

Visual functions in phenylketonuria—evaluating the dopamine and long-chain polyunsaturated fatty acids depletion hypotheses

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ABSTRACT

Background: In phenylketonuria presymptomatic treatment following newborn screening prevents severe mental and physical impairment. The reasons for subtle impairments of cerebral functions despite early treatment remain unclear. We assessed a broad spectrum of visual functions in early-treated patients with phenylketonuria and evaluated two hypotheses—the dopamine and the long-chain polyunsaturated fatty acids (LCPUFAs) depletion hypotheses.

Methods: Contrast sensitivity, colour vision, electroretinography, frequency doubling technology campimetry (FDT), and their relation with blood phenylalanine and docosahexaenoic acid levels were assessed in 36 patients with phenylketonuria and 18 age-matched healthy controls.

Results: Contrast sensitivity was significantly lower and total error scores in colour vision significantly higher in patients than controls. Electroretinography results differed significantly between patients and controls. We found a trend for the effect of phenylalanine-levels on contrast sensitivity and a significant effect on colour vision/FDT results. Docosahexaenoic acid levels in erythrocytes were not associated with visual functions.

Conclusion: This is the first evaluation of visual functions in phenylketonuria using a comprehensive ophthalmological test battery. We found no evidence supporting the long-chain polyunsaturated fatty acids depletion hypothesis. However, the effect of phenylalanine-levels on visual functions suggests that imbalance between phenylalanine and tyrosine may affect retinal dopamine levels in phenylketonuria. This is supported by the similar patterns of visual functions in patients with phenylketonuria observed in our study and patients with Parkinson's disease.

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1. Introduction

Phenylketonuria (PKU, OMIM ID: 261600) is an inborn error of metabolism resulting from deficiency of the enzyme phenylalanine hydroxylase (PAH, EC 1.14.16.1) which converts phenylalanine into

Abbreviations: DHA, docosahexaenoic acid; ERG, electroretinography; FDT, frequency doubling technology campimetry; LCPUFA, long chain polyunsaturated fatty acid; MD, mean deviation; PKU, phenylketonuria; SF, spatial frequency; VEPs, visual evoked potentials.

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tyrosine. In untreated patients accumulation of phenylalanine in blood and consecutively in brain leads to mental retardation and neurological symptoms. Nowadays PKU can be detected by newborn screening. Treatment with a low-protein diet supplemented with a phenylalanine-free amino acid mixture leads to overall normal neurological and intellectual development when initiated early in life [1].

However, it has been described that even in well-treated patients imbalance of phenylalanine and tyrosine affects the central nervous system (CNS), e.g. selective cognitive functions [2,3] or contrast sensitivity as a visual function [4].

Two hypotheses are postulated to explain these findings: On the one hand, increased phenylalanine-levels in plasma might lead to decreased dopamine-levels in brain and eye by lack of tyrosine in the CNS [4]. Dopamine plays an important role in retinal signal transduction [5]. On the other hand, outer segments of rods contain high concentrations of long-chain polyunsaturated fatty acids (LCPUFAs) [6]. The dietary intake of LCPUFAs is often deficient in PKU patients [7].

In this study the dopamine and LCPUFAs depletion hypotheses are assessed by evaluation of contrast sensitivity, colour vision, electroretinography, and frequency doubling technology campimetry (FDT). This test battery was chosen as it comprises a broad spectrum of visual functions which could all presumably be affected by dopamine or LCPUFAs depletion. The application of electroretinography particularly allows for objective measurements of retinal functions.

2. Patients and methods

2.1. Study population

Thirty-six PKU patients (15 male, 21 female) and 18 healthy controls (7 male, 11 female) participated in the study. PKU and control subjects were matched for age and sex. Mean age in years was 17.2 (SD 10.1) for patients and 17.9 (SD 9.9) for controls. Participants were assigned to subgroups of 'children', 'adolescents' and 'adults', with mean age in years for patients of 8.0 (SD 1.9; $n = 14$), 14.2 (SD 1.7; $n = 8$) and 28.2 (SD 6.5; $n = 14$) and for controls of 9.7 (SD 2.6; $n = 6$), 15.4 (SD 1.9; $n = 6$), and 28.6 (SD 10.0; $n = 6$), respectively.

