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Altered visual functions, macular ganglion cell and papillary retinal nerve fiber layer thickness in early-treated adult PKU patients

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ABSTRACT

Purpose: Retinal changes are poorly described in early treated phenylketonuria (ETPKU). We aimed to investigate possible visual functional and ocular microstructural changes in adult patients with ETPKU. Optical coherence tomography (OCT) and its angiography (OCTA) data from patients with PKU were compared to healthy controls.

Methods: In this prospective, monocentric, cross-sectional, case-control study 50 patients with ETPKU and 50 healthy subjects were evaluated with OCT and OCTA. Measurements were performed on right eyes. The following visual function parameters were studied: best corrected visual acuity (BCVA), spherical equivalent (SE), contrast sensitivity and near stereoacuity; microstructural parameters: retinal nerve fiber layer thickness (RNFLT), ganglion cell layer (GCC) *thickness*, focal loss of volume (FLV), global loss of volume (GLV), peripapillary, papillary vessel density (VD), ocular axial length (AL) and intraocular pressure (IOP).

Results: Among functional tests there were significant differences in contrast sensitivity at 1.5 ($p < 0.001$), 6 ($p < 0.013$), 12 ($p < 0.001$), 18 ($p < 0.003$) cycles per degree, in near stereoacuity (Titmus Wirt circles, $p < 0.001$) and in best corrected visual acuity (BCVA, $p < 0.001$). A statistically significant, moderate positive linear correlation was observed between BCVA and average Phe levels over the last ten years ($\beta = 0.49$, $p < 0.001$). The average ($p < 0.001$), superior ($p < 0.001$) inferior GCC ($p < 0.001$), the FLV ($p < 0.003$), GLV ($p < 0.001$) and the average RNFLT ($p < 0.004$) values of the PKU group were significantly lower than the controls. The serum phenylalanine level (Phe) in the PKU group negatively correlated with inferior (-0.32, $p < 0.007$), *superior ($r = -0.26$, $p < 0.028$)* and average (-0.29 $p < 0.014$) RNFL and with AL (-0.32, $p < 0.026$). In AL we detected a significant difference ($p < 0.04$) between the good and suboptimal dietary controlled group. There was no significant difference between the ETPKU and control group in the measured vessel density parameters and in IOP.

Conclusions: Our results suggest that functional and ocular microstructural defects are present in patients with PKU, and some of them may depend on dietary control. The mechanism is unclear, but the correlation indicates the importance of strict dietary control in terms of preservation of retinal functions.

1. Introduction

Phenylketonuria (PKU, OMIM 261600) is a rare, *autosomal recessively* inherited disorder of the phenylalanine hydroxylase gene (PAH; EC 1.14.16.1). The incidence of PKU in Europe is about 1:10000 [1]. Symptoms are consequences of the insufficient phenylalanine to tyrosine conversion [2]: Phenylalanine (Phe) and its metabolites accumulate in the blood and *brain*, and have toxic effects. Before the

Abbreviations: PKU, phenylketonuria; ETPKU, early-treated phenylketonuria; Phe, phenylalanine; BH4, Tetrahydrobiopterine; GMP, Glycomacropeptide; AAS, aminoacid supplements; AL, axial length; SE, spherical equivalent; D, diopters; BCVA, best corrected visual acuity; ETDRS, early treatment diabetic retinopathy study; SWCT, sine wave contrast test; IOP, intraocular pressure; OCT, optical coherence tomography; OCTA, optical coherence tomography angiography; LogMAR, logarithm of the minimum angle of resolution; SD, spectral domain; RNFL, retinal nerve fiber layer; RNFLT, retinal nerve fiber layer thickness; GCC, ganglion cell layer thickness; FLV, focal loss of volume; GLV, global loss of volume; VD, vessel density; PD, Parkinson's disease; DA, dopamine; VEPs, Visual evoked potentials

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implementation of neonatal screening in the 1960s, untreated PKU caused severe intellectual disability, emotional disturbances, psychosocial disabilities, and irreversible neurological abnormalities [3,4].

