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Evaluation of Clinical and Genetic Characteristics of the Inherited Retinal Dystrophies

SUMMARY OF DOCTORAL DISSERTATION

Biomedical science,
Medicine 06B

VILNIUS 2018

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TABLE OF CONTENTS

ABBREVIATIONS

1000 G	—	1000 Genomes Project
ACHM	—	achromatopsia
AD	—	autosomal dominant
AF	—	autofluorescence
Alt	—	alternative
AMD	—	age-related macular degeneration
AR	—	autosomal recessive
BCVA	—	best corrected visual acuity
BE	—	both eyes
CA	—	central atrophy
CD	—	cone dystrophy
CME	—	cystoid macular edema
CNV	—	choroidal neovascularisation
CRD	—	cone-rod dystrophy
CRT	—	central retinal thickness
SD	—	standard deviation
SCT	—	subfoveal choroidal thickness
DA 3.0 ERG	—	dark-adapted 3.0 ERG
EOG	—	electrooculography
ERG	—	electroretinography
ERM	—	epiretinal membrane
EZ	—	ellipsoid zone
FAF	—	fundus autofluorescence
FDD	—	familial dominant drusen
HWE	—	Hardy-Weinberg equation
LE	—	left eye
LITGEN	—	acronym for the project called „Genetic diversity of the population of Lithuania and changes of its genetic structure related with evolution and common diseases“
mfERG	—	multifocal electroretinography

N	—	number
OND	—	optic nerve disc
RD	—	retinal dystrophy/ retinal dystrophies
RE	—	right eye
Ref	—	reference
RNFL	—	retinal nerve fiber layer
RP	—	retinitis pigmentosa
RPE	—	retinal pigment epithelium
RS1	—	retinoschisin 1
SD-OKT	—	spectral domain optical coherence tomography
SNP	—	single nucleotide polymorphism
STGD	—	Stargardt disease
VA	—	visual acuity
VMT	—	vitreomacular traction
XLRS	—	X linked retinoschisis
µm	—	mikrometer (10-6 m)
µV	—	mikrovolt (10-6 V)

GENE ABBREVIATIONS

ABCA4	—	ATP-binding cassette, subfamily a, member 4
CNGB3	—	cyclic nucleotide-gated channel, beta-3
EFEMP1	—	EGF-containing fibulin-like extracellular matrix protein 1
PRPH2	—	peripherin 2, mouse, homolog of
RHO	—	rhodopsin

INTRODUCTION

Inherited retinal dystrophies (IRD) constitute a vast group of genetically determined diseases with different patterns of inheritance, vision disturbance and eye fundus presentation. Consequently, both clinical and genetic heterogeneity is characteristic to the disease. To date, changes causing IRD are identified in more than 190 genes and more than 40 genetic loci. Most of these conditions share common feature: loss or dysfunction of photoreceptors as primary or secondary cause [1]. Most of these diseases result in progressive loss of vision up to blindness. IRD is a serious problem, since these diseases are the most common causes of genetic blindness in the Western countries [2]. It should be noted that the disease is caused by genetic changes related to retinal degeneration only for some of the patients [3]. Due to complex and intricate structure and function of the retina even bigger heterogeneity of the disease is expected as well as discovery of new yet unknown genes and pathogenic variants.

Earlier retinal dystrophies were diagnosed and grouped according to clinical features (e. g., retinitis pigmentosa (RP), Stargardt disease). However, improvement of diagnostic capabilities in ophthalmology and impementation of genetic testing caused better understanding of aforementioned heterogeneity of diseases; for example, it became clear that retinitis pigmentosa is the same clinical presentation of a number of different genetic diseases. Nowadays more accurate classification of retinal diseases based on information provided by molecular genetic research is possible (e. g., *ABCA4* (ATP-binding cassette, subfamily a, member 4) retinopathy, rhodopsin RP, ciliopathies). For a long period of time genetic testing was limited due to its availability, cost and time consumption, but intense development of genetics causes sequential reduction of testing costs and increasing of tests availability.

Diagnostic of IRD frequently is intricate. Careful analysis of genealogy and comprehensive ophthalmologic examination including examination of color vision, optical coherent tomography (OCT),

fundus autofluorescence (FAF) and electrophysiological testing s appropriate in these diseases.

However, even when all possible diagnostic tests are performed exact diagnosis of the disease and thus it's prognosis may remain unclear. In such case, genetic testing remains the only possibility to clarify diagnosis and provide patients with comprehensive information about his/her disease, its course and further life (work, hobby) prospects. Although these diseases, except some special cases, are incurable, patient seeks definite diagnosis in order to find out prognosis of the disease, risk of its transmission to his/her descendants, and hopes that new treatment modalities shall occur in the future.

IRD may present both as isolated and multisystemic lesions.

Genetic testing of patient frequently is complicated. In some monogenic diseases such as X-linked retinoschisis which is usually caused by changes in *RS1* gene testing is rather simple. However, in the most common retinal dystrophy, RP, genetic testing becomes a real challenge as presently more than 2800 mutations in more than 100 genes are identified, and this number is constantly increasing. Therefore, diagnosis of RP is established only in 50% of patients. Furthermore, often presentation of the same genetic factor may be phenotypically differently expressed and thus diagnostic of the disease or genetic change may be complicated [4].

Multiple pre-clinical and clinical trials in various scientific centers around the world are being conducted in order to identify new modalities of the treatment of IRD. In 2007, gene therapy was applied for the first time in 3 patients with Leber's congenital amaurosis [5]. Comprehensive knowledge in the field of genetics allows to identify mutation-specific clinical features in several different phenotypes and develop pathophysiology hypothesis for experimental research [6].

To date there is no data concerning incidence of the IRD in Lithuania, and only global and European data may be used.

The following issues will be analyzed and discussed in this research study: which IRD are prevalent in Lithuania, whether their

phenotype and genotype agree with literature data.

Aim of the research

To evaluate the clinical and genetic characteristics of inherited retinal dystrophies in a Lithuanian patient group.

Objectives of the research

1. To develop the monitoring system of the biomedical research in accordance with standardized patient inclusion criteria, to perform data analysis.
2. To identify known and diagnose new pathogenic variants of known genes in individuals with retinitis pigmentosa.
3. To assess phenotype and genotype-phenotype correlation in subjects with retinitis pigmentosa in the Lithuanian patient group.
4. To assess phenotype of *ABCA4* retinopathies, to characterize identified pathogenic variants.
5. To identify known and diagnose new pathogenic variants of known genes, to characterize phenotype in individual cases of rare retinal dystrophy.

Statements to be defended

1. The continuous monitoring system of the inherited retinal dystrophies helps to analyze the clinical data systematically and set up the base for the new research methods and the evaluation of their effectiveness.
2. The visual electrophysiology investigations are mandatory for the identification and progression evaluation of the inherited retinal dystrophies.
3. Inherited retinal dystrophies are characterized by a high genetic and phenotypic heterogeneity.

Novelty of the scientific research

This research is the first research performed in Lithuania to identify, analyze and classify IRD, and to perform their genetic testing and assess phenotype-genotype correlation.

According to global literature, every country seeks to identify phenotype and genotype of the IRD in their inhabitants because gene pool differs in different nations causing different type and course of the IRD.

During this research a non-invasive visual electrophysiological test was introduced into clinical practice, and one of the testing modes, multifocal electroretinography (mfERG) was applied in Lithuania for the first time. Visual electrophysiological testing is necessary for the identification and analysis of IRD because in the early stages of the disease differences between various diseases could be difficult to recognize; test is also important for the assessment of progression of the disease.

It was sought to assess character and prevalence of the IRD in the Lithuanian patients' group. For the first time in Lithuania group of patients with IRD was formed with comprehensive ophthalmological examination and genetic clinical testing of these patients was performed. For the first time genetic causes of wide spectrum of IRD were identified in the Lithuanian population. To the best of our knowledge, no research evaluating phenotype and genotype in patients with IRD were performed both in Lithuania and Baltic states.

A local monitoring systems of IRD was intended to be established in the Center of Eye Diseases, Clinic of ENT and Eyed Diseases, Institute of Clinical Medicine, Faculty of Medicine (Vilnius University) during the research.

Practical significance of the research

Identification of the causes of IRD allows to assess phenotype of the disease more accurately based on gene mutations described in the literature, and assess course of the disease. This also contributes to the rapidly emerging gene therapy area. Multiple pre-clinical and

clinical studies in the field of gene therapy bring hope to develop treatments for the patients with IRD. However, to achieve this identification of precise phenotype and genotype of the disease is necessary which have been done in this study.

Scientific benefits of the research:

- ✓ Molecular genomic testing allows the identification of new candidate genes that are important for the etiopathogenesis of disturbance of vision due to retinal dystrophy.
- ✓ Analysis of medical data could allows the identification of relation between genes and disease characteristics.
- ✓ Analysis of medical data will allow the identification of etiologic structure of IRD in study population.

Benefits for patients:

- ✓ In this scientific research genetic changes causing the development of IRD in a patient may be identified, prognosis of the disease and risk for patient and for descendants of his family is assessed.

1. SCOPE AND METHODOLOGY OF THE RESEARCH

1.1. Study design

Inclusion criteria:

- ✓ Patients with clinically diagnosed IRD.
- ✓ Patients with clinically suspected IRD.
- ✓ Age: 6-99 years.
- ✓ Completed full ophthalmological examination, specimen for molecular genetic testing was obtained.

As Lithuania has no database of patients with eye diseases including inherited eye diseases, patients were selected and invited to participate in this study according to the following procedure:

- ✓ Patients who have previously visited Consultation Polyclinic of the Vilnius University Hospital Santaros

Klinikos and were diagnosed with the disease coded H35.5 (inherited retinal dystrophies) according to ICD (patients were selected according to the data stored in in the information database).

- ✓ Ophthalmologists and clinical geneticists working at Ophthalmology division of the Vilnius University Hospital Santaros Klinikos and Center of Medical Genetics referred patients with the diagnostic code H35.5 according to ICD for the consultation concerning participation in the research.
- ✓ Ophthalmologists working in hospitals of Vilnius region referred patients with the diagnostic code H35.5 according to ICD for the consultation concerning participation in the research.

Upon confirmation of the diagnosis patients were included into research.

All patients were interviewed, and comprehensive ophthalmological and genetic examination was conducted.

Approval for this research No. 158200-15-770-287 of Vilnius Regional Bioethics Committee was obtained, Protocol No. 2, version 2 (February 3, 2015).

1.2. Methodology of the research

1.2.1. Counselling

The following data on clinical signs were collected: onset of the disease, course of the disease (non-progressive/progressive; rapid/slow progress), complaints and symptoms (nyctalopia, loss of visual field (tunnel vision), nystagmus, deterioration of vision, nature of deterioration of vision, photophobia), history of other eye diseases or ophthalmological drugs use, eye injuries and surgeries; patient was also asked about family history of similar symptoms or other inherited eye diseases. Demographic data were collected: place of residence, age and gender.

1.2.2. Ophthalmological examination

1. Objective refraction in both eyes was identified by auto kerato-refractometer KR-1 (Topcon, Japan) in the presence of mydriasis.
2. Assessment of the best corrected visual acuity (BCVA) according to Snellen decimal system.
3. Color vision testing (Ishihara test, 38 plates, Kanehara Trading Inc., Tokyo, Japan) at the distance of 40 cm.
4. Intraocular pressure (IOP) was measured using Goldmann applanation method (HAAG-STREIT International, tonometer AT-900, Bern Switzerland) under local anesthesia with 0.5% Proxymetacaine (Alcon-Couvreur, Belgium) when patient was sitting at slit-lamp. If not possible, non-contact tonometry was employed (Topcon CT-80A, Japan).
5. Standard automatic perimetry (SAP) was conducted using *Humphrey Field Analyzer* (Carl Zeiss MediTek, Dublin, CA, USA) in *central threshold 30-20 mode*. Only reliable results were assessed (fixations, false-negative and false-positive results were observed in ≤ 33%). In other cases, kinetic *Goldmann spherical* visual field test was conducted.
6. Ophthalmobiomicroscopy (SL-Zoom-bon, Jen-ophthalmo, Jena, Germany) was conducted in the presence of mydriasis.
7. Images of the fundi of both eyes (BE) were taken in the presence of mydriasis (Topcon TRC retinal camera, type IA, 50DX).
8. Optical coherence tomography was conducted using Spectralis SD-OCT apparatus and Heidelberg Eye Explored operating system operating in HRA + OCT and enhanced depth imaging (EDI) mode (Heidelberg Spectralis Engineering, Dossenheim, Germany). The following characteristics were assessed:
    - 8.1. Thickness of the retinal nerve fiber layer (RNFL) (μm);
    - 8.2. Central retinal thickness (CRS) at fovea) (μm);
    - 8.3. Subfoveal choroidal thickness (SCT) (μm) (to evaluate structure of chorioidea enhanced depth imaging (EDI) mode was employed);

8.4. Anatomical integrity and structure of retina was assessed.

8.5. Testing of autofluorescence of the fundi of BE was conducted in the presence of mydriasis using Spectralis HRA + OCT apparatus in AF mode (Heidelberg Engineering, Heidelberg, Germany).

9. Visual electrophysiological examination: subjects underwent full-field electroretinogram (ERG); subjects with sufficient visual acuity (VA) and ability to fixate the gaze underwent additional mfERG. Visual electrophysiological examination was conducted using Reti-port/scan 21 plus apparatus (Roland Consult Stasche & Finger GmbH, Brandenburg, Germany). Examination was conducted according to the protocols of the International Society for Clinical Electrophysiology of Vision (ISCEV).

Study was conducted in April 2015 – November 2017 in the Center of Eye Diseases of the Vilnius University Hospital Santariškių Klinikos (Since May 1, 2017 – Vilnius University Hospital Santaros Klinikos). The majority of tests were conducted by the author of the study. Tests were conducted during the daytime, from 8:00 to 16:00. Tests were conducted in accordance with principles of Helsinki Declaration, 1975 (amended in 2000).

1.3. Molecular genetic testing

Peripheral blood samples were collected for all study participants for molecular genetic testing. DNA extracted using phenol-chloroform method by the standardised protocol (no. 461) at the Laboratory for Molecular Genetics and Cytogenetics of Vilnius University Hospital Santaros Klinikos Center for Medical Genetics. DNA concentration and purity was detected using NanoDrop® spectrophotometer using NanoDrop® ND-1000 Spectrophotometer User Manual. Selected target IRD genes (their coding and non-coding sequences) have been sequenced using *Sanger* sequencing method on 3130xl Genetic Analyzer (Applied Biosystems™, Thermo Fisher Scientific, USA) and next generation sequencing method on Ion

TorrentTM Personal Genome MachineTM (PGM)“ (Ion Torrent™, Thermo Fisher Scientific, USA) according to system user manuals optimized at the Department of Human and Medical Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University and the Laboratory for Molecular Genetics and Cytogenetics of Vilnius University Hospital Santaros Klinikos Center for Medical Genetics.

In the RP group, 9 SNP were determined and were compared Lithuanian, European and global data on healthy patients, distribution of reference and alternative alleles and genotypes in the RP subjects and LITGEN groups was examined, and SNP and phenotype correlations were calculated in RP subjects.

To compareIRD SNP genotypes with data on Lithuanian self-reported healthy individuals from LITGEN project were included. LITGEN (Lithuanian “Lietuvos populiacijos genetinė įvairovė ir sandaros kitimai, susiję su evoliucija ir dažniausiai paplitusiomis ligomis” (Genetic Diversity of the Lithuanian Population and Structural Alterations Related to Evolutions and Most Common Diseases)) is a project completed in 2011-2015 intended to conduct large scale genomic testing and identify genomic areas characteristic to hypothetic Lithuanians that are significant for their health (project scientific leader prof. V.Kučinskas).

To compare SNP with European and global data on healthy subjects’ data from the 1000G project were used. 1000G project (1000 genomes) is a catalogue of the genomic variations created within the collaboration project carried out by the USA, United Kingdom, China and Germany.

1.4. Statistical data analysis

Statistical calculations were performed using free statistical software R (version 4.3.2). Shapiro-Wilks test was used to test normality of quantitative variables of phenotype. Distribution of all variables, except age, were not normally distributed, therefore descriptive statistics of these quantitative variables are presented:

arithmetical mean and standard deviation (SD) as well as median and interquartile differences. Gender differences in phenotype characteristics were evaluated using non-parametric Wilcoxon test for independent samples. Differences between left and right eyes were evaluated using non-parametric Wilcoxon test for depending samples. Spearman's correlation coefficient was calculated to measure correlations between variables. Boxplots were presented to evaluate association between phenotypic and genotypic quantitative variables.

Chi-square criterion was used to test genotype frequency complying with Hardy-Weinberg equation (HWE). Chi-square criterion was also used to evaluate differences in frequencies in RP and LITGEN groups. When no rare allele was identified, Yate's correction for continuity was applied. Proportion differences between LITGEN (www.litgen.mf.vu.lt) and 1000 genome project (http://www.internationalgenome.org) markers were tested using proportion criterion. In this dissertation differences were considered statistically significant when p-value was lower than 0.05.

1.5. Author's contribution

Approval of Vilnius Regional Bioethics Committee	—	100%.
Development of inclusion criteria	—	100%.
Subjects' screening and enrollment	—	95%.
Subjects ophthalmological examination	—	90%.
Assessment of the ophthalmological data	—	100%.
Subjects' genetic consultation	—	0%.
Analysis and interpretation of molecular genetic testing	—	30%.
Statistical data analysis	—	50%.
Publications, poster presentations and oral presentations	—	90%.

2. RESULTS AND DISCUSSION

2.1. Study groups

One hundred and thirty-seven subjects participated in the research, 18 of which did not meet the inclusion criteria. The remaining subjects were divided into three large groups: RP, ABCA4 retinopathies, and other rare IRD. Group of rare dystrophies was divided into 10 subgroups according to different inherited retinal or vitreoretinal diseases. Figure 1 shows study groups.

35 study subjects were related – totally 16 familial cases were included in the study.

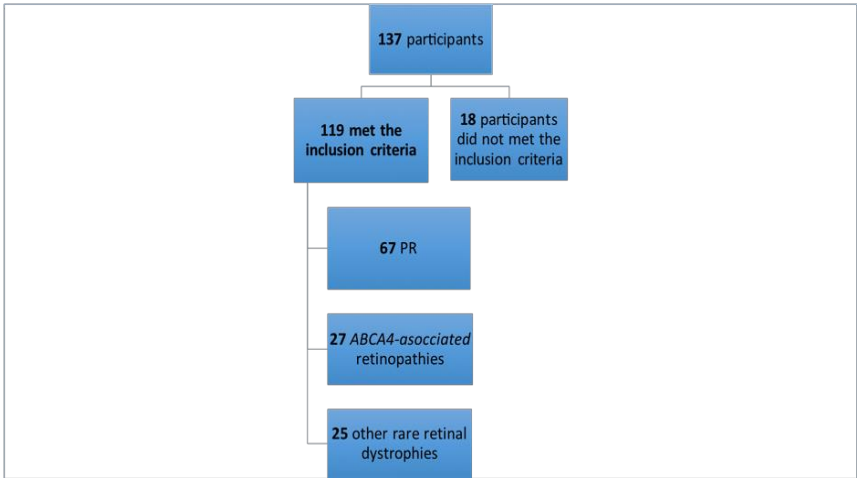

Figure 1. *Study groups*

18 subjects did not meet inclusion criteria due to following reasons:

- ✓ Did not match age criterion.
- ✓ Insufficient evidence of suspected IRD.
- ✓ Another (not retinal) hereditaryinherited eye disease is suspected.
- ✓ Examination is not completed.