Minimum age for participation was 5 years because of the cooperation required for ophthalmological examinations. All patients had been diagnosed in the neonatal period following newborn screening and had received early dietary treatment. Study participants/parents gave written informed consent before participation. The study adhered to the tenets of the Declaration of Helsinki and was approved by the University Hospital Heidelberg ethical committee (EC Number 075/2006).

2.2. Methods

2.2.1. Ophthalmological examination

Participants underwent examination for both eyes including assessment of visual acuity (Landolt signs), slit lamp examination and dilated pupil funduscopy. Refraction was assessed by autorefractometer (Humphrey Instruments, 597/S/N 597 0955, Zeiss, Jena, Germany) and results were converted to spherical equivalents (extent of myopia/hyperopia + 0.5 × extent of astigmatism). Due to risk of amblyopia for the eye with more severe hyperopia/astigmatism in anisometropic subjects, data of the eye with refraction closer to emmetropia were used for statistical analysis. If objective refraction was identical in both eyes one data set was randomly chosen.

Applicants with history of cataract, visual field defects, myopia of more than -6 dioptre (dpt), family history of impaired colour vision or on medication interfering with visual functions were not included. Colour vision, contrast sensitivity and FDT were tested monocularly, if required with optical correction according to best corrected visual acuity. ERG recording was done without corrective glasses.

2.2.2. Contrast sensitivity

Contrast sensitivity was evaluated using the Vistech chart [8], which consists of five rows of three inch patches of gratings, each row showing a different spatial frequency (SF): 1.5, 3, 6, 12 or 18 cycles per degree (cpd).

Each row contains one sample patch and eight test patches with sinusoidal gratings of identical SF with decreasing contrast from left to right. The task is to judge the orientation of each grating as vertical, right or left oblique, or no grating discernible. For each spatial frequency the value of the last correctly identified target was documented according to the test manual [8].

2.2.3. Colour vision

Colour vision was evaluated using the saturated 28 HUE de Roth test selon Farnsworth-Munsell (Luneau, Chartres). Twenty-seven caps have to be arranged according to colouration [9]. Confusions of caps along axes (tritan, protan, tetartan, deutian) indicate different

types of dyschromatopsias. A total error score is calculated by the distances of caps arranged incorrectly [10].

2.2.4. Frequency doubling technology campimetry

Frequency doubling technology campimetry (FDT) was performed by complete C 20 threshold test (Zeiss Humphrey® Systems, Jena, Germany), which measures spatio-temporal contrast sensitivity using high-frequency counterphase flicker with a low spatial frequency sinusoidal grating [11]. Mean deviations (MD) in decibel per eye represent average sensitivity of 17 visual field locations in comparison to age-adjusted norms. Positive (negative) values indicate a result better (worse) than the norm. Results were classified as probably unreliable if a proband showed more than 33% errors in at least one of three criteria (fixation loss, false positive or false negative reactions) [12].

2.2.5. Electroretinography (ERG)

To correspond to the capabilities of the youngest and least compliant participant, ERG recording deviated from the ISCEV (International Society for Clinical Electrophysiology of Vision) standard procedure. After pupil dilation (Tropicamide 0.5%, Phenylephrine 5%), luminance ERGs were recorded by skin electrodes using temporal contrast (flicker) stimuli and single exposures [13]. Dark adaptation was limited to 10 min.

Due to a change in technical equipment in December 2009 from Multiliner® Vision (Toennies, Höchberg, Germany) to Medelec Synergy (Viasys Healthcare Inc., Höchberg, Germany) for recording and BN 940 Strobotest (Knott Elektronik, Hohenschäftlarn, Germany) to 211 Drelloskope® (Drello, Mönchengladbach, Germany) for generation of light stimuli, comparability of raw data was limited. Therefore, individual ERG responses were rated by two experts in electrophysiology (HK and PW) as normal (0), showing mild (1), medium (2) or severe (3) pathology, or lacking any detectable response (4). This was done in consideration of reference values for responses to stimuli for both recording equipments. Responses were obtained for three scotopic (blue 0.3 Hz intensity level E1, white 1 Hz E1, white 1 Hz E2) and four photopic light stimuli (white 10 Hz E1, white 30 Hz E1, white 60 Hz E1, red 1 Hz E3). Red 1 Hz E3 was classified as "photopic single response." E1 was 3.0 lx and 6.3 lx (elder and newer stroboscope, respectively), E2 51.8 lx and 52.8 lx, and E3 117 lx and 120.1 lx.

