PA >1 cm. This result is in agreement with that of Elgamal et al. [18], who reported VA impairment in 54.8% of patients diagnosed with PA, although the authors did not analyze the dependence of VA on PA diameter or hormone activity. In their study, blindness was determined in three eyes, VA was diagnosed when a patient could only see fingers in front of their eyes, and the results were based on a total of six eyes [18]. There were no blind patients in our study, and the most progressed VA was 0.01. In the study by Elgamal et al. [18], a total of 68 of the eyes with PA were affected either by decreased VA and/or change in the visual field. The main visual presentation in 32 patients was impaired vision (VA, 20 / 50 or greater; 38.7%), and it was bilateral in 16 patients. In the remaining 76 eyes (61.3%), there were no visual symptoms related to the presence of PA. Visual field abnormality was detected in

55 eyes of 29 patients (44.4%), and bitemporal hemianopia represented strikingly abnormal VF in 19 patients (69%) [18]. In a case series by Dhar and Pehere [19], 66 of 114 eyes (57 patients) had normal VA. Additionally, only 16 patients (28.1%) had normal visual fields in both eyes [19]. Therefore, the visual field changes were very similar across all of the studies. A review by Hollenhorst and Young [20] of 1,000 cases of pituitary tumors over a 22-year period indicated that 70% of these patients had either VA loss, visual defects, or both, while only 20% of patients had reduced VA and 32% had visual field defects. The results of this analysis differ only from the case series studied by Anderson et al. [21], where the authors found that only 16% of patients had decreased VA and 32% had visual field defects. Out of 36 eyes studied, 24 (66.7%) had visual field defects at presentation, including 12 eyes (33.3%) with temporal defects, 10 eyes (27.8%) with nonspecific defects, and two eyes with peripheral constriction [21]. The most common pattern of visual field loss was bitemporal defects, present in six patients (33.3%) [21]. In another study, eight eyes of four patients (median age, 41.50 years; interquartile range, 33 to 51 years) with PA that caused visual field defects were reviewed [22]. The transverse dimensions of the tumor on MRI ranged from 2.5 to 4.5 cm in all subjects. All the subjects presented with progressive VA loss, headache, and visual field defects. The VA deficits ranged from 20 / 60 to 20 / 30 [22]. In our research, when VA was <0.00, the average PA diameter was 3.1 ± 0.95 cm [22].

Optic nerve changes are common in patients with PA. Longstanding compression by pituitary macroadenoma leads to optic atrophy [23]. Dhasmana et al. [23] reported five patients (27.8%) in their series with optic nerve head changes; three patients had unilateral optic atrophy, and two patients had bilateral disc pallor. In our research, there were 12 individuals (22.22%) with OND atrophy among patients with PA >1 cm, and four (16.66%) among individuals with PA ≤1 cm. Elgamal et al. [18] determined that a funduscopic sign of longstanding chiasmal compression from pituitary macroadenoma is primary optic atrophy (secondary to retrograde axonal degeneration). In this group of patients, optic atrophy was seen clearly in 21 eyes (17%), and all of them were found to have significantly affected vision (VA, 20 / 100 or worse). This also reflects the degree of visual recovery following transsphenoidal decompression [18].

In the literature, information about color contrast sensitivity impairment can only be found in conjunction with VA impairment, i.e., in the later stages of PA. Researchers have determined great color contrast sensitivity impairment in patients diagnosed with PA [24]. In Grochowicki et al. [25]'s opinion, the contrast sensitivity examination is a suitable method to determine visual pathway compression. However, it should not be used separately from other examination methods of the visual system.

The decreasing MCCS test results in our study indicated some of the earliest signs of PA in patients with intact VA. It is interesting to note that, even when VA was normal in patients with PA, the MCCS test results were worse compared to those of healthy persons. Furthermore, a decrease in color contrast sensitivity in patients with PA was found in a study using a Farnsworth-Munsell 100 hue test. In the present study, the results were determined according to PA diameter. The PA diameter was 131.79 in patients with PA ≤1 cm (SD, 30.62), while that of patients with PA >1 cm was 244.68 (SD, 51.56; $p = 0.011$) [26]. Jayaraman et al. [22] also found color vision impairment in one or both eyes in patients with PA. Gupta et al. [27] interpreted the alterations of color contrast sensitivity in patients with hypophysis adenoma as a decrease in the myelination of the vision fibers due to altered nutrition. This can be a primary cause of color sensitivity changes, especially when the tumor is localized in the region of the optic chiasma. In our research, the MCCS was affected in 73.17% of individuals with PA, and the results of the MCCS test were 1.9 times better in patients with PA ≤1 cm compared to patients with PA >1 cm ($p < 0.01$); even when the VA was normal in PA patients, the group error score was 2.35 times worse compared to that of healthy persons ($p < 0.01$).

In conclusion, results of a new MCCS test TES were 1.9 times better in patients with PA ≤1 cm compared to patients with PA >1 cm ($p < 0.01$). Even when VA was normal in the PA patients group, their TES was 2.35 times worse than that of healthy persons. To our knowledge, this is the first study to analyze color vision abnormalities in patients with PA based on diameter. Therefore, this is the first analysis of visual function dependence on PA diameter. However, there were limitations to this study. Although the TES for the hue test was determined, we did not determine the partial error score for the red-green axis

or blue-yellow axis. Future studies can use these parameters to assess the impact of PA on color vision abnormalities.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgements

This work was supported by Lithuanian Science Council (grant no. MIP-008/2014).

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Hindawi Publishing Corporation
Scientifica
Volume 2016, Article ID 2839697, 9 pages
http://dx.doi.org/10.1155/2016/2839697

Hindawi

Research Article

Role of MMP-2 (-1306 C/T) Polymorphism in Pituitary Adenoma

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Received 7 December 2015; Revised 10 February 2016; Accepted 11 February 2016

Academic Editor: Cornelis F. Sier

Purpose. To determine if the frequency of the genotype of *MMP-2 (-1306 C/T) Rs243865* has an influence on the development of pituitary adenoma (PA). *Methods*. The study enrolled $n = 84$ patients with PA and a random sample of the population $n = 318$ (reference group). The genotyping test of *MMP-2 (-1306 C/T)* was carried out using the real-time polymerase chain reaction method. *Results*. Analysis of *MMP-2 (-1306 C/T)* gene polymorphism has not revealed any differences in the genotype (*C/C*, *C/T*, and *T/T*) distribution between the PA patients and the reference group (as follows: 50%, 44%, and 6% versus 59.75%, 33.96%, and 6.29%). *MMP-2 (-1306) C/C* genotype was rarely observed in noninvasive PA compared to healthy controls: 35.1% versus 59.75%; $p = 0.0049$, as well C/C genotype being more frequently detected in nonrecurrence PA compared to healthy controls: 46.5% versus 59.75%; $p = 0.0468$. *MMP-2 (-1306) C/T* genotype was more frequently present in PA females compared to healthy controls females: 49.1% versus 33.66%; $p = 0.041$. *Conclusion*. Patients with noninvasive and nonrecurrence pituitary adenoma were the carriers of the C/C genotype significantly more frequently than their control counterparts and the C/T genotype in females was more frequent.

1. Introduction

Pituitary adenoma (PA) is a common benign monoclonal neoplasm accounting for approximately 15% to 20% of primary intracranial tumours [1]. Ezzat et al. [2] reported the estimated prevalence rates of pituitary adenomas to be 14.4% to 22.5% in pooled autopsy and radiological series, respectively. The pituitary gland is localized in a dural bag attached to the inferior aspect of the diaphragm of the sella and surrounded by venous spaces that correspond laterally to the cavernous sinuses [3]. PA may grow large and extend into the surrounding structures resulting in neurological complications including visual impairment. 6% to 10% of pituitary adenomas involve the cavernous sinus [4–9]. PA is a disease of multifactorial etiology, the occurrence of which is influenced by alterations in hormonal regulation and hormone receptors, dysregulated growth factors and alterations in their receptors, abnormalities in signaling proteins that transduce the signals of these stimuli, and changes in cell-cycle regulators. In addition, the neoplastic process is associated with altered cell-stromal interactions that have a role in the morphogenesis of pituitary tumours [10]. Recently, great attention in the PA pathogenesis has been drawn to the search of new epigenetic and genetic factors. To invade, tumour cells must undergo several changes in molecular pathways in accordance with invasion-associated cellular activities, namely, cell-cell adhesion, cell-matrix adhesion and ectopic survival, migration, and proteolysis [11]. Matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidases, also called matrixins, play an important role in the process of degradation of the extracellular matrix (ECM) and basement membrane (BM) in relation to tumour invasiveness, metastasis, and angiogenesis [12–18]. Many factors might induce MMPs production: cytokines, growth factors, physical stress, cell-extracellular matrix, and cell-cell interaction [19].

MMP-2 is a member of the MMP family and is capable of hydrolyzing type IV collagen, which is the main component of the BM [13, 15]. Several studies have shown that MMP-2 plays an important role not only in tumour

invasion and metastasis, but also in cancer development [20, 21].

Numerous studies have shown that MMP-2 is overexpressed in various human tumours, including breast cancer [22–27], lung cancer [28], colorectal tumours [29], pancreatic carcinoma [30], and gastric and esophageal cancers [31–35]. Some studies have also showed the expression of MMP-2 in human gliomas [36–38]. There are not many studies analyzing MMP-2 expression in PA [5, 39, 40] and to the best of our knowledge there are no studies that have examined the *MMP2* -1306 C/T polymorphism in patients with PA.

MMP polymorphisms can be caused by nucleotide changes within the promoter region by insertions, substitutions, or microsatellite instability [41]. Price et al. [42] reported a single nucleotide polymorphism in the promoter of the *MMP2* gene (-1306 C/T). -1306 C→T transition is located in a core recognition sequence of Sp1 (CCACC box), which abolishes the Sp1-binding site and consequently diminishes promoter activity. Another C to T transition located at nucleotide -735 in the promoter region of *MMP-2* has been identified [43].

Numerous studies have been carried out to look for the possible association between the MMP2 -1306 C>T polymorphism and risk of human cancers (colorectal, breast, gastric, esophageal, prostate, lung, and oral cancer) (reviewed in [44, 45]).

To our knowledge, no studies have investigated the association between the *MMP-2 (-1306 C/T)* gene polymorphism and PA development. Therefore, the aim of this study was to determine the association between the *MMP-2 (-1306 C/T)* gene polymorphism and the development of PA.

2. Materials and Methods

Permission (Number P2-9/2003) to undertake the study was obtained from the Kaunas Regional Biomedical Research Ethics Committee. The study was conducted in the Departments of Ophthalmology and Neurosurgery, Lithuanian Health Sciences University Hospital.

Study participants comprised 84 subjects with a diagnosis of pituitary adenoma and 318 persons from the reference group.

Reference Group Formation. The reference group involved 318 subjects according to their age and gender, considering the pituitary adenoma group structure. It was constructed from the following:

(1) A random sample of the Kaunas population aged 45–74 years collected within the international HAPPIE (Health, Alcohol and Psychosocial Factors in Eastern Europe) project (1) by the Laboratory of Population Research at the Institute of Cardiology of the Lithuanian University of Health Sciences (LUHS).

(2) A random sample of the Lithuanian population aged 25–65 years collected within the international CINDI (Countrywide Integrated Non-Communicable Disease Intervention) project (2) by the Laboratory of Preventive Medicine at the Institute for Biomedical Research of the LUHS.

(3) A random sample of the Kaunas population older than 65 years collected within the "Kaunas Healthy Ageing Study" by the Geriatric Clinic and Laboratory of Molecular Cardiology, Institute of Cardiology of the LUHS (3).

Table 1: Demographic characteristics of patients with pituitary adenoma (PA) and reference group subjects.

Group	N	Age, year (min./max. median)	Males, n (%)
PA	84	19/87/52.5	29 (34.5)
Reference	318	25/87/51	113 (35.5)
p value	—	0.88	0.86

The reference group was created by taking into consideration the distribution of age and gender in the pituitary adenoma group. Therefore, the medians of the patient age of the reference group and the pituitary group did not differ statistically significantly ($p < 0.05$).

Demographic data of the study subjects are presented in Table 1.

The inclusion criteria were as follows: (1) determined and confirmed PA via MRI; (2) patient's general good condition; (3) patient's consent to take part in the study; (4) age ≥ 18 years, (5) no other brain or other localization tumours.

2.1. Radiological Evaluation. All pituitary adenomas were analyzed based on MR imaging findings. The suprasellar extension and sphenoid sinus invasion by PAs were classified according to Hardy classification, modified by Wilson [46]. The degree of suprasellar and parasellar extension was graded as stages A–E. The degree of sellar floor erosion was graded as grades I–IV. Grades I-II mean that sellar floor is intact and was considered as noninvasive PA, grade III shows localized sellar perforation, and grade IV shows diffuse destruction of sellar floor which is the sign of invasive PA. Knosp classification system was used to quantify the invasion of the cavernous sinus. Grade 0: no involvement of cavernous sinus represents the normal condition; grades 1 and 2: the tumour pushes into the medial wall of the cavernous sinus but does not go beyond a hypothetical line extending between the centres of the two segments of the internal carotid artery (grade 1) or it goes beyond such a line, but without passing a line tangent to the lateral margins of the artery itself (grade 2); grade 3: the tumour extends laterally to the internal carotid artery within the cavernous sinus; grade 4: total encasement of the intracavernous carotid artery [47]. According to Knosp classification, only grades 3 and 4 pituitary tumours were considered to be invasive.

2.2. DNA Extraction and Genotyping. The DNA extraction and analysis of the gene polymorphism of MMPs were carried out at the Laboratory of Molecular Cardiology at the Institute of Cardiology of the LUHS for control group and at the Laboratory of Ophthalmology at the Institute of Neuroscience of the LUHS for the PA patient group. The DNA was extracted from the venous blood of patients

Table 2: Frequency of *MMP-2 (-1306 C/T)* genotype in the patients with pituitary adenoma (PA) and in the control group.

Gene marker	Genotype/allele	Frequency (%) Control group n (%) (n = 318)	p HWE	PA group n (%) (n = 84)	p HWE	p value
MMP-2 (-1306) Rs243865	Genotype					
	C/C	190 (59.75)		42 (50.00)		$\chi^2 = 2.980$
	C/T	108 (33.96)		37 (44.00)		$p = 0.225$
	T/T	20 (6.29)	0.383	5 (6.00)	0.390	
	Total	318 (100)		84 (100)		
	Allele					
	C	0.767		0.720		
	T	0.233		0.280		

MMP: matrix metalloproteinase; p value: significance level (alfa = 0.05); p-value HWE: significance level (alfa = 0.05) by Hardy-Weinberg equilibrium.

using the Genomic DNA Purification Kit (Thermo Fisher Scientific) according to the recommendations of the manufacturer or the silica gel column method utilizing the genomic DNA extraction kit SorpoClean™ Genomic DNA Extraction Module (SORPO Diagnostics) according to the recommendations of the manufacturer.

The genotyping test of MMP-2 (-1306 C/T) was carried out using the real-time polymerase chain reaction (PCR) method. Applied Biosystem (USA) kits were used for the genotyping of MMP-2 (-1306 C/T) (rs243865). To ensure internal control, 20 samples were sequenced at the Sequencing Center of the Institute of Biotechnology, and the received results confirmed the reiteration and precision of the data. The genotyping was performed using the HT 7900 real-time PCR quantification system (Applied Biosystems, USA). The real-time PCR reagents (2x Maxima™ Probe/ROX qPCR Master mix buffer, fluorescent dye labeled markers, sterile ddH2O) were taken out from an environment of −20°C and were thawed at room temperature. The thawed reagents were centrifuged (10,000 rpm) and stored in an ice tub. An appropriate real-time PCR mixture of MMP-2 (-1306 C/T) was prepared for determining single nucleotide polymorphism (SNP).

9 μL of the PCR reaction mixture was poured into each well of the microtiter plate with 96 wells and then 1 μL of matrix DNA of the samples (~10 ng) and 1 μL of negative control (−K) were added. An optic film was pasted on the microtiter with 96 wells and the microtiter was centrifuged for 15 seconds at 10,000 rpm.

During the genotyping the following real-time PCR programs were used: Allelic Discrimination and Absolute quantification. Then, the work was continued following the manual provided by the manufacturer (http://www.appliedbiosystems.com/ Allelic Discrimination Getting Started Guide). After that, the Allelic Discrimination program was completed, the genotyping results were received. The program determined the individual genotypes according to the fluorescence intensity rate of different detectors. A molecular marker labeled with VIC fluorescent dye or Yakima Yellow was chosen for the x axis and a molecular marker labeled with FAM fluorescent dye was selected for the y-axis.

