Ph. D. Thesis

Multidisciplinary diagnostic approach and surgical ophthalmologic therapy for progressive high myopia

Noémi Széll M. D.

Szeged

2021

Introduction

Myopia has become a leading cause of blindness and therefore a serious world health issue recently. This can be attributed to its extreme phenotypes on the „upper end of the scale", namely high and pathologic myopia. Cases of high myopia with a rapid progression carry the risk of advancing into pathologic myopia, a condition that is associated with potentially blinding complications. Even with all the recent developments in many areas of ophthalmology have been encountered lately, progressive high myopia continues to remain an unjustly neglected field in many parts of the world.

Because of their markedly different visual consequences, it is indispensable to distinguish between the two main classes of myopia. Common forms account for the vast majority of the cases. These are practically simple refractive errors that may be well corrected with the conventional visual aids like spectacles, contact lenses or refractive laser surgery. Early onset progressive high myopia (eoHM), in contrast, is not simply a refractive error that can be rescued with optical lenses or refractive surgery, but is potentially a vision threatening disease.

The disparate genetic backgrounds also point to the basic differences between common versus early- onset high myopia (eoHM) forms. The manner of inheritance of the common forms is polygenic or multifactorial, i.e. both environmental factors and genetic predisposition are almost equally responsible for these cases. As opposed to this, early-onset high myopia is inherited in a Mendelian manner with one single causative, highly penetrant gene mutation, practically with minimal influence of environment or behaviour. The monogenic manner of inheritance further underlines the severity of this condition, and its specific mode of inheritance covers a wide range of forms including autosomal dominant, autosomal recessive or X-linked recessive. One of the most curious and exceptional modes of transmission is that seen for Myopia-26, displaying X-linked dominant, female-limited inheritance.

Due to an explicit increase in the prevalence of such conditions lately, an urgent need for genuine, targeted treatment in the form of gene therapy is recognized. To devise such treatment options however, we need to thoroughly understand the exact molecular mechanisms of refractive errors and myopia development. Albeit nearly 270 genes associated with myopia have been identified so far, the underlying pathways through which these genes influence refractive error development remain obscure in most of the cases. To our current knowledge, eye growth, i.e. refractive development is guided locally within the eye, and the general pathomechanism of refractive error development is assumed to be based on a retina-

to-sclera signalling cascade guided by light stimuli in the retina. Myopia genes may accordingly act at any point of this route; and dysfunction of either (disorder of retinal cell function, or signal processing as well as changes in the target tissue) may lead to the derailment of emmetropization, i.e. to refractive error development. The trait of myopia is quite complex, however, and the genes responsible for the myopic trait are accordingly also multiple. Therefore a genuine solution for treating myopia in its complexity is a challenge for the future.

At present we only have the alternative of halting the progression of high myopia in order to prevent the development of vision threatening pathological complications. Four main classes of myopia control currently exist, i.e. *pharmacological, optical, environmental/ behavioural, and surgical* options. Since the underlying causes of myopia onset and progression are diverse; the treatment approaches should likewise be combined from the different groups to reach optimum results.

Pathognomic feature of early-onset, progressive myopia is an uncontrolled, life-long elongation of the eyeball. Characteristically, the scleral tissue is biomechanically weakened in progressive high myopic eyes. As a result, the mechanical stretching and thinning of all three layers of the eye occurs along with gradual and excessive axial elongation; and this leads to the formation of vision-threatening degenerative lesions on the retina with age. Logically, the weakened scleral tissue needs to be the primary treatment target in such cases in order to retard excessive axial elongation, and to prevent the potentially blinding complications of pathological myopia. Posterior scleral reinforcement surgery was devised to this end almost a hundred years ago; and notwithstanding the novel alternative trials, this *surgical* procedure remains the only method of scleral reinforcement for now.

In our clinical practice we encounter numerous cases of early-onset progressive high myopia (eoHM). Besides providing these children with adequate optical correction for their myopic refractive error; we perform posterior scleral reinforcement in order to prevent the development of irreversible visual loss due to retinal degenerations.

In the course of this routine ophthalmological work, we found multiple interrelated patients displaying eoHM. Compilation of their pedigree revealed a family of five generations comprising numerous affected patients, all of whom are females. Assuming a monogenic trait, this pattern seemed to be indicative of X-linked heredity where the mutant allele is dominant in females, but has no penetrance in males, i.e. it is female limited. We found only a single paper describing such transmission of eoHM in three Asian families, referred to as Myopia-26.

Aims

I. Myopia-26

- To identify the causative pathogenic mutation in a family of five generations comprising numerous high myopic patients, all of whom are females.
- To explore the exact phenotype matching the identified mutation using a detailed ophthalmologic and electrophysiological testing.
- To provide hypotheses concerning the potential pathomechanism of refractive error development based on the results.

II. PSR

- To evaluate the efficacy, applicability and safety of scleral reinforcement surgery in a progressive high myopic Caucasian children cohort from Central Europe.
- To use a latest optical biometry method, based on swept source optical coherence tomography, to evaluate myopia progression in terms of the most objective parameter, i.e. axial length-changes; in order to assess the efficacy of scleral reinforcement surgery.

