Molecular Genetics and Metabolism Reports 20 (2019) 100498

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Determining factors of the cognitive outcome in early treated PKU: A study of 39 pediatric patients

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ARTICLE INFO

Keywords:
Phenylketonuria
PKU
Intellectual quotient
Cognitive outcome
Multivariate regression

ABSTRACT

Phenylketonuria (PKU) is a disorder of phenylalanine metabolism, characterized by a neurotoxic phenylalanine (Phe) accumulation, and treatable with a life-long Phe-restricted diet. Though early and continuously treated PKU (ETPKU) patients exhibit normal IQ, their cognitive outcome remains suboptimal. In this longitudinal study, we aimed at assessing the determinants of IQ subscales and quality of metabolic control in ETPKU children.

We collected blood Phe levels, numbers of blood samples for Phe determination, parents' socio-professional categories and school achievement data of 39 classical and moderate ETPKU patients who underwent two cognitive evaluations performed by the same neuropsychologist (at 6.5 and 10y of mean age). We then sought to evaluate the determinants of 1) the changes in their IQ between the two testings (delta IQ) and 2) the quality of metabolic control (evaluated by the median Phe levels during the year before the second test) with multivariate regression analysis.

Though in the normal range, mean total IQ slightly decreased between the two evaluations, and we observed a better verbal than performance outcome. Modeling the determining factors of the delta IQ, we found a significant influence of the number of blood samples ($\beta = 0.46$, 95%CI = 0.13 to 0.79, $p < 0.01$) and the moderate type of PKU ($\beta = 12.40$, 95%CI = 3.69 to 21.11, $p < 0.01$) on verbal outcome. We failed to find any determining factors that would statistically influence metabolic control.

In conclusion, ETPKU cognitive outcome is influenced by a network of metabolic and environmental factors, which is not reflected by the sole metabolic control.

1. Introduction

Phenylketonuria (PKU; MIM#261600) is an inherited, autosomal recessive, disorder of phenylalanine (Phe) metabolism responsible for a neurotoxic elevation of blood Phe levels. The discovery of phenylalanine hydroxylase (PAH) deficiency in the early 1950's [1] led to the major means of treatment based on a strict restriction of dietary Phe intake [2] and, subsequently, to the current general newborn screening (NBS) program for congenital diseases [3].

PKU is a treatable disease: early and continuously treated PKU (ETPKU) patients maintained under a strict dietary observance until adolescence are expected to have a normal intellectual quotient (IQ) [4]. A well-obeyed Phe-restricted diet efficiently prevents the severe intellectual disability inherent to untreated PKU; tetrahydrobiopterin (BH_4), the cofactor of PAH is sometimes used as an additional treatment [5]. Blood Phe is considered as the best predictor for the IQ outcome and for guiding treatments [6].

However, the pathophysiology of PKU is not fully understood. Recent data suggest that Phe concentration in brain could result in dopamine and serotonin deficit, leading to brain damage and progressive neuropsychiatric impairment in adult PKU patients [7]. Despite early dietary treatment and adequate compliance, there is growing evidence of suboptimal neurocognitive outcomes in ETPKU patients, underlying the difficulties in predicting the outcomes in PKU. Brain imaging studies in ETPKU patients show white matter anomalies despite appropriate treatment compliance [8]. Recent studies reveal lower IQ scores compared to healthy controls, as well as attention and mood problems in children [9] and adult patients [10]. Additionally, a

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https://doi.org/10.1016/j.ymgmr.2019.100498
Received 19 July 2019; Accepted 19 July 2019
Available online 25 July 2019

Table 1
Description of the cohort.

