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First-year metabolic control guidelines and their impact on future metabolic control and neurocognitive functioning in children with PKU

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ABSTRACT

There is a consensus on the importance of early and life-long treatment for PKU patients. Still, differences exist on target blood phenylalanine (Phe) concentrations for children with PKU in different countries and treatment centers. For the first time, long-term metabolic control and child development and cognitive functioning is compared between children with mean phenylalanine concentrations under 240 μmol/L (group A), between 240 and 360 μmol/L (group B) or over 360 μmol/L (group C) during their first year of life. METHODS: 70 patients diagnosed with PKU through neonatal screening with Phe > 900 μmol/L, were divided into 3 groups: A, B and C, according to mean Phe concentrations and standard deviation (SD). Metabolic control during childhood, psychomotor development and IQ were compared. RESULTS: In group A, Phe was maintained within the recommended range until 6 years of age, in Group B, until 3 years of age, and in group C, Phe was always over the recommended range. No significant differences were found between the three groups in mental development index (MDI) and motor development index (PDI) scores at 12, 24, and 30 months of age, but group C had the lowest scores on MDI at all age periods. At preschool and school age, IQ was higher in group A compared to group C. CONCLUSION: Results show that mean blood Phe concentrations between 120 and 240 μmol/L during first year of life have a positive impact in metabolic control and cognitive functioning during childhood.

1. Introduction

Phenylketonuria (PKU) is an inherited autosomal recessive metabolic disorder that causes elevated blood phenylalanine (Phe) concentrations. If left untreated, irreversible neurological damage, developmental delay and cognitive impairment are generated. Early diagnosis and maintained treatment through a Phe-restricted diet enable normal development, but with a slight decrease in IQ score when compared with controls (Berry, O'Grady et al. [3], Waisbren, Noel et al. [19], [4]).

Cognitive outcome is inversely correlated to blood Phe levels, especially over 6 mg/dL (360 μmol/L) during the early years of development ([5], Griffiths, Demellweek et al. [9]). A systematic review and meta-analysis by Waisbren et al. [19] showed that blood Phe levels provide a reliable method for monitoring the metabolic status in individuals with PKU and predicting cognitive functioning. From ages 0–12 years of age, each 100 μmol/L increase in Phe predict a 1.3- to 3.1-point reduction in IQ.

Available data also show that positive outcomes can be expected by maintaining lifelong blood Phe concentrations between 2 and 6 mg/dL (120–360 μmol/L) which is the target for current treatment guidelines in North America (Singh et al. [23], Singh [15], Vockley [18]). The European guidelines recommend Phe concentrations between 2 and 6 mg/dL (120–360 μmol/L) until 12 years of age and upper targeted Phe concentrations of 10 mg/dL (600 μmol/L) for all individuals older than 12 years (Van Spronsen, van Wegberg et al. [16]). Despite current existing guidelines, differences among treatment centers can be identified in Phe blood target ranges, especially regarding higher Phe target levels. In the published results of diagnostic and management practice for PKU in 19 countries in Southern and Eastern Europe, the median upper target limit was 4 mg/dL (240 μmol/L) in infants, 6 mg/dL (360 μmol/L) in young children, and 10 mg/dL (600 μmol/L) in adolescents and in adults (Gizewska, MacDonald et al. [8]).

Recent evidence on neurocognitive functioning showed that individuals with PKU having mean lifetime Phe levels 4 mg/dL (240 μmol/L) or lower perform as well as unaffected controls (Jahja, Huijbregts et al. [10]). Those with mean lifetime levels between 4 and 6 mg/dL (240–360 μmol/L) and over 6 mg/dL (360 μmol/L) performed less accurately on inhibition task, cognitive flexibility task and motor control, prompting a review of current guidelines (Jahja, van Spronsen

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et al. [11]). Since data are limited, current guideline recommendations do not account for differences in outcomes at Phe concentrations between 2 and 4 mg/dL (120–240 μmol/L) and 4–6 mg/dL (240–360 μmol/L).

