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RESEARCH REPORT

Maternal Phenylketonuria: Long-term Outcomes in Offspring and Post-pregnancy Maternal Characteristics

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Abstract Maternal phenylketonuria (MPKU) is a well-recognized complication of PKU and one of the most potent teratogenic syndromes of pregnancy. Virtually all offspring from untreated pregnancies in women with classic PKU have intellectual disabilities and microcephaly. Congenital heart disease and intrauterine growth retardation occur many times more often than expected in the general population. Control of maternal blood phenylalanine during pregnancy prevents most if not all of these complications. Previous studies demonstrated the benefits of treatment in terms of birth parameters and early development. In this study, physical examinations, a medical history, and neuropsychological evaluation were obtained in 47 children from 24 mothers with PKU who received treatment during pregnancy. Mothers were interviewed and administered an abbreviated IQ test. Associations between maternal factors and offspring outcomes were also analyzed.

The 21 male and 26 female offspring ranged in age from 1 month to 26 years with 21 (62%) over 6 years. Results indicated mean intercanthal distances above the 70th percentile. Microcephaly was present in 19% of offspring, with head circumference below the third percentile. None of the offspring had cardiac anomalies. Mean offspring IQ was 94 ± 19, with 12% performing in the range of intellectual disability (IQ < 70). Among children >5 years of age, 25% had learning disabilities, 31% had attention deficit hyperactivity disorder (ADHD), 22% were on ADHD medication, and 34% had a diagnosis of anxiety and/or depression. Among the 24 mothers, 12 reported following the diet for PKU. Only one woman on diet had a blood phenylalanine concentration <360 μmol/L (recommended range) and the majority had indications of poor nutritional status. Mean maternal Full Scale IQ was 94 ± 16 (range = 61–117), with 25% performing in the borderline intellectual range (IQ < 85). Verbal IQ was significantly lower than Performance IQ ($p = 0.01$, CI 2.7, 16.1). On the self-report Beck Depression Inventory, Second Edition, 25% received scores indicating mild to moderate depression, and on the Beck Anxiety Inventory, 46% reported mild to moderate anxiety. Offspring IQ correlated with maternal metabolic control during pregnancy ($r = 0.51$), maternal IQ ($r = -0.62$), and socioeconomic position ($r = -0.48$). Offspring with ADHD, learning disabilities, or emotional disturbances were more likely to have mothers with anxiety and/or depression. To ensure optimal offspring outcomes, healthcare providers need to assess maternal nutrition, blood phenylalanine concentrations, cognitive abilities, and socioeconomic position. Interventions can then be initiated that reduce psychosocial stressors and enhance adherence to diet and positive parenting, which in turn can lead to better cognitive functioning, behavior, and emotional well-being in their children.

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Introduction

Maternal phenylketonuria (MPKU) is a well-recognized complication of PKU and one of the most potent teratogenic syndromes of pregnancy. Virtually all offspring from untreated pregnancies in women with severe PKU have intellectual disabilities and microcephaly. Congenital heart disease and intrauterine growth retardation occur many times more often than expected in the general population (Lenke and Levy 1980). The frequencies of these abnormalities in offspring are lower when the woman has a milder form of PKU but are still much greater than normally expected (Lenke and Levy 1980; Levy et al. 2003; Güttler et al. 2003).

Control of the maternal blood phenylalanine during pregnancy prevents most if not all of these complications (Lenke and Levy 1980; Rohr et al. 1987; Koch et al. 2003). The International Maternal PKU Collaborative Study (MPKUCS), a prospective, longitudinal study, showed that this was especially true if dietary therapy with control of maternal phenylalanine began before pregnancy or within the first 6 weeks of gestation (Koch et al. 2003.) In the MPKUCS, 228 children who were born to mothers with treated PKU or untreated mild hyperphenylalaninemia were compared to 70 control subjects at 7 years of age. Offspring cognitive outcome negatively correlated with the number of gestational weeks that elapsed until maternal metabolic control was achieved ($r = -0.61$). Behavioral outcome was similarly affected. Postnatal measurement of stimulation in the home was also related to offspring IQ (Waisbren and Azen 2003). However, the MPKUCS followed offspring only to age 7 years, thus was not able to evaluate cognitive performance into the more challenging school years.

The ability of the mother with PKU to provide a secure and intellectually stimulating environment for her offspring needs further examination. Poor metabolic control in adults with PKU is associated with deficits in executive functioning, including planning, organization, and behavioral inhibition as well as fatigue, health problems, depression, and anxiety (Koch et al. 2010; Brumm et al. 2010), all of which may limit parenting ability and result in a suboptimal environment for the offspring.