		Total	c-PKU	m-PKU	Non-compliant	Compliant	Uncontrolled	Controlled
Size		39	23	16	9	30	23	16
Yearly no. of blood samples (mean ± SD)		20.26 (± 11.64)	21.08 (± 13.00)	17.88 (± 9.97)			18.57(± 9.64)	22.69 (± 14.01)
Metabolic control in µmol/L (mean ± SD)		366.50 (± 176.87)	383.77 (± 185.87)	341.67 (± 165.72)	378.04 (± 173.97)	363.04 (± 180.52)		
Type of PKU	c-PKU	23 (58.97%)			6 (66.67%)	17 (56.67%)	13 (56.52%)	10 (62.50%)
	m-PKU	16 (41.03%)			3 (33.33%)	13 (43.33%)	10 (43.48%)	6 (37.50%)
Sex	Male	17 (43.59%)	10 (43.48%)	7 (43.75%)	5 (55.56%)	12 (40.00%)	9 (39.13%)	8 (50.00%)
	Female	22 (56.41%)	13 (56.52%)	9 (56.25%)	4 (44.44%)	18 (60.00%)	14 (60.87%)	8 (50.00%)
Learning disability [n (%)]		7 (17.95%)	4 (17.39%)	3 (18.75%)	4 (44.44%)	3 (10.00%)	4 (17.39%)	3 (18.75%)
No. of repeated years [n (%)]	No	31 (79.49%)	17 (73.91%)	14 (87.5%)	5 (55.56%)	26 (86.67%)	19 (82.61%)	12 (75.00%)
	1 year	7 (17.95%)	6 (26.09%)	1 (6.25%)	4 (44.44%)	3 (10.00%)	4 (17.39%)	3 (18.75%)
	2 years	1 (2.56%)	0	1 (6.25%)	0	1 (3.33%)	0	1 (6.25%)
Mother's SPC [n (%)]	Unemployed/retired	9 (23.08%)	8 (34.78%)	1 (6.25%)	2 (22.22%)	7 (23.33%)	6 (26.09%)	3 (18.75%)
	Middle profession	30 (76.92%)	15 (65.22%)	15 (93.75%)	7 (77.78%)	23 (76.67%)	17 (73.91%)	13 (81.25%)
	Superior profession	0	0	0	0	0	0	0
Father's SPC [n (%)]	Unemployed/retired	3 (7.69%)	3 (13.04%)	0	2 (22.22%)	1 (3.33%)	1 (4.35%)	2 (12.50%)
	Middle profession	31 (79.49%)	20 (86.96%)	11 (68.75%)	7 (77.78%)	24 (80.00%)	18 (78.26%)	13 (81.25%)
	Superior profession	5 (12.82%)	0	5 (31.25%)	0	5 (16.67%)	4 (17.39%)	1 (6.25%)
BH_4 supplementation [n (%)]	Yes	5 (12.82%)	0	5 (31.25%)	0	5 (16.67%)	2 (8.70%)	3 (18.75%)
	No	34	23 (100.00%)	11	9 (100%)	25 (83.33%)	21 (91.30%)	13 (81.25%)
Test 1	mean age (± SD)	6.50 ± 0.61	6.47 ± 0.68	6.54 ± 0.51	6.27 (± 0.60)	6.57 (± 0.60)	6.62 (± 0.67)	6.32 (± 0.47)
	WPPSI-R [n (%)]	16 (41.03%)	10 (43.48%)	6 (37.50%)	6 (66.67%)	10 (33.33%)	11 (47.83%)	5 (31.25%)
	WPPSI-III [n (%)]	22 (56.41%)	13 (56.52%)	9 (56.25%)	3 (33.33%)	19 (63.33%)	11 (47.83%)	11 (68.75%)
	WISC-III [n (%)]	1 (2.56%)	0	1 (6.25%)	0	1 (3.33%)	1 (4.35%)	0
	TIQ1 (mean ± SD)	98.28 (± 14.44)	92.22 (± 13.00)	107 (± 11.97)	91.89 (± 16.48)	100.2 (± 13.49)	98.13 (± 15.27)	98.5 (± 13.65)
	VCI1 (mean ± SD)	100.13 (± 15.67)	93.65 (± 15.26)	109.44 (± 11.14)	99.89 (± 15.32)	100.2 (± 16.04)	100.35 (± 17.42)	99.81 (± 13.31)
	PIQ1 (mean ± SD)	98.64 (± 13.10)	95.01 (± 12.84)	103.75 (± 12.09)	89.67 (± 15.44)	101.33 (± 11.26)	97.43 (± 12.34)	100.38 (± 14.36)
Test 2	mean age (± SD)	9.64 ± 0.64	9.72 ± 0.60	9.52 ± 0.71	9.87 (± 0.40)	9.87 (± 0.69)	9.60 (± 0.71)	9.69 (± 0.55)
	WISC-III [n (%)]	15 (38.46%)	9 (39.13%)	6 (37.50%)	6 (66.67%)	9 (30.00%)	11 (47.83%)	4 (25.00%)
	WISC-IV [n (%)]	24 (61.54%)	14 (60.87%)	10 (62.50%)	3 (33.33%)	21 (70.00%)	12 (52.17%)	12 (75.00%)
	TIQ2 (mean ± SD)	95.36 (± 17.32)	87.74 (± 14.66)	106.31 (± 15.09)	87.89 (± 15.14)	97.6 (± 17.53)	91.52 (± 17.70)	100.88 (± 15.65)
	VCI2 (mean ± SD)	102.44 (± 17.96)	93.87 (± 15.09)	114.75 (± 14.45)	94.89 (± 15.33)	104.7 (± 18.29)	99.09 (± 18.56)	107.25 (± 16.43)
	PIQ2 (mean ± SD)	95.97 (± 15.92)	91.00 (± 15.60)	103.13 (± 13.88)	88.78 (± 16.14)	98.13 (± 15.47)	92.13 (± 16.70)	101.50 (± 13.34)
ΔIQ	ΔTIQ (mean ± SD)	(−)2.92 (± 14.03)	(−)4.48 (± 15.61)	(−)0.69 (± 11.48)	(−)4 (± 10.94)	(−)2.6 (± 14.98)	(−)6.61 (± 12.99)	2.38 (± 14.15)
	ΔVCI (mean ± SD)	2.31 (± 13.68)	0.22 (± 14.75)	5.31 (± 11.78)	5 (± 11.74)	4.5 (± 13.62)	(−)1.26 (± 12.96)	7.44 (± 13.42)
	ΔPIQ (mean ± SD)	(−)2.67 (± 14.06)	(−)4.09 (± 15.00)	(−)0.63 (± 12.79)	0.89 (± 15.43)	(−)3.2 (± 13.86)	(−)5.30 (± 14.70)	1.13 (± 12.59)