Since 1992, 226 early-diagnosed phenylketonuria and 189 mild hyperphenylalaninemia patients have entered a follow-up program for PKU patients at the Laboratory of Genetic and Metabolic Diseases of INTA, University of Chile. Since 2010 the recommendation regarding targeted blood Phe concentration was modified from ≥ 2 to < 6 mg/dL (≥ 120 to < 360 μmol/L) to ≥ 2 to < 4 mg/dL (≥ 120 to < 240 μmol/L), for infants during their first year of life. Children in this age group are considered to be more susceptible to common diseases in our population, which impacts metabolic control [6]. Also, in 2009 the recommendation for children over 8 years of age, was modified from ≥ 2 to < 8 mg/dL (≥ 120 to < 480 μmol/L) to ≥ 2 to < 6 mg/dL (≥ 120 to < 360 μmol/L) considering the evidence available during that period [12].

The present study seeks to generate information for a better understanding the effect that a more strict metabolic control during the first 12 months of life has on later metabolic control, psychomotor development and cognitive functioning in individual with PKU.

This paper is the first that we are aware of to focus on the differences between children with classic and moderate PKU with mean Phe blood concentrations under 4 mg/dL (< 240 or under), ≥ 4 to < 6 mg/dL (≥ 240 to < 360 μmol/L) and ≥ 6 mg/dL (≥ 360 μmol/L) in the first 12 months of life.

2. Sample and methods

We reviewed the medical records of 138 patients with PKU diagnosed through neonatal screening between 1995 and 2009 at our center. Inclusion criteria included patients diagnosed by neonatal screening with Phe > 15 mg/dL (> 900 μmol/L), who participated continuously in the follow-up program with IQ assessment data available at school age. Children with a severe history of child neglect, comorbidities, insufficient blood samples sent for Phe concentration analysis or who discontinued treatment were excluded. Seventy patients met the inclusion criteria, that where divided in three groups of 30, 20 and 20 patients according to first-year metabolic control.

The recommended Phe levels during the first year of life for the complete sample were 2–6 mg/dL (120–360 μmol/L). Children were grouped according to metabolic control into three groups considering mean blood Phe and variability between samples in this period. **Group A** (Very good metabolic control): mean Phe < 4 mg/dL (< 240 μmol/L), and SD < 4.5 (n = 30). **Group B** (Good metabolic control): mean Phe ≥ 4 mg/dL to < 6 mg/dL (≥ 240 to < 360 μmol/L), and SD < 4.5 (n = 20). **Group C** (Poor metabolic control): Phe ≥ 6 mg/dL (≥ 360 μmol/L) or Phe ≥ 4 with a SD > 4.5 (n = 20). No significant differences were found between the mean blood Phe concentrations at diagnosis among the three groups (Table 1). Data on metabolic control were analyzed until 10 years of age.

The definitions of the three groups were established considering the recommended range of 2–4 mg/dL (120–240 μmol/L) was used in some clinical centers and the recent evidence on the positive correlation of this range in executive functioning (Gizewska, MacDonald et al. [8]) (Jahja, van Spronsen et al. [11]).

Blood samples were taken by the patient's caregiver at their homes after overnight fasting. The plasma Phe concentrations were analyzed using a fluorometric method based on McCaman and Robins' technique (Gerasimova, Steklova et al. [7]). The phenylalanine is extracted from the sample with ethanol. Ninhydrin and the dipeptide L-leucyl-L-alanine under optimal conditions of pH and temperature form a fluorescent complex. The complex is stabilized by the addition of copper ions. The intensity of the emitted fluorescence is proportional to the concentration of Phe in the sample. The use of calibrators and controls validate the assays.

All patients were on a Phe-restricted diet in combination with protein-free Phe substitutes, minerals and essential fatty acids supplementation. Phe intake was initiated according to PKU Guidelines for age (Singh RH, Cunningham AC et al. [15]), and adjusted by plasma Phe concentrations. > 80% of protein intake was provided by protein-free Phe substitutes, and also according to the guidelines and adjusted to growth and plasma tyrosine levels. The individual Phe intake and natural protein during different ages was not analyzed as part of the investigation.