Figure 2 shows demographic data of 119 subjects who met the inclusion criteria. Urban and rural inhabitants of 9 counties (Vilnius, Kaunas, Utena, Alytus, Panevėžys, Klaipėda, Tauragė, Telšiai and Šiauliai) participated in the study. 67 females (56.30%) and 52 males (43.70%) participated in the study. Age of subjects ranged from 6 to 77 years.

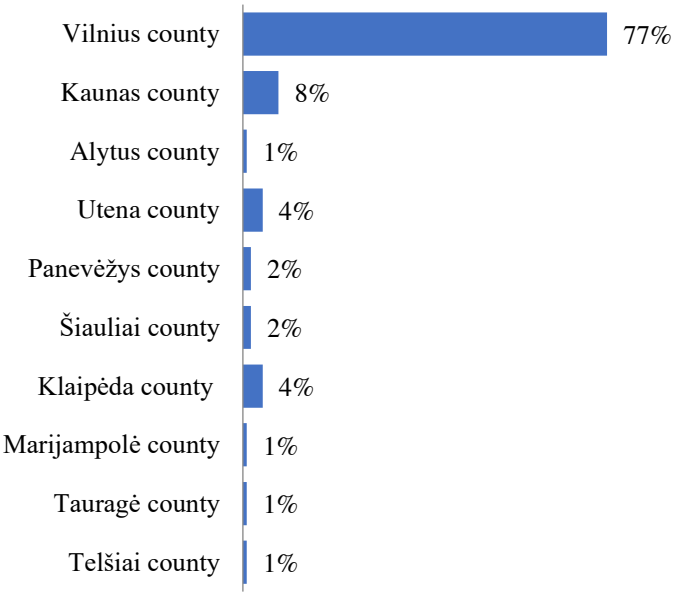

Figure 2. *Subject distribution by county.*

2.2. Subjects with retinitis pigmentosa

Sixty-seven patients were allocated in the RP group. After comprehensive ophthalmological examination, 60 subjects out of total 67 were considered as typical and 7 subjects as atypical RP. Five patients in the RP group were diagnosed with sensorineural hearing loss and syndromic RP - Usher syndrome was suspected, but were included in the typical RP group because of classical clinical RP

symptoms. Atypical RP presented as unilateral, sectoral and RP sine pigmento. Figure 3 shows distribution of all subjects with RP.

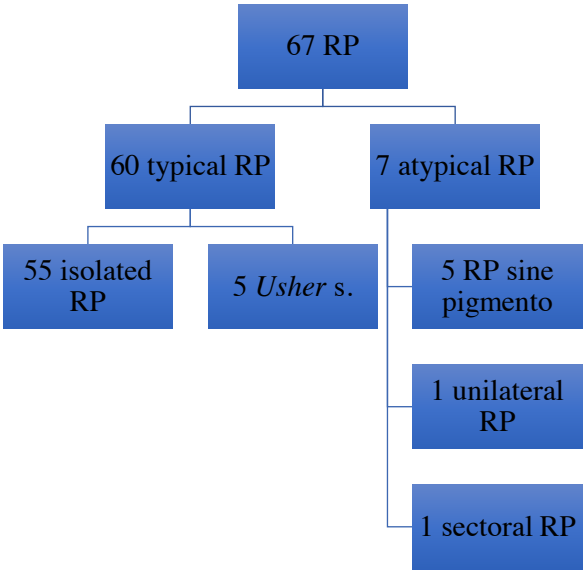

Figure 3. *RP subject classification based on clinical signs*

Demographics of RP group

Thirty-one female (57, 41%) and 23 males (42.59%) participated in the RP group. Mean age of the participants was 46.1 years (SD 16.2, median 47.5). Most of the subjects, 41 (75.93%) were inhabitants of Vilnius city of Vilnius county.

Only unrelated typical RP subjects – 54 individuals – were involved into further statistical analysis.

2.2.1. Analysis of the ophthalmological data

Subjects' BCVA ranged from 0.0 to 1.0 (Table 1). BCVA in most subjects was 0.01 and more (RE 88.9%, LE 90.74%). In these subjects mean BCVA was 0.6 (SD 0.3; median 0.55) in right eye (RE) and 0.5 (SD 0.3; median 0.5) in left eye (LE).

Table 1. *RP patients BCVA data.*

		Number (%)	
		RE	**LE**
BCVA	Blindness (VA – 0,0)		1 (1,85 %)
	Hand movement	4 (7,41 %)	2 (3,7 %
	Finger counting	2 (3,7 %)	2 (3,7 %)
	BCVA ≥ 0,01	48 (88,89 %)	49 (90,74 %)

BCVA – best corrected visual acuity, RE – right eye, LE – left eye

Assessment of refraction showed that only 1 (1.96%) subject had no refraction abnormalities. Myopic astigmatism was the most common refraction abnormality among subjects with RP, in 28 subjects (53.85) it was abnormality of the RE and in 29 subjects (58.86%) – in the LE.

Examination of the anterior segment using slit lamp revealed nystagmus only in 4 subjects (7.41%). Cataract was diagnosed in more than a half of subjects: 32 cataracts (53.85%) in the RE and 34 (62.96%) in the LE. During this study 18 subjects implanted IOL's were observed, 9 (12.12%) in the RE and 9 (26.74%) in the LE.

Full field ERG was carried out in the majority of subjects and and all subjects demonstrated scotopic ERG of no or significantly decreased amplitudes, as well as abnormalities of various degree of the photopic ERG.

2.2.1.1. Assessment of visual field

Visual field was assessed in 48 out of 54 subjects. In 6 subjects visual field was not assessed due to insufficient visual function. In 28 out of 48 subjects who underwent assessment of visual field Humphrey static perimetry was carried out, and in subjects with insufficient visual acuity, slow orientation and only residual visual

field (20 subjects, 37.04%) Goldmann dynamic visual field was assessed. Detailed results are presented in Table 2.

Table 2. *The results of assessment of visual field in RP patients.*

		Number (%)	
		RE	**LE**
Humphrey visual field (N-28)	Single peripheral scotomas / other	4 (14,28%)	4 (14,28%)
	Ring scotoma	1 (3,57%)	1 (3,57%)
	Concentric constriction to 30º	5 (17,85%)	4 (14,28%)
	Concentric constriction to 20º	2 (7,14%)	2 (7,14%)
	Concentric constriction to 10º	10 (50,00%)	2 (10,00%)
Goldmann visual field (N-20)	Single peripheral scotomas / other	6 (30,00%)	5 (25,00%)
	Concentric constriction to 30º	1 (5,00%)	1 (5,00%)
	Concentric constriction to 20º	2 (10,00%)	1 (5,00%)
	Concentric constriction to 10º	17 (85,00%)	18 (90,00%)

RE – right eye, LE – left eye

2.2.1.2. Assessment of the central retinal thickness

Central retinal thickness and correlations

In subjects examined, CRT in the RE was 223.2 μm (SD 110.2 μm), and in the LE was 232.2 μm (SD 120.8 μm). CRT was 227.2 μm in the RE and 237.4 μm in the LE in male subjects, and 218.3 μm in the RE and 226.0 μm in the LE in female subjects (Figure 4).

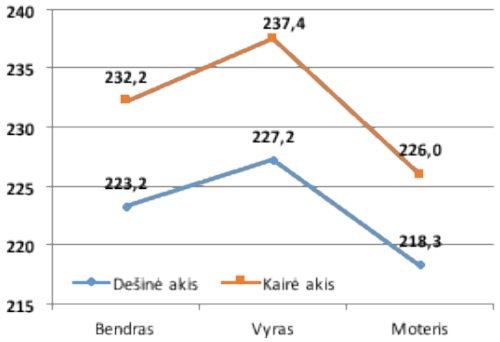

Figure 4. *RP patients mean CRT compared by gender and between eyes.*

Weak but statistically significant correlation between CRT and BCVA was found. This means that thicker central retina is associated with better BCVA. Detailed results are presented in Table 3.

Table 3. *CRT and BCVA correlation.*

	Central retinal thickness					
	RE			**LE**		
	Spearman's correlation coefficient	**N**	**p**	**Spearman's correlation coefficient**	**N**	**p**
BCVA RE	0,35	48	0,0148	0,3422	48	0,0173
BCVA LE	0,3498	49	0,0138	0,4117	49	0,0033

BCVA – best corrected visual acuity, RE – right eye, LE – left eye

Macular changes in patients with RP

Macular changes were assessed in all subjects using SD-OCT, and various changes were found (CRT was discussed previously and

is not discussed in this section). Results that did not match technical requirements were not involved. No structural changes of the macula were found in 90.24% of the RE and 36% of the LE. ERM (including ERM that does not access fovea) was found in 36 RE (87.8%) and 36 LE (100%) of subjects. Combination of CGDE and ERM was the most common; it was found in 25% of right eyes and 37.84% of left eyes.

2.2.1.3. Subfoveal choroidal thickness and correlations

In subjects examined, SCT was 213.8 μm (SD 92.0 μm) in the RE and 220.1 μm (SD 100.7 μm) in the LE. CST in males (RE - 210.8 μm, LE - 221.6 μm) and in females (RE - 217.3 μm, LE -218.5 μm) showed in Figure 5.

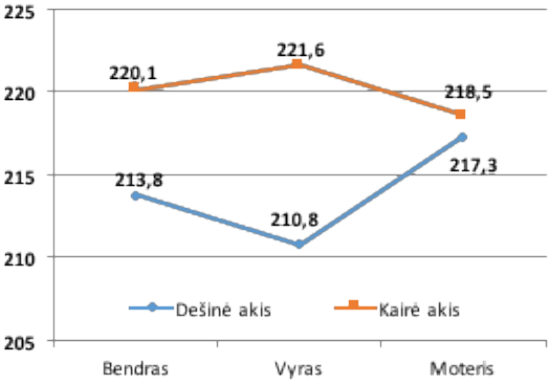

Figure 5. *RP patients mean SCT compared by gender and between eyes.*

Statistically significant association is observed between SCT and age. Spearman‘s correlation coefficient for RE was -0.292, and for LE it was -0.2936, showing weak association between variables (table 4). SCT decreases with age.

Table 4. *SCT and age correlation.*

	Age		
	Spearman's correlation coefficient	**N**	***p***
SCT RE	-0,292	50	0,0396
SCT LE	-0,2936	48	0,0428

SCT – subfoveal choroidal thickness, RE – right eye, LE – left eye

2.2.1.4. Assessment of the ellipsoid zone and correlations

The structure of ellipsoid zone (EZ) in the macular area was assessed using SD-OCT. First, its presense was identified and later its length was measured in the area of the macula. In 8 (15.38 percent) RE and 10 (20 percent) LE no EZ was identified (table 5).

Table 5. *Macular EZ structure assessed by SD-OCT.*

		Number (%)	
		RE	**LE**
Ellipsoid zone (EZ)	Absent	8 (15,38%)	10 (20%)
	Present	44 (84,62%)	40 (80%)
	Present <1mm	7 (16,28%)	5 (12,82%)
	Present >1mm with clear borders	33 (76,74%)	32 (82,05%)
	Present through the whole scan	3 (6,98%)	2 (5,13%)

RE – right eye, LE – left eye

Correlation analysis showed statistically significant moderate correlation between length of intact EZ and BCVA and findings of Ishihara color test. BCVA and results of color test improve with the increase of length of extant EZ. This association could be observed in

BE. Values of Spearman's correlation coefficient, subjects' samples and level of significance are presented in Table 6.

Table 6. *Correlations between EZ length, BCVA and Ishihara test results.*

	EZ length					
	RE			**LE**		
	Spearman's correlation coefficient	**N**	***p***	**Spearman's correlation coefficient**	**N**	***p***
BCVA RE	0,3837	41	0,0133	0,4062	37	0,0126
BCVA LE	0,4791	42	0,0013	0,5269	38	0,0007
Ishihara RE	0,5001	41	0,0009	0,4676	37	0,0035
Ishihara LE	0,4225	41	0,0059	0,4804	37	0,0026

BCVA – best corrected visual acuity, RE – right eye, LE – left eye, EZ – ellipsoid zone

2.2.1.5. Retinal nerve fiber layer

RNFL was 111.1 μm (SD 23.0 μm) in the RE and 114.5 μm (SD 29.8 μm) in the LE in the subjects examined. RNFL in males (118.1 μm in the RE and 121.0 μm in the LE) and females (102.8 μm in the RE and 105.9 μm in the LE) is presented in Table 6.

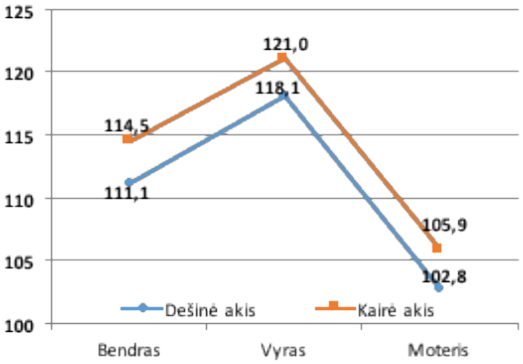

Figure 6. *RP patients mean RNFL compared by gender and between eyes.*

2.2.1.6. Assessment of findings of fundus autofluorescence imaging

During FAF imaging the following hyperautofluorescence (hyper AF) ring characteristics were assessed: horizontal and vertical diameters of external ring boundaries (or one of them when only one could be measured). Hyper AF rings were found in 27 RE (52.94%) and 24 LE (47.06%) of subjects (detailed results are presented in Figure 7). The figure presented below (Fig. 7) shows that horizontal diameters of hyper AF rings were large in BE and in both genders.

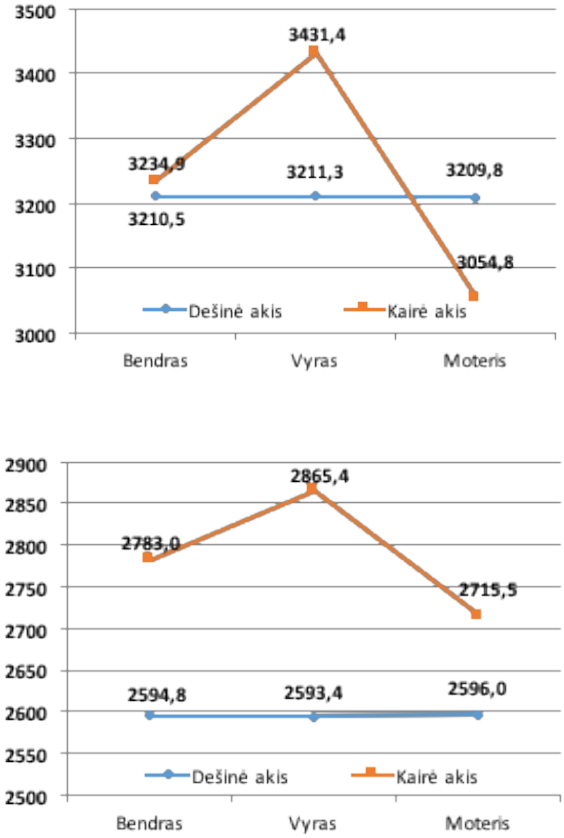

Figure 7. *HyperAF ring horizontal (upper image) and vertical (lower image) diameters in subjects with RP compared between gender and eyes.*

2.2.2. Genetically confirmed cases of typical RP

TD112 typical RP case

In the TD112 (III:1) genealogy RP cases were identified in all generations, and therefore it was established that RP is inherited by AD inheritance. Genotype NM_000539.3:c.1039[C>T];[G=](NP_000530.1:p.[(Pro347Ser)];[(P

ro347=)]) was identified by the analysis of coding and adjacent sequences of *RHO* gene. Heterozygote genotype of CM900200, rs29001637 variant in the *RHO* gene confirms the clinical diagnosis of AD inherited RP.

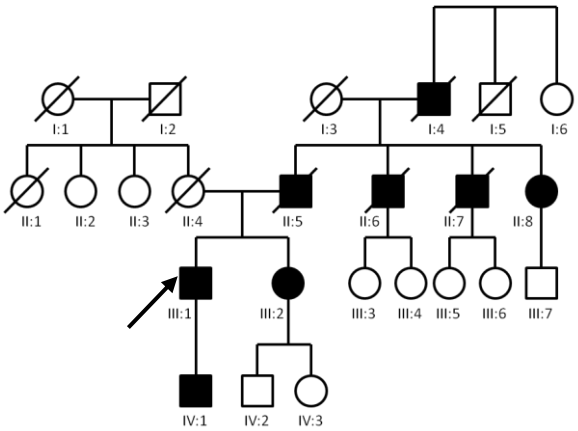

Figure 8. *TD112 (III:1) pedigree.*

TD019 familial typical RP case

Tree female members of one family participated in our study, they underwent molecular genetic testing: TD019 (V:1), TD030 (IV:2) ir TD127 (III:3); RP cases are known in all generation of this family (Figure 9), and therefore it was determined that RP was of AD inheritance. Pathogenic variant CM930659, rs104893779 was identified in the *RHO* gene by Sanger sequencing in all tested individuals, heterozygote genotype confirming clinical diagnosis of AD inherited RP.

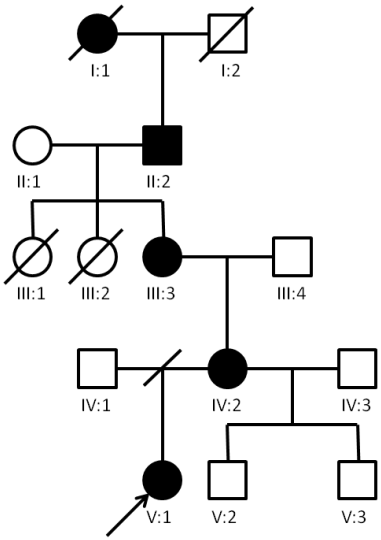

Figure 9. *TD019 (V:1), TD030 (IV:2) ir TD127 (III:3) pedigree.*

TD120 familial RP case

In the TD120 (III:4) genealogy RP cases are identified in most generations, and therefore AD inherited RP was determined. Analysis of coding and adjacent sequences of *RHO* gene and *PRPH2* (peripherin 2, mouse, homolog of) gene revealed heterozygote genotype NM_000322.4:c.[647C>T];[647C=] (NP_000313.2:p.[(Pro216Leu)];[(Pro216=)]). Molecular genetic testing confirmed AD inherited RP in this family.

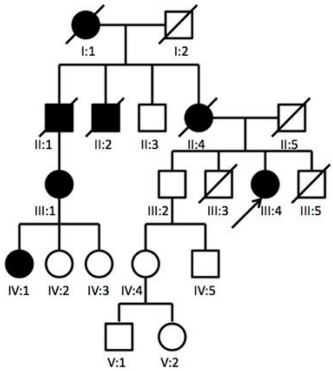

Figure 10. *TD120 (III:4) pedigree.*

TD015 case

Molecular genetic testing of subject TD015 (IV:6) identified genetic changes NM_000260:[494C>T];[596T>G;1969C>T] in the *MYO7A* gene. Familial analysis of genetic variants confirms heterozygote genotype and heterogeneity of sequence variants. Changes identified using molecular genetic testing were included in the *Deafness Variation Database* and confirm diagnosis of AD inherited RP.

