Psychological Medicine (2017), **47**, 2854–2865. © Cambridge University Press 2017
doi:10.1017/S0033291717001398

ORIGINAL ARTICLE

Cerebral dopamine deficiency, plasma monoamine alterations and neurocognitive deficits in adults with phenylketonuria

E. Boot[1–4]*, C. E. M. Hollak[5], S. C. J. Huijbregts[6], R. Jahja[7], D. van Vliet[7], A. J. Nederveen[8], D. H. Nieman[9], A. M. Bosch[10], L. J. Bour[11], A. J. Bakermans[8], N. G. G. M. Abeling[12], A. S. Bassett[2,4,13], T. A. M. J. van Amelsvoort[14], F. J. van Spronsen[7] and J. Booij[1]

[1] *Department of Nuclear Medicine, Academic Medical Center, Amsterdam, The Netherlands;* [2] *The Dalglish Family 22q Clinic for Adults with 22q11.2 Deletion Syndrome, and Center for Mental Health, University Health Network, Toronto, Ontario, Canada;* [3] *Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada;* [4] *Clinical Genetics Research Program, Center for Addiction and Mental Health, Toronto, Ontario, Canada;* [5] *Division of Endocrinology and Metabolism, Department of Internal Medicine, Academic Medical Center, Amsterdam, The Netherlands;* [6] *Department of Clinical Child and Adolescent Studies & Leiden, Institute for Brain and Cognition, Leiden University, Leiden, The Netherlands;* [7] *Division of Metabolic Diseases, University of Groningen, University Medical Center Groningen, Beatrix Children's Hospital, Groningen, The Netherlands;* [8] *Department of Radiology, Academic Medical Center, Amsterdam, The Netherlands;* [9] *Department of Psychiatry, Academic Medical Center, Amsterdam, The Netherlands;* [10] *Department of Pediatrics, Emma Children's Hospital, Academic Medical Center, Amsterdam, The Netherlands;* [11] *Department of Neurology and Clinical Neurophysiology, Academic Medical Center, Amsterdam, The Netherlands;* [12] *Laboratory for Genetic Metabolic Diseases, Academic Medical Center, Amsterdam, The Netherlands;* [13] *Toronto General Research Institute, University Health Network, Toronto, Ontario, Canada;* [14] *Department of Psychiatry and Psychology, Maastricht University, Maastricht, The Netherlands*

Background. Phenylketonuria (PKU), a genetic metabolic disorder that is characterized by the inability to convert phenylalanine to tyrosine, leads to severe intellectual disability and other cerebral complications if left untreated. Dietary treatment, initiated soon after birth, prevents most brain-related complications. A leading hypothesis postulates that a shortage of brain monoamines may be associated with neurocognitive deficits that are observable even in early-treated PKU. However, there is a paucity of evidence as yet for this hypothesis.

Methods. We therefore assessed *in vivo* striatal dopamine $D_{2/3}$ receptor ($D_{2/3}R$) availability and plasma monoamine metabolite levels together with measures of impulsivity and executive functioning in 18 adults with PKU and average intellect (31.2 ± 7.4 years, nine females), most of whom were early and continuously treated. Comparison data from 12 healthy controls that did not differ in gender and age were available.

Results. Mean $D_{2/3}R$ availability was significantly higher (13%; $p = 0.032$) in the PKU group ($n = 15$) than in the controls, which may reflect reduced synaptic brain dopamine levels in PKU. The PKU group had lower plasma levels of homovanillic acid ($p < 0.001$) and 3-methoxy-4-hydroxy-phenylglycol ($p < 0.0001$), the predominant metabolites of dopamine and norepinephrine, respectively. Self-reported impulsivity levels were significantly higher in the PKU group compared with healthy controls ($p = 0.033$). Within the PKU group, $D_{2/3}R$ availability showed a positive correlation with both impulsivity ($r = 0.72$, $p = 0.003$) and the error rate during a cognitive flexibility task ($r = 0.59$, $p = 0.020$).

Conclusions. These findings provide further support for the hypothesis that executive functioning deficits in treated adult PKU may be associated with cerebral dopamine deficiency.

Received 14 February 2017; Revised 27 April 2017; Accepted 3 May 2017; First published online 29 May 2017

Key words: Dopamine, executive function, impulsivity, monoamines, phenylketonuria.

Introduction

Phenylketonuria (PKU; OMIM 261600) is a genetic metabolic disorder, caused by insufficient activity of the hepatic enzyme phenylalanine hydroxylase (PAH). PAH converts the essential amino acid phenylalanine (Phe) to tyrosine (Tyr). Untreated patients with classical PKU demonstrate several cerebral complications including intellectual disability, seizures, and psychiatric symptoms. Neonatal screening and a Phe-restricted diet initiated directly afterwards, prevent most, but not all, of these complications (Blau *et al.* 2010).The neurocognitive outcome of early- and continuously treated PKU patients is generally within the normal range, although variations in outcome exist. Some patients may still suffer from a wide range of symptoms, including difficulties with

* Address for correspondence: E. Boot, Department of Nuclear Medicine, Academic Medical Center, University of Amsterdam, P.O. Box 22700, 1100 DE Amsterdam, The Netherlands.
(Email: h.j.boot@amc.uva.nl)

https://doi.org/10.1017/S0033291717001398 Published online by Cambridge University Press

social skills, lower intelligence quotient (IQ), impairments in executive functioning, and symptoms of attention-deficit/hyperactivity disorder (Huijbregts *et al.* 2002*b*, 2013; Waisbren *et al.* 2007; Antshel, 2010; Christ *et al.* 2010).

Shortages of brain dopamine and other monoamines like serotonin are thought to play a key role in the origin of neurocognitive sequelae in early-treated PKU (Surtees & Blau, 2000; van Spronsen *et al.* 2010). Two mechanisms have been proposed for this theory (online Supplementary Fig. S1). First, high blood Phe levels competitively inhibit the transport of Tyr and tryptophan (Trp), amino acid precursors of catecholamines and serotonin, respectively, across the blood–brain barrier. Second, high brain Phe levels inhibit the activity of Tyr and Trp hydroxylases, the enzymes being responsible for the rate-limiting steps in catecholamine and serotonin synthesis (Pascucci *et al.* 2002; van Spronsen *et al.* 2009).

However, most evidence for monoamine deficiencies in PKU is indirect, relying mainly on: (1) measurements of monoamine (metabolite) levels in body fluids like urine or cerebrospinal fluid (e.g. Douglas *et al.* 2013), (2) postmortem data (McKean, 1972), and (3) studies in rodents (e.g. Puglisi-Allegra *et al.* 2000; Joseph & Dyer, 2003; van Vliet *et al.* 2016; Winn *et al.* 2016). A limitation of these studies is that the degree and locus of monoaminergic abnormalities in the living human PKU brain remain unclear. Molecular imaging studies, however, allow the *in vivo* quantification of many aspects of neurotransmitter functions in the brain. Only two such studies have been performed in PKU. The findings of one positron emission tomography (PET) study in three PKU adults suggested an increased number of available striatal dopamine D_2 receptors (Paans *et al.* 1996). Another PET study reported lower presynaptic striatal 6-[^{18}F] fluoro-L-dopamine uptake in seven adults with PKU compared with seven healthy controls (Landvogt *et al.* 2008). To date, no one has demonstrated, to our knowledge, a link between monoaminergic markers and the neurocognitive impairments in patients with PKU.

In the present study, we tested the hypothesis that adults with PKU have higher striatal dopamine $D_{2/3}$ receptor ($D_{2/3}$R) availability with [^{123}I]iodobenzamide (IBZM) single-photon emission computed tomography (SPECT), as a proxy marker of a reduced brain synaptic dopamine concentration, and lower peripheral monoamine metabolite levels, relative to healthy controls. We also hypothesized that adults with PKU demonstrate relationships between $D_{2/3}$R availability with brain and blood Phe levels, and with self-reported levels of impulsivity and neurocognitive performances.