Psychomotor development was assessed with the Bayley Scales of Infant Development Second Edition [2] at 12 (mean age of the group 12.8 ± 1.7 months), 24 (mean age of the group 22.98 ± 4.3) and 30 (mean age of the group 35.5 ± 4.79) months of age. This test evaluates psychomotor development from the first month of life until 3.5 years of age. It has two scales, mental and motor, and reports results as a mental development index (MDI) and a motor development index (PDI) (means = 100, SD = 15). Cognitive performances at preschool age (mean age of the group 54.9 ± 7.6 months) and school age (mean age of the group 96 ± 14 months) were assessed with the age-appropriate Wechsler Scale. The Wechsler Preschool and Primary Scale of Intelligence (WPPSI) (Wechsler, D [21]) was used for children from 4 to 6 years of age and the Wechsler Intelligence Scale for Children, Revised and third edition (WISC-R or WISC-III) (Wechsler, D [22], Ramírez, et al. [14]) for children from 6 to 16 years. The results of WPPSI and WISC-R/III are expressed in verbal IQ (VIQ), performance IQ (PIQ) and full scale (FSIQ) (IQ mean = 100, SD = 15).

All evaluations were carried during outpatient clinic visits by a staff of two psychologists, with equivalent professional training, one them having > 20 years of experience in the assessment of patients with inborn errors of metabolism.

Psychomotor development assessments last from 30 to 45 min, depending on age. Cognitive assessments duration was between 40 and 60 min. When children were observed to be uncooperative due to hunger, sleepiness or other factors, the assessment was reschedule for next outpatient clinic visit.

Mean Phe concentrations were analyzed by year during the first

Table 1
Sample characteristics.

	Group A (very good metabolic control)	Group B (good metabolic control)	Group C (poor metabolic control)
N°	30	20	20
N° male/N° female	18/12	12/8	7/13
Mean blood Phe concentrations at diagnosis	23.8 ± 6 mg/dL (1428 ± 363 μmol/L),	24.1 ± 6.8 mg/dL (1446 ± 412 μmol/L)	25.5 ± 6.2 mg/dL (1530 ± 375 μmol/L)
Range of blood Phe concentrations at diagnosis	15–46 mg/dL	15–35.5 mg/dL	15–38.5 mg/dL
Classical PKU	73%	70%	85%
Moderate PKU	27%	30%	15%
Mean blood Phe concentrations during the first year of life	2.76 ± 0.7 mg/dL (165.6 ± 42 μmol/L),	4.36 ± 0.5 mg/dL (261.6 ± 30 μmol/L)	6.26 ± 1.1 mg/dL (375.6 ± 67 μmol/L)

4 years of life. From age 4 to 10 years of age, mean Phe concentrations where analyzed in two-year periods.

2.1. Statistical analysis

The Shapiro-Wilk test was used to verify normal distribution of the data. To compare data on Phe blood concentrations, psychomotor development and IQ scores that had normal distribution parameters, the ANOVA test was used, differentiating according to homoscedasticity. When normal distribution of a variable could not be proven, the non-parametric de Kruskal-Wallis test was used to compare the three groups. Significance level was set at $p < 0.05$. Statistical analysis was performed using STATA 13.

