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Systematic Review and Meta-Analysis of Neuropsychiatric Symptoms and Executive Functioning in Adults With Phenylketonuria

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ABSTRACT

This systematic review and meta-analysis (MA) investigates the impact of elevated blood phenylalanine (Phe) on neuropsychiatric symptoms in adults with phenylketonuria (PKU). The meta-analysis of PKU is challenging because high-quality evidence is lacking due to the limited number of affected individuals and few placebo-controlled, double-blind studies of adults with high and low blood Phe. Neuropsychiatric symptoms associated with PKU exceed general population estimates for inattention, hyperactivity, depression, and anxiety. High Phe is associated with an increased prevalence of neuropsychiatric symptoms and executive functioning deficits whereas low Phe is associated with improved neurological performance. Findings support lifelong maintenance of low blood Phe.

Introduction

Phenylketonuria (PKU, OMIM 261600, also referred to as phenylalanine hydroxylase deficiency) is a rare autosomal recessive disorder characterized by a deficiency in the phenylalanine hydroxylase enzyme necessary for the conversion of the amino acid phenylalanine (Phe) to tyrosine. In the United States, the reported incidence of PKU ranges from 1 per 13,500 to 1 per 19,000 newborns and varies by ethnic group being higher in Whites and Native Americans and lower in Blacks, Hispanics, and Asians (National Institutes of Health Consensus Development Conference Statement: Phenylketonuria, 2001). The metabolic block in PKU causes excess blood Phe concentrations that result in increased brain Phe levels (Kreis, Zwygart, Boesch, & Nuoffer, 2009; Leuzzi et al., 2007; Rupp et al., 2001; Scarabino et al., 2009), decreased tyrosine brain influx and reduced cerebral protein synthesis (de Groot et al., 2013; Hoeksma et al., 2009). An association between long-term high blood Phe concentrations and neurocognitive impairment and psychological disturbances has been established, primarily based on pediatric studies (Lindegren et al., 2012). Current guidelines recommend that PKU treatment should be life long, with a goal of maintaining blood Phe between 120 and 360 μmol/L (Camp et al., 2014; Lindegren et al., 2012; Vockley et al., 2014). Citing several studies, a PKU Scientific Review Conference working group on long-term outcomes reported: "serious neurological impairments are now preventable in treated individuals with PKU. However, there is increasing recognition of more subtle physical, cognitive, and behavioral findings in these individuals" (Camp et al., 2014, p. 93; Gentile, ten Hoedt, & Bosch, 2010).

Designing a robust observational or interventional study for PKU is challenging. One of the major difficulties underlying the study design is that PKU is a rare disease resulting in a limited number of

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affected individuals within study catchment areas requiring active participation. Allelic variation in the phenylalanine hydroxylase locus leads to a wide range of phenotypes (Santos et al., 2010) and significant inter-individual variation in blood Phe. Clinical treatment is often variable with some individuals treated from birth after the introduction of newborn screening, some born prior to newborn screening beginning treatment later in life, and most have varying degrees of dietary and treatment adherence. This variability is amplified further by the changes that have occurred in the standards of care for PKU over time, across geographic locations, and during the lifespan. Individuals with PKU report substantial difficulty in maintaining recommended blood Phe levels; Phe levels tend to rise as individuals age (C. S. Brown & Lichter-Konecki, 2016); over 70% of adults do not access regular clinical therapy despite recommendations for treatment for life (Berry et al., 2013).

Designing a meta-analysis from a systematic review of the PKU literature presents additional challenges. Most studies are small, single-center studies with possible attendant selection bias that can overstate treatment effects (Dechartres, Boutron, Trinquart, Charles, & Ravaud, 2011). Few placebo-controlled, double-blind studies have compared high and low Phe states to provide high-quality evidence. Study methodologies are often flawed and lack rigorous recruitment, intervention, and analytical controls. Lindegren et al. (2012) found only one of 17 studies evaluating blood Phe concentrations relative to IQ in PKU to meet criteria for good quality (Lindegren et al., 2012). The remaining 16 studies were classified as having fair (5) or poor quality (11) primarily attributed to unclear documentation for participant recruitment, high attrition rates, excluding eligible partici-pants, and/or improperly assessing confounders in a valid manner (Lindegren et al., 2012).

Two prior meta-analyses (MA) examining blood Phe concentrations and intellectual functioning (e.g. IQ) in pediatric PKU populations (Lindegren et al., 2012; Waisbren et al., 2007) found a strong inverse relationship between historical blood Phe measurements and IQ. One of these studies also assessed the feasibility of performing a similar meta-analysis on the association between blood Phe and executive functioning; however, the absence of consistent outcome measures across studies preempted such an analysis (Lindegren et al., 2012). Indeed, few neurocognitive assessment tools were common to multiple studies. The variability in specific measurements selected for reporting (e.g., time vs. accuracy) further impeded direct comparison of results.