Materials and methods

Participants

Eighteen patients with PKU (nine females; online Supplementary Table S1) were recruited through treating physicians. The mean (±S.D.) age was 31.2 ± 7.4 (range 18–42) years. Full Scale IQ (FSIQ) ranged from 88 to 113 (n = 17, one patient declined testing), as assessed with the Wechsler Adult Intelligence Scale, 3rd ed. Seventeen patients had been diagnosed by neonatal screening. One patient (patient 3, online Supplementary Table S1) was missed by neonatal screening and was identified at 3 years of age after her brother (patient 16) was diagnosed. Sixteen patients were continuously treated with a Phe-restricted diet since diagnosis with Tyr-enriched protein substitutes. Four patients (patients 3, 6, 7, and 16, online Supplementary Table S1) were also proven responsive to, and treated with, oral tetrahydrobiopterin (BH_4), a pharmacological chaperone that promotes correct folding and stability of the PAH enzyme (Strisciuglio & Concolino, 2014), which has been found to lower blood Phe levels in a subset of PKU patients (Hegge *et al.* 2009). No patient received extra free Tyr supplementation. The mean levels on the study day for the 18 subjects were 706.8 ± 347.1 μmol/l for blood Phe and 42.2 ± 17.1 μmol/l for blood Tyr.

To compare the data obtained in PKU patients, we used historical data available for 12 healthy controls (seven females, aged 20–39 years with a mean of 27.0 ± 6.1 years) (Boot *et al.* 2008, 2010). There were no significant between-group differences in gender (p = 0.72) or age (p = 0.12).

All assessments were conducted in the Academic Medical Center in Amsterdam, The Netherlands. Exclusion criteria for all participants included (i) a current or past psychiatric history, (ii) current or previous exposure to anti-psychotic or psychostimulant medication; (iii) a lifetime history of alcohol or substance abuse or dependence; (iv) a concomitant or past severe medical condition; (v) pregnancy; and (vi) iodine allergy. Additional exclusion criteria for PKU patients included (vii) lack of fluency in Dutch. The study protocol was approved by the Institutional Review Board, i.e. the Medical Ethics Committee of the Academic Medical Center of Amsterdam. Each participant gave written informed consent.

Study procedures

A schematic representation of the study procedures is shown in Fig. 1. All PKU patients were asked to complete a dietary record on the 2 days prior (D_{-2} and D_{-1}) to the study day (D_0) to evaluate daily consumption of natural protein and amino acid supplements, in

https://doi.org/10.1017/S0033291717001398 Published online by Cambridge University Press

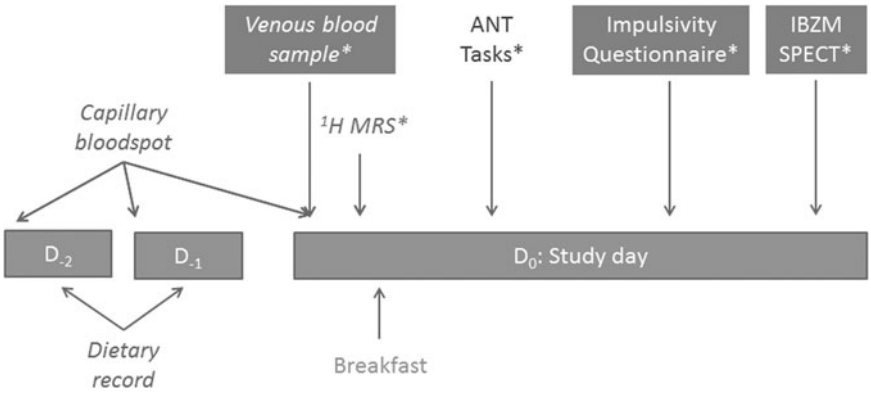

Fig. 1. Schematic representation of study procedures. In the patients with PKU, all the assessments, except for two of three capillary bloodspots (D_{-2}, D_{-1}), were performed on the same day. Data from healthy controls were available from historical records (indicated in upper three boxes). Text in italics represents assessments that provide information on the study population (data are provided in the online Supplementary material). *Indicates assessments that were conducted to test the study hypotheses. D_{-2}, 2 days prior to the study day; D_{-1}, 1 day prior to the study day; ^{1}H MRS, proton magnetic resonance spectroscopy; ANT, the Amsterdam neuropsychological tasks program; IBZM SPECT, [^{123}I]iodobenzamide single-photon emission computed tomography.

particular with regard to the intake of large neutral amino acids (LNAAs). The PKU patients presented at 6:30 or 7:30 AM on the study day. Venous blood samples were drawn approximately 15–30 min after arrival (D_0) for determination of plasma monoamine metabolite and blood amino acid levels. All blood samples were obtained after overnight fasting to eliminate prandial effects on amino acid concentrations. Blood sample collections were immediately followed by a proton magnetic resonance spectroscopy (^{1}H MRS) scan to assess brain Phe levels. All participants then completed a selection of executive function tasks from the Amsterdam neuropsychological tasks (ANT) program (de Sonneville, 2009), completed the Barratt Impulsiveness Scale (BIS), version 11 (Patton *et al.* 1995), and underwent an assessment of postsynaptic striatal $D_{2/3}$R availability with [^{123}I]IBZM SPECT.

Previously published data from healthy controls (SPECT data, plasma monoamine metabolite levels, and self-reported levels of impulsivity) were available for comparison records (Boot *et al.* 2008, 2010).

Amino acid levels in blood and capillary bloodspots

To provide information on the study population, amino acid levels were assessed in all PKU patients. Blood levels of amino acids were measured with automated ion-exchange liquid chromatography followed by postcolumn derivatization with ninhydrin and photometric detection (JEOL JLC-500W Aminotac Amino Acid Analyzer, JEOL Ltd, Tokyo, Japan) (Moore *et al.* 1958). Fasting capillary bloodspot samples were obtained on 3 consecutive days (D_{-2}, D_{-1}, D_0). Phe and Tyr levels were determined using tandem mass spectrometry (standard neutral loss method) (Rashed *et al.* 1995). Six bloodspot samples from four patients did not meet the quality criteria on the Quattro Premier XE (Waters, Milford, Massachusetts, USA) and were rejected by the laboratory.

Plasma monoamine metabolite levels

Fasting venous blood was used to obtain plasma levels of four monoamine metabolites. Homovanillic acid (pHVA; the predominant dopamine metabolite), vanillylmandelic acid (pVMA) and 3-methoxy-4-hydroxyphenylglycol (pMHPG) levels were measured using reverse-phase high-performance liquid chromatography and coulometric electrochemical detection, with a modified method as previously described (Boot *et al.* 2008; Hartleb *et al.* 2013). 5-Hydroxyindoleacetic acid (p5HIAA; the predominant serotonin metabolite) levels were measured in 17 PKU patients. Patient 10 was excluded due to bromocriptine (dopamine D_2 agonist) use that has been found to produce a significant effect on pHVA (Kendler *et al.* 1982). Theoretically, flunarizine, a calcium channel blocker that may affect striatal $D_{2/3}$R availability (Brücke *et al.* 1995), could potentially also influence plasma monoamine metabolite levels. However, to the best of our knowledge, this has not been reported. Therefore, we repeated the analyses of plasma monoamine metabolites after excluding the single patient taking flunarizine (patient 6).

Proton magnetic resonance spectroscopy

Fasting ^{1}H MRS scans were obtained for 18 PKU patients to measure brain Phe levels. They were examined on a Philips Ingenia 3.0 Tesla MR system (Philips Medical Systems, Best, The Netherlands) equipped

https://doi.org/10.1017/S0033291717001398 Published online by Cambridge University Press

with a 16-channel head coil (Philips) using a point-resolved spectroscopy sequence to select a voxel of interest in the parietal white matter. Details are given in the online Supplementary material.