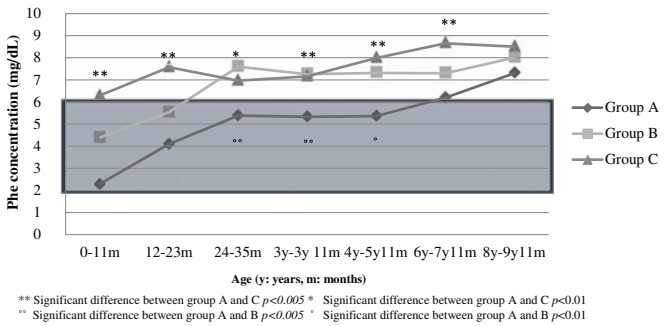
3. Results

3.1. Metabolic control over time

Mean Phe concentrations increased in all groups over the years. In group A, mean Phe concentrations were maintained within the recommended range until the period ranging from 6 years to 7 years 11 months. In Group B, mean Phe concentrations were over the recommended range by the third year of life. In all analyzed periods, mean Phe concentrations in group C were over the recommended range (> 6 mg/dL/≥360 μmol/L). In the period between 8 years and 9 years 11 months of age, all three groups showed mean Phe over the recommended range (Fig. 1). Statistically significant differences were identified between mean Phe blood concentration of groups A and C from ages 12 to 23 months ($p < 0.0000$), 24 to 35 months ($p < 0.0025$), 3 years to 3 years 11 months ($p < 0.0082$), 4 years to 5 years 11 months ($p < 0.0003$) and 6 years to 7 years 11 moths ($p < 0.0002$) of age. Also, statistically significant differences were identified between groups A and B from ages 24 to 35 months $p < 0.0010$, 3 years to 3 years 11 months ($p < 0.0039$) and ages 4 years to 5 years 11 months ($p < 0.0064$).

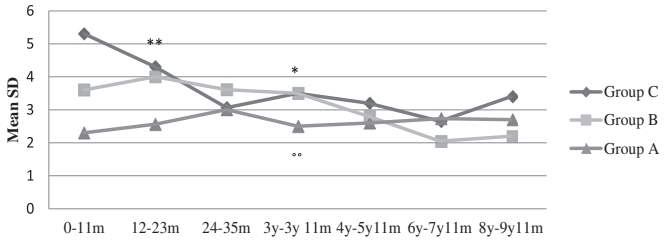
No statistically significant differences were found between mean blood Phe concentrations between group B and C at any age period analyzed.

To analyze variability in blood Phe concentrations among the three groups, mean standard deviations (SD) in blood samples at different ages were compared. Statistically significant differences were found between groups A and C during the 12 to 23 months of age period (mean SD 2.6 ± 1.6 vs. 4.3 ± 1.2, $p < 0.0002$) and during the 3 years to 3 years and 11 months period (mean SD 2.5 ± 1.2 vs. 3.5 ± 1.4 $p < 0.0076$). During this period, significant differences were also identified between groups A and B (mean SD 2.5 ± 1.2 vs. 3.5 ± 1.6 $p < 0.0035$). There was no increase in variability over time (Fig. 2).



** Significant difference between group A and C $p<0.005$ * Significant difference between group A and C $p<0.01$
°° Significant difference between group A and B $p<0.005$ ° Significant difference between group A and B $p<0.01$

Fig. 1. Mean Phe blood concentrations from first year of life to 9 years 11 months of age.



** Significant difference between group A and C $p<0.005$ * Significant difference between group A and C $p<0.01$
°° Significant difference between group A and B $p<0.005$

Fig. 2. Variability of blood Phe concentration, expressed as mean standard deviation of the three groups at different age ranges.

3.2. Child psychomotor development

No statistically significant differences were found between the three groups in score obtained on the Mental Development Index (MDI) and Performance Development Index (PDI) at 12, 24, and 30 months of age. However, the group with poor metabolic control during the first years of life had the lowest scores on the MDI at all age periods assessed (Fig. 3).

3.3. Cognitive functioning

Between ages 4–6 years, results were significantly higher in performance IQ (PIQ) and in full scale IQ (FSIQ) in group A when compared to group C (Fig. 4).

At school age, results in verbal IQ, performance IQ, and full scale IQ were significantly better in group A compared with group C,. with a difference of 11.4 in mean FSIQ between these two groups (Fig. 5).

No significant IQ differences were found between group B and groups A or C in any of the scales at two assessed age periods.