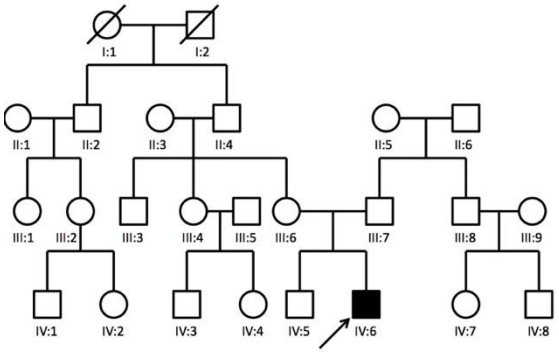

Figure 11. *TD015 (IV:6) pedigree.*

2.2.3. Retinitis pigmentosa sine pigmento

Six subjects with RP sine pigmento were enrolled into subgroup of subjects with all signs characteristic to RP except retinal pigmentation - accumulation of so called „bone spicules“, found during comprehensive ophthalmological examination.

Clinical data of subjects are presented in Table 7. All subjects, except TD119, had myopia. It is likely that no signs of cataract were noticed because of young age of subjects. It also could be that with ageing and progression of the disease “bone spicules” could occur in the peripheral part of retina.

Table 7. *Demographic and clinical data of subjects with RP sine pigmento.*

	TD034	**TD035**	**TD037**	**TD040**	**TD079**	**TD119**
Age	28	29	16	19	28	20
Gender	M	M	M	M	V	M
BCVA RE/L	0,4/ 0,4	1,0/ 1,0	1,0/ 1,0	0,02/ 0,1	0,5/ 0,4	0,63/ 0,63
Ishihara test RE LE	36/ 38 36/ 38	38/ 38 38/ 38	38/ 38 38/ 38	30/ 38 33/ 38	N N	38/ 38 38/ 38

BCVA – best corrected visual acuity, RE – right eye, LE – left eye

SD-OCT revealed diverse abnormalities: in TD040 significantly thickened retina was observed; in TD0798 significant CME was found, and in other subjects moderate decrease in CRT was observed, SCT ranged from 152 to 450 μm (Table 8)

Table 8. *CRT, SCT and macular EZ length results (μm) in subjects with RP sine pigmento obtained with SD-OCT.*

	TD034	**TD035**	**TD037**	**TD040**	**TD079**	**TD119**
CRT						
RE	179	240	226	132	870	229
LE	237	232	226	132	760	225
SCT						
RE	293	158	301	389	355	318
LE	288	152	247	450	293	443
EZ length						
RE	1640	3257	7860	N	8032	3019
LE	1660	3049	7921	N	7368	2932

BCVA – best corrected visual acuity, RE – right eye, LE – left eye, CRT – central retinal thickness, SCT – subfoveal choroidal thickness, EZ – ellipsoid zone

All subjects underwent full-field ERG that showed that *a* and *b* waves were absent or of very low amplitude in the scotopic phase; waves of significantly lower amplitude were registered also in the photopic phase.

2.2.4. Unilateral retinitis pigmentosa

In subject TD062, 40 years of age, signs characteristic to RP were found only in on – left – eye. Diagnosis of unilateral RP is exceptionally rare; less than 100 cases are documented [7]. Diagnosis of unilateral RP shall meet François and Verriest criteria [7]. TD062 noticed first signs, seeing spots, at the age of 20. Her medical history revealed that at the age of 25 years ophthalmologist have found „retinal scars". Since then subject suffers from gradually deteriorating vision of the LE. Subjects BCVA of the RE was 1.25, in the LE - 0.3.

No significant disorders of refraction were found, color vision was normal. Fluffy posterior subcapsular opacification was observed in the LE. According to subject, she had no infectious or non-infectious inflammations, traumas or systemic use of medications. Laboratory testing of possible infectious cause of the disease were negative.

Examination of visual field revealed concentric narrowing of visual field in the LE correlating with the image of fundus of eye (Figure 12)

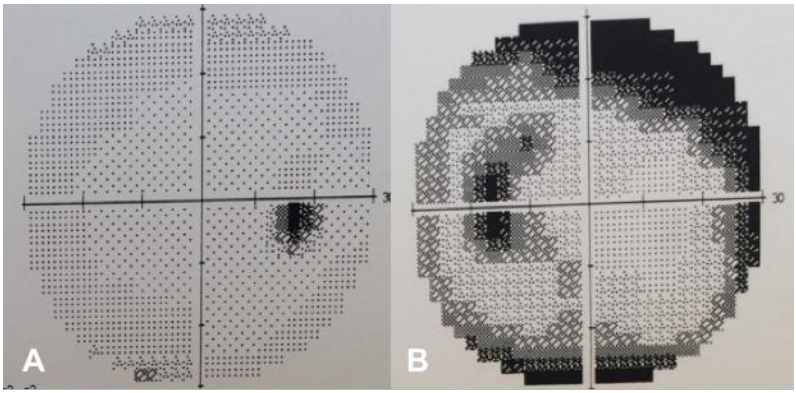

Figure 12. *Visual field results*. RE (A) – normal, LE (B) – concentric visual field constriction.

No abnormalities were found during ophthalmobiomicroscopy if the RE while changes characteristic to the RP were observed in the LE: optic nerve disc (OND) is paler compared with the right eye, abundant „bone spicules“ are observed in the mid-peripheral portion, blood vessels are narrowed (Figure 13); furthermore, SD-OCT confirmed developing ERM et the macula slightly deforming foveal contour and mild parafoveal and cystic swelling. The latter abnormalities in the macular area and opacifications of the lens of the LE are the main courses of deterioration of VA.

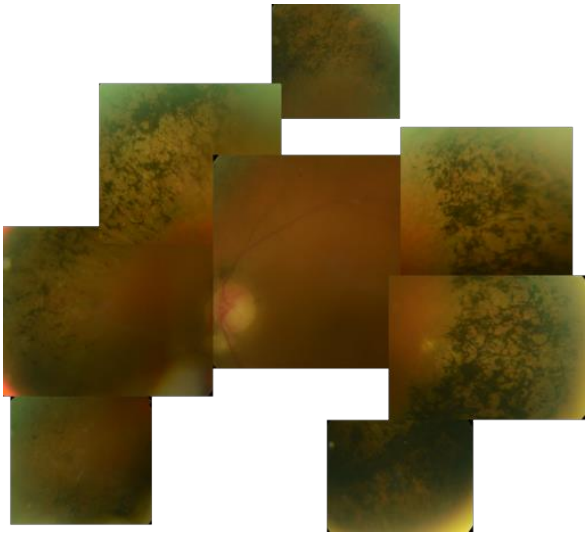

Figure 13. *Typical RP signs in the RE of TD062.*

Full-field ERG also revealed asymmetric responses: normal results in the right eye, and in left eye, a and b waves were absent in scotopic mode and of significantly lower amplitude in photopic mode (Figure 14).

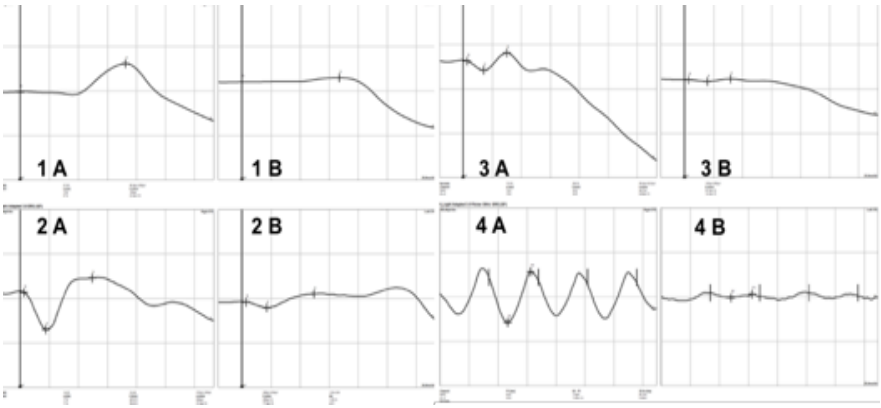

Figure 14. *TD062 results of scotopic (1-2 A-B) and photopic (3-4 A-B) full field ERG.*

Based on data collected unilateral RP was diagnosed. However, monitoring of presently intact RE for possible RP signs over time is indicated.

2.2.5. Results of the genetic testing

2.2.5.1. SNP in the RP subjects

Molecular genetic testing of coding and adjacent sequences of two genes, *RHO* and *RPRH2,* was performed in subjects with RP by Sanger sequencing. Nine different SNPs were identified: rs7984, rs56340615, rs2071092, rs2071093, rs361524, rs434102, rs425876, rs390659, rs7764439. SNPs reference and alternative alleles and genotype distribution between RP and LITGEN groups is presented in Table 9.

Table 9. *SNPs reference and alternative alleles and genotype distribution between RP and LITGEN groups.*

	Ref, n (%)	Alt, n (%)	p value compared to LITGEN	Ref/Ref, n (%)	Ref/Alt, n (%)	Alt/Al, n (%)	HWE p-value	p value compared to LITGEN
rs7984								
RP	85 (85,0)	15 (15,0)	0,227	36 (72,0)	13 (26,0)	1 (2,0)	0,890	0,212
LITGEN	176 (89,8)	20 (10,2)		81 (82,7)	14 (14,3)	3 (3,1)	0,029	
rs361524								
RP	72 (72,0)	28 (28,0)	0,248	25 (50,0)	22 (44,0)	3 (6,0)	0,519	0,000
LITGEN	153 (78,1)	43 (21,9)		70 (71,4)	13 (13,3)	15 (15,3)	0,000	
rs390659								
RP	24 (24,0)	76 (76,0)	0,615	4 (8,0)	16 (32,0)	30 (60,0)	0,385	0,783
LITGEN	42 (21,4)	154 (78,6)		5 (5,1)	32 (32,7	61 (62,2)	0,764	
rs425876								
RP	11 (11,0)	89 (89,0)	0,518	2 (4,0)	7 (14,0)	41 (82,0)	0,044	0,774
LITGEN	17 (8,7)	179 (91,3)		2 (2,0)	13 (13,3)	83 (84,7)	0,107	

rs434102								
RP	24 (24,0)	76 (76,0)	0,000	4 (8,0)	16 (32,0)	30 (60,0)	0,385	0,000
LITGEN	89 (45,4)	107 (54,6)		37 (37,8)	15 (15,3)	46 (46,9)	0,000	
rs2071092								
RP	98 (98,0)	2 (2,0)	0,206	48 (96,0)	2 (4,0)	0 (0,0)	0,885	0,450
LITGEN	195 (99,5)	1 (0,5)		97 (99,0)	1 (1,0)	0 (0,0)	0,960	
rs2071093								
RP	99 (99,0)	1 (1,0)	0,057	49 (98,0)	1 (2,0)	0 (0,0)	0,943	0,294
LITGEN	185 (94,4)	11 (5,6)		90 (91,8)	5 (5,1)	3 (3,1)	0,000	
rs7764439								
RP	52 (52,0)	48 (48,0)	0,215	15 (30,0)	22 (44,0)	13 (26,0)	0,402	0,416
LITGEN	87 (44,4)	109 (55,6)		20 (20,4)	47 (48,0)	31 (31,6)	0,777	
rs56340615								
RP	92 (92,0)	8 (8,0)	0,872	42 (84,0)	8 (16,0)	0 (0,0)	0,539	0,902
LITGEN	178 (90,8)	18 (9,2)		80 (81,6)	18 (18,4)	0 (0,0)	0,317	

Statistical analysis showed that only rs434102 results in RP group statistically significantly ($p = 0.000$) differ from LITGEN, Lithuanian group of healthy subjects, i. e., alternative alleles of rs434102 statistically significantly are more frequently found in subjects with RP.

Comparison between RP and LITGEN groups showed statistically significant differences between rs434102 and rs361524. Alternative genotypes were significantly more frequent in RP subjects group compared with LITGEN population ($p = 0.000$) (Table 9).

In the RP group, distribution of all genotypes, except rs425876 showed equilibrium when assessed according to HWE.

Data from RP subject group were compared with self-reported healthy population 1000G European and global studies.

In the Lithuanian population, compared with global population the following were statistically more frequent: rs434102 ($p=0,000$, $p<0,05$), rs425876 ($p=0,000$, $p<0,05$), rs390659 ($p=0,000$, $p<0,05$), rs7764439 ($p=0,000$, $p<0,05$). rs7984 ($p=0,000$, $p<0,05$) and rs2071093 ($p=0,000$, $p<0,05$) were significantly rarer. In Lithuanian population, compared with European population rs7984 ($p=0,000$, $p<0,05$) and rs361524 ($p=0,036$, $p<0,05$) were rarer. rs434102 ($p=0,000$, $p<0,05$), rs425876 ($p=0,000$, $p<0,05$), rs390659 ($p=0,000$, $p<0,05$), rs7764439 ($p=0,000$, $p<0,05$) were significantly more frequent. Detailed data are presented in the Figure 15.

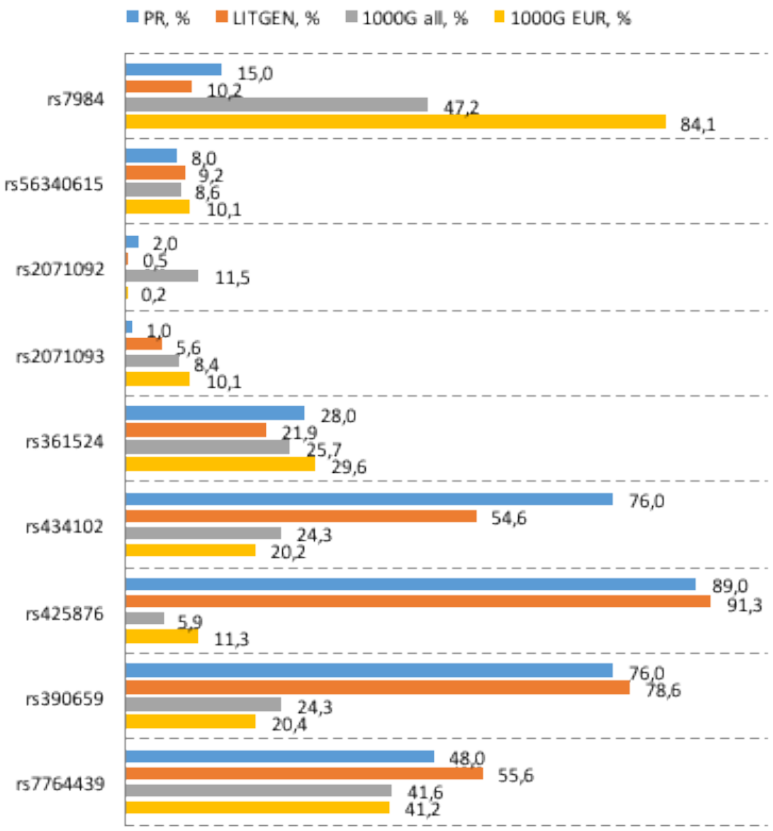

Figure 15. *SNPs frequency distribution in Lithuanian (LITGEN), world (1000G all) and Europe (1000G Eur) populations.*

2.2.5.2. SNP and phenotype correlations

Association of genetic factors with CRT

CRT association with different SNPs was analyzed. Detailed results are presented in the Figure 16 A-B. CRT median of BE in rs425876 and in RE rs361524 decreases with every rare allele, whereas in rs7984 the largest mode was observed in two rare alleles. In rs7764439 minor decrease of CRT median is observed in the LE in

every rare allele. To clarify if alternative alleles of aforementioned rs425876, rs361524 and rs7764439 are associated/cause decrease of CRT comprehensive functional testing is needed.

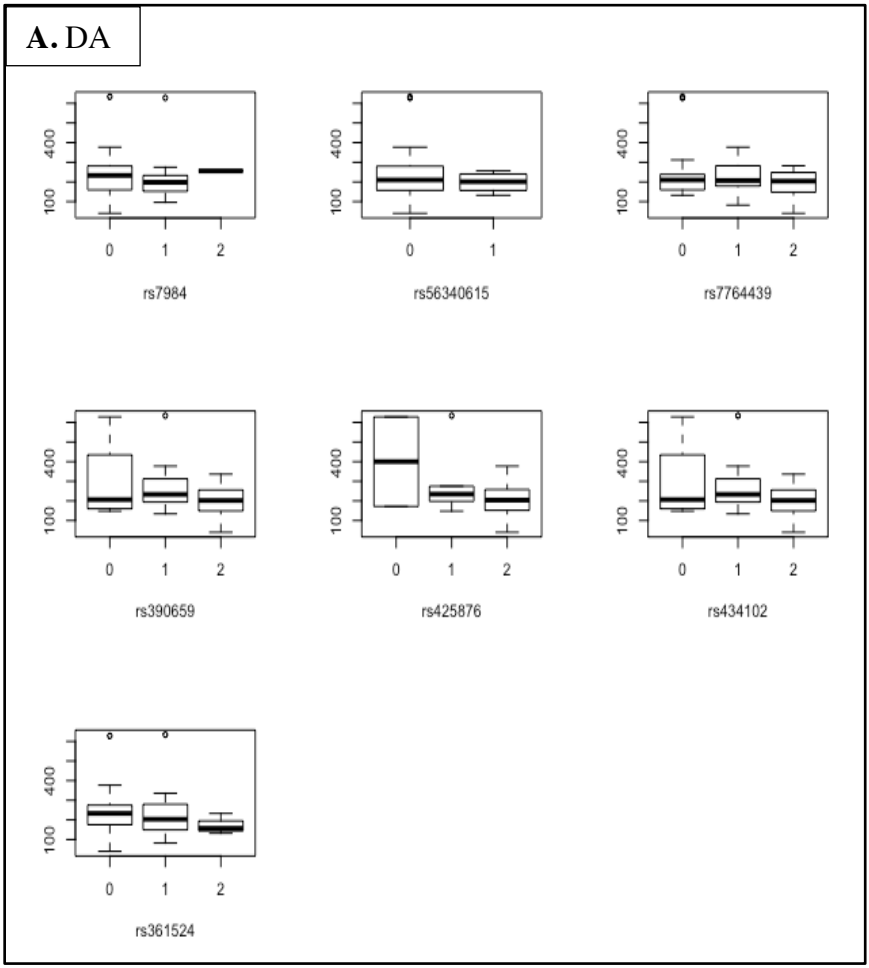

Figure 16. *(A) The association analysis of RE and CRT with different SNPs. 0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles, y-axis – CRT (μm).*

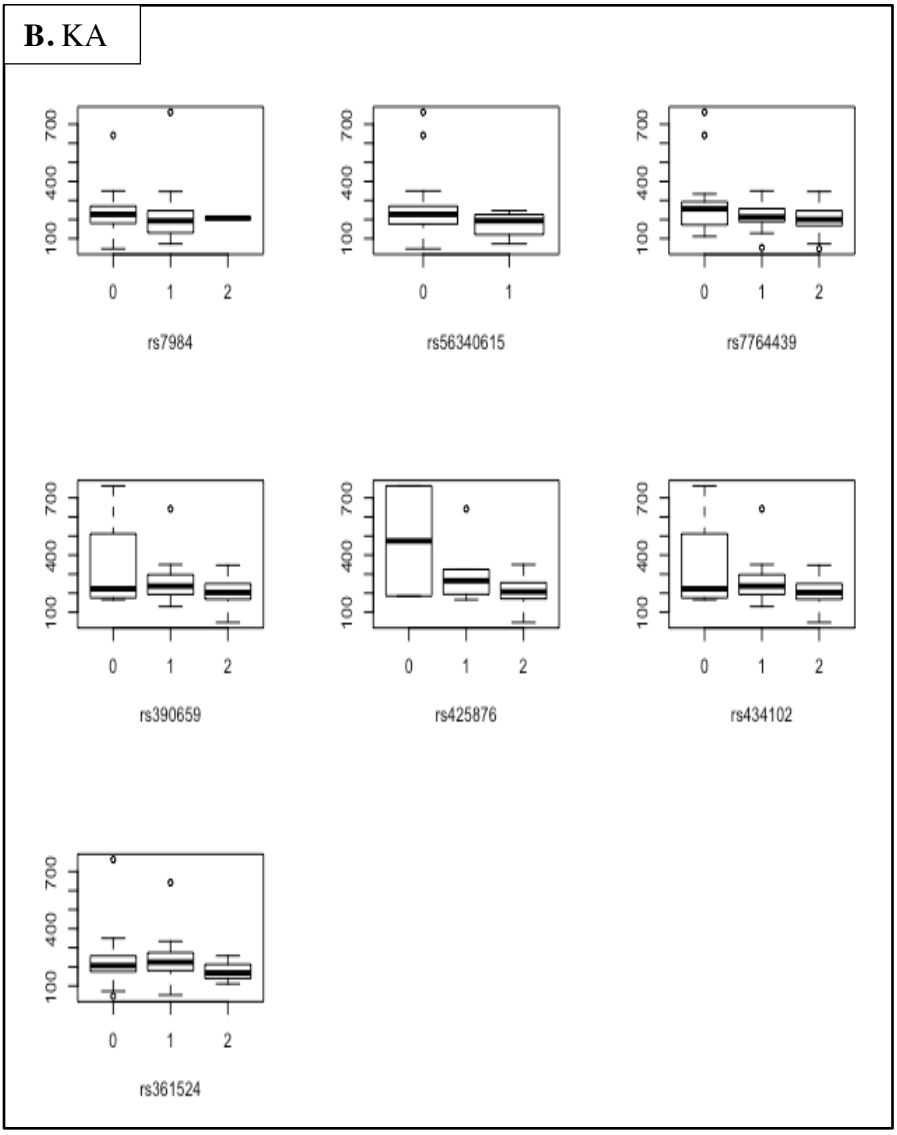

Figure 17. (B) *The association analysis of LE and CRT with different SNPs. 0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles, y-axis – CRT (μm).*

Genetic factors association with EZ length

Association of the intact macular EZ with SNP was analyzed. Detailed results are presented in the Figure 17 A-B. Analysis of this association showed that two SNPs, rs7984 and rs361524 in BE were associated with the highest mode meaning that in cases of alternative genotypes rs7984 and rs361524 EZ layer remains intact for e longest period of time.