Patients and Methods

I. Myopia-26

Patients and Ethics

In our genetic study of eoHM we investigated a five-generation family displaying numerous affected individuals in each generation. Blood samples were taken from 18 family members representing four generations, eight of whom went through comprehensive ophthalmological and electrophysiological testing.

Written informed consent was obtained from all individual participants included. This study was approved by the National Scientific and Research Ethics Committee of the Medical Research Council of Hungary. All procedures performed were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Genetic analyses

We compiled the pedigree of the affected family, and blood samples of 18 family members were taken. Human genomic DNA was prepared from blood samples using the MagCore Genomic Whole Blood Kit. Exomes were enriched using the SureSelect XT Human All Exon + UTRs v.5 Exome Kit, and whole exome sequencing was carried out on an Illumina NextSeq500 sequencer. Raw sequence data analyses, including base calling, de-multiplexing, alignment to the hg19 human reference genome, and variant calling were performed. For variant filtration, all disease-causing variants reported in various databases (HGMD, ClinVar, CentoMD, ExAc) were considered. Extreme rare variants affecting the X-chromosome that could possibly impair the protein sequence were prioritized. The genomic segment comprising the proposed pathogenic mutation was amplified by PCR, and was submitted to Sanger sequencing for all 18 DNA samples.

Ophthalmological investigations

- Patients' own and family medical history was registered regarding other ophthalmological disorders than eoHM as well as any systemic diseases.
- Best corrected visual acuity (BCVA) was recorded (Snellen chart) and refractive error expressed as spherical equivalent (SE). High myopia was specified as SE > -6.0 dioptres (D) on at least one of the eyes.
- Slit lamp biomicroscopy with applanation tonometry and fundus ophthalmoscopy in mydriasis was carried out (Topcon SL-D701, Topcon, Tokyo, Japan).
- Digital fundus photography (TRC-501X; Topcon, Tokyo, Japan) and in some cases also ultra-wide field (200°) fundus images (Optos® California, Optos, Marlborough, MA) were taken.
- Spectral domain optical coherence tomography (Heidelberg Engineering, Heidelberg, Germany) was performed where possible.
- Axial length measurements were executed with a swept source OCT (SS OCT)- based optical biometry device (IOLMaster 700, Carl Zeiss, Jena, Germany).
- Automated kinetic full-field perimetry was carried out with Humphrey Field Analyzer (Carl Zeiss Meditec, Jena, Germany).

Electrophysiological tests

- Pattern Visual Evoked Potential (pVEP)
- Pattern Electroretinography (PERG)
- Standard Full-Field ERG
- Multifocal ERG

All electrophysiology tests were performed according to the ISCEV standards and using the Roland Electrophysiological Test Unit with the RETIport 32 software (Roland Consult, Brandenburg a.d. Havel, Germany).

Colour Vision testing

Colour vision deficiencies were assessed using the Lanthony Desaturated D-15-hue Panel tests where possible and the Isihara pseudoisochromatic plates (Isihara 24 plates edition, 2006) in the rest of the cases.

Statistical analyses of electrophysiological data

Unpaired, two-tailed t-tests were used for the statistical analysis of the measurements obtained with pVEP and PERG tests concerning the ARR3 mutant individuals as compared to healthy controls, as well as within the group of ARR3 mutant individuals. Measurements obtained with mfERG (R1, R2, R3, R4 and R5 amplitudes) concerning the ARR3 mutant individuals were compared to those of healthy controls using unpaired, two-tailed t-tests. The relative R1, R2, R3, R4 and R5 amplitudes of the ARR3 mutant individuals were compared to each other using a one-way analysis of variance test. To test the correlation between R wave amplitudes (for each ring) and BCVA or refractive error (SE), the respective parameters were plotted against each other and the Pearson product-moment correlation coefficient was calculated. To compare the extent of amplitude changes observed with pERG and mfERG within the group of ARR3 mutant individuals, an unpaired, two-tailed t-test was used.

II. PSR

Patients and Ethics

38 eyes of 32 children underwent scleral reinforcement surgery (PSR group). A control group of 14 eyes of 9 age- and myopia-matched subjects was built for comparison.

Indication for surgery (inclusion criteria) were: progressive (myopic shift $\geq$ 1D/ year), high (SE $\geq$ -6.0D) myopia with or without incipient pathological fundus alterations (META-PM stage 1-2); especially if the above criteria are associated with high anisometropia ($\geq$ 4D).

Written informed consent was signed by parents or guardians, as patients were under the age of 18. All procedures carried out were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Surgical method

Operations were carried out by two surgeons using the same method in all cases. The Snyder-Thompson simplified, single-band method was applied under general anaesthesia, and halves of a 10 mm wide (5mm) lyophilized human fascia lata band (Tutogen GmbH, Neunkirchen am Brand, Germany) were implanted to reinforce the posterior pole sclera.

