Oznaka poročila: ARRS-RPROG-ZP-2014/65

ZAKLJUČNO POROČILO O REZULTATIH RAZISKOVALNEGA PROGRAMA

A. PODATKI O RAZISKOVALNEM PROGRAMU

1.Osnovni podatki o raziskovalnem programu

Šifra programa	P3-0333
Naslov programa	Očesne bolezni odraslih in otrok
Vodja programa	9154 Marko Hawlina
Obseg raziskovalnih ur	10200
Cenovni razred	B
Trajanje programa	01.2009 - 12.2013
Izvajalci raziskovalnega programa (javne raziskovalne organizacije - JRO in/ali RO s koncesijo)	312 Univerzitetni klinični center Ljubljana 481 Univerza v Ljubljani, Biotehniška fakulteta
Raziskovalno področje po šifrantu ARRS	3 MEDICINA 3.03 Nevrobiologija
Družbeno-ekonomski cilj	07. Zdravje
Raziskovalno področje po šifrantu FOS	3 Medicinske vede 3.02 Klinična medicina

B. REZULTATI IN DOSEŽKI RAZISKOVALNEGA PROGRAMA

2.Povzetek raziskovalnega programa[1]

SLO

Raziskovalni program "Očesne bolezni odraslih in otrok" zajema praktično vse segmente klinične oftalmologije, od bolezni roženice, leče, glavkoma, bolezni mrežnice in vidnega živca. Pri teh boleznih so bile v času trajanja programa ARRS izvedene številne izboljšave diagnostičnih in terapevtskih postopkov, kar smo objavili v domači in mednarodni strokovni javnosti. Poleg tega je v okviru programa doslej dokseglo stopnjo doktorja znanosti kar 6 kolegic, ena pa je doseglo stopnjo magistrice znanosti. Število objav je v zadnjih letih eksponentno naraščalo, prav tako pa tudi število citatov.

Raziskovalni program je razdeljen v osem sklopov, ki so podrobneje opisani v točki 3.
V vseh sklopih je delo potekalo po načrtu, objavljeno ali pripravljeno za objavo je bilo več člankov, izsledki pa so bili predstavljeni na več mednarodnih konferencah.

SIklopi so smiselno razdeljeni glede na poglavitne očesne bolezni:

1. SKLOP: DISTROFIJE IN DEGENERACIJE TER VNETNE BOLEZNI MREŽNICE IN VIDNEGA

ŽIVCA (nosilec prof.dr.M.Hawlina)

2.SKLOP:ELEKTROFIZIOLOŠKA FUNKCIJA MREŽNICE IN VIDNE POTI (nosilka znanstvena svetnica J.Brecelj).

3.SKLOP: KLINIČNI ŠTUDIJ OTROKOVEGA VIDA (nosilka prof.dr. B.StirnKranjc)

4.SKLOP: KLINIČNE IN ULTRASTRUKTURNE ZNAČILNOSTI ROŽENICE IN LEČE (nosilec prof.dr. M.Hawlina)

5.SKLOP: DIABETIČNA RETINOPATIJA (nosilka prof.dr.Mojca Globočnik Petrovič)

6.SKLOP: GLAVKOM (nosilka doc.dr.Barbara Cvenkel)

7.SKLOP: VPLIV LASERSKIH ŽARKOV NA TKIVA OČESA IN PERIOKULARNEGA PODROČJA (nosilka prof.dr.B.DrnovšekOlup)

8.SKLOP: BAZIČNE EKSPERIMENTALNE ŠTUDIJE OČESNE LEČE IN MREŽNICE (nosilca: doc.dr. G.Zupančič, prof.dr.Marko Hawlina)

Skupno so po podatkih SICRISa člani raziskovalne skupine skupaj s preglednimi in kratkimi prispevki objavili preko 100 člankov v zadnjih 5 letih.

ANG

Research programme "Eye diseases of adults and children" comprises practically all segments of clinical ophthalmology, starting with the diseases of the cornea, towards lens, glaucoma, retina and optic nerve. With all these diseases, we were able to improve diagnostic and therapeutical methods and published that in national and international peer reviewed articles. In the duration of the last 5 years, six colleagues reached their PhD and one MSc. Three of PhD's were young researchers completing their programme. The number of publications as well as the number of citations exponentially grew during last years.

Our research programme consists of eight sections led by the key experts in respective fields of research. Work on the programme was conducted according to the plan and was divided in the following 8 sections:

1. SECTION: DYSTROPHIES AND DEGENERATIONS AND INFLAMMATORY DISEASES OF UVEA, RETINA AND OPTIC NERVE (leader prof.dr.M.Hawlina)
2.SECTION: ELECTROPHYSIOLOGICAL FUNCTION OF THE RETINA AND VISUAL PATHWAY (leader dr. J.Brecelj)
3.SECTION: CLINICAL STUDIES OF CHILD'S VISION (leader prof.dr. B.Stirn-Kranjc)
4.SECTION: CLINICAL AND ULRASTRUCTURAL CHARACTERISTICS OF THE CORNEA AND LENS (leader prof.dr. M.Hawlina)
5.SECTION: DIABETIC RETINOPATHY (leader doc.dr.Mojca Globočnik Petrovič)
6.SECTION: GLAUCOMA (leader doc.dr.Barbara Cvenkel)
7.SECTION: INFLUENCE OF LASER IRRADIATION ON THE OCULAR AND PERIOCULAR TISSUES (leader prof.dr.B.Drnovšek-Olup)
8.SECTION: BASIC AND EXPERIMENTAL STUDIES OF THE LENS AND RETINA (leaders: doc.dr. G.Zupančič, prof.dr.Marko Hawlina)

In all sections, relevant papers were published, a number were submitted and works were presented on the international conferences. According to SICRIS, members of our research group published over 100 papers from the programme topics in the last 5 years.

3.Poročilo o realizaciji predloženega programa dela na raziskovalnem programu[2]

SLO

Natančnejša realizacija zastavljenih colev je navedenaspodaj po posameznih sklopih:

1.SKLOP: DISTROFIJE IN DEGENERACIJE TER VNETNE BOLEZNI MREŽNICE IN VIDNEGA ŽIVCA (nosilec prof.dr.M.Hawlina)
Proučevali smo metodologijo avtofluorescence mrežnice (AF), optične koherentne tomografije (OCT), mikroperimetrije (MP) ter elektrofizioloških metod za oceno morfologije in funkcije mrežnice. Ob tem smo uvedli tudi gensko analizo teh bolezni ter analizo vnetnih parametrov v

nih tekočinah.
Ugotovili smo pomembne značilnosti najpogostejših distrofij in degeneracij mrežnice in sicer: pigmentne retinopatije, Stargardtove makulopatije, Bestove makulopatije, S-cone sindroma, Usherjevega sindroma, starostne degeneracije makule ter različnih vrst uveitisov.

2.SKLOP:ELEKTROFIZIOLOŠKA FUNKCIJA MREŽNICE IN VIDNE POTI (nosilka znanstvena svetnica J.Brecelj)
V raziskavi križanja vlaken vidnega živca v kiazmi pri otrocih s prirojenimi nistagmusom smo prikazali značilnosti vidnih evociranih potencialov (VEP) in magnetno resonančne tomografije (MRI) v skupini otrok z akiazmijo, pri kateri se vlakna vidnega živca ne križajo v kiazmi, in jih primerjali z značilnostmi prekomernega križanja pri otrocih z albinizmom. Nadalje smo preučevali prevajanje barvnega vida po parvocelularni vidni poti. Proučevali smo tudi delovanje elektrofiziološke funkcije mrežnice, prikazano z odgovori on-off, fotopičnim negativnim odgovorom-PhNR in odgovorom S-čepnic). Izsledki so pokazali pomembnost novih metod pri diagnostiki glavkoma, S-cone sindroma ter prirojene stacionarne nočne slepote.

3.SKLOP:KLINIČNI ŠTUDIJ OTROKOVEGA VIDA (nosilka prof.dr. B.StirnKranjc)
Proučevali smo genotipsko-fenotipske korelacije in raziskovali funkcionalno in morfološko očesno stanje pri otrocih s kongenitalnim nistagmusom pri sindromih in kromosomopatijah. Raziskovali smo genetsko klinične povezave retinalnih distrofij pri otrocih z optično koherentno tomografijo (OCT). Barvni vid smo preučevali z dodatnimi psihofizičnimi testi in sočasnim razvijanjem elektrofizioloških metod. Za študij vida močno nezrelih in bolnih otrok smo ugotavljali ogrožujoče dejavnike za retinopatijo nedonošenčka in za okvaro vidne funkcije in možne morfolološke spremembe. Pri agresivni posteriorni retinopatiji nedonošenčka s »plus« spremembami smo pričeli zdravljenje z zaviralcem žilnega endotelnega rastnega faktorja (antiVEGF) bevacizumabom.

4.SKLOP: KLINIČNE IN ULTRASTRUKTURNE ZNAČILNOSTI ROŽENICE IN LEČE (nosilec prof.dr. M.Hawlina)
Ugotavljali smo vzroke keratokonusa in preverjali možne načine zdravljenja. Naredili smo molekularno-genetsko analizo pri dednih in nedednih oblikah. Pri raziskavah leče smo se osredotočili na proučevanje ultrastrukture sprednje lečne ovojnice pri intumescentnih belih kataraktah, in ktaraktah povezanih z uveitisom.

5.SKLOP: DIABETIČNA RETINOPATIJA (nosilka doc.dr.Mojca Globočnik Petrovič)
Z asociacijskimi študijami smo analizirali kandidatne gene za diabetično retinopatijo: IL-8, IL-18, ICAM, glikoproteina III, BFGF, GSTT1, eNOS in ugotovili nekatere nove povezave. Analizirali smo vpliv kliničnih dejavnikov in ravni citokinov in VEGF v steklovini bolnikov na vid. Ugotovili smo povezavo med višjo ravnijo IL-8 s slabo pooperativno vidno ostrino, medtem ko vpliva VEGF na vidno ostrino ni bilo. Analizirali smo celice, ki proliferirajo iz fibrovaskularne membrane odvzete bolnikom s proliferativno diabetično retinopatijo. V teh celicah smo ugotovili potencial sproščanja vnetnih citokinov in angiogenih dejavnikov. Pri analizi vpliva IL-8 in VEGF v steklovini bolnikov z diabetično retinopatijo na prognozo po vitrektomiji smo povezali porast IL-8 v steklovini s slabšo pooperativno vidno ostrino bolnikov.

6.SKLOP: GLAVKOM (nosilka doc.dr.Barbara Cvenkel)
Analizirali smo vpliv sestave prekatne vodke na uspeh glavkomske operacije. Ugotovili smo, da so višje koncentracije vnetnih molekul – TNF-alfa in IL-6 pred operacijo povezane z večjim tveganjem za neuspeh operacije, ki je posledica zabrazgotinjenja kirurško napravljene fistule. Raziskovali smo korelacijo med uspešnostjo glavkomske operacije in kliničnim izgledom filtracijske blazinice ter subkliničnim vnetje veznice opredeljenim z izražanjem molekul HLA-DR. Ugotovili smo, da so uspešne operacije povezane z večjo površino in manjšo vaskularizacijo filtracijskih blazinic, na uspeh operacije pa ne vpliva subklinično vnetje veznice.
Pri glavkomskih bolnikih smo raziskovali korelacijo med debelino plasti retinalnih živčnih vlaken in različno stopnjo okvare vidne funkcije. Ugotovili smo pomembno stanjšanje debeline plasti živčnih vlaken pri 35% bolnikov, pri katerih ni bilo sprememb v vidnem polju in zaključili, da je preiskava s SD-OCT-jem pomembna za zgodnjo diagnostiko glavkoma.

7.SKLOP: VPLIV LASERSKIH ŽARKOV NA TKIVA OČESA IN PERIOKULARNEGA PODROČJA (nosilka prof.dr.B.DrnovšekOlup)
Razvili smo klinično metode zdravljenja zapore solznih izvodil z dakriocistorinostomijo z diodnim

laserjem 980 nm. Gre za nov način zdravljenja z minimalno invazivnim pristopom, pri katerem dosežemo mesto zapore po anatomski - poti preko solznega kanalčka, solzne vrečke in nosne kosti namesto s kožnim rezom, kot je to primer pri klasičnem kirurškem posegu oz. transnazalni dakriocistorinostomiji. Opravili smo preko 300 posegov in spremljali dolgoročno stopnjo uspešnosti, ki je bila med 85 - 90% in je primerljiva z uspešnostjo klasične kirurške metode. Ppseg za odpravo zapore solznih izvodil z diodnim laserjem je bistveno hitrejši od klasičnega kirurškega posega, z manj zapleti, s krajšim časom okrevanja bolnika po posegu in za bolnika manj boleč.

8.SKLOP: BAZIČNE EKSPERIMENTALNE ŠTUDIJE OČESNE LEČE IN MREŽNICE (nosilca: doc.dr. G.Zupančič, prof.dr.Marko Hawlina)
Analizirali smo morfologijo lečnih epitelnih celic pri različnih patologijah s sken elektronskim mikroskopom, v sodelovanju z Biotehnično fakulteto v Ljubljani. Vidne so bile tudi kontrakcije celic, ki bi lahko bile podlaga razvoja sive mrene. Analizirali smo tudi vpliv barvila gentiane violet, ki jo rutinsko uporabljamo, in ugotovili, da nima citotoksičnih učinkov, ki bi vplivali na homeostazo koncentracije kalcija v lečnih epitelijskih celicah.

4.Ocena stopnje realizacije programa dela na raziskovalnem programu in zastavljenih raziskovalnih ciljev[3]

SLO

V vseh sklopih je delo potekalo po načrtu, objavljeno ali pripravljeno za objavo je bilo več člankov, izsledki pa so bili predstavljeni na več mednarodnih konferencah.

1. SKLOP: DISTROFIJE IN DEGENERACIJE TER VNETNE BOLEZNI MREŽNICE IN VIDNEGA ŽIVCA (nosilec prof.dr.M.Hawlina)
Iz tega sklopa smo objavili 12 člankov v mednarodnih revijah in 3 v Zdravniškem Vestniku-ZV) in izsledke predstavili na več mednarodnih konferencah. V letih 2009-2013 sta iz tega sklopa doktorirali 2 kolegici, še ena pa je pred zagovorom doktorske naloge. Pri tem smo sodelovali s kolegi iz Anglije, Francije, Nizozemske in Hrvaške.

2.SKLOP:ELEKTROFIZIOLOŠKA FUNKCIJA MREŽNICE IN VIDNE POTI (nosilka znanstvena svetnica doc.dr.J.Brecelj).
Izsledke smo objavili v 8 mednarodnih člankih in enem poglavju v knjigi ter v 3 člankih v ZV. Končana je bila 1 doktorska disertacija.

3.SKLOP: KLINIČNI ŠTUDIJ OTROKOVEGA VIDA (nosilka prof.dr. B.StirnKranjc)
Izsledke smo objavili v 8 člankih v mednarodnih in 5 člankih v Zdravniškem Vestniku.