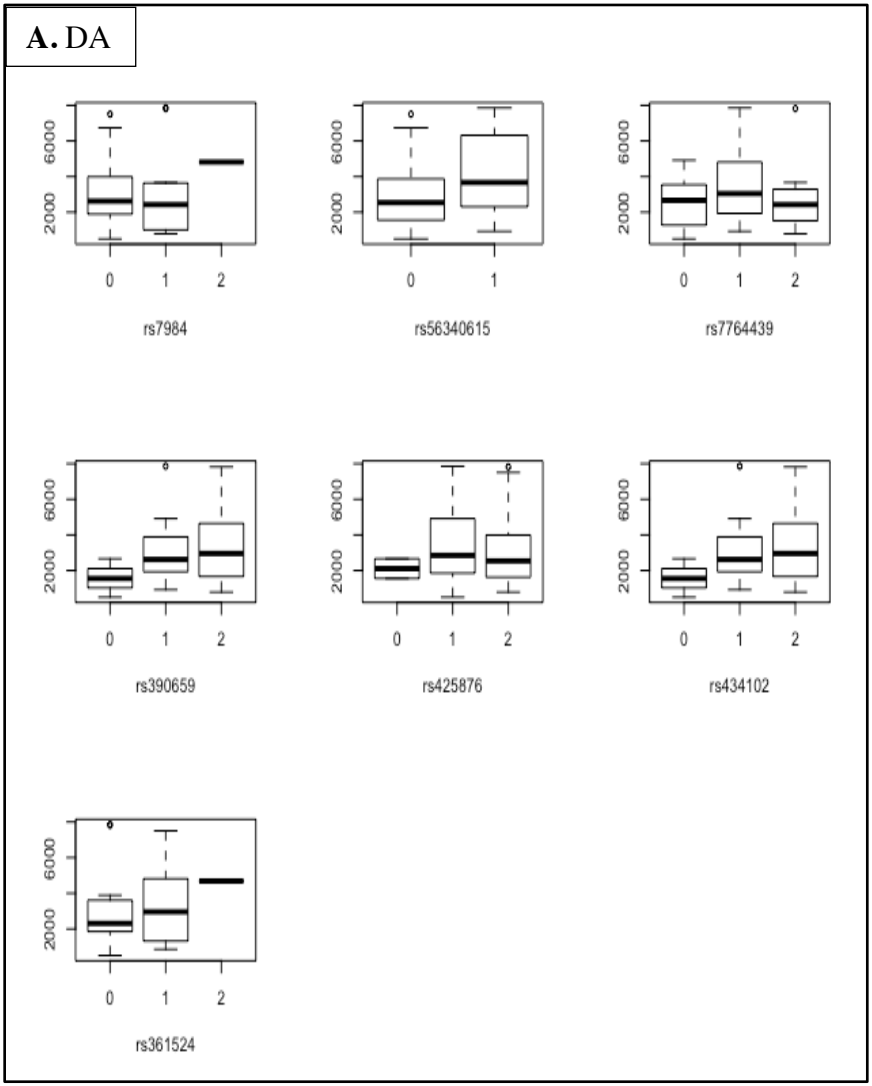

Figure 18. (A) The association analysis of RE and macular EZ length with different SNPs. 0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles, y-axis – macular EZ lentgh (μm).

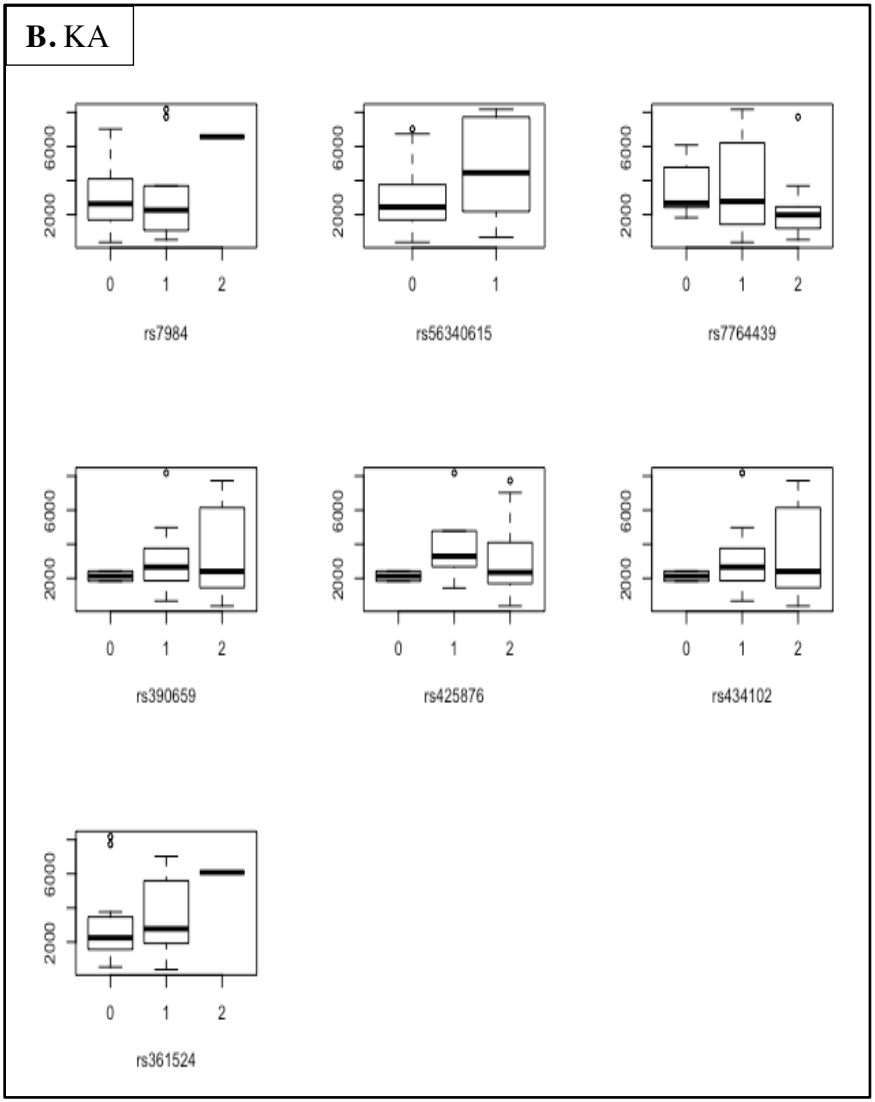

Figure 19. *(B) The association analysis of RE and LE macular EZ length with different SNPs. 0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles, y-axis – macular EZ lentgh (μm).*

Genetic factors association with SCT

BE SCT association with genotype was analyzed. In rs361524 SCT decrease in any rare allele was wound. Meanwhile, two rare alleles of SNP rs7984 were associated with the lowest mode (Figure 18 A-B). It could be summarized that alternative alleles may be associated with thinnest subfoveal choroid in cases of RP. Results are presented in the Figure 18.

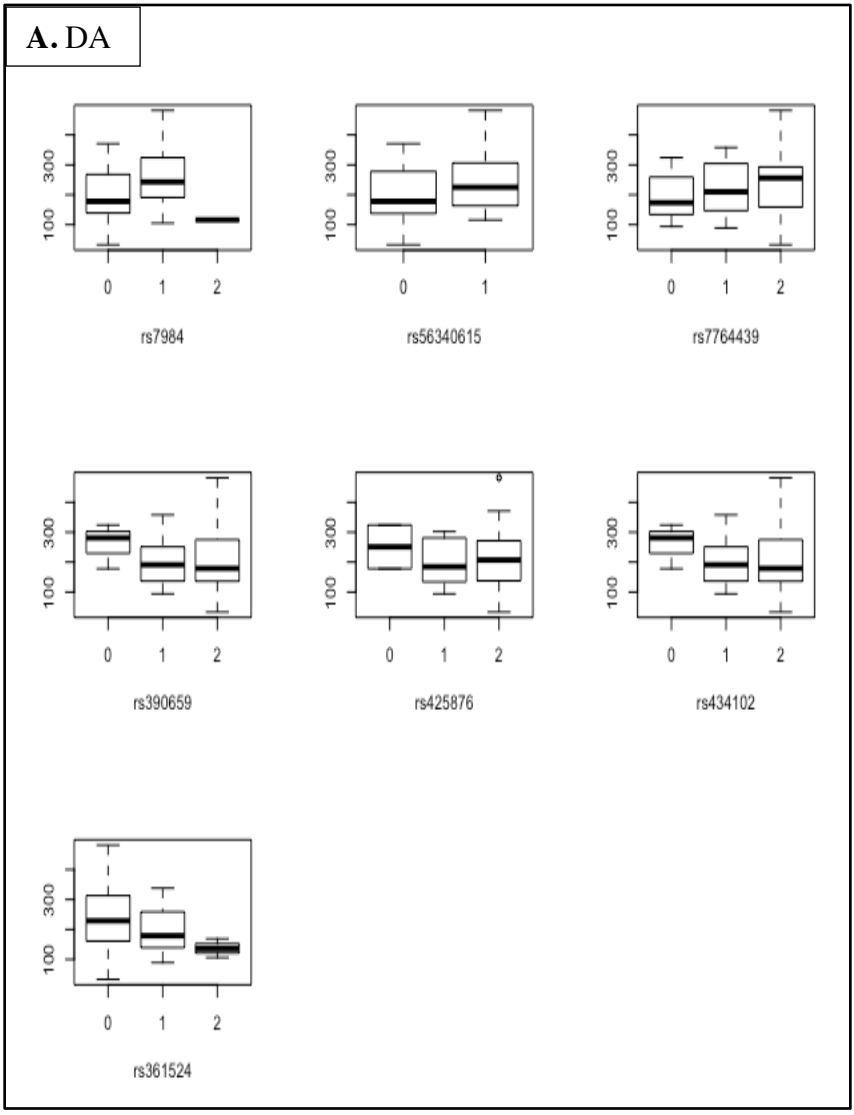

Figure 20. *(A) The association analysis of RE and SCT with different SNPs. 0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles, y-axis – SCT (μm).*

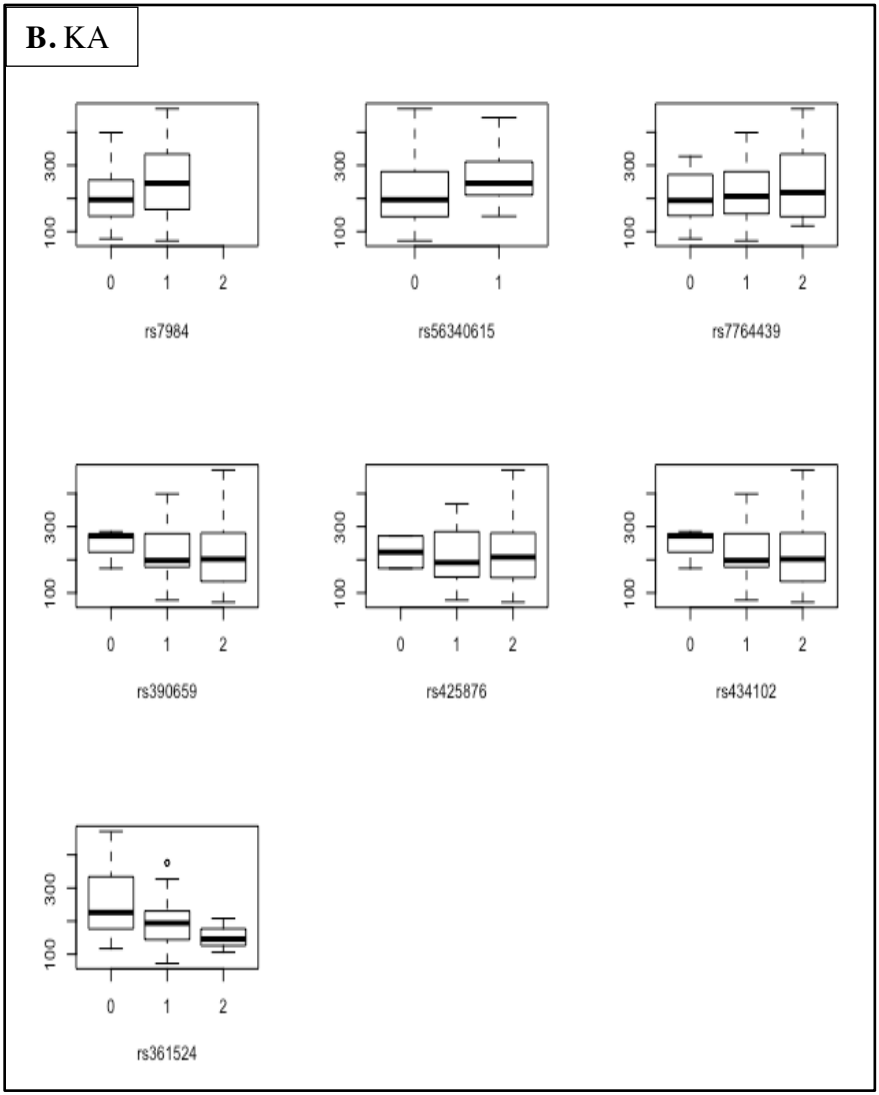

Figure 21. (B) *The association analysis of LE and SCT with different SNPs. 0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles, y-axis – SCT (μm).*

Genetic factors association with RFNL

RFNL association with SNPs in both eyes was analyzed. In SNPs rs7984 and rs361524 RFNL thickness increased in both eyes with every rare allele and in case of rs425846 RFNL in the left eye RFNL thickness decreased with every rare allele (Figure 19 A-B). It could be summarized that rs7984 and rs361524 alternative alleles may be associated with thicker RFNL, and rs425876 with thinner RFNL.

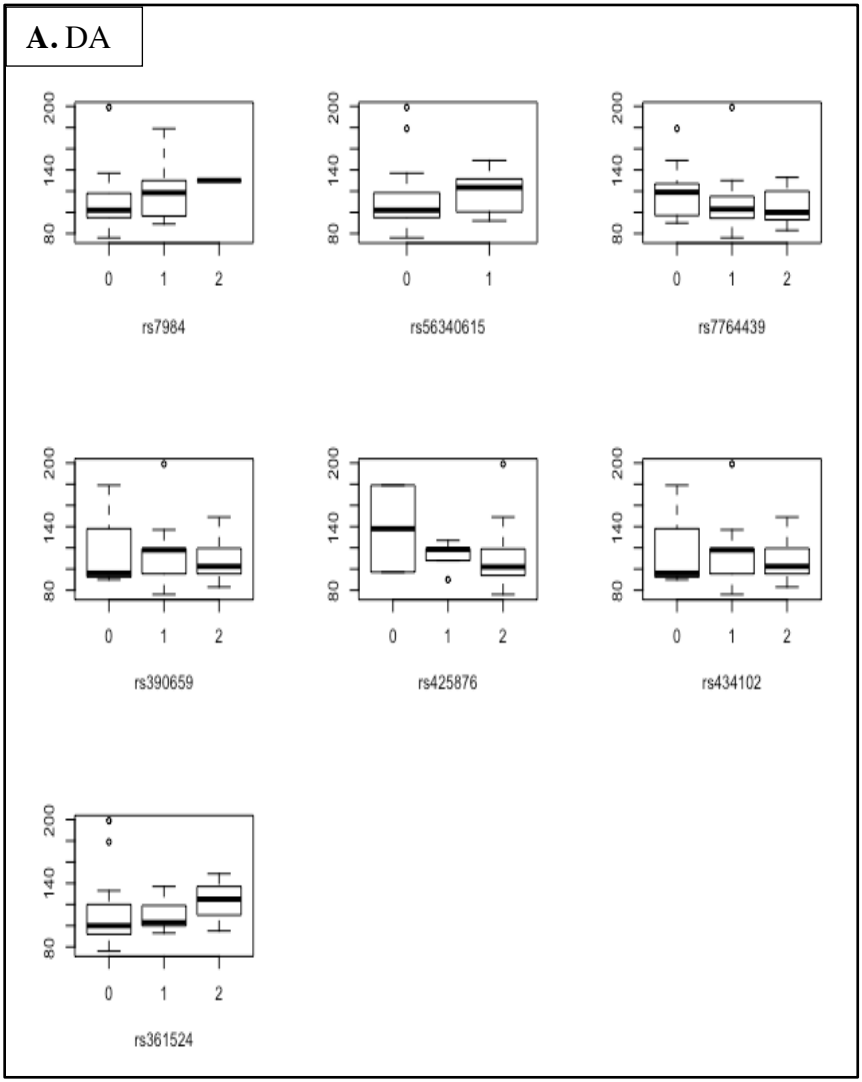

Figure 22. (A) *The association analysis of RE and RNFL thickness with different SNPs. 0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles, y-axis – RNFL thickness (μm).*

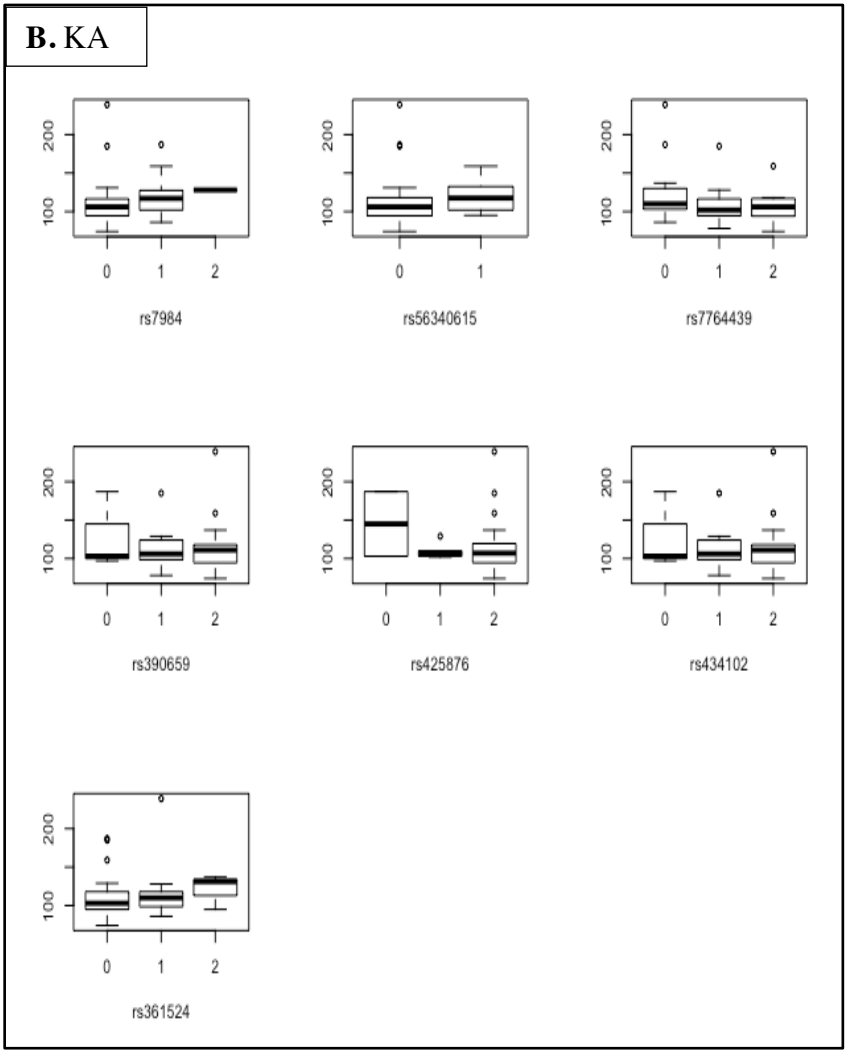

Figure 23. (B) *The association analysis of RE and LE RNFL thickness with different SNPs. 0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles, y-axis – RNFL thickness (μm).*

Genetic factors association with MD

BE visual field MD (mean deviation of visual field) association with SNP was analyzed. Different results were obtained in different SNPs. Analysis of the association of RE and rs56340615 and rs425876 showed significant differences in different alleles, and in cases of rs390659 and rs434102 analysis showed MD increase in every rare allele (Figure 20 A). In the LE, only rs361524 analysis showed MD increase in every rare allele (Figure 20 B). In summary, results are different in different SNPs, and no uniform trend is observed.

