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Neurocognitive and neuroimaging outcome of early treated young adult PKU patients: A longitudinal study

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ABSTRACT

The aim of the study was to explore the outcome of neurocognitive deficits and neuroimaging correlates in young adult early treated phenylketonuric (PKU) patients. We conducted a longitudinal study of 14 PKU patients that were assessed for IQ and neuropsychological functioning including executive functions (EF) over 14 years of follow-up (age range at 1st and 2nd assessments were 7.8–13.5 and 22.2–27.7 years, respectively). The IQ of all 14 PKU patients was within the normal range. With respect to the 1st assessment, mean IQ at follow-up did not decrease significantly. Compared to control subjects (n = 14), mean IQ of patients was significantly lower (p = .0005). Throughout adolescence and early adulthood there was an improvement of neuropsychological functioning of PKU patients in spite of the relaxation of diet, however some deficits were still detectable when compared to controls. All patients that underwent a second MRI scan showed white matter alterations ranging from mild to severe which was correlated neither with IQ nor with EF scoring. Cognitive, neuropsychological and neuroimaging outcome was influenced from life-long and/or second decade of life metabolic control. Nevertheless patients' developmental trajectories were in some cases independent from metabolic control. Our results support the hypothesis of an individual vulnerability to phenylalanine. However, as long as individual factors that account for the vulnerability to Phe are not recognized, strict dietary control is recommended for all the patients also in the second decade of life.

1. Introduction

Despite the favourable clinical outcome of early treated phenylketonuric (PKU; OMIM #261600) patients when compared with late- or untreated patients, a lower than expected intelligence quotient (IQ) and minor neuropsychological and psychiatric problems [1–5] remain challenging aspects of the disease and the possible targets for future improvement of the treatment.

Moreover, in the last 20 years several studies have showed that for almost all early treated PKU (ETPKU) subjects, brain Magnetic Resonance Imaging (MRI) shows white matter (WM) alterations in the second decade of life [6]. The relationship (if any) between this neuroimaging alterations and neuropsychological disorders remains controversial.

Diet remains the mainstay of the treatment for PKU patients. Current literature and available guidelines recommend life-long treatment [7,8]. Nevertheless the restrictive diet for PKU causes a psychosocial burden for the patients and their families. During childhood adherence to the diet lies on parents, but as children get older staying in the target phenylalanine (Phe) levels might become difficult. From adolescence on compliance with the diet is often poor indeed [9–11], disclosing the need of PKU patients for alternative therapies [12].

In the present knowledge there is a lack of longitudinally designed studies assessing the outcome of neuropsychological and neuroimaging abnormalities in PKU patients. About 14 years ago we have performed a case–control study focusing on neuropsychological functioning in young early and continuously treated PKU patients with normal IQ and brain MRI [13]. In comparison with controls, we have found an impairment in a number of tests exploring neuropsychological functioning with special focus on executive functions (EF). With the present longitudinal study we intended to explore the neurocognitive outcome and

Abbreviations: PKU, phenylketonuria; Phe, phenylalanine; EF, executive function; WAIS-R, Wechsler Adult Intelligence Scale-Revised; EPMT, Elithorn's Perceptual Maze Test; WCST, Wisconsin Card Sorting Test; IDC, Index of Dietary Control; MRI, Magnetic Resonance Imaging; WM, white matter.

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neuroimaging correlates in the same small group of ETPKU subjects over 14 years of follow-up and the factors possibly affecting them.

2. Patients and methods

2.1. Patients

All ETPKU patients (n = 14; 12 females, 2 males; mean age 24.97 years, SD 1.57; range 22.2–27.7) that took part of the previous study [13] were enrolled in the present study. All the patients were affected by PKU due to phenylalanine hydroxylase (PAH) deficiency: 9 patients with classic and 5 with mild PKU, defined as blood Phe at diagnosis or under free diet persistently > 1200 μM and between 600 and 1200 μM respectively.

Performances on neuropsychological and cognitive assessment of PKU patients were compared to those of 14 healthy subjects of comparable age (mean age 23.73 years, SD 2.59; range 21.0–28.2) and sex (12 females, 2 males). Control subjects enrolled in this study are not the same 14 subjects examined in the previous study [13]. Patients' academic achievement at the time of the 2nd evaluation was also chosen as an outcome measure.

2.2. Metabolic control measures

Various measures of metabolic control were taken into account: concomitant Phe level of the day of testing (PHED); the life-long Index of Dietary Control (IDC0-2), calculated as the mean of all yearly medians from the beginning of the dietary treatment to the day of the 2nd assessment; the IDC during the interval between the two evaluations (IDC1-2); the IDC from the beginning of the diet to the day of the 1st assessment (IDC0-1); the IDC from the beginning of the diet to the fourth year of life (PHE04), as index of early dietary control quality.