[^{123}I]IBZM SPECT

We assessed striatal $D_{2/3}$R availability with [^{123}I]IBZM, using the validated equilibrium/constant infusion technique (Laruelle *et al.* 1995), and a brain-dedicated SPECT system (Neurofocus). The SPECT protocol was performed as described in our previous report (Boot *et al.* 2010). To optimize image quality, PKU patients received a bolus of approximately 80 MBq instead of 56 MBq, followed by a bolus to hourly infusion ratio of approximately 4.0, identical to the above-mentioned study. Fifteen PKU patients completed the SPECT protocol. Data of the 12 age-matched controls were published before (Boot *et al.* 2010). All SPECT data were reconstructed and analyzed blind to clinical data by the same investigator (E.B.) as described in our previous report (Boot *et al.* 2010). Two PKU patients (patients 6 and 10, online Supplementary Table S1) who received medication (flunarizine and the dopamine agonist bromocriptine, respectively) that influences $D_{2/3}$R availability (Brücke *et al.* 1995; Lam, 2012), and one other (patient 15) because of a technical failure of the infusion pump during imaging, were excluded from [^{123}I]IBZM SPECT.

Impulsivity

The BIS-11, Dutch version 11 (Patton *et al.* 1995), a validated 30-item self-report questionnaire, widely used as a measure of impulsivity, was administered to all participants on the study day.

Executive function performance

The ANT program (de Sonneville, 2009) was used to evaluate executive function performance in the PKU patients. The ANT is a computer-aided assessment battery that allows for the systematic evaluation of information processing capacities. It has been used successfully to determine cognitive deficit profiles for various clinical conditions, including PKU (Huijbregts *et al.* 2002*a*). For this study, we selected three subtasks: (i) the Memory Search Two-Dimensions (MS2D) task, to assess working memory; and (ii) the Shifting Attentional Set – visual (SSV) task, to assess inhibitory control (subtask, part 2) and cognitive flexibility (subtask, part 3). More details on the subtasks can be found in the online supplementary material. One PKU participant (patient 9, online Supplementary Table S1) did not complete the MS2D task.

Statistical analysis

All statistical analyses were conducted using IBM SPSS Statistics 22 for Windows (SPSS Inc., Chicago, Illinois, USA). A probability value of <0.05 was selected as the level of significance for all tests. Kolmogorov–Smirnov tests were used to examine normality. Between-group differences for gender distribution were tested using a χ^2 test. Between-group differences were tested using an independent-samples t test or Mann–Whitney U test as appropriate. Pearson correlation coefficients or Spearman's rank correlation coefficients were calculated with two-tailed tests of significance to investigate the relationships between biological markers, and between biological markers and neurocognitive performances and impulsivity levels, as appropriate. All data are presented as mean ± 1 S.D. unless indicated otherwise.

Results

Striatal $D_{2/3}$ receptor availability

Consistent with our hypothesis, the striatal $D_{2/3}$ receptor binding potential ($D_{2/3}$R BP_{ND}), as a measure of striatal $D_{2/3}$R availability, was 13% higher in adults with PKU (1.34 ± 0.19 *v.* 1.18 ± 0.17 for controls; $p = 0.032$; Fig. 2). This finding remained significant after excluding the PKU patients who received BH_4 (PKU, $n = 12$, 1.33 ± 0.19; $p = 0.048$). There were no significant correlations between (brain or blood) Phe levels and striatal $D_{2/3}$R BP_{ND} (data not shown). Excluding the patients who received BH_4 did not change these results.

Plasma monoamine metabolite levels

As expected, plasma HVA and pMHPG levels were significantly lower in PKU patients than in controls (41.4 ± 13.6 nmol/l *v.* 76.1 ± 24.8 and 10.7 ± 3.0 nmol/l *v.* 26.4 ± 9.3, respectively; Fig. 3*a*, *b*). After excluding patient 6, who received flunarizine, a compound that may possibly influence peripheral monoamine levels, the differences remained significant for pHVA ($p < 0.001$) and pMHPG ($p = 0.0001$). After excluding the four patients who received oral BH_4, the significance levels for pHVA and pMHPG remained unchanged ($p < 0.001$ and $p < 0.0001$, respectively). In contrast, plasma VMA levels were not significantly different between the two groups (Fig. 3*c*). This result remained unchanged after excluding patient 6 and/or the patients who received BH_4. The mean plasma 5HIAA level in the PKU group was 22.2 ± 7.7 nmol/l. The range (7.4–37.3 nmol/l) indicated that for all participants, levels were lower than the laboratory reference values (44.0–79.0 nmol/l; Fig. 3*d*).

https://doi.org/10.1017/S0033291717001398 Published online by Cambridge University Press

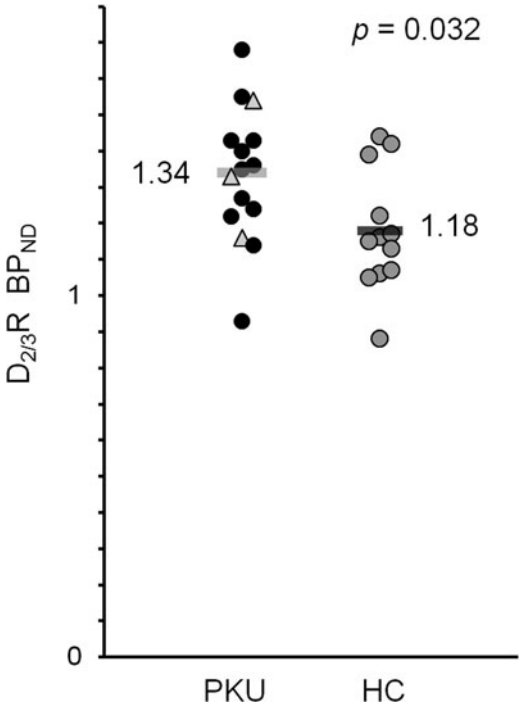

Fig. 2. Increased mean striatal $D_{2/3}$ receptor availability in patients with phenylketonuria (PKU). Horizontal lines indicate mean striatal $D_{2/3}$ receptor binding potential ($D_{2/3}R$ BP_{ND}). Triangles mark the PKU patients who were treated with oral tetrahydrobiopterin. $D_{2/3}R$ BP_{ND}, as a proxy marker of brain synaptic dopamine concentration, was significantly higher in patients with PKU ($n = 15$) compared with healthy controls (HCs; $n = 12$), indicating more available dopamine receptors for the radiopharmaceutical [^{123}I]iodobenzamide (IBZM), which binds selectively to dopamine $D_{2/3}$ receptors.

Impulsivity

Self-reported impulsivity levels were significantly higher in PKU patients compared with healthy controls (60.2 ± 7.7 *v.* $n = 12$, 54.5 ± 5.1, $p = 0.033$; Fig. 4*a*). $D_{2/3}R$ BP_{ND} correlated positively with impulsivity levels in the PKU patients ($p = 0.003$; Fig. 4*b*) but not in the controls ($p = 0.219$). Given that later onset of dietary management could potentially influence impulsivity levels, we reran analyses after excluding patient 3. The between-group differences ($p = 0.042$) and correlation with $D_{2/3}R$ BP_{ND} ($p = 0.002$) remained significant. No significant correlations were found between (blood or brain) Phe levels and scores of impulsivity on the BIS-11 (data not shown).

Executive function performance

$D_{2/3}R$ BP_{ND} also correlated positively with error rate during a cognitive flexibility task (SSV-subtask, part 3; Fig. 5*a*). Results were not significant for error rate during a working memory task (MS2D-task; $p = 0.075$; Fig. 5*b*) or performance on an inhibitory control task (Fig. 5*c*). Excluding patient 3 did not materially change results (data not shown). There were no significant correlations between blood or brain Phe levels and executive function performance (data not shown).