In this study, we examined offspring, ages 1 month to 26 years, from treated maternal PKU pregnancies and analyzed associations between maternal factors, including current adherence to recommendations for treatment, and offspring outcomes, such as cognitive abilities and emotional/behavioral characteristics.

Methods

Participants

Families with children in which the mother has PKU requiring dietary treatment (excluding non-PKU mild hyperphenylalaninemia) were invited to participate in this study. Classification of degree of PKU in the mothers was established as set forth by the European multicenter study (Guldberg et al. 1998) and was based on at least two of the following indictors: confirmatory or untreated blood phenylalanine level; dietary tolerance for phenylalanine; and phenylalanine hydroxylase (PAH) genotype. The study was approved by the institutional review board (IRB) of Boston Children's Hospital. The parents and children in each family were seen in the Clinical and Translational Study Unit (CTSU) of Boston Children's Hospital. The mother in each family as well as all offspring above the age of 6 years gave informed consent/assent. Nine fathers consented to having a photo taken for comparison of offspring physiognomy.

Evaluations

Clinical

A detailed health history was obtained from the mothers and a health and developmental history was obtained on the offspring. The mothers and offspring received a general physical examination as well as a neurologic assessment. Dysmorphology in offspring was evaluated through measurements of inner and outer canthal distances, interpupillary distance, lengths of the palpebral fissures, and the lengths of both ears (Hall et al. 2007). Frontal and side photographs of the face were obtained on the offspring and, for comparison, on the mothers and fathers. Parental photographs allowed for identification of dysmorphic features associated with maternal PKU and not simply reflective of familial characteristics.

Nutrition

Mothers were interviewed about their current diets (including a questionnaire about the use of medical foods, avoidance of protein, and other nutrition therapies) and asked to provide a 24 h diet recall. Laboratory studies included plasma amino acids, hemoglobin and hematocrit, prealbumin, 25-hydroxy vitamin D, red blood cell folate, iron, ferritin, zinc, and vitamin B_{12}. The mother's

adherence to diet during pregnancy was assessed by the review of pregnancy blood phenylalanine results, and assignment was made into one of three categories: (1) Excellent: on diet prior to conception, blood phenylalanine <360 μmol/L throughout pregnancy; (2) Good: on diet prior to conception, blood phenylalanine <360 μmol/L by 10 weeks gestation; (3) Fair: blood phenylalanine <600 μmol/L by second trimester; and (4) Poor: on diet after conception and blood phenylalanine not in control until the second trimester or after.

Neuropsychological

Mothers were administered the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler 1999). They also completed self-report measures of adaptive behavior (Adaptive Behavior Assessment System—Second Edition (ABAS-II)) (Harrison and Oakland 2003), executive functioning (Behavior Rating Inventory of Executive Functioning (BRIEF)) (Gioia et al. 2000), anxiety (Beck Anxiety Index (BAI)) (Beck and Steer 1993), and depression (Beck Depression Index—Second Edition (BDI-II)) (Beck et al. 1996).

The offspring were administered age-appropriate measures of intellectual development: Bayley Scales of Infant and Toddler Development, Third Edition (Bayley 2003), for children less than 36 months of age; Wechsler Preschool and Primary Scales of Intelligence, Third Edition (WPPSI-III) (Wechsler 2002), for ages 4–6 years; and the WASI for offspring ages 7 years and older. In addition, the offspring completed the Beery-Buktenica Developmental Test of Visual Motor Integration, Sixth Edition (VMI) (Beery et al. 2010), which measures visual-spatial abilities and fine motor coordination. The mothers rated their children using age-appropriate forms of the ABAS-II, BRIEF, and a measure of emotional well-being, Behavior Assessment System for Children, Second Edition (BASC-II) (Reynolds and Kamphaus 2003).

Statistical Analyses

We report descriptive statistics for the offspring and mother characteristics and outcomes, including mean and standard deviation or median and range as appropriate. Two-group comparisons of continuous variables use the Wilcoxon rank-sum test of median values, and comparisons of categorical variables use Fisher's exact test. For distributional reasons, we report Spearman's correlation coefficients for rank associations of continuous variables. Because of multiple child observations per mother, we use a Monte Carlo bootstrap approach with 10,000 iterations to calculate 95% confidence intervals (CIs) and p-values for correlations of child and maternal outcomes. Similarly we used linear models fit with generalized estimating equations (GEE) to compare mean values of child outcomes between groups.