2.3. Cognitive and neuropsychological assessment

Patients and controls were assessed for IQ using the Wechsler Adult Intelligence Scale-Revised (WAIS-R). At the 1st assessment IQ was measured with the Wechsler Intelligence Scale for Children-Revised (WISC-R). As for the EF, a battery of tests was chosen on the basis of the results from the previous study. Patients and controls were resubmitted the following tests: a) *Wisconsin Card Sorting Test (WCST)* — this test assesses the ability to form abstract concepts and to shift cognitive set following changes in conceptual rules; b) *Elithorn's Perceptual Maze Test (EPMT)* — this is a test for spatial planning in which visual-spatial and sustained-attention skills can also be evaluated; c) *Rey Figure Test — with copy and from memory*, this test calls into play sustained attention, planning and visual organization of complex data, as well as visual memory; d) *Tower of London (ToL)* — this test gives a measure of planning and organization (for more details about the tests see [13]).

Since neuropsychological testing consisted of several tests and subtests, the results were condensed in two composite scores: EF1, which was the sum of the scores on EPMT, ToL, Rey Figure Test with copy and from memory; EF2, reflecting performances on WCST, computed as the sum of differential score on each WCST subtest, with reference to the score corresponding to the 50th centile of normative data.

2.4. Neuroimaging

Thirteen out of 14 patients underwent brain MRI, which was performed according to the same protocol adopted for the previous study (Fast Spoiled Gradient Recalled (FSPGR) T1-weighted, Fluid-Attenuated Inversion Recovery (T2-FLAIR), Fast Spin Echo (FSE) T2-weighted, T2-short echo proton density) and scored according to the already published criterion [14]. The severity score included: i) the evaluation of the white matter signal alteration on T2-weighted sequences for each localization, rated from 0 (normal) to 4 (markedly increased signal); ii) the number of localizations in cerebral white matter (one more point for each localization); iii) the presence of confluent versus patchy high-signal intensity areas (one more point in case of patchy lesions); iv) the quality of the signal in T1-weighted sequences (one more point in case of decrease). In short, the higher the score the more severe was the involvement of WM on MRI, so that 0 means no involvement and 15 denotes a diffuse and profound WM alteration.

2.5. Statistical analysis

Quantitative data are presented as mean ± standard deviation (SD), while categorical data are summarized by absolute and percent frequencies. Comparisons between independent groups of subjects (i.e., PKUs *vs* controls; PKUs with good *vs* poor IDC; PKUs with good IDC *vs* controls) were performed using Mann–Whitney *U* test and Fisher's exact probability test for quantitative and categorical variables, respectively. For all comparisons, uncorrected p values are reported (N.B.: should Bonferroni's correction be applied, the pairwise p-value would be $p \leq .025$, to take into account the involvement of the control subgroup into two comparisons for any outcome variable, namely *vs* PKUs and *vs* PKUs with good IDC). Comparisons between paired groups of subjects (PKUs at 1st *vs* 2nd assessment) for quantitative variables were performed by Wilcoxon test. Correlation between IQ and EPMT or EF2 was estimated by Spearman's correlation coefficient. The effect of dietary control at different stages of subject life on functional and MRI measures was evaluated by multiple linear regression analysis. The variance inflation factor was computed for any explanatory variable in each model: in case of values greater than 5 (suggesting multicollinearity), the analysis was repeated excluding the highly correlated variables. When significantly different from 0, regression coefficients were reported together with 95% Confidence Interval (95%CI). P values were two-sided in all statistical analyses.

Statistical analyses were performed using STATA Statistical Software (Release 8.1).

After the nature and possible consequences of the study were fully explained, informed written consent was obtained. The local ethics committee gave consent to the study protocol.

3. Results

3.1. Cognitive and neuropsychological assessment

Table 1 summarizes demographic features and IDCs of PKU patients. After a mean of 14.15 years (range 13.5–14.5), IQ of all 14 patients was within the normal range (81–117), defined as 100 ± 2SD. With respect to the 1st assessment, mean IQ at follow-up decreased by 4.5 points, which was not statistically significant (Tables 2 and 3). IQ of 8 patients remained stable (with a delta score ranging from −4 to +2 IQ points). Five patients worsened their global cognitive performances with a loss of IQ points ranging from 6 to 28 points. Only one patient had an improvement of cognitive abilities with an increase of 12 IQ points. As for academic achievement, all 14 patients were High School graduates; 5 of them were attending University courses, 3 had a Bachelor's Degree, and 2 had a Master's Degree (Table 1). Compared to the 14 healthy peers of the control group, mean IQ of PKU patients was significantly lower (p = .0005) (Table 4). The same comparison between patients' and controls' global cognitive performances at the time of the 1st evaluation [13] cannot be done because controls subjects were IQ matched.

The within-group comparison of patients that have had a good (≤600 μM; n = 5) *vs* a poor (>600 μM; n = 9) quality of dietary control for the period in between the two evaluations (IDC1-2) disclosed a significantly lower IQ in patients with worse metabolic control (105.8 ± 8.8 *vs* 93.1 ± 9.2; p = .0327). Four out of the 5 patients with IDC1-2 ≤ 600 μM kept stable or improved their IQ (with a delta score

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