2.3. Statistical Analysis. Statistical analysis was performed using the SPSS/W 20.0 software (Statistical Package for the Social Sciences for Windows, Inc., Chicago, IL, USA). The data are presented as minimum, maximum, and median. The frequencies of genotypes (in percentage) are presented in Table 2. Hardy-Weinberg analysis was performed to compare the observed and expected MMP genotype frequencies using the χ^2 test for all groups. The distribution of the MMPs (SNP) in the PA and control groups was compared using the χ^2 test or Fisher exact test. Binomial logistic regression analysis was performed to estimate the impact of genotypes on PA development. Odds ratios (OR) and 95% confidence intervals are presented. The selection of the best genetic model was based on the Akaike Information Criterion; therefore, the best genetic models were those with the lowest Akaike Information Criterion values. Differences were considered statistically significant when $p < 0.05$.

3. Results

The genotyping of *MMP-2 (-1306) C/T* was performed in patients with PA and in the control group subjects (Table 2). The distribution of the analyzed MMP genotypes and allele frequencies in patients with PA and in the control group matched the Hardy-Weinberg equilibrium. *MMP-2 (-1306) C/T* gene polymorphism analysis in the overall group has not revealed any differences in the genotypes distribution between patients with PA and control group patients (Table 2).

MMP-2 (-1306) C/T gene polymorphism analysis in males and females with PA has not revealed any statistical significant differences in the genotype (*C/C*, *C/T* and *T/T*) distribution (as follows: 45.5%, 49.1%, and 5.5% versus 58.6%, 34.5%, and 6.9%) (Table 3). When comparing *MMP-2* genotype distribution in healthy females and females with PA we have revealed significant differences. *MMP-2 (-1306) C/T* genotype was more frequently present in PA females compared to healthy controls females: 49.1% versus 33.66%; $p = 0.041$. *MMP-2 (-1306)* C/C and C/T genotypes have not revealed any statistically significant differences when healthy females and females with PA were compared: *MMP-2 (-1306)* C/C genotype 59.02% versus

Table 3: Frequency of *MMP-2 (-1306 C/T)* genotype in the patients with pituitary adenoma (PA) and in the control group by gender.

Gene marker	Genotype/allele	Frequency (%) Control group n (%) Females $N = 205$	Males $N = 113$	P HWE	p value	PA group n (%) Females $N = 55$	Males $N = 29$	P HWE	p value
MMP-2 (-1306) Rs243865	Genotype								
	C/C	121 (59.02)	69 (61.06)		0.811	25 (45.5)	17 (58.6)		0.359
	C/T	**69 (33.66)***	39 (34.51)		0.803	**27 (49.1)***	10 (34.5)		0.251
	T/T	15 (7.32)	5 (4.42)	0.488	0.347	3 (5.5)	2 (6.9)	0.394	1.0
	Total	205 (100)	113 (100)			55 (100)	29 (100)		
	Allele								
	C	311 (75.85)	177 (78.32)			77 (70)	44 (75.86)		
	T	99 (24.15)	49 (21.68)			33 (30)	14 (24.14)		

*$p = 0.0412$.
MMP: matrix metalloproteinase; p value: significance level (alfa = 0.05); p-value HWE: significance level (alfa = 0.05) by Hardy-Weinberg equilibrium.

Table 4: Binomial logistic regression analysis in the patients with pituitary adenoma (PA) and in the control group.

Model	Genotype	OR (CI 95%)	p value	AIC
Codominant	T/T	1		415.177
	T/C	0.645 (0.391–1.065)	0.087	
	C/C	0.884 (0.314–2.490)	0.816	
Dominant	CC	1		413.540
	T/C + T/T	0.647 (0.416–1.092)	0.109	
Recessive	C/C + C/T	1		416.093
	T/T	1.060 (0.386–2.914)	0.909	
Overdominant	T/T + C/C	1		413.231
	C/T	0.653 (0.400–1.066)	0.088	
Additive	T allele	0.784 (0.535–1.148)	0.211	414.568

45.5%; $p = 0.09$; and T/T genotype 15% versus 3%, $p = 0.772$.

Binomial logistic regression analysis in the patients with PA and in the control group was performed (Table 4). This analysis revealed that there were no statistically significant variables in the models of the patients with PA and in the control group.

Binomial logistic regression analysis in the patients with PA and in the control group by gender was performed (Table 4). There were no statistically significant variables in the models of the pituitary adenoma and control groups. Binomial logistic regression analysis in the patients with PA and in the control group by gender was performed as well (Table 5). There were no statistically significant variables in the models of males. In females this analysis revealed that the codominant (p value = 0.043) and overdominant (p value = 0.037) variables were statistically significant.

MMP-2 (-1306) C/C genotype was rarely observed in noninvasive PA compared to healthy controls: 35.1% versus 59.75%; $p = 0.0049$, as well C/C genotype being more frequently detected in nonrecurrence PA compared to healthy controls: 46.5% versus 59.75%; $p = 0.0468$ (Tables 6 and 7). These results could be explained by increased expression of *C/C* genotype.

Binomial logistic regression analysis in noninvasive PA and in the control group was performed (Table 8). In noninvasive PA group this analysis revealed that the codominant (p value = 0.003), dominant (p value = 0.005), overdominant (p value = 0.003), and additive (p value = 0.028) variables were statistically significant. Binomial logistic regression analysis in the patients with nonrecurrence PA and in the control group was performed as well (Table 8). In nonrecurrence PA group this analysis revealed that the codominant (p value = 0.039), dominant (p value = 0.042), and overdominant (p value = 0.049) variables were statistically significant.

4. Discussion

Pituitary tumours are benign but do not uncommonly invade locally into adjacent tissues such as the cavernous sinus and dura. Early prediction of which pituitary tumours will recur and/or exhibit an invasive phenotype remains difficult despite the introduction of several tissue-based molecular markers [48].

The importance of *MMP-2 (-1306)* gene polymorphism in the susceptibility of various tumours has been shown in numerous studies [47–50]. In addition, MMP-2 has been shown to be overexpressed in PA [5, 39, 40]. On the

Table 5: Binomial logistic regression analysis in pituitary adenoma (PA) and the control women by gender.

Model	Genotype	OR (CI 95%)	p value	AIC
Males				
Codominant	CC	1		149.476
	CT	0.961 (0.401–2.303)	0.929	
	TT	0.616 (0.110–3.452)	0.582	
Dominant	CC	1		147.705
	T/C + T/T	0.903 (0.394–2.072)	0.810	
Recessive	C/C + C/T	1		147.484
	T/T	0.625 (0.115–3.298)	0.586	
Overdominant	T/T + C/C	1		147.762
	T/C	1.001 (0.424–2.362)	0.998	
Additive	—	0.870 (0.441–1.717)	0.688	147.603
Females				
Codominant	CC	1		271.981
	CT	0.528 (0.284–0.981)	0.043	
	TT	1.033 (0.278–3.837)	0.961	
Dominant	CC	1		269.092
	T/C + T/T	0.579 (0.318–1.053)	0.073	
Recessive	C/C + C/T	1		272.068
	T/T	1.368 (0.382–4.907)	0.630	
Overdominant	T/T + C/C	1		267.927
	T/C	0.526 (0.288–0.961)	0.037	
Additive	—	0.748 (0.471–1.187)	0.218	270.822

Table 6: Frequency of *MMP-2 (-1306 C/T)* genotype in the patients with pituitary adenoma (PA) and in the control group by PA invasiveness.

Gene marker	Genotype/allele	Frequency (%)					
		Control group n (%) (n = 318)	p HWE	Noninvasive PA group n (%) (n = 37)	p HWE	Invasive PA group n (%) (n = 47)	p HWE
MMP-2 (-1306) Rs243865	Genotype						
	C/C	**190*** (59.75)	0.383	**13*** (35.1)	0.064	29 (61.7)	0.5823
	C/T	**108**** (33.96)		**22**** (59.5)		15 (31.9)	
	T/T	20 (6.29)		2 (5.4)		3 (6.4)	
	Total	318 (100)		37 (100)		47 (100)	
	Allele						
	C	488 (76.72)		48 (64.86)		73 (77.66)	
	T	148 (23.33)		26 (35.14)		21 (22.34)	

* $p = 0.0049$.
** $p = 0.0035$.
MMP: matrix metalloproteinase; p value: significance level (alfa = 0.05); p-value HWE: significance level (alfa = 0.05) by Hardy-Weinberg equilibrium.

basis of these findings, we sought to examine whether the polymorphism in the *MMP2 (-1306)* promoter could have an impact on the risk of PA development. We analyzed 84 PA patients and 318 age- and sex-matched controls for the -1306 C/T polymorphism in the *MMP-2* promoter. Our results demonstrated that *MMP-2 (-1306 C/T)* gene polymorphism has not revealed any differences in the genotype (*C/C*, *C/T*, and *T/T*) distribution between the PA patients and the reference group (as follows: 50%, 44%, and 6% versus 59.75%, 33.96%, and 6.29%), but *MMP-2 (-1306) C/T* genotype was more frequently present in PA females compared to healthy controls females: 49.1% versus 33.66%; $p = 0.041$.

To our knowledge, there are no studies which have explored the relationship between the polymorphisms in *MMP2* -1306 C/T and the development of PA. However, several studies have analyzed MMP-2 expression in PA [5, 39, 40]. Liu et al. [39] have found that the MMP-2 score of PAs with cavernous sinus invasion (3.9 ± 0.5) was significantly higher than those without invasion (2.3 ± 0.2; $p < 0.01$). There was no difference in the MMP-2 score between macroadenomas (3.0 ± 0.3) and microadenomas (2.1 ± 0.4;

Table 7: Frequency of *MMP-2 (-1306 C/T)* genotype in the patients with pituitary adenoma (PA) and in the control group by PA recurrences.

Gene marker	Genotype/allele	Frequency (%) Control group n (%) (n = 318)	p HWE	Nonrecurrence PA group n (%) (n = 71)	p HWE	Recurrence PA group n (%) (n = 13)	p HWE
MMP-2 (-1306) Rs243865	Genotype						
	C/C	**190*** (59.75)		**33*** (46.5)		9 (69.2)	
	C/T	108 (33.96)		33 (46.5)		4 (30.8)	
	T/T	20 (6.29)	0.383	5 (7.0)	0.3958	0 (0)	0.5121
	Total	318 (100)		71 (100)		13 (100)	
	Allele						
	C	488 (76.72)		99 (69.72)		22 (84.62)	
	T	148 (23.33)		43 (30.28)		4 (15.38)	

* $p = 0.0468$.
MMP: matrix metalloproteinase; p value: significance level (alfa = 0.05); p-value HWE: significance level (alfa = 0.05) by Hardy-Weinberg equilibrium.

Table 8: Binomial logistic regression analysis in noninvasive and nonrecurrence pituitary adenoma (PA) and in the control group.

Model	Genotype	OR (CI 95%)	p value	AIC
Noninvasive				
Codominant	CC	1		234.221
	CT	2.977 (1.442–6.148)	**0.003**	
	TT	1.462 (0.308–6.944)	0.633	
Dominant	CC	1		233.197
	T/C + T/T	2.740 (1.346–5.581)	**0.005**	
Recessive	C/C + C/T	1		241.285
	T/T	0.851 (0.191–3.797)	0.833	
Overdominant	T/T + C/C	1		232.431
	T/C	2.852 (1.422–5.721)	**0.003**	
Additive	—	1.782 (1.065–2.982)	**0.028**	236.667
Nonrecurrence				
Codominant	CC	1		371.421
	CT	1.759 (1.028–3.011)	**0.039**	
	TT	1.439 (0.505–4.102)	0.496	
Dominant	CC	1		369.564
	T/C + T/T	1.709 (1.019–2.868)	**0.042**	
Recessive	C/C + C/T	1		373.646
	T/T	1.129 (0.409–3.117)	0.815	
Overdominant	T/T + C/C	1		369.859
	T/C	1.689 (1.003–2.843)	**0.049**	
Additive	—	1.423 (0.953–2.123)	0.084	370.788

$p > 0.05$), and also no difference between the functioning adenomas (2.8 ± 0.3) and nonfunctioning adenomas (2.8 ± 0.3; $p > 0.05$) [5]. MMP-2 mRNA expression was also intense in invasive pituitary adenomas and was significantly higher in invasive pituitary adenomas than those without invasion (68.2 ± 15.3; 21.8 ± 8.2; $p < 0.05$). Pereda et al. [40] have observed the activities of MMP-2 and MMP-9 together with the expression of membrane-type MMP and tissue inhibitor of metalloproteinase-1 in all types of human pituitary adenomas. They found high levels of MMP activity and low levels of tissue inhibitor of metalloproteinases, indicating a high level of extracellular matrix-degrading activity in PAs.

Numerous studies have demonstrated overexpressed MMP-2 in various tumours: breast cancer [22–27], lung cancer [28], colorectal tumours [29], gastric and esophageal cancers [31–35], and pancreatic carcinoma [30]. Some studies have reported the expression of MMP-2 in human gliomas [36–38].

Numerous studies have also been carried out to look for an association between the MMP-2 -1306 C/T polymorphism and risk of other human tumours, but the results remain controversial [49–61].

Yu et al. [49] in their research have found that the allele frequency of *MMP2* -1306 C was significantly higher among cases of lung cancer than among controls (0.91 versus

0.83). Subjects with the *CC* genotype had an overall 2-fold increased risk of developing lung cancer [adjusted OR 2.18; 95% confidence interval (CI), 1.70–2.79] compared with those with the *CT* or *TT* genotype. In another study Yu et al. [55] have reported that the C_{-1306}–C_{-735} haplotype in the *MMP-2* promoter contributes to risk of the occurrence and metastasis of esophageal squamous cell carcinoma by increasing the expression of MMP-2. In another study this group of researches has found that subjects with the *CC* genotype had a more than 3-fold increased risk [adjusted OR 3.36, 95% confidence interval 2.34–4.97] for developing gastric cardia adenocarcinoma compared with those with the variant *CT* or *TT* genotype. The increased risk was found to be more pronounced in smokers and younger subjects. No significant association was demonstrated between the *MMP2* polymorphism and the risk of metastasis of the cancer at the time of diagnosis, with the OR being 0.90 (95% confidence interval 0.36–2.20) for the *CC* genotype [50]. In breast cancer research Miao et al. [50] have found that the variant *MMP2* genotype (-1306 CT or TT) was associated with substantially reduced risk of breast cancer [OR 0.46; 95% confidence interval (95% CI), 0.34–0.63], compared with the CC genotype. Grieu et al. [62] have also reported that MMP-2 TT homozygous patients had smaller breast tumours (p = 0.006) and contained lower concentrations of the estrogen receptor (ER; p = 0.002) compared to patients with the MMP-2 CC or CT genotype. Homozygosity for the MMP-2 -1306 T allele was associated with markedly different patient survival depending upon tumour ER status. For patients with ER negative tumours, the MMP-2 TT genotype was associated with poor survival (2/8 patients alive at end of study, 25%) compared to the CC or CT genotypes (59/70, 84%; $p < 0.001$). For patients with ER positive tumours, the MMP-2 TT genotype was associated with a trend for very good survival (10/10, 100%) compared to the CC or CT genotypes (130/157, 83%; $p = 0.16$).

Lin et al. in their study [52] provided evidence that -1306 $C \rightarrow T$ polymorphism in the MMP-2 promoter is a susceptibility factor for the development of oral squamous cell carcinoma, with the *CC* genotype being associated with the increase of risk. O-charoenrat and Khantapura [58] have reported that subjects with the MMP2 CC genotype were associated with a significantly increased risk [adjusted OR 1.97; 95% confidence interval (95% CI), 1.23–3.15] for developing HNSCC compared with those with the variant genotype (-1306 CT or TT).

Xu et al. [60] have found that the frequency of MMP-2 CC genotype was significantly higher in colorectal cancer patients when compared with controls (OR, 1.959; 95% CI, 1.055–3.637). Srivastava et al. [61] have found that patients with MMP2 (-1306) CT genotype as well as T allele were at higher risk of prostate cancer (p = 0.018; OR = 1.68 and p = 0.015; OR = 1.52). This effect was even more evident in the case of the T allele carrier (CT + TT) (p = 0.011; OR = 1.71). Shao et al. [63] have reported that the risk of nasopharyngeal carcinoma was significantly increased in young (<60 years) subjects with the -1306 CC genotype (OR = 1.52, 95% CI = 1.01–2.29). Wieczorek et al. [56] have found that the combined genotype MMP2 -1306 C/T (rs243865) allele T with MMP9 -1562 C/T (rs3918242) allele T increased bladder cancer risk (OR 2.00, 95% CI 1.10–3.62; p = 0.022).

On the basis of these findings, we hypothesized that the -1306 C/T polymorphism in *MMP-2* might also have impact on individual susceptibility to PA.