The capacity to conduct a meta-analysis on the association of executive functioning and blood Phe levels is particularly important for understanding the experiences of adults with early-treated PKU. Executive functioning is a collection of cognitive skills that are required to self-regulate and organize mental efforts in order to achieve goals. For most adults with early-treated PKU who have an IQ within normal range, executive functioning provides a relevant measure of cognitive ability as it relates to daily functioning. The heterogeneity among executive functioning measurement tools and reporting methods used in prior studies necessitates a meta-analysis method that can account for these challenges. Moyle, Fox, Arthur, Bynevelt, and Burnett (2007) demonstrated the feasibility of applying a Hedges g meta-analysis method to executive functioning reported in 11 studies of adolescents and adults with early-treated PKU. Hedges g allows for the inclusion of studies with small participant numbers (e.g., less than 10) through an adjustment for small sample sizes and accounts for variability in measurement tools by analyzing standard mean differences for each set of mean test scores for PKU cohorts and comparison groups.

Although Phe control for life is now considered standard of care, adherence and outcomes associated with a Phe-restricted diet in adulthood have not previously been reviewed in a systematic manner to encompass diverse treatment histories and a broad range of neuropsychiatric symptoms and executive functioning. The purpose of the current study is to establish a clearer understanding of neuropsychiatric and cognitive outcomes in adults with PKU by: (1) completing a thorough systematic review; (2) performing a meta-analysis on neuropsychiatric symptoms stratified by treatment history; and (3) conducting a meta-analysis on executive functioning domains in adults with early-treated PKU using Hedges g inclusive of studies published through 2013.

Methods

Systematic review

The systematic review (SR) was conducted on all literature of individuals ≥ 16-years-old with PKU, including case reports, and observational and interventional studies. The SR was performed using best methods SR research (Cook, 1997; Mulrow & Oxman, 1997) and in compliance with Meta-analysis of Observational Studies in Epidemiology (MOOSE) (Stroup et al., 2000) and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Welch et al., 2012) guidelines. Electronic searches were performed in MEDLINE via the PubMed interface, Embase Drugs and Pharmacology, and the Cochrane Collection database of systematic reviews from January 1, 1980 to June 30, 2013. Electronic searches were supplemented by manual searches of accepted studies and recent reviews including the Agency for Healthcare Research and Quality Comparative Effectiveness Review of Adjuvant Treatment for PKU (AHRQ Review) (Lindegren et al., 2012). Landmark studies published prior to 1980 were added to search results.[1]

Two reviewers independently evaluated each document, accepting those publications including adults with PKU and neuropsychiatric symptoms and/or executive function assessments, with a mean (or median) age ≥18 years or, for ≥16 years, if results were reported individually. Publications of overlapping participants were identified as "kinned" citations, and data was extracted and only counted once under the "parent" study. Two independent researchers extracted data from each accepted study. Early-treated, late-treated, and untreated individuals with PKU were defined as initiating a Phe-restricted diet ≤ 90 days of birth, >90 days of birth, or never, respectively.

The AHRQ Review was used to categorize executive functioning measurement tools for this study (Lindegren et al., 2012). The executive functioning domains examined were as follows: attention (focusing), planning, inhibitory control (self-regulation), cognitive flexibility (shifting focus), and working memory (transiently maintaining and processing information). Executive function assessments not classified within the AHRQ Review were placed into domains most consistent with their primary focus as reported by the primary author. Where there were multiple assessments for an executive function domain, the assessment most closely reflecting the executive functioning domain was selected. When a measure could be categorized under multiple domains, it was placed under the domain that best described its function that did not already have a tool from the study categorized within that domain.

Meta-analysis

MA were performed using random effects models based on the DerSimonian and Laird method (DerSimonian & Laird, 1986). When unable to determine the percentage of participants ≥ 16-years-old, the following steps were taken: (1) all studies with mean population ≥ 18-years-old were included to be as inclusive as possible and (2) a sensitivity analysis was performed including only those studies that reported a minimum age of ≥16-years-old. Results were stratified by PKU treatment history: only studies indicating treatment history were included.

For the executive function MA, a standardized mean difference for each set of mean test scores within each executive function domain was calculated, using Hedges g adjustment for small sample bias (Hedges, 1981). This method accounts for the variability among testing measures and results reporting within the PKU literature. The directionality of the test result was taken into account in the MA by multiplying the standardized mean difference by −1 if a higher score indicated better performance. Within each executive function domain, results were pooled across studies based on the standardized mean differences. For the neuropsychiatric symptoms MA, study-specific proportions were first transformed using the Freeman-Tukey transformation (Freeman & Tukey, 1950) to correct for over-dispersion.