4.SKLOP: KLINIČNE IN ULTRASTRUKTURNE ZNAČILNOSTI ROŽENICE IN LEČE (nosilec prof.dr. M.Hawlina)
Iz tega področja smo objavili 5 člankov v mednarodnih revijah ter 2 članka v ZV. Iz tega področja sta bili končani dve doktorski disertaciji.

5.SKLOP: DIABETIČNA RETINOPATIJA (nosilka prof.dr.Mojca Globočnik Petrovič)
Dognanja smo objavili v 5 člankih v mednarodnih revijah in 4 člankih v ZV.

6.SKLOP: GLAVKOM (nosilka doc.dr.Barbara Cvenkel)
Rezultate smo objavili v 4 člankih v mednarodnih revijah ter 3 člankih v Zdravniškem Vestniku.

7.SKLOP: VPLIV LASERSKIH ŽARKOV NA TKIVA OČESA IN PERIOKULARNEGA PODROČJA (nosilka prof.dr.B.DrnovšekOlup)
Rezultate smo objavili v 3 člankih v mednarodnih revijah ter 8 člankih v ZV. Iz tega področja je bilo narejeno eno magistrsko delo.

8.SKLOP: BAZIČNE EKSPERIMENTALNE ŠTUDIJE OČESNE LEČE IN MREŽNICE (nosilca: doc.dr. G.Zupančič, prof.dr.Marko Hawlina)
Iz tega sklopa smo objavili 3 članke v mednarodnih revijah in 2 članka v Zdravniški vestnik.

5.Utemeljitev morebitnih sprememb programa raziskovalnega programa oziroma sprememb, povečanja ali zmanjšanja sestave programske skupine[4]

Bistvenih odmikov od začrtanih vsebinskih ciljev ni bilo.
Glede na sestavo programske skupine v letu 2004, ko smo bili nova programska skupina s 6 doktorji znanosti in 1.2 FTE odobrenega financiranja imamo danes 16 doktorjev znanosti, od tega jih je bilo 5 mladih raziskovalcev. Eksponentno nam narašča število objav in citatov. Žal pa je sistem financiranja ARRS povsem statičen in tako imamo tudi danes še vedno le 1.2 FTE ob skoraj potrojenem številu doktoratov. Sredstva zato ne zadoščajo za sprotno delo in objave na tujih konferencah

6.Najpomembnejši znanstveni rezultati programske skupine[5]

	Znanstveni dosežek			
1.	COBISS ID		547244	Vir: COBISS.SI
	Naslov	*SLO*	Avtofluorescenca in optična koherentna tomografija v povezavi z vidno funkcijo pri Usherjevem sindromu tipa 1 in 2.	
		ANG	Fundus autofluorescence and optical coherence tomography in relation to visual function in Usher syndrome type 1 and 2	
	Opis	*SLO*	Pomen te študije je bila karakterizacija slovenskih bolnikov s sindromom Usher, ki je najpogostejša bolezen, ki hkrati prizadane vid in sluh. Zajeli smo večino slovenskih bolnikov s to boleznijo. Z analizo slikovnih preiskav smo prišli do novih ugotovitev na področju naravnega poteka bolezni v mrežnici. Pri tem je pomembna predvsem izpopolnjena klasifikacija vzorcev avtofluorescence mrežnice. V sodelovanju z laboratorijem dr. Bonnet v Parizu smo genetsko potrdili 63 bolnikov, kar je bistvenega pomena pri kasnejšem vključevanju v genetsko terapijo, ki je trenutno v razvoju.	
		ANG	Purpose of this study was to characterize retinal disease in Usher syndrome using fundus autofluorescence and optical coherence tomography. Study included54 patients (26 male, 28 female) aged 7-70 years. There were 18 (33%) USH1 and 36 (67%) USH2 patients. 49/52 (94%) patients were found to carry at least one mutation in Usher genes. Ophthalmological examination included assessment of Snellen visual acuity, color vision with Ishihara tables, Goldmann visual fields (targets II/1-4 and V/4), microperimetry, fundus autofluorescence imaging and optical coherence tomography. Average age at disease onset (nyctalopia) was significantly lower in USH1 than USH2 patients (average 9 vs. 17 years, respectively; $p < 0.01$); however no significant differences were found regarding type of autofluorescence patterns, frequency of foveal lesions and CME, rate of disease progression and age at legal blindness. All representative eyes had abnormal fundus autofluorescence of either hyperautofluorescent ring (55%), hyperautofluorescent foveal patch (35%) or foveal atrophy (10%). Disease duration of more than 30 years was associated with a high incidence of abnormal central fundus autofluorescence (patch or atrophy) and visual acuity loss.	
	Objavljeno v		Pergamon Press.; Vision Research; 2012; Vol. 75, iss. 12; str. 60-70; Impact Factor: 2.137;Srednja vrednost revije / Medium Category Impact Factor: 1.771; A': 1; WoS: RU, SU; Avtorji / Authors: Fakin Ana, Jarc-Vidmar Martina, Glavač Damjan, Bonnet Crystel, Petit Christine, Hawlina Marko	
	Tipologija		1.01 Izvirni znanstveni članek	
2.	COBISS ID		29828313	Vir: COBISS.SI
	Naslov	*SLO*	Lastnosti VEP odzivov pri otrocih z akiazmijo v primerjavi z albino in zdravimi otroci	
		ANG	VEP characteristics in children with achiasmia, in comparison to albino and	

			healthy children
	Opis	*SLO*	V raziskavi nenormalnega križanja vlaken vidnega živca v kiazmi smo prikazali značilnosti vidnih evociranih potencialov (VEP) in magnetno resonančne tomografije (MRI) v skupini otrok z do sedaj redko opisano akiazmijo, pri kateri se vlakna vidnega živca ne križajo v kiazmi, in jih primerjali z značilnostmi prekomernega križanja vlaken pri otrocih z okularnim albinizmom.
		ANG	Achiasmia is a rare disorder of visual pathway maldevelopment that can show diverse clinical and magnetic resonance imaging spectra. The aim of this studywas to define the characteristics of visual evoked potentials (VEPs) thatdifferentiate abnormal optic-nerve-fibre decussation in children with achiasmia versus children with albinism and healthy children. In four childrenwith achiasmia, the following VEP characteristics were studied and compared to children with ocular albinism and with healthy control children: (a) flash and pattern onset VEP interhemispheric asymmetry; (b) flash N2, P2 and onset C1 amplitudes and latencies; (c) interocular polarity differences ininterhemisphere potentials; and (d) chiasm coefficients (CCs). In the children with achiasmia, VEPs were related to an absence of or reduced optic-nerve-fibre decussation at the chiasm and showed: ipsilateral asymmetry,significantly higher VEP amplitudes over the ipsilateral hemisphere ($p < 0.05$), interocular inverse polarity and negative CC. Other VEP features (uncrossed asymmetry and positive CC) were also seen if additional visual pathway maldevelopment (such as severe optic nerve hypoplasia and/or absence of the optic tractus on one side) were associated with achiasmia. In the children with albinism, the VEPs were related to excess optic-nerve-fibre decussation at the chiasm and showed: contralateral asymmetry, significantly higher VEP amplitudes over the contralateral hemisphere ($p < 0.001$), interocular inverse polarity and negative CC. In achiasmia and albinism, the VEPs to flash stimulation were more robust and more clearly distinguished between the conditions compared with the VEPs to pattern onset stimulation. VEPs in achiasmia are associated with absent or reduced optic-nerve-fibre decussation, where ipsilateral interhemispheric asymmetry is associated with interocular inverse polarity and a negative CC.
	Objavljeno v		Junk; Documenta ophthalmologica; 2012; Vol. 124, no. 2; str. 109-123; Impact Factor: 1.542;Srednja vrednost revije / Medium Category Impact Factor: 1.771; WoS: SU; Avtorji / Authors: Brecelj Jelka, Šuštar Maja, Pečarič-Meglič Nuška, Škrbec Miha, Stirn-Kranjc Branka
	Tipologija		1.01 Izvirni znanstveni članek
3.	COBISS ID		26132953 Vir: COBISS.SI
	Naslov	*SLO*	Histokemični in imunohistokemični profil notranje preme mišice pri človeku in podgani
		ANG	Histochemical and immunohistochemical profile of human and rat ocular medial rectus muscles
	Opis	*SLO*	Primerjalna študija histološke organizacije notranje preme mišice očesa pri človeku in podgani, ki kaže na razlike v vrstah vlaken.
			Purpose: To compare the organization of human and rat ocular medial recti muscles (MR). Methods: The cryosections of human and rat MR were processed formyofibrillar ATPase (mATPase), succinate dehydrogenase and glycerol-3-phosphate dehydrogenase. To reveal myosin heavy chain (MyHC) isoforms, specific monoclonal antibodies against MyHC-1/beta-slow, alpha-cardiac (-alpha), -2a, -2x, -2b, -extraocular (eom), -embryonic (-emb) and -neonatal (-neo) were applied. The MyHC gene expression was studied by in situ hybridization in human muscle. Results: The muscle fibers were arranged in two distinct layers in both species. In the orbital layer most fibers were highly oxidative and expressed fast MyHC isoforms,

		ANG	whereas slow and oxidative fibers expressed MyHC-1 and -alpha, some of them also MyHC-2a, -2x, -eom, very rarely -emb, and -neo. In the global layer, slow fibers with very low oxidative and glycolytic activity and three types of fast fibers, glycolytic, oxidative and oxidative-glycolytic, could be distinguished. The slow medium-sized fibers with mATPase activity stable at pH 4.4 expressed mostly MyHC-1 and -alpha in rat, while in humans they co-expressed MyHC-1 with -2b, -2x, -eom, and -neo. In both species, the fast fibers showed variable mATPase activity after preincubation at pH 9.4, and co-expressed various combinations of MyHC-2b, -2x, -2a and -eom but not -emb and -neo. MyHC-2b expressing fibers were larger and glycolytic, while MyHC-2a expressing fibers were smaller and highly oxidative in both species. To our knowledge, the present study is the first that demonstrated the expression of MyHC-2b in any of humanskeletal muscles. Though the expression of MyHC genes did not correlate with the immunohistochemical profile of fibers in human MR, the expression of MyHC-2b gene was undoubtedly confirmed. (Abstract truncated at 2000 characters)
	Objavljeno v		Springer; Graefe's archive for clinical and experimental ophthalmology; 2009; Letn. 247, št. 11; str. 1505-1515; Impact Factor: 2.102;Srednja vrednost revije / Medium Category Impact Factor: 1.931; WoS: SU; Avtorji / Authors: Stirn-Kranjc Branka, Smerdu Vika, Eržen Ida
	Tipologija		1.01 Izvirni znanstveni članek
4.	COBISS ID		27754201 Vir: COBISS.SI
	Naslov	*SLO*	Povezanost preoperativne koncentracije IL-8 in VEGF z ostrino vida po vitrektomiji zaradi proliferativne diabetične retinopatije
		ANG	Association of preoperative vitreous IL-8 and VEGF levels with visual acuity after vitrectomy in proliferative diabetic retinopathy
	Opis	*SLO*	Študija kaže pomen citokina IL-8 pri poteku in VEGF po vitrektomiji zdravljenju proliferativne diabetične retinopatije.
		ANG	Purpose: To determine whether the vitreous levels of interleukin 8 (IL-8) and vascular endothelial growth factor (VEGF) of patients with proliferative diabetic retinopathy (PDR) were associated with poor visual acuity after vitrectomy. Methods: Observational cross-sectional study. Patient clinical characteristics and preoperative eye characteristics (63 eyes): visual acuity,iris neovascularization, vitreous haemorrhage, macular detachment, macular oedema, active retinal neovascularization, neovascularization of the disc, burned out PDR (defined as natural end stage of PDR with inactive membranes without previously performed laser photocoagulation) and panretinal photocoagulation were registered prior to vitrectomy for each patient. Vitreous VEGF and IL-8 levels were measured using the cytometric bead array method. Poor postoperative visual acuity was defined as visual acuity of <20/200 and was checked 2 years after vitrectomy. Results: Twenty-one of the 63 eyes (33.3%) had poor visual acuity after vitrectomy. Univariate analysis showed that vitreous levels of IL-8, the absence of panretinal photocoagulation, preoperative macular detachment and poor preoperative visualacuity were significantly associated with poor final visual acuity aftervitrectomy. A stepwise multiple logistic regression analysis showed thatelevated vitreous levels of IL-8 ($p < 0.0001$), macular detachment ($p = 0.011$) and the absence of panretinal photocoagulation ($p = 0.03$) were independent predictors for poor visual outcome. Conclusions: Elevated vitreousIL-8 level could either be a marker of ischaemic inflammatory reaction, or it could play a role in deteriorating visual acuity by DR progression or both. Further studies are needed to provide better understanding of IL-8 and inflammation involvement in visual prognosis in PDR.
			Blackwell; Acta ophthalmologica; 2010; Letn. 88, št. 8; str. e311-e316;

	Objavljeno v		Impact Factor: 2.809;Srednja vrednost revije / Medium Category Impact Factor: 1.791; A': 1; WoS: SU; Avtorji / Authors: Globočnik Petrovič Mojca, Korošec Peter, Košnik Mitja, Hawlina Marko	
	Tipologija		1.01 Izvirni znanstveni članek	
5.	COBISS ID		2425679	Vir: COBISS.SI
	Naslov	*SLO*	Kontrakcija epitelnih celic sprednje lečne ovojnice pri človeku	
		ANG	Human anterior lens capsule epithelial cells contraction	
	Opis	*SLO*	Gre za opis pojava kontrakcije lečnih epitelnih celic na podlagi kemične ali mehanične stimulacije, ki doslej še ni bil opisan. Kontrakcija povzroči večanje špranj med lečnimi epitelnimi celicami skozi katere bi lahko vdrla voda. To bi lahko bil eden od mehanizmov nastanka katarakte.	
		ANG	Human anterior lens epithelial cells, attached to surgically isolated capsules, were found to contract upon stimulation. The purpose of this study was to characterize these contractions, which create gaps between cells, and to assess the underlying physiological mechanisms and their possible association with cataract formation. METHODS: Lens capsules obtained during cataract surgery were stained with fluorescent dye Fura-2. Its fluorescence, upon excitation at 360 and 380 nm, was imaged to monitor changes in cell morphology and cytosolic free Ca(2+) concentrations ([Ca(2+)](i)) in response to pharmacological stimulation by acetylcholine (ACh) and to mechanical stimulation by flow of saline or direct contact. RESULTS: Epithelial cells contracted in approximately a third of preparations when stimulated by either ACh application, fluid movement or direct mechanical contact. Contractions started either before or at best simultaneously with the rise in [Ca(2+)](i). Contractions also occurred when there was hardly any change in [Ca(2+)](i) upon application of physiological saline alone. The probability of contractions occurring did not differ significantly among cortical, nuclear and combined cortical + nuclear cataract. CONCLUSIONS: This study provides the evidence that contractions of the anterior lens epithelial cells take place in significant portion of human lens anterior capsule postoperative preparations after non-specific stimulation. Contractions are at least partially independent of changes in [Ca(2+)](i). They can be mechanically induced, are localized and reversible and have a fast response and did not differ among different types of cataract. Physiological and clinical significance of this phenomenon remains to be elucidated.	
	Objavljeno v		Blackwell Publishing; Acta ophthalmologica; 2011; Vol. 89, issue 8; str. e645-e653; Impact Factor: 2.629;Srednja vrednost revije / Medium Category Impact Factor: 1.841; A': 1; WoS: SU; Avtorji / Authors: Andjelić Sofija, Zupančič Gregor, Perovšek Darko, Hawlina Marko	
	Tipologija		1.01 Izvirni znanstveni članek	