Some authors have not found an association of MMP-2 (-1306 C/T) polymorphism with tumours. Rollin et al. [53] have found no difference in -1306 C/T MMP-2, -735 C/T MMP-2, and -1562 C/T MMP-9 genotypes between cases of non-small cell lung cancer and controls. Eftekhary et al. [54] have found no statistically significant differences in genotype and allele frequencies of MMP-2 (-1306 C/T) between patients with esophageal squamous cell carcinoma and controls ($p > 0.05$). A significant association of the MMP-2 (-1306 C/T) polymorphism with GBM (p = 0.475) was not found by Kumar et al. [59] suggesting that MMP-2 (-1306 C/T) polymorphism is not associated with increased GBM susceptibility. Kawal et al. [57] have not found a significant association of MMP-2 (-1306 C/T) polymorphism with oligodendroglioma (p = 0.54).

To the best of our knowledge, this is the first study to examine the relationship between the *MMP-2* (-1306 C/T) polymorphism and PA risk. Our study showed a significantly greater prevalence of the C/T genotype in females than their control counterparts. Further studies with a larger number of patients, however, are necessary in order to better understand the real value of such a normative database in developing of PA.

Our study suggests that the effects of polymorphisms of *MMPs* on PA risk deserve further investigation.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

The authors thank Silvija Jakstiene for radiological evaluation and Professor Abdonas Tamosiunas for control group data. This study was supported by Lithuanian Science Council (Grant no. MIP-008/2014) and the Wellcome Trust (Grant no. 064947/Z/01/Z); the US National Institute on Aging (Grant no. IR0I AG23522-01), the MacArthur Foundation (Health and Social Upheaval network); Oslo University College (Grant no. 57116).

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Hindawi Publishing Corporation
Disease Markers
Volume 2017, Article ID 5839528, 9 pages
https://doi.org/10.1155/2017/5839528

Hindawi

Research Article

Does MMP-9 Gene Polymorphism Play a Role in Pituitary Adenoma Development?

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Received 1 August 2016; Accepted 23 November 2016; Published 17 January 2017

Academic Editor: Robert Pichler

Purpose. To determine if the MMP-9 genotype has an influence on development of pituitary adenoma (PA). *Methodology*. The study enrolled $n = 86$ patients with PA and $n = 526$ healthy controls (reference group). The genotyping of MMP-9 was carried out using the real-time polymerase chain reaction method. *Results*. Our data demonstrated that the MMP-9 (–1562) C/C genotype was more frequent in PA group than in healthy controls (81.4% versus 64.6%, $p = 0.002$); C/C genotype was more frequently present in PA females compared to healthy control females, 81.5% versus 64.6%, $p = 0.018$, as well. MMP-9 (–1562) C/C genotype was frequently observed for all subgroups: noninvasive and invasive, nonrecurrence, and inactive PA compared to healthy controls: 81.8% versus 64.6%, $p = 0.021$; 81.0% versus 64.6%, $p = 0.041$; 81.8% versus 64.6%, $p = 0.005$; 100.0% versus 64.6%, $p < 0.001$, respectively. MMP-9 (–1562) C/C genotype was more frequent in inactive PA compared to active PA: 100.0% versus 71.4%; $p < 0.001$. *Conclusion*. MMP-9 (–1562) C/C genotype plays a role in nonrecurrence, inactive, and invasive as well as in nonivasive PA development.

1. Introduction

Most of the pituitary tumours are pituitary adenomas (PAs): benign, slow-growing neoplasms that arise from cells of the pituitary gland. Pituitary adenomas have the high incidence, following the gliomas and meningiomas. In recent years, the frequency of PA has been increasing, particularly in younger age groups [1, 2]. PA accounts for 15 to 20% of primary brain tumours. PA may grow large and extend into the surrounding structures resulting in neurological complications including visual impairment [3]. In addition, pituitary adenomas may be distinguished anatomically as intrapituitary, intrasellar, diffuse, and invasive [4]. Invasive adenomas which account for approximately 35% of all pituitary neoplasms may invade the dura mater, cranial bone, or sphenoid sinus [5]. PA is a disease of multifactorial etiology, the occurrence of which is influenced by genetic factors, hormonal stimulation, growth factors, and so forth. Recently, great attention in the PA pathogenesis has been drawn to the search for new epigenetic and genetic factors [6]. To invade, tumour cells must undergo several changes in molecular pathways in accordance with invasion-associated cellular activities, namely, cell-cell adhesion, cell-matrix adhesion and ectopic survival, migration, and proteolysis [7]. Invasive factors involve the heparinase, serine proteinases, cathepsins, and matrix metalloproteinases *(MMPs)* [8]. *MMPs* are capable of degrading most of the components of the extracellular matrix which may play an important role in the extracellular matrix remodeling during angiogenesis. A particular interest has been focused on *MMP-9* due to its ability to degrade components of the basement membrane components, such as type IV collagen. There are 24 different genes defined that code the expression

of proteases of *MMP* family [9]. The expression in transcription level depends on gene promoter mutations and various transcription factors. A transition of C to T at the 1562-bp position upstream of the transcription initiation site (–1562 C/T) of *MMP-9* (NCBI SNP identification number rs3918242) has shown having an effect on promoter activity. Transition of C nucleotide to T nucleotide causes more difficulties for nucleic protein complex binding to DNA strain in the presence of T allele. It was determined that once C allele mutates to T allele, a promoter activity increases 1.5 times [10].

Few studies have analyzed MMP-9 expression in PA [11–13] and in other tumours such as prostate and gastric cancer [14–16]. MMP-9 expression was reported to be significantly higher in invasive pituitary adenomas [17–20]. Also, some studies have been carried out to look for the possible association between the MMP-9 (–1562 C/T) gene polymorphism and prostate [14], breast [21], gastric [22–24], colorectal [25, 26], and lung cancer [27]. However, results of the published studies are controversial. To our knowledge, there have been no studies that investigated the association between the MMP-9 (–1562 C/T) gene polymorphism and PA development. Therefore, the aim of this study was to determine the association between the MMP-9 (–1562 C/T) gene polymorphism and the development of PA.

2. Materials and Methods

Permission (number P2-9/2003) to undertake the study was obtained from the Kaunas Regional Biomedical Research Ethics Committee. The study was conducted in the Departments of Ophthalmology and Neurosurgery, the Hospital of Lithuanian University of Health Sciences Kaunas Clinics.

The study comprised 86 subjects with a diagnosis of pituitary adenoma and 526 healthy subjects in the control group.

The control group was designed by taking into consideration the distribution of age and gender in the pituitary adenoma group. Therefore, the medians of the patients' age of the control group and the pituitary group did not differ statistically significantly ($p > 0.05$).

Demographic data of the study subjects are presented in Table 1.

The inclusion criteria of the PA group were as follows: (1) determined and confirmed PA via MRI; (2) a patient's general good condition; (3) a patient's consent to take part in the study; (4) age ≥ 18 years, (5) no other brain or other localization tumours.

2.1. Invasiveness Evaluation. The analysis of all pituitary adenomas was based on MR imaging findings. The suprasellar extension and sphenoid sinus invasion by PAs were classified according to the Wilson-Hardy classification (the Hardy classification, modified by Wilson) [28]. The degree of suprasellar and parasellar extension was graded as stages A–E. The degree of sellar floor erosion was graded as grades I–IV. Grade III, localized sellar destruction, and grade IV, diffuse destruction, were considered as invasive PA. The Knosp classification system was used to quantify invasion of the cavernous sinus, in which only grades 3 and 4 define true invasion of the tumour into the cavernous sinus. In grade 0, there is no cavernous sinus involvement; in grades 1 and 2, the tumour pushes into the medial wall of the cavernous sinus but does not go beyond a hypothetical line extending between the centres of the two segments of the internal carotid artery (grade 1) or it goes beyond such a line, but without passing a line tangent to the lateral margins of the artery itself (grade 2); in grade 3, the tumour extends laterally to the internal carotid artery within the cavernous sinus; in grade 4, there is total encasement of the intracavernous carotid artery [29]. Thus grade III and IV tumours were considered to be invasive.

Table 1: Demographic characteristics of patients with pituitary adenoma (PA) and the control group subjects.

Characteristics	Patients	Controls	p
Sample size	86	526	
Age (year) (min./max. median)	19/87/52.5	25/87/51	0.88
Gender			
Females, n (%)	54 (62.8)	316 (60.1)	0.633
Males, n (%)	32 (37.2)	210 (39.9)	
Invasiveness			
Invasive, n (%)	42 (48.8)		
Noninvasive, n (%)	44 (51.2)		
Recurrence			
Recurrence, n (%)	20 (23.3)		
Nonrecurrence, n (%)	66 (76.7)		
Activity			
Active, n (%)	56 (65.1)		
Inactive, n (%)	30 (34.9)		

2.2. Activeness and Recurrence Evaluation. The analysis of all pituitary adenomas was based on histopathological findings of PA and hormone levels in the blood serum before surgery. All 100 subjects were categorized into two groups, active or inactive PA. Active PA group was not distributed to smaller groups by increase of specific hormone because dominant tumours were prolactinomas and others would not fill the optimal space in our study. Since some of the 100 subjects had already had surgery in recent years, we categorized them by recurrence of pituitary adenoma into two groups, with PA and without recurrence.

2.3. Control Group Formation. The age- and gender-matched control group comprised 526 subjects randomly selected from the following projects:

(1) The international Health, Alcohol and Psychosocial Factors in Eastern Europe (HAPPIE) project involving the Kaunas population aged 45–74 years run by the Laboratory of Population Research, Institute of Cardiology, LUHS.

(2) The international Countrywide Integrated Noncommunicable Disease Intervention (CINDI) project involving the Lithuanian population aged 25–65 years run by the Laboratory of Preventive Medicine, Institute for Biomedical Research, LUHS.

Table 2: Frequency of MMP-9 (–1562 C/T) genotype in the patients with pituitary adenoma (PA) and in the control group.

Gene marker	Genotype/allele	Frequency (%) Control group n (%) (n = 526)	p HWE	Frequency (%) PA group n (%) (n = 86)	p HWE	p value
MMP-9 (–1562) Rs3918242	Genotype					
	C/C	**340 (64.6)***	0.469	**70 (81.4)***	**0.0029**	$X^2 = 11.788$ $p = \mathbf{0.003}$
	C/T	**169 (32.1)****		**12 (14.0)****		
	T/T	17 (3.2)		4 (4.7)		
	Total	526 (100)		86 (100)		
	Allele					
	C	849 (80.70)		152 (88.37)		
	T	203 (19.30)		20 (11.63)		

MMP, matrix metalloproteinase; p value, significance level (alpha = 0.05); p value HWE, significance level (alpha = 0.05) by Hardy-Weinberg equilibrium.
* $P = 0.002$.
** $P < 0.001$.

(3) The Kaunas Healthy Ageing Study involving the Kaunas population older than 65 years run by the Clinic of Geriatrics and the Laboratory of Molecular Cardiology, Institute of Cardiology, LUHS.

2.4. DNA Extraction and Genotyping. The DNA extraction and analysis of the gene polymorphism of *MMP-9* were carried out at the Laboratory of Molecular Cardiology at the Institute of Cardiology of the LUHS for control group and at the Laboratory of Ophthalmology at the Institute of Neuroscience of the LUHS for the PA patient group. DNA was extracted from 200 μL venous blood (white blood cells) using a DNA purification kit based on the magnetic beads method (MagJET Genomic DNA Kit, Thermo Scientific) or the silica-based membrane technology utilizing a genomic DNA extraction kit (GeneJET Genomic DNA Purification Kit, Thermo Scientific), according to the manufacturer's recommendations.

The genotyping of *MMP-9* (–1562 C/T) was carried out using the real-time polymerase chain reaction (PCR) method. For the genotyping of *MMP-9* (–1562 C/T) (Rs3918242), the following were used: 2x TaqMan® Universal Master Mix, nuclease-free water, and the following primers and fluorescently labeled allele specific probes: forward primer 5′-CAGATCACTTGAGTCAGAA-3′, reverse primer 5′-GGTGTAGTATCACTCTGTCA-3′, probe "C" allele 5′ FAM-TGGCGCACGCCTATAATACCA-MGB 3′, and probe "T" allele 5′ VIC-TGGCGCATGCCTATAATACCAGC-MGB 3′ (Applied Biosystems, Warrington, UK). PCR conditions included predenaturation at 95°C for 10 minutes followed by 40 cycles of 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds. Genotyping was performed using the HT 7900 real-time PCR quantification system (Applied Biosystems, USA).

To ensure an internal control, 20 samples were sequenced at the Sequencing Center of the Institute of Biotechnology.

2.5. RNA Extraction and MMP-9 mRNA Expression Analysis. Twenty-eight PA samples were surgically resected in the Department of Neurosurgery, Hospital of Lithuanian University of Health Sciences Kaunas Clinics (Lithuania), and were confirmed histologically. PA tissue samples were snap-frozen in liquid nitrogen prior to RNA extraction. Total RNA was purified using TRIzol Reagent (Ambion, Life Technologies). cDNA synthesis was performed using total RNA (2 μg) and random hexamer primers (ThermoFisher Scientific) with the RevertAid H Minus M-MuLV Reverse Transcriptase (ThermoFisher Scientific) in a final volume of 40 μL, according to the manufacturer's recommendations. For inhibition of mRNA degradation RiboLock RNase inhibitor (ThermoFisher Scientific) was used. MMP-9 mRNA expression was analyzed using quantitative real-time RT-PCR TaqMan probe assay (assay number Hs00234579_m1) in 3 replicates on 7500 Fast Real-Time PCR detection system (Applied Biosystems). One endogenous control (GAPDH TaqMan probe assay number Hs0 2758991_g1) was used. MMP-9 expression levels in PA were determined by the comparative Ct method ($2^{-\Delta\Delta Ct}$). The expression of MMP-9 was normalized to GAPDH and calibrated using reference sample ("FirstChoice Human Brain Reference RNA" (Ambion)).

2.6. Statistical Analysis. Statistical analysis was performed using the SPSS/W 20.0 software (Statistical Package for the Social Sciences for Windows, Inc., Chicago, Illinois, USA). The data are presented as absolute numbers with percentages in brackets and as medians with min. and max. values. The frequencies of genotypes (in percentage) are presented in Table 2.

Hardy-Weinberg analysis was performed to compare the observed and expected frequencies of *MMP-9* using the χ^2 test in all groups. The distributions of *MMP-9* SNPs in the PA and control groups were compared using the χ^2 test or the Fisher exact test. Binomial logistic regression analysis was performed to estimate the impact of genotypes on PA development. Odds ratios and 95% confidence intervals are presented. The selection of the best genetic model was based on the Akaike Information Criterion (AIC); therefore, the best genetic models were those with the lowest AIC values.

Table 3: Frequency of MMP-9 (–1562 C/T) genotype in the patients with pituitary adenoma (PA) and in the control group by gender.

Gene marker	Genotype/allele	Frequency (%) Control group n (%) Females $N = 316$	Control group n (%) Males $N = 210$	p HWE	p value	PA group n (%) Females $N = 54$	PA group n (%) Males $N = 32$	p HWE	p value
MMP-9 (–1562) Rs3918242	Genotype								
	C/C	**204 (64.6)**[1]	136 (64.8)	0.991	0.868	**44 (81.5)**[1]	26 (81.2)	0.836	1.00
	C/T	**10 (32.3)**[2]	**67(31.9)**[3]		0.928	**8(14.8)**[2]	**4 (12.5)**[3]		1.00
	T/T	10 (3.2)	7 (3.3)		0.915	2 (3.7)	2 (6.2)		0.626
	Total	316 (100)	210 (100)			54 (100)	32 (100)		
	Allele								
	C	510 (80.7)	339 (80.71)			96 (88.89)	56 (87.5)		
	T	122 (19.3)	81 (19.29)			12 (11.11)	8 (12.5)		

MMP, matrix metalloproteinase; p value, significance level (alpha = 0.05); p value HWE, significance level (alpha = 0.05) by Hardy-Weinberg equilibrium.
[1] $P = 0.018$.
[2] $P = 0.01$.
[3] $P = 0.023$.

Table 4: Binomial logistic regression analysis in the patients with pituitary adenoma (PA) and in the control group.

Model	Genotype	OR (CI 95%)	p value	AIC
Codominant	C/C	1		489.539
	C/T	0.345 (0.182–0.654)	**0.01**	
	T/T	1.143 (0.373–3.5)	0.815	
Dominant	C/C	1		490.616
	C/T + T/T	0.418 (0.236–0.740)	**0.003**	
Recessive	C/C + C/T	1		50.423
	T/T	1.461 (0.479–4.449)	0.505	
Overdominant	C/C + T/T	1		478.593
	C/T	0.343 (0.181–0.648)	**0.001**	
Additive	—	0.551 (0.337–0.901)	**0.018**	494.457

Kruskal–Wallis test was used to reveal the difference across medians of MMP-9 mRNA expression in all hormone groups. Differences were considered statistically significant when $p < 0.05$.