The early initiation of therapy radically improved life expectancy and quality of life among patients with PKU [5]. Therapy is traditionally based on a lifelong diet which is poor in Phe, with daily supplementation of Phe-free amino acids. There are emerging novel therapeutic methods which provide further options to improve metabolic control, including *tetrahydrobiopterine* (BH4) for responsive patients or *glycomacropeptide* (GMP) which can partially substitute amino acid supplements (AAS). In the upcoming years phenylalanine ammonia lyase (PegPal) may also become increasingly available to more patients with PKU [6].

Long-term optimal therapy adherence with blood Phe levels in the target range is associated with better intellectual capabilities in adults [7]. PKU can be considered as a medical success but social and neuropsychological consequences still occur in some patients. *Several studies found mean neurocognitive level among patients with PKU to be decreased compared with control groups* [8,9]*, although normal cognitive outcome - by patients with a history of well-controlled therapy – had been reported aswell* [10]. *White matter abnormalities are also apparent in some early and late treated patients* [11].

The exact pathomechanism of neurotoxicity in PKU is poorly understood. *Decreased concentration of serotonin and dopamine (DA) in the brain and highly variable Phe transport across blood-brain barrier are reported* [12,13]. *Altered DA level, as in other neurodegenerative diseases, may have detectable effect on the retina in patients with PKU* [14]. *In contrast to the well-known cognitive deficit, there is limited data available about the potential ocular abnormalities (hypopigmentation of iris and retina, cataract, corneal opacities), or functional visual impairment (deficit in contrast sensitivity and in color vision, electroretinography alterations, latency increase and amplitude decrease in visual evoked potentials (VEPs) caused by PKU* [15–21].

Therefore, in this study we intend to evaluate ocular functional and microstructural characteristics of early-treated patients with PKU, reveal potential correlations between ocular alterations and therapy adherence, and compare findings with an age- and gender-matched control group from the general population. We also aimed to investigate the connection between metabolic parameters potentially affecting dopamine signaling and myopia development.

2. Patients and methods

2.1. Study design

In this prospective, monocentric, cross-sectional, matched case-control study, 50 patients with ETPKU (early-treated phenylketonuria) and 50 controls were evaluated between November 2018 and March 2020. This study was conducted according to the Declaration of Helsinki, relevant national and local requirements, approved by the Semmelweis University's Regional, Institutional Scientific and Research Ethics Committee (ETT registration number: SE RKEB 171/2018). Subjects participated voluntarily in the study and written informed consent was obtained from all.

2.2. Study population

2.2.1. PKU patients

Patients who were diagnosed with PKU by neonatal screening, and who had attended the Inborn Error of Metabolism Adult Metabolic Centre (former 2nd Department of Medicine, Semmelweis University, Budapest) for at least 10 years were enrolled in this study. Inclusion criteria were the following: a.) classical or mild/moderate PKU diagnosis at the time of birth; b.) early and continuous treatment initiated after diagnosis; c.) older than 18 years of age; d.) Caucasian origin; e.) good compliance regarding regular checkups at the Adult Metabolic Centre; f.) none of the patients had history of other systemic or ocular disease, no previous ocular trauma or operation. Exclusion criteria were $> +/- 5.5$ diopters [D] of spherical equivalent refraction or over $+/- 3$D of astigmatism. *Genotype was not known by most patients. To obtain proper phenotype classification we used a combination of methods: those, who had Phe levels over 1200 μmol/l before therapy initiation were classified as classical PKU. By patients, whose pretreatment blood Phe level was not unequivocal (mostly because of early introduction of therapy) course of the disease and phenylalanine tolerance were also assessed for classification* [22] *forty-one (82%) of our patients had classical PKU, six had moderate and 3 had mild PKU.* Patients' mean age was 30.66 ± 8.00 years, the sex ratio was 22 female/28 male. The control group consisted of 50 healthy individuals with a mean age of 30.45 ± 7.18 years.

2.2.2. Case-matched controls

The control group met the same exclusion criteria as the PKU patient group. Controls were case-matched to the PKU patient group with respect to age [23,24], axial length (AL) [25], spherical equivalent (SE), ethnicity, sex and race, because all these parameters can have an effect on the measured data [26–29].