Follow-up

To assess myopia progression we routinely used the relatively subjective methods of recording BCVA (best corrected visual acuity) and SE (spherical equivalent) of refractive errors. To evaluate myopia progression in a more objective way, axial lengths (AL) were measured with an optical biometry device (IOLMaster 700, Zeiss, Jena, Germany).

Statistical analyses

Changes from baseline to the end of the follow-up period within each group were analysed using one-sample or paired t-test. To assess differences between the two groups, a two-sample or group t-test was used. To identify potential correlations between different parameters the „rho" value of Spearman's rank correlation coefficient (ρ) was calculated. Numerical data are presented in the form of mean ± standard deviation (range).

Results

I. Myopia-26

Genetic analyses

To identify the causative mutation, DNA prepared from the blood samples of patients III/3 and V/8 (a male carrier and a symptomatic female, respectively) were submitted to whole exome sequencing. We identified the same variant (NM_004312.2:c.214C>T NP_004303.2:p.Arg72Ter) in the X chromosome-based ARR3 gene in both individuals in hemizygous and heterozygous form, respectively. The presence of this candidate pathogenic variant was confirmed by conventional PCR amplification and Sanger sequencing as well. Segregation of this change with the disease was assessed for all available family members. We confirmed the presence of this nonsense variant in heterozygous state in all available symptomatic female members of the family (II/1, II/3, III/8, III/13, IV/1, IV/2, IV/6, IV/7, IV/10 and IV/18). We have also confirmed the absence of this ARR3 variant from all studied asymptomatic females (IV/4, IV/13, IV/14, IV/17 and V/5). Patient V/6, a healthy male was found to carry the wild type allele (Figure 1).

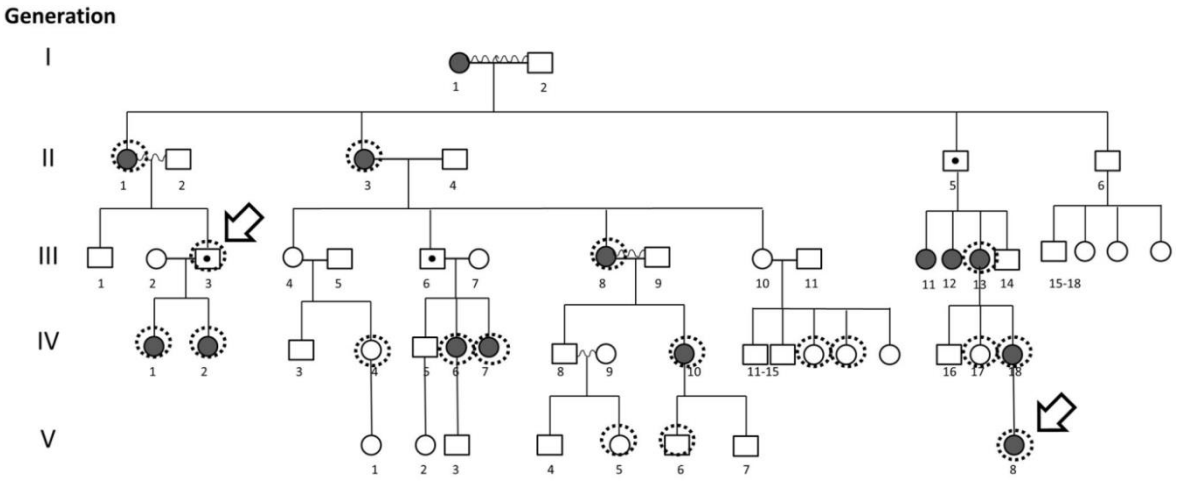

Figure 5. Pedigree displaying the X-linked dominant, female-limited heredity pattern.
Shaded colour marks eoHM phenotype. Circles mark blood sampling, arrows mark the two patients whose DNA went through exome sequencing. Dotted squares mark potential male carriers. Wavy lines mark consanguinity.

To date, this variant has not been described in the Human Gene Mutation Database, the Exome Aggregation Consortium, the Exome Sequencing Project, ClinVar or the 1000 Genome Browser. Prediction programs Polyphen2, SIFT, and MutationTaster predicted pathogenicity of the nonsense variant. Overall, these results confirmed the diagnosis of Myopia-26.

Clinical assessment

Ophthalmology findings (fundus-, OCT-, and visual field alterations) overall showed no characteristics of cone dystrophy (such as „bull's eye" appearance on the central fundus, outer retinal changes with OCT or a central scotoma with visual field testing) contrary to that expected based on X-arrestin knockout animal models. Rather they were characteristic of high myopia: META-PM1-2 fundus appearance and thinner or incipient atrophic sensory retina on macular OCT scans of patients with META-PM 1-2 fundus appearance.

The possibility of an association of POAG with high myopia in our patients also arose due to a couple IOP- and visual field- alterations; however available data do not provide sufficient and inarguable evidence to support the diagnosis of POAG at present.