3. Results

The genotyping of MMP-9 (–1562) C/T was performed in patients with PA and in the control group subjects (Table 2). The distribution of the analyzed MMP genotypes and allele frequencies in the control group but not in PA group matched the Hardy-Weinberg equilibrium. MMP-9 (–1562) C/T gene polymorphism analysis in the overall group revealed differences in the genotype distribution between patients with PA and control group patients ($p = 0.003$). The genotype C/C was more frequent in PA group than in healthy controls (81.4% versus 64.6%, $p = 0.002$) and the genotype C/T was less frequent in PA group compared to healthy control group (14.0% versus 32.1%, $p < 0.001$) (Table 2).

MMP-9 (–1562) C/T gene polymorphism analysis between females and males with PA did not reveal any statistically significant differences in the genotypes (C/C, C/T, and T/T) distribution (as follows: 81.5%, 14.8%, and 3.7% versus 81.2%, 12.5%, and 6.2%) (Table 3). A comparison of MMP-9 genotype distribution in healthy females and females with PA revealed significant differences. MMP-9 (–1562) C/C genotype was more frequently present in PA females compared to healthy control females: 81.5% versus 64.6%; $p = 0.018$; and C/T was less frequent in PA females compared to healthy females: 14.8% versus 32.3%; $p = 0.01$. MMP-9 (–1562) T/T genotype did not show any statistically significant differences when healthy females and females with PA were compared. When analyzing genotypes distribution in men, only MMP-9 (–1562) C/T genotype distribution showed statistically significant difference among men with PA and healthy men: 12.5% versus 31.9%; $p = 0.023$.

Binomial logistic regression analysis in the patients with PA and in the control group was performed (Table 4). This analysis revealed that there were statistically significant variables in the codominant ($p = 0.01$), dominant ($p = 0.003$), overdominant ($p = 0.001$), and additive ($p = 0.018$) models of the patients with PA and in the control group.

Binomial logistic regression analysis in the patients with PA and in the control group by gender was also performed

Table 5: Binomial logistic regression analysis in pituitary adenoma (PA) and the control by gender.

Model	Genotype	OR (CI 95%)	p value	AIC
Males				
Codominant	C/C	1		189.034
	C/T	0.312 (0.105–0.931)	**0.037**	
	T/T	1.495 (0.294–7.601)	0.628	
Dominant	C/C	1		189.340
	C/T + T/T	0.424 (0.167–1.077)	0.071	
Recessive	C/C + C/T	1		190.485
	T/T	1.933 (0.384–9.745)	0.424	
Overdominant	C/C + T/T	1		187.253
	C/T	0.305 (0.103–0.904)	**0.032**	
Additive	—	0.603 (0.278–1.310)	0.201	191.239
Females				
Codominant	C/C	1		306.013
	C/T	0.364 (0.165–0.801)	**0.012**	
	T/T	0.927 (0.196–4.381)	0.924	
Dominant	C/C	1		305.045
	C/T + T/T	0.414 (0.201–0.854)	**0.017**	
Recessive	C/C + C/T	1		311.512
	T/T	1.177 (0.251–5.525)	0.836	
Overdominant	C/C + T/T	1		304.022
	C/T	0.365 (0.166–0.802)	**0.012**	
Additive	—	0.520 (0.275–0.983)	**0.044**	306.912

Table 6: Frequency of MMP-9 (–1562 C/T) genotypes in the patients with pituitary adenoma (PA) and in the control group by PA invasiveness.

Gene marker	Genotype/allele	Frequency (%) Control group n (%) (n = 526)	p HWE	Noninvasive PA group n (%) (n = 44)	p HWE	Invasive PA group n (%) (n = 42)	p HWE
MMP-9 (–1562) Rs3918242	Genotype						
	C/C	**340 (64.6)**[1,2]	0.469	**36 (81.8)**[1]	0.507	**34 (81.0)**[2]	**<0.001**
	C/T	**169 (32.1)**[3]		8 (18.2)		**4 (9.5)**[3]	
	T/T	17 (3.2)		0 (0)		4 (9.5)	
	Total	526 (100)		44 (100)		42 (100)	
	Allele						
	C	849 (80.70)		80 (90.91)		72 (85.71)	
	T	203 (19.30)		8 (9.09)		12 (14.29)	

MMP, matrix metalloproteinase; p value, significance level (alpha = 0.05); p value HWE, significance level (alpha = 0.05) by Hardy-Weinberg equilibrium.
[1] $p = 0.021$.
[2] $p = 0.041$.
[3] $p = 0.001$.

(Table 5). There were statistically significant variables in the codominant ($p = 0.037$) and overdominant ($p = 0.032$) models of males. In females this analysis revealed that the codominant ($p = 0.012$), dominant ($p = 0.017$), overdominant ($p = 0.012$), and additive ($p = 0.044$) variables were statistically significant.

MMP-9 (–1562) C/C genotype was frequently observed in noninvasive, nonrecurrence, and inactive PA subgroups compared to healthy controls: 81.8% versus 64.6%; $p = 0.021$; 81.0% versus 64.6%; $p = 0.041$; 81.8% versus 64.6%; $p = 0.005$; 100.0% versus 64.6%; $p < 0.001$, respectively. On the other hand, there were no differences between noninvasive and invasive and nonrecurrence and recurrence PA subgroups except when compared with inactive and active PA. There was statistically significant difference between patients with inactive PA and active PA. MMP-9 (–1562) C/C genotype was more frequent in inactive PA compared to active PA: 100.0% versus 71.4%; $p < 0.001$ (Tables 6, 7, and 8).

Table 7: Frequency of MMP-9 (–1562 C/T) genotype in the patients with pituitary adenoma (PA) and in the control group by PA recurrences.

Gene marker	Genotype/allele	Frequency (%) Control group n (%) (n = 526)	p HWE	Nonrecurrence PA group n (%) (n = 66)	p HWE	Recurrence PA group n (%) (n = 20)	p HWE
MMP-9 (–1562) Rs3918242	Genotype						
	C/C	**340 (64.6)**[1]	0.469	**54 (81.8)**[1]	0.103	16 (80.0)	0.007
	C/T	**169 (32.1)**[2,3]		10 **(15.2)**[2]		**2 (10.0)**[3]	
	T/T	17 (3.2)		2 (3.0)			
	Total	526 (100)		66 (100)		2 (10.0)	
	Allele					20 (100)	
	C	849 (80.70)		118 (89.39)		34 (85.0)	
	T	203 (19.30)		14 (10.61)		6 (15.0)	

MMP, matrix metalloproteinase; p value, significance level (alpha = 0.05); p value HWE, significance level (alpha = 0.05) by Hardy-Weinberg equilibrium.
[1] $P = 0.005$.
[2] $P = 0.004$.
[3] $P = 0.047$.

Table 8: Frequency of MMP-9 (–1562 C/T) genotype in the patients with pituitary adenoma (PA) and in the control group by PA activity.

Gene marker	Genotype/allele	Frequency (%) Control group n (%) (n = 526)	p HWE	Inactive PA group n (%) (n = 30)	p HWE	Active PA group n (%) (n = 56)	p HWE
MMP-9 (–1562) Rs3918242	Genotype						
	C/C	**340 (64.6)**[1]	0.469	**30 (100)**[1,3]	0.007	**40 (71.4)**[3]	0.044
	C/T	**169 (32.1)**[2]		**0 (0)**[2,4]		**12 (21.4)**[4]	
	T/T	17 (3.2)		0 (0)		4 (7.1)	
	Total	526 (100)		30 (100)		56 (100)	
	Allele						
	C	849 (80.70)		60 (100)		92 (82.14)	
	T	203 (19.30)		0 (0)		20 (17.86)	

MMP, matrix metalloproteinase; p value, significance level (alpha = 0.05); p value HWE, significance level (alpha = 0.05) by Hardy-Weinberg equilibrium.
[1] $P < 0.001$.
[2] $P < 0.001$.
[3] $P < 0.001$.
[4] $P < 0.007$.

Further analysis revealed that MMP-9 (–1562) C/T genotype was less frequent in invasive, nonrecurrence, recurrence, and inactive PA subgroups compared to healthy controls: 9.5% versus 32.1%; $p = 0.001$; 15.2% versus 32.1%; $p = 0.004$; 10.0% versus 32.1%; $p = 0.047$; 0% versus 32.1%; $p < 0.001$, respectively (Tables 6, 7, and 8).

The analysis of PA subgroups showed only one statistically significant difference between inactive and active PA. MMP-9 (–1562) C/T genotype was more frequent in active PA subgroup compared to inactive one: 21.4% versus 0%; $p = 0.007$ (Table 8).

Binomial logistic regression analysis in noninvasive PA and in the control group was performed (Table 9). In noninvasive PA group this analysis revealed that the codominant ($p = 0.045$), dominant ($p = 0.025$), overdominant, and additive ($p = 0.028$) variables were statistically significant. Binomial logistic regression analysis in the patients with invasive PA and in the control group was performed as well (Table 9, shown in Supplementary Data available online at https://doi.org/10.1155/2017/5839528). In invasive PA group this analysis showed that the codominant ($p = 0.007$), dominant ($p = 0.036$), and overdominant ($p = 0.005$) variables were statistically significant.

Binomial logistic regression analysis in inactive PA and in the control group was performed as well as in active PA and in the control group (Table 10, shown in Supplementary Data). There was no statistical significance of variables in analysis of MMP-9 (–1562) in both groups by activity of PA.

Binomial logistic regression analysis performed in nonrecurrence PA and in the control group showed that the codominant ($p = 0.006$), dominant ($p = 0.007$), overdominant ($p = 0.006$), and additive ($p = 0.016$) variables were statistically

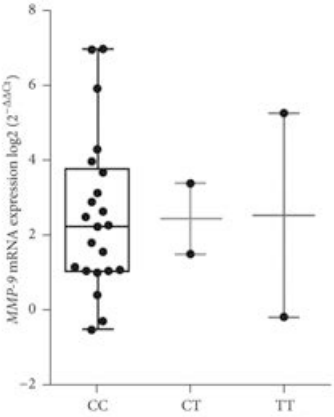

FIGURE 1: MMP-9 mRNA expression in pituitary adenoma (PA).

significant (Table 11, shown in Supplementary Data). There was no statistical significance of variables in analysis of MMP-9 (–1562) in PA with recurrence.

MMP-9 mRNA expression analysis in pituitary adenomas was performed. Therefore, the results of MMP-9 mRNA expression did not reveal any statistically significant differences between three genotypes of MMP-9 (C/T) polymorphism (C/C, C/T, and T/T). Lower expression was noticed in patients with MMP-9 C/C genotype (36.4% (8/22)), but differences between groups were not statistically significant ($p = 0.9$) (Figure 1).

MMP-9 expression in different groups of genotypes (CC, CT, and TT) of MMP-9 gene in patients with PA: the horizontal bars represent the mean values; the spots represent the amount of samples.

4. Discussion

The impact of MMP-9 (–1562) C/T gene polymorphism on the development of various tumours was analyzed in few studies [14, 21–27]. In addition, *MMP-9* expression has been shown to be significantly higher in invasive PAs compared to noninvasive ones [17–20]. In our previous study we analyzed 84 PA patients and 318 age- and sex-matched controls for the –1306 C/T polymorphism in the *MMP-2* promoter. Our results demonstrated that *MMP-2 (–1306) C/T* genotype was more frequently present in PA females compared to healthy control females: 33.66% versus 49.1%; $p = 0.041$ [30].

On the basis of these findings, we sought to examine whether the polymorphism in the *MMP-9 (–1562)* promoter could have an impact on the risk of PA development.

Our data demonstrated that the MMP-9 (–1562) C/C genotype, which causes lower gene expression, was more frequent in PA group than in healthy controls (81.4% versus 64.6%, $p = 0.002$); C/C genotype was more frequently present in PA females compared to healthy control females: 81.5% versus 64.6%; $p = 0.018$, as well. It is very interesting that MMP-9 (–1562) C/C genotype was frequently observed for all subgroups: noninvasive and invasive, nonrecurrence, and inactive PA compared to healthy controls: 81.8% versus 64.6%; $p = 0.021$; 81.0% versus 64.6%; $p = 0.041$; 81.8% versus 64.6%; $p = 0.005$; 100.0% versus 64.6%; $p < 0.001$, respectively. MMP-9 (–1562) C/C genotype was more frequent in inactive PA compared to active PA: 100.0% versus 71.4%; $p < 0.001$. Therefore, we may hypothesize that MMP-9 gene polymorphism plays a role in noninvasive/invasive, nonrecurrence, and inactive PA development.

To our knowledge, no studies have been carried out analyzing the impact of MMP-9 (–1562) C/T gene polymorphism on the development of PA. Previous studies on the morphogenesis of PA have drawn attention to the role of MMP-9 expression but not to the MMP-9 C/T gene polymorphism in the development of PA, especially considering PA invasiveness.

Kawamoto et al. have observed the incidence of tumour cells secreting MMP-9 to be significantly higher in invasive pituitary adenomas than in noninvasive ones [17]. Gong et al. [20] have analyzed 73 pituitary tumour specimens and have found MMP-9 mRNA expression to be significantly increased in the majority of invasive pituitary adenomas. Liu et al. have found MMP-9 score of invasive case (4.1 ± 0.4) to be significantly higher than those (2.6 ± 0.2; $p < 0.01$) without invasion [18]. Hussaini et al. have demonstrated an increase in the expression level and activity of MMP-9 in invasive nonfunctioning PAs and HP75 cell line [19]. Turner et al. also have reported MMP-9 expression to be higher in invasive macroprolactinomas ($p = 0.003$) when compared with noninvasive macroprolactinomas or normal anterior pituitary gland [31]. Significantly higher MMP-9 expression was detected in invasive prolactinomas ($p = 0.004$) by Gültekin et al. [32]. Some authors have not found an association of MMP-9 expression and tumour invasiveness [33]. Few studies have also been carried out to look for an association between the MMP-9 –1562 C/T polymorphism and the risk of other human tumours. Schveigert et al. in their research have found MMP-9 –1562 polymorphism CC variant to be associated with prostate cancer tumour differentiation grade [14]. Zheng et al. have reported CT and TT genotypes of the MMP-9 gene (C–1562T) as high-risk genotypes for solid breast tumour invasion and metastasis progression [15]. In another study Matsumura et al. have found that the presence of T allele at MMP-9 –1562 site was significantly associated with tumour progression and invasive phenotype of gastric cancer among Japanese population [23]. In another study Kubben et al. did not find any significant association between MMP-9 –1562 C/T polymorphism and gastric cancer risk among Caucasian population [24]. Jafari et al. in their study concluded that T allele may be the risk factor for lung cancer progression [27]. Xing et al. have reported that the MMP-9–1562C>T polymorphism affects lymph node metastasis of colorectal cancer [26]. On the basis of these findings, we hypothesized that the –1562 C/T polymorphism in MMP-9 might also have impact on individual susceptibility to PA.

It is the first study to examine MMP-9 (–1562 C/T) polymorphism in patients with PA. Numerous genetic studies have been carried out to assess an association between

the MMP-9 expression and PA clinical features, but results remain controversial [17–20, 31, 32]. Additionally, we analyzed MMP-9 expression using mRNA determination in resected PA of 28 patients and explored the correlation between MMP-9 gene mRNA expression and different genotypes of MMP-9 (–1562 C/T) polymorphism. In our study we found that MMP-9 (–1562) C/C genotype was more frequently observed in PA group than in healthy controls with a tendency for lower mRNA activity in most of PA samples with CC genotype. Unfortunately, these findings did not show any statistical significance ($p = 0.9$). Zhang and colleagues determined that once C allele mutates to T allele a promoter activity increases [10], and in such way CC genotype may cause lower promoter activity resulting in lower MMP-9 expression. These findings correlate with our study results which show higher frequency of CC genotype in inactive, noninvasive, and nonrecurrence PA groups suggesting that CC genotype could be associated with decreased MMP-9 expression, as well. However, there are more MMP-9 gene polymorphisms which may interact and influence MMP-9 expression, so further research with the bigger sample size is needed to analyze other MMP-9 gene polymorphisms as risk factors for PA development.

To the best of our knowledge, this is the first study that examined the relationship between the MMP-9 (–1562) C/T gene polymorphism and development of PA. For the first time, we have found that MMP-9 (–1562) C/C genotype was frequently observed for all subgroups: noninvasive and invasive, nonrecurrence, and inactive PA compared to healthy controls. Therefore, MMP-9 (–1562) C/T gene polymorphism might play an important role in the development on PA but further research is required to be repeated in the bigger sample size.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This study was supported by Research Council of Lithuania (Grant no. MIP-008/2014).