2.3. Laboratory tests

All blood samples were drawn from the antecubital vein under standardized conditions. Phenylalanine and tyrosine levels were measured by API2000 LC/MS/MS (Perkin-Elmer Sciex, Ontario, Canada) at the 1st Department of Pediatrics, Semmelweis University, Budapest. The analysis was performed according to methods previously described [30].

2.4. Ophthalmological examinations

Participants underwent complete ophthalmological examination at the Department of Ophthalmology, Semmelweis University, including best corrected visual acuity (BCVA) detection with Early Treatment Diabetic Retinopathy Study (ETDRS) chart, near Titmus stereo acuity and sine wave contrast test (SWCT), *non-contact intraocular pressure (IOP) measurement, slit lamp and dilated fundus examinations using slit lamp biomicroscopy and optical coherence tomography (OCT) imaging.*

2.4.1. Refraction measurement, visual acuity testing

The refractive assessment was based on objective non-cycloplegic refraction with conventional auto refractor (Accuref-K 9001, Shin Nippon, Japan), followed by subjective visual acuity detection with ETDRS (2000 series revised) letter chart for testing at 4 m. This chart with Sloan letter optotypes is a conventional method for research purposes worldwide, it is designed to enable more accurate measurement of BCVA than other acuity charts [31,32]. *BCVA is scored with reference to the Logarithm of the Minimum Angle of Resolution (LogMAR). Zero LogMAR means visual acuity equal to the reference standard (1.0 in decimal scale, or 20/20).*

When necessary, BCVA was assessed with the best spectacle correction. Analysis was based on spherical equivalent, which means the algebraic sum of the value of the sphere and half the cylindrical value.

2.4.2. Contrast sensitivity

Measurements were conducted using SWCT (Arthur P. Ginsburg, Stereo Optical Co.) [33]. This test has alternating light and dark bars with sinusoidal transition from light to dark. The interval between the contrasting lines (the spatial frequency) is varied, contrast sensitivity is a measurement of the threshold contrast for seeing a target. Sinusoidal gratings vary in frequency *(1.5, 3, 6, 12, and 18 cycles/degree),* contrast, and phase. The logarithmic transformed average contrast sensitivity values of each grating were recorded according to spatial frequency at all five frequencies [34,35]. We excluded subjects who had strange crossing of the right and left sided measured curves or strange deviation

of the curves at highest spatial frequency [36].

2.4.3. Stereoacuity

We assessed stereoacuity using the Titmus Wirt circles stereotest (Stereo Optical Co., Inc. Chicago, IL) at a distance of 40 cm, with the participants own near correction in place if needed, and cross polarized filters are required. Patients with a score of zero circles were excluded because this test is not able to give a precise estimate in such cases [37]. Stereoacuity was transformed to log units for analysis. This is one of the most well-known clinical test of stereopsis [38,39].

2.4.4. Axial length

AL was measured five times using partial coherence interferometry (Zeiss IOL Master, Carl Zeiss, Germany Version 5.4.4.0006), average AL was calculated by the machine. This routinely used noncontact biometry device is favourable to assess AL because of its high accuracy (± 0,01 mm) and reproducibility [40,41].

2.4.5. Intraocular pressure

IOP was measured three times by non-contact Tonometer (Topcon CT-1P) with auto alignment and shot function [42]. Average IOP was calculated by the machine.

2.4.6. Spectral domain optical coherence tomography

We used an ultrahigh resolution spectral domain OCT (SD-OCT) system AngioVue™ (RTVue-XR Avanti; Optovue Inc., Fremont, CA, USA, software version 2018.0.0.18) to measure retinal parameters. It has an axial scanning speed of 70,000/s, a resolution of 5 μm, a light source centered at 840 nm, and a full-width at half maximum (FWHM) bandwidth of 45 nm and for obtaining angiographic information uses the SSADA algorithm, which reduces the effect of bulk-motion to improve signal-to-noise ratio in the axial direction [43–45].