Discussion

A longstanding pathophysiological theory postulates that high blood Phe concentrations in PKU patients may lead to neurocognitive deficits by impairing brain dopamine and other monoamine synthesis (Pascucci *et al.* 2002; Christ *et al.* 2010). In support of this theory, the present study shows, for the first time, significantly higher striatal $D_{2/3}R$ availability in adult PKU patients in comparison with control participants, suggesting that there may be reduced concentrations of dopamine in the synapse. These findings can be explained by the dopamine receptor competition model that predicts that a lower dopamine concentration in the synapse will lead to a lower occupancy of dopamine $D_{2/3}R$ (and possibly also a compensatory upregulation of the presence of dopamine $D_{2/3}R$) and consequently higher binding of the radiotracer [^{123}I] IBZM to dopamine $D_{2/3}R$ (see discussion in Boot *et al.* 2010). The present study complements the two previous molecular imaging studies in PKU that suggested reduced dopamine synthesis (Paans *et al.* 1996; Landvogt *et al.* 2008), by providing further support for the assumption of dopamine deficiencies at the level of the synapse of these treated patients.

Results of our study found no significant correlations between blood or brain Phe levels and striatal $D_{2/3}R$ BP_{ND}. Several possible mechanisms may have contributed. In addition to power issues, these include between-subject variability in $D_{2/3}R$ availability (Kegeles *et al.* 1999), challenging aspects of measuring *in vivo* brain Phe levels (Kreis *et al.* 2009), and blood concentrations of other LNAAs competing with Tyr and Phe for transport into brain (van Vliet *et al.* 2015*b*). Reduced availability of monoaminergic precursors in the brain may be an even greater limiting factor in monoamine biosynthesis than increased brain Phe levels (van Vliet *et al.* 2015*b*; van Vliet *et al.* 2016) (online Supplementary Fig. S1).

Our results add to previous human studies of PKU that assessed monoamine (metabolite) levels in body fluids such as urine, and found lower levels compared with controls (e.g. Nadler & Hsia, 1961; Douglas *et al.* 2013). In the present study, PKU patients showed lower levels of pHVA and pMHPG, and p5HIAA levels below the lower limit of the reference range, suggesting reduced synthesis of dopamine, norepinephrine, and serotonin, respectively. It however remains

https://doi.org/10.1017/S0033291717001398 Published online by Cambridge University Press

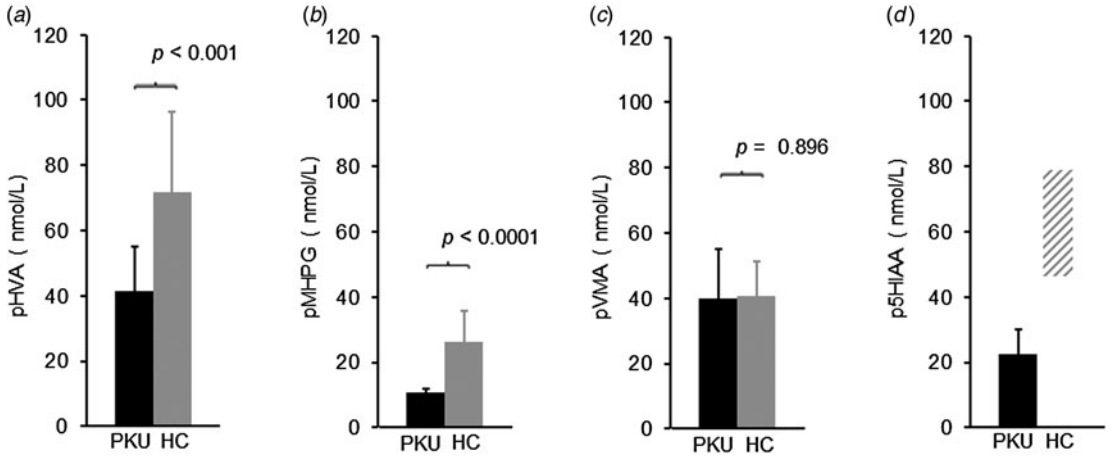

Fig. 3. Plasma monoamine metabolite levels in 17 adults with phenylketonuria (PKU) and 12 healthy controls (HCs). (*a*) Mean plasma homovanillic acid levels (pHVA, nmol/l). (*b*) Mean plasma 3-methoxy-4-hydroxy-phenylglycol levels (pMHPG, nmol/l). (*c*) Mean plasma vanillylmandelic acid levels (pVMA, nmol/l). (*d*) Mean plasma 5-hydroxyindoleacetic acid levels (p5HIAA); p5HIAA was not assessed in the HCs. Laboratory reference values for p5HIAA are indicated with diagonal stripes. Error bars indicate ± 1 S.D.

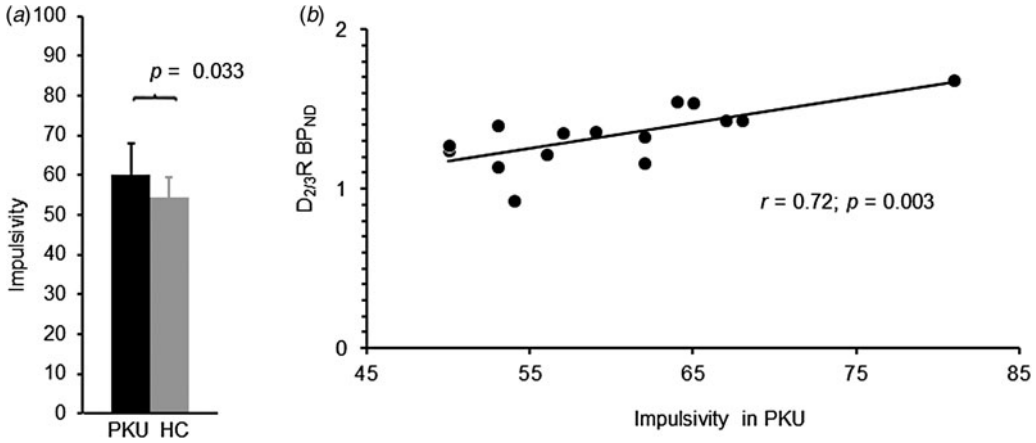

Fig. 4. Relationship between striatal $D_{2/3}$ receptor availability [$D_{2/3}$ receptor binding potential ($D_{2/3}R$ BP_{ND})] and impulsivity. (*a*) Patients with phenylketonuria (PKU; $n = 18$) reported higher levels of impulsivity than healthy controls (HCs; $n = 12$). (*b*) In patients with PKU, $D_{2/3}R$ BP_{ND} correlated positively with impulsivity levels.

unclear to what extent these abnormalities may reflect brain monoaminergic alterations (Elsworth *et al.* 1987; Pickar *et al.* 1988).