Table 1 Sample demographics

	Mean ± SD, range or *n* (%)
Mothers (n = 24)	
Age (years)	39 ± 7, 24–49
Education (years)	14 ± 3, 9–18
Hollingshead–Redlich social position	39 ± 19, 15–73
Marital status	
Married	19 (79)
Single	3 (13)
Divorced	2 (8)
Offspring (n = 48)	
Mean ± SD age (years)	8.5 ± 6.2, 0–26
Ages 0–3 years (infancy)	12 (26)
Ages 4–5 years (preschool)	6 (13)
Ages 6–18 years (school age)	26 (55)
Ages 19+ years (adult)	3 (6)
Sex	21 males (44)
	27 females (56)

Results

Sample Demographics

The 24 mothers were well into their childbearing years (Table 1) and 10/24 (42%) completed a bachelor's degree in college. The majority were married and had middle-class socioeconomic status. The Hollingshead Index of Social Position (Hollingshead 1957) ranged from 15 to 73, where low scores indicate higher levels of education and employment. In our sample, 9/23 (39%) families were in the lowest two Hollingshead social positions (scores >43).

There were 77 pregnancies among the mothers with 56/77 (73%) of pregnancies resulting in live births (including 2 sets of twins), 16/77 (21%) spontaneous abortions, and 5/77 (6%) terminations. Of the 58 live births, one offspring suffered asphyxia at birth, with associated cerebral palsy, and was excluded from the study since her condition was judged by her physicians as not related to her mother's PKU. One offspring with PKU was included since he had been in excellent metabolic control since birth. An additional 10/58 (17%) were unavailable due to living in another state, working or no longer in contact with the mother. A total of 47 offspring (81%) were available for study. The majority were of school age or older, with 29/47 (62%) ages 6–18 years. Also included in the sample were

Table 2 Offspring cognitive and emotional outcomes

Outcome	Mean ± SD	Range	*n* (%) outside normative range[a]
Bayley scales of infant and Toddler development, third edition (ages < 3 years)			
Bayley cognitive	113 ± 12	95–130	0/9 (0)
Bayley language composite	112 ± 12	94–132	0/9 (0)
Bayley motor composite	101 ± 11	82–124	1/9 (11)
Intelligence test (WPPSI-III or WASI) (ages > 3 years)[b]			
Full Scale IQ	94 ± 19	53–138	9/36 (25)
Verbal IQ	94 ± 20	55–150	12/36 (33)
Performance IQ	97 ± 17	56–127	7/36 (19)
Vocabulary subtest (WASI)	45 ± 13	20–69	8/24 (33)
Block design subtest (WASI)	48 ± 11	23–65	4/24 (17)
Similarities subtest (WASI)	44 ± 12	20–66	8/24 (33)
Matrix reasoning subtest (WASI)	48 ± 14	20–65	5/24 (21)
Visual-spatial skills			
Visual motor integration test (VMI)	89 ± 16	45–118	10/33 (30)
Adaptive behavior assessment system, second edition (ABAS-II)			
General adaptive composite (GAC)	97 ± 19	43–130	9/45 (20)
Conceptual	99 ± 20	49–133	11/46 (24)
Social	102 ± 17	61–133	8/46 (17)
Practical	92 ± 18	42–120	11/46 (24)
Behavior assessment system for children, second edition (BASC-2)			
BASC somatization	46 ± 11	33–69	6/38 (16)
BASC attention problems	50 ± 10	33–76	6/38 (16)

[a] Thresholds indicating performance within normative range on neuropsychological measures:
– >85 for BSID, IQ, VMI, and ABAS-II scales
– >40 for WASI subtest scores
– <60 for BASC-2
[b] 24 offspring received a WASI and 12 preschool children received the WPPSI-III

12 infants (<3 years), 6 preschool-aged children (ages 4–5 years), and 3 young adults (ages 19–26 years). Among the 47 offspring, 18 (38%) were from pregnancies in which adherence to medical recommendations was rated as excellent, 15 (32%) in which adherence was good, 10 (21%) in which adherence was fair, and 4 (9%) in which adherence was poor.

Offspring Outcomes

Dysmorphology

The only facial dysmorphology noted in the offspring was hypertelorism; the mean percentile distances were 73 ± 25% for the inner canthal distance, 89 ± 12% for outer canthal distance, and 83 ± 22% for the interpupillary distance. Other features of facial dysmorphology that have been reported in treated MPKU such as epicanthal folds, elongated and smooth philtrum, and high-arched palate were not observed. Comparisons between facial photographs of maternal PKU offspring and their parents revealed no other dysmorphic findings. None of the offspring had congenital heart disease. The mean head circumference of the offspring was in the 48th percentile but with the wide range of <3–99%. Nine (19%) of the 47 offspring had head circumference <3 percentile, indicating microcephaly.