With this device we detected the macular ganglion cell complex (GCC) thickness, and the circumpapillary retinal nerve fiber layer thickness (RNFLT) in microns. Subsequently, we performed spectral domain OCTA examinations of the papillary region. In GCC measurements, three thickness parameters; lower and upper quadrant and mean GCC measurements, focal and global loss volume values were taken. GCC scanning protocol represents the innermost retinal layers: the retinal nerve fiber layer (RNFL), the ganglion cell layer (GCL), the inner plexiform layer (IPL), while 7 × 7 mm macular cube is centered on the fovea.

RNFLT was measured for evaluating structural change in a peripapillary two-dimensional (2D) section, which is typically obtained along a 3.45 to 3.46 mm diameter circle centered on the disc. The section was divided into four sectors – measurement of the temporal, superior, nasal, and inferior quadrants and the mean nerve thickness and papillary rim and cup/disc area were taken. For accurate analyses well-centered scans with high image quality are needed [46]. RNFL contains the retinal ganglion cell axons, which are covered by astrocytes and bundled by processes of Müller glial cells [47]. Even in patients with cognitive impairment, RNFLT has high repeatability and reproducibility [48]. Peripapillary vessel density can be calculated from *en face* angiograms (percentage area occupied by vessels and microvasculature) [44], previous studies described high accuracy and reproducibility [49–51]. Data for the right eye were evaluated. Each scan was automatically segmented by the software and measurements were performed by a single, well-trained examiner on the same day. In order not to provide misleading or false information, we excluded poor-quality scans with lower signal strength or local weak signal (GCC and optic nerve head signal strength index under 50, angio scan quality under 7/10), image processing or motion-related artifacts, and off-centered pictures [52].

Table 1
Comparison of demographic data and structural biometric characteristics between ETPKU patients and healthy controls at baseline. SD, standard deviation; SE, spherical equivalent; IOP, intraocular pressure; AL, axial length. We used ANOVA for Age, SE, IOP, and AL and Chi-square test for the sex comparison.

Characteristic, mean ± SD	ETPKU (n = 50)	CONTROL (n = 50)	p-value
Age (years)	30.66 ± 8.00	30.45 ± 7.18	0.890
SE (Dioptre)	−0.72 ± 1.2	−0.42 ± 1.0	0.173
IOP *(mmHg)*	17.15 ± 2.19	17.25 ± 2.15	0.800
AL (mm)	23,37 ± 0.81	23.44 ± 0.62	0.625
Sex, n (%)			
Female	28 (56%)	28 (56%)	0.999
Male	22 (44%)	22 (44%)	0.999

2.5. Statistical analysis

Statistical analyses were carried out using the software SPSS version SPSS Statistics for Windows, version 17.0 (SPSS Inc., Chicago, Ill., USA). For normally distributed variables we used parametric, for non-normally distributed variables non-parametric tests. Continuous variables were analyzed with Kendall's Tau-b and we compared the means within groups with Mann-Whitney *U* test. Continuous data are reported as mean ± SD. A p-value of < 0.05 was considered statistically significant.

3. Results

Demographic information and structural biometric characteristics of the study population are summarized in Table 1. In the patient group, the mean age was 30.66 ± 8.00 years, compared with 30.45 ± 7.18 years in the case-matched controls. The sex ratio was 22 female/28 male. We found no significant differences in age, SE, IOP or sex between the groups, confirming successful matching. SE was between +1.8 and − 5.5 D in the ETPKU and between +1.37 and − 4.5 D in the control group.

3.1. Ophthalmological and metabolic parameters in ETPKU group

In the ETPKU patient group the mean blood Phe level over the last ten years was 619.79 ± 225.8 μmol/l. In the ETPKU patient group there was a negative correlation between the AL of the globe and mean Phe level ($r = -0.315$, $P < 0.026$).

Mean Phe levels were negatively correlated with inferior (Kendall's t-test $r = -0.32$, $p < 0.007$), superior ($r = -0.26$, $p < 0.028$) and average ($r = -0.24$, $p < 0.014$) RNFLT, other parameters (nasal and temporal RNFLT, superior, inferior and average GCC thickness, GLV, FLV, age and gender) did not show significant correlation.