7.Najpomembnejši družbeno-ekonomski rezultati programske skupine[6]

	Družbeno-ekonomski dosežek			
1.	COBISS ID		292268	Vir: COBISS.SI
	Naslov	*SLO*	Razvojna uspešnost v oftalmologiji in njena cena	
		ANG	Development effectiveness in ophtalmology and its price	
			Gre za uvodnik k oftalmološki številki Zdravniškega Vestnika, ki opredeljuje	

	Opis	SLO	razvojno raziskovalno delo v oftalmologiji in ga opredeljuje v soodnosu s klinično oftalmologijo.	
		ANG	Editorial introducing ophthalmological number of Zdravniški Vestnik, the journal of Slovene Medical Association, that summarizes the achievements of research in ophthalmology in relation to clinical work.	
	Šifra		C.05 Uredništvo nacionalne revije	
	Objavljeno v		Slovensko zdravniško društvo; 120 let Očesne klinike v Ljubljani; Zdravniški vestnik; 2012; Str. I-3-I-4; Impact Factor: 0.167;Srednja vrednost revije / Medium Category Impact Factor: 2.548; WoS: PY; Avtorji / Authors: Hawlina Marko	
	Tipologija		1.20 Predgovor, spremna beseda	
2.	COBISS ID		262329856	Vir: COBISS.SI
	Naslov	SLO	Program in zbornik povzetkov	
		ANG	Programme and book of abstracts	
	Opis	SLO	Slovenski oftalmološki kongres z mednarodno udeležbo (9 ; 2012 ; Portorož) Simpozij oftalmologov Slovenije in Hrvaške (32 ; 2012 ; Portorož) Kongres združenja oftalmologov jugovzhodne Evrope (SEEOS) (9 ; 2012 ; Portorož) Mednarodni simpozij oftalmologov Alpe Adria (34 ; 2012 ; Portorož)	
		ANG	Programme and book of abstracts / 9th Slovenian Congress of Ophthalmology with International Participation, Portorož, 2830 June 2012 ... [et al.] ; [Editors Marko Hawlina, Branka StirnKranjc]	
	Šifra		C.02 Uredništvo nacionalne monografije	
	Objavljeno v		Združenje oftalmologov Slovenije = Slovenian Society of Ophthalmology; 2012; 363 str.; Avtorji / Authors: Hawlina Marko, Stirn-Kranjc Branka	
	Tipologija		2.25 Druge monografije in druga zaključena dela	
3.	COBISS ID		382380	Vir: COBISS.SI
	Naslov	SLO	oftalmologije.	
		ANG	History and future of the European Board of Ophthalmology Diploma examination	
	Opis	SLO	Gre za članek, ki opisuje način izvedbe in ocenjevanja na evropskem izpitu iz oftalmologije, ki se ga od leta 2005 udeležujejo tudi naši oftalmologi. M Hawlina je bil predsednik EBO v letih 20102011, ko so bile uvedene te metode, ki pripomorejo k objektivnosti izpita.	
		ANG	Purpose: The European Board of Ophthalmology Diploma (EBOD) examination has evolved over the last few years, especially with the introduction of negative marking (-0.5 points) for incorrect or blank answers (0 points for don't know option), which aimed to improve the quality and reliability of the examination. Methods: In 2010, negative marking at the written part of the EBOD examination has been introduced in an attempt to improve not only the reliability of the examination as entity but also the statistical performance parameters of the individual questions. As lower pass rates and discriminationof female candidates are feared by the general public when negative marking is concerned, these parameters have been explicitly investigated. Results: Introduction of negative marking has not only lead to improved reliability of the EBOD examination (increased Cronbach's alpha value: Ž0.80 without and Ž0.90 with negative marking), but also to improved statistical performance parameters of the individual questions. The pass rate of the EBOD examination has proven to remain at the same high level as withoutnegative marking (around 90%). Furthermore, although female candidates do seem to have different answering strategies (pŽ<Ž0.01, use	

			of don't know option), no statistically significant difference has been found between total scores of male and female candidates (pŽ>Ž0.05). Conclusion: Introduction of negative marking at the written EBOD examination has proven to be beneficial, not only for the organizers (improvement of the statistical performance of theexamination and its questions), but also for candidates (better discrimination with borderline candidates). These results have been obtained without evidence of lower pass rates or discrimination of female candidates.
	Šifra		D.10 Pedagoško delo
	Objavljeno v		Blackwell; Acta ophthalmologica; 2013; Vol. 91, iss. 6; str. 589-593; Impact Factor: 2.345;Srednja vrednost revije / Medium Category Impact Factor: 1.771; A': 1; Avtorji / Authors: Mathysen D. G., Hawlina Marko
	Tipologija		1.01 Izvirni znanstveni članek
4.	COBISS ID		1002668 Vir: COBISS.SI
	Naslov	*SLO*	Marko Hawlina nagrajen s strani EBO
		ANG	Marko Hawlina honoured by EBO
	Opis	*SLO*	Gre za najvišjo nagrado evropskega odbora za oftalmologijo za prispevek k izobraževanju na področju oftalmologije podeljena Marku Hawlina v maju 2013
		ANG	This is the highest award of European Board of Ophthalmology given for contribution to European ophthalmological teaching, awarded to Marko Hawlina in May 2013
	Šifra		E.02 Mednarodne nagrade
	Objavljeno v		ESCRS; Euro times; 2013; Vol. 18, iss. 7/8; str. 34; Avtorji / Authors: Hawlina Marko, McGrath Dermot
	Tipologija		1.25 Drugi sestavni deli
5.	COBISS ID		625580 Vir: COBISS.SI
	Naslov	*SLO*	Multimodalno snemanje očesa
		ANG	Multimodal imaging of the eye
	Opis	*SLO*	Multimodalno snemanje očesa: vabljeno predavanje na 1. evropskem nevrooftalmološkem tečaju, 1415. april 2012, Budimpešta, Madžarska
		ANG	Multimodal imaging of the eye : invited lecture at the 1st European Neuro-Ophthalmology Society Update Course, 1415 April 2012, Budapest, Hungary / Marko Hawlina
	Šifra		B.04 Vabljeno predavanje
	Objavljeno v		2012; Avtorji / Authors: Hawlina Marko
	Tipologija		3.16 Vabljeno predavanje na konferenci brez natisa

8. Drugi pomembni rezultati programske skupine[7]

Raziskovalna skupina ima jasno razvojno vizijo, ki je pomembno umeščena v domač in mednarodni raziskovalni prostor. Razvili smo klinične elektrofiziološke metode, metode slikanja mrežnice z skenirajočim laserskim oftalmoskopom in metodo slikanja avtofluorescence, mikroperimetrijo ter v zadnjem času, optično koherenčno tomografijo in druge metode. V zvezi s tem smo pridobili akreditacijo European Board of Ophthalmology za referenčni učni center in akreditacijo European Vision Institute za referenčni raziskovalni center. Organizirali smo tudi več evropskih znanstvenih konferenc, Z mislijo na pomen translacijske znanosti smo opremili 4 raziskovalne laboratorije, ki omogočajo sodobne morfološke in fiziološke metode na celičnem nivoju in za 70 % zaposlili eno raziskovalko z doktoratom, kolikor nam dopušča minimalni obseg programa.

Člani programske skupine so imeli številna vabljena predavanja na mednarodnih kongresih in so na njih nastopali kot moderatorji. Sodelovali so kot soavtorji pri izdelavi mednarodnih standardov in bili so-uredniki tematskih številk mednarodnih revij. Člani raziskovalne skupine so bili povabljeni v uredniške odbore mednarodnih revij O svojih dosežkih smo redno obveščali tudi širšo javnost s prispevki v prilogi Dela Znanost.

Mlade kolege aktivno spodbujamo v sodelovanje v mednarodnem prostoru.V okviru tega programa so naziv izrednega profesorja dosegli 4 člani programske skupine, štiri članice pa so postale docentke. Vse navedeno omogoča dobre napovedi za razvoj znanosti našega področja v bodoče.

9.Pomen raziskovalnih rezultatov programske skupine[8]

9.1.Pomen za razvoj znanosti[9]

SLO

Znanstveni pomen programskih sklopov je v prepoznavanju patofizioloških procesov v diagnostiki in zdravljenju najpomembnejših očesnih bolezni.Vsi predlagani segmenti projekta prinašajo nova znanstvena spoznanja, ki imajo tudi klinični pomen. Gre za uvajanje novih metod tudi v mednarodnem merilu, pri tem v marsičem odpiramo nova spoznanja in predlagamo rešitve. Velik pomen je v zelo zgodnji diagnostiki retinalnih distrofij in degeneracij, diabetične retinopatije, glavkoma in otroških nevrooftalmoloških bolezni, pri čemer v zgodnjih fazah še lahko preprečimo propadanje vida. Prav tako so pomembne elektrofiziološke preiskave pri otrocih, ki so edina objektivna informacija o vidni funkciji. Raziskave o genetiki retinalnih distrofij ter diabetične retinopatije so pomembni napovedni dejavniki pri prognozi teh bolezni. Raziskave laserskega sevanja na tkiva očesa in periokularnega področja so pomembna za razvoj medicinskega laserja od teoretičnih konceptov preko izdelave naprave do predkliničnih in kliničnih testiranj.
Izsledki raziskav imajo neposredno uporabno vrednost in ponudile boljšo diagnostiko in zdravljenje očesnih bolezni.

ANG

Scientific impact of our research is in recognition of pathophysiological processes in diagnosis and management of most important eye diseases. All proposed segments of this program yielded new scientific findings with clinical impact. This relates to introduction of new diagnostic and therapeutic modalities that have become available for our patients. Large importance of this is in early dyagnosis of retinal dystrophies and degenerations, diabetic retinopathy, glaucoma and pediatric neuroophthalmic diseases, in order to prevent visual loss. The importance of electrophysiological testing in children is emphasised as these tests give objective information about visual function. Research in genetics of eye diseases give important insight in mechanisms and prognosis. Research of effects of laser irradiation on the ocular and periocular tissues are important for development of medical lasers from the concept phase to the phase of pre-clinical and clinical testing. The results of these studies have direct applicability and are useful for better diagnosis and management of ocular diseases.

9.2.Pomen za razvoj Slovenije[10]

SLO

V slovenskem prostoru se z našimi raziskavami neposredno uvajajo metode, ki so enake tistim, ki so dostopne na najmodernejših inštitucijah v tujini. Na očesni kliniki smo zagotovili možnosti vrhunskega znanstvenega razvoja z moderno opremo omogoča vrhunsko diagnostiko in terapijo. Rezultate dela smo objavili že v štirih tematskih številkah Zdravniškega vestnika, ki smo jih uredili ob naših kongresih z mednarodno udeležbo. Gre za uvajanje novih metod s katerimi lahko slovenskim bolnikom nudimo kvalitetno in moderno diagnostiko in zdravljenje očesnih bolezni. Temeljne genetske raziskave pa so pomembne za ugotavljanje genetskih značilnosti in mutacij v Sloveniji in pomenijo doprinos k prepoznavanju dejavnikov, ki lahko vplivajo oziroma napovedujejo izid zdravljenja in izboljšanje uspeha zdravljenja, oz. upočasnitev napredovanja očesnih bolezni.

ANG

With this research, the modern methods that were once available only in most modern

European institutions have been introduced in Slovenia. At the University Eye Hospital, we have succeeded to establish the possibilities of scientific development that allows most advanced diagnostics and treatment. The results of these novel methods have been published in Slovene and foreign literature and presented at congresses. Slovene patients can thus have access to the most contemporary management. In addition, genetic testing has provided the characteristics of these diseases in Slovenian population which give insight in mechanisms, course and prognosis. These findings are fundamental in attempts for revealing scientific avenues to treat or retard the progression of eye diseases.

10.Zaključena mentorstva članov programske skupine pri vzgoji kadrov v obdobju 1.1.2009-31.12.2013[11]

10.1. Diplome[12]

vrsta usposabljanja	število diplom
bolonjski program - I. stopnja	
bolonjski program - II. stopnja	1
univerzitetni (stari) program	

10.2. Magisterij znanosti in doktorat znanosti[13]

Šifra raziskovalca	Ime in priimek	Mag.	Dr.	MR	
29596	Eva Lenassi	○	◉	☑	
20708	Nataša Vidovič Valentinči	○	◉	☐	
31505	Špela Štunf	○	◉	☐	
25616	Maja Šuštar	○	◉	☑	
26333	Ingrid Rahne	◉	○	☐	
33340	Ana Fakin	○	◉	☑	

Legenda:
Mag. - Znanstveni magisterij
Dr. - Doktorat znanosti
MR - mladi raziskovalec

11.Pretok mladih raziskovalcev – zaposlitev po zaključenem usposabljanju[14]

Šifra raziskovalca	Ime in priimek	Mag.	Dr.	Zaposlitev	
29596	Eva Lenassi	○	◉	D - Javni zavod	
25616	Maja Šuštar	○	◉	D - Javni zavod	
33340	Ana Fakin	○	◉	D - Javni zavod	

Legenda zaposlitev:
A - visokošolski in javni raziskovalni zavodi
B - gospodarstvo
C - javna uprava
D - družbene dejavnosti
E - tujina
F - drugo

12.Vključenost raziskovalcev iz podjetij in gostovanje raziskovalcev, podoktorandov ter

študentov iz tujine, daljše od enega meseca, v obdobju 1.1.2009-31.12.2013

Šifra raziskovalca	Ime in priimek	Sodelovanje v programski skupini	Število mesecev	
0	Goran Petrovski	B - uveljavljeni	12	

Legenda sodelovanja v programski skupini:
A - raziskovalec/strokovnjak iz podjetja
B - uveljavljeni raziskovalec iz tujine
C - študent – doktorand iz tujine
D - podoktorand iz tujine

13. Vključevanje v raziskovalne programe Evropske unije in v druge mednarodne raziskovalne in razvojne programe ter drugo mednarodno sodelovanje v obdobju 1.1.2009-31.12.2013 z vsebinsko obrazložitvijo porabe dodeljenih sredstev iz naslova dodatnega letnega sofinanciranja mednarodnega sodelovanja na podlagi pozivov za EU vpetost.[15]

SLO

Člani programske skupine se mednarodno povezujejo s kolegi iz tujine. Prof.Hawlina je povezan z Moorfields Eye Hospital, pa tudi s kolegi v Parizu, Gentu in drugod, kar dokazujejo članki objavljeni s tujimi avtorji. Npr. mlada raziskovalka, Eva Lenassi je trenutno na usposabljanju pri prof.Andrewu Websterju na Moorfields Eye Hospital. Mlada raziskovalka Ana Fakin je predstavila svoje delo v mednarodnem konzorciju Teatrush, ki povezuje evropske raziskovalne skupine, ki proučujejo Usherjevo bolezen. V sodelovanju z evropskim projektom Treatrush je bila opravljena tudi genotipizacija bolnikov, ki je omogočila analizo genotipfenotip korelacij. Dr.Brecljeva in prof.Stirnova je povezana s skupino dr.Dorothy Thompson z Hospital for Sick Children, Great Ormond Street London. Dr.Cvenklova sodeluje v mednarodnih študijah na področju glavkoma. Pri člankih dr.Nataše Vidovič Valentinčič je bila soavtorica prof.Aniki Rothova iz Utrechta, Nizozemska.