Electrophysiology test results overall indicated a macular dysfunction in our patients with ARR3 mutation apparently affecting both the inner and outer retinal structures of the central retina, as opposed to a generalized cone dysfunction expected based on X-arrestin knockout animal models. These electrophysiological alterations were detected in all patients with ARR3 mutation irrespective of their affected or carrier genetic status, and at the same time showed no correlation with either the VA, SE or the age of the patients. Accordingly, these alterations are most likely attributable to the genetic defect itself, and are not secondary consequences of the high myopic refractive error.

Standard Ganzfeld ERG

Both scotopic and photopic responses were normal, indicating an overall normally functioning cone system in all affected and unaffected patients.

A general cone system dysfunction could not be evidenced in our patients with ARR3 mutation, in contrast to that seen in animal models. Taken together with the PERG and mfERG results, which were both reduced in amplitude, full-field ERGs in our patients point to a central rather than general alteration of the cone system.

Pattern VEP

P100 latency (or implicit time) was significantly increased in nearly all cases as compared to normal controls (t test: $p<0.00005$ for 60' and $p<0.00001$ for 15'). P100 implicit times to 15' stimulation were significantly more delayed than responses to 60' stimulations (t test: $p<0.001$). No significant correlation of P100 delay with either visual acuity (VA) or the refractive error (SE) could be detected for our patients.

Pattern VEP results, as evaluated together with reduced PERG and mfERG responses, reflect a central macular deficit in our patients with ARR3 mutation. Correlation could not be evidenced between patients' VA, SE, age or affected/ carrier genetic status and the pVEP results, these alterations are accordingly most probably attributable not to the patients' high myopia, but rather to the genetic mutation in ARR3 evidenced in all these patients- irrespective of their VA, SE or affected/ carrier genetic status.

Pattern ERG

Amplitudes of both the P50 and N95 waves were significantly reduced as compared to normal controls (t test: $p<0.000001$ for both). In numerous cases the amplitudes of P50 and N95 waves were reduced to the nanovolt domain, which implies extremely low or even undetectable responses. The amplitudes of P50 and N95 waves were reduced in our patients with ARR3 mutation to mean values of 29.8 % and 20.8 % of the controls, respectively, and the difference of the extent of their reduction was significant (t test: $p<0.005$). There was also a statistically significant difference between the measure of reduction in mfERG and PERG responses, i.e. the amplitudes of N95 were reduced in our patients with ARR3 mutation to mean values of 20.8 % of the controls, the amplitudes of R1, R2, R3, R4 and R5 were reduced

to an overall mean of 40.2%. The difference in the extent of their reduction was highly significant (t test: $p<1E-9$).

The significant, robust general PERG disturbance along with mfERG alterations seen for our patients with ARR3 mutation reflects a macular dysfunction. The significant discrepancy between the extent of reduction in amplitudes of the P50 and N95 waves of PERG along with the significant difference between mfERG and PERG disturbances, however (PERGs are more prominently reduced than mfERGs are) may point to a disturbance inherent also to the RGCs themselves (inner retinal, postreceptoral problem) besides a receptoral problem originating from the photoreceptor cells.

Multifocal ERG

Trace arrays with 61 hexagons were analyzed in the form of a ring analysis for our patients. In each ring (1-5) there was a significant reduction in amplitudes as compared to normal controls (t tests: $p<0.000005$ for R1, $p<0.000001$ for R2 to R5). There was no significant difference between any pairs of the individual rings in amplitude as evidenced by analysis of variance (ANOVA). There was no significant correlation between the amplitude and the patients' VA or SE within each individual ring.

MfERGs indicated a central macular deficit in our patients with ARR3 mutation along with significantly reduced PERG recordings (Figure 10). There were no spatial differences in alteration within the central 30° of the macular area as evidenced by the similarly reduced responses in rings 1 to 5. These alterations –similarly to pVEP alterations-are most probably also attributable to our patients' genetic defect (ARR3 mutation) rather than to their high myopia, as these alterations showed no correlation with either the VA or the SE.

Colour Vision tests

Colour vision test results revealed a diffuse colour vision discrimination error with no specific axis in our patients tested with the Lanthony Desaturated D-15-hue Panel test. This is again consistent with the central macular deficit suggested by the electrophysiology tests of our patients.

II. PSR

To meet ethical norms, we built no control group of fellow-eyes, rather a control group of 14 eyes of 9 age- and myopia-matched subjects was built for comparison. There were accordingly no significant differences between the two groups in respect of preoperative age, AL, SE as well as follow-up period.

In respect of mean annual change of AL and SE there were significant differences ($p=0.002$ and $p=0.001$; respectively) encountered between the PSR and control group, demonstrating a significantly lower rate of myopia progression in the PSR than in the control group over the whole follow-up period as well.

BCVA improvement was 0.15±0.09 (range: 0-0.5) on average during the overall follow-up period in the PSR group, whereas it was overall practically negligible in control eyes (0.01±0.1, range: 0-0.2). It is of note that the extent of BCVA improvement was even more explicit in the six amblyopic eyes operated: 0.35±0.12 (range: 0.2-0.45).