This study is the first to demonstrate a relationship between a neurochemical monoaminergic brain marker and measures of executive functioning in adult PKU patients, most of whom were treated early and continuously. Previous studies have found that in early treated adult PKU patient, executive functioning is particularly affected (Christ *et al.* 2010). The prefrontal cortex and striatum play a crucial role in executive functioning, and cognitive processes are believed to be largely modulated by monoamines, including dopamine (Cropley *et al.* 2006; Christ *et al.* 2010). Consistent with the potential importance of this in PKU is the finding from the present study that there was a significant positive correlation between striatal $D_{2/3}R$ availability and error rate on a cognitive flexibility task in the adults with PKU. The correlation between $D_{2/3}R$ BP_{ND} and error rate during a working memory task did not however reach statistical significance ($p = 0.075$). Performance on the inhibitory control task was also not correlated with $D_{2/3}R$ BP_{ND}, although it is possible that ceiling effects associated with this relatively easy task could have hampered the possibility of finding a correlation. Notably, it is possible that associations between neuropsychological test performance and $D_{2/3}R$ BP_{ND} could be accounted for by differences in

https://doi.org/10.1017/S0033291717001398 Published online by Cambridge University Press

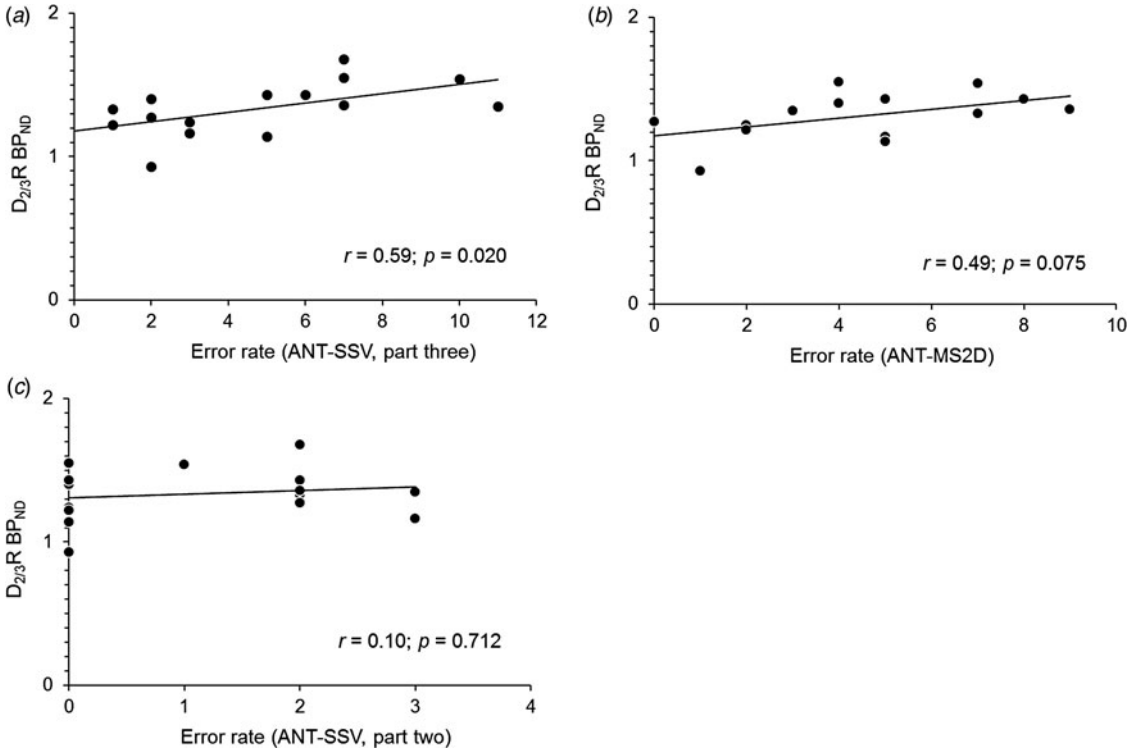

Fig. 5. Relationship between striatal $D_{2/3}$ receptor availability and executive function performance in patients with phenylketonuria (PKU). (*a*) Relationship between striatal $D_{2/3}$ receptor availability [$D_{2/3}$ receptor binding potential ($D_{2/3}R$ BP_{ND})] and error rate during the Shifting Attentional Set – visual task (SSV), part 3; a subtask of the Amsterdam neuropsychological tasks (ANT) program that requires cognitive flexibility ($n = 15$). (*b*) Relationship between $D_{2/3}R$ BP_{ND} and error rate during the Memory Search Two-Dimensions task (MS2D); a working memory task ($n = 14$). (*c*) Relationship between $D_{2/3}R$ BP_{ND} and error rate during the ANT-SSV, part 2, that requires inhibitory control ($n = 15$).

general attention performance. In this study, different from other studies (e.g. Jahja *et al.* 2014), a correlation with executive functioning was not observed with (blood or brain) Phe levels, nor with other biochemical markers assessed. We note that Ullrich *et al.* did not find an effect of levodopa treatment on visual-evoked potentials or neuropsychological test performance in adults with PKU (Ullrich *et al.* 1994, 1996).

Higher levels of impulsivity in patients with PKU than in healthy controls, consistent with previous reports (Hendrikx *et al.* 1994; Stemerdink *et al.* 2000), and the strong positive correlation of self-reported levels of impulsivity with striatal $D_{2/3}R$ BP_{ND} within the PKU group, are also in line with a possible 'hypodopaminergic state' in PKU. Here, it would be surmised that patients with a higher striatal dopamine $D_{2/3}$ receptor ($D_{2/3}R$) availability may have a more pronounced shortage of synaptic dopamine that could then lead to increased impulsivity. Impulsivity is believed to be mediated by dopaminergic and serotonergic activity (Dalley & Roiser, 2012) and to be directly related to dysfunctional inhibitory processes (Bari & Robbins, 2013).

While previous studies in PKU patients have also shown reduced peripheral monoamine (metabolite) levels and negative correlations between blood Phe levels and peripheral monoamine (metabolite) levels (Nadler & Hsia, 1961; Douglas *et al.* 2013), their correlation with availability of monoamines in the brain, and their relationship with neurocognitive performance, remain unclear. Hence, it is uncertain to what extent these peripheral markers could be of value for monitoring brain pathophysiology. This is also the case for plasma prolactin, a neuroendocrine marker for cerebral dopamine (van Vliet *et al.* 2015*a*; Juhász *et al.* 2016). In this regard, it is of interest that in the present study, no significant correlations were found between $D_{2/3}R$ BP_{ND} and plasma monoamine levels.

The results of the present study may have implications for the monitoring of PKU patients that has thus far focused primarily on reducing blood Phe levels (Weglage *et al.* 2001). While blood Phe levels can relatively easily be monitored, and blood Phe is the major marker for dietary adherence, its utility as a biomarker for brain pathophysiology and clinical monitoring appears suboptimal. A significant

https://doi.org/10.1017/S0033291717001398 Published online by Cambridge University Press

correlation between blood and brain Phe levels, as found in this and other studies (Pietz *et al.* 1995; Leuzzi *et al.* 2007; Kreis *et al.* 2009), is not supported by other studies (Weglage *et al.* 2001; Moats *et al.* 2003; Sijens *et al.* 2004). A linear relationship between blood and brain Phe levels has also been deemed unlikely under the assumption of a saturable transport mechanism (Weglage *et al.* 2001) and there have been anecdotal reports of PKU patients achieving normal intellect with low brain Phe levels, despite elevated blood Phe levels (Weglage *et al.* 1998, 2001; Koch *et al.* 2000). In addition, findings concerning relationships between blood Phe and neurocognitive performance are mixed (for an overview see Christ *et al.* 2010). In the present study, neither blood nor brain Phe levels correlated significantly with any of the clinical outcome measures. Given the finding that striatal dopamine $D_{2/3}R$ availability, however, was significantly correlated with measures of executive functioning in this study, we propose that brain monoaminergic markers may be potential biomarkers of long-term cognitive outcome in PKU (van Vliet *et al.* 2015*b*). In this context, a parabolic ('inverted *U*') relationship between cerebral dopamine concentrations and neurocognitive performance (Cools & D'Esposito, 2011), and between striatal dopamine $D_{2/3}R$ availability and impulsivity (Gjedde *et al.* 2010), should be considered.