14. Vključenost v projekte za uporabnike, ki v so obdobju trajanja raziskovalnega programa (1. 1. 2009 – 31. 12. 2013), potekali izven financiranja ARRS[16]

SLO

Člani programske skupine so vodili več terciarnih projektov UKC Ljubljana in sodelovali tudi pri izvedbi kliničnih študij, ki so jih vodile farmacevtske družbe. V letošnjem letu bo iz teh projektov kupljenih nekaj prepotrebnih aparatur.

15. Ocena tehnološke zrelosti rezultatov programa in možnosti za njihovo implementacijo v praksi (točka ni namenjena raziskovalnim programom s področij humanističnih ved)[17]

SLO

16. Ocenite, ali bi doseženi rezultati v okviru programa lahko vodili do ustanovitve spin-off podjetja, kolikšen finančni vložek bi zahteval ta korak ter kakšno infrastrukturo in opremo bi potrebovali

možnost ustanovitve spin-off podjetja	○ DA ◉ NE
potrebni finančni vložek	
ocena potrebne infrastrukture in opreme[18]	

17. Izjemni dosežek v 2013[19]

17.1. Izjemni znanstveni dosežek

Fundus autofluorescence and optical coherence tomography in relation to visual function in Usher syndrome type 1 and 2.
Pergamon Press.; Vision Research; 2012; Vol. 75, iss. 12; str. 60-70; Impact Factor: 2.137;Srednja vrednost revije / Medium Category Impact Factor: 1.771; A': 1; WoS: RU, SU;

Avtorji / Authors: Fakin Ana, Jarc-Vidmar Martina, Glavač Damjan, Bonnet Crystel, Petit Christine, Hawlina Marko

Pomen te študije je bila karakterizacija slovenskih bolnikov s sindromom Usher, ki je najpogostejša bolezen, ki hkrati prizadane vid in sluh. Zajeli smo večino slovenskih bolnikov s to boleznijo. Z analizo slikovnih preiskav smo prišli do novih ugotovitev na področju naravnega poteka bolezni v mrežnici. Pri tem je pomembna predvsem izpopolnjena klasifikacija vzorcev avtofluorescence mrežnice. V sodelovanju z laboratorijem dr. Bonnet v Parizu smo genetsko potrdili 63 bolnikov, kar je bistvenega pomena pri kasnejšem vključevanju v genetsko terapijo, ki je trenutno v razvoju.

17.2. Izjemni družbeno-ekonomski dosežek

Peter Eustace Medal, ki jo podeljuje European Board of Ophthalmology za izjemne dosežke v evropskem izobraževanju iz oftalmologije.

Gre za najvišjo nagrado evropskega odbora za oftalmologijo za prispevek k izobraževanju na področju oftalmologije podeljena Marku Hawlina v maju 2013. intervju o tem in o organizaciji izobaževanja v UKCL je bil objavljen v februarski številki glasila Interno (dodano v priponki). Gre za nagrado za razvoj izobraževanja v evropskem merilu in v Sloveniji. Naši specializanti že od leta 2004 dosegajo odlične rezultate na specialističnih izpitih EBO v Parizu. Organizacija specialističnega izobraževanja je neločljivo povezana tudi z raziskovalnim in strokovnim delom.

C. IZJAVE

Podpisani izjavljam/o, da:

- so vsi podatki, ki jih navajamo v poročilu, resnični in točni
- se strinjamo z obdelavo podatkov v skladu z zakonodajo o varstvu osebnih podatkov za potrebe ocenjevanja in obdelavo teh podatkov za evidence ARRS
- so vsi podatki v obrazcu v elektronski obliki identični podatkom v obrazcu v papirnati obliki
- so z vsebino poročila seznanjeni in se strinjajo vsi izvajalci raziskovalnega programa

Podpisi:

zastopnik oz. pooblaščena oseba JRO in/ali RO s koncesijo:	in	*vodja raziskovalnega programa:*
Univerzitetni klinični center Ljubljana		Marko Hawlina
_____		_____

ŽIG

Kraj in datum:	Ljubljana	15.4.2014

Oznaka prijave: ARRS-RPROG-ZP-2014/65

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[1] Napišite povzetek raziskovalnega programa v slovenskem jeziku (največ 3.000 znakov vključno s presledki – približno pol strani, velikost pisave 11) in angleškem jeziku (največ 3.000 znakov vključno s presledki – približno pol strani, velikost pisave 11). Nazaj

[2] Napišite kratko vsebinsko poročilo, v katerem predstavite raziskovalno hipotezo in opis raziskovanja. Navedite ključne ugotovitve, znanstvena spoznanja, rezultate in učinke raziskovalnega programa in njihovo uporabo ter sodelovanje s tujimi partnerji. Največ 12.000 znakov vključno s presledki (približno dve strani, velikosti pisave 11). Nazaj

[3] Realizacija raziskovalne hipoteze. Največ 3.000 znakov vključno s presledki (približno pol strani, velikosti pisave 11). Nazaj

[4] V primeru bistvenih odstopanj in sprememb od predvidenega programa dela raziskovalnega programa, kot je bil zapisan v predlogu raziskovalnega programa oziroma v primeru sprememb, povečanja ali zmanjšanja sestave programske skupine v zadnjem letu izvajanja raziskovalnega programa, napišite obrazložitev. V primeru, da sprememb

ni bilo, to navedite. Največ 6.000 znakov vključno s presledki (približno ena stran, velikosti pisave 11). Nazaj

[5] Navedite znanstvene dosežke (največ pet), ki so nastali v okviru tega programa. Raziskovalni dosežek iz obdobja izvajanja programa (do oddaje zaključnega poročila) vpišete tako, da izpolnite COBISS kodo dosežka – sistem nato sam izpolni naslov objave, naziv, IF in srednjo vrednost revije, naziv FOS področja ter podatek, ali je dosežek uvrščen v A'' ali A'. Nazaj

[6] Navedite družbeno-ekonomske dosežke (največ pet), ki so nastali v okviru tega programa. Družbeno-ekonomski dosežek iz obdobja izvajanja programa (do oddaje zaključnega poročila) vpišete tako, da izpolnite COBISS kodo dosežka – sistem nato sam izpolni naslov objave, naziv, IF in srednjo vrednost revije, naziv FOS področja ter podatek, ali je dosežek uvrščen v A'' ali A'.

Družbeno-ekonomski dosežek je po svoji strukturi drugačen kot znanstveni dosežek. Povzetek znanstvenega dosežka je praviloma povzetek bibliografske enote (članka, knjige), v kateri je dosežek objavljen.

Povzetek družbeno-ekonomskega dosežka praviloma ni povzetek bibliografske enote, ki ta dosežek dokumentira, ker je dosežek sklop več rezultatov raziskovanja, ki je lahko dokumentiran v različnih bibliografskih enotah. COBISS ID zato ni enoznačen, izjemoma pa ga lahko tudi ni (npr. prehod mlajših sodelavcev v gospodarstvo na pomembnih raziskovalnih nalogah, ali ustanovitev podjetja kot rezultat programa … - v obeh primerih ni COBISS ID). Nazaj

[7] Navedite rezultate raziskovalnega programa iz obdobja izvajanja programa (do oddaje zaključnega poročila) v primeru, da katerega od rezultatov ni mogoče navesti v točkah 6 in 7 (npr. ker se ga v sistemu COBISS ne vodi). Največ 2.000 znakov vključno s presledki (približno 1/3 strani, velikost pisave 11). Nazaj

[8] Pomen raziskovalnih rezultatov za razvoj znanosti in za razvoj Slovenije bo objavljen na spletni strani: http://sicris.izum.si/ za posamezen program, ki je predmet poročanja. Nazaj

[9] Največ 4.000 znakov vključno s presledki. Nazaj

[10] Največ 4.000 znakov vključno s presledki. Nazaj

[11] Upoštevajo se le tiste diplome, magisteriji znanosti in doktorati znanosti (zaključene/i v obdobju 1. 1. 2009 – 31. 12. 2013), pri katerih so kot mentorji sodelovali člani programske skupine. Nazaj

[12] Vpišite število opravljenih diplom v času trajanja raziskovalnega programa glede na vrsto usposabljanja. Nazaj

[13] Vpišite šifro raziskovalca in/ali ime in priimek osebe, ki je v času trajanja raziskovalnega programa pridobila naziv magister znanosti in/ali doktor znanosti ter označite doseženo izobrazbo. V primeru, da se je oseba usposabljala po programu Mladi raziskovalci, označite MR. Nazaj

[14] Za mlade raziskovalce, ki ste jih navedli v tabeli 11.2. točke (usposabljanje so uspešno zaključili v obdobju od 1. 1. 2009 do 31. 12. 2013), ustrezno označite, kje so se zaposlili po zaključenem usposabljanju. Nazaj

[15] Navedite naslove projektov in ime člana programske skupine, ki je bil vodja/koordinator navedenega projekta. Točko izpolnijo tudi izvajalci raziskovalnega programa, prejemniki sredstev iz naslova dodatnega letnega sofinanciranja raziskovalnega programa zaradi mednarodnega sodelovanja (sodelovanja v projektih okvirnih programov Evropske unije). Izvajalec, ki je na podlagi pogodbe prejel sredstva iz navedenega naslova, vsebinsko opiše porabo prejetih sredstev za financiranje stroškov blaga in storitev ter amortizacije, nastalih pri izvajanju tega raziskovalnega programa. V primeru, da so bili v okviru raziskovalnega programa prejemniki sredstev različni izvajalci, vsak pripravi vsebinsko poročilo za svoj delež pogodbenih sredstev. Vodja raziskovalnega programa poskrbi, da je vsebinsko poročilo, ločeno za vsakega izvajalca, vključeno v navedeno točko poročila.
Največ 6.000 znakov vključno s presledki (približno ena stran, velikosti pisave 11). Nazaj

[16] Navedite naslove projektov, ki ne sodijo v okvir financiranja ARRS (npr: industrijski projekti, projekti za druge naročnike, državno upravo, občine idr.) in ime člana programske skupine, ki je bil vodja/koordinator navedenega projekta. Največ 3.000 znakov vključno s presledki (približno pol strani, velikosti pisave 11). Nazaj

[17] Opišite možnosti za uporabo rezultatov v praksi. Opišite izdelke oziroma tehnologijo in potencialne trge oziroma tržne niše, v katere sodijo. Ocenite dodano vrednost izdelkov, katerih osnova je znanje, razvito v okviru programa oziroma dodano vrednost na zaposlenega, če jo je mogoče oceniti (npr. v primerih, ko je rezultat izboljšava obstoječih tehnologij oziroma izdelkov). Največ 3.000 znakov vključno s presledki (približno pol strani, velikosti pisave 11). Nazaj

[18] Največ 1.000 znakov vključno s presledki (približno 1/6 strani, velikost pisave 11) Nazaj

[19] Navedite en izjemni znanstveni dosežek in/ali en izjemni družbeno-ekonomski dosežek raziskovalnega programa v letu 2013 (največ 1000 znakov, vključno s presledki, velikost pisave 11). Za dosežek pripravite diapozitiv, ki vsebuje sliko ali drugo slikovno gradivo v zvezi z izjemnim dosežkom (velikost pisave najmanj 16, približno pol strani) in opis izjemnega dosežka (velikost pisave 12, približno pol strani). Diapozitiv/-a priložite kot priponko/-i k temu poročilu. Vzorec diapozitiva je objavljen na spletni strani ARRS http://www.arrs.gov.si/sl/gradivo/, predstavitve dosežkov za pretekla leta pa so objavljena na spletni strani http://www.arrs.gov.si/sl/analize/dosez/. Nazaj

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Fundus autofluorescence and optical coherence tomography in relation to visual function in Usher syndrome type 1 and 2

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ABSTRACT

Purpose of this study was to characterize retinal disease in Usher syndrome using fundus autofluorescence and optical coherence tomography. Study included 54 patients (26 male, 28 female) aged 7–70 years. There were 18 (33%) USH1 and 36 (67%) USH2 patients. 49/52 (94%) patients were found to carry at least one mutation in Usher genes. Ophthalmological examination included assessment of Snellen visual acuity, color vision with Ishihara tables, Goldmann visual fields (targets II/1–4 and V/4), microperimetry, fundus autofluorescence imaging and optical coherence tomography. Average age at disease onset (nyctalopia) was significantly lower in USH1 than USH2 patients (average 9 vs. 17 years, respectively; $p < 0.01$); however no significant differences were found regarding type of autofluorescence patterns, frequency of foveal lesions and CME, rate of disease progression and age at legal blindness. All representative eyes had abnormal fundus autofluorescence of either hyperautofluorescent ring (55%), hyperautofluorescent foveal patch (35%) or foveal atrophy (10%). Disease duration of more than 30 years was associated with a high incidence of abnormal central fundus autofluorescence (patch or atrophy) and visual acuity loss.

1. Introduction

Usher syndrome is a group of recessively inherited diseases, characterized by a combination of retinitis pigmentosa (RP) and sensorineural hearing loss. It has a prevalence of 3-6/100.000 (Millan et al., 2011) and represents 18% of all RP (Boughman, Vernon, & Shaver, 1983). Nine causative genes have been identified and three clinical types are known based on severity of hearing loss. Usher type 1 (USH1; *MYO7A*, *CDH23*, *PCDH15*, *USH1C* and *USH1G* genes) is characterized by severe congenital hearing loss while Usher type 2 (USH2; *USH2A*, *GPR98* and *DFNB31* genes) is characterized by moderate congenital hearing loss (Cohen, Bitner-Glindzicz, & Luxon, 2007). Usher type 3 (USH3; *USH3A* gene) is rare and characterized late onset progressive hearing loss (Saihan et al., 2009). Among all Usher genes, the three most commonly affected are *USH2A* (36–59%), *MYO7A* (10–18%) and *PCDH15* (4–6%) (Bonnet & El-Amraoui, 2011). Usher proteins were found to be integrated into a network in photoreceptor ciliary region, that could explain the common retinal phenotype (Lefevre et al., 2008; Maerker et al., 2008; Williams, 2008). Early histopathologic changes in RP are shortening of rod outer segments followed by rod cell death leading to nyctalopia (Milam, Li, & Fariss, 1998). With advancement, the retinal degeneration gives rise to a characteristic ring-shaped scotoma in the mid-periphery, which can expand to the periphery and macula. As the disease progresses, the cone photoreceptors also degenerate leading to loss of central vision (Milam, Li, & Fariss, 1998; van Soest et al., 1999). Fundus autofluorescence imaging (FAF) and optical coherence tomography (OCT) are used for morphological assessment of photoreceptors in the macular area (Mitamura et al., 2012). Various patterns of abnormal FAF have been reported in RP patients. Hyperautofluorescent ring can be seen in up to 60% of patients (Popovic, Jarc-Vidmar, & Hawlina, 2005; Robson et al., 2003) while central hyperautofluorescence was observed in 18% (Murakami et al., 2008). It is unclear whether any differences exist in retinal disease between different Usher types or genotypes. The most consistent

Abbreviations: FAF, fundus autofluorescence; OCT, optical coherence tomography; ISe, inner segment ellipsoid (also known as IS/OS junction); ELM, external (outer) limiting membrane; RP, retinitis pigmentosa; USH, Usher; VA, visual acuity; ISH, Ishihara color vision test; OPL, outer plexiform layer; IPL, inner plexiform layer; ONL, outer nuclear layer.