2.2.6. Blood laboratory investigations

Blood samples were drawn from patients and controls after a minimum fasting period of 3 h. Phenylalanine and tyrosine were analysed from dried blood spots by electrospray ionization tandem-mass spectrometry (Micromass Ultima). LCPUFAs were measured as described previously [14]. Docosahexaenoic acid level in erythrocyte phosphatidylethanolamine was chosen for further calculations, assuming that LCPUFA-levels in erythrocyte membranes show closer resemblance to levels in photoreceptor membranes than plasma levels. Vitamins A/E were determined using high-performance liquid chromatography. Long-term phenylalanine-levels in PKU patients were calculated as the mean of all values during 12 months preceding study participation.

2.2.7. Dietary regimen

Current diet and amino acid mixture (if applicable) were documented using a standardized questionnaire.

2.2.8. Standardized psychological testing

IQ data were available for 35 patients from the most recent routine psychological testing using tests appropriate for age (WPPSI III; WISC-R, III, IV; WAIS and WAIS-R; CFT20; K-ABC). For control subjects no IQ data were available.

2.2.9. Statistical evaluation

Two research questions were analysed with SPSS® (Version 18, IBM®). The first question compares visual functions of patients and

controls by two-factorial ANOVAs with repeated measurement of five spatial frequencies for contrast sensitivity and scotopic, photopic, and photopic single response for ERG. Colour vision and FDT were compared by one-sided *t*-tests. Significance level of 0.05 was Bonferroni adjusted to 0.0125 for multiple testing.

The second question tests the dopamine and LCPUFAs depletion hypotheses. Data of docosahexaenoic acid and visual function were converted to z-scores using control subjects' means and standard deviations of the respective age group. Z-standardisation allowed to assess influence of patients' IQ although no IQ data were available for control subjects. Principal component analysis followed by Varimax rotation with Kaiser normalisation was used to reduce the number of dependent variables. Relation between the independent variables (phenylalanine, docosahexaenoic acid, IQ) and factor scores of the dependent variables of visual function was analysed by multivariate regression analysis. As this is only one test, no further adjustment of the significance level was made. A *p*-value ≤ 0.05 was considered statistically significant, values > 0.05 and < 0.1 are reported as trends.

2.2.10. Role of the funding source

Sponsors of the study did not have any role in collection, analysis, and interpretation of data, writing the report or decision to submit for publication. The corresponding author has full access to all data and had final responsibility for decision to submit for publication.

3. Results

3.1. Study population

There was no significant age difference between patients and controls in the whole cohort ($t(52) = 0.22$; $p = 0.826$) and the three age groups (ANOVA: $F(1,48) = 0.56$; $p = 0.458$).

3.2. Ophthalmological examination

Visual acuity, mean spherical equivalent, frequency of myopia, hyperopia, astigmatism, or abnormalities of anterior/posterior eye segments were not significantly different between patients and controls (data not shown). All participants had sufficient visual acuity to perform complete testing.

3.3. Contrast sensitivity

Mean contrast sensitivity of patients and control subjects was 47.1 (SD 20.2) vs. 62.8 (SD 31.0) in spatial frequency (SF) 1.5 cycles per degree (cpd); 112.7 (SD 49.6) vs. 139.7 (SD 46.4) in SF 3 cpd; 119.3 (SD 43.9) vs. 155 (SD 30.9) in SF 6 cpd; 79.5 (SD 30.6) vs. 105.6 (SD 30.5) in SF 12 cpd, and 28.9 (SD 15.4) vs. 46.2 (SD 12.5) in SF 18 cpd (Fig. 1).

Patients showed significantly lower contrast sensitivity than controls [ANOVA: $F(1,52) = 15.97$; $p < 0.001$]. No interaction was found between group (patients/controls) and spatial frequency of the Vistech test [$F(3,162) = 0.91$; $p = 0.439$; Greenhouse–Geiser corrected degrees of freedom for violation of assumption of sphericity].

3.4. Colour vision

Abnormal results for colour vision (defined as one or more confusions along axes for dyschromatopsias) were found in 9 patients (25%) and none of the controls. Eight patients showed at least one permutation in the tetartan (blue–yellow) axis. Mean total error scores were significantly higher [Welch test (1,45) = 7.05; $p = 0.006$] in patients (57.3; SD 94.5, 0–336) than controls (12; SD 27.9, 0–108).

