Clinical and genetic examinations of progressive photoreceptor dystrophies

PhD thesis

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Introduction

Inherited retinal dystrophies (RD) are a group of rare heterogenous degenerative diseases of progressive nature and primary involvement of photoreceptor morphology and function. In advanced stages there is widespread remodeling in the retinal structure with consequential severe dysfunction. Regarding onset, genetic background, pathomechanism and clinical symptoms RD-s vary in many ways. The main symptoms are mostly characterized by rod dysfunction but later on, there is always cone involvement (rod-cone disease or „retinitis pigmentosa") (RP, OMIM: 268000), as well. The most severe form of retinal dystrophies, Leber congenital amaurosis (LCA, OMIM 204000) causes symptoms earlier than one year of age and was first described by Theodor Leber in 1869. A distinct form with slightly less severe symptoms and onset in childhood, mostly earlier than 10 years of age is called early onset retinal dystrophy (EORD)**.** EORDs have distinct genetic background compared to classic LCA.

The worldwide prevalence of RP is 1:4000 with around 2500 affected Hungarian patients, while the worldwide prevalence of LCA-EORD is around 1:50-80000. The number of Hungarian LCA-EORD patients is around 125-200. LCA-EORD takes approximately 5-7% of all RD cases.

Some forms of RP may affect several members in families, while other forms are sporadic. Due to the development in genetic diagnostics the number of identified RP genes is nowadays more

than 300, but genetic tests are to date not a part of routine examinations in RP.

LCA-EORD show mainly an autosomal recessive inheritance trait with usually two symptomless parents having an affected child. The first LCA-associated gene *(retinal guanyl cyclase2D, GUCY2D)* was identified by French researchers in 1996. Today the number of LCA-EORD associated genes are more than 20. Unlike in RP, genetic tests are available and are a part of an accurate diagnosis for LCA-EORD patients.

Late stages of RP are characterized with diffuse retinochoroidal atrophy, pigmentary deposits involving the macular area, attenuated vessels and atrophic optic discs. Some patients lose central acuity, as well. For these patients the quality of life is highly influenced by the presence or lack of the morphological and functional integrity of the macula lutea.

The first histopathological alteration in RP retinas is the shortening of rod outer segments and thinning of the outer nuclear layer with loss of cell nuclei, independently form inheritance trait and age of onset.

Optical coherence tomography (OCT) was introduced in ophthalmological diagnostics more than twenty five years ago, providing the first possibility to examine retinal structure in vivo. Due to the technical improvements in the last two decades, the latest equipment have a minimal resolution of approximately 5-7 microns which is almost equal to that of histopathological methods.

One of the aims of my work was to examine macular morphology in advanced RP patients with the assumption that the information of topographic intraretinal thickness changes may help in optimal patient selection for retinal implantation procedures in the future.

Hungarian LCA-EORD patients have not been examined earlier with modern clinical and genetic methods. My second aim was to collect these patients and perform clinical and genetic tests on them.

Retinal dystrophies are not treatable yet, yet there is intensive ongoing international research to develop new therapies such as gene therapy and stem cell therapy to which our studies may hopefully also contribute.

Aims

Alterations in the fine macular morphology can be revealed by segmentation analysis of OCT images, while function can be assessed by multifocal ERG recordings. Previous literature data report about the thinning of the outer nuclear layer (ONL), but the intraretinal layer thickness changes in relation to excentricity and function were not examined in vivo earlier.

In LCA-EORD patients some genotype-phenotype correlations were reported as multiplex yellow deposits in some genetic subtypes, while in others intraretinal pigmentation in early childhood. The presence or lack of maculopathy is also associated with some genotypes.

Based on the above we had the following aims in our work:

1. Retinitis pigmentosa patients

As RP is primarily the disease of the peripheral retina and according to histological findings primarily the outer retinal layers are involved, it is likely that the peripheral macular region is more affected compared to foveal and perifoveal regions.

Our aim was to examine:

- Whether there was a demonstrable difference in changes of the intraretinal layers' thickness according to excentricity (foveal, central, paracentral and pericentral macular regions) measured

by segmentation analysis of the OCT images. (Does thinning in the **peripheral** macular layers exceed that in the **central** macula?)