We divided our patients into two subgroups - good (n = 24) and suboptimal (n = 26) diet adherence, according to the European guidelines' recommended target blood Phe-value for adults. There was a significant difference in AL between patients with good and with suboptimal diet adherence, patients with long-term optimal blood Phe levels had longer AL. (Table 2.)

Table 2
Kendall's T Correlation of the mean ± SD. Values are shown as mean ± SD. Phe level of the last ten years with the mean ± axial length (AL), Phe: phenylalanine, AL: axial length.

Metabolic group	Good diet adherence	Suboptimal diet adherence	p value
Phe level (μmol/l)	440.42 ± 107.0	782.58 ± 153	< 0.001
AL (mm)	23.65 ± 0.77	23.11 ± 0.76	< 0.016

Table 3
Mean BCVA in LogMAR and mean stereoacuity (Log Units) differences in the ETPKU and control groups, ANOVA and Mann-Whitney *U* tests were executed. Values are mean ± SD. BCVA: best corrected visual acuity in LogMAR: Logarithm of the Minimum Angle of Resolution.

	ETPKU	CONTROL	p-value
BCVA (LogMAR)	−0.02 ± 0.17	−0.16 ± 0.06	< 0.01
stereoacuity (Log)	1.96 ± 0.30	1.67 ± 0.10	< 0.001

Table 4
Mean contrast sensitivity (Log Units) of the ETPKU and control groups. Tests are two-tailed, using Mann Whitney *U* test. Values are shown as mean ± SD.

Spatial frequency (cycles/degree)	ETPKU	CONTROL	p-value
1.5	1.69 ± 0.19	1.90 ± 0.16	< 0.001
3	1.94 ± 0.09	1.97 ± 0.13	< 0.400
6	2.02 ± 0.17	2.09 ± 0.82	< 0.013
12	1.72 ± 0.27	1.93 ± 0.12	< 0.001
18	1.36 ± 0.24	1.53 ± 0.17	< 0.003

3.2. ETPKU vs controls

Univariate linear regression analyses were performed to assess the relationship in BCVA between the ETPKU group and the control group (ß = 0.489, r^2 = 0.24, p < 0.001).

Patients with ETPKU had significantly lower BCVA and near stereoacuity compared with the control group (Table 3.)

Contrast sensitivity values in patients with ETPKU were generally lower than those of the control group at all spatial frequencies except 3 cyl/deg. (Table 4).

Based on the OCT measurements there was no significant difference between groups in terms of papillary cup to disc ratio or rim area. Furthermore, there were no myopic optic disc features such as severe peripapillary atrophy or disc tilt in the groups. Average RNFLT in patients with ETPKU was significantly reduced compared with controls (Table 5). There were no significant differences between groups when quadrants were analyzed separately.

Macular GCC thickness values are detailed in Table 6. The average, superior and inferior GCC thickness of ETPKU eyes were significantly thinner, and the focal and global loss volume values were significantly lower in the healthy controls.

No significant differences were observed in vessel density (VD) parameters of quantified OCTA measurements between the groups in the whole 4.5 × 4.5 mm scan area: both inside the optic disc and in the entire peripapillary region.

4. Discussion

Eye abnormalities in untreated patients with PKU have been previously described [21,53], but there *is* limited data available about the ophthalmological manifestations in early-treated patients with PKU. *Jones* et al. *detected subclinical visual pathway involvement in older children and adults in PKU, VEPs were abnormal in 80%. Among older patients there was no significant correlation between the reduction of VEP amplitudes and plasma Phe or tyrosine levels and the authors supposed, that alterations may reflect events occurring during early development* [20].

We aimed to study the ophthalmological functional and structural

Table 5
Mean RNFLT in ETPKU and control group. Data were analyzed using ANOVA. Values are shown as mean ± SD. RNFLT: retinal nerve fiber layer thickness.

	ETPKU	CONTROL	p-value
RNFLT ave. (microns)	96.34 ± 9.89	101.94 ± 7.19	< 0.004

Table 6
Mean macular GCC of the ETPKU and control groups. Values are shown as mean ± SD. For GCC measurements ANOVA test was used, for FLV and GLV two-tailed Mann Whitney *U* test. GCC: ganglion cell complex, FLV: focal loss of volume, GLV: global loss of volume.