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difference that is found is earlier onset of night blindness in USH1 than in USH2 (Tsilou et al., 2002). More severe form of retinitis pigmentosa in USH1 was reported by some authors (Edwards et al., 1998; Fishman et al., 1995; Hope et al., 1997; Piazza et al., 1986), however others did not share those findings (Kimberling et al., 1989; Seeliger et al., 1999; Tsilou et al., 2002).

The main aims of the study are to characterize the natural course of macular involvement in Usher syndrome using fundus autofluorescence and to compare FAF and OCT phenotypes associated with USH1 and USH2 genotypes.

2. Material and methods

2.1. Patients and study design

This prospective cross-sectional study included 54 patients with Usher syndrome (26 male, 28 female) aged from 7–70 years (average 44 ± 15). Six pairs of patients were siblings. Diagnosis of RP was based on nyctalopia, visual field constriction, pigmentary retinal changes, full-field scotopic and photopic electroretinography and genetic data where available. All patients had sensorineural hearing loss previously confirmed by audiometry. Ophthalmological examination included assessment of Snellen visual acuity (VA), color vision with Ishihara tables, Goldmann visual fields (targets II/1–4 and V/4), microperimetry, fundus autofluorescence imaging (FAF) and optical coherence tomography (OCT). Eyes with better quality of FAF and OCT images were chosen as representative eyes. Patients were asked about age when they first noticed night vision problems (nyctalopia). This age was used as disease onset in our study. Disease duration was calculated as time between onset of nyctalopia and examination date.

Molecular diagnosis was performed in 52 patients by sequencing of all exons and flanking intronic regions of nine known Usher genes. Statistical analysis was performed using PASW statistical software (PASW 18.0; PASW Inc., Chicago, IL). Study was approved by Slovenian Ethics Committee for Research in Medicine and all research procedures have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Informed consent was obtained from all subjects.

2.2. Fundus autofluorescence and spectral-domain optical coherence tomography

Fundus autofluorescence imaging of 30° field of view and 8-mm Spectral-domain OCT scan through the fovea were performed simultaneously with a confocal scanning laser ophthalmoscope (OCT-SLO Spectralis, Heidelberg Engineering, Dossenheim, Germany) after pupil dilation with topical 1% Tropicamide. Patterns of FAF were determined in 51/54 patients. In three patients this was not possible due to imaging difficulties or severe cystoid macular edema (CME). Measurements of structures on FAF and OCT were performed using Spectralis software with micrometer caliper. Temporal horizontal radius of the hyperautofluorescent ring (distance from fovea to inner and outer border) or patch was measured on FAF image. Distance from the fovea along the horizontal midline to the location of disappearance of inner segment ellipsoid (ISe) junction and external limiting membrane (ELM) were measured on OCT. Thicknesses of the outer nuclear layer (from upper border of ELM/ONL to the upper border of OPL/INL) was measured in the fovea. Measurements of FAF structures, ELM and ISe were rounded to 0,01 mm to compensate for measurement error. Conversion of 1 mm of retina to 3.5° of visual field was used. Longitudinal changes in FAF were evaluated in 18 patients who have previously undertaken autofluorescence imaging. Visual function and retinal structure were compared between different FAF patterns in patients without media opacities or large CME ($N = 43$).

2.3. Microperimetry

Static microperimetry was performed with the Nidek MP1 (Nidek Technologies, Padua, Italy) after pupil dilation with topical 1% tropicamide. Minimum of 12 stimuli in the central 8° of retina were used. Stimuli of the size of Goldmann III target appeared for 200 ms and changed intensity in 4–2 strategy. Average retinal sensitivity of central 12 stimuli (8° of retina) was calculated using MP1 software. Additionally, kinetic microperimetry using 0 dB stimulus, size Goldmann III, moving in eight directions from 10° to the foveal center with velocity of 2.4°/s was performed on 10 patients with hyperautofluorescent rings.

3. Results

3.1. Clinical data

There were 18 (33%) USH1 patients with average age of 33 ± 16 years and 36 (67%) USH2 patients with average age of 49 ± 12 years. 49/52 (94%) analyzed patients were found to carry at least one mutation in Usher genes. Among those, there were

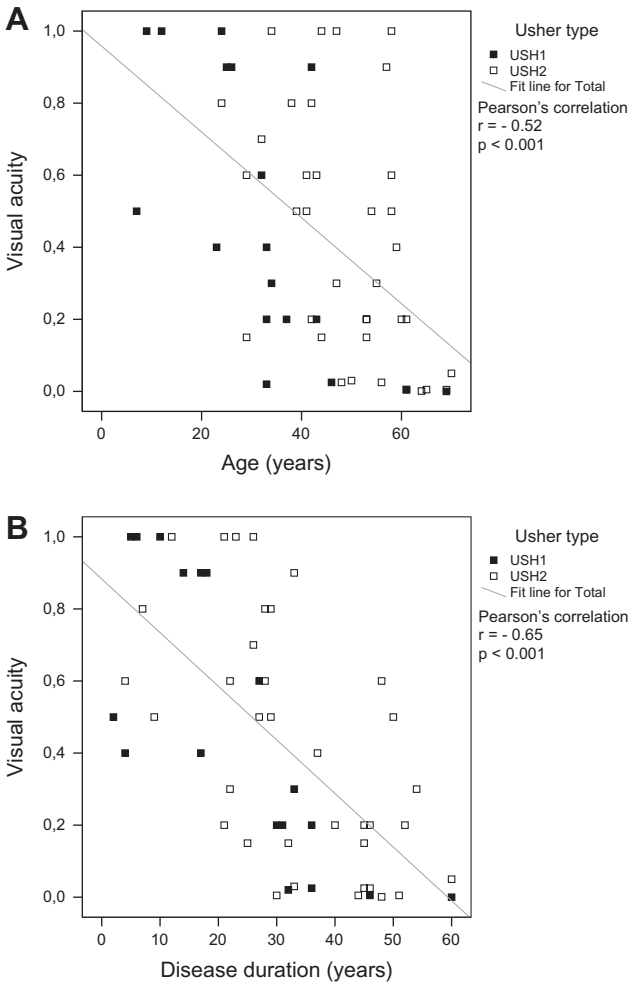

Fig. 1. Visual acuity in association with age (A) or disease duration (B) in Usher patients ($N = 43$). Patients with media opacities or large CME were excluded. Correlation was stronger between visual acuity and disease duration ($r = -0.65$) than between visual acuity and age ($r = -0.52$). It was significant in both cases ($p < 0.001$, Pearson's correlation).

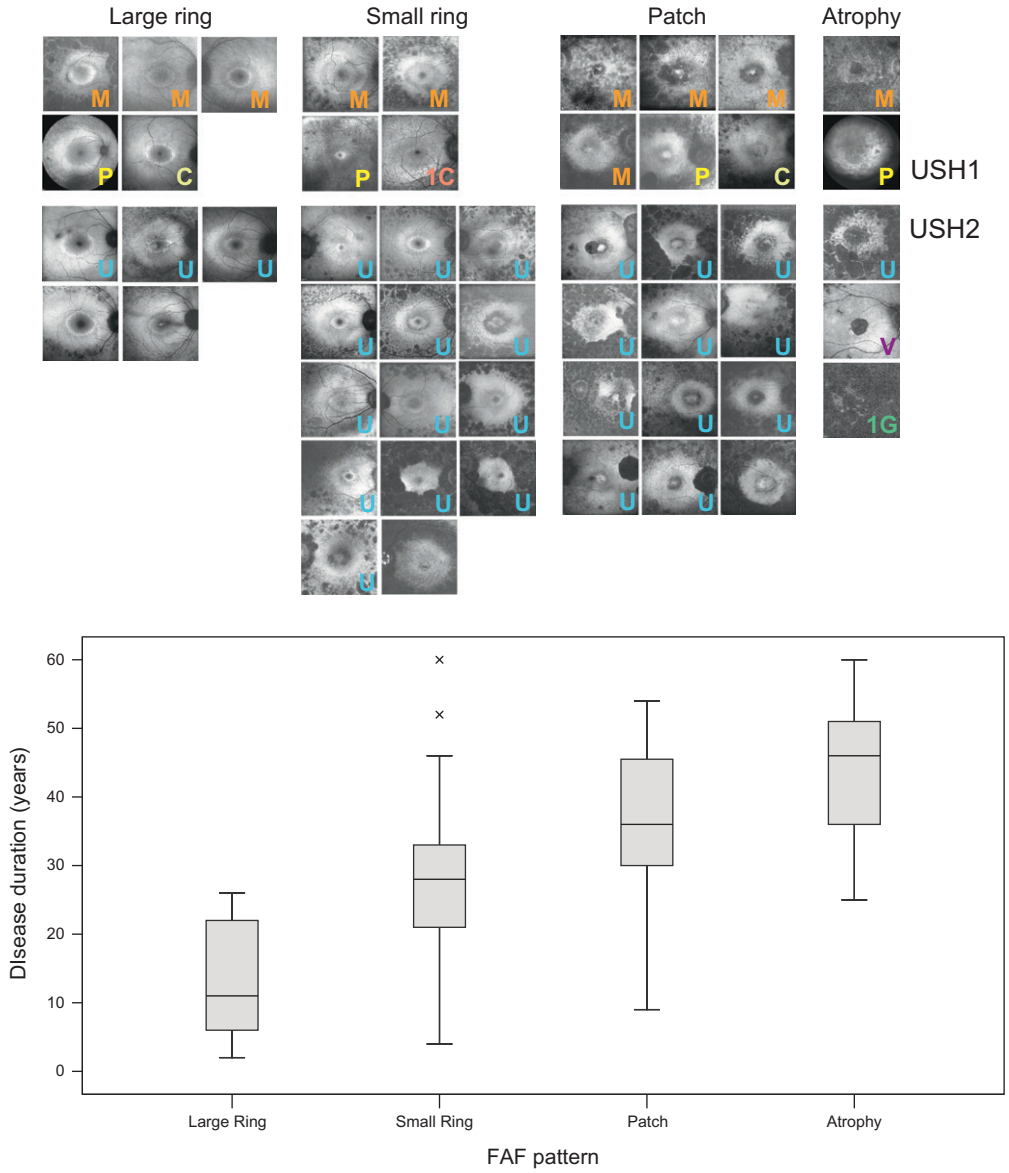

Fig. 2. Fundus autofluorescence of 51 patients with different types of Usher syndrome. Eyes with better image quality are presented. Three patients were excluded due to large CME or imaging difficulties. FAF patterns were divided into categories of large ring (radius $\geqslant 3°$), small ring (radius $< 3°$), foveal patch and atrophy. Genotyped patients are marked with capital letters (M = *MYO7A*, P = *PCDH15*, C = *CDH23*, 1C = *USH1C*, U = *USH2A*, V = *VLGR1*, 1G = *USH1G*). Below is a box plot graph showing disease duration in different FAF categories.

Table 1
Frequency of FAF patterns of ring, patch and atrophy in Usher patients with known genotypes. All three patterns were observed in the three most commonly affected genes (*USH2A*, *MYO7A* and *PCDH15*). In one USH2A patient and one MYO7A patient pattern was unable to be determined due to cystoid macular edema and imaging difficulties, respectively. FAF = fundus autofluorescence.

USH type	USH2	USH1					
Genotype	USH2A	MYO7A	PCDH15	CDH23	VLGR1	USH1C	USH1G
N	29	11	4	2	1	1	1
Avg. age (years)	50	33	40	20	29	23	48
Avg. age at onset (years)	17	9	10	4	4	19	2
Avg. disease duration (years)	33	24	30	17	25	4	46
FAF pattern frequency							
Ring (percent, *N*)	48% (14)	46% (5)	50% (2)	50% (1)		100% (1)	
Patch (percent, *N*)	31% (9)	36% (4)	25% (1)	50% (1)			
Atrophy (percent, *N*)	3% (1)	9% (1)	25% (1)		100% (1)		100% (1)
Ring on one eye and patch on the other	10% (3)						
Patch on one eye and atrophy on the other	4% (1)						

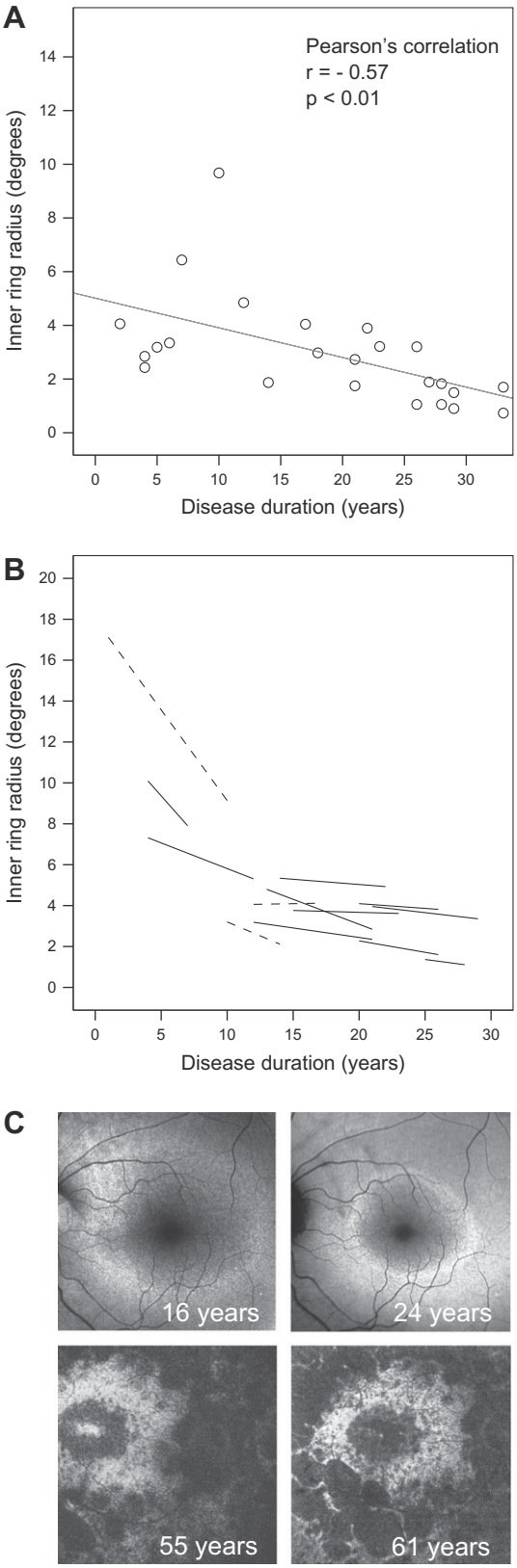

Fig. 3. (A) Inner ring radius in association with disease duration ($N = 24$). Four patients were excluded because of difficulties in FAF analysis due to CME (3) or cataract (1). (B) Spaghetti graph showing decrease of inner ring radius over the years in 13 patients with Usher syndrome. Dashed lines represent USH 1 patients. (C) Sequential fundus autofluorescence imaging of two patients showing ring constriction (up) and progression from patch to atrophy (bottom). Age at the time of examination is marked on FAF images.