Cognitive Functioning and Emotional Well-Being

Child cognitive and emotional outcomes are presented in Table 2. The mean Cognitive, Language, and Motor Composite scores on the Bayley Scales of Infant and Toddler Development, Third Edition, were well within the average range (85–115). Infants received a mean DQ of 113 ± 12, while preschool children attained a mean IQ of 96 ± 19 and older children attained the same mean Full Scale IQ of 96 ± 19. The mean IQ for the three adults in the study was 81 ± 15, considerably lower than the IQ of the school-aged children. The correlation between age and

Full Scale IQ in offspring over age 3 years was -0.32 ($p = 0.06$). Overall, 6/26 (23%) offspring attained a Full Scale IQ in the borderline range (IQ 70–85) and 3/26 (12%) performed in the range of intellectual disabilities (IQ < 70). Difficulties in fine motor coordination and visual-spatial skills were noted on the VMI, for which the child is asked to copy a series of increasingly complex geometric figures. The mean score on the VMI was 89 ± 16, with 10/33 children (30%) at least one standard deviation below the normal mean of 100.

The mean score on the ABAS-II General Adaptive Composite (GAC) was comparable to Full Scale IQ. The mother's responses indicated that children had relative weaknesses in the practical domain and strengths in the social realm.

Scores on the Behavior Assessment System for Children, Second Edition, were generally within the average range. These mothers perceived their children as well adjusted and well behaved. The only two scales with moderately elevated scores were Somatization (physical complaints) and Attention Problems, both with 6/38 (16%) in the at-risk range.

However, the mother's answers to direct questions related to school functioning and medical intervention suggest that maternal PKU offspring experience troublesome symptoms not readily detected by intelligence testing or the parent questionnaires we administered. Among the children at least 5 years of age, 8/32 (25%) had learning disabilities, 10 had attention deficit hyperactivity disorder (ADHD) (31%) with 7 on ADHD medication (22%), and 11 had been diagnosed with anxiety and/or depression (34%). These percentages are above rates reported for the general population in which 1.9% of children are known to have learning disabilities (Brault 2012), 5.1% are labeled as having ADHD (2014a), 6.5% are on medication for ADHD in Massachusetts (ADHD 2014b), and 15–20% of children suffer from anxiety or depression (Beesdo et al. 2009). In addition, two children in our sample had been diagnosed with bipolar disorder, and one teenaged boy suffered from substance abuse.

Maternal Outcomes

Maternal Health and Nutrition

Among the 24 mothers, 16 (67%) had severe PKU, 6 (25%) had moderate PKU, and 2 (8%) had mild PKU. History and physical examination of the mothers did not reveal any major abnormalities. One mother had a papular erythematous rash on both arms, another mother had recurrent basal cell carcinomas, and a third mother had mild eczema. Among 22 mothers whose height and weight were measured, 5 (23%) had body mass index (BMI) in the normal range, 10 (45%) were overweight, 6 (27%) were obese, and 1 (5%) was underweight. All 24 women answered the questionnaire about diet practices, and 12 (50%) reported following a diet for PKU although 14 (58%) reported taking a PKU formula/medical food. Among 12 women responding to a question about formula consumption, 9 (75%) reported taking at least 75% of the amount of prescribed medical food. Overall, 16/23 mothers (70%) reported restricting protein intake.

Of the 10 women who reported being off-diet, 3 (30%) stated that they restricted protein, and one of them also reported taking medical food. Among the entire sample, 3/24 women (13%) were treated with tetrahydrobiopterin (sapropterin dihydrochloride or Kuvan®), a cofactor for phenylalanine hydroxylase. No women were treated with large neutral amino acid (LNAA) therapy.

Laboratory indices of maternal nutritional intake are presented in Table 3. Results are stratified by self-report of phenylalanine-restricted diet. Laboratory values for phenylalanine, tyrosine, vitamin B12, and RBC folate differed significantly between the on- and off-diet groups. While the on-diet group had significantly lower blood phenylalanine compared to the off-diet group, overall only 3/23 mothers (15%) had blood phenylalanine levels less than 600 μmol/L at time of the study and none had blood phenylalanine <360 μmol/L.

Vitamin D was abnormally low in 1/3 (33%) of on-diet mothers and 5/7 (71%) of those off-diet. Prealbumin values were predominantly in the normal range, suggesting adequate protein intake. Hemoglobin and hematocrit values were normal and did not differ significantly between the groups. Vitamin B12 and RBC folate were significantly higher in the on-diet group.