We also strove to identify factors that could have an influence on the extent of myopia progression (AL- and SE-change). We found, however, that neither the number of follow-up years, nor the age at the time of surgery correlated with either the extent of axial elongation (ρ: -0.373, $p=0.072$ and ρ: -0.231, $p=0.277$, respectively) or the extent of myopic shift of SE (ρ: -0.031, $p=0.886$ and ρ: -0.089, $p=0.678$, respectively). Here, the correlation coefficient was considered as clinically significant at the level of $p<0.05$. At the same time, as could be expected, the preoperative age showed correlation with both the baseline axial length and subjective myopic refraction error (SE): ρ: 0.819, $p=0.001$ and ρ: 0.689, $p=0.001$; respectively. Here, the correlation coefficient was considered as clinically significant at the level of $p<0.01$.

As for the adverse events, conjunctival chemosis was encountered in all patients, and diplopia in three cases as mild and transient consequences of the surgery; whereas no rejection of the transplanted material or any other severe, lasting complication such as IOP elevation, optic nerve compression, retinal detachment or retinal haemorrhage could be observed.

Discussion

I. Myopia-26

Due to the increasingly overwhelming socioeconomic burden posed by early-onset progressive high myopia globally; an urgent need for targeted treatment options besides the longer introduced myopia control options has also been recognized lately. To devise such treatment potentials, however, we need to identify further genes responsible for the disease, as well as to get deeper insight into their specific roles in the pathogenesis of refractive error development. Our genetic study of Myp-26 is a step forward in these fields.

We report a family displaying a heritable form of eoHM, where the disease is manifested only in females. Compilation of the pedigree permitted the identification of carrier males, and revealed that their female offspring is exclusively affected, which suggested an X-linked dominant, female-limited inheritance. We carried out whole exome sequencing of two individuals, which indeed revealed a nonsense-mutation within the coding region of a gene on the X-chromosome, namely ARR3. Sanger sequencing of the respective locus in a total of 16 female family members unveiled a perfect correlation between the presence of the mutant allele and the high myopia phenotype. This is the first report of a mutation in ARR3 causing hereditary eoHM, called Myopia-26 in a Caucasian family. In the three Chinese families that have been reported earlier to display a similar, X-linked dominant, female-limited transmission of eoHM; the ARR3 was found to carry c.893C>A (p.Ala298Asp), c.298C>T (p.Arg100*) and c.239T>C (p.Leu80Pro) mutations, respectively. The mutant allele identified in our study (c.214C>T, p.Arg72*) is therefore novel. The earlier publication on Myopia-26 lacked a detailed phenotypic description of the patients, and did not attempt to explain the pathomechanism of the disease. Our main goals from this point onwards were therefore to carry out a thorough ophthalmologic investigation of the family and use the acquired information, along with literature data to build reasonable hypotheses on the molecular mechanism of pathogenesis.

ARR3 encodes a 388 amino acid-long visual arrestin with multiple names (Arrestin 3, Arrestin 4, Cone-arrestin, Retinal cone arrestin-3, X-arrestin), we refer to it as X-arrestin. Besides its key role in the phototransduction process in retinal cones, it is also expressed in pinealocytes of the pineal gland. Arrestins make up an important family of proteins, with the primary function of desensitizing phosphorylated G-protein coupled receptors (GPCRs). Our present knowledge about arrestins, along with the electrophysiology test results in our patients

affected with ARR3 mutation; permitted the formulation of two hypotheses, i.e. the cone- and the ganglion cell hypotheses, respectively to explain the development of myopia.

The **cone-hypothesis** assumes that an X-arrestin defect would lead to a selective (red-green) cone dysfunction which would mean their increased activity, and the "sensitization" to red/green visual stimuli. Due to the physical phenomenon of chromatic aberration this in turn would lead to increased accommodation, and a hyperopic retinal defocus. These alterations have been shown to provoke ocular elongation in numerous animal studies. Briefly, since blue light is claimed to have a protective effect against myopia, the relative weakening of the blue light stimulus upon the loss of X-arrestin can explain the eventual development of myopia in these patients. The selectively altered function of various cone types, however, cannot be tested with standard photopic 3.0 ERGs. Photopic 3.0 ERGs indeed, were normal and showed no alteration in our patients, reflecting an overall normally functioning cone system. L, M and S-cones responses can be isolated electrophysiologically by recording the light adapted ON/OFF-ERG and the S-cone ERG, neither of which has been available in Hungary at the time of our investigations.

Our **ganglion cell-hypothesis** attributes the development of refractive error to the dysfunction of a subset of the retinal ganglion cells (RGC), called intrinsically photosensitive retinal ganglion cells (ipRGCs); for pattern ERG test results in our patients indicated a postreceptoral (inner retinal) dysfunction besides the primary photoreceptoral disturbance due to the ARR3 mutation. Apart from their primary role of transmitting visual information from photoreceptors to higher cerebral visual centers ("image forming pathway"), ipRGC cells also contribute to the signal transduction of the so-called „non-image forming" pathway. Both these pathways play key roles in the emmetropization of the eye, and a disturbance of either may lead to the development of refractive error.