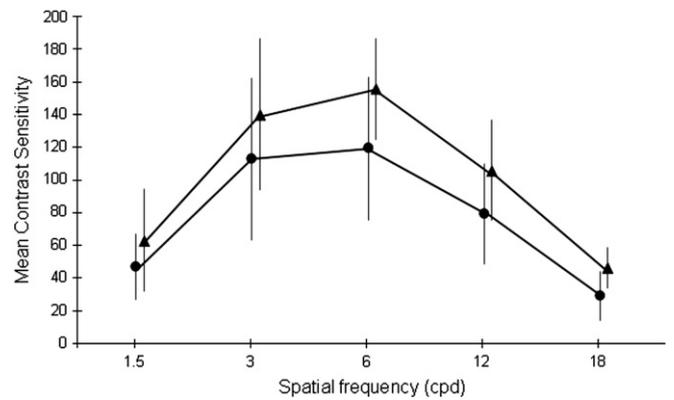


Fig. 1. Contrast sensitivity assessed by Vistech test in PKU patients and control subjects. Mean contrast sensitivity results are shown for patients (●) and control subjects (▲) for spatial frequencies 1.5–18 cycles per degree (cpd).

3.5. Frequency doubling technology campimetry (FDT)

Two patients (age 5 and 6 years) and one control subject (age 5 years) could not perform FDT because of insufficient cooperation. For three patients FDT was not available on the day of investigation. Absolute mean deviation (MD) was not significantly greater in patients (−2.63 dB, SD 2.66) than in controls (−0.99 dB, SD 2.28; $t(1,45) = 4.59$; $p = 0.019$). Percentage of probably unreliable FDT results was similar in patients and controls.

3.6. Electroretinography (ERG)

Mean scores of pathology for scotopic ERG (patients 0.56, SD 0.71; controls 0.11, SD 0.32), photopic ERG (patients 0.41, SD 0.77; controls 0.19, SD 0.08) and photopic single response (patients 0.38, SD 0.70; controls 0, SD 0) were significantly higher in patients than controls [$F(1,50) = 7.78$; $p = 0.007$]. Examples of representative ERG recordings in one PKU patient and control subject are shown in Fig. 2.

3.7. Laboratory investigations

3.7.1. Phenylalanine-levels

Mean concurrent phenylalanine-level was 677 $\mu\text{mol/l}$ (SD 506; 42–1943) in patients and 11 $\mu\text{mol/l}$ (SD 8; 0.7–32) in controls. For patient subgroups 'children', 'adolescents' and 'adults' mean levels were 242 $\mu\text{mol/l}$ (SD 138; 97–557), 980 $\mu\text{mol/l}$ (SD 414; 393–1719), and 919 $\mu\text{mol/l}$ (SD 523; 42–1943).

3.7.2. Vitamin A/E

Vitamin A and E levels did not differ significantly between patients and controls (data not shown) with results within normal range for all controls and 34/36 patients. In one patient vitamin A, in another vitamin A and E levels were slightly decreased, but normalized upon follow-up without need for supplementation.

3.8. Dietary regimen

Questionnaires showed a balanced diet in all participants. Thirty-three patients (92%) and none of the controls adhered to a protein-restricted diet at the time of study participation. Three patients had stopped the diet 2, 6, and 9 years before study participation, respectively. LCPUFA containing foods were consumed by none of the patients and 14/18 controls.

3.9. Standardized psychological testing

Mean IQ of patients was 104.6 (SD 14.7; 73–130). Median interval between IQ-test and study participation was 19 months.