Metabolic control: mean and SD of median Phe levels; SPC: Socio-Professional Category; PKU: Phenylketonuria; m-PKU: moderate PKU; c-PKU: classical PKU; BH_4: tetrahydrobiopterin; IQ: Intellectual Quotient; TIQ: Total IQ; PIQ: performance IQ; VCI: Verbal Comprensive Index.

notably impaired executive function in ETPKU patients has been well documented [11]. A recent review summarizes neuropsychological findings in ETPKU patients [12]. In response to these findings, recent guidelines recommend life-long Phe-restricted diet and new treatment targets for ETPKU patients [13].

In this longitudinal study performed in 39 ETPKU patients who underwent 2 cognitive evaluations at 6.5 and 10 years of age (on average), our aim was to assess the major determinants of 1) the changes in IQ between the two evaluations and 2) the median of blood Phe levels (reflecting metabolic control). We hypothesized that the type of PKU (classic or moderate) and the patient's environment (of which the number of blood samples for Phe determination and parents' socio-professional [SPC] category) could influence the IQ outcome along with the quality of metabolic control. We conducted an analysis of the relationship between the IQ variations and metabolic, environmental and academic achievement data on the one hand. On the other hand, we analyzed the relationship between the quality of metabolic control and the type of PKU, the patient's environment, academic achievement and neurocognitive evaluation results.

2. Materials and methods

2.1. Data record

2.1.1. Patients

Thirty-nine patients were recruited in the Reference Center for Inherited Metabolic Disorders of the Robert Debré University Hospital, Paris, from 1996 to 2012. The ethics committee of Robert Debré University Hospital (APHP, 75019 Paris, France) approved the study. All procedures conformed to ethical standards. Informed consent was obtained from parents.

Patients were neonatally screened according to the NBS program in France and then early and continuously treated from the neonatal period immediately at the time of NBS results. The traditional classification was employed to determine the type of PKU: classical PKU (c-PKU), defined by blood Phe levels at screening > 1200 μmol/L, and moderate PKU (m-PKU) defined by blood Phe levels at screening between 600 and 1200 μmol/L. Mild hyperphenylalaninemia patients (300–600 μmol/L) were excluded. A BH_4 loading test was performed for each patient at screening and consisted of an administration of 20 mg/kg of BH_4 followed by regular measurements of Phe levels at T0, T2, T4, T6, T8 and T24 hours after BH_4 administration [14]. An average supplementation of 10 mg/kg/day of BH_4 was given in case of Phe decrease ≥30% at T24. In total, 5 patients received a BH_4 supplementation at the time of the study. BH_4 metabolism defects were excluded by the DHPR erythrocyte activity measurement and urinary pterins profile. Molecular sequencing of *PAH* was not performed.

2.1.2. Neurocognitive assessment

Total IQ (TIQ), performance IQ (PIQ) and verbal comprehension index (VCI) were assessed for the 39 patients at two different ages by the same neuropsychologist: between 5 and 7 years for the first test and between 8 and 10 years for the second test (at 6.5 and 10 years of mean age respectively).

For the first test, the Wechsler Preschool and Primary Scale for Intelligence-Revised (WPPSI-R) [15] and third edition [16], together with the Wechsler Intelligence Scale for Children (WISC) third edition [17], were used. For the second test, the WISC-III and fourth edition [18] were used.

No control group was constituted, as all tests are standardized and follow a normal distribution with 100 as the mean and a standard deviation (SD) of ± 15.

2.1.3. Metabolic control measures

Blood Phe levels were determined on dried blood spots. The number of blood samples and the median of Phe levels were recorded during the year preceding the second cognitive evaluation. The number of blood samples was used as an indication of the compliance. French recommendations at the moment of the study recommended one blood sample per month for patients younger than 10 years of age. Target therapeutic blood Phe concentration was below 300 μmol/L according to French recommendations prior to the recent European guidelines [13]. Metabolic control was computed as the median Phe levels during the year before the second test.

Complete metabolic, environmental, academic achievement and neurocognitive data are presented in Table 1. The parents SPC are presented in Supplementary Data. Learning disabilities were defined as a neurodevelopmental disorder that impedes the ability to learn or use specific academic skills (e.g., reading, writing, or arithmetic) and were documented by the psychologist on the basis of her own evaluation and school reports. As a guide, patients are grouped in c-PKU vs m-PKU, controlled (median of Phe levels < 300 μmol/L) vs uncontrolled (median of Phe levels > 300 μmol/L) and compliant (number of blood samples > 12) vs non-compliant (number of blood samples < 12) patients. Due to the small number of participants, we avoid comparisons in the table with descriptive statistics.