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Association of *FGFR2 rs2981582*, *SIRT1 rs12778366*, *STAT3 rs744166* gene polymorphisms with pituitary adenoma

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DOI: 10.3892/ol_xxxxxxxx

Abstract. The aim of the present study was to determine the association between sirtuin 1 (*SIRT1*), fibroblast growth factor receptor 2 (*FGFR2*) and signal transducer and activator of transcription 3 (*STAT3*) polymorphisms, and pituitary adenoma (PA) development, invasiveness, hormonal activity and recurrence. The present study included 143 patients with a diagnosis of PA. The reference group involved 808 healthy subjects. The genotyping of *SIRT1 rs12778366, FGFR2 rs2981582* and *STAT3 rs744166* was performed using the quantitative polymerase chain reaction method. The SIRT1 rs12778366 polymorphism analysis in the overall group revealed differences in the genotype distribution between patients with PA and control group subjects. The *rs12778366* T/C genotype was observed to be different in non-invasive, non-recurrent and inactive PA subgroups compared with the control group, while the C/C genotype was observed to be different in invasive, recurrent and active PA subgroups compared with the control group. STAT3 rs744166 polymorphism analysis in the overall group revealed differences in the genotype distribution between patients with PA and the control groups. The *rs744166* G/G genotype was observed to be different in invasive, non-recurrent and active PA subgroups compared with the control group, while the *rs744166* A/A genotype was observed to be different in the active PA subgroup compared with the control group, and was also different in terms of invasiveness and recurrence in PA subgroups. The present study demonstrated that SIRT1 *rs12778366* is associated with pituitary adenoma development while STAT3 *rs744166* is associated with PA invasiveness, hormonal activity and recurrence.

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Key words: pituitary adenoma, gene polymorphism, fibroblast growth factor receptor 2 *rs2981582*, sirtuin 1 rs12778366, signal transducer and activator of transcription 3 rs744166

Introduction

Pituitary adenomas (PAs), located in a bone cavity termed the sella turcica, are one of the most common types of intracranial neoplasms, with reported estimated prevalence rates ranging between 14.4 and 22.5% in pooled autopsy and radiological series, respectively (1). Although the majority of PAs are benign, it is not uncommon for them to grow large and extend locally into the surrounding structures, invading the sphenoid bone inferiorly, the cavernous sinus laterally (2-7,8) and/or compressing the optic chiasm, if the direction of expansion is suprasellar, thus resulting in neurological complications, including headache and visual impairment (9-17). Certain types of PA are extremely invasive and may cause extensive destruction of the skull base (18). Investigation of tumour invasiveness is required, as this affects the management and prognosis of PA (19). The aim of the present study was to identify possible genes involved in PA tumourigenesis, which may serve as potential diagnostic and prognostic molecular markers. The present study selected 3 genes, sirtuin 1 (*SIRT1*), fibroblast growth factor receptor 2 (*FGFR2*) and signal transducer and activator of transcription 3 (*STAT3*), which are associated with different types of cancer, but are connected in pathogenic processes (20-23).

SIRT1 is a nicotinamide adenine dinucleotide-dependent histone deacetylase (HDAC) (24), which serves an important role in maintaining the balance between cell death and survival through targeting the Ku70-B-cell lymphoma-like protein 4 pathway (25), p53 (26,27) and forkhead box O3 (28), among others. A significant increase in the level of *SIRT1* in hepatocellular carcinoma (29), breast cancer (30), prostate cancer (31), ovarian cancer (32), gastric cancer (33), colon cancer (34), glioblastoma (35) and lymphoma (36) was previously suggested to be associated with the development and invasion of these tumours. Furthermore, the *rs12778366* polymorphism of the *SIRT1* gene was found to be associated with breast cancer (37).

FGFR2 is a member of the *FGFR* family of tyrosine kinase receptors and participates in the process of tumourigenesis by inducing mitogenic and survival signals, and promoting invasiveness and angiogenesis (38). If cancer cells overexpress an FGFR with altered ligand-binding specificity, FGFs, secreted from neighbouring cells, stimulate the cancer cells, creating a paracrine loop (38). *FGFR2* was previously revealed to be

overexpressed in bladder (39) and lung cancer (40). Additionally, the importance of the *FGFR2 rs2981582* gene polymorphism was investigated in breast (41-50) and prostate cancer (51).

STAT3 is activated in tumour cells and numerous immune cells of the tumour microenvironment, and is associated with tumour cell proliferation, invasion and angiogenesis (52-54). The effect of *STAT3* was previously studied in the tumour development of colorectal adenocarcinoma (55), hepatocellular carcinoma (56), multiple myeloma (57), glioblastoma (58), prostate cancer (59), and head and neck cancer (60). The *STAT3* rs744166 polymorphism was also evaluated in gastric (61,62), colon (63) and lung cancer (64).

These findings support the hypothesised role of *SIRT1, FGFR2* and *STAT3* as tumour promoters. However, an association between *SIRT1, FGFR2* and *STAT3* polymorphisms, and PA development, invasiveness, PA activity and recurrence has not yet been reported. The aim of the present study was to determine these associations.

Materials and methods

Patients and selection. Permission to undertake the present study was obtained from the Biomedical Research Ethics Committee of Lithuanian Health Sciences University (Kaunas, Lithuania). The study was conducted in the Departments of Ophthalmology and Neurosurgery, Lithuanian Health Sciences University Hospital (Kaunas, Lithuania).

The participants comprised of 143 patients with a diagnosis of PA. The reference group involved 808 healthy subjects. The reference group was created by taking into consideration the distribution of age and gender in the PA group. Therefore, the median patient age of the control group and the PA group did not differ significantly (P<0.05). Demographic data of the study subjects are presented in Table I.

The inclusion criteria were as follows: Determined and confirmed PA via magnetic resonance imaging (MRI); general good condition of the patient; consent of the patient to take part in the study; age ≥18 years; and no other brain tumours or tumours with other localizations.

All PAs were analysed based on MRI findings. The pre-operative MRI investigations were performed with 1.5T MRI scanners (Siemens MAGNETOM Avanto: Siemens AG, Munich, Germany; 1.5 T Philips ACHIEVA: Philips Healthcare, DA Best, The Netherlands) using a head coil and a standard pituitary scanning protocol, obtaining T1-weighted (T1W) sagittal and coronal and T2W/turbo spin echo coronal pre-contrast images, and T1W coronal and sagittal *gadolinium-enhanced* MR images with the intravenous agent gadodiamide (Omniscan; GE Healthcare Life Sciences, Chalfont, UK). The retrospective analysis of MRI data was conducted by an experienced radiologist. The suprasellar extension and sphenoid sinus invasion by PAs were classified according to Wilson-Hardy classification (Hardy classification, modified by Wilson) (19). The degree of suprasellar and parasellar extension was graded as stages A-E. The degree of sellar floor erosion was graded between I and IV. Grade III, localized sellar destruction, and grade IV, diffuse destruction, were considered to be invasive PAs. The Knosp classification system (4) was used to quantify invasion of the cavernous sinus, in which only grades 3 and 4 define true invasion of the tumour into the cavernous sinus: Grade 0, no cavernous sinus involvement; grades 1 and 2, the tumour pushes into the medial wall of the cavernous sinus, but does not go beyond a hypothetical line extending between the centres of the two segments of the internal carotid artery (grade 1) or it goes beyond such a line, but without passing a line tangential to the lateral margins of the artery itself (grade 2); grade 3, the tumour extends laterally to the internal carotid artery within the cavernous sinus; and grade 4, total encasement of the intracavernous carotid artery.

Table I. Demographic characteristics of patients with PA and reference group subjects.

Group	n	Min/max/median age, years	Females, n (%)
PA	143	19/87/52.5	88 (65.67)
Control	808	20/90/58	510 (63.12)
P-value	-	0.793[a]	0.882

[a]P-value for comparison of the median age between the PA and control groups. PA, pituitary adenoma; min, minimum; max, maximum.

DNA extraction and genotyping. The DNA extraction and analysis of the gene polymorphisms of *SIRT1 rs12778366, FGFR2 rs2981582* and *STAT3 rs744166* were performed at the Laboratory of Ophthalmology at the Institute of Neuroscience of the Lithuanian University of Health Sciences (Kaunas, Lithuania). DNA was extracted from 200 μl venous blood (white blood cells) using a DNA purification kit based on the magnetic beads method (MagJET Genomic DNA kit; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to manufacturer's instructions.

The genotyping of *SIRT1 rs12778366, FGFR2 rs2981582* and *STAT3 rs744166* was performed using the quantitative polymerase chain reaction (qPCR) method with a Rotor-Gene Q Real-Time PCR Quantification system (Qiagen, Inc., Valencia, CA, USA). All 3 single-nucleotide polymorphisms were determined using TaqMan® Genotyping assays (Applied Biosystems; Thermo Fisher Scientific, Inc.), C_1340370_10 (rs12778366), C_2917302_10 (rs2981582) and C_3140282_10 (rs744166), according to the manufacturer's protocols.

The Allelic Discrimination program (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used during the qPCR. The assay was then continued following the manufacturer protocols. The Allelic Discrimination program was completed, and the genotyping results were received. The program determined the individual genotypes according to the fluorescence intensity rate from different detectors: Molecular marker labeled with VIC fluorescent dye was chosen for the X axis and a molecular marker labeled with FAM fluorescent dye was selected for the Y axis. These dy-labeled probes were included in the TaqMan® Genotyping assays.

Statistical analysis. Statistical analysis was performed using SPSS 20.0 software (IBM SPSS, Armonk, NY, USA).

Table II. Frequency of single nucleotide polymorphisms in patients with PA and the control group.

Gene marker	Control group, n (%)	P-value HWE	PA group, n (%)	P-value HWE	χ^2	P-value
SIRT1 rs12778366						
Genotype						
T/T	647 (80.1)	<0.01	116 (81.1)	<0.001	91.139	<0.001
T/C	141 (17.5)[a]		0 (0.0)[a]			
C/C	20 (2.5)[b]		27 (18.9)[b]			
Total	808 (100.0)		143 (100)			
Allele						
T	1,435 (88.8)		232 (81.1)			
C	181 (11.2)		54 (18.9)			
FGFR2 rs2981582						
Genotype						
G/G	336 (41.6)	<0.001	56 (39.2)	<0.001	3.502	0.174
G/A	429 (53.1)		84 (58.7)			
A/A	43 (5.3)		3 (2.1)			
Total	808 (100.0)		143 (100.0)			
Allele						
G	1,101 (68.1)		196 (68.53)			
A	515 (31.9)		90 (31.47)			
STAT3 rs744166						
Genotype						
G/G	154 (19.1)[c]	<0.001	13 (9.1)c	0.354	8.825	0.012
G/A	363 (44.9)		68 (47.6)			
A/A	291 (36.0)		62 (43.4)			
Total	808 (100.0)		143 (100.0)			
Allele						
G	671 (41.5)		94 (32.9)			
A	945 (58.5)		192 (67.1)			

[a]*SIRT1 rs1277836* T/C genotype was significantly less frequent (P<0.001) in the PA group compared with the control group. [b]*SIRT1 rs1277836* C/C genotype was significantly more frequent (P<0.001) in the PA group compared with the control group. [c]*STAT3 rs744166* G/G genotype was significantly less frequent (P=0.003) in the PA group compared with the control group. PA, pituitary adenoma; *SIRT1*, sirtuin 1, *FGFR2*, fibroblast growth factor receptor 2; *STAT3*, signal transducer and activator of transcription 3; HWE, Hardy-Weinberg equilibrium.

The data are presented as absolute numbers with percentages in brackets, and as median with minimum/maximum values. The frequencies of genotypes are presented as percentages.

Hardy-Weinberg analysis was performed to compare the observed and expected frequencies of rs12778366, rs2981582 and rs744166 using the χ^2 test in all groups. The distribution of rs12778366, rs2981582 and rs744166 single-nucleotide polymorphisms (SNPs) in the PA and control groups was compared using the χ^2 test or Fisher's exact test. Binomial logistic regression analysis was performed to estimate the impact of genotypes on PA development. Odds ratios (ORs) and 95% confidence intervals (CIs) are presented. Only statistically significant variables are presented in the tables. The selection of the most suitable genetic model was based on the Akaike Information Criterion (AIC), whereby the best genetic models were those with the lowest AIC values (65). P<0.05 was considered to indicate a statistically significant difference.

Results

Genotype distribution in the PA patients and the control group. The genotyping of *SIRT1 rs12778366, FGFR2 rs2981582* and *STAT3 rs744166* was performed in the PA group and the control group subjects (Table II).

The distribution of analysed SIRT1 genotypes and allele frequencies in the control and PA groups did not match the Hardy-Weinberg equilibrium. The *SIRT1 rs12778366* polymorphism analysis in the overall group revealed differences in the genotype distribution between patients with PA and control group subjects (P<0.001). The genotype T/C was significantly less frequent in the PA group compared with the healthy controls (0 vs. 17.5%; P<0.001) and the genotype C/C was significantly more frequent in the PA group compared with the healthy control group (18.9 vs. 2.5%, respectively; P<0.001) (Table II).

The distribution of analysed *FGFR2* genotypes and allele frequencies in the control and PA groups did not match the

Table III. Frequency of single nucleotide polymorphisms in patients with PA and control group according to gender.

	Control group, n (%)				PA group, n (%)			
Gene marker	Females	Males	P-value HWE	P-value	Females	Males	P-value HWE	P-value
SIRT1 rs12778366								
Genotype								
T/T	407 (79.8)	240 (80.5)	0.811	0.801	71 (80.7)	45 (81.8)	0.866	0.866
T/C	89 (17.5)[a]	52 (17.4)[b]		1.000	0 (0.0)[a]	0 (0.0)[b]		1.00
C/C	14 (2.7)[c]	6 (2.0)[d]		0.518	17 (19.3)[c]	10 (18.2)[d]		0.866
Total	510 (100.0)	298 (100.0)			88 (100.0)	55 (100.0)		
Allele								
T	903 (88.5)	532 (89.3)			142 (80.7)	90 (81.8)		
C	117 (11.5)	64 (10.7)			34 (19.3)	20 (18.2)		
FGFR2 rs2981582								
Genotype								
G/G	218 (42.7)	118 (39.6)	0.675	0.381	39 (44.3)	17 (30.9)	0.197	0.117
G/A	265 (52.0)	164 (55.0)		0.398	48 (54.5)	36 (65.5)		0.224
A/A	27 (5.3)	16 (5.4)		1.0	1 (1.1)	2 (3.6)		0.559
Total	510 (100)	298 (100)			88 (100)	55 (100)		
Allele								
G	701 (68.7)	400 (67.1)			126 (71.6)	70 (63.6)		
A	319 (31.3)	196 (32.9)			50 (28.4)	40 (36.4)		
STAT3 rs744166								
Genotype								
G/G	104 (20.4)[e]	50 (16.8)	0.378	0.207	7 (8.0)[e]	6 (10.9)	0.815	0.563
G/A	229 (44.9)	134 (45.0)		0.986	43 (48.9)	25 (45.5)		0.733
A/A	177 (34.7)	114 (38.3)		0.312	38 (43.2)	24 (43.6)		1.00
Total	510 (100)	298 (100)			88 (100)	55 (100)		
Allele								
G	437 (42.84)	234 (39.3)			57 (32.4)	37 (33.6)		
A	583 (57.2)	362 (60.7)			119 (67.6)	73 (66.4)		

[a]*SIRT1 rs12778366* T/C genotype is significantly less frequent (P<0.001) in PA females compared with control females. [b]*SIRT1 rs12778366* T/C genotype is significantly less frequent (P<0.001) in PA males compared with control males. [c]*SIRT1 rs12778366* C/C genotype is significantly more frequent (P<0.001) in PA females compared with control females. [d]*SIRT1 rs12778366* C/C genotype is significantly more frequent (P<0.001) in PA males compared with control males. [e]*STAT3 rs744166* G/G genotype is significantly less frequent (P=0.004) in PA females compared with control females. PA, pituitary adenoma; *SIRT1*, sirtuin 1; *FGFR2*, fibroblast growth factor receptor 2; *STAT3*, signal transducer and activator of transcription 3; HWE, Hardy-Weinberg equilibrium.

Hardy-Weinberg equilibrium. Statistical analysis did not reveal significant genotype (G/G, G/A and A/A) distribution differences between the control and PA groups: 41.6 vs. 39.2%, 53.1 vs. 58.7%, and 5.3 vs. 2.1%, respectively (P=0.174) (Table II).

The distribution of the analysed *STAT3 rs744166* genotypes and allele frequencies did not match the Hardy-Weinberg equilibrium in the control group, but it did in the group of patients with PA. *STAT3 rs744166* polymorphism analysis in the overall group revealed differences in the genotype distribution between the patients with PA and the control group (P=0.012). The genotype G/G was less frequent in the PA group compared with the healthy controls (9.1 vs. 19.1%, respectively; P=0.003) (Table II).

Genotype distribution in the PA patients and the control group by gender. All 3 SNPs were analysed in the PA and control groups according to gender (Table III). *SIRT1 rs12778366* polymorphism analysis did not revealed any statistically significant differences between females and males with PA in genotype (T/T, T/C and C/C) distribution (80.7, 0 and 19.3% vs. 81.8, 0 and 18.2%, respectively; Table III). Comparing *SIRT1 rs12778366* genotype distribution in healthy females and females with PA, significant differences were revealed. The T/C genotype was less frequently present in females with PA compared with the healthy control females (0 vs. 17.5%, respectively; P<0.001) and C/C was more frequent in PA females compared with healthy females (19.3 vs. 2.7%,

Table IV. Binomial logistic regression analysis in patients with pituitary adenoma and the control group.