Maternal Cognitive and Emotional Outcomes

As noted in Table 4, mean scores were within the average range (85–115) on the Wechsler Abbreviated Scale of Intelligence (WASI). The mean maternal Full Scale IQ was 94 ± 16, with 6/24 mothers (25%) having a Full Scale IQ < 85 (one standard deviation below the population normal mean.) Maternal Performance IQ was on average 9.4 points higher than Verbal IQ, reflecting reduced vocabulary and verbal reasoning abilities ($p = 0.01$, 95% CI 2.7–16.1 points higher).

The ABAS-II measures self-reported functioning in a variety of domains, including cognitive, social and practical. The mothers rated themselves slightly above the population norm of 100 on all dimensions, with a mean overall score of 110 ± 10. Among the 23 mothers completing this questionnaire, only 1 (4%) received a score <85, which on this test similarly represents more than one standard deviation below the population mean.

Table 3 Median and range of dietary intake and laboratory findings in mothers with PKU on and off a phenylalanine-restricted diet (figure in parenthesis indicates number of women with values out of recommended range)

	On-diet ($n = 12$)	Off-diet ($n = 10$)	Reference values	p-value[a]
Phenylalanine (μmol/L)	816; 389–1,610 (10/12)	1,319; 395–1,934 (9/10)	120–600[b]	0.04
Tyrosine (μmol/L)	47; 30–112 (1/12)	32; 25–36 (5/9)	32–122	0.03
Prealbumin (g/dL)	27; 23–43 (1/12)	25; 20–35 (0/10)	20–40	0.64
Vitamin D (ng/mL)	40.2; 25.3–43.4 (1/3)	28.7; 21.2–39.2 (5/7)	30–80	0.34
Vitamin B12 (pg/mL)	844.5; 197–1,336 (6/12)	328; 223–859 (0/9)	211–946	0.02
Ferritin (mg/dL)	53.5; 18–108 (0/12)	67; 15–198 (2/9)	13–150	0.11
Hemoglobin (g/dL)	13.5; 12.4–14.3 (0/12)	13.2; 12.3–14.7 (0/9)	11.5–16	0.52
Hematocrit (%)	39.5; 35.8–43.1 (0/12)	38.5; 35.6–41.4 (0/9)	34–44	0.27
Plasma Zinc (ng/mL)	119; 58–193 (4/12)	128.5; 94–158 (1/8)	70–150	0.42
RBC folate (ng/mL)	922; 579–1,468 (3/12)	828; 417–862 (1/9)	468–1,258	0.03

[a] p-values from the Wilcoxon rank-sum test
[b] Recommended blood phenylalanine range for adults with PKU

Table 4 Maternal neuropsychological outcomes

	Mean ± SD	Range	Outside normal bounds[a] (%)
All mothers			
Full Scale IQ[b]	94 ± 16	61–117	6/24 (25)
Verbal IQ	90 ± 16	59–113	7/18 (39)
Performance IQ	100 ± 16	69–126	3/18 (17)
ABAS GAC	110 ± 11	83–128	1/23 (4)
ABAS Conceptual	106 ± 18	39–120	2/23 (9)
ABAS Social	104 ± 21	26–120	4/23 (17)
ABAS Practical	108 ± 14	63–120	1/23 (4)
BRIEF GEC	46 ± 10	35–76	1/23 (4)
Beck depression inventory	8.3 ± 9.8	0–39	6/24 (25)
Beck anxiety inventory	6.7 ± 7.0	0–30	11/24 (46)
Mothers on formula			
Full Scale IQ	100 ± 11	79–117	1/14 (7)
Verbal IQ	96 ± 13	80–109	3/9 (33)
Performance IQ	105 ± 14	83–126	1/9 (11)
Beck depression inventory	5.8 ± 7.2	0–26	2/14 (14)
Beck anxiety inventory	3.9 ± 4.3	0–12	5/14 (36)
Mothers not on formula			
Full Scale IQ	86 ± 19	61–115	5/10 (50)
Verbal IQ	85 ± 18	59–113	4/9 (44)
Performance IQ	95 ± 18	69–123	2/9 (22)
Beck depression inventory	11.7 ± 12.1	0–39	4/10 (40)
Beck anxiety inventory	10.6 ± 8.3	0–30	6/10 (60)

ABAS GAC Adaptive Behavior Assessment System, General Adaptive Composite; *BRIEF GEC* Behavior Rating Inventory of Executive Function, Global Executive Composite
Thresholds for normal neuropsychological test measures used:
– >=85 for IQ measures
– <=64 for BRIEF GEC
– <=13 for Beck Depression Inventory
– <=7 for Beck Anxiety Inventory
[a] Six mothers received the 2-subtest form of the WASI, which yields only a Full Scale IQ

The mothers did not report themselves as having difficulties in executive functioning, as measured by the BRIEF. Of the 23 mothers completing this self-report questionnaire, 1 (4%) received a score >65 on the Global Executive Composite (GEC).