2.2. Data analysis

For each patient, we calculated the delta TIQ (ΔTIQ = TIQ2-TIQ1), the delta VCI (ΔVCI = VCI2-VCI1) and the delta PIQ (ΔPIQ = PIQ2-PIQ1), as the variation of IQ between the first and the second test. We performed a multiple regression analysis to explain the relationship between the delta IQ as dependent variable and the metabolic control, the sex, the type of PKU, the environment (parents' SPC and compliance) and academic achievement data as independent variables. Univariate linear regressions were first used and then stepwise multivariate linear regression with backward elimination by Akaike information criterion (AIC).

We also analyzed the relationship between the quality of metabolic control assessed by the median of Phe levels during the year preceding the second neurocognitive test as dependent variable, and the type of PKU, the sex, neurocognitive, environment and academic achievement data as independent variables, with a second multiple regression analysis. Again, univariate linear regressions were first used and then stepwise multivariate linear regression with backward elimination by AIC.

A 0.05 α-error level was used for testing the statistical significances. Statistical analyses were performed with R software, version 3.3.2.

3. Results

As a whole, when comparing the two tests, there was a slightly negative ΔTIQ and ΔPIQ, and positive ΔVCI though IQ and IQ subscales remained in the normal range (Table 1). Boxplots of IQ and IDC in the different groups are presented in Supplementary Data.

The delta IQ was analyzed as a dependent variable. According to the cohort description, we selected the median of Phe levels and the number of blood samples during the year before the second test, the mother and father's SPC, the sex, the occurrence of learning disabilities, the number of repeated school years and the type of PKU as independent variables of interest. BH_4 supplementation was excluded from the multiple regressions due to its collinearity with the type of PKU. As a major known determinant of the IQ outcome in PKU, the median of Phe levels during the year preceding the second test was forced into the model. Regression coefficients, their 95% confidence interval and their p-values are presented in Tables 2 and 3.

The independent variables were not significantly correlated with the ΔTIQ at the univariate linear regression and after the stepwise multivariate linear regression. The mother's SPC showed a negative influence on the ΔTIQ (β = −10.42, 95%CI = −21.44 to 0.59, p = 0.063), whereas the m-PKU type (β = 8.31, 95%CI = −0.68 to

Table 2
Determinants of the cognitive outcome after regression analysis.

Univariate linear regression					Stepwise multivariate linear regression				
Variable	β	95% confident interval		P-Value	Variable	β	95% confident interval		P-Value
		Lower limit	Upper limit				Lower limit	Upper limit	
ΔTIQ									
Yearly no. of blood samples	0.3603327	−0.0226001	0.7432655	0.06	Yearly no. of blood samples	0.3314765	−0.0476878	0.7106407	0.08
Yearly median of blood Phe levels	−0.0081772	−0.0344541	0.0180998	0.53	Yearly median of blood Phe levels	−0.0099193	−0.0345494	0.0147108	0.42
Mother's SPC	−8.5666667	−19.1349912	2.0016578	0.11	Mother's SPC	−10.4235261	−21.4398111	0.5927590	0.06
Father's SPC	−2.1720779	−12.3957977	8.0516418	0.67	Type of PKU (m-PKU)	8.3065900	−0.6791796	17.2923597	0.07
Type of PKU (m-PKU)	3.7907609	−5.5004232	13.0819449	0.41	No. of repeated school years	8.4612041	−0.2251084	17.1475165	0.06
Sex (male)	−2.6390374	−11.8984810	6.6204062	0.57	Intercept	−3.3444989	−21.5672534	14.8782556	0.71
No. of repeated school years	7.3189655	−2.0100072	16.6479383	0.12					
Learning disabilities	−0.7901786	−12.8056534	11.2252962	0.89					
ΔVCI									
Yearly no. of blood samples	0.5462405	0.1997677	0.8927133	< ***0.01*** **	Yearly no. of blood samples	0.4626103	0.1313478	0.7938729	< ***0.01*** **
Yearly median of blood Phe levels	−0.0146036	−0.0399024	0.0106952	0.25	Yearly median of blood Phe levels	−0.0112829	−0.0329748	0.0104089	0.30
Mother's SPC	−8.5555556	−18.8432764	1.7321653	0.10	Mother's SPC	−8.8237161	−18.3760040	0.7285718	0.07
Father's SPC	−3.6233766	−13.5451926	6.2984393	0.46	Father's SPC	−7.4339333	−16.6914317	1.8235651	0.11
Type of PKU (m-PKU)	5.0951087	−3.8899224	14.0801398	0.26	Type of PKU (m-PKU)	12.4020775	3.6934828	21.1106722	< ***0.01*** **
Sex (male)	−2.3181818	−11.3555284	6.7191647	0.61	Learning disabilities	−7.6203668	−17.1581768	1.9174431	0.11
No. of repeated school years	−1.0948276	−10.4907587	8.3011035	0.81	Intercept	7.9544282	−8.6747486	24.5836049	0.34
Learning disabilities	−10.9955357	−22.1286542	0.1375828	0.05					
ΔPIQ									
Yearly no. of blood samples	0.2990752	−0.0907695	0.6889199	0.13	Yearly no. of blood samples	0.3581242	−0.0223314	0.7385799	0.06
Yearly median of blood Phe levels	−0.0089074	−0.0352289	0.0174141	0.50	Yearly median of blood Phe levels	−0.0022545	−0.0274430	0.0229340	0.86
Mother's SPC	−5.7777778	−16.5836939	5.0281383	0.29	Father's SPC	−7.6362880	−18.8302239	3.5576478	0.17
Father's SPC	−5.4025974	−15.5203955	4.7152007	0.29	Type of PKU (m-PKU)	8.8083465	−1.4644305	19.0811235	0.09
Type of PKU (m-PKU)	3.4619565	−5.8686312	12.7925442	0.46	No. of repeated school years	8.3522570	−0.7674572	17.4719711	0.07
Sex (male)	−3.7192513	−12.9624561	5.5239534	0.42	Intercept	−6.6079398	−23.4560137	102,401,341	0.43
No. of repeated school years	8.4051724	−0.8485148	17.6588596	0.07					
Learning disabilities	3.5982143	−8.3924106	15.5888392	0.55					