- Whether thickness alterations of the **outer** retinal layers are more pronounced than those of the **inner** retinal layers in the same region?
- How the thickness changes of macular layers relate to retinal function? Is there a difference in intraretinal layer structure between eyes with detectable and extinguished central multifocal ERG responses?

2. LCA-EORD patients

Aims:

- To describe our patients' phenotype;
- To identify patients genotype, to reveal pathogenic mutations;
- To analyze the effect of identified mutations and to reveal allele frequency of previously known mutations in the given genetic LCA-EORD subtype;
- To evaluate the effect of novel mutations using „in silico" predilection software;
- To compare geno-phenotype correlations of Hungarian LCA-EORD patients with literature data;
- OCT analysis of our LCA-EORD patients using a Spectralis SD-OCT-device.

Methods

Retinitis pigmentosa patients

Patients diagnosed with RP and who had received both OCT and mfERG at the same visit between November 2006 and March 2010 at the Department of Ophthalmology, Semmelweis University, Budapest, Hungary, were retrospectively reviewed. Exclusion criteria were the presence of any other ocular or optic nerve disease, including glaucoma or of any systemic disease other than controlled hypertension. Exclusion criteria based on OCT imaging were the following: cystoid macular edema, with or without epiretinal membrane formation, a low signal strength of the OCT images and foveal decentration. Twenty-nine eyes of 22 from 57 RP patients were included (16 males and 6 females, median age: 32 years; range: 14 to 63 years). Diagnostic criteria of RP included progressive night blindness and visual field constriction, a rod–cone pattern of ERG abnormality, atrophic optic discs, and intraretinal bone spicule pigmentary depositions.

For the *OCT control group* 17 eyes from 17 age-matched controls were randomly selected from the normative database (median age: 31 years; range: 21 to 59 years). Eligibility criteria for control subjects were best-corrected Snellen visual acuity (VA) of 20/20 and the lack of any ophthalmic, neurologic, or systemic diseases. All control subjects gave informed consent and the study conformed to the tenets of the Declaration of Helsinki. No Institutional Review Board approval was required for the study.

LCA-EORD patients

Fourteen patients (8 male and 6 female) with the clinical symptoms of LCA-EORD were examined at the Department of Ophthalmology, Semmelweis University Budapest between 2005 - 2015. The age of our patients ranged between 6 months and 25 years at the first visit in our Department. Diagnosis of LCA-EORD was established on the basis of severe visual impairment since birth or early infancy, nystagmus and lack of fixation ability and residual or extinguished full-field ERG responses. Genetic tests were performed in ten patients. Detailed clinical examination and positive genetic results with identified biallelic pathogen mutations resulted in six patients.

Clinical evaluation

Routine ophthalmological examinations and full-field electro-retinography (ff-ERG) (Retiscan Roland Consult GmbH, Wiesbaden, Germany) were performed in both RP and LCA-EORD patients. Retinitis pigmentosa patients underwent multifocal electro-retinography examinations (mf-ERG), as well. (Retiscan, Roland Consult GmbH, Wiesbaden, Germany). OCT was performed using a time-domain(TD)–OCT device (Stratus OCT; Carl Zeiss Meditec, Dublin, CA). Each eye was scanned using the "macular thickness map" protocol, The raw OCT data were exported from the device and further processed by our custom-built optical coherence tomography retinal image analysis (OCTRIMA) software.

The thickness values for the retinal nerve fiber layer (RNFL), ganglion cell layer and inner plexiform layer (GCL+IPL) complex,

inner nuclear layer and outer plexiform layer (INL+ OPL) complex, outer nuclear layer (ONL), and the total retina were recorded for each eye in each Early Treatment Diabetic Retinopathy region. The intraretinal layers in various eccentricities from the fovea were assessed by calculating the mean thickness of the layers for the central (R1), pericentral (R2–R5), and peripheral (R6–R9) ETDRS regions. The central ETDRS subfield corresponds mainly to the central hexagon area on mf-ERG, the pericentral subfield corresponds mainly to the second ring of hexagons and the paracentral subfield corresponds to the third ring of hexagons on mfERG, which allows an assessment of layers thickness values according to both excentricity and function.