Heterogeneity (i.e., variation in study outcomes between studies) was assessed by calculating the 95% confidence interval for the I^2 statistic (Higgins & Thompson, 2002). If evidence of high heterogeneity was detected, potential causes were assessed by recalculating I^2 removing one study at a time to assess the impact of each study; performing MAs for PKU treatment subgroups and excluding studies with a minimum age <16 years. Meta-regressions using generalized linear mixed models evaluated the relationships between PKU treatment, country of publication (USA vs. other), and publication year on the outcome of interest. For MA of controlled studies reporting executive function test scores, the potential for publication bias was assessed by calculating a Rosenthal fail-safe N (Rosenthal, 1979).

Statistical analyses were performed using SAS statistical software (SAS v.9.2, Cary, NC), Comprehensive Meta-Analysis (Biostat Inc. v.2.0, Englewood, NJ) and MedCalc (MedCalc Software, Belgium).

Results

The SR screened 1327 citations and accepted 82 primary studies (with 13 "kins"), including 42 primary noninterventional studies and 40 primary intervention reports (e.g., blinded cross-over studies, cohort studies, and case reports).

Systematic review: Neuropsychiatric symptoms

Table 1 summarizes 10 interventional studies (253 total participants) that assessed effects of changing blood Phe concentrations for ≥3 weeks in adults with PKU on neuropsychiatric symptoms. Blood Phe concentrations changes were induced by introducing a Phe-restricted diet in untreated adults (M. C. Brown & Guest, 1999; Fitzgerald et al., 2000; Yannicelli & Ryan, 1995), by re-introducing a Phe-restricted diet in early- and late-treated adults who had discontinued diet (Bik-Multanowski et al., 2008; Finkelson, Bailey, & Waisbren, 2001; Gassió et al., 2003; Lee et al., 2009; Marholin et al., 1978; Schuett, Brown, & Michals, 1985), or by introducing a Phe load in early-treated adults (ten Hoedt et al., 2011). Effects on neuropsychiatric symptoms were generally based on clinical observations, not test scores. Although most studies reported neuropsychiatric symptom improvement associated with better metabolic control, two blinded cross-over studies in late/untreated adults found little evidence of symptom reduction during low Phe periods (Lee et al., 2009; Marholin et al., 1978).

The SR identified case reports in which a Phe-restricted diet (or tetrahydrobiopterin treatment) was introduced to ameliorate neurologic or neuropsychiatric symptoms in symptomatic adults with PKU.[2] Of 20 adults with PKU and late-onset neurologic symptoms (11 cases of spastic paraparesis/ quadriplegia, 3 cases of muscle weakness/difficulty walking, 3 cases of vision loss) and/or psychiatric symptoms (3 cases of disabling depression, 4 cases of difficulty communicating, 1 case of agoraphobia), reducing blood Phe resulted in marked improvement of symptoms in 13 of 20 cases. In an additional 8 adults with PKU, intellectual disability, and severely disruptive behavior, 7 adults showed marked reduction in disruptive behaviors on a Phe-restricted diet.

Systematic review: Executive function

Table 2 shows data from 9 studies that assessed effects of blood Phe on executive function in adults with PKU. Four of these studies (Lou, Lykkelund, Gerdes, Udesen, & Bruhn, 1987; Pietz et al., 1993; Schmidt et al., 1994; ten Hoedt et al., 2011) used a single cohort intervention approach and found improvements with reduced Phe on reaction time (1 study), attention (3 studies), and cognitive flexibility (1 study). Five studies compared an on-diet (or low Phe) cohort with an off diet (or high Phe) cohort (Bik-Multanowski, Pietrzyk, & Mozrzymas, 2011; Brumm et al., 2004; Burgard, Rey, Rupp, Abadie, & Rey, 1997; Channon, Goodman, Zlotowitz, Mockler, & Lee, 2007; Dawson et al., 2011); although measures and results varied, better scores were associated with on-diet and/or low Phe in two or more studies for attention, working memory, and psychomotor speed/reaction time. An additional study plotted test results for working

Table 1. Studies assessing effects of reducing blood Phe for ≥3 weeks in adults with PKU and neuropsychiatric symptoms.