Emotionally, a different picture emerged, with 6/24 mothers (25%) receiving scores on the self-report Beck Depression Inventory, Second Edition (BDI-II) > 13, indicating mild to moderate depression, and 11/24 mothers (46%) reporting mild to moderate anxiety on the Beck Anxiety Inventory (BAI) with scores >7. These percentages are higher than reported in the general population, where annual rates are 9.5% for depression and 18.1% for anxiety (Kessler et al. 2005).

Mean scores on all measures of cognitive functioning were directionally higher for mothers on-diet (defined as taking formula). Moreover, 2/14 on-diet mothers (14%) self-reported depression on the BDI-II compared to 4/10 off-diet mothers (40%) (Fisher $p = 0.19$), and 5/14 on-diet mothers (27%) self-reported anxiety on the BAI compared to 6/10 off-diet mothers (60%) (Fisher $p = 0.41$).

Correlations Between Offspring Outcome and Maternal Characteristics

As expected, offspring IQ correlated highly with maternal metabolic control during pregnancy ($r = 0.51$, $p = 0.002$, 95% CI (0.19, 0.74)), maternal IQ ($r = 0.62$, $p = 0.0001$, 95% CI (0.33, 0.81)), and the Hollingshead Index of Social Position (Hollingshead 1957) ($r = -0.48$, $p = 0.005$, 95% CI (−0.68, −0.12)). Offspring IQ was not associated with current maternal blood phenylalanine level ($r = -0.10$, $p = 0.55$, 95% CI (−0.44, 0.25)), diet status (on- or off-diet) (mean difference 0.6, $p = 0.96$), or maternal marital status (mean difference 4.6, $p = 0.38$).

Children whose mothers had depression or anxiety were at risk for behavioral difficulties, psychiatric problems, and learning disabilities: 7/11 (64%) of children with anxiety or depression, 6/10 (60%) of children with ADHD, and 6/8 (75%) children with learning disabilities had mothers with anxiety or depression.

Discussion

The purpose of this study was to obtain a longer-term assessment of offspring from treated MPKU pregnancies and to identify maternal and environmental characteristics associated with offspring outcome. Published results of offspring outcome from treated maternal PKU have been limited to developmental studies of the neonatal and early infancy periods or to cognitive assessments in the first few years of childhood. The oldest offspring from treated maternal PKU pregnancies in our previous New England study was 4 years old (Rohr et al. 1987), and the evaluations of offspring in the MPKUCS ended at age 7 years (Koch et al. 2003; Waisbren and Azen 2003). In this study, 62% of the offspring were older than age 5 years with the oldest age 26 years. This study extended the length of follow-up into adolescence and early adulthood and also examined emotional and behavioral characteristics of these offspring. Particular attention was given to those factors of PKU in the mothers that might affect their ability to provide a secure and stimulating environment for their offspring.

None of the maternal PKU offspring in our study had evidence of heart disease or a history of having had congenital heart disease. The mean head circumference of the 48th percentile was within the expected range for the general population although in several instances, MPKU might have had a lingering adverse effect on head growth since 19% had a head circumference below the 3rd percentile, lower than the 31% for microcephaly among offspring in the MPKUCS. Facial dysmorphology included evidence of hypertelorism, as previously reported (Rouse et al. 1997, 2000), but other facial features reported as due to MPKU were absent. In summary, we found growth and somatic development in the offspring similar to the general population.

Despite the variability in current adherence to the diet for PKU, the majority of women who participated in this study perceived themselves as functioning well in their daily lives. Nonetheless, 25% performed in the borderline intellectual range, much higher than the 12.3% reported in epidemiological studies (e.g., Hassiotis et al. 2008) and higher than the 15.9% expected based on the normal population curve for IQ. Their mean IQ was 94, lower than that of adults with PKU recently studied in Europe (Weglage et al. 2013). In the study of 57 adults with PKU, the mean IQ was 100.6 compared to an IQ of 110.4 in a matched control group. However, the group of mothers in our study taking their medical food (formula) was 100, the same as that in the European study. Nearly 40% of mothers in our study were in the two lowest categories of socioeconomic position. Closer examination of their scores on tests of verbal expression, verbal reasoning, depression, and anxiety revealed vulnerabilities that may have an important impact on their parenting skills and hence on their children's development and well-being.