SPC: Socio-Professional Category; PKU: Phenylketonuria; m-PKU: moderate PKU; IQ: Intellectual Quotient; TIQ: Total IQ; PIQ: performance IQ; VCI: Verbal Comprensive Index;
** $P < 0.01$.

Table 3
Determinants of the metabolic control (median of yearly blood Phe levels) after regression analysis.

Univariate linear regression					Stepwise multivariate linear regression				
Variable	β	95% confident interval		P-Value	Variable	β	95% confident interval		P-Value
		Lower limit	Upper limit				Lower limit	Upper limit	
Yearly no. of blood samples	−0.0125865	−0.0960635	0.0708905	0.76	Mother's SPC	−1.677778	−3.888186	0.5326308	0.13
Mother's SPC	−1.6777778	−3.8881864	0.5326308	0.13	Intercept	8.892654	6.545328	11.2399788	0
Father's SPC	0.6448052	−1.4790524	2.7686628	0.54					
Type of PKU (m-PKU)	−0.6953804	−2.6345238	1.2437629	0.47					
Sex (male)	−0.7002674	−2.6233727	1.2228379	0.47					
No. of repeated school years	−0.1439655	−2.1516564	1.8637254	0.89					
Learning disabilities	0.4218750	−2.0773240	2.9210740	0.73					
QIT1	−0.0395403	−0.1056241	0.0265435	0.23					
ICV1	−0.0256546	−0.0871557	0.0358465	0.40					
QIP1	−0.0427046	−0.1155952	0.0301861	0.24					

SPC: Socio-Professional Category; PKU: Phenylketonuria; m-PKU: moderate PKU; IQ: Intellectual Quotient; TIQ: Total IQ; PIQ: performance IQ; VCI: Verbal Comprensive Index;

17.29, p = 0.069) and the number of repeated school years (β = 8.46, 95%CI = −0.23 to 17.15, p = 0.056) had a positive influence on the ΔTIQ (Table 2). Considering the ΔVCI, the yearly number of blood samples before the second test showed a significant positive influence from the univariate linear regression (β = 0.55, 95%CI = 0.20 to 0.89, p < 0.01) (Table 2). After the multivariate regression, the yearly number of blood samples before the second test (β = 0.46, 95%CI = 0.13 to 0.79, p < 0.01) and the m-PKU type (β = 12.40, 95%CI = 3.69 to 21.11, p < 0.01) had a positive and statistically significant influence on the ΔVCI. The mother and father's SPC had a negative nonsignificant influence on ΔVCI, with a lower p for the mother's SPC (β = −8.82, 95%CI = −18.38 to 0.73, p = 0.069) (Table 2). Considering the ΔPIQ, the independent variables were not significantly correlated with the ΔPIQ at the univariate regression and neither after the stepwise multivariate regression. The father's SPC displayed after stepping a negative insignificant influence on ΔPIQ (β = −7.64, 95%CI = −18.83 to 3.56, p = 0.17) whereas the number of repeated school years had a trend towards positive influence (β = 8.35, 95%CI = −0.77 to 17.41, p = 0.071) (Table 2). For the ΔTIQ, the ΔVCI and the ΔPIQ, the yearly metabolic control before the second test had a negative but not significant influence.