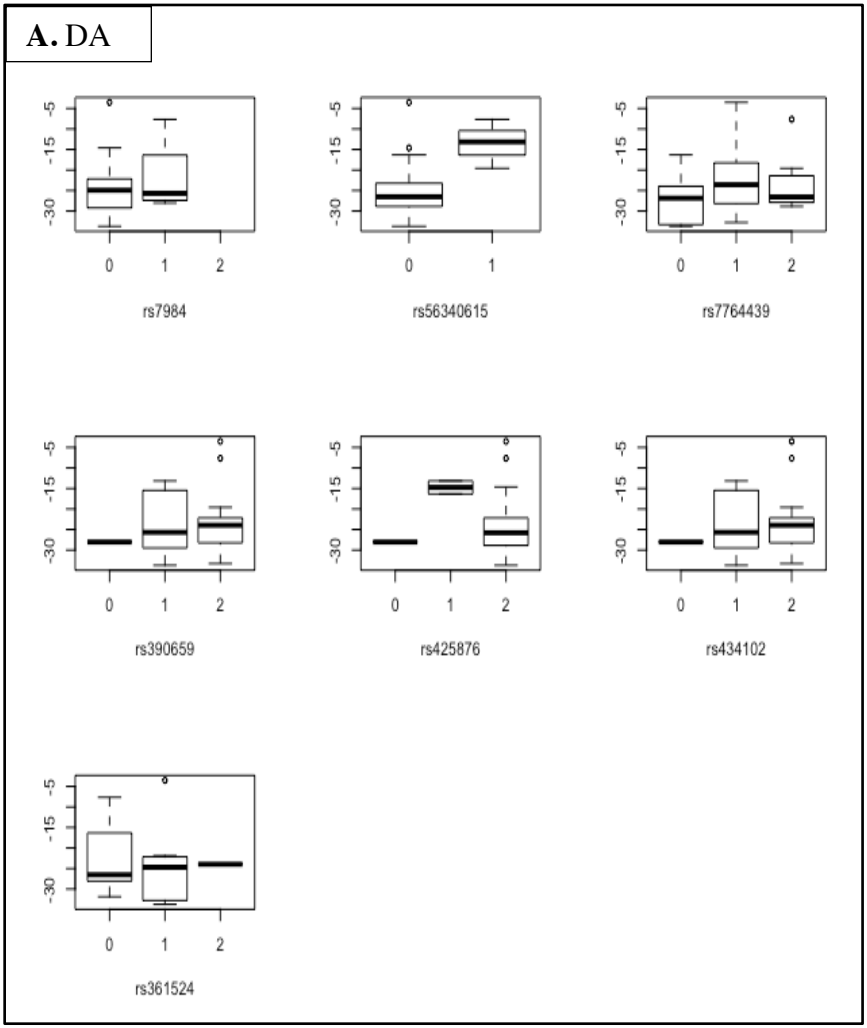

Figure 24. *(A) The association analysis of RE and MD value with different SNPs. 0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles, y-axis – MD value (dB).*

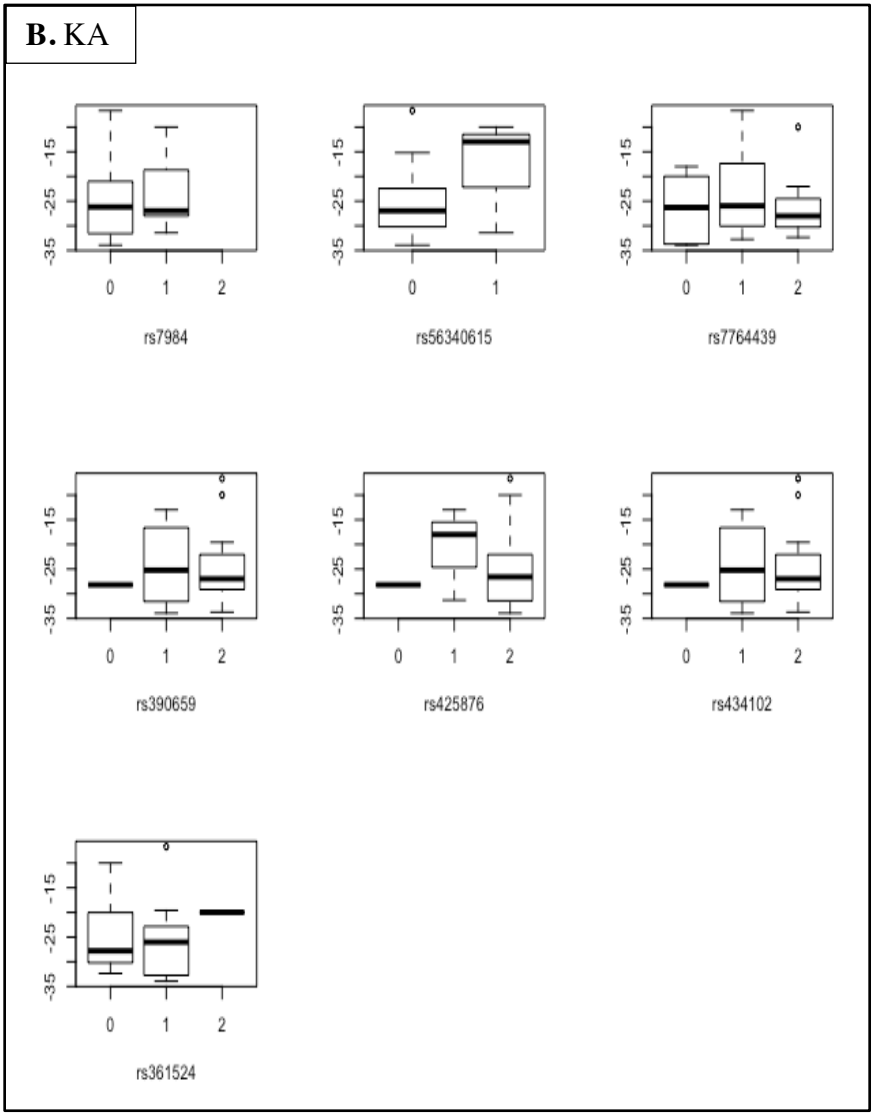

Figure 25. (B) *The association analysis of LE and MD value with different SNPs. 0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles, y-axis – MD value (dB).*

Discussion

Correlation analysis showed statistically significant moderately strong positive correlation relationship between EZ length and BCVA which is in line with findings reported by *Liu et al*. that BCVA improves with the increase of EZ length [8].

Konieczka et al. study also confirmed that shortened or lost its integrity EZ is related to poorer BCVA [9].

Sodi et al. did not find any SCT correlations with any parameters, including age, age at the onset of the disease, duration of the disease, CRT, BCVA, field of visions and ERG responses [10]. At the same time in our study we observed statistically significant association between SCT and age and we made a conclusion that SCT decreases with age. However, clinical conclusions concerning importance of choroid in the course of the RP are unclear yet, and more comprehensive studies are necessary [10].

Of the SNPs examined in our study, SNPs rs434102, rs7984 and rs56340615 were described in the literature in greater details. According to study data presented by *Shankar SP et al*. [11], SNP rs434102 (*PRPH2* gene) is found in 2% of families with AD inherited RP and in 10% of families with various clinical diagnoses such as pattern dystrophy, macular or generalised dystrophies.

Guo et al. [12] studied subjects with AD RP and established abnormal mutation of *RHO* gene as well as healthy persons belonging to one of the smallest Chinese ethnic groups, Bai family, and described two non-pathogenic SNPs, rs7984 and rs2269736. The most common rs7984 genotype among patients with RP in Bai family was found to be AA - 61.5% (n = 13), which was similar to our study, 72.0% (n = 50). In healthy persons belonging to Bai family an alternative G allele was more frequent and was found in 66.7% of subjects; however, according to LITGEN, A allele was more frequent in healthy population in Lithuania accounting for 89.8% (n = 98).

Ma Li et al. [13] studies prevalence of different sequences of *RHO* gene SNP rs56340615, and their proportion was similar to that

found in our study: in *Ma Li et al*. study, in the RP sine pigmento group the proportion of genotype/heterozygous genotype/reference genotype was 0/8/12, while in our RP subject group it was 0/8/42; reference genotype was the most common in both studies.

Close cooperation of ophthalmologists, geneticists and epidemiologists in the large-scale clinical studies is necessity to analyze clinical and genetic complexity of RP [14].

2.3. *ABCA4* retinopathies

According to recent molecular studies, STGD, cone dystrophies (CD) and CRD (cone-rod dystrophies) shall be considered as inherited diseases related to *ABCA4* gene, and therefore it is more correctly to call them *ABCA4*-related retinal degenerations [15]. Since symptoms of these diseases are overlapped, establishment of their diagnosis is often possible only upon assessment of the character their progression, furthermore, the same gene abnormalities may be related to difference phenotypes and vice versa [16]. Subjects allocated to *ABCA4* group were divided into two subgroups based on clinical signs, subjects with STGD and subjects with CD/CRD.

2.3.1. Subjects with Stargardt disease

Fifteen subjects were allocated in STGD group. Nine (60%) females and 6 (40%) males participated in the study. Their age ranged from 9 to 53 years (Table 10), mean age was 35 years (SD 14, Median 31). Majority of these subjects (86.67%) were from Vilnius county. Age at the onset of the disease was considered as the beginning of the deterioration of vision, mean age was 19 years (SD 13, median 15). Mean duration of the disease was 13 years (SD 11, median 10).

In majority of subjects' refraction assessment showed myopic astigmatism (86.67%). BCVA results were the same BE, mean was 0.16 (SD 0.25, median 0.10). Disorders of color visions varied: in 3 (20%) subjects with BCVA is was too little to see test tables, 4 (26.67) subjects were able to see only test tables, and 8 subjects (53.33%)

color vision still persisted or they had moderately disorders of color vision. Visual field was examined in all subjects, and central scotomas were found. Detailed information about the patients is presented in Table 10.

In all subjects ophthalmobiomicroscopy showed macular characteristic to STGS, they were especially visible during FAF test (Table 11), as well as „beaten bronze“ pattern and central atrophy. Various changes were observed in subjects during FAF test (Table 11).

Macular OCT results showed that VMT was observed in 8 subjects (53.33%). EZ structure and integrity was assessed, and in 11 (73.33%) out of 15 subjects its structure was significantly damaged in EZ area, its layer could not be identified, various stages of “optical gap” were observed. In these cases, length of the defect of EZ layer was measured (Table 11). Mean CRT was 69.4 μm (SD 35.7 μm, median 56 μm) in the RE and 77.8 μm (SD 42.0 μm, median 63 μm) in the LE. Mean SCT was 315.4 μm (SD 124.0 μm, median 338.5 μm) in the RE and 305.1 μm (SD 126.5 μm, median 303.5 μm) in the LE. OCT data, Fishmann and Lois classification FAF data are presented in Table 11. Full-field ERG results were presented according to Lois classification (Table 11). MfERG was conducted in 8 subjects (53.33%): weakened responses in central zone were observed in 6 subjects (75%), and weakened responses in all mfERG zones were observed in 2 (25%) subjects.

Table 10. *Demographic, color vision and visual field data (N/A – not performed) in STGD subjects.*

Nr.	TD number	Gender	Age of VA deterioration onset (y)	Duration of the disease	Symptoms, other eye diseases	BCVA RE/ LE	Refraction RE/ LE	Ishihara test RE/ LE	Visual field
1	TD004	V	17	36	Dyschromatopsia; floaters	0,16/0,16	None/ Myopic astigmatism	2/2	Central scotomas within 10° of visual field
2	TD005	M	7	19	None	0,03/0,04	Myopic astigmatism/ Myopic astigmatism	0/0	Central scotomas within 20° of visual field
3	TD006	M	30	19	Dyschromatopsia	0,1/0,1	None/ None	5/4	Central scotomas within 20° of

									visual field
4	TD014	M	8	22	None	0,1/0,1	Myopic astigmatism/ Myopic astigmatism	31/31	Central scotomas within 10° of visual field
5	TD021	M	15	15	Photophobia	0,06/0,06	Mixed astigmatism/ Mixed astigmatism	0/0	Central scotomas within 20° of visual field
6	TD024	V	27	3	None	0,1/0,1	Mixed astigmatism/ Mixed astigmatism	24/33	Central scotomas within 10° of visual field

7	TD026	M	10	10	None	0,1/0,1	Myopia/ Myopic astigmatism	8/12	Central scotomas within 20° of visual field
8	TD027	V	6	36	Uveitis	0,02/0,02	Myopic astigmatism/ Myopic astigmatism	0/0	Central scotomas extend beyond the 20° range of visual field
9	TD050	V	45	7	None	0,12/0,16	Hypermetropic astigmatism/ Hypermetropic astigmatism	38/38	Central scotomas within 10° of visual field

10	TD081	V	33	6	Floaters	0,1/0,05	Myopic astigmatism/ Myopia	31/33	Central scotomas within 20° of visual field
11	TD090	V	7	2	None	0,4/0,5	Myopic astigmatism/ Myopic astigmatism	18/12	Central scotomas within 20° of visual field
12	TD091	M	34	0,5	Uveitis	1/0,95	Mixed astigmatism/ Mixed astigmatism	38/38	Central scotomas within 10° of visual field
13	TD093	M	13	5	None	0,09/0,09	Myopic astigmatism/ Myopic astigmatism	2/2	Central scotomas within 20° of

									visual field
14	TD114	M	30	28	Glaucoma	0,02/0,02	Myopic astigmatism/ Myopic astigmatism	2/2	Central scotomas within 10° of visual field
15	TD123	M	10	21	None	0,005/0,005	Myopic astigmatism/ Myopic astigmatism	2/2	N/A

STGD – Stargardt disease, BCVA – best-corrected visual acuity, RE – right eye, LE – left eye

Table 11. *Fishman classification, FAF, SD-OCT and Lois classification data in STGD subjects (NA – no data, N - normal).*

Nr.	TD nr	*Fishman* classification	FAF categories	More detailed FAF evaluation	Macular appearance on SD-OCT RE/LE	CRT RE/LE	SCT RE/LE	Macular EZ BE	EZ defect length (µm) RE/LE	*Lois*
1	TD004	1	HypoAF fovea surrounded by homogenous AF	Foveal granularity, hyperAF ring	VMT/VMT	38/38	446/454	N	2077/1594	I
2	TD005	3	Multiple hypoAF areas at the posterior pole on a heterogenous AF background	CA, hyperAF flecks beyond the arcades	VMT/N	92/104	N/N	Disrupted		III
3	TD006	1	HypoAF fovea surrounded by homogenous AF	Foveal granularity, hyperAF flecks at the fovea	N/N	51/60	450/452	N	2269/2665	II
4	TD014	1	HypoAF fovea surrounded by homogenous AF	CA, hyperAF ring, hyperAF flecks at the fovea	N/VMT	40/174	335/340	N	2281/1525	I

Nr.	TD nr	*Fishman* classification	FAF categories	More detailed FAF evaluation	Macular appearance on SD-OCT RE/ LE	CRT RE /LE	SCT RE/LE	Macular EZ BE	EZ defect length (µm) RE/LE	*Lois*
5	TD021	3	HypoAF macula in the heterogeneous AF background, multiple uneven AF lesions	CA, hiperAF flecks extended out of arches	N/VMT	82/39	226/233	Disrupted	-	II
6	TD024	2	HypoAF fovea surrounded by homogenous AF	RE - CA, hyperAF flecks at the posterior pole/ LE - CA, hyperAF flecks beyond the arcades	VMT/N	54/78	405/254	N	2868/2702	II
7	TD026	1	HypoAF fovea surrounded by homogenous AF	Foveal granularity, hyperAF flecks beyond the arcades	VMT/N	56/58	352/298	N	2230/2099	II
8	TD027	4	Normal	Normal/ Normal	N/N	81/43	89/78	N		III
9	TD050	2	HypoAF fovea surrounded by homogenous AF	CA, hyperAF ring, hyperAF flecks at the posterior pole	N/N	56/50	511/538	N	2007/1698	I

Nr.	TD nr	*Fishman* classification	FAF categories	More detailed FAF evaluation	Macular appearance on SD-OCT RE/LE	CRT RE/LE	SCT RE/LE	Macular EZ BE	EZ defect length (µm) RE/LE	*Lois*
10	TD081	3	HypoAF macula in the heterogeneous AF background, multiple uneven AF lesions	CA, hyperAF flecks beyond the arcades	N/VMT	44/44	271/243	N	5922/6957	III
11	TD090	2	HypoAF macula in the heterogeneous AF background, multiple uneven AF lesions	Foveal granularity, hyperAF flecks beyond the arcades	N/N	104/118	292/309	N	2087/1764	I
12	TD091	1	HypoAF fovea surrounded by homogenous AF	Foveal granularity, hyperAF flecks ta the fovea	N/N	176/158	381/412	N	2556/2883	I
13	TD093	2	HypoAF macula in the heterogeneous AF background, multiple uneven AF lesions	CA, hyperAF flecks beyond the arcades	N/N	61/63	342/309	N	3034/3331	I

Nr.	TD nr	*Fishman* classification	FAF categories	More detailed FAF evaluation	Macular appearance on SD-OCT RE/LE	CRT RE/LE	SCT RE/LE	Macular EZ BE	EZ defect length (µm) RE/LE	*Lois*
14	TD114	2	HypoAF macula in the heterogeneous AF background, multiple uneven AF lesions	CA, hyperAF flecks beyond the arcades	N/N	39/49	166/189	N	3821/4101	I
15	TD123	4	Multiple hypoAF areas at the posterior pole on a heterogenous AF background	CA, hyperAF flecks beyond the arcades	VMT/VMT	67/66	149/162	Disrupted	-	III

AF – autofluorescence, CA – central atrophy, CRS – central retinal thickness, EZ – ellipsoid zone, FAF – fundus autofluorescence, Lois – Lois classification, RE – right eye, LE – left eye, SCT – subfoveal choroidal thickness, SD-OCT – spectral – domain optical coherence tomography

2.3.2. Subjects with cone/cone-rod dystrophy

Subjects' ophthalmological data

Twelve subjects, 5 males (41.67%) and 7 females (58.33%) were allocated into con/cone-rod dystrophy group. Subjects' age ranged from 17 to 66 years. Majority of subjects (85.71%) were from Vilnius county, and 3 patients (27.27%) were from other counties.