Methodological considerations

Neurochemical studies provide the most direct method to assess monoamine systems in the living human brain currently available. In the present study, we performed [^{123}I]IBZM SPECT, a well-validated reproducible modality to assess striatal $D_{2/3}R$ availability that has been used successfully for several disorders by our group (e.g. Boot *et al.* 2010). We used a bolus/infusion technique; this approach is not sensitive to changes in cerebral blood flow and measures $D_{2/3}R$ availability in a state of equilibrium (Booij *et al.* 1997). Nevertheless, differences in neuroreceptor density in PKU patients and healthy controls cannot be ruled out with this modality and may have influenced the results observed. In addition, [^{123}I]IBZM SPECT is not suited to examining the dopamine system in extrastriatal regions. It would be of interest to examine other brain areas in PKU, in particular the prefrontal cortex, given its crucial role in executive functioning and its dependence on dopamine activity, with other PET or SPECT radioligands (Cropley *et al.* 2006; Barnes *et al.* 2011). The caudate nucleus has widespread connections with the prefrontal cortex. Future study design could thus be improved by using magnetic resonance imaging to co-register the SPECT images, which would permit delineation of [^{123}I]IBZM binding in the caudate nucleus from that in the putamen (Cropley *et al.* 2006). Evaluating the left and right caudate nucleus separately would also allow assessment of lateralization of $D_{2/3}R$ availability in relation to neurocognitive deficits. Future studies investigating absolute quantification of brain Phe concentrations using ^{1}H MRS (Kreis *et al.* 2009), and those studying other neurotransmitters in human PKU brain *in vivo*, in particular serotonin (Pascucci *et al.* 2002; van Spronsen *et al.* 2009; Christ *et al.* 2010), and neurotransmitter system interactions, are also needed.

As in another Dutch study (Schmaal *et al.* 2012), in the present study, the levels of self-reported impulsivity in healthy controls were relatively low (and the levels in patients with PKU were similar) to those previously reported for two US community samples (Patton *et al.* 1995; Reise *et al.* 2013). This raises the possibility of cultural differences and/or differences due to translation of the questionnaire. Considerations for future studies with respect to assessment of impulsivity include task-based tests instead of self-reported impulsivity scores, and accounting for the clinical psychiatric state of the patient since impulsivity ratings may be state dependent (e.g. Corruble *et al.* 2003).

Extraneous factors may have influenced the outcome of the assessments. For example, one patient took his Phe-free amino acid mixture 2 h prior to the blood sample (patient 11). Theoretically, this could have led to increased monoamine synthesis, and consequently higher monoamine metabolite levels in this patient. Still, pHVA, pMHPG, and p5HIAA levels in this patient were all lower than the mean levels in the PKU group. One patient had also stopped smoking 17 days prior to the study (patient 7), and one patient smoked approximately five cigarettes per day (patient 8). Although cerebral dopamine is released when smoking, these two patients had $D_{2/3}R$ BP_{ND} values close to the mean (1.33 and 1.36 *v.* 1.34) in the PKU group. This is consistent with one study reporting that there is no effect of smoking on striatal $D_{2/3}R$ availability over the long term (Yang *et al.* 2006).

Some data collected in the PKU patients were not available for the control participants, e.g. p5HIAA levels. However, the fact that none of the PKU patients reached a p5HIAA level above the lower limit of the reference range makes the null hypothesis (no between-group differences in p5HIAA levels) unlikely. Because formal executive functioning was not assessed in controls, and in the absence of normative data for the ANT, it is unclear to what extent the executive deficits present in our PKU cohort would be clinically relevant. It should be noted that all patients had an FSIQ score within the normal range. Also, blood samples for plasma monoamine metabolite levels were not necessarily drawn in the fasted state in the controls.

https://doi.org/10.1017/S0033291717001398 Published online by Cambridge University Press

Although this could have influenced the results for between-group differences in plasma monoamine metabolite levels (Doran *et al.* 1990), the other findings would not be affected.

There was no between-group difference in sex distribution; however, we cannot rule out an effect of sex on our findings. Males and females are equally likely to be affected with PKU. However, one could speculate that sexually dimorphic characteristics of the monoaminergic system of the brain (e.g. Harrison and Tunbridge, 2008) could produce differing effects on executive functioning in men and women with PKU.

The present study did not evaluate the effect of any dietary intervention. A challenge study with equal assessments in a diet *v.* placebo condition would allow testing the hypothesis that dietary factors may affect cerebral monoamine synthesis and availability in PKU, and consequently neurocognitive functioning (e.g. Mehta *et al.* 2005).

Although the present study is to date the largest involving neurochemical imaging techniques in PKU, the sample sizes were small and the number of outcome variables relatively large. On the one hand, the results should therefore be interpreted with caution and this work needs to be replicated in an independent larger cohort of PKU patients. On the other hand, the range of blood Phe levels, striatal $D_{2/3}R$ binding, and other variables enabled the performance of correlational analyses within the PKU group. Future studies, including a prospective design, are required to elucidate the effects of aging on monoamine systems in PKU.

Conclusions

This study provides further support for the hypothesis of cerebral dopamine deficiency as a possible pathophysiological explanation of executive functioning deficits in early and continuously treated adult PKU.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S0033291717001398.

Acknowledgements

This work was financially supported by the Dutch Brain Foundation (E.B., grant number KS2012(1)-31); the Brain & Behavior Research Foundation (T.A.M.J.v.A., formerly NARSAD); the Dutch PKU Research Foundation (F.J.v.S.); NutsOhra Fund (F.J.v.S., grant number 1003–061); and the Division of Metabolic Diseases of the Beatrix Children's Hospital of the University Medical Centre Groningen, The Netherlands. The funding agencies had no role in study design, in the collection, analysis and interpretation of data, in the writing of the report, or in the decision to submit the paper for publication. The authors are grateful to all study participants. They thank the staff of the Department of Nuclear Medicine, including Bastiaan Kee for excellent technical assistance and Corry Stoelman-Andreas for administrative support, Raschel van Luijk-Snoeks and Sandra van den Berg for conducting the ^{1}H MRS scans, Lydia Veerhuis for administrative support, and Corrie Timmer and Cora Jonkers for evaluating the patient's dietary records. Parts of this study have been presented as an oral presentation at the 19th International Research Symposium of the Society for the Study of Behavioural Phenotypes (SSBP) in Siena, Italy in September 2016 and as posters at the annual symposium of the Society for the Study of Inborn Errors of Metabolism (SSIEM) in Rome, Italy in September 2016, the 23rd Annual Meeting & Exhibition of the International Society for Magnetic Resonance in Medicine (ISMRM), Toronto, Ontario, Canada in June 2015, and the Society of Biological Psychiatry's (SOBP) 70th annual meeting in Toronto in May 2015.

Declaration of Interest

None.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

References

Antshel KM (2010). ADHD, learning, and academic performance in phenylketonuria. *Molecular Genetics and Metabolism* **99** (Suppl. 1), S52–S58.

Bari A, Robbins TW (2013). Inhibition and impulsivity: behavioral and neural basis of response control. *Progress in Neurobiology* **108**, 44–79.

Barnes JJ, Dean AJ, Nandam LS, O'Connell RG, Bellgrove MA (2011). The molecular genetics of executive function: role of monoamine system genes. *Biological Psychiatry* **69**, e127–e143.

Blau N, van Spronsen FJ, Levy HL (2010). Phenylketonuria. *Lancet* **376**, 1417–1427.

Booij J, Korn P, Linszen DH, van Royen EA (1997). Assessment of endogenous dopamine release by methylphenidate challenge using iodine-123 iodobenzamide single-photon emission tomography. *European Journal of Nuclear Medicine* **24**, 674–677.

Boot E, Booij J, Zinkstok J, Abeling N, de Haan L, Baas F, Linszen D, van Amelsvoort T (2008). Disrupted

https://doi.org/10.1017/S0033291717001398 Published online by Cambridge University Press

dopaminergic neurotransmission in 22q11 deletion syndrome. *Neuropsychopharmacology* **33**, 1252–1258.

Boot E, Booij J, Zinkstok JR, de Haan L, Linszen DH, Baas F, van Amelsvoort TA (2010). Striatal D_2 receptor binding in 22q11 deletion syndrome: an [^{123}I] IBZM SPECT study. *Journal of Psychopharmacology* **24**, 1525–1531.