Gene	Model	Genotype	OR (95% CI)	P-value	AIC
SIRT1 rs12778366	Co-dominant	T/T	1.000		720.516
		T/C	0 (0.000)	0.995	
		C/C	7.530 (4.087-13.873)	<0.001	
	Recessive	T/T+T/C	1.000		780.895
		C/C	9.171 (4.982-16.881)	<0.001	
	Additive	-	1.584 (1.187-2.115)	0.002	780.214
STAT3 rs744166	Co-dominant	A/A	1.000		801.223
		G/G	0.879 (0.603-1.282)	0.504	
		G/G	0.396 (0.211-0.743)	0.004	
	Recessive	A/A+G/A	1.000		799.670
		G/G	0.425 (0.234-0.771)	0.005	
	Additive	-	0.702 (0.541-0.911)	0.008	801.881

OR, odds ratio; CI, confidence interval; AIC, Akaike Information Criterion; *SIRT1*, sirtuin 1; *STAT3*, signal transducer and activator of transcription 3.

Table V. Binomial logistic regression analysis in patients with pituitary adenoma and control subjects according to gender.

Gene	Gender	Model	Genotype	OR (95% CI)	P-value	AIC
SIRT1 rs12778366	Male	Co-dominant	T/T	1.000		275.783
			T/C	0.000 (0.000)	0.997	
			C/C	8.889 (3.076-25.683)	<0.001	
		Recessive	T/T+T/C	1.000		290.082
			C/C	10.815 (3.748-31.205)	<0.001	
	Female	Co-dominant	T/T	1.000		450.358
			T/C	0.000 (0.000)	0.996	
			C/C	6.961 (3.285-14.750)	<0.001	
		Recessive	T/T+T/C	1.000		474.428
			C/C	8.483 (4.008-17.955)	<0.001	
		Additive	-	1.580 (1.100-2.271)	0.013	497.979
STAT3 rs744166	Female	Co-dominant	A/A	1.000		496.249
			G/A	0.875 (0.542-1.411)	0.583	
			G/G	0.314 (0.135-0.727)	0.007	
		Recessive	A/A+G/A	1.000		494.549
			G/G	0.337 (0.151-0.752)	0.008	
		Additive	-	0.654 (0.469-0.912)	0.012	497.077

OR, odds ratio; CI, confidence interval; AIC, Akaike Information Criterion; *SIRT1*, sirtuin 1; *STAT3*, signal transducer and activator of transcription 3.

respectively; P<0.001). The T/T genotype did not exhibit any significant differences when healthy females and females with PA were compared. When analysing genotype distribution in males, T/C genotype distribution showed statistically significant difference between males with PA and healthy males (0 vs. 17.4%, respectively; P<0.001) and the C/C genotype was more frequent in males with PA compared with the control group (18.2 vs. 2.0%, respectively; P<0.001) (Table III).

FGFR2 rs2981582 polymorphism analysis by gender was performed, but it did not reveal any genotype distribution differences between females and males.

STAT3 rs744166 polymorphism analysis did not reveal any significant differences between females and males with PA in the genotype (G/G, G/A and A/A) distribution (8.0, 48.9 and 43.2% vs. 10.9, 45.5 and 43.6%, respectively; Table III) either. When comparing *STAT3* genotype distribution between healthy

Table VI. Frequency of SNPs in patients with PA and in control group according to PA invasiveness.

Gene marker	Control group, n (%)	P-value HWE	Non invasive PA group, n (%)	P-value HWE	Invasive PA group, n (%)	P-value HWE
SIRT1 rs12778366						
Genotype						
T/T	647 (80.1)	<0.001	47 (81.0)	<0.001	69 (81.2)	<0.001
T/C	141 (17.5)[a,b]		0 (0.0)[a]		0 (0.0)[b]	
C/C	20 (2.5)[c,d]		11 (19.0)[c]		16 (18.8)[d]	
Total	808 (100.0)		58 (100.0)		85 (100.0)	
Allele						
T	1,435 (88.8)		94 (81.0)		138 (81.2)	
C	181 (11.2)		22 (19.0)		32 (18.8)	
FGFR2 rs2981582						
Genotype						
G/G	336 (41.6)[e]	<0.001	16 (27.6)[e,f]	<0.001	40 (47.1)[f]	0.043
G/A	429 (53.1)[g]		42 (72.4)[g,h]		42 (49.4)[h]	
A/A	43 (5.3)		0 (0.0)		3 (3.5)	
Total	808 (100.0)		58 (100.0)		85 (100.0)	
Allele						
G	1,101 (68.1)		74 (63.8)		122 (71.8)	
A	515 (31.9)		42 (36.2)		48 (28.2)	
STAT3 rs744166						
Genotype						
G/G	154 (19.1)[i]	<0.001	9 (15.5)[j]	0.313	4 (4.7)[i,j]	0.031
G/A	363 (44.9)		23 (39.7)		45 (52.9)	
A/A	291 (36.0)		26 (44.8)		36 (42.4)	
Total	808 (100.0)		58 (100.0)		85 (100.0)	
Allele						
G	671 (41.5)		41 (35.3)		53 (31.2)	
A	945 (58.5)		75 (64.7)		117 (68.8)	

[a]*SIRT1 rs12778366* T/C genotype is significantly less frequent (P=0.021) in non-invasive PA compared with the control group. [b]*SIRT1 rs12778366* T/C genotype is significantly less frequent (P<0.001) in invasive PA compared with the control group. [c]*SIRT1 rs12778366* C/C genotype is significantly more frequent (P=0.041) in non-invasive PA compared with the control group. [d]*SIRT1 rs12778366* C/C genotype is significantly less frequent (P<0.001) in invasive PA compared with the control group. [e]*FGFR2 rs2981582* G/G genotype is significantly less frequent (P=0.038) in non-invasive PA compared with the control group. [f]*FGFR2 rs2981582* G/G genotype is significantly more frequent (P=0.024) in invasive PA compared with the non-invasive PA group. [g]*FGFR2 rs2981582* G/A genotype is significantly more frequent (P=0.004) in non-invasive PA compared with the control group. [h]*FGFR2 rs2981582* G/A genotype is significantly less frequent (P=0.009) in invasive PA compared with the non-invasive PA group. [i]STAT3 rs744166 G/G genotype is significantly less frequent (P<0.001) in invasive PA compared with the control group. [j]*STAT3 rs744166* G/G genotype is significantly less frequent (P=0.038) in invasive PA compared with the non-invasive PA group. PA, pituitary adenoma; *SIRT1*, sirtuin 1, *FGFR2*, fibroblast growth factor receptor 2; *STAT3*, signal transducer and activator of transcription 3; HWE, Hardy-Weinberg equilibrium.

females and females with PA, there were significant differences. The *STAT3 rs744166* G/G genotype was less frequently present in PA females compared with healthy control females (8.0 vs. 20.4%, respectively; P=0.004). The *STAT3 rs744166* G/A and A/A genotype distribution did not exhibit any significant differences when healthy females and females with PA were compared. *STAT3 rs744166* analysis between male groups did not reveal any statistically significant differences (Table III).

Binomial logistic regression analysis of the patients with PA and the control group. Binomial logistic regression analysis of the patients with PA and the control group was performed (Table IV). *SIRT1 rs12778366* analysis revealed that there were significant variables in the co-dominant (OR=7.530; 95% CI: 4.087-13.873; P<0.001), recessive (OR=9.171; 95% CI: 4.982-16.881; P<0.001) and additive (OR=1.584: 95 % CI: 1.187-2.115; P=0.002) models of the patients with PA and the control group (Table IV).

FGFR2 rs2981582 analysis did not reveal any significant variables.

STAT3 rs744166 analysis revealed that there were significant variables in the co-dominant (OR=0.396; 95% CI: 0.211-0.743; P=0.004), recessive (OR=0.425; 95% CI:

Table VII. Frequency of single nucleotide polymorphisms in patients with PA and in the control group according to PA recurrences.

Gene marker	Control group, n (%)	P-value HWE	Non-recurrent PA group, n (%)	P-value HWE	Recurrent PA group, n (%)	P-value HWE
SIRT1 rs12778366						
Genotype						
T/T	647 (80.1)	<0.001	91 (81.3)	<0.001	25 (80.6)	<0.001
T/C	141 (17.5)[a,b]		0 (0.0)[a]		0 (0.0)[b]	
C/C	20 (2.5)[c,d]		21 (18.8)[c]		6 (19.4)[d]	
Total	808 (100)		112 (100.0)		31 (100.0)	
Allele						
T	1,435 (88.8)		182 (81.3)		50 (80.6)	
C	181 (11.2)		42 (18.8)		12 (19.4)	
FGFR2 rs2981582						
Genotype						
G/G	336 (41.6)	<0.001	44 (39.3)	<0.001	12 (38.7)	0.067
G/A	429 (53.1)		66 (58.9)		18 (58.1)	
A/A	43 (5.3)		2 (1.8)		1 (3.2)	
Total	808 (100.0)		112 (100.0)		31 (100.0)	
Allele						
G	1,101 (68.1)		154 (68.8)		42 (67.7)	
A	515 (31.9)		70 (31.3)		20 (32.3)	
STAT3 rs744166						
Genotype						
G/G	154 (19.1)[e]	<0.001	7 (6.3)[e,f]	0.083	6 (19.4)[f]	0.305
G/A	363 (44.9)		56 (50.0)		12 (38.7)	
A/A	291 (36.0)		49 (43.8)		13 (41.9)	
Total	808 (100.0)		112 (100.0)		31 (100.0)	
Allele						
G	671 (41.5)		70 (31.3)		24 (38.7)	
A	945 (58.5)		154 (68.8)		38 (61.3)	

[a]*SIRT1 rs12778366* T/C genotype is significantly less frequent (P<0.001) in non-recurrent PA compared with the control group. [b]*SIRT1 rs12778366* T/C genotype is significantly less frequent ([b]P=0.005) in recurrent PA compared with the control group. [c]*SIRT1 rs12778366* C/C genotype is significantly more frequent (P<0.001) in non-recurrent PA compared with the control group. [d]*SIRT1 rs12778366* C/C genotype is significantly more frequent (P=0.047) in recurrent PA compared with the control group. [e]*STAT3 rs744166* G/G genotype is significantly less frequent (P<0.001) in non-recurrent PA compared with the control group. [f]*FGFR2 rs2981582* G/G genotype is statistically more frequent (P=0.036) in recurrent PA compared with non-recurrent PA. PA, pituitary adenoma; *SIRT1*, sirtuin 1, *FGFR2*, fibroblast growth factor receptor 2; *STAT3*, signal transducer and activator of transcription 3; HWE, Hardy-Weinberg equilibrium.

0.234-0.771; P=0.005) and additive (OR=0.702; 95% CI: 0.541-0.911; P=0.008) models of the patients with PA and the control group (Table IV).

Binomial logistic regression analysis in the patients with PA and the control group according to gender was performed (Table V). In the *SIRT1 rs12778366* analysis there were statistically significant variables in the co-dominant (P<0.001) and recessive (P<0.001) models of males. The co-dominant (P<0.001), recessive (P<0.001) and additive (P=0.013) variables were also significant in females.

Binomial logistic regression analysis of *FGFR2 rs2981582* in the patients with PA and in the control group according to gender was performed, but no significant variables were observed.

However, binomial logistic regression analysis of *STAT3 rs744166* in the patients with PA and in the control group according to gender showed statistically significant variables only in the co-dominant (P=0.007), recessive (P=0.008) and additive (P=0.012) models of females (Table V).

Genotype distribution in the control group and the PA patients by different PA subgroups. Analysis of *SIRT1 rs1277836, FGFR2 rs2981582 and STAT3 rs744166* polymorphisms was performed by different PA subgroups (Tables VI-VIII).

The *SIRT1 rs12778366* T/C genotype was less frequently observed in non-invasive, non-recurrent and inactive PA subgroups compared with healthy controls (0 vs. 17.5%, P=0.021; 0 vs. 17.5%, P<0.001; 0 vs. 17.5%, P<0.001, respectively). However, no differences were observed between non-invasive and invasive, non-recurrent and recurrent, and inactive and active PA subgroups (Tables VI-VIII).

Table VIII. Frequency of single nucleotide polymorphisms in patients with PA and in the control group according to PA activity.

Gene marker	Control group, n (%)	P-value HWE	Inactive PA group, n (%)	P-value HWE	Active PA group, n (%)	P-value HWE
SIRT1 rs12778366						
Genotype						
T/T	647 (80.1)	<0.001	48 (76.2)	0.007	68 (85.0)	0.044
T/C	141 (17.5)[a,b]		0 (0.0)[a]		0 (0.0)[b]	
C/C	20 (2.5)[c,d]		15 (23.8)[d]		12 (15.0)[c]	
Total	808 (100.0)		63 (100.0)		80 (100.0)	
Allele						
T	1,435 (88.8)		96 (76.2)		136 (85.0)	
C	181 (11.2)		30 (23.8)		24 (15.0)	
FGFR2 rs2981582						
Genotype						
G/G	336 (41.6)	<0.001	25 (39.7)	<0.001	31 (38.8)	0.005
G/A	429 (53.1)		38 (60.3)		46 (57.5)	
A/A	43 (5.3)		0 (0.0)		3 (3.8)	
Total	808 (100.0)		63 (100.0)		80 (100.0)	
Allele						
G	1,101 (68.1)		88 (69.8)		108 (67.5)	
A	515 (31.9)		38 (30.2)		52 (32.5)	
STAT3 rs744166						
Genotype						
G/G	154 (19.1)[e]	<0.001	6 (9.5)	0.193	7 (8.8)[e]	0.915
G/A	363 (44.9)		34 (54.0)		34 (42.5)	
A/A	291 (36.0)[f]		23 (36.5)		39 (48.8)[f]	
Total	808 (100.0)		63 (100.0)		80 (100.0)	
Allele						
G	671 (41.5)		46 (36.5)		48 (30.0)	
A	945 (58.5)		80 (63.5)		112 (70.0)	

[a]*SIRT1 rs12778366* T/C genotype is significantly less frequent (P<0.001) in inactive PA compared with the control group. [b]*SIRT1 rs12778366* T/C genotype is significantly less frequent (P<0.001) in active PA compared with the control group. [c]*SIRT1 rs12778366* C/C genotype is significantly less frequent (P<0.001) in active PA compared with the control group. [d]*SIRT1 rs12778366* C/C genotype is significantly more frequent (P<0.001) in inactive PA compared with the control group. [e]*FGFR2 rs2981582* G/G genotype is significantly less frequent (P=0.022) in active PA compared with the control group. [f]*FGFR2 rs2981582* A/A genotype is significantly more frequent (P=0.029) in active PA compared with the control group. PA, pituitary adenoma; *SIRT1*, sirtuin 1, *FGFR2*, fibroblast growth factor receptor 2; *STAT3*, signal transducer and activator of transcription 3; HWE, Hardy-Weinberg equilibrium.

Additional analysis revealed that the C/C genotype was more frequent in invasive, recurrent and active PA subgroups compared with the healthy controls (18.8 vs. 2.5%, P=0.041; 19.4 vs. 2.5%, P=0.047; 15.0 vs. 2.5%, P<0.001, respectively; Tables VI, VII and VIII).

The *FGFR2 rs2981582* G/G genotype was less frequently observed in the non-invasive PA subgroup compared with the healthy controls (27.6 vs. 41.6%, respectively; P=0.038), but the G/A genotype was more frequently observed in the non-invasive PA subgroup compared with the control group (72.4 vs. 53.1%, respectively; P=0.004) and the invasive PA subgroup (72.4 vs. 49.4% respectively; P=0.009) (Table VI).

Statistical analysis was performed to evaluate the *FGFR2 rs2981582* association with PA activity and recurrence (Tables VII and VIII). This analysis did not reveal any association between SNP and active or non-active PA, and PA without recurrence or with recurrence.

The *STAT3 rs744166* G/G genotype was less frequently observed in invasive, non-recurrent and active PA subgroups compared with healthy controls (4.7 vs. 19.1%, P<0.001; 6.2 vs. 19.1%, P<0.001; 8.8 vs. 19.1%, P=0.022, respectively).

The *STAT3 rs744166* A/A genotype was more frequent in the active PA subgroup compared with the control group (48.8 vs. 36.0%, respectively; P=0.029). There were

Table IX. Binomial logistic regression analysis in non-invasive and invasive PA, and the control group.