When analyzing the proportion of children with high average FSIQ (≥110) at school age, a total of nine children were identified, eight from group A (27% of the total of 30 children in the group) and one from group B (5% of the 20 children of the group). Of the 70 children, 35 had an average FSIQ (90–109) at school age, 13/30 from group A, 12/20 from group B and 10/20 from group C. Twenty-one children had a low average IQ, 8/30 from group A, 5/20 from group B and 8/20 from group C.

When analyzing the percentage of children with suboptimal intellectual functioning (borderline or mental retardation, FSIQ ≤ 79) at school age, only one child in group A, two children in group B, and three children in group C showed suboptimal IQ scores (Fig. 6).

4. Conclusion

In this study, the effect of a more strict metabolic control during this first year of life was maintained during the subsequent stages of development, both from a metabolic perspective as well as from a cognitive functioning perspective.

Regarding metabolic control, the group A, had a significantly better metabolic control than group C from the first year of life and than the two groups through the following years until almost eight years of age.

Phe levels in the group with mean Phe concentrations from ≥ 4 mg/dL to < 6 mg/dL (≥240 to < 360 μmol/L) and ≥ 6 mg/dL (≥360 μmol/L) during the first year, were not significantly different during any of the following assessment periods. Also, by age 24 months, the average of the mean Phe concentrations of groups B and was over the recommended upper limit (≥360 μmol/L).

Regarding psychomotor development during infancy and early childhood, no significant differences were identified among the three groups. However, the group with highest mean Phe concentrations

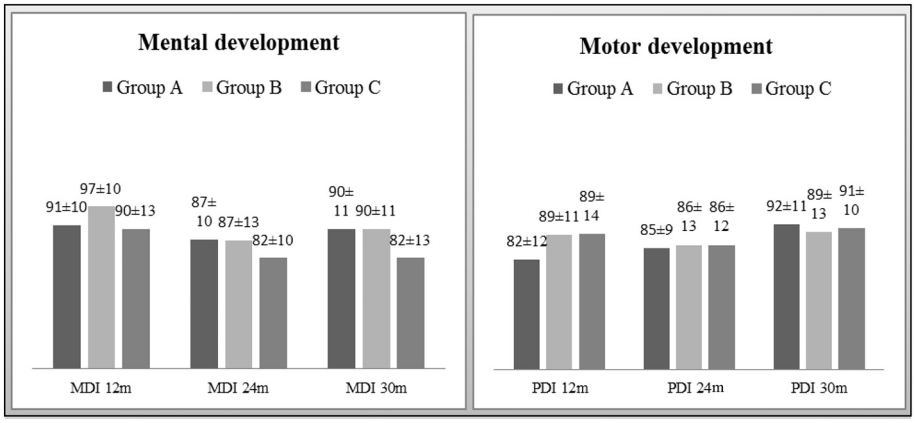


Fig. 3. Mean Mental and Motor development scores in three compared groups.

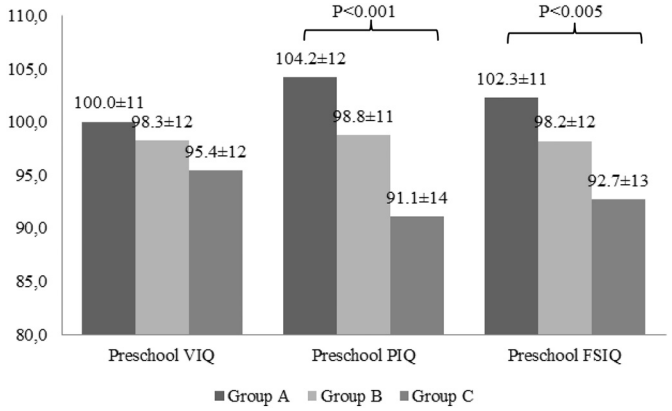


Fig. 4. Mean verbal, performance and full scale IQ in preschool period, between four and six years of age.