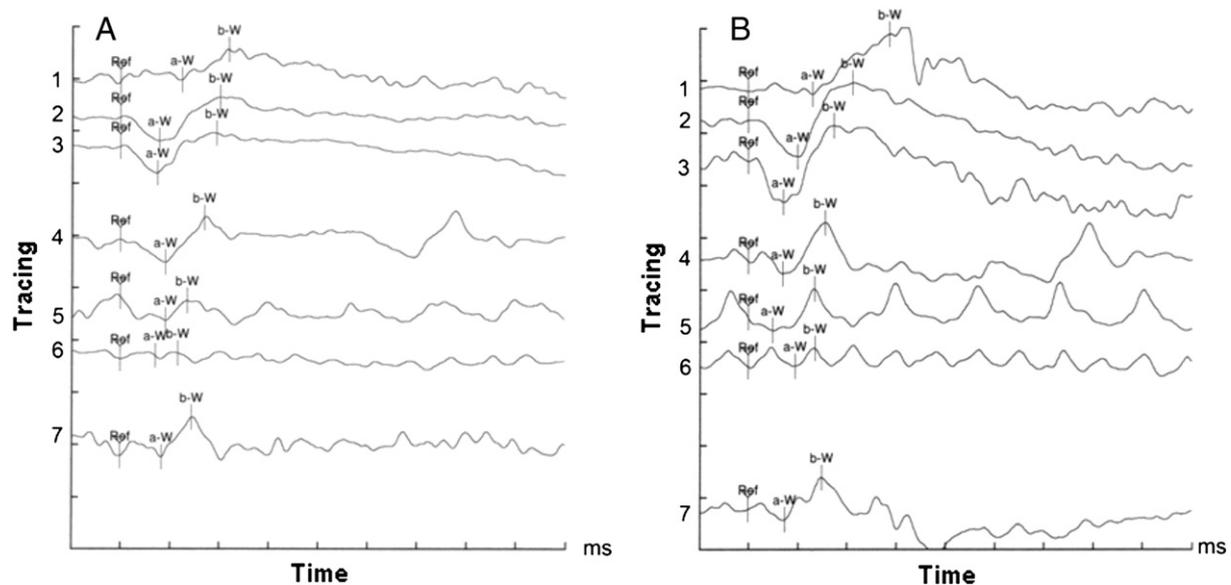


Fig. 2. Electroretinography results in a PKU patient (A) and a healthy control subject (B). Responses were obtained for three scotopic (1 = blue 0.3 Hz intensity level E1, 2 = white 1 Hz E1, 3 = white 1 Hz E2) and four photopic light stimuli (4 = white 10 Hz E1, 5 = white 30 Hz E1, 6 = white 60 Hz E1, 7 = red 1 Hz E3). Red 1 Hz E3 (tracing 7) was classified as “photopic single response.” For details on response evaluation see text. Note that scotopic and photopic amplitudes are reduced in the PKU patient. In scotopic conditions (upper-most tracing) b-wave implicit time is shorter in the PKU patient compared to the control subject. This finding points to an impairment of the scotopic system. The difference shrinks with higher stimulus intensities, which account for more cone input into the b-wave. If, however, the photopic system is asked for a higher output by flicker stimuli, it is unable to comply with these demands, since flicker responses (tracings 5 and 6) are low.

3.10. Evaluation of the dopamine and long-chain polyunsaturated fatty acids (LCPUFAs) depletion hypotheses

Factor analysis of 10 dependent variables (five spatial frequencies for contrast sensitivity, three ERG variables, colour vision, FDT) revealed four rotated factors, explaining 76% of total variance: Factor 1 contrast sensitivity spatial frequencies 3–18, Factor 2 ERG, Factor 3 colour vision/FDT and Factor 4 contrast sensitivity spatial frequency 1.5 (explaining 25%, 22%, 16% and 12% of total variance).

As concurrent and long-term phenylalanine-levels ($r = 0.923$; $p < 0.001$), as well as concurrent phenylalanine-levels and phenylalanine/tyrosine ratios ($r = 0.882$, $p < 0.001$) were highly correlated, only concurrent phenylalanine-levels were used for multivariate regression analysis. Concurrent tyrosine-levels and phenylalanine/tyrosine ratios also were significantly correlated ($r = -0.348$, $p = 0.037$).

Multivariate regression analysis (Fig. 3) showed a trend for influence of phenylalanine-levels on contrast sensitivity spatial frequencies 3–18 ($p = 0.084$) and a significant effect on colour vision/FDT results ($p = 0.046$). Phenylalanine-levels did not significantly influence contrast sensitivity spatial frequency 1.5 ($p = 0.368$) and ERG ($p = 0.158$). Only colour vision/FDT results were significantly associated with patients' IQ ($p = 0.005$). No association was found between IQ and contrast sensitivity results (spatial frequency 1.5 $p = 0.92$; spatial frequency 3–18 $p = 0.975$) and between IQ and ERG results ($p = 0.316$). A relation between concurrent phenylalanine-levels and IQ is not to be expected, and was not significant ($r = 0.188$, $p = 0.280$). No association was found between long-term phenylalanine-levels and IQ ($r = 0.234$, $p = 0.177$). Therefore, phenylalanine-level and IQ can be regarded as variables with independent influence on visual function results in this analysis. Levels of docosahexaenoic acid were not associated with any variable of visual function.