Gene	PA subgroup	Model	Genotype	OR (95% CI)	P-value	AIC
SIRT1 rs12778366	Non-invasive	Co-dominant	T/T	1.000		390.145
			T/C	0.000 (0.000)	0.996	
			C/C	7.571 (3.426-16.734)	<0.001	
		Recessive	T/T+T/C	1.000		406.092
			C/C	9.221 (4.175-20.367)	<0.001	
		Additive	-	1.649 (1.065-2.554)	0.025	425.148
	Invasive	Co-dominant	T/T	1.000		509.448
			T/C	0.000 (0.000)	0.996	
			C/C	7.501 (3.715-15.147)	<0.001	
		Recessive	T/T+T/C	1.000		533.418
			C/C	9.136 (4.528-18.434)	<0.001	
		Additive	-	1.616 (1.120-2.330)	0.010	459.515
FGFR2 rs2981582	Non-invasive	Co-dominant	G/G	1.000		419.170
			G/A	2.056 (1.136-3.721)	0.017	
			A/A	0.000 (0.000)	0.998	
		Dominant	G/G	1.000		425.028
			G/A+A/A	1.869 (1.033-3.380)	0.039	
		Over-dominant	G/G+A/A	1.000		420.961
			G/A	2.319 (1.283-4.193)	0.005	
STAT3 rs744166	Invasive	Co-dominant	A/A	1.000		553.313
			G/A	1.002 (0.630-1.595)	0.993	
			G/G	0.210 (0.073-0.601)	0.004	
		Recessive	A/A+G/A	1.000		551.313
			G/G	0.210 (0.076-0.581)	0.003	
		Additive	-	0.651 (0.467-0.908)	0.011	558.771

PA, pituitary adenoma; *SIRT1*, sirtuin 1; *FGFR2*, fibroblast growth factor receptor 2; *STAT3*, signal transducer and activator of transcription 3; OR, odds ratio; CI, confidence interval; AIC, Akaike Information Criterion.

differences between non-invasive and invasive, non-recurrent and recurrent PA subgroups as well, with the exception of comparing inactive and active PA. The *STAT3 rs744166 G/G* genotype was more frequent in non-invasive PA compared with invasive PA (15.5 vs. 4.7%, respectively; P=0.038) and in recurrent PA group comparing to non-recurrent PA (19.4 vs. 6.2%, respectively; P=0.036) (Tables VI-VIII).

Binomial logistic regression analysis of the control group and the PA patients by different PA subgroups. Binomial logistic regression analysis in the non-invasive PA, invasive PA and control groups was performed (Table IX). Analysing the *SIRT1* polymorphism in non-invasive PA group and control group this analysis showed that the co-dominant (P<0.001), recessive (P<0.001) and additive (P=0.025) variables were significant. Binomial logistic regression analysis in the patients with invasive PA and the control group revealed significance of the same co-dominant (P<0.001), recessive (P<0.001) and additive (P=0.010) variables (Table IX).

Binomial logistic regression analysis of *FGFR2 rs2981582* in the non-invasive PA and control groups showed that the co-dominant (P=0.017), dominant (P=0.039) and over-dominant (P=0.005) variables were significant, but this analysis in the patients with invasive PA and the control group did not reveal any significance of these models.

Binomial logistic regression analysis of *STAT3 rs744166* was also performed (Table IX). The analysis showed that the co-dominant (P=0.004), recessive (P=0.003) and additive (P=0.011) variables were statistically significant only in the invasive PA and control groups (Table IX).

Binomial logistic regression analysis in the inactive PA and control groups, and in the active PA and control groups, was performed for all 3 SNPs (Table X).

Inactive PA group analysis of *SIRT1 rs12778366* showed that the co-dominant (P<0.001), recessive (P<0.001) and additive (P<0.001) variables were significant. The analysis of the active PA group revealed significance in the co-dominant (P<0.001) and recessive (P<0.001) models (Table X).

Binomial logistic regression analysis of *FGFR2 rs2981582* was performed in the inactive PA, active PA and control groups, but this analysis did not reveal significance in these models.

STAT3 rs744166 analysis in the inactive PA group showed that there were no significant variables. Analysing the active PA group, the present study revealed significance

Table X. Binomial logistic regression analysis in inactive and active PA, and control groups.

Gene	PA subgroup	Model	Genotype	OR (95% CI)	P-value	AIC
SIRT1 rs12778366	Inactive	Co-dominant	T/T	1.000		402.990
			T/C	0.000 (0.000)	0.996	
			C/C	10.109 (4.868-20.996)	<0.001	
		Recessive	T/T+T/C	1.000		419.306
			C/C	12.312 (5.933-25.552)	<0.001	
		Additive	-	2.045 (1.388-3.015)	<0.001	444.842
	Active	Co-dominant	T/T	1.000		497.635
			T/C	0.000 (0.000)	0.996	
			C/C	5.709 (2.675-12.183)	<0.001	
		Recessive	T/T+T/C	1.000		521.245
			C/C	6.953 (3.260-14.828)	<0.001	
STAT3 rs744166	Active	Co-dominant	A/A	1.000		535.474
			G/A	0.669 (0.430-1.135)	0.148	
			G/G	0.339 (0.148-.0776)	0.010	
		Dominant	A/A	1.000		536.765
			G/A+G/G	0.592 (0.373-0.939)	0.026	
		Recessive	A/A+G/A	1.000		535.576
			G/G	0.407 (0.184-0.902)	0.027	
		Additive	-	0.622 (0.442-0.887)	0.007	533.914

PA, pituitary adenoma; *SIRT1*, sirtuin 1; *STAT3*, signal transducer and activator of transcription 3; OR, odds ratio; CI, confidence interval; AIC, Akaike Information Criterion.

Table XI. Binomial logistic regression analysis in non-recurrent and recurrent PA and control groups.

Gene	PA subgroup	Model	Genotype	OR (95% CI)	P-value	AIC
SIRT1 rs12778366	Non-recurrent	Co-dominant	T/T	1.000		614.042
			T/C	0.000 (0.000)	0.995	
			C/C	7.465 (3.896-14.307)	<0.001	
		Recessive	T/T+T/C	1.000		645.812
			C/C	9.092 (4.748-17.411)	<0.001	
		Additive	-	1.592 (1.153-2.199)	0.005	678.223
	Recurrent	Co-dominant	T/T	1.000		247.718
			T/C	0.000 (0.000)	0.996	
			C/C	7.764 (2.868-21.019)	<0.001	
		Recessive	T/T+T/C	1.000		255.407
			C/C	9.456 (3.495-25.586)	<0.001	
STAT3 rs744166	Non-recurrent	Co-dominant	A/A	1.000		673.559
			G/A	0.916 (0.606-1.385)	0.678	
			G/G	0.270 (0.119-0.610)	0.002	
		Recessive	A/A+G/A	1.000		671.731
			G/G	0.283 (0.129-0.621)	0.002	
		Additive	-	0.653 (0.487-0.876)	0.005	677.047

PA, pituitary adenoma; *SIRT1*, sirtuin 1; *STAT3*, signal transducer and activator of transcription 3; OR, odds ratio; CI, confidence interval; Akaike Information Criterion.

in the co-dominant (P=0.010), dominant (P=0.026), recessive (P=0.027) and additive (P=0.007) models (Table X).

Additional binomial logistic regression analysis of SNPs was performed in non-recurrence, recurrence and control

groups. The analysis in the non-recurrent PA and control groups showed that the co-dominant (P<0.001), recessive (P<0.001) and additive (P=0.005) variables were statistically significant (Table XI). In the analysis of PA with recurrence, the co-dominant (P<0.001) and recessive (P<0.001) variables were also significant.

FGFR2 rs2981582 polymorphism analysis did not show any statistical significance.

Binomial logistic regression analysis of *STAT3 rs744166* in the non-recurrent and recurrent PA groups, and the control group was performed. This revealed that in the non-recurrent PA and control groups, the co-dominant (P=0.002), recessive (P=0.002) and additive (P=0.005) variables were significant (Table XI). In the analysis of PA with recurrence there were no statistically significant variables.

Discussion

The impact of *SIRT1, FGFR2* and *STAT3* gene polymorphisms on the development of various tumours has been analysed in numerous studies (38,42-52,60,63-65), but no studies have investigated the associations with PA development, invasiveness, activity and recurrence.

A study conducted by Rizk *et al* (37) investigated *SIRT1* gene single nucleotide polymorphism rs12778366 in patients with breast cancer, revealing that the *SIRT1 rs12778366* T/T genotypes were more frequent, exhibited higher SIRT1 levels than the C/C and C/T genotypes, and were associated with histological grade and lymph node status. The T allele frequency was higher in patients with breast cancer compared with that in normal subjects.

The present study was the first to assess the association between *SIRT1 rs12778366* and PA. It was found that the T/C genotype was less frequent in the PA group compared with the healthy controls (0 vs. 17.5%, respectively; P<0.001) and that the C/C genotype was more frequent in the PA group compared with the healthy control group (18.9 vs. 2.5%, P<0.001).

Numerous studies have investigated the *FGFR2 rs2981582* polymorphism in breast cancer patients, and have provided controversial data on the impact of this polymorphism on tumour development. Chen *et al* (43) revealed that the G/A and A/A genotypes of *FGFR2* rs2981582 were associated with lower mammographic density and a reduced risk of breast cancer, and Butt *et al* (42) revealed a statistically significant association between the *FGFR2 rs2981582* A/A genotype and breast cancer risk. Shan *et al* (66) also revealed that patients with the A/A genotype of *FGFR2 rs2981582* exhibited an increased risk of breast cancer, while Ledwoń *et al* (47) revealed that the *rs2981582* SNP showed significant association with the familial and sporadic types of breast cancer. On the basis of these findings, the present study aimed to examine whether the polymorphism in the *FGFR2* promoter may affect the risk of PA development, activity, recurrence or invasiveness. No differences in genotype (G/G, G/A and A/A) distribution were observed between the control and PA groups (41.6 vs. 39.2%, 53.1 vs. 58.7%, and 5.3 vs. 2.1%, respectively; P=0.174). No significant differences were observed between genotype distribution according to gender, PA activity, invasiveness or recurrence.

Several studies have analysed the *STAT3 rs744166* polymorphism in association with various types of tumour, but none have investigated the association between *STAT3 rs744166* and PA. Rocha *et al* (61) reported that the *rs744166* polymorphic G allele was associated with gastric cancer, and a significantly decreased risk of non-small cell lung cancer was observed for carriers of *STAT3 rs744166* in a study by Jiang *et al* (64). The present study demonstrated the differences in the distribution of the *STAT3 rs744166* polymorphism between patients with PA and control group subjects (P=0.012). The G/G genotype was less frequent in the PA group compared with the healthy controls (9.1 vs. 19.1%, respectively; P=0.003). Analysis in different PA subgroups showed that the *STAT3 rs744166* G/G genotype was more frequent in non-invasive PA compared with invasive PA (15.5 vs. 4.7%; P=0.038) and in recurrent PA compared with the non-recurrent PA (19.4 vs. 6.2%, respectively; P=0.036).

Overall, the present study demonstrated that the SNPs *SIRT1 rs12778366* and *STAT3* require replication in future larger studies, particularly with increased sample sizes to confirm the association of SIRT1 and STAT3 in patients with PA.

Acknowledgements

The present study received funding from the Research Council of Lithuania (grant no. MIP-008/2014).

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SUMMARY

Introduction

Pituitary adenoma (PA) is an intracranial tumor arising from the anterior pituitary with the reported prevalence rate of 16.7%. The pituitary gland is located in a dural bag attached to the inferior aspect of the diaphragm of the sella and surrounded by venous spaces that correspond laterally to the cavernous sinuses. PAs can easily expand laterally, because the medial wall of the cavernous sinus is a lateral part of the thin dural bag, so 6–10% of PAs can involve the cavernous sinus. The optic chiasm is directly above the pituitary gland and, when the tumor grows beyond the sella, it frequently causes visual disturbances such as a decrease of visual acuity, visual field defects, and impaired color vision and contrast sensitivity due to chiasmal compression. PA can be the cause of retinal nerve fiber layer (RNFL) thinning which reflects axonal degeneration caused by compression of the anterior visual pathways and can be objectively evaluated by optical coherence tomography (OCT) by quantifying the thickness of the RNFL.

It is important to evaluate the tumor's invasiveness because it can influence the management and prognosis of PA. PA is a disease of multifactorial etiology, the occurrence of which is influenced by various genetic factors, hormonal stimulation, growth factors, etc. Recently, a great attention in the PA pathogenesis has been drawn to the search for new epigenetic and genetic factors. Several studies have analyzed the *MMP-2* rs24386, *MMP-9* rs3918242, *STAT3* rs744166, *SIRT1* rs12778366, *FGFR2* rs2981582 polymorphisms in association with various types of tumors, but no studies have investigated the associations with PA development, invasiveness and recurrence. This is the only study that analyzed the IL-17A impact on the PA invasiveness in Lithuania. Numerous studies have shown that Ki-67 labeling index (LI) is associated with tumor invasiveness, but results remain controversial.

The aim of the present study was to identify possible genes involved in PA tumorigenesis, which may serve as potential diagnostic and prognostic molecular markers.

Aim and objectives of the study

Aim of the study

To determine pituitary adenoma molecular markers and associations with visual functions.

Objectives of the study

1. To evaluate associations between results of ophthalmological evaluation and pituitary adenoma clinical and morphological characteristics.
2. To determine if the Ki-67 LI reflects invasiveness of pituitary adenoma, and to evaluate IL-17A concentration in blood serum of pituitary adenoma patients.
3. To determine the association between *MMP-2* rs243865 and *MMP-9* rs3918242 gene polymorphisms, and pituitary adenoma invasiveness and recurrence.
4. To determine the association between *FGFR2* rs2981582, *SIRT1* rs12778366, *STAT3* rs744166 gene polymorphisms and pituitary adenoma development.

Methods

Permission to undertake the present study (Number P2-9/2003) was obtained from the Biomedical Research Ethics Committee of Lithuanian Health Sciences University (Kaunas, Lithuania). The study was conducted in the Departments of Ophthalmology, Endocrinology and Neurosurgery, Lithuanian Health Sciences University Hospital (Kaunas, Lithuania).

Ophthalmological evaluation

Non-corrected and the best-corrected visual acuity (VA) (measured in decimals from 0.1 to 1.0) was evaluated using Landolt rings (C optotypes) by Snellen test types at a 5 meter distance from the chart. Intraocular pressure measurement, biomicroscopy and fundoscopy were performed in order to assess corneal and lenticular transparency, and to evaluate the eye fundus. The subjects' pupils were dilated with 1% of tropicamide. Standard automated perimetry was conducted using a visual field analyzer (Humphrey Field Analyser, Model 745i, Carl Zeiss Meditec Inc. Dublin, CA, USA). The visual field test routinely assesses only the central 24–30°, however, we performed Full Field Screening (135 points, 87 degrees temporally). In this test, the patient needs to fixate on a central point with each eye separately, while a light of variable intensity is flashed in the peripheral field of vision. The patient has to acknowledge the flash of light by pressing a button. VF testing was considered unreliable if the fixation losses, false negative or false positive errors exceeded 20%.

RNFL thickness was analyzed with spectral domain OCT (RS 3000 Advance Nidec Co., Japan) after pupil dilation. Fundus surface images were captured with the confocal laser scanning using a near-infrared light source

with a wavelength of 785 nm. Cross-sectional images of the retina were captured with the optical interferometer using an infrared light source with a wavelength of 880 nm. OCT image capture mode was a disk circle mode: the patient's fundus was scanned circularly around the optic disk in the order "Temporal", "Superior", "Nasal", and "Inferior" to obtain OCT image. Peripapillary RNFL average thickness and each quadrant thickness were calculated.

Contrast sensitivity was measured by employing a Ginsburg Box, VSCR-CST-6500 with a FACT chart at photopic (in the daytime, 85 cd/m^2) and mesopic (in the nighttime, 3 cd/m^2) luminance with and without glare at 5 standard spatial frequencies: 1.5, 3, 6, 12, and 18 cycles per degree.

The F-M 100 hue test requires the arrangement of colored caps of similar lightness and saturation between the two fixed caps (one at the beginning of the tray and one at the end) in order to form a consistent transition of tones between the fixed caps. The test consists of 85 colored caps, numbered on the back and arranged in four trays. The result is estimated by the total number of differences between the number of the color sample chosen by a subject and the number of the color sample actually belonging to the position. In each group the average number of errors is assessed. The color contrast sensitivity is considered to be very high (when the number of mistakes is up to 20), normal (up to 100), or impaired (more than 100).