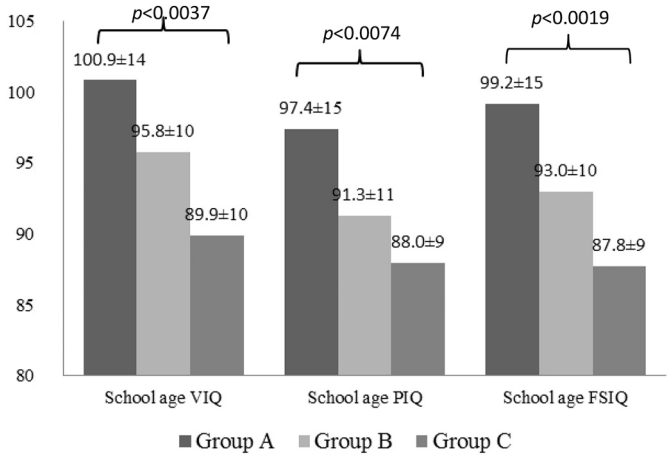


Fig. 5. Mean verbal, performance and full scale IQ between four and six years of age.

during the first 12 months had the lowest average scores in the mental development scale at 12, 24 and 30 months of age. However, during the preschool and school age periods, significant differences in cognitive functioning were found between the groups with lower and higher Phe blood concentrations in the first year of life.

As for group B with mean Phe concentration between ≥ 4 mg/dL to < 6 mg/dL (≥ 240 to < 360 μmol/L), no significant difference in cognitive functioning could be identified when compared to the other two groups. Still a tendency can be seen, since this group showed a poorer cognitive functioning than the group with mean Phe concentrations lower than 4 mg/dL (< 240 μmol/L) during the first year and better performance than the group with mean Phe concentrations

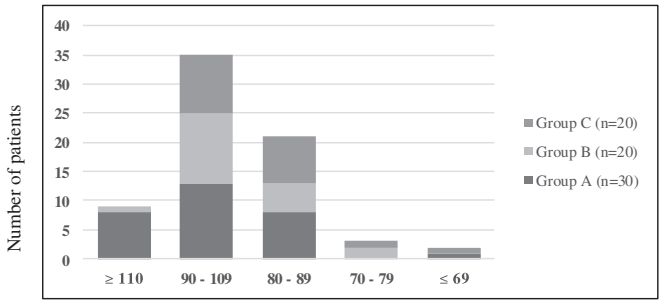


Fig. 6. Number of individuals grouped according to Wechsler Intelligence Scale FSIQ scores in high average (≥ 110), average (90–109), low average (80–89), Very low or borderline (70–79) and extremely low or intellectually disabled (≤ 69).

≥ 6 mg/dL (≥ 360 μmol/L) during the first year. The results of our study open the possibility that a more strict initial metabolic control might have a positive impact in metabolic control during the following years of childhood, thereby protecting cognitive functioning.

A progressive deterioration in metabolic control could be seen over time in all three groups, similar to previous reports, ([13], Ahring, Belanger-Quintana et al. [1], [17]). By age 8–10 years the average mean Phe concentrations of the three groups was over the upper recommended level. Walter et al. [20] found in an audit of 4 centers in the UK and Australia on a group of 330 patients that in the 0–4 year and 5–9 year age groups, about a quarter of the sample had phenylalanine concentrations above the maximum recommended range, and by age 15–19 the proportion increased to 80%. Many barriers have been described for treatment compliance, such as difficulties in food preparation, educational background, family and social characteristics among others (Macdonald 2010). These might explain the difficulties individuals with PKU face to maintain optimal metabolic control.

One of the strengths of our study was that only patients with Phe levels > 15 mg/dL (900 μmol/L) at the time of diagnosis were included, excluding patients with mild forms of the disease. Results show that maintaining Phe concentrations between 2 and 4 mg/dL (120–360 μmol/L) during the first year of life not only benefit cognitive outcome, but are achievable in individuals with moderate and classical PKU. Dependency on care providers and less likelihood of dietary transgressions may facilitate dietary adherence during this period. Currently, our center and others have adopted guidelines based on the results of diagnostic and management practice for PKU in 19 countries of Southern and Eastern Europe in which the median upper target limit in infants is 4 mg/dL (240 μmol/L) (Gizewska [8]).