Brücke T, Wöber C, Podreka I, Wöber-Bingöl C, Asenbaum S, Aull S, Wenger S, Ilieva D, Harasko-van der Meer C, Wessely P, Deecke L (1995). D_2 receptor blockade by flunarizine and cinnarizine explains extrapyramidal side effects. A SPECT study. *Journal of Cerebral Blood Flow and Metabolism* **15**, 513–518.

Christ SE, Huijbregts SC, de Sonneville LM, White DA (2010). Executive function in early-treated phenylketonuria: profile and underlying mechanisms. *Molecular Genetics and Metabolism* **99** (Suppl. 1), S22–S32.

Cools R, D'Esposito M (2011). Inverted-U-shaped dopamine actions on human working memory and cognitive control. *Biological Psychiatry* **69**, 113–125.

Corruble E, Benyamina A, Bayle F, Falissard B, Hardy P (2003). Understanding impulsivity in severe depression? A psychometrical contribution. *Progress in Neuropsychopharmacology and Biological Psychiatry* **27**, 829–833.

Cropley VL, Fujita M, Innis RB, Nathan PJ (2006). Molecular imaging of the dopaminergic system and its association with human cognitive function. *Biological Psychiatry* **59**, 898–907.

Dalley JW, Roiser JP (2012). Dopamine, serotonin and impulsivity. *Neuroscience* **215**, 42–58.

de Sonneville LM (2009). Amsterdam neuropsychological tasks: a computer-aided assessment program. In *Computers in Psychology: Cognitive Ergonomics, Clinical Assessment and Computer-Assisted Learning* (ed. B. P. L. M. den Brinker, P. J. Beek, A. N. Brand, S. J. Maarse, L. J. M. Mulder), pp. 187–203. Swets & Zeitlinger: Leiden.

Doran AR, Labarca R, Wolkowitz OM, Roy A, Douillet P, Pickar D (1990). Circadian variation of plasma homovanillic acid levels is attenuated by fluphenazine in patients with schizophrenia. *Archives of General Psychiatry* **6**, 558–563.

Douglas TD, Jinnah HA, Bernhard D, Singh RH (2013). The effects of sapropterin on urinary monoamine metabolites in phenylketonuria. *Molecular Genetics and Metabolism* **109**, 243–250.

Elsworth JD, Leahy DJ, Roth RH, Redmond DE (1987). Homovanillic acid concentrations in brain, CSF and plasma as indicators of central dopamine function in primates. *Journal of Neural Transmission* **68**, 51–62.

Gjedde A, Kumakura Y, Cumming P, Linnet J, Møller A (2010). Inverted-U-shaped correlation between dopamine receptor availability in striatum and sensation seeking. *Proceedings of the National Academy of Sciences* **107**, 3870–3875.

Harrison PJ, Tunbridge EM (2008). Catechol-O-methyltransferase (COMT): a gene contributing to sex differences in brain function, and to sexual dimorphism in the predisposition to psychiatric disorders. *Neuropsychopharmacology* **33**, 3037–3045.

Hartleb J, Eue S, Kemper A (1993). Simultaneous analysis of homovanillic acid, 5-hydroxyindoleacetic acid, 3-methoxy-4-hydroxyphenylethylene glycol and vanilmandelic acid in plasma from alcoholics by high-performance liquid chromatography with electrochemical detection. Critical comparison of solid-phase and liquid-liquid extraction methods. *Journal of Chromatography* **622**, 161–171.

Hegge KA, Horning KK, Peitz GJ, Hegge K (2009). Sapropterin: a new therapeutic agent for phenylketonuria. *Annals of Pharmacotherapy* **43**, 1466–1473.

Hendrikx MM, van der Schot LW, Slijper FM, Huisman J, Kalverboer AF (1994). Phenylketonuria and some aspects of emotional development. *European Journal of Pediatrics* **153**, 832–835.

Huijbregts SC, de Sonneville LM, Licht R, van Spronsen FJ, Sergeant JA (2002*a*). Short-term dietary interventions in children and adolescents with treated phenylketonuria: effects on neuropsychological outcome of a well-controlled population. *Journal of Inherited Metabolic Disease* **25**, 419–430.

Huijbregts SC, de Sonneville LM, van Spronsen FJ, Licht R, Sergeant JA (2002*b*). The neuropsychological profile of early and continuously treated phenylketonuria: orienting, vigilance, and maintenance *v.* manipulation-functions of working memory. *Neuroscience & Biobehavioral Reviews* **26**, 697–712.

Huijbregts SC, Gassio R, Campistol J (2013). Executive functioning in context: relevance for treatment and monitoring of phenylketonuria. *Molecular Genetics and Metabolism* **110** (Suppl), S25–S30.

Jahja R, Huijbregts SC, de Sonneville LM, van der Meere JJ, van Spronsen FJ (2014). Neurocognitive evidence for revision of treatment targets and guidelines for phenylketonuria. *Journal of Pediatrics* **164**, 895–899.

Joseph B, Dyer CA (2003). Relationship between myelin production and dopamine synthesis in the PKU mouse brain. *Journal of Neurochemistry* **86**, 615–626.

Juhász E, Kiss E, Simonova E, Patocs A, Reismann P (2016). Serum prolactin as a biomarker for the study of intracerebral dopamine effect in adult patients with phenylketonuria: a cross-sectional monocentric study. *European Journal of Medical Research* **21**, 22.

Kegeles LS, Zea-Ponce Y, Abi-Dargham A, Rodenhiser J, Wang T, Weiss R, van Heertum RL (1999). Stability of [123I]IBZM SPECT Measurement of amphetamine-induced striatal dopamine release in humans. *Synapse* **31**, 302–308.

Kendler KS, Heninger GR, Roth RH (1982). Influence of dopamine agonists on plasma and brain levels of homovanillic acid. *Life Sciences* **30**, 2063–2069.

Koch R, Moats R, Guttler F, Guldberg P, Nelson Jr. M (2000). Blood-brain phenylalanine relationships in persons with phenylketonuria. *Pediatrics* **106**, 1093–1096.

Kreis R, Zwygart K, Boesch C, Nuoffer JM (2009). Reproducibility of cerebral phenylalanine levels in patients with phenylketonuria determined by ^{1}H-MR spectroscopy. *Magnetic Resonance in Medicine* **62**, 11–16.

Lam YW (2012) Clinical pharmacology of dopamine agonists. *Pharmacotherapy* **20**, 17S–25S.

Landvogt C, Mengel E, Bartenstein P, Buchholz HG, Schreckenberger M, Siessmeier T, Scheurich A,

https://doi.org/10.1017/S0033291717001398 Published online by Cambridge University Press

Feldmann R, Weglage J, Cumming P, Zepp F, Ullrich K (2008). Reduced cerebral fluoro-L-dopamine uptake in adult patients suffering from phenylketonuria. *Journal of Cerebral Blood Flow and Metabolism* **28**, 824–831.

Laruelle M, Abi-Dargham A, van Dyck CH, Rosenblatt W, Zea-Ponce Y, Zoghbi SS, Baldwin RM, Charney DS, Hoffer PB, Kung HF, Innis RB (1995). SPECT imaging of striatal dopamine release after amphetamine challenge. *Journal of Nuclear Medicine* **36**, 1182–1190.

Leuzzi V, Tosetti M, Montanaro D, Carducci C, Artiola C, Carducci C, Antonozzi I, Burroni M, Carnevale F, Chiarotti F, Popolizio T, Giannatempo GM, D'Alesio V, Scarabino T (2007). The pathogenesis of the white matter abnormalities in phenylketonuria. A multimodal 3.0 tesla MRI and magnetic resonance spectroscopy (^{1}H MRS) study. *Journal of Inherited Metabolic Disease* **30**, 209–216.

McKean CM (1972). The effects of high phenylalanine concentrations on serotonin and catecholamine metabolism in the human brain. *Brain Research* **47**, 469–476.