In the maximum color contrast sensitivity test (MCCS) the subject's task was to correctly determine the direction of a bar in the circle and indicate it by pressing a corresponding button. If the direction was unclear, a blank button was pressed. Each time the button was pressed, a blank screen appeared, and then, after one second, a circle with a randomly chosen bar direction was presented. If the direction of the bar in the circle was chosen incorrectly, its color was automatically highlighted. After the correct choice of the direction of the bar, the intensity of its color was automatically dulled; due to the change in intensity of the bar, the brightness of the circle's background appeared to change. The first correct answer after a series of incorrect answers or the first incorrect answer after a series of correct answers was accepted as the subject's maximum sensitivity to the target color of the bar. When this maximum sensitivity was determined, the color of the bar was changed, and the test was started again. The bar was presented in a total of six colors: red, green, blue, teal violet, and yellow. Once a subject's sensitivity to all these colors had been assessed, all findings were recorded in a database, and the results of the test were presented in a result window. The luminance of the grey background of the monitor was 350 cd/m^2. The surrounding area luminance was 400 cd/m^2.

Brain imaging

All pituitary adenomas were analyzed based on MR imaging findings. The preoperative MRI investigations were performed with 1,5 T MRI scanners (Siemens MAGNETOM Avanto, 1,5 T Philips ACHIEVA) using a head coil and a standard pituitary scanning protocol, obtaining T1W sagittal and coronal and T2W/TSE coronal pre-contrast images, and T1W coronal and sagittal Gadolinium-enhanced MR images with the intravenous agent gadodiamide (Omniscan, GE Healthcare). The retrospective analysis of MRI data was conducted by an experienced radiologist. The coronal T2W/TSE sequence was chosen as a standard for the best optic chiasm resolution. The optic chiasm thickness values were obtained by measuring the vertical diameter on the right side, left side and the middle part. The deformations or signal changes of the optic chiasm were evaluated and documented. The distance between the superior margin of the tumor and the inferior surface of the optic chiasm was measured as well.

The suprasellar extension and sphenoid sinus invasions by PA were classified according to Hardy classification, modified by Wilson. The degree of suprasellar and parasellar extension was graded as stages A–E. The degree of sellar floor erosion was graded as grades I–IV. Grades I–II mean that sellar floor is intact and are considered as non-invasive PA; grade III shows localized sellar perforation, and grade IV shows diffuse destruction of sellar floor, which are the signs of invasive PA. Knosp classification system was used to quantify the invasion of the cavernous sinus: grade 0, no involvement of cavernous sinus, represents the normal condition; grades 1 and 2, the tumor pushes into the medial wall of the cavernous sinus but does not go beyond a hypothetical line extending between the centers of the two segments of the internal carotid artery (grade 1); or it goes beyond such a line but without passing a line tangent to the lateral margins of the artery itself (grade 2); grade 3, the tumor extends laterally to the internal carotid artery within the cavernous sinus; grade 4, total encasement of the intracavernous carotid artery. Thus, grade 3 and 4 pituitary tumors were considered to be invasive.

DNA extraction and genotyping

The DNA extraction and analysis of the gene polymorphism of *MMP-9* were carried out at the Laboratory of Molecular Cardiology at the Institute of Cardiology of the LUHS for control group and at the Laboratory of Ophthalmology at the Institute of Neuroscience of the LUHS for the PA patient group. DNA was extracted from 200 μL venous blood (white blood cells) using a DNA purification kit based on the magnetic beads method

(*MagJET Genomic DNA Kit*, Thermo Scientific) or the silica-based membrane technology utilizing a genomic DNA extraction kit (*GeneJET Genomic DNA Purification Kit,* Thermo Scientific), according to the manufacturer's recommendations.

The genotyping of *MMP-2* (-1306 C/T) (rs243865), *MMP-9* (-1562 C/T), *SIRT1* (rs12778366), *FGFR2* (rs2981582) and *STAT3* (rs744166) was carried out using the real-time polymerase chain reaction (PCR) method. TaqMan® SNP Genotyping Assays (Applied Biosystem,USA) for *MMP-2* (-1306 C/T) (rs243865), *SIRT1* (rs12778366), *FGFR2* (rs2981582) and *STAT3* (rs744166), as well as primers and fluorescently labeled allele specific probes for *MMP-9* (-1562 C/T) (rs3918242) (Applied Biosystems, USA) were used to determine the genotypes. The genotyping of *MMP-2* (-1306 C/T) and *MMP-9* (-1562 C/T) was performed using the 7900HT real-time PCR quantification system (Applied Biosystems, USA), and the genotyping of *SIRT1* (rs12778366), *FGFR2* (rs2981582) and *STAT3* (rs744166) was performed using a Rotor-Gene Q Real-Time PCR Quantification system (Qiagen, Inc., Valencia, CA, USA).

The real-time PCR reaction mixtures were prepared according the manufacturer's protocols and the Allelic Discrimination program (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used during the qPCR. The program determined the individual genotypes according to the fluorescence intensity rate from different detectors: molecular marker labeled with VIC fluorescent dye was chosen for the X axis and a molecular marker labeled with FAM fluorescent dye was selected for the Y axis.

IL-17A

IL-17A analysis was performed in the Department of Laboratory Medicine, Lithuanian Health Sciences University Hospital (Kaunas, Lithuania). The serum IL-17A level was measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions („*ThermoFisher Scientific Human IL17A ELISA Kit*").

Ki-67 labeling index

The Ki-67 labeling index (LI) was obtained by performing an immunohistochemical analysis with the monoclonal antibody (clone SP6; Spring Bioscience Corporation). The Ki-67 LI was defined as the percentage of positive staining tumor cells.

Statistical analysis

Statistical analysis was performed using the SPSS / W 23.0 software (Statistical Package for the Social Sciences for Windows, Inc., Chicago, Illinois, USA). Normality of the distribution was tested with Shapiro-Wilk and Kolmogorov-Smirnov statistics. Kruskal-Wallis and Mann-Whitney U test was conducted to compare non parametric values. The data are presented as absolute numbers with percentages in brackets and as median with min and max values. Hardy-Weinberg analysis was performed to compare the observed and expected frequencies of *MMP-9* using the χ^2 test in all groups. The distribution of genotypes of *MMP-9, MMP-2, SIRT1, FGFR2* and *STAT3* SNPs in the PA and control groups was compared using the χ^2 test or the Fisher exact test. Binomial logistic regression analysis was performed to estimate the impact of genotypes on PA development. Odds ratios and 95% confidence intervals are presented. The selection of the best genetic model was based on the Akaike Information Criterion (AIC); therefore, the best genetic models were those with the lowest AIC values. Differences were considered statistically significant when $p < 0.05$.

Results

PA characteristics

Seventy seven patients diagnosed with pituitary adenoma by MRI were examined. Forty-six patients (59.7%) were diagnosed with invasive PA and 31 patients (40.3%) with noninvasive PA. Recurrant PA was found in 12 patients (15.6%). Suprasellar extension of PA was diagnosed in 55 patients (71.4%). Paraintracranial extension was diagnosed only in 10 patients (13.0%). Paracavernous extension was diagnosed in 56 persons (73.7%), true cavernous sinus invasion (grades 3–4 by Knosp classification) was found in 26 PA patients (33.8%). Sphenoid extension (grade III-IV) was diagnosed in 40 PA patients (51.9%).

Optic chiasm evaluation

The optic chiasm and its contact with the pituitary adenoma were evaluated in 73 MR images of PA patients. Four images could not be evaluated because of the bad MRI quality. The median of the height of the right side of chiasm was found to be 2.0 mm (min 0.1; max 3.7), the left side 2.0 mm (min 0; max 4.0), and the middle part median 1.7 mm (min 0.1; max 3.7). The median of the distance between the optic chiasm and the pituitary adenoma was 0 mm (min 0; max 7.6).

Visual acuity and visual field changes

Median of VA in PA patients was found to be 0.95 (min 0; max 1.0). VA in the group with suprasellar PA extension (mean rank 71.40) and without it (mean rank 92.76) differed statistically significantly ($p = 0.004$). A weak positive correlation was found between the height of the optic chiasm right side, middle part, left side and visual acuity ($r = 0.349$; 0.276; 0.307), ($p < 0.001$).

Normal visual field was found in 82 eyes (53.2%), 13 eyes (18.4%) presented with partial temporal hemianopy, 51 (33.1%) eyes with complete hemianopy and six eyes (3.9%) with concentric visual field narrowing. Two eyes could not be examined because of blindness.

Contrast sensitivity and color vision disturbances

Functional acuity contrast sensitivity test results showed a significant decrease in the nighttime and daytime conditions with and without glare in low, medium and high spatial frequencies (cycles per degree) in patients with PA.

In the control group the average number of errors of the F-M 100 test was 87.39 ± 24.106, while in the group of patients with PA it was 201.95 ± 06.071 ($p < 0.001$). The F-M 100 test showed better results in patients with non-recurrent PA compared to the patients who had the recurrence of PA (the errors score of 63.62 (mean rank) vs. 84.72 (mean rank), respectively; $p=0.03$), and in patients with noninvasive PA compared to invasive PA (the errors score of 54.88 (mean rank) vs. 72.76 (mean rank), respectively; $p=0.041$).

The average error score of MCCS test was 1.33 ± 0.649 in the control group, and 3.806 ± 3.425 in the group with PA ($p < 0.001$). There was no significant difference between the group with recurrent PA and the group with non-recurrent PA (63.03 vs. 66.33 (mean rank), respectively; $p = 0.72$), and between the group with noninvasive PA and the group of invasive PA (60.87 vs. 65.41 (mean rank), respectively; $p=0.49$). There was a significant increase in the error scores of MCCS test for PA patients with suprasellar extension compared with PA patients without suprasellar extension (73.95 vs. 39.29 (mean rank), respectively; $p<0.001$).

Retinal nerve fiber layer thickness changes

RNFL thickness around the optic nerve disk measured preoperatively was reduced significantly in all four quadrants in PA patients compared with the control group ($p < 0.001$). The temporal RNFL thickness showed the

strongest positive correlation with the distance between optic chiasm and PA ($r = 0.401$, $p < 0.001$). The chiasmal right side, middle part, and left side heights correlated significantly with the RNFL thickness in all quadrants ($p < 0.05$). RNFL thickness in PA patients with suprasellar extension was reduced significantly only in the temporal quadrant compared with the patients without suprasellar extension ($p=0.001$).

Ki-67 labeling index

Immunohistochemistry for Ki-67 revealed a LI 1% in 49.3% of patients with PA, Ki-67 LI 1% in 24.6% and Ki-67 LI > 1% in 26.1% of patients. Analysis showed statistical significance in relation to tumor invasiveness ($p = 0.039$).

The concentration of IL-17 A in blood serum

Median serum IL-17A level in the PA patients was 42.12 (min 1.95; max 76.80) and 8.19 (min 0.39; max 74.57) in the control group ($p < 0.001$), but the analysis did not show any statistical significance comparing different types of PA ($p > 0.05$).

***MMP-2* gene polymorphism**

MMP-2 (-1306 C/T) gene polymorphism analysis in the overall group has not revealed any genotype distribution differences between patients with PA and control group patients ($p > 0.05$). *MMP-2* (-1306 C/T) C/T genotype was more frequently present in PA females compared to healthy females (49.1% vs. 33.66%; $p = 0.041$). Subjects with the *MMP-2* (-1306 C/T) C/T genotype had 3-fold increased risk of noninvasive pituitary adenoma compared with those with the C/C genotype, and 2.8-fold increased risk compared with those with C/C + T/T genotypes. Subjects with the C/T + T/T genotypes had 2.7-fold increased risk of noninvasive pituitary adenoma compared with those with the C/C genotype.

***MMP-9* gene polymorphism**

The *MMP-9* (-1562 C/T) C/C genotype was more frequent in PA group than in healthy controls (81.4% vs. 64.6%, $p = 0.002$); C/C genotype was more frequently present in PA females compared to healthy control females (81.5% vs. 64.6%, $p = 0.018$) as well. *MMP-9* (-1562 C/T) C/C genotype was frequently observed for all subgroups: noninvasive, invasive and nonrecurrence PA compared to healthy controls (81.8% vs. 64.6%, $p = 0.021$; 81.0% vs. 64.6%, $p = 0.041$; 81.8% vs. 64.6%, $p = 0.005$; respecti-

vely). Subjects with the *MMP-9* (-1562 C/T) T/T genotype had 3.2-fold increased risk of invasive pituitary adenoma compared to those with the C/C + C/T genotypes.

FGFR2 rs2981582, *SIRT1* rs12778366, *STAT3* rs744166 gene polymorphisms

FGFR2 rs2981582 G/G genotype was less frequently observed in non-invasive PA subgroup compared to healthy controls (27.6% vs. 41.6%, p = 0.038), but the G/A genotype was more frequently observed in the non-invasive PA subgroup compared with the control group (72.4 vs. 53.1%, respectively; p = 0.004) and the invasive PA subgroup (72.4 vs. 49.4% respectively; p = 0.009).

We found that *SIRT1* rs12778366 genotype T/C was less frequent in PA group than in healthy controls (0% vs. 17.5%, p < 0.001), and the genotype C/C was more frequent in PA group comparing to healthy controls group (18.9% vs. 2.5%, p < 0.001). Subjects with the *SIRT1* C/C genotype had a more than 7-fold increased risk of PA compared with those with T/T genotype, and 9-fold increased risk of PA compared with the variant T/T + T/C genotypes.

Analysis demonstrated the differences in distribution of genotypes of *STAT3* rs744166 polymorphism between patients with PA and control group subjects (p = 0.012). The genotype G/G was less frequent in PA group than in healthy controls (9.1% vs. 19.1%, p = 0.003). Analysis in different PA subgroups showed that *STAT3* rs744166 G/G genotype was more frequent in non-invasive PA comparing to invasive PA (15.5% vs. 4.7%; p = 0.038), and in recurrent PA comparing to not recurrent PA (19.4% vs. 6.2%; p = 0.036).

The combined genotype *SIRT1* T/T + *FGFR2* G/G + *STAT3* G/G had 3.5-fold increased risk of PA, while the combined genotype *SIRT1* T/T + *STAT3* G/G had 2.7-fold increased risk of PA.

Conclusions

1. The ophthalmological evaluation showed the following:
    - There was observed a significant decrease of functional acuity contrast sensitivity in patients with PA.
    - The F-M 100 test results were worse for patients with recurrent PA and for patients with invasive PA. There was a significant increase in the error scores of MCCS test for PA patients with suprasellar extension.
    - RNFL thickness around the optic nerve disk measured preoperatively was reduced significantly in all four quadrants

in PA patients. RNFL thickness in PA patients with suprasellar extension was reduced significantly only in the temporal quadrant compared with the patients without suprasellar extension.

2. Analysis revealed statistically significantly higher Ki-67 labeling index in invasive compared to noninvasive pituitary adenomas.
   Median serum IL-17A level in the PA patients was higher than in the control group.

3. Subjects with the *MMP-2* (-1306 C/T) C/T genotype had 3-fold increased risk of noninvasive pituitary adenoma compared with those with the C/C genotype, and 2.8-fold increased risk compared with those with C/C + T/T genotypes. Subjects with the C/T + T/T genotypes had 2.7-fold increased risk of noninvasive pituitary adenoma compared with those with the C/C genotype.
   Subjects with the *MMP-9* (-1562 C/T) T/T genotype had 3.2-fold increased risk of invasive pituitary adenoma compared to those with the C/C + C/T genotypes.

4. Subjects with the *SIRT1* C/C genotype had a more than 7-fold increased risk of pituitary adenoma compared with those with T/T genotype, and 9-fold increased risk of pituitary adenoma compared with the variant T/T + T/C genotypes. The combined genotype *SIRT1* T/T + *FGFR2* G/G + *STAT3* G/G had 3.5-fold increased risk risk of pituitary adenoma, while the combined genotype *SIRT1* T/T + *STAT3* G/G had 2.7-fold increased risk of pituitary adenoma.

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PADĖKA

Dėkoju mokslinei vadovei prof. Daliai Žaliūnienei už pagalbą, pasitikėjimą ir šiltą bendravimą visos doktorantūros metu.

Noriu padėkoti visam LSMUL Kauno klinikų Akių ligų klinikos, taip pat Neurochirurgijos, Endokrinologijos, Laboratorinės medicinos ir Patologinės anatomijos klinikų kolektyvui už pagalbą tiriant ligonius.

Ypatingai noriu padėkoti už pagalbą ir be galo daug sugaišto laiko gydytojai radiologei Silvijai Jakštienei bei gydytojai patologei Ingai Gudinavičienei. Taip pat dėkoju genetikei Alvitai Vilkevičiūtei už bendradarbiavimą atliekant imunogenetinius tyrimus.

Atskiras ačiū visam LSMU MA Mokslo centro kolektyvui ir jo vadovei prof. Ingridai Ulozienei už visapusišką pagalbą, vertingus patarimus bei nuoširdų bendravimą.

Ačiū visiems draugams, ypač Rasai, už pagalbą ir supratingumą visos doktorantūros metu.

Taip pat esu dėkinga tėvams Vandai ir Algiui Bielskams už palaikymą ir skatinimą siekti užsibrėžtų tikslų.

Didžiausia padėka vyrui Arimantui, sūnui Daumantui ir dukrytei Aistei už pagalbą ir kantrybę visą šį laiką! Ačiū!