One of the limitations of the study was that it is a retrospective study and all individuals included were recommended the same target blood Phe concentrations 2–6 mg/dL (120–360 μmol/L). The configuration of the groups occurred spontaneously, so it is possible that other

confounding variables that could not be identified (family characteristics, access to low protein foods, educational background, genotype, etc.) might also be influencing the results. Also data were not available on concurrent Phe concentrations when psychometric assessments were performed. Finally, another limitation, similar to those found in other reports is the limited size of the sample, due to low prevalence of this condition. Future researches should address these limitations.

In conclusion, a more restricted metabolic control in the first year of life is possible in classic and moderated PKU patients and might promote a better metabolic control in the following years, protecting their cognitive development.

Conflict of interest

Alicia de la Parra declares that she has no conflict of interest.
María Ignacia García declares that she has no conflict of interest.
Valerie Hamilton declares that she has no conflict of interest.
Juan Francisco Cabello declares that she has no conflict of interest.
Verónica Cornejo declares that she has no conflict of interest.

Informed consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Institute of Nutrition and Food Technologies Ethics Committee approved a waiver of consent for registries and chart reviews.

Details of the contributions of individual authors:

Alicia de la Parra contributed to the study conception and design; acquisition, analysis and interpretation of the data, and revising of the manuscript.

María Ignacia García contributed in the acquisition, analysis and interpretation of the data, and drafting the manuscript.

Valerie Hamilton contributed analysis and interpretation of the data, and revising the manuscript.

Carolina Arias contributed revising the manuscript.

Juan Francisco Cabello contributed revising the manuscript.

Verónica Cornejo contributed revising the manuscript.

All authors gave final approval of the present manuscript.