Within the general population, individuals with IQ in the borderline range are significantly more likely to be at social disadvantage, experience neurotic disorders (such as anxiety and depression), and suffer from substance misuse. They take psychotropic medications and seek emergency services at a higher rate but are not more likely to seek psychotherapy

(Hassiotis et al. 2008). Other studies report that adults with borderline IQ are at risk for poor occupational attainment and depression (Seltzer et al. 2009).

Children of parents with borderline IQ, anxiety, or depression also seem to be negatively affected, not so much during the preschool period but after age 7 years, with increased rates of conduct disorders and emotional and attention problems (Whitely et al. 2011). Likewise in our study of maternal PKU offspring, children under age 3 years exhibited fewer cognitive or behavioral deficits than children older than 3 years. By school age, the children were much more likely to have ADHD, learning disabilities, and anxiety or depression than children in the general population. This suggests that the deficits noted later in childhood may be related not only to prenatal effects but also to environmental circumstances, including the home environment, maternal depression, anxiety, and educational opportunities. We found that the majority of school-aged maternal PKU offspring with behavioral disturbances, emotional difficulties, or low IQ had mothers who were depressed or anxious. The percentage of children with psychiatric symptoms or low IQ may be even higher than we reported, given that one mother appeared to be an "outlier." Her 5 offspring had a range of problems including anxiety and depression, ADHD, low IQ, and learning disabilities, but she rated herself as having no anxiety or depression. Based on clinical observation and prior medical reports, however, she experiences both anxiety and depression. If she had rated herself as such, all offspring with ADHD, all with low IQ, all with learning disabilities, and 82% of those with anxiety and depression would have mothers who were experiencing anxiety or depression.

While there was overlap between current maternal blood phenylalanine results for the on- and off-diet groups, median blood phenylalanine concentrations were lower in the on-diet group. Notably, only one woman on diet had blood phenylalanine concentration <360 μmol/L, which is considered to be safe for an MPKU pregnancy (Vockley et al. 2014). The typical "on-diet" approach for this population consisted of taking about 75% of the prescribed phenylalanine-free or low-phenylalanine medical food (formula) for PKU and avoiding high-protein foods such as meat, eggs, nuts, and dairy products. Few women strictly reduced their phenylalanine intake, as evidenced by elevated plasma phenylalanine concentrations.

The women off-diet were at significantly higher risk of having low blood tyrosine, which may be counterintuitive since the off-diet group consumes more natural protein. However, medical food is a source of tyrosine and may account for the significantly higher blood tyrosine in the on-diet group. Other discrepancies between amino acid values in the two groups are notable, especially higher blood concentrations of the large neutral amino acids—valine, leucine, and tryptophan—in the on-diet group. Valine and leucine are large neutral amino acids (LNAAs) which compete for transport of phenylalanine into the brain. High amounts of LNAAs in the blood block phenylalanine uptake as well as promote neurotransmitter synthesis (Pietz et al. 1999).

Half of the women had 25-OH vitamin D results below the lower limit of 30 ng/mL, but only one had severe vitamin D deficiency (<20 ng/mL) (Christesen et al. 2012). A recent Cochrane review indicates that vitamin D supplementation and higher levels of 25-OH vitamin D in pregnant women have been associated with increased birth weight. Moreover, observational studies show a positive effect of vitamin D status on other health outcomes in children. Indices of iron nurtiture (hemoglobin, hematocrit, ferritin) were normal in both groups, as were zinc and folate. Plasma vitamin B12 was significantly lower in the off-diet group compared to the on-diet group, again indicating that the medical food is a significant source of vitamin B12 in the diets of mothers with PKU. Vitamin B12 deficiency in adults with PKU is a well-established phenomenon arising from diets that do not contain either medical food or animal products (Hvas et al. 2006).

The prevalence of overweight women with PKU (45%) and obesity (27%) was somewhat above national norms that indicate 64% of the population is either overweight or obese Fryar and Ogden 2014; Fryar et al. 2014. Female children with PKU have been reported to have a higher incidence of being overweight or obese in a single study (Burrage et al. 2012). In a previous study of pregnant women, a bimodal distribution of prepregnancy weight was observed with nearly equal numbers of overweight and underweight women (Rohr et al. 2004).

The suboptimal nutritional status of these women with PKU may have implications for their emotional well-being. For example, recent studies demonstrate associations between low vitamin D or low vitamin B12 and depression (Anglin et al. 2013; Kalita et al. 2013), and low tyrosine has been implicated in depressive symptoms in PKU (Sharman et al. 2012). Moreover, anxiety and agoraphobia have long been recognized as symptoms of poor metabolic control in PKU patients (Waisbren and Levy 1991; Brumm et al. 2004).