Mehta MA, Gumaste D, Montgomery AJ, McTavish SFB, Grasby PM (2005). The effects of acute tyrosine and phenylalanine depletion on spatial working memory and planning in healthy volunteers are predicted by changes in striatal dopamine levels. Psychopharmacology *(2005)* **180**, 654–663.

Moats RA, Moseley KD, Koch R, Nelson Jr. M (2003). Brain phenylalanine concentrations in phenylketonuria: research and treatment of adults. *Pediatrics* **112**, 1575–1579.

Moore S, Spackman DH, Stein WH (1958). Chromatography of amino acids on sulfonated polystyrene resins: an improved system. *Analytical Chemistry* **30**, 1185–1190.

Nadler HL, Hsia DY (1961). Epinephrine metabolism in phenylketonuria. *Proceedings of the Society for Experimental Biology and Medicine* **107**, 721–723.

Paans AM, Pruim J, Smit GP, Visser G, Willemsen AT, Ullrich K (1996). Neurotransmitter positron emission tomographic-studies in adults with phenylketonuria, a pilot study. *European Journal of Pediatrics* **155** (Suppl. 1), S78–S81.

Pascucci T, Ventura R, Puglisi-Allegra S, Cabib S (2002). Deficits in brain serotonin synthesis in a genetic mouse model of phenylketonuria. *Neuroreport* **13**, 2561–2564.

Patton JH, Stanford MS, Barratt ES (1995). Factor structure of the Barratt impulsiveness scale. *Journal of Clinical Psychology* **51**, 768–774.

Pickar D, Breier A, Kelsoe J (1988). Plasma homovanillic acid as an index of central dopaminergic activity: studies in schizophrenic patients. *Annals of the New York Academy of Sciences* **537**, 339–346.

Pietz J, Kreis R, Boesch C, Penzien J, Rating D, Herschkowitz N (1995). The dynamics of brain concentrations of phenylalanine and its clinical significance in patients with phenylketonuria determined by *in vivo* ^{1}H magnetic resonance spectroscopy. *Pediatric Research* **38**, 657–663.

Puglisi-Allegra S, Cabib S, Pascucci T, Ventura R, Cali F, Romano V (2000). Dramatic brain aminergic deficit in a genetic mouse model of phenylketonuria. *Neuroreport* **11**, 1361–1364.

Rashed MS, Ozand PT, Bucknall MP, Little D (1995). Diagnosis of inborn errors of metabolism from blood spots by acylcarnitines and amino acids profiling using automated electrospray tandem mass spectrometry. *Pediatric Research* **38**, 324–331.

Reise SP, Moore TM, Sabb FW, Brown AK, London ED (2013). The Barratt Impulsiveness Scale-11: reassessment of its structure in a community sample. *Psychological Assessment* **25**, 631–642.

Schmaal L, Veltman DJ, Nederveen A, van den Brink W, Goudriaan AE (2012). *N*-acetylcysteine normalizes glutamate levels in cocaine-dependent patients: a randomized crossover magnetic resonance spectroscopy study. *Neuropsychopharmacology* **37**, 2143–2152.

Sijens PE, Oudkerk M, Reijngoud DJ, Leenders KL, de Valk HW, van Spronsen FJ (2004). ^{1}H MR chemical shift imaging detection of phenylalanine in patients suffering from phenylketonuria (PKU). *European Radiology* **14**, 1895–1900.

Stemerdink BA, Kalverboer AF, van der Meere JJ, van der Molen MW, Huisman J, de Jong LWA, Slijper FME, Verkerk PH, van Spronsen FJ (2000). Behaviour and school achievement in patients with early and continuously treated phenylketonuria. *Journal of Inherited Metabolic Disease* **23**, 548–562.

Strisciuglio P, Concolino D (2014). New strategies for the treatment of phenylketonuria (PKU). *Metabolites* **4**, 1007–1017.

Surtees R, Blau N (2000). The neurochemistry of phenylketonuria. *European Journal of Pediatrics* **159** (Suppl. 2), S109–S113.

Ullrich K, Weglage J, Oberwittler C, Pietsch M, Funders B, van Eckhardstein H, Colombo JP (1994). Effect of L-dopa on pattern visual evoked potentials (P-100) and neuropsychological tests in untreated adult patients with phenylketonuria. *Journal of Inherited Metabolic Disease* **17**, 349–352.

Ullrich K, Weglage J, Oberwittler C, Pietsch M, Funders B, van Eckhardstein H, Colombo JP (1996). Effect of L-dopa on visual evoked potentials and neuropsychological tests in adult phenylketonuria patients. *European Journal of Pediatrics* **155** (Suppl. 1), S74–S77.

van Spronsen FJ, de Groot MJ, Hoeksma M, Reijngoud DJ, van Rijn M (2010). Large neutral amino acids in the treatment of PKU: from theory to practice. *Journal of Inherited Metabolic Disease* **33**, 671–676.

van Spronsen FJ, Hoeksma M, Reijngoud DJ (2009). Brain dysfunction in phenylketonuria: is phenylalanine toxicity the only possible cause? *Journal of Inherited Metabolic Disease* **32**, 46–51.

van Vliet D, Anjema K, Jahja R, de Groot MJ, Liemburg GB, Heiner-Fokkema MR, van der Zee EA, Derks TGJ, Kema IP, van Spronsen FJ (2015*a*). BH4 treatment in BH4-responsive PKU patients: preliminary data on blood prolactin concentrations suggest increased cerebral dopamine concentrations. *Molecular Genetics and Metabolism* **114**, 29–33.

van Vliet D, Bruinenberg VM, Mazzola PN, van Faassen MH, de Blaauw P, Kema IP, Heiner-Fokkema RM, van Anholt RD, van der Zee EA, van Spronsen FJ (2015*b*). Large neutral amino acid supplementation exerts its effect

https://doi.org/10.1017/S0033291717001398 Published online by Cambridge University Press

through three synergistic mechanisms: proof of principle in phenylketonuria mice. *PLoS ONE* **10**, e0143833.

van Vliet D, Bruinenberg VM, Mazzola PN, van Faassen MH, de Blaauw P, Pascucci T, Puglisi-Allegra S, Kema IP, Heiner-Fokkema MR, van der Zee EA, van Spronsen FJ (2016). Therapeutic brain modulation with targeted large neutral amino acid supplements in the Pah-enu2 phenylketonuria mouse model. *American Journal of Clinical Nutrition* **104**, 1292–1300.

Waisbren SE, Noel K, Fahrbach K, Cella C, Frame D, Dorenbaum A, Levy H (2007). Phenylalanine blood levels and clinical outcomes in phenylketonuria: a systematic literature review and meta-analysis. *Molecular Genetics and Metabolism* **92**, 63–70.

Weglage J, Moller HE, Wiedermann D, Cipcic-Schmidt S, Zschocke J, Ullrich K (1998). *In vivo* NMR spectroscopy in patients with phenylketonuria: clinical significance of interindividual differences in brain phenylalanine concentrations. *Journal of Inherited Metabolic Disease* **21**, 81–82.

Weglage J, Wiedermann D, Denecke J, Feldmann R, Koch HG, Ullrich K, Harms E, Möller HE (2001). Individual blood-brain barrier phenylalanine transport determines clinical outcome in phenylketonuria. *Annals of Neurology* **50**, 463–467.

Winn SR, Scherer T, Thony B, Harding CO (2016). High dose sapropterin dihydrochloride therapy improves monoamine neurotransmitter turnover in murine phenylketonuria (PKU). *Molecular Genetics and Metabolism* **117**, 5–11.

Yang YK, Yao WJ, McEvoy JP, Chu CL, Lee IH, Chen PS, Yeh TL, Chiu NT (2006). Striatal dopamine D2/D3 receptor availability in male smokers. *Psychiatry Research* **146**, 87–90.

https://doi.org/10.1017/S0033291717001398 Published online by Cambridge University Press