First Author, Year, [Reference]	N	Population and Treatment	Blood Phe (μmol/L) Mean ± SD (range)	Effects on Low-Phe Diet
Blinded Cross-over Studies				
Lee et al. (2009)	17	Late-treated, off-diet, intellectual disability, severe challenging behavior Returned to low-Phe diet for 24 weeks	off diet: 1570 ± 222 on diet: 553 ± 158	"Challenging behaviors" varied with individual but included aggression, agitation, self-injury, and screaming. No significant changes in frequency of challenging behaviors from participant diaries or in Aberrant Behavior Checklist, but 76% of caregivers gave positive comments on behavior during low-Phe diet
Marholin et al. (1978)	5	Late/untreated–treated, off diet, intellectual disability, severe challenging behavior Returned to low-Phe diet for 8 weeks	off diet: 2119–2906 on diet: 180–600	"Challenging behaviors" varied with individual but included aggression, self-injury, and stereotyped behavior. Few significant desirable behavior changes occurred in 4 adults in which proper methodological controls were employed. Tremor and stereotypy were ameliorated in 2 of 5 adults.
ten Hoedt et al. (2011)	9	Early and continuous treatment 4-week Phe load	on diet: 709 ± 332 Phe load: 1259 ± 332	Self-reported mood states were significantly less favorable (Profile of Mood States, $p = 0.017$) during Phe loading
Interventional Studies (Single Cohorts)				
Bik-Multanowski et al. (2008)	13	Early-treated, off-diet, psychiatric distress Returned to low-Phe diet for 3 months	off diet: 1160 (780–1620) on diet: 180–600	While on diet, 77% (10/13) showed significant improvement in Psychological Well Being Index, particularly in domains of anxiety and depression
M. C. Brown and Guest (1999)	8	Untreated, intellectual disability, severe challenging behaviors Introduced to low-Phe diet for 12 months	off diet: 1614 ± 355 on diet: 617 ± 139	"Challenging behaviors" varied with individual but included aggression, screaming, self-injury, and stubbornness. Caregiver diaries reported 59% reduction in frequency of negative behaviors. Significant reduction in medication use and hospitalization for asthma and respiratory tract infection.
Finkelson et al. (2001)	21	Early-treated, off-diet, then returned to diet as adults	not reported	While on diet, 86% (18/21) reported improvement in symptoms (mood lability, energy level, concentration, obesity)
Fitzgerald et al. (2000)	5	Untreated, intellectual disability, severe challenging behaviors Introduced to low-Phe diet for 12 months	off diet: 1676 (1535–1880) on diet: 479 (401–640)	"Challenging behaviors" varied with individual but included aggression, agitation, screaming, hyperactivity, and self-injury. Caregivers reported improved communication skills, alertness and attention while on diet and 4 of 5 participants showed marked reduction in challenging behaviors (aggression, hyperactivity, agitation).
Gassió et al. (2003)	15	Early/late-treated, off-diet, then returned to low-Phe diet for 12 months	on diet: 717 (330–1342)	While on diet, 40% reported improved health, 53% were calmer and less easily upset, 40% were more alert/better able to maintain attention, and 29% were less impulsive/aggressive
Schuett et al. (1985)	72	Early-treated, off-diet, then returned to low-Phe diet for ~10 months	not reported	While on diet, 58% showed positive changes in behavior, 78% showed marked decrease in hyperactivity, 83% showed loss of moodiness

(Continued)

Table 1. (Continued).

First Author, Year, [Reference]	N	Population and Treatment	Blood Phe (μmol/L) Mean ± SD (range)	Effects on Low-Phe Diet
Yannicelli and Ryan (1995)	88	Untreated, intellectual disability Introduced to low-Phe diet for ≥ 2 months	off diet: 1659 (1000–2330) on diet: 617 (182–1495)	Within 3 to 8 weeks of introducing low-Phe diet, 46% showed improved behavior, including reductions in degree of irritability (67%), attention to task (59%), hyperactivity (51%), aggressive behavior (46%), moodiness (46%), and incidence of neurologic symptoms (26%). Reduction in use of psychotropic medications was reported in 40% of those who showed improved behavior.

Table 2. Studies assessing effects of blood Phe on executive function in adults with PKU.