GCC thickness (microns)	ETPKU	CONTROL	p-value
superior	91.41 ± 5.76	97.42 ± 6.4	< 0.001
inferior	92.77 ± 5.44	98.84 ± 6.09	< 0.001
average	91.91 ± 5.52	98.12 ± 6.01	< 0.001
FLV	1.46 ± 1.81	0.34 ± 0.46	< 0.003
GLV	4.66 ± 3.56	1.93 ± 2.39	< 0.001

features of early-treated adult patients. Guideline recommendations focus on avoiding brain damage with neurological and psychiatric consequences caused by long-term high Phe levels. *However, there are further potential consequences of living with phenylketonuria, such as alterations in bone metabolism, obesity or aortic stiffness, psychosocial difficulties* [54–56]. In this context, the eye is a very complex and interesting organ. As a part of the sensory nervous system, one might expect that neuronal damage caused by high Phe levels can have an impact on the retinal part of the eye.

Previous studies have demonstrated that patients with PKU may have lower BCVA and contrast sensitivity compared with healthy individuals of the same age [16,17]. This is in accordance with our functional test results; we detected significantly lower BCVA and contrast sensitivity in the ETPKU group. Furthermore, patients with PKU had significantly lower near stereoacuity compared with healthy individuals. Interestingly, mean Phe levels were not found to be statistically associated with any of the detected visual functions.

Hopf et al. found stereopsis to be normal in 17 out of 19 patients, and reduced in two patients, but they did not find foveal hypoplasia in the macula OCT in patients with PKU [18]. To the best of our knowledge, this is one of the first studies investigating detailed retinal structural changes by OCT in PKU. RNFL and GCC measurements have been known as a good structural parameter to detect neuroretinal degeneration. Our data showed that ETPKU patients have significantly thinner average RNFLT compared with healthy controls. Moreover, we found that the average and the inferior RNFLT loss correlate with blood Phe-level. There are several studies which detected a significant trend in peripapillary RNFL thinning and Parkinson's disease (PD) progression and therefore suppose that average RNFL might be an adequate biomarker for detecting and later following progression of PD [57,58]. The potential linkage is the substantially reduced intracerebral *DA* availability in both diseases.

The importance and complexity of dopamine, as a chemical messenger in the visual system is well known. It has a role in retina development, visual signaling, circadian rhythmicity, cell survival and eye growth [59]. Retinal *DA* is synthesized in and released by subtypes of amacrine and interplexiform cells [60,61]. The *DA* neurons receive excitatory [62] and inhibitory inputs [63], have receptors for a variety of peptides, for melatonin, for neurotrophins [64], and for growth factors [59]. Reduced retinal DA leads to loss of dopaminergic amacrine cells, which are a part of the neuro-optic pathway and act as an interneuron between bipolar and retinal ganglion cells [65,66]. In diseases with alterations in the DA system, like in PD, DA dysfunction has been shown in the retina as well [67]. *Diminished retinal DA concentration in PD was first described by Harnois and Di Paolo, based on post mortem data* [68]. Inzelberg et al. suggested that DA depletion causes dysfunctions and impoverished dopaminergic input to a subset of ganglion cells and contributes to abnormal production of glutamate and atrophy of these selected fibers [69]. Previous data suggest significant RNFL thinning in different regions of PD patients compared with healthy controls, but others did not demonstrate any significant difference. The inconsistency could be explained by the limitations of the studies; axial lengths were not considered, devices were different, patients' age and disease

duration were heterogeneous in most studies [58].

Beyond the average RNFL loss, we found several significant alterations in various retinal compartments by OCT. All measured GCC values were found to be significantly thinner in the ETPKU group. GCC thinning may also demonstrate anterograde or retrograde axonal loss. Macular GCC thickness may be a more sensitive marker of potential inner retinal thinning than RNFL [70]. Interestingly, in contrast to anatomical alterations there was no statistically significant correlation between GCC or RNFL thinning and the measured functional deficits, like BCVA, stereoacuity or contrast sensitivity. Structural and functional changes are related to each other, but the structure–function relationship is highly variable and imperfect in other diseases as well [71]. Although we found a correlation between average, inferior RNFLT and blood Phe, there was no significant relationship between GCC thinning and blood Phe level. It is also important to note that we did not detect any significant microvascular impairment in the papillar and peripapillar region of ETPKU patients with OCTA.