59% (29) *USH2A*, 22% (11) *MYO7A*, 8% (4) *PCDH15*, 4% (2) *CDH23*, 2% (1) *VLGR1*, 2% (1) *USH1C* and 2% (1) *USH1G* patients. Average age at disease onset (nyctalopia) was 14 years (range 1–39) and was significantly lower in USH1 patients (average 9 years) than USH2 patients (average 17 years), $p < 0.01$. Average disease duration was 30 years (range 2–60 years). VA on the better eye ranged from 1.0 (20/20) to light perception. Correlation between visual acuity and disease duration was stronger than correlation between visual acuity and age. It was significant in both cases (Fig. 1). VA less than <0.05 (20/400) on the better eye, classified as blindness by World Health Organization, was found in 9/54 (17%) patients (3 USH1, 6 USH2). They were 60 ± 8 years old with 45 ± 9 years of disease duration. Age and disease duration of those patients was not significantly different between USH1 and USH2 patients. There were three children with Usher type 1, ages 7, 9 and 12 years. All three children had a hyperautofluorescent ring and CME at the time of presentation.

3.2. Fundus autofluorescence patterns

All patients had abnormal fundus autofluorescence. Three main patterns were recognized, namely hyperautofluorescent ring, hyperautofluorescent foveal patch and abnormal central hypoautofluorescence (atrophy). Ring was further divided into two subcategories (large and small) in respect to radius of inner ring border (Fig. 2). All FAF patterns could be seen in USH1 and USH2 patients and also in patients with the three most commonly affected genes (*USH2A*, *MYO7A* and *PCDH15*) (Table 1 and Fig. 2). Asymmetric FAF was observed in 5/51 (10%) patients. One *USH1C* patient had rings with large difference in size, three USH2A patients had a combination of unilateral ring and unilateral patch (two are shown in Fig. 4E) and one *USH2A* patient had a combination of unilateral patch and unilateral central hypoautofluorescence.

3.2.1. Hyperautofluorescent ring

Hyperautofluorescent ring was seen in 28/51 (55%) representative eyes. 22/28 (79%) of rings were surrounded with a ring of decreased autofluorescence (Fig. 2) which in some cases correlated with RPE atrophy on OCT. All rings were located inside vascular arcades. Location of inner and outer ring border could be determined in 24/28 patients; in four patients this was not possible due to cataract and/or CME. Internal radii ranged from 0.21 to 2.77 mm and external radii from 0.52 to 3.32 mm. Average ring width was 0.56 ± 0.32 mm. Inner ring radius was smaller in patients with longer disease duration (Fig. 3A). Average visual acuity in patients without media opacities or CME ($N = 20$) was 0.8 ± 0.2 (range, 0.3–1.0).

3.2.2. Hyperautofluorescent foveal patch

Hyperautofluorescent foveal patch was seen in 18/51 (35%) eyes. It was surrounded by a ring of decreased or absent autofluorescence (Fig. 2) which in 17/18 patients correlated with RPE atrophy on OCT (examples in Fig. 6, third and fourth column). Presence of patch was associated with longer disease duration (average 37 years) than presence of ring (average 23 years) (*t*-test, $p = 0.001$). Radius of patch ranged from 0.22 to 0.86 mm (average 0.52 mm) and did not correlate with disease duration (Pearson's correlation, $r = 0.16$, $p = 0.56$). Average visual acuity in patients without media opacities or CME was 0.2 ± 0.2 (range, 0.001–0.6).

3.2.3. Central hypoautofluorescence (atrophy)

Abnormal foveal hypoautofluorescence (atrophy) was seen in 5/51 (10%) eyes. It was surrounded with variable amount of residual perifoveal autofluorescence (Fig. 2). VA ranged from 0.15 to no light perception (average 0.04 ± 0.06).

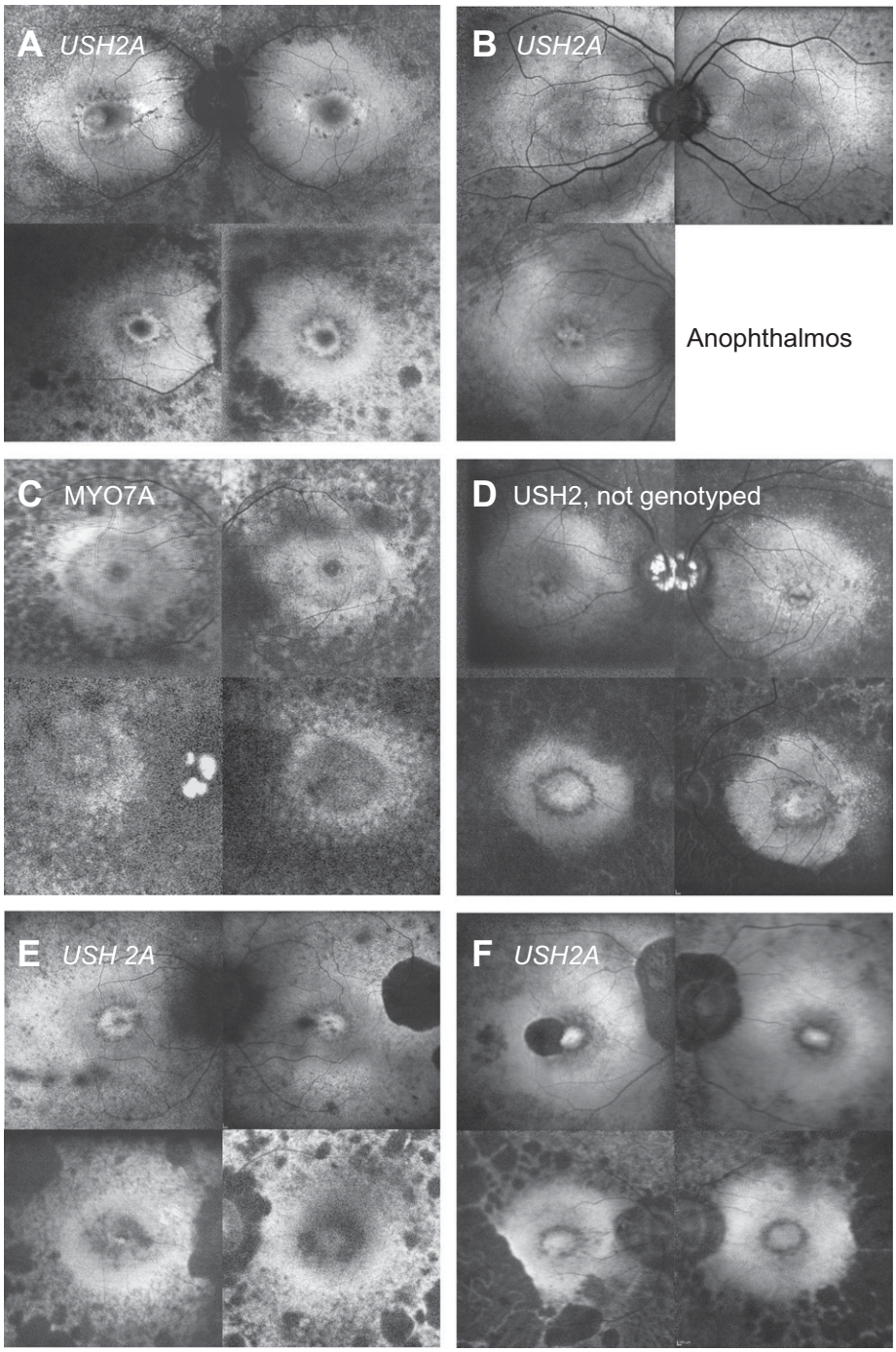

Fig. 4. Fundus autofluorescence in six pairs of siblings (A–F) with Usher syndrome. Specific details appeared similar between siblings, such as small dark spots on the outer ring border (A), large spots of atrophy (E and F) and peripapillary atrophy (F). Autofluorescence pattern on the periphery was also similar in all pairs. Differences between siblings included presence of cystoid macular edema (B bottom and D up) and optic disc drusen (D up and C bottom). Bottom sibling in pair B has had left eye enucleated due to neovascular glaucoma.

3.3. Fundus autofluorescence in siblings with Usher syndrome

Six pairs of siblings with Usher syndrome were included in this study (Fig. 4). Because siblings have presumably the same mutations (confirmed in five pairs), we analyzed their FAF patterns for genotype specific characteristics. Four pairs presented with matching patterns, including bilateral ring (Fig. 4A and B), bilateral patch (Fig. 4F) or combination of unilateral ring and unilateral patch (Fig. 4E). Two pairs presented with different FAF patterns (Fig. 4C and D), possibly as a result of different disease duration (pair C 14 and 27 years, pair D 46 and 54 years). Common characteristics included similar pattern of peripheral autofluorescence, small dark spots on the outer ring border, large spots of atrophy and/or peripapillary atrophy. Differences that were observed between siblings included presence of cystoid macular edema and optic disc drusen (Fig. 4).

3.4. Visual field in relation to fundus autofluorescence

Residual visual field (tested with Goldmann V/5 target) was detected in average of 7° eccentrically to the outer ring or patch border. Goldmann II/4 target was detected eccentrically to the outer ring/patch border in all patients with large rings, 83% of patients

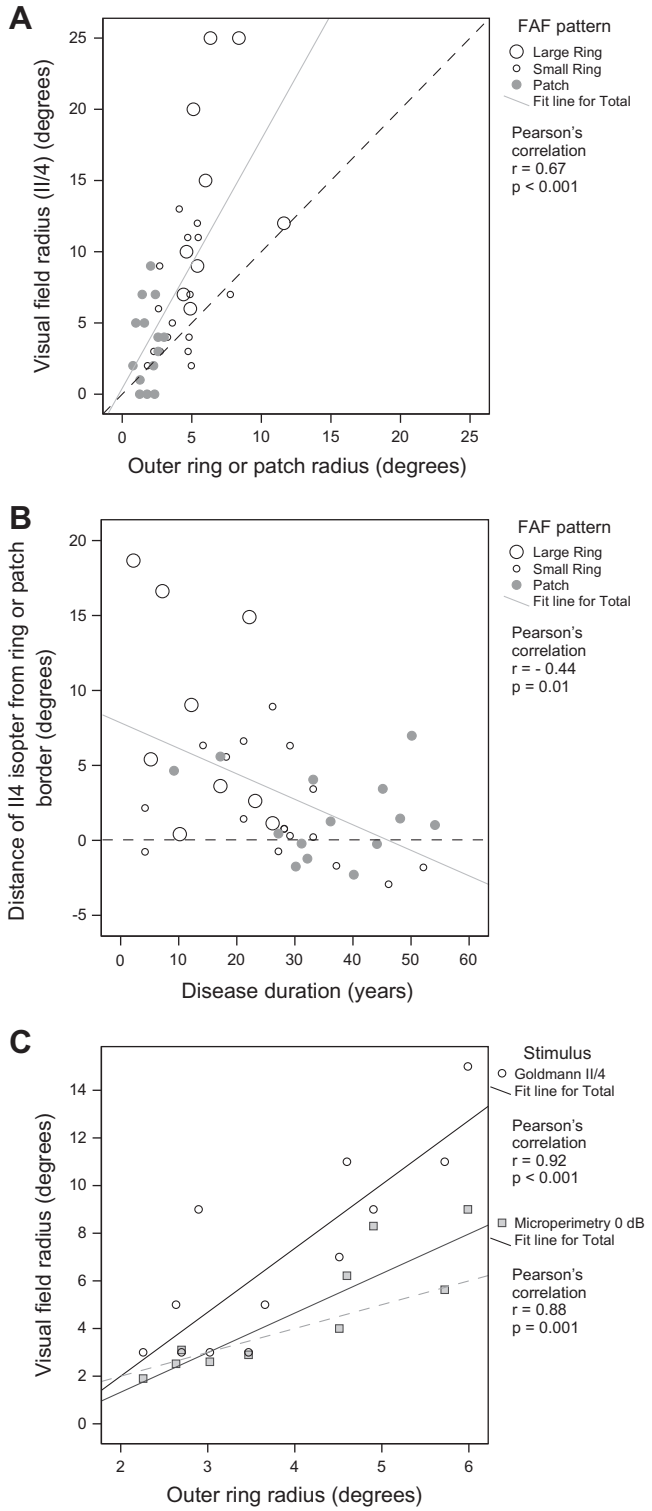

Fig. 5. Visual field in relation to fundus autofluorescence. (A) In majority of patients, visual field measured with Goldmann II/4 stimulus distended over larger area than the ring or patch on FAF. (B) Eccentricity of visual field from outer ring/patch border in association with disease duration. Border of preserved visual field was closer to the ring/patch in patients with long-standing disease. (C) Comparison of microperimetry and Goldmann perimetry in relation to ring radius. Goldmann II/4 target was usually detected further away than the highest intensity stimulus (0 dB) on microperimetry. Dashed line represents a situation where testing stimulus is detected exactly on the outer border of ring/patch (radius of visual field equals radius of autofluorescence).

with small rings and 64% of patients with patches (in average 7°, 3° and 2° eccentrically to border of hyperautofluorescence) (Fig. 5A). Eccentricity of II/4 visual field in relation to AF border decreased with disease duration (Pearson's correlation, $r = -0.4$, $p = 0.01$) (Fig. 5B). Goldmann II/1 target was detected by all patients with large rings and 58% of patients with small rings, in average 1° internally to the inner ring border. Ten patients with hyperautofluorescent rings additionally underwent kinetic microperimetry with stimulus of highest intensity (0 dB). In those patients temporal radii of visual fields measured by Goldmann perimetry (II/4 and II/3) and microperimetry were compared. Goldmann visual field using II/4 stimulus was in average 2.4 ± 2.3° larger than MP visual field (Fig. 5C). Goldmann visual field using II/3 stimulus was in average 0.4 ± 1.3° smaller than MP visual field.