First Author, Year, [Reference]	N	Population and Treatment	Blood Phe (µmol/L) Mean ± SD (range)	Results in Low Blood Phe vs. High Blood Phe Cohorts
Intervention Studies (Single Cohort)				
Lou et al. (1987)	9	Early- (5) and late-treated (4), off diet	1477 (980–2050)	6 of 7 participants with abnormally long continuous visual reaction times on regular diet showed improved reaction times on low-Phe diet (means: 34.6 vs. 31.6 1/100 sec)
		After 3 weeks on low-Phe diet	758 (456–1052)	
Pietz et al. (1993)	5	Early-treated, off diet	1600 (1290–2130)	On low-Phe diet, participants showed improved scores for attention (Dot Pattern Exercise: 8.8 vs. 11.7) and cognitive flexibility (Color Pattern Exercise)
		After 4 weeks on low-Phe diet	753 (515–1023)	
Schmidt et al. (1994)	14	Early-treated, off diet	1332 (569–1949)	On low-Phe diet, participants showed significantly improved scores for attention (Dot Pattern Exercise: 8.1 vs. 10.1, $p < 0.001$)
		After 4 weeks on low-Phe diet	636 (121–1017)	
ten Hoedt et al. (2011)	9	Early and continuous treatment	on diet: 709 ± 332	Phe loading resulted in a significant larger fluctuation in tempo during sustained attention ($p = 0.029$), but had no significant effect on other measures (Amsterdam Neuropsychological Tasks)
		4-Week Phe load	Phe load: 1259 ± 332	
Studies Comparing Cohorts with Low and High Blood Phe				
Bik-Multanowski et al. (2011)	22	Early-treated	≤720	Low Phe cohort showed improved scores for working memory (Spatial Span: −1.09 SD vs. −2.92 SD), attention (Rapid Visual Info: −1.59 SD vs. −2.49 SD), and inhibitory control (Stop Signal accuracy: 0.68 vs. 0.60)
	27	Early-treated	>720	
Brumm et al. (2004)	11	Early-treated	<1000	When comparing low vs. high Phe cohorts the following reached or neared statistical significance: Attention (CPT Omission Errors 49.7 vs 65.5 and CPT response rate 57.8 vs. 37.3), language function (BNT 40.8 vs 13.1, COWAT 22.7 vs. 5.3, Animal Naming Test 21.9 vs. 16.9, PPVT-R 67.5 vs. 39.9, WAIS-R Vocabulary 67.3 vs. 47.7), psychomotor speed (WAIS-R Digit Symbol 68.8 vs. 48.7).
	13	Early-treated	>1000	
Burgard et al. (1997)	8	Early, continuously treated	870 (569–1150)	Low Phe cohort showed significantly improved scores for attention (Dot Pattern Exercise: 8.7 vs. 10.2)
	8	Early-treated, off diet	1350 (1029–1876)	
Channon et al. (2007)	25	Early, continuously treated	759 (221–1233)	Low Phe cohort showed significantly improved scores for working memory (2-back [% accuracy]: 88.9 vs. 84.6, p < 0.01) and attention (0-back [% accuracy]: 98.8 vs. 97.1, p < 0.01), but not cognitive flexibility (Object Alteration [% correct trials]: 66.5 vs. 60.2). Low Phe cohort results were mixed for inhibitory control, with significantly better results for speed (Flanker [speed]: 0.45 vs. 49, .47 vs. .52; $p < 0.01$) but deficits in accuracy (Flanker [% accuracy]: 99.4 vs. 98.0)
	25	early-treated, off diet	1286 (990–1651)	
Dawson et al. (2011)	21	Early, continuously treated	640 ± 103	Low Phe cohort showed improved scores for reaction time (saccadic latency: 6.0 vs. 5.6). Reaction times for low Phe cohort did not differ from unaffected controls ($p = 0.82$), while those for high Phe cohort were significantly worse ($p = 0.02$)
	56	Early-treated, off diet	1461 ± 185	

memory (Spatial Working Memory test), attention (Rapid Visual Information Processing test), and inhibitory control (Stop Signal Task) as a function of blood Phe level in 50 patients with classic PKU without intellectual disability (Bik-Multanowski & Pietrzyk, 2011). Plots demonstrated a visual trend of increasing executive function deficits with increasing blood Phe levels, though correlation coefficients were not reported.

Meta-analysis: Neuropsychiatric symptoms

Of 17 studies reporting psychiatric symptoms in adults with PKU, the 10 reporting prevalence of inattention, hyperactivity, anxiety, or depression were included in the MA (Anjema et al., 2011;

Table 3. Meta-analyses of reported neuropsychiatric complications and executive function deficits in adults with PKU.

	Number of Study Arms	Number of PKU Participants Tested	
Psychiatric Symptoms			**Symptom Prevalence (95% CI)**
Inattention			
Overall	5	805	49% (26%–73%)
Early-treated PKU	2	586	20% (17%–23%)
Late/untreated PKU	3	219	68% (54%–81%)
Hyperactivity			
Overall	8	945	20% (14%–28%)
Early-treated PKU	6	745	16% (12%–22%)
Late/untreated PKU	2	200	34% (20%–51%)
Anxiety			
Overall	8	889	22% (11%–36%)
Early-treated PKU	5	670	8% (6%–11%)
Late/untreated PKU	3	219	49% (26%–72%)
Depression			
Overall	8	889	18% (8%–31%)
Early-treated PKU	6	689	12% (5%–22%)
Late/untreated PKU	2	200	35% (16%–58%)
Neurologic Symptoms			**Symptom Prevalence (95% CI)**
Epilepsy/seizures			
Overall	14	1,028	10% (5%–17%)
Early-treated PKU	7	745	3% (1%–5%)
Late/untreated PKU	7	283	21% (17%–26%)
Tremors			
Overall	14	1,028	29% (16%–44%)
Early-treated PKU	7	745	18% (9%–29%)
Late/untreated PKU	7	283	40% (17%–65%)
Executive Function Deficits (Early-treated PKU only)			**Effect Size: Standardized Mean Difference (95% CI) [p-value]**
Working memory	5	112	0.08 (−0.45 to +0.61) [$p = 0.77$]
Attention	11	252	0.74 (0.55 to 0.93) [$p < 0.0001$]
Cognitive flexibility	7	157	0.43 (0.12 to 0.74) [$p = 0.006$]
Inhibitory control	6	119	0.41 (0.005 to 0.81) [$p = 0.047$]