The "image forming pathway" contributes to emmetropization under physiological circumstances by the anti-myopic effect of blue light stimuli through imposing myopic defocus on the retina (as mentioned before in context with the cone hypothesis); and also by acting against myopia development through a dopamine-mediated pathway.

The non-image forming visual functions of ipRGCs, such as circadian rhythm photoentrainment also play an important role in eye development. As their name suggests, ipRGC cells can detect light directly through their photosensitive protein called melanopsin. IpRGCs and melanopsin mediate circadian cycles both endogenously in the retina and via a systemic route.

Currently, the most obviously missing piece of both the cone- and the ganglion cell-hypothesis is the cause of RGC dysfunction displayed on the PERG recordings, as ARR3 expression in RGCs was not detectable in mice. However, the promoter of the human ARR3 and its murine orthologue are markedly different, which may result in disparate cell type specific expression as well. Another possibility would be the secondary malfunction of RGCs, resulting from the altered activity of pinealocytes. This could be mediated by the humoral control of retinal dopaminergic transmission by the pineal gland (described above), or the direct effect of melatonin on RGCs via their MT1 and MT2 melatonin receptors.

Another major shortcoming of both the cone- and the ganglion cell hypothesis is the lack of explanation for the female-limited heredity pattern of myopia. It is especially curious that the central macular dysfunction seems to be present also in males, without leading to eoHM. We assume the presence of a "rescue mechanism" in males, or in other words, the lack of a pathological process that would lead to an axial length elongation in response to the central retinal dysfunction. Sex-dependent differences in retina function have been described in mice, and the risk of certain retinal diseases have been shown to be sex hormone-dependent in humans. Further physiology and molecular biology studies are required however to unveil the exact mechanisms responsible for the observed female-limited phenotype. Such research may also shed light on why the mutant allele is dominant in females.

Additional investigative measures are needed to confirm our hypotheses, such as i) cone-specific ERGs (S-cone ERGs and ON/OFF ERGs) to isolate individual (L, M, or S) cone responses, and thus support or exclude our selective cone dysfunction hypothesis; ii) post-illumination pupil response (PIPR) to test melanopsin expressing ipRGC function [59], and thus shed light on the extent of ipRGC damage; and iii) long-term follow-up to reveal any evidence of progression of glaucoma parameters (IOPs, visual field defects, optic nerve head appearances and RNFL OCTs) that could also be expected in glaucoma.

Evidencing the existence of the Myopia-26 in the Caucasian race for the first time through genetic testing along with providing thorough phenotypic characterization of the disease inarguably brings us one step closer to understanding the molecular development of myopic refractive error.

II. PSR

As long as a targeted treatment potential for progressive high myopic patients is not available, we need to rely on myopia control options in order to possibly save these patients from the vision-threatening complications of pathological myopia. Pathognomic feature of early-onset, progressive forms is an uncontrolled, life-long elongation of the eyeball. Posterior scleral reinforcement surgery was developed to strengthen the weakened scleral tissue. We have been applying this surgical procedure successfully in our clinical practice for 30 years now.

The availability of PSR surgery has become rather limited worldwide by now, however. One of the main reasons for this might be the scarcity of convincing evidence supporting its long-term efficacy. It is not simple to make a comparison between different trials, due to the great variability in study designs. Firstly, inclusion criteria already vary among different studies. Some authors leave this procedure mostly for adult cases where pathological alterations and visual loss have already been encountered. In our clinical practice, however, similarly to others, we most widely typically operate children with progressive high myopia, as the goal of the surgery would be to stop axial elongation yet before the onset of degenerative lesions and severe visual impairment.

Reason for the disregard of the surgery might be that several authors claim it to be difficult to learn and execute, as well as to provoke severe adverse events. In our clinical practice, however, the Snyder-Thompson procedure has been proved to be relatively simple and well applicable, and also safe at the same time. Experienced professionals are nevertheless of great value to learn special maneuvers to execute the surgery successfully. Safety of the procedure is supported by our results, inasmuch as only the usual mild and transient consequences of the surgery, such as conjunctival chemosis and mild diplopia occurred in our patients postoperatively, whereas no other severe, lasting complications were encountered. Besides the sort of technique and surgical expertise, a good choice of supporting material is also indispensable for PSR surgical success. Materials with high collagen fiber content provide the best support. We used allograft fascia lata preparations in our PSR study.

The Snyder-Thompson posterior scleral reinforcement procedure also proved to be effective in our clinical practice. Myopia progression is best reflected by the changes of two parameters: the myopic shift of the refractive error (i.e. spherical equivalent of spectacle diopter) and more objectively the increase in axial length, i.e. axial elongation. In order to assess the efficacy of the surgery we evaluated the changes of these two parameters in the

operated eyes as compared to a control group. As opposed to previous studies, we laid great emphasis on the accuracy, reliability and comparability of AL measurements in our study; and consequently used the exact same, highly reliable device availing an optical biometer (IOLMaster 7000) to achieve this goal. Our results evidenced the efficacy of the surgery, i.e. the myopic progression was significantly lower over the follow-up period in the non-operated control eyes as compared to the operated eyes.