4. Discussion

We found significant differences in contrast sensitivity, colour vision, and electroretinography between PKU patients and controls. Although the observed changes in visual functions are of an extent, which will most probably not result in impairment of visual functions

required in daily activities, the pattern of abnormalities and its relation with the independent variables (phenylalanine-level, docosahexaenoic acid, IQ) can help to elucidate the underlying mechanism.

4.1. Dopamine depletion hypothesis

Impaired contrast sensitivity in PKU patients was first described by Diamond and Herzberg [4] who hypothesized that even mild elevation in plasma phenylalanine-levels will result in reduction of tyrosine in the CNS, because phenylalanine and tyrosine use the same transporter to pass the blood–brain barrier [15], and will lead to a reduction of dopamine in CNS and retina [4]. However, the study left questions unanswered, as no objective measurements of retinal function by ERG had been conducted and to our knowledge it has never been systematically investigated whether colour vision is also impaired in PKU patients. This is particularly significant for evaluation of the dopamine depletion hypothesis, since rods as well as cones are connected to dopaminergic neurons [5].

In PKU patients increased latencies of visual evoked potentials (VEPs) have been reported [16], which were equivalent to delays of VEPs demonstrated in a rat model after application of dopamine antagonists [17]. The essential role of dopamine in retinal function is also documented in patients with Parkinson's disease [18], which is characterized by degeneration of dopaminergic neurons. Post-mortem retina analyses of patients with this disease proved diminished retinal dopamine content [19]. As rods as well as cones are connected to dopaminergic neurons [5], dopamine depletion should presumably affect colour vision, contrast sensitivity, scotopic and photopic ERG. Comparable effects have been demonstrated for Parkinson's disease [20–24].

Several studies, especially pharmacological studies in animal models using selective dopamine receptor agonists and antagonists, have led to the assumption that dopamine plays an essential role in receptive field organization [25]. A study by Domenici et al. on the effect of dopamine on contrast sensitivity in humans found that dopaminergic drugs improve visual contrast sensitivity at medium to high spatial frequencies in healthy individuals [26]. In Parkinson's