As the influence of BH_4 supplementation on the delta IQ could not be modeled due to its collinearity with the PKU group, we assessed its influence on the delta IQ adjusted for the metabolic control (median of Phe levels during the year preceding the second test) in the m-PKU group only in a separated multivariate regression without stepping (Table 4). We observed a trend towards positive influence of BH_4 on ΔVCI (β = 14.20, 95%CI = −0.86 to 29.27, p = 0.063), whereas ΔPIQ was negatively but not significantly correlated with the BH_4 supplementation (β = −10.40, 95%CI = −28.04 to 7.24, p = 0.22).

The median of Phe levels during the year preceding the second evaluation was then analyzed as a dependent variable. The number of blood samples during the year before the second test, the mother and father's SPC, the sex, the occurrence of learning disabilities, the number of repeated school years, the type of PKU and the TIQ, VCI and PIQ at the first test were selected as independent variables of interest. We did not observe a statistically significant correlation between these variables and the median of Phe levels neither at the univariate regression nor at the stepwise multivariate regression (Table 3). After stepping, only the mother's SPC was retained in the model, with a negative but not significant regression coefficient.

Table 4
Influence of the BH_4 treatment adjusted for the yearly median of blood Phe levels on the delta IQ.

Variable	β	95% confident interval		P-Value
		Lower limit	Upper limit	
ΔTIQ				
BH_4	1.8057651	−14.933533	18.545064	0.82
Yearly median of blood Phe levels	−0.0051708	−0.053525	0.0431834	0.82
Intercept	0.5149185	−20.333695	21.363532	0.96
ΔVCI				
BH_4	14.203785	−0.8628344	29.270405	0.06
Yearly median of blood Phe levels	0.0182297	−0.0252927	0.0617521	0.38
Intercept	−5.3547564	−24.1200669	13.410554	0.55
ΔPIQ				
BH_4	−10.401774	−28.038942	7.235394	0.22
Yearly median of blood Phe levels	−0.0238213	−0.0747691	0.0271265	0.33
Intercept	10.7646405	−11.2022602	32.7315411	0.31

BH_4: tetrahydrobiopterin; IQ: Intellectual Quotient; TIQ: Total IQ; PIQ: performance IQ; VCI: Verbal Comprensive Index;

4. Discussion

In this longitudinal study, we document cognitive, environmental and metabolic data of ETPKU patients between 6.5 and 10 years. Modeling the relationship between the delta IQ and the metabolic, environmental and academic achievement features, we observed a significant positive influence of the compliance and the m-PKU type on the ΔVCI, as well as a positive but nonsignificant influence of these variables on ΔTIQ and ΔPIQ. The metabolic control assessed by the median of Phe levels during the year preceding the second neurocognitive evaluation did not significantly influence the delta IQ in our regression model. We finally failed to build a strong model explaining the metabolic control in our cohort.

During the 1980s and the 1990s, collaborative longitudinal studies performed in PKU aimed at characterizing the performance of patients following early and continuous Phe-restricted diet. For example, the German Collaborative Study reported IQ performance of 89 ETPKU patients at 3 different pediatric ages [19]. A slight decrease in TIQ between ages 5 and 9 years was observed, but the authors concluded to a possible artifact related to the use of different scales. One study aimed to further delineate cognitive profiles in ETPKU and found a worse outcome in PIQ compared to VCI [20]. This pattern had been already discussed in the German Collaborative Study but was also found in the healthy controls [19]. Noteworthy, an opposite pattern was described in adult patients in an American collaborative study [21]. In accordance with Griffiths and colleagues, our study describes a better outcome of verbal IQ in ETPKU patients [20].

Relationships between cognitive outcomes and Phe levels have been clearly established in ETPKU. Starting from a meta-analysis of 40 studies, Waisbren and colleagues highlighted the inverse relationship between global IQ and mean Phe levels [6]. They found a 1.3 to 3.1 points decrease in IQ for each increase of 100 μmol/L in Phe concentrations between 6 and 12 years. For each 100 μmol/L increase of the yearly median of blood Phe level, our results show a decrease – yet nonsignificant – of 1.0 TIQ point (95%CI ranging from −3.5 to +1.5), a decrease of 1.1 VCI points (95%CI ranging from −3.3 to +1.0) and a decrease of 0.2 PIQ points (95%CI ranging from −2.7 to +2.3). A recent longitudinal study assessed the relationship between IQ variations and metabolic control during the first two decades in ETPKU patients [22]. It documented a mean decrease of 0.74 IQ points, with a high inter-individual variability. IQ at the first evaluation was the strongest determinant of IQ outcome at the second test, and, when modeling the IQ outcome without considering the first IQ, authors found a significant negative influence of the metabolic control as well as the variability in Phe levels during the second decade. Using a treatment target of 360 μmol/L, Manti and colleagues found no significant differences in the IQ outcome between the well-controlled group and the uncontrolled group in the first decade, though correlation studies found a significant correlation. We used a slightly stricter Phe target of 300 μmol/L, and we observed a significant difference in ΔTIQ and ΔVCI between controlled and uncontrolled patients.