Although it is no primary goal of the surgery to improve visual acuity, quite a few patients clearly experienced an improvement in their eyesight after surgery. This finding is in accordance with other reports, and two possible explanations for this may exist. Firstly, photoreceptor cells get closer to each other i.e. the “minimum separabile” decreases due to the relative tightening effect of the implanted band. Secondly, the blood supply of the macular region is improved owing to the mechanical stimulus of surgical manipulation. Of note is that the extent of BCVA improvement encountered was even more substantial in our six amblyopic cases. It is therefore especially important to consider PSR surgery in anisometropic high myopic cases as early as possible, before amblyopia is finalized in such eyes in the lack of adequate intervention

To sum it up, we think that supporting the posterior sclera surgically in progressive high myopia is proved to be an effective and safe procedure in our clinical practice, in agreement with former as well as current international trials.

Summary of new results

- Myopia-26 or female limited early onset high myopia is the first human disease associated with ARR3. Prior to our study, it has been described only in three Asian families. Using whole exome sequencing, we identified the pathogenic mutation of the female-limited early onset high myopia observed in our patients, i.e. evidenced the existence of the disease in the Caucasian race for the first time.

- Previously, the disease has not gone through detailed investigation concerning collateral symptoms. The present study is the first to carry out phenotypic characterization, i.e. a thorough ophthalmological and electrophysiological testing in humans. ARR3 has been investigated phenotypically only in animal models previously, where a generalized cone dysfunction was suggested according to the accomplished electrophysiological testing. In our study, however, we could not evidence a generalized cone dysfunction but rather a central macular dysfunction affecting both the inner and outer (postreceptoral and photoreceptoral) retinal structures attributable to ARR3 mutation.

- This study is also the first to offer potential mechanisms explaining the pathogenesis of this disease. Electrophysiology test results in our patients suggested two hypotheses, i.e. the ganglion cell- and the cone hypotheses for myopia development.

- The application of PSR surgery has become rather limited globally by now, and the epicentre has also been shifted from the former Soviet Union, Central Europe and the United States to East Asia for today. In Hungary, we are in a unique position to apply this surgical technique at present; therefore our results practically cover the Hungarian results with PSR surgery. Our work also remedies the paucity of international results in this field.

- Our study is the first to assess efficacy of PSR surgery in terms of axial length changes measured with a latest method of optical biometry, i.e. a swept source OCT. We evidenced the rate of axial elongation to be significantly lower after an average of 3.4 years after PSR surgery in the oprated eyes as compared to the control group.

Acknowledgements

First I would like to express my sincere gratitude to my supervisors, Prof. Dr. Andrea Facskó and Dr. Zoltán Sohajda for inspiring my clinical and scientific interest from the very beginnings, furthermore for guiding and supporting me all the way through my work, and providing the all-important professional and institutional backgrounds to conduct my study.

I also wish to convey my profound thanks to my collaborator and co-author in the clinical genetic study, molecular biologist Dr. Tamás Fehér, who made this multidisciplinary research possible. I am most grateful for his fundamental and unfailing assistance and help throughout this fruitful cooperation.

I have great pleasure in thanking my colleague, Dr. Adrienn Boross for her amazing professional and altruistic human guidance and enthusiasm to teach me the almost forgotten, by now exceptional surgical technique, which served as the starting point of this whole study.

I am grateful to Prof. Dr. Márta Janáky and Dr. Zsuzsanna Z. Orosz for rendering their valuable help with the electrophysiology examinations.

Mapping and compiling the pedigree serving for the basis of the genetic study are due to Gabriella Őrsy and Ibolya Lakatos, whereas drawing the schematic figures of the surgery is owing to Adrienn Sallai.

I am also much obliged to Dr. Mirella Telles Salgueiro Barboni, whose help proved to be indispensable in interpreting our electrophysiology test results. I learned a lot from her in this field that was completely unknown to me earlier. Her person did not only reveal a devoted scientist, but also awarded me a very precious friend.

My work was followed with attention and supported from the beginnings by Dr. Ágoston Tóth, former member of Debrecen University's Centre for Informatics and Computing and current instructor of the Institute of English and American Studies. I am indebted to him for his indispensable and kind support.

Last, but not least I owe my dearest family the debt of gratitude for their endless support and patience as well as for providing me the peaceful background for my work. They placed trust in my final success all the way long. I am happy to earn their trust now with this thesis.

Publications

List of full papers related to the subjects of the Thesis

1. **Széll N,** Boross A, Sohajda Z. Scleramegtámasztás progresszív, nagyfokú myopiában. (Scleral reinforcement surgery in progressive, high myopia.) Szemészet 2019; 156:39-45.