Very few women were being treated with tetrahydrobiopterin supplementation and none with large neutral amino acids. The women who were enrolled in the study were older, and many were off-diet and not seen frequently in clinic. Thus, they may be part of the "lost generation" of adults with PKU who do not have the opportunity to learn about updated therapies for PKU (Burton and Leviton 2010). However, this MPKU follow-up study offered an opportunity to update women about treatment options, and yet none chose to change their current treatment. Responses

to the nutrition questionnaire may provide insight into this behavior. About half of the women who were off-diet reported that they had tried to return to diet at some point in the postpartum period. When asked about obstacles to returning to diet, the most common responses were not the expected ones (difficulty with formula, protein restriction, or insurance) but rather that they did not perceive a need for any treatment. Only one mother reported lack of access to treatment as an obstacle.

One limitation of this study was the use of self-report and parental report instruments. Rates of offspring difficulties may be underestimated in our study, since the mothers tended not to report themselves or their children as functioning below the average range while cognitive test scores tended to suggest otherwise. Subgroup comparisons of on-diet versus off-diet women or children with versus without cognitive deficits or mood disorders involved small sample sizes.

Conclusion

The primary conclusion of this study is that maternal diet influences offspring outcome—before, during, and after pregnancy. Mothers who are well treated from the very beginning of their lives have a higher IQ and therefore often a higher SES, both of which were found to correlate with offspring outcome. They are also less likely to be anxious or depressed which was a determining factor in offspring with ADHD, anxiety, depression, and low IQ. While maternal metabolic control during pregnancy explains much about offspring outcomes, other parental characteristics may also contribute to the increased rates of low IQ and attention problems in these children, and these appear to be directly related to diet and medical food intake. Moreover, it takes time for the maternal PKU offspring's problems to emerge. Most social-emotional problems were not evident until the children were school-aged. For this reason, it is important that MPKU offspring have psychological evaluations throughout childhood and adolescence and that mothers continue to receive therapy for PKU throughout the lifespan. Evidence for positive maternal PKU pregnancy outcomes is now well established. This study illustrates that risks associated with maternal PKU do not end with the birth of the infant, but continue throughout the child's life. To ensure optimal offspring outcomes, healthcare providers need to assess maternal nutrition, blood phenylalanine concentrations, cognitive abilities, mood, and socioeconomic position. Interventions can then be initiated that reduce psychosocial stressors and enhance adherence to medical recommendations and positive parenting, which in turn can lead to better cognitive functioning, behavior, and emotional well-being in the children.

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Compliance with Ethics Guidelines

Conflict of Interest

We wish to draw the attention of the editor to the following facts which may be considered as potential conflicts of interest and to significant financial contributions to this work.

Dr. Susan Waisbren receives grant support from BioMarin Pharmaceuticals and has, in the past, consulted to the company with regard to psychological assessment of individuals with PKU. She also receives funds from the National Institutes of Health for the study of genomic sequencing in newborn screening.

Dr. Harvey Levy receives grant support from BioMarin Pharmaceuticals for a Phase 3 clinical trial of PEG-PAL enzyme therapy for PKU and for a PKUDOS study of outcome of Kuvan therapy in PKU. He also receives funds from the National Institute of Health for a Phase 2 crossover trial of glycomacropeptide in dietary therapy for PKU and for the study of genomic sequencing in newborn screening.

Frances Rohr, Vera Anastasoaie, Matthew Brown, Dr. David Harris, Al Ozonoff, Stephanie Petrides, and Ann Wessel have no conflicts of interest to disclose.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki

Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from Susan.Waisbren@childrens.harvard.edu.

Author Contributions

Dr. Waisbren conceived and designed this study. She oversaw data collection, conducted data analyses, interpreted data and drafted the manuscript.

Frances Rohr, MS, RD, LDN assisted in data collection, analysis and interpretation. She contributed to the manuscript drafts.

Vera Anastasoaie, BA assisted in data collection, analysis and interpretation. She critically reviewed the manuscript.

Matthew Brown, BA assisted in data analysis and interpretation and critically reviewed the manuscript.

David Harris, MD assisted in data collection, analysis and interpretation and contributed to the writing and review of the manuscript.

Al Ozonoff: Assisted in data analysis and interpretation. He critically reviewed and revised the manuscript.

Stephanie Petrides, BA assisted in data collection, analysis and interpretation. She critically reviewed the manuscript

Ann Wessel, MD RD, LDN assisted in data collection, analysis and interpretation. She contributed to the manuscript drafts.

Harvey Levy, MD collected data, analyzed and interpreted the findings and contributed to the drafting of the manuscript.

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