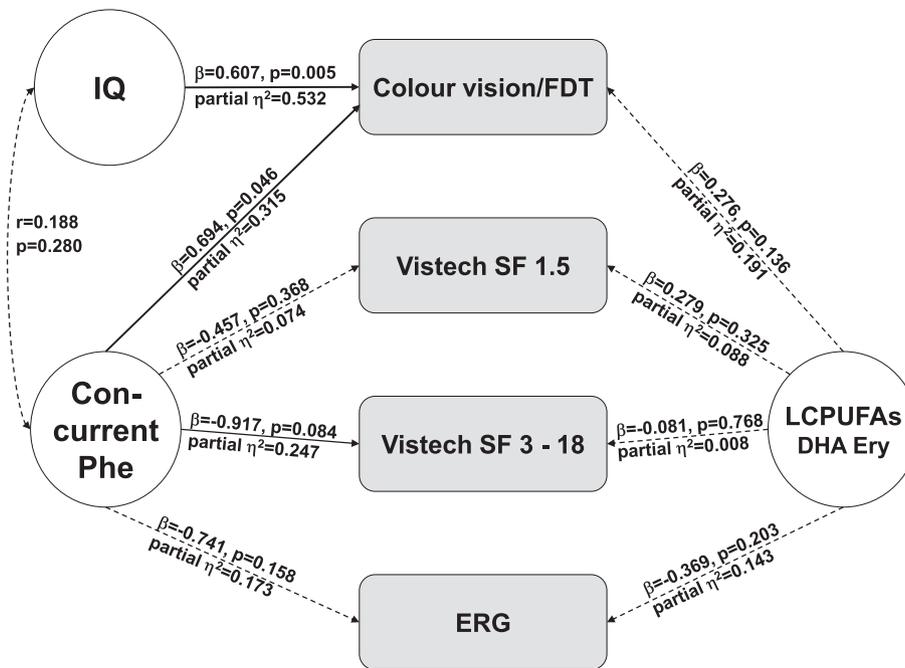


Fig. 3. Influence of phenylalanine-level, docosahexaenoic acid level, and IQ on variables of visual function. Multivariate regression analysis of factor scores was performed to test the influence of concurrent phenylalanine-level, docosahexaenoic acid level, and IQ on variables of visual function. IQ (intelligence quotient), Phe (phenylalanine-level), DHA Ery (docosahexaenoic acid level in erythrocyte phosphatidylethanolamine), LCPUFAs (long-chain polyunsaturated fatty acids), FDT (frequency doubling technology campimetry), Vistech (Vistech test for assessment of contrast sensitivity), SF (spatial frequency).

disease, diminished contrast sensitivity was also detected mainly at midrange spatial frequencies, with low frequencies left almost unaffected [20,27]. In our study we found overall diminished contrast sensitivity in PKU patients and a statistical trend for the effect of phenylalanine-levels on contrast sensitivity for higher but not the lowest spatial frequency, thus paralleling the results in Parkinson's disease.

Our patients showed significantly higher total error scores in colour vision than controls and a high number of errors along the tetartan (blue-yellow) axis. Impairments of blue-green and blue-yellow colour vision are frequently reported in acquired dyschromatopsias [28,29] and were also found in patients with Parkinson's disease [22]. Blue cones represent the smallest fraction of all cones in diurnal primates [30]. Thus, acquired impairments of colour vision might affect the blue system first. In PKU impairment of colour vision could be acquired due to long-term retinal deficiency in dopamine caused by elevated phenylalanine-levels.

Evaluation of the relation of visual functions with the variables phenylalanine, docosahexaenoic acid and IQ revealed that results of FDT and colour vision were significantly associated with phenylalanine-levels. Patients' IQ was significantly associated with colour vision/FDT results. This is not surprising, as these tasks are the most cognitively demanding ones in our test battery. As expected, no significant correlation was found between concurrent phenylalanine-levels and IQ. Thus phenylalanine-level and IQ independently influence FDT and colour vision results.

Impairment of magnocellular function has been demonstrated in patients with Parkinson's disease using a frequency doubling illusion [31]. In our study difference in FDT mean deviation between PKU patients and control subjects closely missed statistical significance on the Bonferroni adjusted significance level.

ERG in PKU patients was, to the best of our knowledge, performed systematically for the first time in our study. Whereas standard pattern VEPs reflect the function of ganglion cells and axons responsible for paracentral and central retinal areas, luminance ERG reflects activities of the outer and middle layers throughout the retina. With regard to dopamine as intraretinal transmitter and to LCPUFAs as components of retinal structures, ERG recording in PKU patients makes sense in a twofold way. Although ERG recording by skin electrodes does not correspond to

ISCEV standards, it was the method of choice to obtain ERG tracings in our study participants due to variation of age and compliance.

Alterations in photopic and scotopic ERG were significantly more frequent in PKU patients than controls, in accordance with the dopamine depletion hypothesis, as ERG results were also shown to be altered in Parkinson's disease with diminished scotopic and photopic luminance responses [32].

A representative ERG tracing in a PKU patient (Fig. 2) displays reduced scotopic and photopic amplitudes. Latencies behave in a discordant way. In dark adapted conditions, b-wave implicit time is reduced in the purely scotopic response in comparison to the control subject. This points to an impairment of the scotopic system, since the difference to the control subject diminishes with increased stimulus intensity. Temporal processing within the photopic (flicker) responses appears again discordant. In the PKU patient, slow flicker b-waves are faster, fast flicker b-waves are slower than in the healthy control. These findings may support a possible deficit of temporal retinal signal processing in patients with PKU. A deficit in temporal processing has also been suggested for patients with Parkinson's disease in some studies [33,34], whereas others did not find evidence for this [35].

An additional aspect that has been postulated to contribute to subtle deficits in contrast sensitivity in PKU patients is the age at which the dietary treatment was started in the neonatal period. This has been addressed by Diamond and colleagues [36]. These authors state that in addition to dopamine depletion, high phenylalanine levels in the first 2 weeks of life may cause lasting impairments of the developing visual system. This aspect was not part of our study concept and our data were not detailed enough for the neonatal period to test this third hypothesis.