However, the metabolic control plays a nonsignificant role in our model after regression, which highlights the influence of the compliance and the m-PKU type on the delta IQ. These variables have been less studied than blood Phe level, but interestingly in 1981 the US Collaborative Study performed multiple regression analysis to explain the IQ in 6-year-old children and found the mother's IQ, the age at which treatment was introduced and the compliance to the diet as major predictors [23]. We did not assess the parent's IQ in our study but we collected their SPC, and found a negative nonsignificant influence of their SPC on the delta IQ. In our study, the lowest SPC means unemployment: the presence of one or both parents at home may help the care, and, therefore the cognitive outcome, of young PKU children. Another predictor of IQ outcome is the number of repeated school years, which has a positive influence on the total and performance IQ. We assume that making children repeat a year aim at improving their

learning process, and thus might explain a better performance outcome. Few studies compare IQ performances between c-PKU and m-PKU: Chien and colleagues studied 21 ETPKU children and reported better outcome in m-PKU patients [24]. We strive further in this direction and show that the m-PKU type is a protective factor on the IQ outcome, especially for verbal performances with a significant positive correlation between the ΔVCI and the m-PKU type, even though no significant differences in the delta IQ were apparent when first comparing c-PKU and m-PKU with a *t*-Test. Both together, the compliance and the type of PKU have a stronger influence than the metabolic control in our study. This emphasizes the need for adequate initial therapeutic education of the patients especially regarding Phe levels that have to be regularly assessed during childhood.

Interestingly, though there was no clear correlation between the BH_4 treatment and the global IQ outcome, we found an inverse influence of the BH_4 on verbal and performance outcome in the m-PKU group. There is no published data on a negative influence of the BH_4 on performance IQ. The recent PKU-COBESO study published several reports on adult ETPKU patient, of which one study aiming to investigate social and cognitive functioning. They found no difference comparing patients using BH_4 and those who did not [25].

Our study presents limitations. Due to the long period of recruitment, IQ was assessed using different editions of Wechsler scales. Although the differences between each scale are minor according to the manuals, these differences may carry bias to our conclusions. A healthy control group would have been definitely useful to get rid of this putative bias but has not been included in this study. Furthermore, we focused on IQ as main indicator for the cognitive outcome and didn't take into account executive functions, although it would have been interesting to assess potential difference in executive outcome especially between c-PKU and m-PKU patients. Recent studies have indeed shown specific impairment of executive function in ETPKU patients [25] and the pathophysiology of this dysfunction is not fully understood. Additionally, molecular analysis of *PAH* was not performed in our patients, precluding any possibility of genotype-phenotype analysis in the multivariate regression. We assumed that the yearly median of blood Phe levels was the best indicator of the metabolic control, as it is largely used in the literature. However, some authors highlight the variability in Phe levels as a stronger predictor of cognitive performance in ETPKU patients than the yearly median of blood Phe levels [22,26]. This impact of Phe levels fluctuations could be even especially meaningful for m-PKU patients [27]. Finally, our sample size remained limited to 39 patients. The few statistically significant results and the failure to build a model explaining metabolic control in our cohort might be attributable to a lack of a large enough sample size.

5. Conclusion

Though blood Phe levels play a crucial role on IQ in ETPKU patients, the final cognitive outcome is likely to be influenced by a network of metabolic and environment factors, rather than a single metabolic parameter. In well-controlled ETPKU patients, the compliance and the type of PKU appear to be determining factors of cognitive outcomes.

Authors' contributions

YH performed the analyses, wrote the manuscript; EM performed the analyses, wrote the manuscript; LF drafted the manuscript; JP performed statistics; JB drafted the manuscript; SP drafted the manuscript; HOB initiated the work and drafted the manuscript; MS wrote the manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2019.100498.