Brumm et al., 2004; Campistol, González, Gutiérrez, & Vilaseca, 2012; Fisch et al., 1995; González et al., 2011; Kalkanoğlu et al., 2005; Koch et al., 2002; Pietz, Fatkenheuer, Armbruster, Esser, & Schmidt, 1997; Ris et al., 1997; Schuett et al., 1985). Of 19 studies reporting neurologic symptoms, the 10 reporting prevalence of both epilepsy/seizures and tremors were included in the MA (Campistol et al., 2012; Crowley, Koch, Fishler, Wenz, & Ireland, 1990; González et al., 2011; Leuzzi, Bianchi, Tosetti, Carducci, & Antonozzi, 2000; Leuzzi, Trasimeni, Gualdi, & Antonozzi, 1995; McDonnell, Esmonde, Hadden, & Morrow, 1998; Pfaendner et al., 2005; Pitt & Danks, 1991; Schuett et al., 1985; Thompson et al., 1993).

Table 3 presents MA results that show higher than expected prevalence estimates for neuropsychiatric symptoms in adults with PKU (based on clinical observation) when compared with prevalence estimates (based on a structured diagnostic interview) established by the U.S. National Comorbidity Survey Replication (Kessler, Chiu, Demler, & Walters, 2005).[3] Studies showed significant heterogeneity overall, but no heterogeneity was observed for inattention, hyperactivity, and epilepsy/seizures within subsets stratified by PKU treatment history. Heterogeneity in anxiety, depression, and tremors was largely explained by blood Phe level and PKU treatment (early vs. late/untreated).

Meta-analysis: Executive function

Of 15 studies reporting executive function assessments for adults with early-treated PKU and age/gender matched controls without PKU, 13 studies reported mean test scores with variance estimates and were subsequently included in the MA (Antenor-Dorsey et al., 2013; Burgard et al., 1997; Channon et al., 2007; Feldmann, Denecke, Grenzebach, & Weglage, 2005; Griffiths, Paterson, & Harvie, 1995; Luciana, Sullivan, & Nelson, 2001; Moyle, Fox, Bynevelt, Arthur, & Burnett, 2006, 2007; Pietz et al., 1998, 1995; Ris et al., 1997; Sundermann et al., 2011; Ullrich et al., 1996). The MA effect sizes using Hedges g found significant differences between adults with early-treated PKU and unaffected controls of 0.74 for attention (11 study arms; 252 participants, $p < 0.0001$), 0.41 for inhibitory control (6 study arms; 119 participants, $p = 0.047$), and 0.43 for cognitive flexibility (7 study arms; 157 participants, $p = 0.006$). The working memory domain of executive function failed to show a difference between adults with PKU and unaffected controls (5 study arms; 112 participants, $p = 0.77$).

Studies included in the MA for attention showed no evidence of heterogeneity ($I^2 = 0$), while moderate heterogeneity was observed for studies included in the MAs for working memory ($I^2 = 74$), cognitive flexibility ($I^2 = 46$), and inhibitory control ($I^2 = 60$). Given the low and moderate heterogeneity observed between studies, a meta-regression was performed on mean blood Phe and effects size for each domain. Although a positive correlation was observed, that is, increase in blood Phe was associated with more pronounced deficits in each executive function domain, results did not reach statistical significance. Sensitivity analyses, calculating effect size by removing one study at a time, showed that no individual study had a significant impact on pooled effect size. Results for fail safe N tests for publication bias found the following number of negative studies required to overturn the significant results of each respective executive functioning domain: attention, 167; inhibitory control, 11; and cognitive flexibility, 22.