Duration of the diseases ranged from 1 to 33 years, mean duration was 13.2 years (SD 9.7, median 12). As in patients with STGD, subjective assessment of the onset of the disease, beginning of the deterioration of vision was selected; median age at the beginning of the disease was 17.2 years (SD 10.8, median 15). Other complaints of subjects are presented in Table 12.

In majority of subjects refraction assessment showed myopic astigmatism (71.42%). BCVA in the RE was 0.37 (SD 0.22, median 0.45), in the LE - 0.37 (SD 0.27, median 0.36). Disorders of color visions varied: in 2 (14.28%) subjects with BCVA is was too little to see test tables, 4 (28.57%) subjects were able to see only test tables, and other subjects (57.14%) color vision still persisted or they had moderately disorders of color vision. In all patients with assessed visual field central scotomas were found. Detailed information about the patients is presented in Table 12.

Assessment of EZ layer structure showed that in 6 subjects (50%) out of 12 macular structure was significantly damaged in one or both eyes, layer could not be identified, and optical caps of various stages were observed. When optical gap was identified length of EZ defect was measured (Table 13). Mean CRT in the RE was 139.5 μm (SD 47.9 μm, median 142.5 μm), in the LE - 142.3 μm (SD 52.1 μm, median 155 μm). Mean CRT was 304.5 μm (SD 140.1 μm, median 292 μm) in the RE and 303.3 μm (SD 119.3 μm, median 308 μm) in the LE. Unlike STGD subjects, ophthalmobiomicroscopy did not showed significant macular changes; usually depigmentation or

normal fundus of eye were observed. OCT data are presented in Table 13.

Findings of full-field ERG were normal in subjects with CD, and in subjects with CRD both scotopic and photopic responses were lowered or absent. MfERG was not conducted in one subject due to low BCVA, in all other subjects, abnormalities were found: flattening of the central hill or decrease of various degrees of central amplitudes.

Table 12. *Demographic, color vision and visual field results in CD and CRD subjects (VF – visual field).*

Nr.	TD nr.	Gender	Age of VA deterioration onset (y)	Disease duration (y.)	Disease	Symptoms, other eye/systemic diseases	BCVA RE/ LE	Refraction RE/ LE	Ishihara test RE/ LE	Visual field
1	TD003	M	8	27	CD	N	0,5/0,45	Myopia/N	38/38	Ny/Ny
2	TD007	M	14	12	CD	N	0,16/0,16	Myopia/ Myopia	38/38	Central scotomas within 10° of VF
3	TD010	M	17	1	CD	Floaters	0,63/0,1	N/ Hypermetropic ast	38/38	Central scotomas within 10° of VF
4	TD029	M	35	14	CD	Dyschromatopsia	0,2/0,1	Hypermetropia/ Myopic ast	2/2	Central scotomas within 10° of VF
5	TD036	M	16	9	CD	N	0,25/0,25	Myopic ast/ Myopia	12/10	Central scotomas within 10° of VF
6	TD053	W	17	5	CD	Migraine without aura	0,4/0,4	Hypermetropic ast/ Hypermetropic ast	22/7	Central scotomas within 10° of VF
7	TD058	W	6	13	CD	N	0,75/0,7	Myopic ast/ Hypermetropic ast	37/38	Central scotomas extends beyond

Nr.	TD nr.	Gender	Age of VA deterioration onset (y)	Disease duration (y.)	Disease	Symptoms, other eye/systemic diseases	BCVA RE/ LE	Refraction RE/ LE	Ishihara test RE/ LE	Visual field
										the 20° range of VF
8	TD061	W	12	10	CD	Dyschromatopsia	0,16/0,32	Myopia/ Myopic ast	2/2	RE – central scotomoas within 20° of VF/ LE - within 10°
9	TD067	M	30	33	CD	N	0,5/0,95	N/ Myopic ast	38/38	DA - N/ KA – central scotomas within 10° VF
10	TD095	M	5	12	CRD	Photophobia; Renal polycystosis	0,5/0,45	Hypermetropic ast/ Hypermetropic ast	2/2	central scotomas within 10° VF
11	TD113	W	36	1	CD	N	0,5/0,5	Myopic ast/ Myopic ast	17/20	RE -central s scotomos within 10° of VF/ LE - >20°

Nr.	TD nr.	Gender	Age of VA deterioration onset (y)	Disease duration (y.)	Disease	Symptoms, other eye/systemic diseases	BCVA RE/ LE	Refraction RE/ LE	Ishihara test RE/ LE	Visual field
12	TD123	W	10	21	CRD	N	0,005/0,005	Myopic ast/ Myopic as	2/2	N

BCVA – best-corrected visual acuity, RE – right eye, LE – left eye, CD – cone dystrophy, CRD – cone-rod dystrophy, ast – astigmatism

Table 13. *Findings of fundus ophtalmobiomicroscopy, AF, OCT and mfERG in subjects with cone/come-rod dystrophy*

Nr.	TD nr	Macular appearance	More detailed FAF evaluation	Macular appearance on SD-OCT RE/ LE	CRT RE/ LE	SCT RE/ LE	EZ at the macula BE	EZ defect length (µm) RE/ LE	mfERG
1	TD003	Very mild dyspigmentation	Foveal granularity	ERM+VMT/ ERM+VMT	140/158	286/258	Normal	-	Slightly lowered central amp
2	TD007	Dyspigmentation	CA, hyperAF ring	N/N	72/59	245/277	Absent	1283/1554	Slightly lowered central amp
3	TD010	Dyspigmentation	HyperAF ring	N/N	83/70	312/310	Absent	1451/1508	Lowered central amp, eccentric fixation
4	TD029	Yellow flecks	Foveal granularity	N/N	196/200	298/327	Normal/ Absent	Normal /701	Lowered central amp
5	TD036	Very mild dyspigmentation	Foveal granularity, hyperAF ring/ CA,	CME+lamellar hole/ CME+lamellar hole	145/160	106/306	Absent	662/786	Lowered central amp

Nr.	TD nr	Macular appearance	More detailed FAF evaluation	Macular appearance on SD-OCT RE/ LE	CRT RE/ LE	SCT RE/ LE	EZ at the macula BE	EZ defect length (µm) RE/ LE	mfERG
			hyperAF ring						
6	TD053	Dull macular reflex	Foveal granularity	N/N	202/195	360/360	Normal	-	Lowered central amp
7	TD058	N	Foveal granularity	N/VMT	140/140	464/434	Disrupted	-	Flattened central *hill*
8	TD061	Dyspigmentation, hypopigmented areas at the fovea	CA, hyperAF ring	N/N	146/155	480/536	Absent	1133/1176	Lowered central amp
9	TD067	Dyspigmentation, local RPE atrophy	Foveal granularity/ CA	VMT/ CME+lamellar hole	151/155	254/160	Disrupted/ Absent	- /2065	Flattened central *hill*
10	TD095	N	-	VMT/VMT	208/212	553/384	Normal	-	Lowered amp in all zones
11	TD113	Dyspigmentation	Foveal granularity	N/N	124/138	147/125	Normal	-	Slightly flattened central *hill*

Nr.	TD nr	Macular appearance	More detailed FAF evaluation	Macular appearance on SD-OCT RE/ LE	CRT RE/ LE	SCT RE/ LE	EZ at the macula BE	EZ defect length (µm) RE/ LE	mfERG
12	TD123	Atrophy, dyspigmentations, pigmented lesions	CA, hyperAF extends beyond arcades	VMT/VMT	67/66	149/162	Normal	-	N

AF – autofluorescence, SD-OKT –spectral domain optical coherence tomography, mfERG – multifocal electroretinography, FAF – fundus autofluorescence, CRS – central retinal thickness, SCT – subfoveal choroidal thickness, EZ – ellipsoid zone, BE – both eyes, RE – right eye, LE – left eye, ERM – epiretinal membrane, VMT – vitreomacular traction, CA – central atrophy, CME – cystic macular edema, RPE – retinal pigment epithelium, amp - amplitude

2.3.3. Genetically confirmed cases of *ABCA4* retinopathy

In all subjects with *ABCA4* retinopathy molecular genetic testing of the most common pathogenic variations of *ABCA4* gene was performed. Coding and adjacent sequences of exons 12 and/or 21 and 42 were examined, fragments where most common pathogenic changes are identified in Lithuanian patients group. Molecular diagnosis of *ABCA4* retinopathy was confirmed for five patients; in all subjects compound heterozygous genotype of mutations CM990022 and CM970016 genotype was identified. In seven subjects with ABCA4 retinopathy pathogenic variation CM970016, possibly pathogenic variation HM060037 or complex disease causing allele L541P; A1038V in one allele were found.

During this study, the following sequence changes were identified in subjects with *ABCA4* retinopathy:

- CM970016 mutation frequency in the group of Lithuanian patients with *ABCA4* retinopathy is 11.84%;
- CM990022 mutation frequency in the group of Lithuanian patients with *ABCA4* retinopathy is 5.26%;
- HM060037 mutation frequency in the group of Lithuanian patients with *ABCA4* retinopathy is 1.32%;
- L541P; A1038V complex mutation allele frequency in the group of Lithuanian patients with *ABCA4* retinopathy is 3.95%. Sequence changes presented in this complex two-substituions allele is inherited in cis-configuration. Such complex is more frequently identified in population of German origin and therefore is considered as German founder effect alteration. 12.7% of patients with diagnosed *ABCA4* retinopathy are of German origin compared with 1.1% of non-German subjects.

Subject TD050

Analysis of sequences of exons 12, 21 and 42 and adjacent sequences of *ABCA4* gene identified the following genotype: c.[1622T>C];[5882G>A] (p.[(Leu541Pro)];[(Gly1961Glu)]) - rs61751392, CM990022; rs1800553, CM970016*.

Possibly compound heterozygous genotype of pathogenic mutations c.1622T>C and c.5885F>A was identified in gene *ABCA4* causing autosomal recessive STGD.

Subject TD114

Analysis of sequences of exons 12, 21 and 42 and adjacent sequences of *ABCA4* (MIM#601691) gene identified the following genotype: c.[(1622T>C(;)3200C>T)];[(5882G>A)] (p.(Leu541Pro)(;)(Ala1038Val);(Gly1961Gln)) - rs61751392, CM990022; rs61751374, CM970006; rs1800553, CM970016*.

Molecular genetic testing indentified possibly compound pathogenic heterozygous mutation genotype causing STGD (OMIM#248200, ORPHAN:827). Changes possibly identified in one allele (rs61751392+rs61751374) are reported being inherited together (complex allele).

Discussion

Results of clinical study of two subgroups are rather similar, except the ocular fundus. The most common refraction abnormality in both groups is myopic astigmatism (86.67% ir STGD subgroup and 71.42% in CD/CRD subgroup). *Ishihara* test did not show any or showed only insignificant disorders of color vision in 53.33% of subjects with STGD and 57.14% of subjects with CD/CRD. In both groups central scotomas were found in subjects during the assessment of visual field.

Using SD-OCT technique different stages of „optical gaps“ were identified in 73.33% of subjects with STGD and 50% of subjects with CD/CRD. However, optical gap has been described also in other

diseases such as solar retinopathy, rod monochromatism and maculopathies related to *RP1L1* mutations [17]–[20] and therefore this sign is not pathognomonic to RD related to *ABCA4*.

The importance of electrophysiological testing, ERG and EOG is still disputable. No EOG was conducted in study participants.

According to study conducted by *K. G. Noble and R. E. Carr*, full-field ERG shows decrease of the amplitude only in advanced disease (16%, 50 subjects examined) [21]. Because subjects of various age and duration of the disease underwent full-field ERG in the STGD group, assessment of changes according to *Lois* classification is presented for all three groups: 7 were assigned to group I and by 4 subjects were assigned to groups II and III. In subjects with CD full-field ERG did not identify any changes, and in both subjects from CRD group lowered responses were observed both in photopic and scotopic phases that could be expected in subjects with CRD. Due to small samples both in STGD and CD/CRD groups statistical analysis considered to be inappropriate and was not conducted, therefore comparison is impossible.

U. Kretschmann et all. examined mfERG changes in Stargardt disease. They Identified changes even is subjects with good VA: in 25 eyes out of examined 28 (89.3%) regional cone dysfunction was found even when VA was higher than 0.6 [22]. We conducted mfERG in 8 subjects (53.33%) with STGD and in all subjects (100%) changes in central or all zones were found, weakened or disappeared potentials. Also, in all 11 subjects with CD/CRD who underwent mfERG weakened or disappeared potentials were observed. These findings confirm importance of mfERG, especially in subjects with early stages of the disease when there may be no changes of fundus of eye or disorders of color.

2.4. Other inherited retinal dystrophies

Ten different IRD were identified in the group of other rare IRD (Figure 21). Diseases in which molecular genetic testing was conducted are presented below.

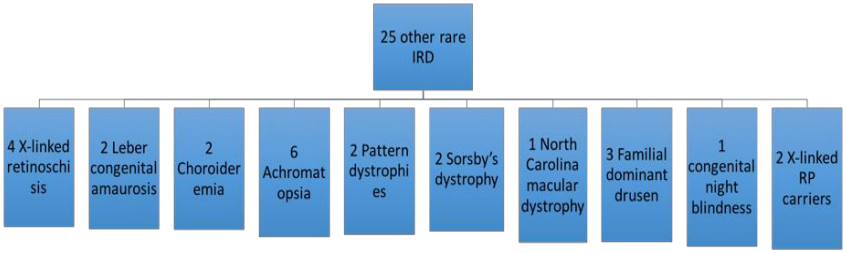

Figure 26. The groups of other rare retinal dystrophies.

2.4.1. Subjects with achromatopsia

Six subjects were allocated in the group of achromatopsia (ACHM).

In all subjects, symptoms of ACHM were present since birth. However, only during the study ACHM diagnosis was clinically suspected and molecular genetic testing showed abnormalities causing ACHM.

Subjects' BCVA was very similar and ranged from 0.07 to 0.16; all subjects except TD089 were hypermetropic. Detailed data are presented in Table 14.

Table 14. ACHM patients age, BCVA, subjective complaints and color vision data (CV – color vision, HM – hypermetropia, Ny – nystagmus, V – vision, Phb - photophobia).

	TD011	**TD012**	**TD013**	**TD089**	**TD121**	**TD122**
Age (y)	28	27	29	13	14	5

Complaints	Phb Low V Low CV	Phb Low V Ny No CV HM	Phb Low V Ny No CV HM	Phb Low V Ny	Phb Low V Ny	Phb Low V Ny
BCVA RA/LA	0,12/ 0,12	0,1/ 0,16	0,1/ 0,1	0,12/ 0,12	0,12/ 0,12	0,08/ 0,07
***Ishihara* test**						
RA	8/ 38	2/38	2/38	5/ 38	6/ 38	2/ 38
LA	7/38	2/38	2/38	3/ 38	7/ 38	2/ 38

Absence of glaze fixation and horizontal nystagmus, symptoms characteristic to ACHM, were observed in all subjects. Due to these symptoms many tests were challenging or impossible to conduct.

No specific changes of eye anterior segment were found at ophthalmobiomicroscopy. As usual in ACHM, no changes or minimum changes were found in the fundi of the eyes: lack of macular reflex (TD011, TD012, TD013) and mild depigmentation (TD011 and TD012) (Figure 22). Due to significant photophobia and age imaging of fundi of the eye was not made in TD012 and TD122.

Fluorescein angiography tests showed no significant changes, only in TD013 mild hyperAF was observed.

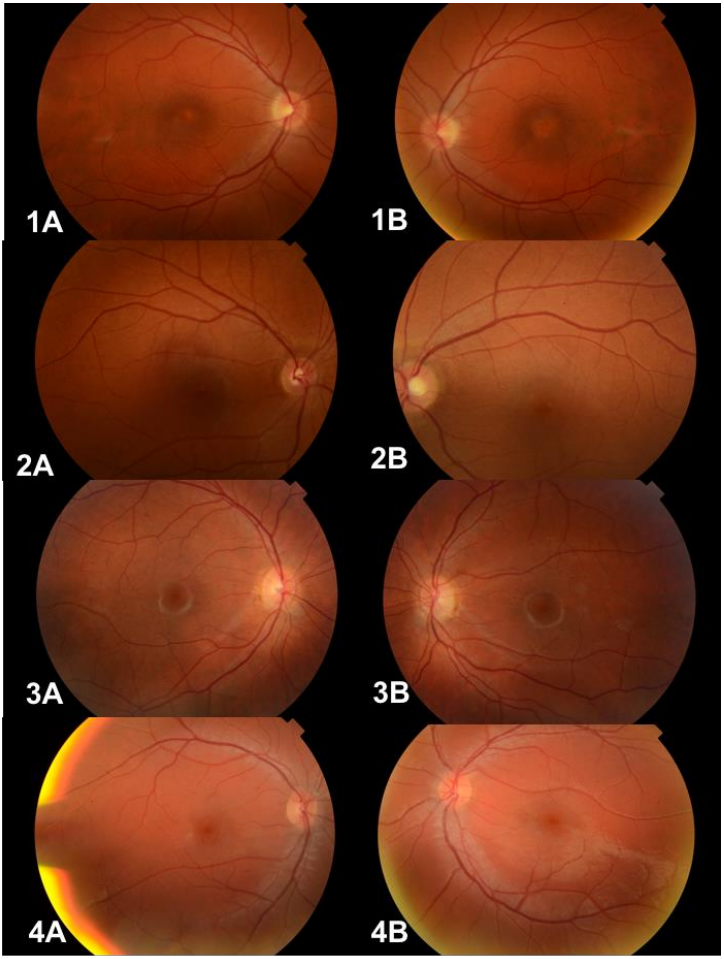

Figure 27. *Images of fundus in ACHM subjects.*
TD011: BE mild dispigmentation and dull macular reflex are found (1A-B).
TD013: dull macular reflex (2A-B).
TD089 ir TD121: without obvious (3A-B ir 4A-B).

SD-OCT testing revealed a variety of macular changes (Figure 23). Foveal hypoplasia of different degree was observed in all patients.

In TD122 SD-OCT testing was not conducted due to subject's young age. EZ defects in the macula were observed in 3 subjects: EZ integrity derangement with defect length in the RE 1269 μm and in the LE 1345 μm was observed in TD011; total loss of EZ in subfoveal area called optical gap with defect length of 715 μm in the RE and 641 μm in the LE was observed in TD012; and small integrity defects were observed in TD089.