4.2. LCPUFAs depletion hypothesis

This hypothesis proceeds on the assumption that deficient dietary intake of LCPUFAs by PKU patients [7] causes changes in retinal photoreceptors. Protein rich foods must be avoided by PKU patients and these foods are particularly rich in LCPUFAs. Outer segments of rods contain high concentrations of LCPUFAs [6], and insufficient dietary

intake may result in impairments of visual functions. In animal models LCPUFAs depletion was shown to interfere with the normal metabolism of photoreceptor membranes and resulted in alterations of ERG responses in some studies [25]. LCPUFAs depletion should presumably concern in particular visual functions based on rod functioning, e.g. dark-adapted electroretinogram. However, we did not find reports on systematic evaluation of colour vision, contrast sensitivity or FDT in association with LCPUFAs depletion. In a study on the effects of long-term parenteral nutrition on patients' visual functions, low LCPUFA levels and abnormalities in flash electroretinograms were observed in a considerable number of patients [37].

In our study we found impairment of a broad spectrum of visual functions in patients with PKU, including functions mediated mainly by rods, as well as functions mediated predominantly by cones. We did not find an association between docosahexaenoic acid levels and any of the variables of visual function. Therefore, no evidence for the LCPUFAs depletion hypothesis was found. It has previously been reported that delayed latencies in VEPs of PKU patients improved significantly after 3 months of LCPUFA supplementation [38]. This could be attributed to a generally positive effect of LCPUFAs on retinal function, regardless of an underlying deficiency, as a benefit of LCPUFAs has also been postulated in ischemia-, inflammation- or age-associated retinal dysfunction [39].

5. Conclusions

Evaluating a broad spectrum of visual functions and their relation to phenylalanine-levels, docosahexaenoic acid levels and IQ in early-treated patients with phenylketonuria, our study provides new information on the pathophysiology of subtle impairments of cerebral functions despite early treatment in PKU.

PKU patients show significant differences from healthy controls in contrast sensitivity, colour vision, and ERG. Our data are in favour of the dopamine depletion hypothesis, postulating that the imbalance between phenylalanine and tyrosine may negatively affect dopamine levels in PKU patients and that decreased concentrations of dopamine in the retina are causal for changes in visual perception. This is supported by the similar patterns of visual functions in patients with phenylketonuria observed in our study and patients with Parkinson's disease. No evidence for the LCPUFAs depletion hypothesis as explanation of these impairments was found in our data.

Competing interest

None.

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Presentation of results

Preliminary data of this study were presented in an oral presentation at the annual meeting of the Society for the Study of Inborn Errors of Metabolism (SSIEM) 2011 in Geneva, Switzerland and the annual meeting of the German working group for Paediatric Metabolic Disturbances (Arbeitsgemeinschaft für Pädiatrische Stoffwechselstörungen, APS) 2012 in Fulda, Germany.

Authors' contributions

G. Gramer: Study design, principal investigator, recruitment and examination of patients/controls, evaluation and interpretation of data, drafting and writing the manuscript

B. Förl: Study design, examination of patients/controls, revision of the manuscript

C. Springer: Examination of patients/controls, revision of the manuscript

P. Weimer: Examination of patients/controls, evaluation of electrophysiology results, revision of the manuscript

G. Haege: Statistical analysis, evaluation and interpretation of results, writing and revision of the manuscript

F. Mackensen: Examination of patients/controls, revision of the manuscript

E. Müller: Evaluation of questionnaires on diet and amino acid mixtures, revision of the manuscript

H.E. Völcker: Advice on study design, revision of the manuscript

G.F. Hoffmann: Advice on study design, revision of the manuscript

M. Lindner: Study design, revision of the manuscript

H. Krastel: Study design, examination of patients/controls, evaluation of electrophysiology results, interpretation of data, writing and revision of the manuscript

P. Burgard: Study design, statistical analysis, evaluation and interpretation of data, writing and revision of the manuscript

Conflicts of interest

G. Gramer received support for travel expenses to a scientific meeting from Merck Serono.

B. Förl, C. Springer, P. Weimer, G. Haege, E. Müller and H.E. Völcker report no disclosures.

H. Krastel is ophthalmic consultant of Oculus Optik Geräte, Wetzlar and of Roland Consult, Brandenburg and received lecture and consultant fees.

F. Mackensen is member of advisory boards/received lecture fees from Abbott, Allergan, Esba Tech, Novartis, Merck Serono and Lux Biosciences.

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