Discussion

The SR-MA showed higher than expected rates for neuropsychiatric comorbidity, even among those with early-treated PKU, when compared to general population estimates. Although useful in quantifying the presence of symptom clusters, observational studies lack the capacity to determine the degree to which potential factors contribute to disease burden associated with PKU such as emotional stress (e.g., from chronic illness, dietary restrictions) and physiologic effects of lifetime and concurrent blood Phe. Intervention studies provide an opportunity to investigate the effects of short-term Phe reduction. All seven single cohort intervention studies reported neuropsychiatric improvement upon introduction or resumption of a Phe-restricted diet. Of three blinded cross-over studies that assessed the effects of blood Phe, one study in early-treated PKU showed a significant worsening of mood state on Phe loading (ten Hoedt et al., 2011), while two studies in late/never-treated adults found no significant change in the frequency of "challenging behaviors" upon resumption of a Phe-restricted diet (Lee et al., 2009; Marholin et al., 1978). The discrepancy between these results may reflect the Hawthorne effect that is the tendency of an individual to improve when followed through study participation. Interestingly, the blinded cross-over study that reported positive results differed from the remaining two studies in regards to participant selection (early and continuously treated) and off diet simulation (Phe load). This illustrates the importance of blinded studies when assessing treatment response and suggests a greater likelihood of demonstrating response to manipulating blood Phe in individuals with good historic and concurrent metabolic control.

Executive functioning impairment impacts daily life by interfering with the ability to perform basic cognitive tasks such as focusing, memory, planning, and impulse control. These tasks play a critical role in fulfilling responsibilities of adulthood such as acquiring/maintaining employment,

managing money, raising a family, and driving. MA of 13 unique studies that reported executive functioning measures and variance estimates for adults with early-treated PKU compared with unaffected controls found significant differences between the two groups in domains of attention, inhibitory control, and cognitive flexibility. Effect sizes found were 0.74 for attention, 0.41 for inhibitory control, and 0.43 for cognitive flexibility. An effect size of 0.4 means that the executive function score for an average person in the PKU group is 0.4 standard deviations below that for an average person in the control group and, hence, is below scores for 66% of the control group. Since the p-value depends on both the size of the effect and the sample size, obtaining a statistically significant effect in a small sample suggests that the effect is large. Furthermore, results for fail safe N tests for publication bias found that it would require a substantial number of negative studies to overturn these significant results.

The MA failed to find a significant difference between adults with early-treated PKU compared with unaffected controls for working memory (effect size of 0.08). The SR identified six study arms that assessed working memory, four of which reported a significant decrease in the PKU cohort for at least one assessment of working memory. However, three of four study arms reporting significant decreases in the PKU cohort were not included in the MA: one study could not be included in the MA because no variance estimates were reported (Wasserstein, Snyderman, Sansaricq, & Buchsbaum, 2006) and results reflecting accuracy rather than speed of the 2-back test were selected to represent working memory in two study arms (Channon et al., 2007). A previous MA in adolescents and adults with PKU (Moyle, Fox, Arthur, et al., 2007) also failed to demonstrate a significant working memory impairment based on three studies that overlapped with the current MA (Channon, German, Cassina, & Lee, 2004; Luciana, Hanson, & Whitley, 2004; Luciana et al., 2001). However, a previous MA in children and adolescents with PKU (DeRoche & Welsh, 2008) found a moderate deficit in working memory in the PKU cohort (effect size of 0.59) based on six studies. One possible explanation for the discrepancy between working memory assessments in children and adults with PKU is that sustained activity and neural efficiency, which are key aspects of working memory function, change across the course of development (Brahmbhatt, White, & Barch, 2010). McAuley and White (2011) found that processing speed, response inhibition, and working memory improved most rapidly between early and late childhood and plateaued in early adulthood; after controlling for processing speed only the effect of age on working memory remained significant.

There is an inherent challenge to identifying objective, consistent, and meaningful outcome measures, particularly for adults with late/untreated PKU. Late/untreated PKU causes substantial, life-long disability through the toxic effect of excess Phe exposure on myelin and dendritic projections during critical postnatal periods of neuronal development. Myelin formation and integrity facilitate the speed of action potentials along the axon and are critical to normal brain functioning. Brain MRI and histopathology studies of individuals with late/untreated PKU demonstrate the irreversible findings of diffuse cortical atrophy, white matter vacuolization, hypomyelination, and astrocystic gliosis (Dyer, 1999; Huttenlocher, 2000); phenotypically, these abnormalities are accompanied by severe cognitive and neurologic deficits (Fitzgerald et al., 2000). Currently available tools to measure psychiatric symptom response to intervention are inadequate in this population because valid administration of established tests requires participants to have expressive language ability. Subsequently, most treatment outcome studies involving adults with severe neurodevelopmental disabilities with disruptive behaviors have been completed by caregivers and are grossly insufficient for capturing the feelings, thoughts, and experiences of this population. The predominant white matter neuropathology in adults with early-treated PKU differs from that of late/untreated PKU. Neuroimaging findings suggest the presence of a demyelinating or dysmyelinating, rather than a hypomyelinating condition, hypothesized to result from abnormal intracellular water content, disruptions in myelin synthesis, and increased myelin turn over (Anderson & Leuzzi, 2010; Dyer, 1999; Leuzzi et al., 2007; Phillips, McGraw, Lowe, Mathews, & Hainline, 2001). These white matter abnormalities have been associated with high concurrent blood Phe levels, and have demonstrated some degree of reversibility following Phe reduction (Cleary et al., 1995; Scarabino et al., 2009), a