3.5. Optical coherence tomography findings

In all eyes with rings, OCT revealed preserved ELM and ISe across the fovea. In eyes with patch, foveal ELM was seen in all cases and remains of ISe were found in 5/18 (28%) cases (average radius 0.21 mm). Eyes with patches that had remains of ISe had significantly better VA than eyes without it (average 0.5 vs. 0.1; $p < 0.001$). Foveal ONL thickness was not significantly different between the two groups (average 55 vs. 56 μm; $p = 0.9$). In eyes with central hypoautofluorescence, OCT revealed severely disorganized retinal structure with areas of RPE atrophy. Neither ISe nor ELM could be recognized (Fig 6, last column). In eyes with preserved ISe (all eyes with ring and 5/18 eyes with patch), visual acuity and color vision were low when ISe loss encroached on fovea (radius 500 μm), regardless of FAF pattern of ring or patch (Fig. 7).

3.5.1. Correlations between structures on FAF and OCT

In aligned photos of FAF and OCT we observed spatial correlations between inner/outer ring borders and ISe/ELM disruption. Inner ring border was located at approximately the same location as ISe disruption and outer ring border was located are approximately the same location as ELM disruption (point where ELM disappeared or fused with RPE) (Fig. 8). We were interested whether the correlations were exact; therefore we calculated average distances between corresponding structures on FAF and OCT. In average, distance between inner ring border and ISe disruption was 0.13 ± 0.21 mm (range, −0.01 to 0.65 mm; inner ring border was closer to the fovea). Out of the four cases with the largest difference (>0.30 mm), one had large CME (Fig. 8D), one had microcysts and two were without CME. Average difference between outer ring border and ELM disruption was 0.01 ± 0.16 mm (range, −0.44 to 0.29 mm; outer ring border was located closer to the fovea than ELM loss). In eyes with patch, there was a spatial correlation between patch border and horizontal span of ELM (Fig. 6, third and fourth column; Fig. 8B). In average, ELM loss appeared 0.04 mm closer to the fovea than patch border.

3.5.2. Cystoid macular edema

Cystic changes on OCT were seen in at least one eye of 56% (30/54) of patients. They were present in 56% (10/18) of USH1 and 56% (20/36) of USH2 patients. Out of patients with CME, 23% (7/30) had large CME, 57% (17/30) mild CME and 20% (6/30) had micro cystic changes on the more affected eye. Cysts were present in INL (60%, 18), INL and ONL (33%, 10) or OPL (7%, 2). Location or frequency of CME was not dependent on USH type.

3.6. Progression of disease in Usher syndrome

Disease progression in the retina was studied with longitudinal and cross-sectional analysis. Visual function and retinal structure were strongly associated with disease duration (Fig. 9).

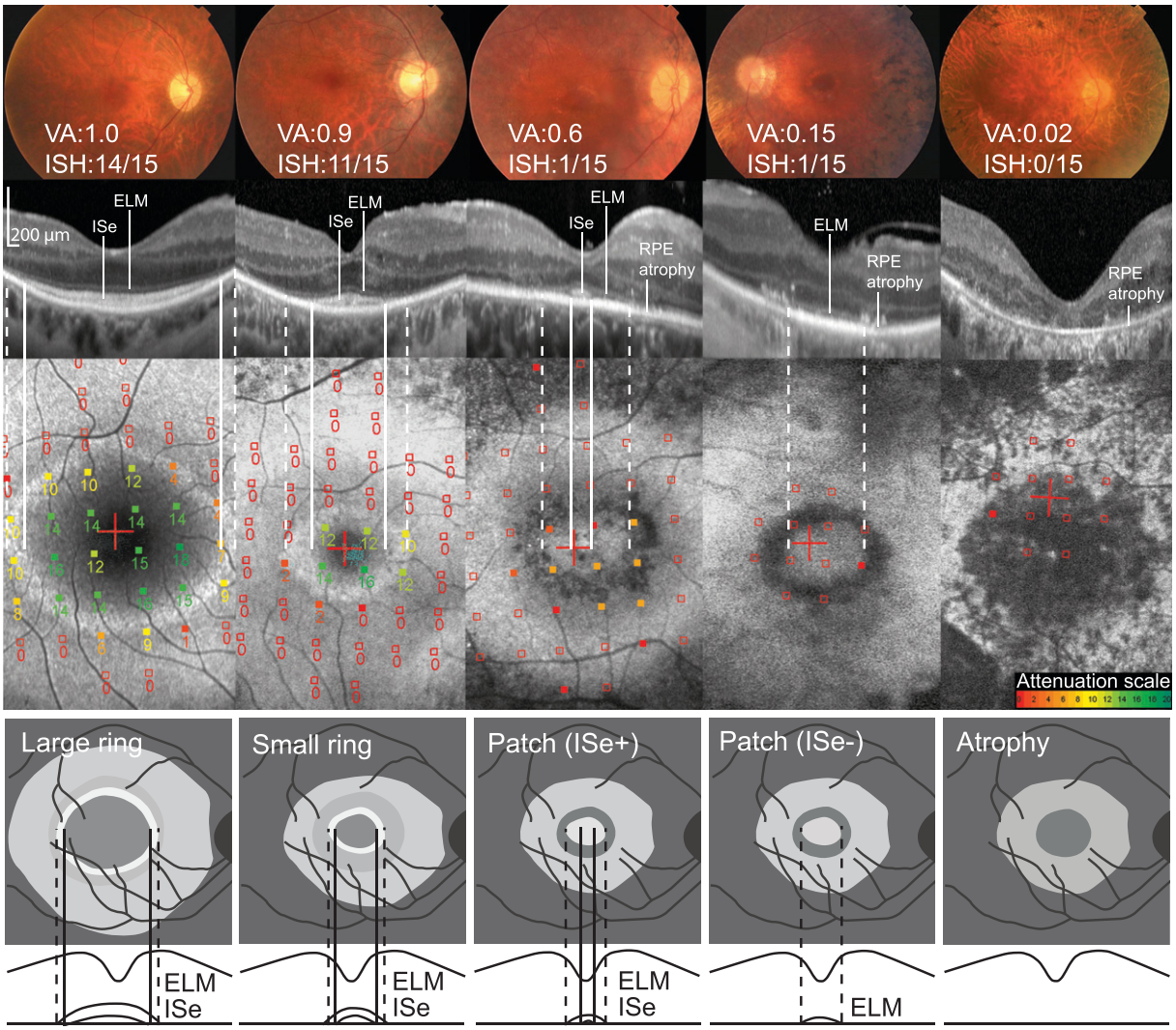

Fig. 6. Examples of Usher patients with different FAF patterns. Fundus photographs, optical coherence tomography and autofluorescence overlaid with microperimetry are shown. White lines show the point of ISe loss and dashed white lines show point of ELM loss. Below is a schematic representation of proposed disease progression. FAF = fundus autofluorescence, ISe = inner segment ellipsoid, ELM = external limiting membrane.

3.6.1. Longitudinal analysis of fundus autofluorescence

Longitudinal changes were assessed in 18 patients who have previously undergone FAF imaging.

Thirteen patients (3 USH1, 10 USH2, without large CME) at baseline imaging presented with hyperautofluorescent ring with average radius of 1.55 ± 1.19 mm. At follow up (7 ± 2 years), ring was seen again in all patients with average inner ring radius of 1.15 ± 0.67 mm. None of the patients developed central patch or hypoautofluorescence. Ring constriction was observed in 12/13 patients (Fig. 3B). Average rate of ring inner border constriction was 0.06 mm (range, 0.00–0.25 mm) or 4% (range, 0–9%) of starting radius per year. Outer ring border could be detected in 12/13 patients. Its average rate of constriction was 0.06 mm (0.00–0.23 mm) or 3% (range, 0–10%) per year. Rate of constriction was not dependent on USH type. After excluding two patients with changes in media opacities and one for lack of visual acuity data, there was a significant drop in average visual acuity in this group (from 0.94 to 0.88, $p < 0.01$). Out of those patients, four were without CME, three had microcysts and three had mild CME.

Five patients at baseline imaging presented with foveal patch. In two of them patch remained at follow-up after 5 years with change in radius from 0.65 to 0.60 and 0.74 to 0.70 mm, respectively. Average rate of patch radius constriction was 0.01 mm or 1% per year. Visual acuity in those patients remained unchanged and was 0.6 in patient with central remains of ISe and 0.015 in patient with absent ISe. In three patients patch was replaced with central hypoautofluorescence (atrophy) at follow-up after 6–9 years (Example in Fig. 3C). Average VA in those patients decreased from 0.28 to 0.08.

3.6.2. Cross-sectional analysis of fundus autofluorescence

FAF images were organized into categories of large ring ($\geqslant 3°$ internal radius), small ring (<3° internal radius), patch and central hypoautofluorescence (atrophy) and average disease duration was calculated for different categories. Ring was observed mainly in patients with up to 30 years of disease (large ring average 13 years, range 2–26; small ring average 28 years, range 4–60) whereas patch and atrophy were observed in patients with longer disease duration (patch average 36 years, range 9–54; atrophy average 44 years, range 25–60) (Fig. 2). Difference in disease duration was significant between patients with small ring and patch and not significant between large and small ring or patch and atrophy (Table 2). Retinal structure (ONL thickness, ELM radius, ISe radius) and function (VA, color vision, visual field, retinal sensitivity) decreased significantly in association with FAF changes (Pearson's correlation, $p < 0.001$ for all) (Table 2, examples in Fig. 6). Foveal

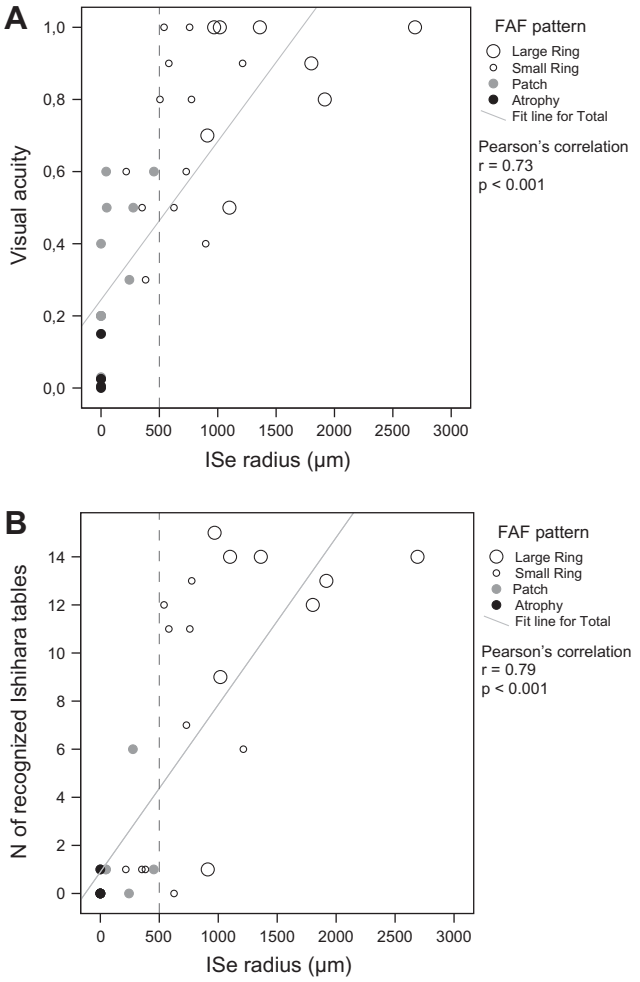

Fig. 7. Visual acuity (A) and color vision (B) in relation to ISe radius. Short ISe radius (<500 μm) was associated with low visual acuity and color vision, regardless of FAF pattern.

lesion (patch or atrophy) in one or both eyes was observed in 3/11 (27%) of USH1 and 4/17 (24%) of USH2 patients with up to 30 years of disease and in 5/6 (83%) of USH1 and 13/17 (76%) of USH2 patients with more than 30 years of disease. Frequencies were not significantly different between USH1 and USH2 patients (Fisher's exact test, $p > 0.5$).

4. Discussion

This study suggests that Usher syndrome patients have common morphological stages of retinal disease in the macula that can be characterized by FAF patterns of ring, patch and foveal atrophy. Ring represents early stage of disease with relatively preserved central visual function while patch and atrophy represent advanced disease with moderate to severe loss of vision.

4.1. Fundus autofluorescence abnormalities

Murakami et al. classified FAF patterns of RP patients including one Usher patient into patterns of ring, central hyperautofluorescence and absence of both. They hypothesized different patterns could be a result of different pathogenesis or different disease stages (Murakami et al., 2008). We have also found patterns of ring and central hyperautofluorescence (patch), however no patients had absence of both and we also had a group of patients with central hypoautofluorescence which they have not described. Difference in our findings could be a result of different stages of the disease or differences between Usher syndrome and nonsyndromic RP. In a few cases it was difficult to distinguish between ring and patch on FAF (examples in Fig. 4E). It is possible that those cases represent intermediary stages between ring and patch pattern, which could be confirmed with longitudinal FAF imaging. While it is known that abnormal FAF can be seen in patients with retinitis pigmentosa, the location of fluorophores in the retina contributing to hyperautofluorescence is not clear. Recently it has been proposed that photoreceptor cells could be a major source of autofluorescence in pathological states (Sparrow et al., 2010). OCT findings in the ring and patch area suggest absence of outer segments in those areas. We propose that photoreceptor inner segments or cell bodies could be the location of abnormal bisretinoid accumulation, possibly contributing to photoreceptor apoptosis (Cottet & Schorderet, 2009; Maeda et al., 2008).

4.2. OCT findings

The hyper-reflective band representing ELM appeared to be intact across the foveal area in all eyes with rings or patches and absent in eyes with central hypoautofluorescence. ELM length correlated closely with outer ring border and patch border, contributing to the idea that patch is a stage following ring. Central remains of ISe were seen in all patients with rings and showed good spatial correlation with the area of normal autofluorescence inside the ring. In patients with patches, ISe was either absent or had radius shorter than 0.5 mm (foveal radius). Spatial correlation between AF and OCT structures was not always exact, i.e. inner ring border was measured in average 0.1 mm closer to the fovea than ISe loss. An explanation for this could be measurement error, especially in recognizing the exact location of inner ring border due to rising signal of autofluorescence from the foveal center towards the periphery or due to presence of CME (Fig. 8C).

4.3. FAF and OCT in relation to visual function

Hyperautofluorescent ring was associated with relatively preserved central visual function, which is in agreement with previous studies (Popovic, Jarc-Vidmar, & Hawlina, 2005; Robson et al., 2003, 2004, 2006). Nevertheless some loss of central vision was observed in majority of patients even at this stage. Visual acuity and color vision were lower in patients with ring radius smaller than 3° and the dimmest (II/1) Goldmann target was often not detected by those patients (see Section 3.4). Patients with ISe radius smaller than radius of fovea were most affected (Fig. 7). Similarly, decrease in VA associated with ring constriction was described in rings smaller than 0.68 mm (2.5°) in radius (Aizawa et al., 2010). These findings could be explained by deterioration of retinal structure inside the rings that has been described in literature, such as shortening of outer segments and ONL thinning in areas of preserved ISe (Hood et al., 2011) and reduced cone numbers inside the rings (Greenstein et al. IOVS, 2012; 53: ARVO E-Abstract 4577).