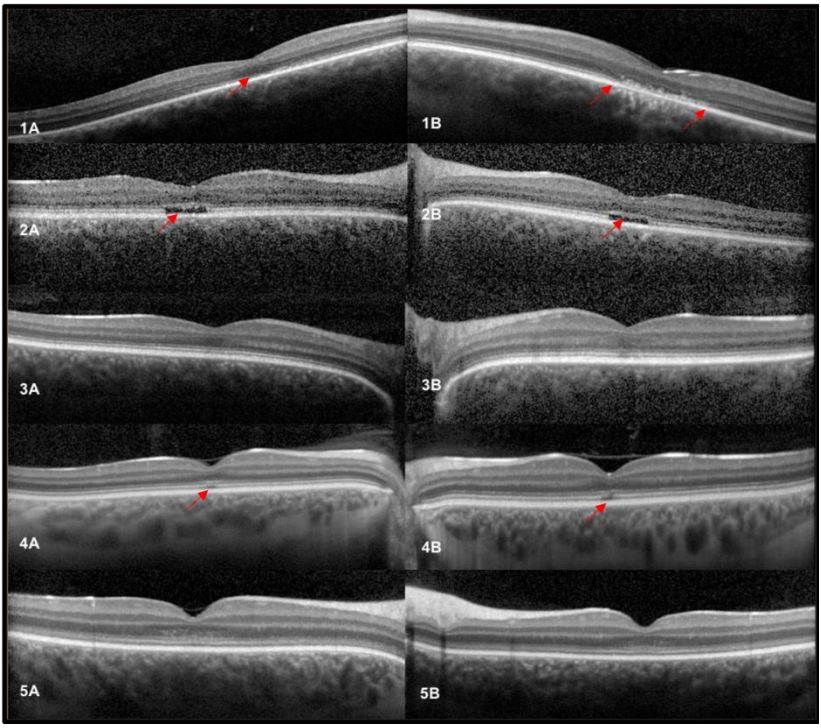

Figure 28. *ACHM patients macular SD-OCT scans.*
TD011: RE EZ loss was found (1A), LE – EZ structure is lost (1B) (red arrows).
TD012: BE optical gap is observed (2A-B) (red arrows). TD013 and TD121 very mild EZ and external limiting membrane structure changes are observed (3A-B ir 5A-B).

TD089: very mild EZ integrity loss (4A-B) (red arrows).

In all subjects, CRT was thinned compared with normal ranges (Table 15). This thinning could be related to the pathogenesis of the disease, lack of cone photoreceptors that are most abundant in the macula.

Table 15. *CRT results* (μm) in *ACHM subjects*.

CRT (μm) / Subject	RE	LE
TD011	192	181
TD012	206	221
TD013	222	216
TD089	185	177
TD121	208	216

It was difficult to conduct SD-OCT due to significant photophobia, and in 2 subjects SCT was measured using the EDI mode. In TD089 it was 280 μm in the RE and 257 μm in LE eye. In TD121 subfoveal choroid was significantly thicker: 418 μm in the RE and 428 μm in the LE.

Full-field ERG was conducted in all subjects; normal, slightly lowered or halved responses were observed in scotopic phase, and in photopic phase lowered amplitudes of waves including *flicker* ERG

showing isolated response of cones were observed almost no responses were seen.

Results of genetic testing

In all subjects with ACHM molecular genetic testing was performed, and pathogenic changes in *CNGB3* (cyclic nucleotide-gated channel, beta-3) gene were identified for 5 patients (Table 16). In the 16th table the references are listed, where the mutations were described for the first time.

Table 16. *ACHM patients pathogenic CNGB3 mutations.*

Patient	Gene	Genotype	Identified mutation	Literature
TD011	*CNGB3*	compound heterozygous	c.31insC (p.Val11fs*9) c.1148delC (p.Thr383Ilefs*13)	*Kohl et al.*, EJHG; 2005 *Sundin et al.*, Nature Genet; 2000
TD012	*CNGB3*	homozygous	c.819_826del (p.Arg274Valfs*13)	*Sundin et al.*, Nature Genet; 2000
TD013	*CNGB3*	homozygous	c.819_826del (p.Arg274Valfs*13)	*Sundin et al.*, Nature Genet; 2000
TD121	*CNGB3*	homozygous	c.1148delC:p.Thr383IlefsX13	*Sundin et al.*, Nature Genet; 2000
TD122	*CNGB3*	homozygous	c.1148delC:p.Thr383IlefsX13	Sundin et al., Nature Genet; 2000

Discussion

Specific instrumental ophthalmologic testing is of extreme importance in the diagnosis of ACHM because ophthalmobiomicroscopy shows no or only very mild non-specific changes. Lack of macular reflex or mild depigmentation was observed in only 4 out of 6 subjects (66.67%). *Genead et al*. found changes in the yellow-spot area in 58.3% of subjects [23], similar results were reported in other studies [24], [25].

OCT also showed a variety of macular changes: hypoplasia of *fovea* of various degrees, loss of EZ structural integrity or optical gap, thinning of CRT. Only in one subject, TD012, optical gap was observed. Prevalence of optical gap on ACHM patients is unknown; it was found in the presence of various genotypes [20], [26], [27]. Different morphological changes are observed even in sibs (TD12 and TD13) with identified pathogenic deletion c.819_826del p.Arg274Valfs*13 in *CNGB3* gene. These results are in line with the literature data arguing that the same abnormal change may cause different phenotype, ant presently the cause of this is unknown [23]. Due to small sample size we are unable to confirm or dispel hypothesis set up by *Thiadens et al*. that different phenotypes of the same genotype could be observed due possible progression of ACHM [20].

FAF images of the subjects showed characteristic hyperAF which is considered to be early sign of ACHM and could be useful to confirm diagnosis [18].

In subjects, pathogenic changes in *CNGB3* genes were identified which combined are responsible for 75% of ACHM cases [27]–[29]. Prevalence of gene mutations causing ACHNM in Lithuanian population is not known yet, and molecular genetic testing in larger samples is necessary.

Identified in subjects TD012 and TD013 8b deletion c.819_826del p.Arg274Valfs*13 in *CNGB3* gene causing frame shift was reported for the first time by *Sundinir et al*. in 2000 [30]. This mutation causes premature termination of translation of *CNGB3*

protein, and therefore necessary areas of protein channel composing pair, S6 transmembrane part and cGMP connecting domain are eliminated [31].

Identified in subjects TD121 and TD122 deletion c.1148delC:p.Thr383IlefsX13 in *CNGB3* gene causing frame shift was reported for the first time by *Sundin et al*. in 2000 [30]. This mutation also causes premature termination of translation of *CNGB3* protein [32].

2.4.2. Subjects with X-linked inherited juvenile retinoschisis

Four subjects with clinically suspected XLRS participated in the study; molecular genetic testing revealed pathogenic mutation of *RS1* gene in these study patients.

All subjects were males, their main complaints were difficulties reading, tiredness of eyes looking from short distances and text flickering while reading.

Subjects' age and data on VA are presented in Table 17. Hypermetropic astigmatisms presented refraction abnormalities in subjects. Anterior segment was unremarkable.

Table 10. *XLRS patients' age and BCVA data.*

	TD002	**TD017**	**TD022**	**TD137**
Age	12	9	17	6
BCVA RE/LE	0,32/ 0,32	0,6/ 0,5	0,6/ 0,3	0,3/ 0,3
Near BCVA RE/ LE	0,6/ 0,6	0,6/ 0,6	0,6/ 0,6	0,4/ 0,4

Images of fundi of the eyes of subjects are presented in Figure 23; ophthalmoscopy shows no changes of OND, lack of macular reflex

is observed as well as macular cystic changes and presentation or „wheel spokes“ which is especially characteristic to XLRS. In 2 subjects out of 4 (50%) changes in the peripheral part of retina were observed: peripheral schisis in the lower and nasal part, mild traction and lamellar gap of internal layers and obliterated blood vessels were observed in TD002 and demarcation lines in the nasal part in the RE and in the paramacular temporal part in the LE were observed in TD017.

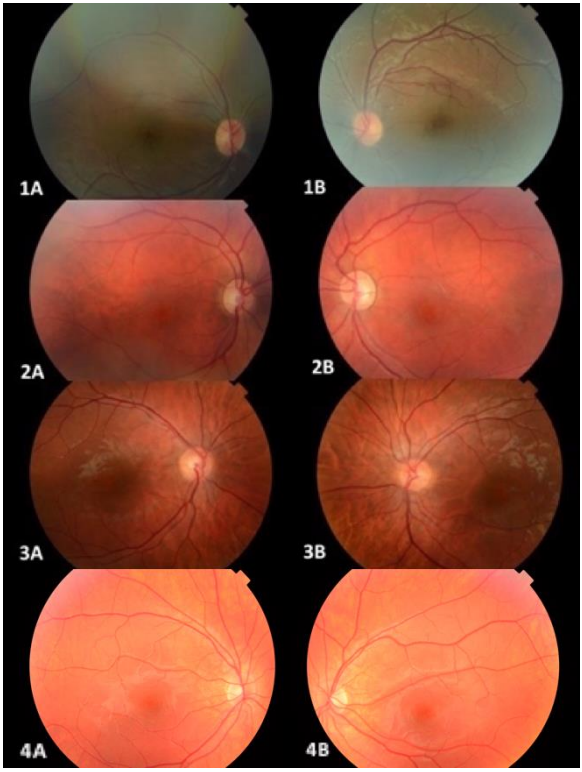

Figure 29. *Images of fundus in XLRS subjects, macular appearance. TD002: dystrophic changes at the macula, dyspigmentation, CME (1A-B); TD017:“wheel spokes” like CME, foveoschisis, “star”-like*

dyspigmentation (2A-B); TD022 – foveoschisis and cystic changes (3A-B); TD137 – CME without dyspigmentation.

SD-OCT revealed retinoschisis in the macula, predominantly in the internal nuclear layer and less prominent in the external retinal, external nuclear and retinal ganglion cells layers; formation of intraretinal gaps of various sizes was also observed (Figure 25). CRT ranged from 373 μm to 777 μm; mean CRT of all subjects was 498.5 μm in the RE, and 498.25 μm in the LE. SCT was evenly distributed among subjects and ranged from 304 μm to 375 μm; mean SCT of all subjects was 323.75 μm in the RE and 340 μm in the LE.

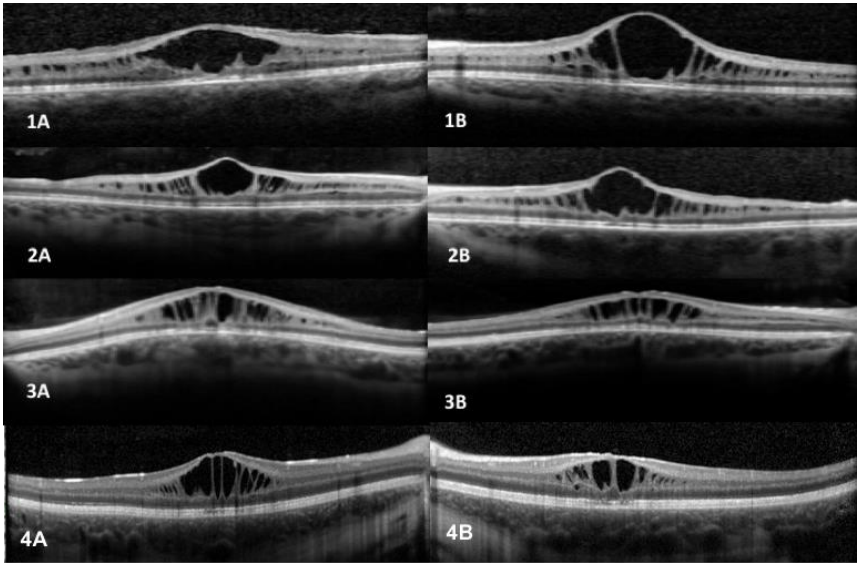

Figure 30. *Macular SD-OCT retinal schisis with large i/r cavities are observed for all patients: TD002 CTS RE – 600 μm, LE – 661 μm (1A-B); TD017 CRT RE – 570 μm, LE – 777 μm (2A-B); TD022 CRT RE – 373 μm, LE – 434 μm (3A-B), TD137 CRT RE – 450 μm, LE - 379 μm (4A-B).*

Ishihara color test was normal in all subjects, 38 out of 38 pictures were assessed correctly.

Full-field ERG was conducted in 3 out of 4 subjects (Figure 26), end 3.0 ERG mode showed negative ERG, pathognomonic sign of XLRS. Mild lowering of amplitude of b-wave was observed in subjects under conditions of photopia. mfERG was additionally conducted in TD017 and TD022, and significant lowering of amplitudes of central responses were observed. In TD137 full-field ERG was unreliable due to young age of the subject; it is not involved into discussion.

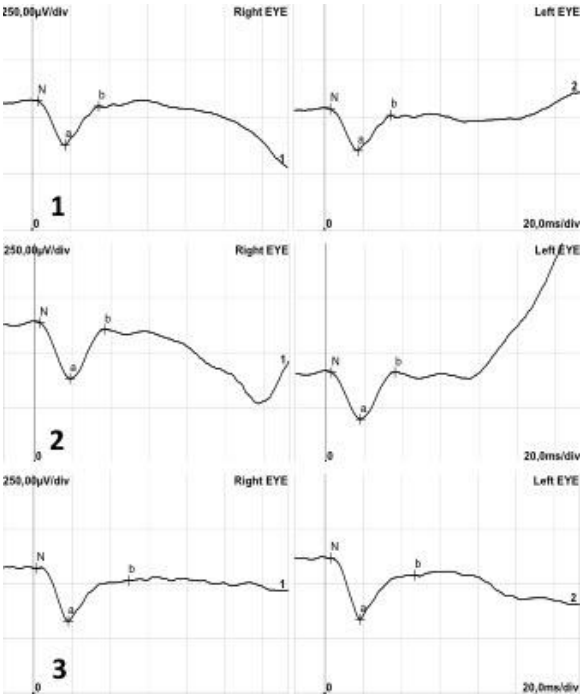

Figure 31. *Full-field ERG combined response shows a negative waveform the plot showed an electronegative ERG with b/a wave ratio*

of: RE-0,876 and LE-0,848 for TD002 (1), RE-0,875 and LE-1,0 for TD017 (2) and RE-0,769 and LE-0,723 for TD022 (3).

Results of genetic testing

Genealogy of subjects is inconclusive.

Mutation NM_000330.3: c.599G>T (p.R200L) was indentified in subjects TD022 and TD137, *in silico* analysis confirmed pathogenicity (disease-causing mutation (mutationtaster.org); PROVEAN score -6.883 – dexterous; probably damaging). Three other different changes of the same aminoacid (CM095237 [33], CM981767 [34], CM981768 [34]) supporting pathogenicity of identified variations are published in the Human Genome Mutation Database (HGMD). NM_000330.3:c.(92_97)insC (p.W33fs) insertion creating frame shift is identified in subject TD002, and *in silico* analysis, again, shows pathogenicity of this variant (mutationtaster.com). Previously reported [34] pathogenic mutation NM_000330.3:c.422G>A (p.R141H), HGMD CM981753 was identified in third subject, TD017. Sequenograms of *Sanger* sequencing results are presented in Picture 27.

Mothers of all patients were examined; they had no clinical ophthalmological signs of XLRS. *Sanger* sequencing confirmed that mothers of all subjects were heterozygous carriers of the identified pathogenic mutations.

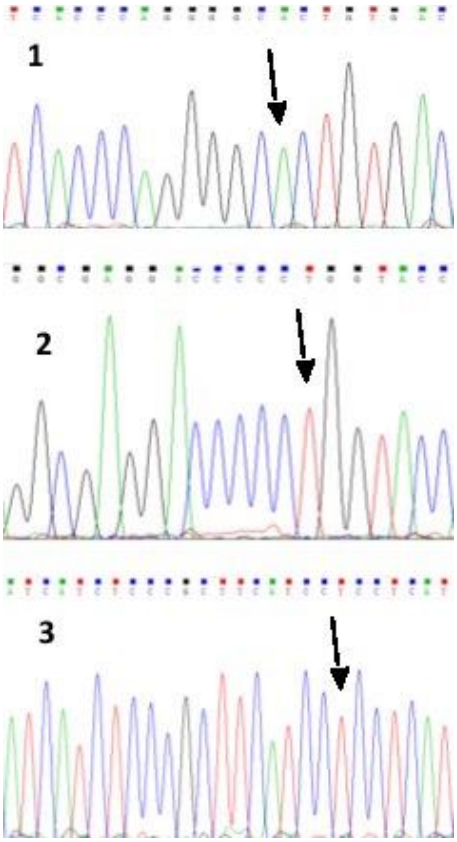

Figure 32. *The Sanger sequencing sequenograms indicating the identified variants:* ***1 – TD017*** *(NM_000330.3:c.422[G>A];[0] (NP_000321.1:p.[(Arg141His)];[(0)]),* ***2 - TD002*** *(NM_000330.3:c.599[G>T];[0] (NP_000321.1:p.[(Arg200Leu)];[(0)])),* ***3 – TD022*** *ir* ***TD137*** *the same variant was identified (NM_000330.3:c.96[insC];[0] (NP_000321.1:p.[(Trp33Leu)];[(0)]).*

Discussion

According to the literature, foveal retinoschisis was observed in 68% to 100% patients with XLRS [35], and detachment of

peripheral retina was observed in 43% to 60% of patients. In our study significant foveal retinoschisis was observed in all four subjects with XLRS, and peripheral retinoschisis was observed in 2 subjects (50%). These results are in line with the literature data. Various other possible abnormalities of peripheral retina are also reported [35], [36] with prevalence ranging from 93% to 38%; peripheral abnormalities were observed in 2 of our subjects.

OCT testing revealed retinoschisis mostly in the internal nuclear layer, and less frequently it was observed in the external retinal, external nuclear and retinal ganglion cells layer, i/r formations of various sizes were also observed in places; these findings were in line with data previously reported by *Padrón-Pérez at al*. and *Yu at al*. [37], [38].

Sign which is reported as pathognomonic, electronegative dark adaptations 3.0 cd*s/m^2 (DA 3.0) ERG, were found in all our subjects; however, these signs is not always seen in subjects with XLRS [39].

At the moment, alterations of single *RS1* gene are responsible for XLRS [40]. Pathogenic alterations of *RS1* gene were identified in all subjects, pathogenic changes that are undocumented yet were identified in 2 subjects [33], [34]. According to *Fahim et al*., patients with identifies pathogenic *RS1* gene mutation and peripheral retinal abnormalities has higher risk of complications of retinoschisis [36].

2.4.3. Subjects with familial dominant drusen

Three subjects are included into this group and all of them are female relatives (sisters) TD100, TD104 and TD110.

It is important to emphasize that subjects had no ophthalmological complaints.

Subjects' age ranged from 38 to 41 years, VA was 1.0-1.5, color vision was normal.

In all subjects, anterior segment was normal, ophthalmobiomicroscopy showed normal OND. Images of the fundi

are presented in Figure 28, yellowish-whitish drusen of various abundance was observed in the macula of all subjects.

Fundus AF showed sporadic hyperfluosescence spots corresponding drusen at fundus.

SD-OCT testing was conducted in all subjects (Figure 29). Most significant changes of SD-OCT were observed in subject TD100 who had the most abundant drusen.

KP 30-2 testing did not show any central scotoma in subjects.