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References

[1] K. Ahring, A. Belanger-Quintana, K. Dokoupil, H. Gokmen-Ozel, A.M. Lammardo, A. MacDonald, K. Motzfeldt, M. Nowacka, M. Robert, M. van Rijn, Blood phenylalanine control in phenylketonuria: a survey of 10 European centres, Eur. J. Clin. Nutr. 65 (2) (2011) 275–278.
[2] N. Bayley, Bayley scales of infant development, Second edition, The Psychological Corporation, San Antonio, TX, 1993.
[3] H.K. Berry, D.J. O'Grady, L.J. Perlmutter, M.K. Bofinger, Intellectual development and academic achievement of children treated early for phenylketonuria, Dev. Med. Child Neurol. 21 (3) (1979) 311–320.
[4] V.L. Brumm, M.L. Grant, The role of intelligence in phenylketonuria: a review of research and management, Mol. Genet. Metab. 99 (Suppl. 1) (2010) S18–21.
[5] P. Burgard, Development of intelligence in early treated phenylketonuria, Eur. J. Pediatr. 159 (Suppl. 2) (2000) S74–79.
[6] G. Castro, V. Hamilton, V. Cornejo, Chilean nutrition management protocol for patients with phenylketonuria, J Inborn Errors Metab & Screen 5 (2017) 1–6.
[7] N.S. Gerasimova, I.V. Steklova, T. Tuuminen, Fluorometric method for phenylalanine microplate assay adapted for phenylketonuria screening, Clin. Chem. 35 (10) (1989) 2112–2115.
[8] M. Gizewska, A. MacDonald, A. Belanger-Quintana, Burlina, M. Cleary, T.F. Coskun, F. Feillet, A.C. Muntau, F.K. Trefz, F.J. van Spronsen, N. Blau, Diagnostic and management practices for phenylketonuria in 19 countries of the South and Eastern European Region: survey results, Eur. J. Pediatr. 175 (2) (2016) 261–272.
[9] 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 (3) (2000) 209–215.
[10] R. Jahja, S.C. Huijbregts, L.M. De Sonneville, J.J. Van Der Meere, F.J. Van Spronsen, Neurocognitive evidence for revision of treatment targets and guidelines for phenylketonuria, J. Pediatr. 164 (4) (2014) 895–899.
[11] R. Jahja, F.J. van Spronsen, L.M. de Sonneville, J.J. van der Meere, A.M. Bosch, C.E. Hollak, M.E. Rubio-Gozalbo, M.C. Brouwers, F.C. Hofstede, M.C. de Vries, M.C. Janssen, A.T. van der Ploeg, J.G. Langendonk, S.C. Huijbregts, Social-cognitive functioning and social skills in patients with early treated phenylketonuria: a PKU-COBESO study, J. Inherit. Metab. Dis. 39 (3) (2016) 355–362.
[12] K. Kono, Y. Okano, K. Nakayama, Y. Hase, S. Minamikawa, N. Ozawa, H. Yokote, Y. Inoue, Diffusion-weighted MR imaging in patients with phenylketonuria: relationship between serum phenylalanine levels and ADC values in cerebral white matter, Radiology 236 (2) (2005) 630–636.
[13] C. Meli, S. Bianca, Dietary control of phenylketonuria, Lancet 360 (9350) (2002) 2075–2076.
[14] V. Ramírez, R. Rosas, Test de Inteligencia para niños de Wechsler WISC-III, Normas de Estandarización Chilena, Ediciones Universidad Católica de Chile, Santiago, Chile, 2007.
[15] R.H. Singh, A.C. Cunningham, S. Mofidi, T.D. Douglas, D.M. Frazier, D.G. Hook, L. Jeffers, H. McCune, K.D. Moseley, B. Ogata, Skrabal J. Pendyal 1, P.L. Splett, A. Stembridge, A. Wessel, F. Rohr, Updated, web-based nutrition management guideline for PKU: An evidence and consensus based approach, Mol. Genet. Metab. 118 (2) (2016) 72–83.
[16] F.J. Van Spronsen, A.M. van Wegberg, K. Ahring, A. Bélanger-Quintana, N. Blau, A. Bosch, M. Burlina, J. Campistol, F. Feillet, M. Gizewska, S. Huijbregts, S. Kearny, V. Leuzzi, F. Maillot, A. Muntau, F. Trefz, M. van Rijn, A. Macdonald, Key European guidelines for the diagnosis and management of patients with phenylketonuria, The Lancet Diabetes & Endocrinology 5 (9) (2017) 669–756.
[17] T.A. Vieira, T. Naline, B.C. Krug, C. Matzenbacher, C. Brickmann, I. Doederlein, Adherence to treatment of phenylketonuria, Journal if Inborn Errors of Metabolism & Screening (2015) 1–7.
[18] J. Vockley, H.C. Andersson, K.M. Antshel, N.E. Braverman, B.K. Burton, D.M. Frazier, J. Mitchell, W. Smith, H. Thompson, S.A. Berry, Phenylalanine hydroxylase deficiency: diagnosis and management guideline, Genetics in Medicine 16 (2) (2013) 188–200.
[19] S.E. Waisbren, K. Noel, K. Fahrbach, C. Cella, D. Frame, A. Dorenbaum, H. Levy, Phenylalanine blood levels and clinical outcomes in phenylketonuria: a systematic literature review and meta-analysis, Mol. Genet. Metab. 92 (1–2) (2007) 63–70.
[20] J.H. Walter, F.J. White, S.K. Hall, A. MacDonald, G. Rylance, A. Boneh, D.E. Francis, G.J. Shortland, M. Schmidt, A. Vail, How practical are recommendations for dietary control in phenylketonuria? Lancet 360 (9326) (2002) 55–57.
[21] D. Wechsler, Escala de Inteligencia de Wechsler Para Preescolar y Primaria (WPPSI), TEA, Madrid, 1981.
[22] D. Wechsler, Escala de Inteligencia de Wechsler Para Niños Revisada (WISC-R), Manual Moderno, México, 1974.
[23] R.H. Singh, et al., Recommendations for the nutrition management of phenylalanine hydroxylase deficiency, Genet. Med. 16 (2) (2014) 121–131.