References

[1] G.A. Jervis, Phenylpyruvic oligophrenia deficiency of phenylalanine-oxidizing system, Proc. Soc. Exp. Biol. Med. Soc. Exp. Biol. Med. N. Y. N. 82 (1953) 514–515.
[2] H. Bickel, J. Gerrard, E.M. Hickmans, Influence of phenylalanine intake on phenylketonuria, Lancet. Lond. Engl. 265 (1953) 812–813.
[3] R. Guthrie, A. Susi, A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants, Pediatrics 32 (1963) 338–343.
[4] P. Burgard, Development of intelligence in early treated phenylketonuria, Eur. J. Pediatr. 159 (Suppl. 2) (2000) S74–S79.
[5] N. Blau, F.J. van Spronsen, H.L. Levy, Phenylketonuria, Lancet 376 (2010) 1417–1427.
[6] S.E. Waisbren, K. Noel, K. Fahrbach, et al., Phenylalanine blood levels and clinical outcomes in phenylketonuria: a systematic literature review and meta-analysis, Mol. Genet. Metab. 92 (2007) 63–70.
[7] A. Pilotto, N. Blau, E. Leks, et al., Cerebrospinal fluid biogenic amines depletion and brain atrophy in adult patients with phenylketonuria, J. Inherit. Metab. Dis. 42 (2019) 398–406.
[8] M.J. González, M.R. Polo, P. Ripollés, et al., White matter microstructural damage in early treated phenylketonuric patients, Orphanet J. Rare Dis. 13 (2018) 188.
[9] G.M. Enns, R. Koch, V. Brumm, E. Blakely, R. Suter, E. Jurecki, Suboptimal outcomes in patients with PKU treated early with diet alone: revisiting the evidence, Mol. Genet. Metab. 101 (2010) 99–109.
[10] J. Weglage, J. Fromm, A. van Teeffelen-Heithoff, et al., Neurocognitive functioning in adults with phenylketonuria: results of a long term study, Mol. Genet. Metab. 110 (2013) S44–S48 Suppl.
[11] F.J. van Spronsen, S.C.J. Huijbregts, A.M. Bosch, V. Leuzzi, Cognitive, neurophysiological, neurological and psychosocial outcomes in early-treated PKU-patients: a start toward standardized outcome measurement across development, Mol. Genet. Metab. 104 (Suppl) (2011) S45–S51.
[12] A.P. Burlina, R.H. Lachmann, R. Manara, C. Cazzorla, A. Celato, F.J. van Spronsen, A. Burlina, The neurological and psychological phenotype of adult patients with early-treated phenylketonuria: a systematic review, J. Inherit. Metab. Dis. 42 (2019) 209–219.
[13] F.J. van Spronsen, A.M. van Wegberg, K. Ahring, et al., Key European guidelines for the diagnosis and management of patients with phenylketonuria, Lancet Diabetes Endocrinol. 5 (2017) 743–756.
[14] A. Ponzone, O. Guardamagna, S. Ferraris, G.B. Ferrero, I. Dianzani, R.G. Cotton, Tetrahydrobiopterin loading test in hyperphenylalaninemia, Pediatr. Res. 30 (1991) 435–438.
[15] D. Wechsler, Forme Révisée (Ed.), WPPSI-R: Echelle d'Intelligence de Wechsler Pour la Période Préscolaire et Primaire, Les Editions du Centre de Psychologie Appliquée, Paris, 1995.
[16] D. Wechsler, WPPSI-III: Echelle d'Intelligence de Wechsler pour la période Préscolaire et Primaire, Troisième édition, Manuel d'interprétation, Eds Les Editions du Centre de Psychologie Appliquée, Paris, 2004.
[17] D. Wechsler, WISC-III: Echelle d'Intelligence de Wechsler pour Enfants, Troisième édition, Eds, Les Editions du Centre de Psychologie Appliquée, Paris, 1996.
[18] D. Wechsler, WISC-IV: Echelle d'Intelligence de Wechsler pour enfants, Quatrième édition, Manuel d'interprétation, Eds Les Editions du Centre de Psychologie Appliquée, Paris, 2006.
[19] P. Burgard, E. Schmidt, A. Rupp, W. Schneider, H.J. Bremer, Intellectual development of the patients of the German collaborative study of children treated for phenylketonuria, Eur. J. Pediatr. 155 (Suppl. 1) (1996) S33–S38.
[20] P.V. Griffiths, C. Demellweek, N. Fay, P.H. Robinson, D.C. Davidson, Wechsler subscale IQ and subtest profile in early treated phenylketonuria, Arch. Dis. Child. 82 (2000) 209–215.
[21] R. Koch, B. Burton, G. Hoganson, et al., Phenylketonuria in adulthood: a collaborative study, J. Inherit. Metab. Dis. 25 (2002) 333–346.
[22] F. Manti, F. Nardecchia, S. Paci, et al., Predictability and inconsistencies in the cognitive outcome of early treated PKU patients, J. Inherit. Metab. Dis. 40 (2017) 793–799.
[23] M.L. Williamson, R. Koch, C. Azen, C. Chang, Correlates of intelligence test results in treated phenylketonuric children, Pediatrics 68 (1981) 161–167.
[24] Y.H. Chien, S.C. Chiang, A. Huang, et al., Phenylalanine hydroxylase deficiency: intelligence of patients after early dietary treatment, Acta. Paediatr. Taiwan. Taiwan 45 (2004) 320–323 Er. Ke. Yi. Xue. Hui. Za. Zhi.
[25] R. Jahja, F.J. van Spronsen, L.M.J. de Sonneville, et al., Social-cognitive functioning and social skills in patients with early treated phenylketonuria: a PKU-COBESO study, J. Inherit. Metab. Dis. 39 (2016) 355–362.
[26] A. Hood, D.K. Grange, S.E. Christ, R. Steiner, D.A. White, Variability in phenylalanine control predicts IQ and executive abilities in children with phenylketonuria, Mol. Genet. Metab. 111 (2014) 445–451.
[27] R. Feldmann, M. Schallert, T. Nguyen, U. Och, F. Rutsch, J. Weglage, Children and adolescents with phenylketonuria display fluctuations in their blood phenylalanine levels, Acta Paediatr. 108 (2019) 541–543.