Microperimetry and fine matrix mapping studies have shown strong spatial correspondence between sensitivity losses and the internal edge of the ring (Lenassi et al., 2012; Robson et al., 2004, 2012). They have shown that sensitivity is well preserved inside the ring of hyperautofluorescence and decreases with eccentricity. We have also found good sensitivity inside the ring and decreased sensitivity outside the ring using Goldmann perimetry (stimulus II/1 was detected only inside while II/4 and V/4 were detected outside the ring in most patients). Large Goldmann target with high intensity (V/4) was used to determine how far from the ring any light stimulus can still be detected. Our results suggest that although abnormal, there usually is some cone function up to

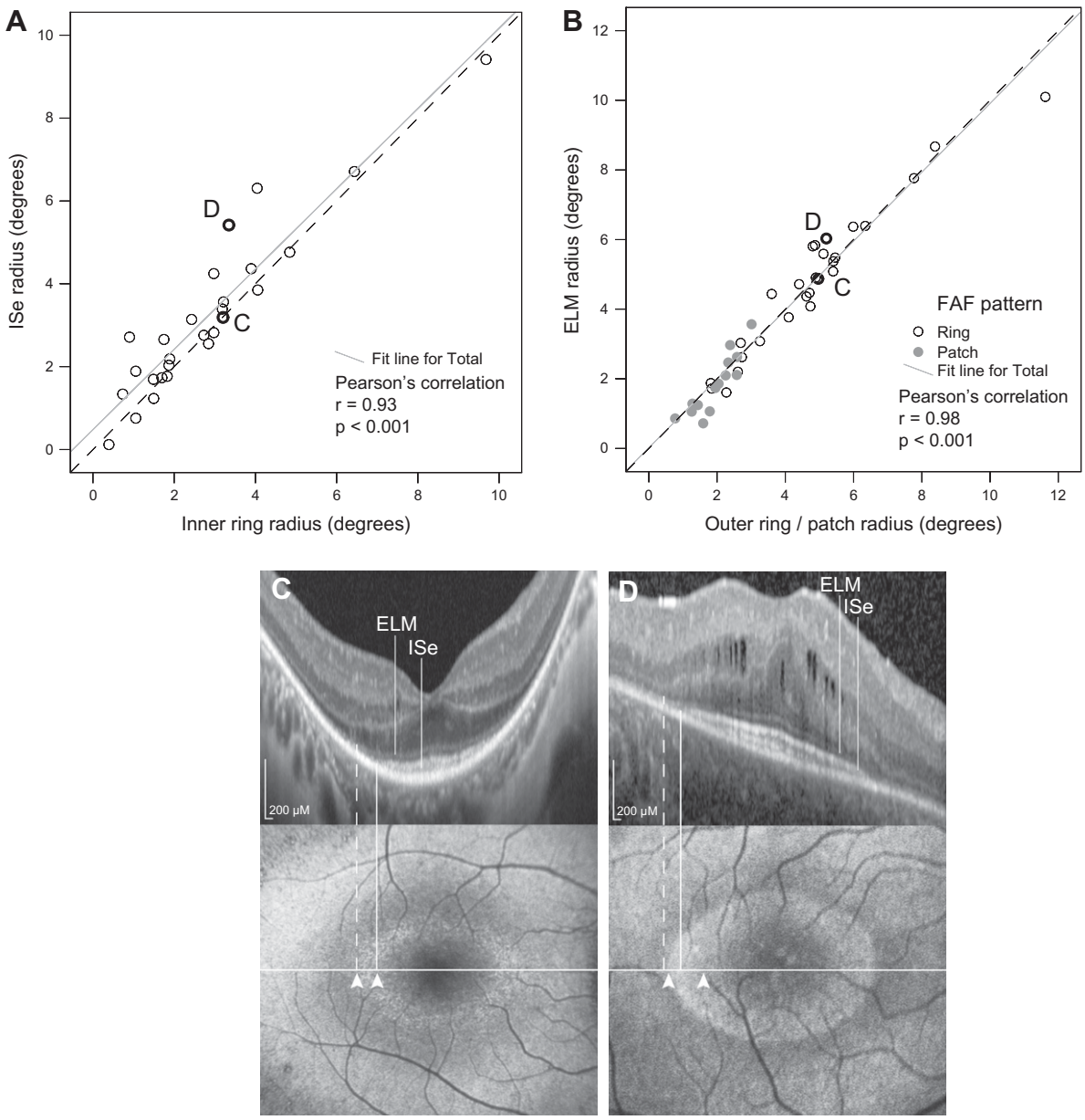

Fig. 8. (A) Correlation between horizontal span of ISe and radius of inner ring border. (B) Correlation between horizontal span of ELM and radius outer ring border. Dashed line on graph represent a situation where radius of measured structure on OCT equals radius of measured ring or patch border. (C and D) Two examples of spatial correlations between FAF and OCT structures (cases are also marked on the graphs with letters C and D). Dashed and full white lines represent point of ELM and ISe disruption, respectively. Arrowheads represent outer and inner border of the hyperautofluorescent ring. ISe = inner segment ellipsoid, ELM = external limiting membrane.

about 7° outside from the ring. Interestingly, histological analysis of RP retinas have shown that when all rods and most of the cones are lost, the macula usually retains a monolayer of cone somata with very short or absent outer segments (Milam, Li, & Fariss, 1998), which might explain this residual cone function.

Hyperautofluorescent foveal patch was seen in patients with several decades of disease duration and was associated with significant loss of central visual function and ONL thinning (Table 2), reflecting loss of photoreceptor cells in the fovea. Visual acuity was relatively preserved only in patients with central remains of ISe, which might represent a transition from ring to patch. Abnormal central hypoautofluorescence (foveal atrophy) was seen in 5 eyes and was associated with the most severe loss of retinal structure and function. Limitation of the current study is that scotopic macular function was not examined as all examinations were performed under photopic conditions and therefore only function of cones could be evaluated.

4.4. Disease progression

Serial FAF imaging of rings in patients with short disease duration and the presence of central FAF abnormalities in cases with long-standing disease suggest that rings constrict with time and may eventually be replaced by central FAF abnormalities (hyperautofluorescent patch and atrophy) associated with visual acuity loss. This is in keeping with published data that demonstrated progression from a ring to central hyperautofluorescence in a few individuals (Robson et al., 2011; Wakabayashi et al., 2010). Additonally, we have observed progression from central hyperautofluorescence (patch) to central hypoautofluorescence (atrophy) in three patients (example in Fig. 3C).

Average rate of disease progression in the macula was estimated by measuring ring constriction and was 4% per year. This is in good agreement with two previously published papers. Lima et al. reported rates of inner border constriction for two patients

Table 2
Differences in retinal structure and function between patients with large ring, small ring, patch or atrophy. Patients with CME, cataract, macular traction or posterior capsular opacification are excluded. Values which are significantly different between each other are marked in bold (ANOVA, LSD post hoc test, $p < 0.05$). *y* = Years, ELM = external limiting membrane, ISe = inner segment ellipsoid, ONL = outer nuclear layer, VA = visual acuity, N/A = not applicable.

FAF pattern	Large ring ($r \geqslant 3°$)	Small ring ($r < 3°$)	Patch	Atrophy	Pearson's correlation (r, p)
N	8	12	18	5	
Age (average, range)	31y (7–58)	37y (23–54)	50y (32–69)	51y (29–69)	0.5, <0.001
Disease duration (average, range)	13y (2–26)	**22y** (4–33)	**37y** (9–54)	44y (25–60)	0.7, <0.001
Retinal structure (average, range)					
Outer ring border/patch radius	**6**° (4–12)	**4**° (2–8)	**2**° (1–3)	N/A	−0.8, <0.001
ELM radius	**6**° (4–10)	**4**° (2–8)	**2**° (1–4)	N/A	−0.8, <0.001
Inner ring border radius	**5**° (3–10)	**2**° (1–3)	NA	N/A	−0.7, <0.001
ISe radius	**5**° (3–9)	**2**° (1–4)	**1**° (0–2)	N/A	−0.8, <0.001
ONL thickness in fovea	114 μm (93–129)	**108 μm** (63–182)	**56 μm** (36–88)	N/A	−0.8, <0.001
Retinal function (average, range)					
VA	0.9 (0.5–1.0)	**0.7** (0.3–1.0)	**0.2** (0.001–0.6)	0.04 (0–0.15)	−0.8, <0.001
Number of recognized Ishihara tables	**12** (1–15)	**6** (0–13)	**1** (0–6)	0 (0–1)	−0.8, <0.001
II/1 visual field radius	**3**° (1–7)	**1**° (0–2)	N/A	N/A	−0.8, <0.001
II/4 visual field radius	**14**° (6–25)	**7**° (2–13)	**3**° (0–9)	3° (0–6)	−0.7, <0.001
Average sensitivity in central 8°	**13 dB** (7–19)	**6 dB** (1–13)	**1 dB** (0–7)	0 dB (0–1)	−0.8, <0.001

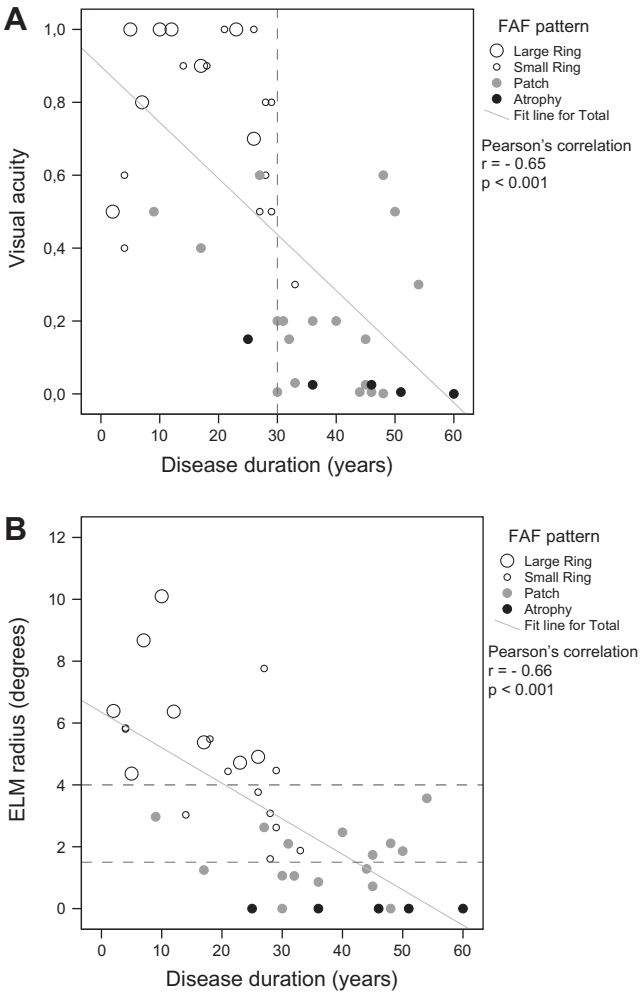

Fig. 9. (A) Visual function (A) and retinal structure (B) in association with disease duration. Different FAF patterns are marked (see legend). (A) Significant loss of visual acuity was seen after 30 years of disease, associated with patch or atrophy on fundus autofluorescence. (B) ELM was shorter in patients with longer disease duration. At ELM radius between 1.5° and 4°, rings or patch could be observed. FAF = fundus autofluorescence, ELM = external limiting membrane.

with recessive RP. Average rate of constriction was calculated from their data to be 4% per year (Lima et al., 2012). Robson et al. reported inner border constriction in 6/12 Usher patients. Taken all 12 patients together, their average rate of constriction was calculated at 5% per year (Robson et al., 2011). Reported rates of visual field constriction are somewhat higher (6–9%) (Fishman et al., 2007; Iannaccone et al., 2004; Sandberg et al., 2008).

4.5. Disease expression in different types of Usher syndrome

The existence of different clinical subtypes of Usher syndrome based on hearing loss have been known for a long time, however possible differences in retinal phenotype have been controversial. Apart from earlier disease onset in USH1 we have not found any other differences in disease expression. We found the same basic FAF patterns in USH1 and USH2 patients as well as in all three the most commonly affected genes (*USH2A*, *MYO7A* and *PCDH15)*. Similarly, Jacobson et al. have not found any differences when analyzing OCT of *MYO7A*, *PCDH15*, *USH2A* and *GPR98* patients (Jacobson et al., 2008). When separately analyzing patients with short and long-standing disease, USH1 patients did not have significantly higher frequency of foveal lesions than USH2. There was also no significant difference between age and disease duration between USH1 and USH2 patients with legal blindness. We cannot exclude a possibility that specific genes or mutations produce variations of disease expression such as some of the features seen in our sibling pairs; however this should be confirmed in large groups of genotype specific patients with the same disease stage.

4.6. Conclusion

The present study provides an opportunity to increase understanding of retinal disease in Usher syndrome. Central vision remained relatively preserved in most Usher patients for up to three decades after onset of nyctalopia. After three decades the fovea was usually involved, reflected by loss of central visual function. Hyperautofluorescent foveal patch was the hallmark of this stage. Understanding the natural history of retinal disease in Usher syndrome will be important in the prospect of clinical trials.

Contributors

Ana Fakin: Microperimetry, OCT, Goldmann, morphological and statistical analysis, concept and article preparation.

Martina Jarc-Vidmar: Performed ophthalmologic examination, morphological analysis and article preparation.

Damjan Glavač: Contributed to genetic analysis and article preparation.

Crystel Bonnet: Performed genetic sequencing of Usher genes.

Christine Petit: Contributed to genetic analysis and article preparation.

Marko Hawlina: Performed ophthalmologic examinations, morphological analysis, concept and article preparation.

All authors have approved the final article.

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Priloga 2

»AKTIVNA UDELEŽBA NA KONGRESIH IN STROKOVNIH SREČANJIH POMENI ZELO RESNO DELO«

Pogovarjali smo se s prof. dr. Markom Hawlino, dr. med., ki je prejel najvišjo nagrado evropskega odbora za izobraževanje – Peter Eustace Medal

Prof. dr. Marko Hawlina, dr. med., je eden od profesorjev, ki so v zadnjih letih prejeli nagrado evropskega odbora za izobraževanje (European Board of Ophthalmology, EBO) za prispevek pri razvoju izobraževanja v evropski oftalmologiji. Nagrada se imenuje Peter Eustace Medal, imenovana pa je po ustanovitelju evropskih specialističnih izpitov na področju oftalmologije. Prof. dr. Marko Hawlina, dr. med., je nagrado prejel za svoje dolgoletno delo na področju izobraževanja v evropskem merilu.

Zaradi katerih dejavnosti ste si prislužili nagrado evropskega odbora za izobraževanje?

> Ob vseh obremenitvah z rutinskim delom, ki so tudi v terciarni ustanovi v veliki meri na sekundarni ravni zahtevnosti, za izobraževanje žal velikokrat zmanjka volje in predvsem časa. Ob težavah in incidentih pa nato vsi ugotavljajo, da je razlog v pomanjkljivem izobraževanju.

Koliko držav povezuje evropski odbor za izobraževanje?

Kakšna pa je splošna vloga evropskega izpita?

Vrnimo se k vašim dejavnostim, zaradi katerih ste prejeli nagrado.

Druga težava je tudi denar ...