We performed subgroup analyses to examine whether metabolic control in the last 10 years can influence eye findings or not. Although some of the examined parameters correlated with Phe levels, we found no significant difference between the visual functional, microstructural properties of the two subgroups (good *vs.* suboptimal diet adherence). The reason might be that the differences between the two subgroups in mean Phe level were not outstanding. In the suboptimal metabolic subgroup patients also had a partial diet lowering blood Phe. A comparison between a good metabolic subgroup with a group consisting of totally off-diet patients might give significant differences in eye parameters, as well.

There is a huge increase in myopia in the general population, and if current trends continue, it will affect half of the world's population by 2050 [72]. Animal models and pharmacological experiments have established the role of DA in myopia progression, *where DA can act as a "stop" signal in refractive eye growth* [73]*, but the exact pathway is still undefined* [74]*. The European Eye Epidemiology Consortium reported 30.6% age-standardized myopia prevalence* [75]*. Among our patients with ETPKU the SE was between + 1.8 and − 5.5 D, the myopia prevalence was 76%, which is higher than in the European population and may confirm the previous hypothesis, that retinal DA might be the major regulator of postnatal eye growth* ([73])*. Further analysing our data, the good metabolic control group had longer eyeball length, which does not support the theory, that DA might slow the development of myopia and underlines the complexity of this mechanism. There are still challenges in understanding the exact role of DA signaling in myopia* [74] *or its altered signaling in PKU.*

It is already known that high Phe can alter the intracerebral dopamine signaling, and upon the available data it might have also an effect on the dopaminergic neurons in the retina. But it is obscure, whether changes in the retinal dopaminergic neuron function are reversible or permanently damaged. Substantial, long term amelioration of Phe metabolism either by enzyme- or future gene therapy can give an answer for these questions.

Ocular and visual abnormalities can have an impact on various activities such as reading, sports or driving. Although we found many functional and microstructural alterations, its influence on PKU patients visual and life quality is not known.

It is important to note some limitations to our study. Since this was a cross-sectional study, we cannot examine causal effects, or explore effects over time. The macular region and its microvasculature, the function of higher visual areas, the lateral geniculate nucleus and visual cortex containing dopaminergic cells might also affect visual functions and should be investigated in the future. Intracerebral DA level measurement could support our results.

In spite of these limitations, this study has several substantial strengths: The rigorous selection of the control group and the thorough ophthalmological examination of all patients with multiple modern diagnostic instruments makes our findings reliable. Ours is the first study to examine eye alterations in adults with ETPKU in such detail.

5. Conclusion

We found that there are structural and functional eye alterations among early-treated patients with PKU. One can postulate that there may be neurodegenerative processes which affect the retina of these patients with PKU. We report the first direct evidence that retinal neurodegeneration in ETPKU can be detected *in vivo* by OCT.

Contributors

CsSe, AGB and PR reviewed the literature and conceived the study. PR was.

in charge of overall direction and planning. CsSe, AGB, CsSu, ZZsN and PR were involved in patient selection, sample and data collection. CsSe interpreted the results and drafted the manuscript.

CsSe, AGB and EH were involved in the statistical analysis. CsSe, BCs, MR, ZZsN performed further analysis and data interpretation. PR reviewed and edited the manuscript.

All authors discussed the results and contributed to the final manuscript.

Financial disclosures

There are no financial conflicts of interest to disclose.

Ethics approval

The study followed the principles of the guidelines in the World Medical.

Association Declaration of Helsinki of 1975 and was approved by the Semmelweis University's Regional, Institutional Scientific and Research Ethics Committee (ETT registration number: SE RKEB 171/2018).

Declaration of Competing Interest

The authors have no conflict of interest to report.

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