Based on mf-ERG responses the 29 eyes were divided into two groups. One with residual (detectable retinal function, DRF, n=15) and one with extinguished (no central retinal function, NCRF, n=14) function. As controls, data of 50 age-matched healthy individuals were selected from the Electrophysiological Laboratory of our Department.

Intraretinal layer thickness values, total retinal thickness and LogMAR visual acuity were measured in all three groups (DRF, NCRF, control).

LCA-EORD patients underwent retinal fundoscopy and photo-documentation (Topcon TRC 50IX retina camera, IMAGEnet 2000 system software, Tokyo, Japan) and OCT imaging by using an SD-OCT device (Heidelberg Spectralis OCT, Heidelberg, Germany).

Genetic analysis

DNA was extracted from peripheral blood in Budapest, Hungary, all further molecular genetic tests were performed by Asper Ophthalmics, Tartu, Estonia. DNA samples were PCR amplified, amplification products were concentrated, purified and used in an LCA genotyping microarray (APEX method). Slides were imaged (Genorama Quattro Imager Detector 003, Genorama Ltd, Tartu, Estonia) and analyzed with Genorama genotyping software (Asper Biotech, Tartu, Estonia). All results were validated by direct sequencing using standard protocols. One patient underwent targeted next generation sequencing (NGS) in order to identify the second mutation in the CEP290 gene. (Illumina kit, TruSight one panel on a Miseq platform, Illumina, San Diego, CA, USA)

Statistical analysis

To compare examination results between RP patient groups and controls, a mixed-model ANOVA test was used with Newman–Keuls post hoc test. Linear correlation analysis was performed to assess the correlation between logMAR visual acuities and the thickness of the intraretinal layers. Statistical analyses were performed using statistical software (SPSS 15.0; SPSS Inc., Chicago, IL; and Statistica 8.0 Software; Statsoft Inc., Tulsa, OK). Because of the number (n=14) of comparisons, Bonferroni adjustment was performed for the level of statistical significance, which was set at $P \leq 0.0036$.

Results

Retinitis pigmentosa patients

1. Alterations of intraretinal layer thicknesses according to excentricity

- The thickness of ONL showed no alterations in the foveal region, in the DRF group compared to the control group; however, it was significantly thinner in the pericentral region.
- Likewise, in the pericentral region the thickness of the GCL+IPL complex did not show significant changes in the DRF group compared to the control group; nevertheless, it was significantly thinner in the peripheral region.

2. Alterations in the intraretinal layer thickness according to function

- In the foveal region the thickness of the ONL was significantly thinner only in the group with extinguished function compared to the control group, but there was no thinning in the DRF group.
- Similarly, in the pericentral region, the thickness of the inner retinal layers (INL+OPL and GCL+IPL) was significantly thinner compared to the control group only in the NCRF group, while it did not differ significantly in the DRF group.

3. Relationships of the thickness value changes in the outer and inner retinal layers

- In the pericentral region, the thickness of the ONL was

significantly decreased in both (DRF and NCRF) patient groups compared to the controls. On contrary, the thickness of the INL+OPL and GCL+IPL layers showed no thinning in the DRF group, only in the NCRF group.

- In the peripheral region, the thickness of ONL was significantly thinned in both patient groups, similarly to that in the pericentral region. On contrary, the thickness of the INL+OPL layers did not show any changes compared to the control group.
- In the peripheral macula, the thickness of GCL+IPL complex was significantly decreased in both patient groups, likewise the thickness of the ONL.