phenomenon that may also contribute to the psychiatric symptom and executive functioning responses measured in the blinded cross-over studies of adults with early-treated PKU.

The effects of elevated Phe on neuropsychiatric functioning extends beyond white matter structural changes and impact the availability of neurotransmitter precursors in the central nervous system. The defective phenylalanine hydroxylase enzyme impairs the peripheral production of tyrosine from Phe. Because Phe shares a common transport system across the blood brain barrier with tyrosine and tryptophan, elevated Phe levels also limit the entry of these amino acids into the central nervous system (Pardridge & Choi, 1986). As precursors to the neurotransmitters dopamine/neuroepinephrine and serotonin, low tyrosine and tryptophan levels, respectfully, in the CNS can impact the critical regulation of mood, anxiety, and cognition provided by these neurotransmitters (Burlina, Bonafé, Ferrari, Suppiej, & Zacchello, 2000; Landvogt et al., 2008; Stahl, 2000). This dynamic phenomenon involving concurrent Phe has the potential to affect adults with early- and late/untreated PKU and may subsequently contribute to improvements associated with Phe reduction in either group that relate to mood, anxiety, behavior, and functioning. The effect of Phe reduction in adulthood, however, occurs within the constraints of intellectual capacity established during the critical window of neurodevelopment.

Limitations of the SR-MA include weaknesses in the source studies. The majority of published reports were small (<50 adults), observational studies of fair/poor quality. Populations in most studies were poorly defined and likely heterogeneous. Published studies in adults with PKU necessarily reflect selection bias because a large percentage of adults with PKU are reported as lost to follow-up by metabolic clinics (Berry et al., 2013). The rarity of studies using a rigorous methodology reflects the challenges of recruiting a sufficient numbers of adults with this rare condition who have the requisite effort and ability to maintain a Phe-restricted diet over a sustained amount of time. Finally, few published reports provided a comprehensive survey of neuropsychiatric and neurologic complications in adults with PKU, including both debilitating complications and less severe complications that necessarily affect quality of life. Despite these weaknesses, the low to moderate heterogeneity observed in many of the MAs, particularly for early-treated subsets, provide confidence in the findings. Evidence quality is low for case reports and series; however, their inclusion in the SR provides a collective description of observations reported by individuals and their caregivers across the broad context of PKU in adulthood.

Although adult PKU treatment practices vary greatly by geographic region, most North American centers are expected to follow treatment for life guidelines as recommended by the American College of Medical Genetics and Genomics (Vockley et al., 2014). The SR-MA findings supported these recommendations whether in the context of maintaining or resuming a Phe-restricted diet. Yet, more than 70% of adults in the United States diagnosed with PKU via newborn screening are not actively treated at a metabolic clinic (Berry et al., 2013). In addition to personal choice, adults may discontinue PKU treatment because of barriers inherent to managing a chronic medical condition as well as those specific to PKU. Strict dietary restriction, medical food and formula intake, treatment costs, and insufficient social and health-care provider support systems challenge efforts to maintain low blood Phe levels over a sustained period of time. Executive functioning impairments may also hinder efforts to maintain low blood Phe by limiting the ability to follow a Phe-restricted diet, monitor Phe intake, plan meals, schedule and keep medical appointments. These issues merit consideration when developing therapeutic intervention strategies to improve metabolic control among adults with PKU.

Conclusions

This comprehensive SR and MA describe the broad range of neuropsychiatric and neurologic complications and executive function deficits in adults with PKU reported in the literature and capture this population's heterogeneity in overall functioning. Additionally, results from this SR suggest that lowering blood Phe levels in symptomatic adults with PKU may reduce neuropsychiatric

and neurologic complications and improve executive functioning. Although study findings support lifelong maintenance of low blood Phe, limitations in study design and sample size, particularly in late-treated cohorts, demonstrate the challenges inherent to establishing evidence-based guidelines in rare disorders with a heterogeneous presentation of symptomatology and impairment.

Notes

1. Files available from authors.
2. Files available from authors.
3. Files available from authors.

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Potential conflicts of interest and competing interests

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