2. **Széll N**, Boross A, Facskó A, Sohajda Z. Results with Posterior Scleral Reinforcement for Progressive Highly Myopic Children in Hungary. Klin Monatsbl Augenheilkd 2021; 238:1-9. **IF:0.605**

3. **Széll N**, Fehér T, Maróti Z, Kalmár T, Latinovics D, Nagy I, Orosz ZZ, Janáky M, Facskó A, Sohajda Z. Myopia-26, the female-limited form of early-onset high myopia, occurring in a European family. Orphanet J Rare Dis 2021; 16:45. **IF:3.71**

List of full papers unrelated to the present Thesis

4. Tsorbatzoglou A, Németh G, **Széll N**, Bíró Z, Berta A. Anterior segment changes with age and during accommodation measured with partial coherence interferometry. J Cat Refract Surg 2007; 33:1597-1601. **IF:3.021**

5. **Széll N**, Kiss M, Sohajda Z. Kontrasztérzékenység vizsgálata két különböző aszferikus műlencse esetén. Szemészet 2007; 144:201-205.

6. **Széll N**, Sohajda Z. Toricus műlencsékkel szerzett tapasztalatok. Szemészet 2012; 149: 24-29.

7. **Széll N**, Sohajda Z. HP Guar-tartalmú, műkönny hatása phacoemulsificatiót követő szemszárazság esetén. Szemészet 2012; 149: 280-284.

8. Sohajda Z, **Széll N,** Hayfron J, Facskó A. Modified phacoemulsification in oculocutaneous albinism to reduce photophobia. Spektrum Augenheilkd. (2020) https://doi.org/10.1007/s00717-020-00452-8. **IF:0.04**

9. Sohajda Z, **Széll N**, Revák Á, Papp J, Tóth-Molnár E. Retinal Nerve Fibre Layer Thickness Change After CO2 Laser-Assisted Deep Sclerectomy Surgery. Clin Ophth 2020; 14: 1749-1757. **IF:2.04**

List of presentations and abstracts related to the subjects of the Thesis

1. **Széll N**, Boross A, Sohajda Z. Scleramegtámasztás- a múlt és a jelen a Kenézy Kórházban. Magyar Szemorvostársaság 2012. évi Kongresszusa, Siófok 2012.06.07-09. Szemészet 2012; 149 (Supplementum I.): p. 63.

2. Facskó A, Deák A, Berkes Sz, **Széll N**, Sohajda Z. Extraokuláris műtétek gyermek és fiatal felnőttkorban- régi és új megoldások. Kurzus. Magyar Szemorvostársaság 2015. évi Kongresszusa, Pécs 2015.06.18-20. Szemészet 2015; 152 (Supplementum I.): p. 79.

3. **Széll N**, Fehér T, Sohajda Z, Facskó A. Izolált, nem szindrómás nagyfokú myopia halmozott esetei és scleramegtámasztás két magyarországi családban. Magyar Szemorvostársaság 2017. évi Kongresszusa, Szeged 2017.06.22-24. Szemészet 2017; 154 (Supplementum I.): p. 51.

4. **Széll N**. Results with Snyder-Thompson Posterior Scleral Reinforcement in Progressive High Myopic Children in Hungary. The 17th International Myopia Conference, Tokyo, Japan 2019.09.12-15. Book of Abstracts p. 66.

5. **Széll N**, Fehér T, Maróti Z, Kalmár T, Latinovics D, Nagy I, Orosz ZZ, Janáky M, Facskó A, Sohajda Z. Egy familiáris, nőkre korlátozódó, progresszív miópia genetikai vizsgálata és potenciális patogenezise. Genetikai Műhelyek Magyarországon, XIX. Konferencia, Szeged 2020. szept.11. p. 5.

List of presentations and abstracts unrelated to the present Thesis

6. **Széll N**, Nagy V, Damjanovich J, Berta A. Differenciáldiagnosztikai kérdések leukémiás beteg látásromlása esetén. Magyar Szemorvostársaság 2006. évi Kongresszusa, Sopron 2006.06.15-17. Szemészet 2006; 143 (Supplementum I.): p. 17.

7. **Széll N**, Kiss M, Sohajda Z. Contrast sensitivity and glare disability with two different types of aspheric intraocular lenses. XXVI. Congress of the ESCRS, Berlin 2008.09.13-17. Book of Abstracts p. 150.

8. **Széll N**, Káldi I, Deák T. Diabeteses maculaödéma kezelése- korszerűen (egyszerűen). Magyar Szemorvostársaság Retina Szekciójának 2017. évi Kongresszusa, Eger 2017. 11. 24-25. p. 45.

9. **Széll N**, Revák Á, Sohajda Z. Kombinált HIV- és lues-fertőzés következtében kialakult ocularis syphilis. Magyar Szemorvostársaság 2019. évi Kongresszusa, Szeged 2019.05.30.-06.01. Szemészet 2019; 156 (Supplementum I.): p. 74.

10. **Széll N**, Sohajda Z. Retinális és chorioideális keringési paraméterek OCT-angiográfiás vizsgálata nagyfokú myopiás gyermekekben. Magyar Szemorvostársaság Retina Szekciójának 2019. évi Kongresszusa, Győr 2019. 11. 22-23. p. 42.

Scientometric parameters:

Full papers:	9
first author:	6
co-author:	3
Cumulative impact factor:	9.461