LCA-EORD patients

Clinical examinations

In seven patients a detailed clinical examination and high quality fundus photodocumentation was done; in six of these cases genetic tests with detection of biallelic mutations were performed. The visual performance (logMAR 2.0-3.0 – hand motion-counting fingers) of four patients (two with AIPL1, one with CEP290, and one with non-identified genotype) corresponded to the classic LCA, while the logMAR scores (logMAR 0.7-1.4) of the three other patients were typical for EORD. The Gf-ERG responses were extinguished in six cases and residual in one patient. In two cases, there was a pigmentary maculopathy and midperipheral hyperpigmentation with apparently normal optic discs and vessels of normal diameter. According to literature data these alterations are typical for the

AIPL1 genetic subtype. In three patients there were diffusely scattered small yellowish deposits, nummular pigmentation of different sizes, and nonpigmentary maculopathy (typical for CRB1 genetic subtype). One patient showed confluent white deposits in the midperiphery without any other apparent retinal changes (typical for CEP290 genetic subtype). In one case, we found intact macula and optic disc with peripheral diffuse salt and pepper pattern. In three cases, we detected the diffuse thickening of the extrafoveal retina with SD-OCT.

Genetic analysis

We identified six different pathogenic mutations in three LCA-EORD genes in six patients: in two patients the **c.834G>A (p.Thr278X)** alteration in homozygous form in the *AIPL1 gene,* in three patients the **c.2843G>A (p.C948Y)** mutation in homozygous or compound heterozygous form in the *CRB1 gene.* Two further variants (**c.2536G>T (p.G846X** and **c.2555T>C (p. Ile852Thr)** were identified in the *CRB1 gene.* In one patient two pathogenic variants (the **c.2991+1655A>G (p.C998X** and the **c.4929delA (p.Lys 1643fsX2)** were detected in the *CEP290 gene*.

Conclusions

The present study correlates retinal structure and function in patients with RP using multifocal electroretinography and optical coherence tomography. The thickness changes of the macula were assessed according to different excentricity and function in advanced stages of the disease. To our knowledge, there were no similar studies reported previously.

1. We concluded that the thickness alterations of a certain layer seems to be more pronounced in the pericentral and peripheral macular regions than that in the central regions. Furthermore, thinning of a layer is already detectable in the DRF group of patients in more peripheral macular regions. It confirms the statement that the thinning of the layers is significantly related to the excentricity in advanced RP.

2. Thickness changes of retinal layers were significantly different between patient groups with preserved and extinguished responses which was more pronounced in the latter group in the macular region.

3. We confirmed that thinning in the outer retinal layers exceeds alterations in the inner layers. These results are in accordance with the histopathological data reported in the literature.

The other aim of my work was to perform detailed examination of Hungarian LCA-EORD patients. Our study provided the first clinical and genetic information regarding this special group of patients in

Hungary and Central Europe.

1. Seven patients with the clinical diagnosis of LCA-EORD underwent molecular genetic screening, among them biallelic mutations were detected in six cases, confirming the clinical diagnosis in all cases.

2. A detailed phenotypic description and photodocumentation was performed and compared to literature data. All the six patients showed similar phenotypes as previously reported.

3. The genes identified in Hungarian LCA-EORD patients were *AIPL1, CRB1* and *CEP290*, all of them are among the earlier most frequently found genes in the Caucasian population.

4. In two Hungarian patients two novel mutations were detected, c.G846X (p.2536GT) in the *CRB1 gene* and the c4929delA (p.lys1643fsX2) variant in *CEP290 gene.*

5. The previously reported mutations identified in our LCA-EORD patients, as **c.834G>A (p.Thr278X)** in the *AIPL1 gene,* **c.2843G>A (p.C948Y)** in the *CRB1 gene* and **c.2991+1655A>G (p.C998X)** in the *CEP290 gene,* have the highest allele frequency in all three given genes.

6. Pathogenicity of the novel mutations was assessed by using the "Mutation Tester" predilection software, which predilected the truncation of the affected protein in both cases.

7. In three EORD patients a diffuse thickening of the extrafoveal retina was revealed, all patients had CRB1 gene associated disease. These data confirm earlier reported findings in the literature.

Modern clinical and genetic examination methods made possible to gain basic new knowledge regarding pathomechanism, retinal remodeling and genetic background in retinal dystrophies.

I do hope that my work contribute to gain access to modern diagnostic tools for Hungarian patients with retinal dystrophy, which is a basic condition in choosing adequate therapy, hopefully in the not too distant future.

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