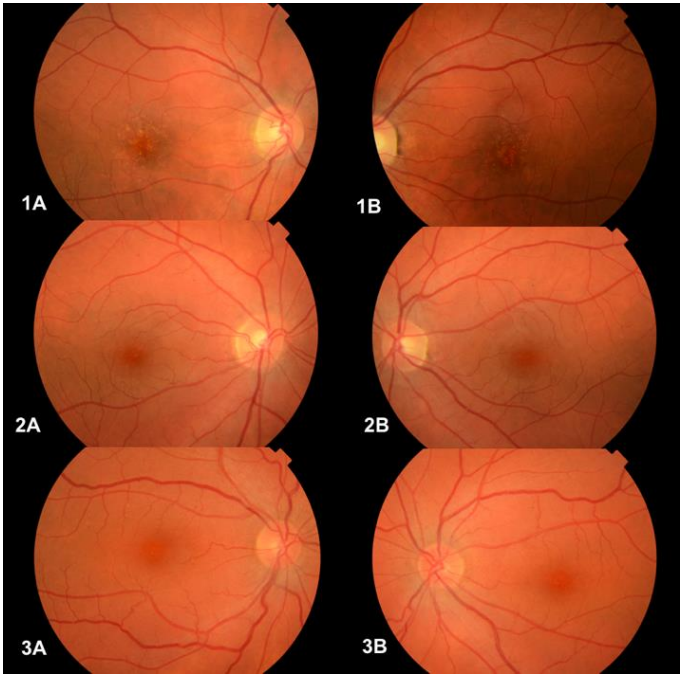

Figure 33. *For all patients yellowish-white macular flecks were observed.*
TD100 – 1A-B
TD104 – 2A-B
TD110 – 3A-B.

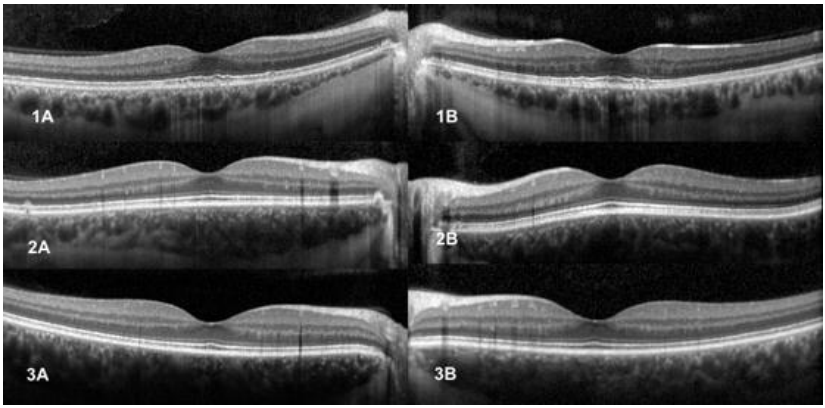

Figure 34. *On macular SD-OCT sub-retinal pigment epithelium deposits are observed for TD100 (1A-B), the OCTs of TD104 (2A-B) and TD110 (3A-B) were without RPE irregularities.*

In all subjects full-field ERG was conducted, results were normal, no data indicating disorder of function of photoreceptors were obtained.

Whereas abnormalities were characteristic to familial dominant drusen disease, targeted molecular genetic testing was conducted.

Results of genetic testing

Molecular genetic testing of *EFEMP1* (EGF-containing fibulin-like extracellular matrix protein 1) gene causing AD inherited FDD disease was performed in all patients. Most common mutation c.1033C>T, p.Arg345Trp of *EFEMP1* gene was analysed and not identified in any of the tested research patients. Promotors, introns and other sequences were not included into analysis, and therefore probability of possible changes of these parts of gene could not be denied. *EFEMP1 :*c.1033C>T, p.Arg345Trp is the most common molecular cause of AD inherited FDD [41].

Discussion

The only known mutation of *EFEMP1* gene causing FDD - non-conservative R345W (arg345trp) - was identified in 1999 [42]. Therefore, sequencing and target analysis of parts of 9th and 10th exons and adjacent sequences of gene *EFEMP1* were conducted in our subjects. Aforementioned tests did not reveal pathogenic mutation .1033C>T, p.Arg345Trp in our subjects. Possibility of FDD could not be denied because cases when FDD diagnosis is based on clinical data are reported (*Tarttelin et al*.) [43], although molecular genetic testing does not reveal pathogenic changes in *EFEMP1* gene. In cases of AMD no changes of *EFEMP1* gene are found despite significant similarity of phenotype; this correlation between inherited and age-related diseases is characteristic to many age-related diseases [44].

Clinical symptoms of subjects are in line with reported in the literature. No one of subjects (their age range from 38 to 41 years) presently has no ophthalmologic complaints; however, according to the literature, symptoms usually occur at the age of 40-50 years [45].

2.4.4. Subjects with North Carolina macular dystrophy

Subject TD071, 50 years of age, several months ago noticed deteriorating and distorted vision with her RE. She has poor VA of her LE for 30 years.

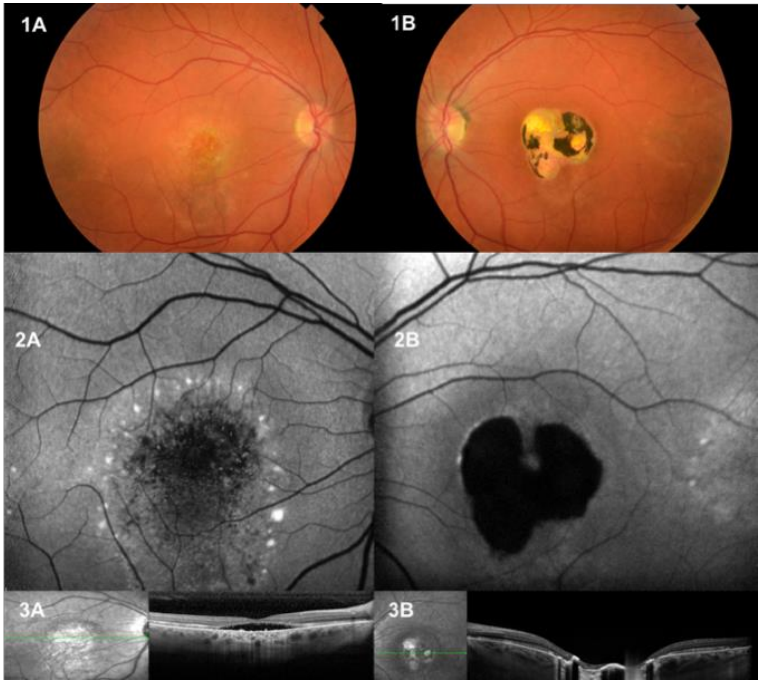

Figure 35. *In the fundus photo of the RE confluent yellow macular deposits are observed (1A), in FAF hypoAF area at the macula surrounded by hyperAF (2A), on SD-OCT SRF is observed (3A).*
In fundus photo of the coloboma-like atrophicmacular lesion is observed (1B), at the FAF large macular hypoAF lesion (2B), on SD-OCT atrophy of the neurosensorial retinal and RPE is observed (3B).

Based on history and clinical findings, North Carolina macular dystrophy was suspected (Figure 30).

Results of genetic testing

Changes in copy number variation in the genomic region involving *PRDM13* gene are related to North Carolina macular dystrophy [46]. SNP comparative genome hybridization was performed with analysis of 220000 probes tag SNPs in the SNP chip

located in the clinically significant genome sites (including subtelomeric and pericentromeric regions and sex chromosome) and in ~400 disease-related gene areas, no pathogenic changes of copy number variantion related to reported diseases and syndromes were identified.

Discussion

North Carolina macular dystrophy penetration is full and it is considered as non-progressive RD with various phenotypic presentation and usually bilateral lesion [47], [48]. Although disease is non-progressive, complications such as CNV may cause deterioration of VA [49].

After intense genetic testing in 1999, abnormalities causing North Carolina macular dystrophy were identified in 6q16 chromosome (MCDR1, MIM:136550) in several families of different ethnic origin [50]–[53]. Similar phenotype was identified in the second locus 5p15-p13 (*MCDR3*, MIM:608850) [47] [54]. Recently, 3 new single nucleotide variations were identified in 11 families, in *MCDR1* locus, DNase hypersensitivity site (DHS), non-coding interval between PRDM13 and adjacent overlapping genes *CCNC/TSTD3* [46].

CONCLUSIONS

1. Registry and monitoring system of the inherited retinal diseases was established and developed in the Center of Eye Diseases, Clinic of Ear, Nose, Throat and Eye Diseases, Institute of Clinical Medicine of the VU Faculty of Medicine.
2. Pathogenic changes confirming molecular diagnosis of inherited retinal dystrophies were identified in 16,81% of subjects. Four pathogenic variants alterations of *RHO*, *PRHP2* and *MYO7A* genes were identified in the largest group of 67 subjects with retinitis pigmentosa.
3. Biostatistical analysis showed statistically significant correlations between central retinal thickness, subfoveal choroidal thickness, remained ellipsoid zone, diameters of hyperautofluorescent ring, visual field and SNPs of the retinitis pigmentosa genes.
4. The group of *ABCA4*-linked retinopathies was divided in two subgroups according the phenotype. In 6 subjects with *ABCA4*-linked retinopathies variants of *ABCA4* gene were identified and the prevalence of these variants were determined: CM970016 (11,84%), CM990022 (5,26%), HM060037 (1,32%), L541P;A1038V (3,95%).
5. In the group of other rare inherited retinal dystrophies pathogenic variants were identified:
   5.1. Variants of *CNGB3* gene were identified in 5 subjects with achromatopsia.
   5.2. In the group of subjects with X-linked hereditary juvenile retinoschisis 3 variants of *RS1* gene were identified, two of them were novel.

PRACTICAL RECOMMENDATIONS

1. Considerable clinical and genetic heterogeneity of IRD requires close cooperation between ophthalmologists and geneticists in

order to establish precise diagnosis. Consultations by other specialists are advisable in syndromic cases.

2. Definite diagnosis of IRD enables to inform patient about course of the disease, collect data on the disease and its pathogenesis in order to develop possible treatment strategies and assess likelihood of inheritance of the disease.
3. Genetical testing of patients using Next generation sequencing may help establish definite clinical diagnosis and conduct more precise clinical examination and therefore it should be recommended for patients.

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THE APPROBATION OF THE RESULTS

Publications

1. **Strupaitė R**, Tumienė B, Kohl S, Utkus A. Achromatopsija, nulemta *CNGB3* geno homozigotiniai pokyčiai: šeiminio atvejo pristatymas. Laboratorinė medicina. 2017, t. 19, Nr. 1, p. 42 – 48.

2. Mayer AK, Cauwenbergh CV, Rother C, Baumann B, Reuter P, De Baere E, Wissinger B, Kohl S, ACHM Study Group (**Strupaitė R**). *CNGB3* mutation spectrum including copy number variations in 552 achromatopsia patients. Hum Mutat. 2017 Nov;38(11):1579-1591.
3. **Strupaitė R**, Ambrozaitytė L, Cimbalistienė L, Ašoklis RS, Utkus A. X-linked juvenile retinoschisis: phenotypic and genetic characterization. Int J Ophthalmol 2018;11(11):1875-1878.

Presentations (international conferences, oral and poster)

1. Strupaitė R, Tumienė B, Utkus A. *Hereditary retinal dystrophies – case series*. Evolutionary medicine: pre-existing mechanisms and patterns of current health issues. 2016, Vilnius, Lithuania.
2. Strupaitė R, Tumienė B, Utkus A. *Hereditary retinal dystrophies - selected cases*. The XV Forum Ophthalmologicum Balticum 2016 (FOB). 2016, Riga, Latvia.
3. Strupaitė R, Cimbalistienė L, Ambrozaitytė L, Utkus A, Ašoklis R. *Stargardt disease phenotype-genotype correlation – first results of a Lithuanian cohort study*. European Association for Vision and Eye Research Congress 2016 (EVER). 2016, Nice, France.
4. Strupaitė R, Tumienė B, Kohl S, Utkus A. *Familial case of achromatopsia*. VIIth international conference "Moksliniai tyrimai – sergančiųjų retomis ligomis viltis". 2017, Vilnius, Lithuania.
5. Strupaitė R. *Paveldimos vaikų akių ligos: genetinio ištyrimo nauda*. Local conference: Lithuanian anual ophthalmologist society conference 2017. 2017, Kaunas, Lithuania.
6. Strupaitė R, Ambrozaitytė L, Cimbalistienė L, Ašoklis R, Utkus A. *X-linked juvenile retinoschisis: different mutations*

– *same phenotype*. European Association for Vision and Eye Research Congress 2017 (EVER). 2017, Nice, France.

7. Kucinskas V, Ambrozaitytė L, Cimbalistienė L, Strupaite R, Asoklis R, Utkus A. *Novel RS1 gene mutations of X-linked retinoschysis Lithuanian patients*. 2017 American Society of Human Genetics (ASHG) Meeting. 2017, Orlando, USA.
8. Strupaitė R, Ambrozaitytė L, Meškienė R, Cimbalistienė L, Strupaitė-šakalienė I, Utkus A. *Overview of the lithuanian retinitis pigmentosa group*. IXth international conference „Inovations of the treatment of rare diseases. 2018, Vilnius, Lithuania.
9. R. Strupaitė, L. Ambrozaitytė, L.Cimbalistienė, R. Ašoklis, L.Šveikauskienė, M.Aukštikalnienė, A.Cimbalas, A.Utkus . *X-linked juvenile retinoschisis*. Update in Neuro-ophthalmology and Retinal Diseases. 2018, Vilnius, Lithuania.
10. Strupaitė R, Ambrozaitytė L, Meškienė R, Cimbalistienė L, Strupaitė-Šakalienė I, Utkus A. *Clinical heterogeneity of the lithuanian retinitis pigmentosa group*. Evolutionary medicine: health and diseases in changing environment. 2018, Vilnius, Lithuania.

CURRICULUM VITAE

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Education:

2014 – 2018 PhD studies at Vilnius University, Faculty of Medicine
2011 – 2014 Vilnius University, Faculty of Medicine, Ophthalmology Residency
2005 – 2011 Vilnius University Faculty of Medicine, Medicine (including 1 year with Erasmus Exchange program at Leipzig University, Faculty of Medicine, Leipzig, Germany)
1999 – 2005 Vilnius Jesuit Gymnasium
1994 – 1999 Vilnius J. Basanavičiaus high school
1993 – 1994 Primary school in Berlin, Germany

Work experience:

Since 2018 – younger assistant at Vilnius University, Faculty for Medicine
Since 2014 – young ophthalmologist at Vilnius University Hospital „Santariškių clinics“, Center of eye diseases
2014 – 2015 – physician assistant at Vilnius University Hospital „Santariškių clinics“, Center of eye diseases
Since 2014 – young ophthalmologist at private company „Pašilaičių JP surgery“
2014 – 2015 – young ophthalmologist at private company „Žiūra“
2011 – 2013 – physician assistant at opticians „Vision“

Other publications):

1. Asoklis R, Cimbalas A, Augyte A, Jasinskiene E, Strupaite R. Late ocular changes after closantel poisoning in five women. Eye (Lond). 2018 Aug 3. doi: 10.1038/s41433-018-0180-6.
2. Galgauskas S, Strupaitė R, Strelkauskaitė E, Ašoklis R. Comparison of IOP measurements with different contact tonometers in young healthy persons“. Int J Ophthalmol. 2016; 9(1):76-80.

3. Mockevičiūtė E, Kizlaitienė R, Budrys V, Strupaitė R, Liveikienė A, Mamontovaitė G, Cimbalas A, Ašoklis R. OCT value for multiple sclerosis patients, „Neurologijos seminarai“ (Neurology papers), 2015, Vol. 19 Issue 1, p50-58. 9p.

Other presentations:

1. Strupaitė R, Laniauskaitė I, Ašoklis R. „Knowledge and attitudes of corneal transplantation among the physicians and medical students in Vilnius“. SOE Congress, 2013, Copenhagen, Danenmark
2. Strupaitė R, Kuolienė K, Strelkauskaitė E, Ašoklis R. Stendinis pranešimas „Comparison of efficacy between focal laser hotocoagulation and photodynamic therapy in central serous chorioretinopathy treatment“. EVER Congress, 2014, Nice, France
3. Galgauskas S, Strupaitė R, Strelkauskaitė E, Ašoklis R. Stendinis pranešimas „Comparison of intraocular pressure measurements with the I-Care, Tono-Pen and Goldmann applanation tonometers including properties of the central corneal thickness in young healthy persons“. ARVO 2014, Orlando, USA
4. Paulavičienė R, Strelkauskaitė E, Strupaitė R, Cimbalas A, Ašoklis R. Stendinis pranešimas „Purtscher-like retinopathy associated with acute pancreatitis at Vilnius University Hospital Santariskiu kliniko“. EVER 2013 Congress, Nice, France

Fellowships and observerships attended:

- **2017 (1 month)** – Observership at Eye Clinic of the University Hospital Erlangen (Erlangen, Germany)
- **2017 (1 week)** – Salzburg seminars of Ophthalmology, (Salzburg, Austria)
- **2016 (1 week)** – Clinical Electrophysiology of Vision courses at Moorfields Eye Hospital (London, UK)
- **2015 (1 week)** – Venice Summer Ophthalmology School (Venice, Italy)

- **2014 (1 month)** – Observership at University Eye Hospital (Ljubljana, Slovenia)
- **2013 (3 months)** – Fellowship at Heidelberg University Clinics Eye Department (including Fellowship at David J Apple Laboratory) (Heidelberg, Germany)
- **2008 – 2009** Fellowship at Leipzig University Hospital Department of Ophthalmology (duration – 2 weeks); fellowship at Institut of Virology, Leipzig (duration - 2 weeks) (Heidelber, Germany)
- **2007 (1 month)** – Observership at Graz University Hospital Department of Ophthalmology, Austria (duration – 1 month)

Courses and international conferences attended in Latvia, Germany, France, United Kingdom, Slovenia, Italy, Austria, Danemark, Belgium, Czech Republik, Lithuania.

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2005 – 2011 Vilniaus Universitetas, Medicinos fakultetas, Medicinos studijos (2008 spalis – 2009 liepa studijų mainai pagal Erasmus programą Leipcigo universiteto Medicinos fakultete (Leipcigas, Vokietija))
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Kitos publikacijos:

1. Asoklis R, Cimbalas A, Augyte A, Jasinskiene E, Strupaite R. Late ocular changes after closantel poisoning in five women. Eye (Lond). 2018 Aug 3. doi: 10.1038/s41433-018-0180-6.
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Kiti stendiniai, e-stendiniai ir žodiniai pranešimai:

5. Strupaitė R, Laniauskaitė I, Ašoklis R. „Knowledge and attitudes of corneal transplantation among the physicians and medical students in Vilnius“, SOE Kongresas 2013, Kopenhaga, Danija
6. Strupaitė R, Kuolienė K, Strelkauskaitė E, Ašoklis R. Stendinis pranešimas „Comparison of efficacy between focal

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7. Galgauskas S, Strupaitė R, Strelkauskaitė E, Ašoklis R. Stendinis pranešimas „Comparison of intraocular pressure measurements with the I-Care,Tono-Pen and Goldmann applanation tonometers including properties of the central corneal thickness in young healthy persons“, ARVO 2014, Orlando, JAV.
8. Paulavičienė R, Strelkauskaitė E, Strupaitė R, Cimbalas A, Ašoklis R. Stendinis pranešimas „Purtscher-like retinopathy associated with acute pancreatitis at Vilnius University Hospital Santariskiu kliniko“, EVER 2013 Kongresas, Nica, Prancūzija.

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