ABSTRACT. Objective. The Maternal PKU Collaborative Study (MPKUCS) was initiated in 1984 by the National Institute of Child Health and Human Development (NICHD). The purpose was to assess the efficacy of dietary restriction of phenylalanine in reducing morbidity in offspring of women with hyperphenylalaninemia (HPA). A contract was awarded to Childrens Hospital Los Angeles as the Coordinating Center to provide implementation of the research protocol, data collection, and analysis.

Methods. The Study included four regional coordinating centers: Childrens Hospital Los Angeles (Western Region), Boston Children’s Hospital (Northeast Region), University of Illinois (Midwest Region), and University of Texas Medical Branch, Galveston (Southeast Region). Within each region, many participating clinics were responsible for obstetric care, treatment, and monitoring protocols. In 1985, Canada joined the MPKUCS, and in 1992, Germany entered. They were selected because they provided dietary supplies and strong professional services. Acquisition began in 1984 and ended in October 1995. The study included 574 pregnancies in women with HPA and 100 control subjects matched on age, race, parity, and weeks of gestation. The sample included women with blood phenylalanine values >240 µmol/L, 66% of whom had classical PKU, 22% had atypical PKU, and 12% had mild HPA. Informed consents were obtained on all participants. The women ranged in age from 15 to 36 years of age, with a mean age at conception of 23 years. Teenage pregnancies accounted for 19%. Seventy-five percent graduated from high school. Offspring included 416 newborns, 317 of whom were evaluated at 4 years of age and 289 at 6 to 7 years. Follow-up involved medical, nutritional, psychosocial, and psychological assessments.

Conclusion. Women with PKU treated before conception and in control of their blood phenylalanine levels between 120 and 360 µmol/L (2–6 mg) exhibited normal pregnancies and neonatal outcome. Surprisingly, women who achieved control in the recommended range by 8 weeks of pregnancy also had a normal fetal outcome.

This study had its beginning in 1980 when Lenke and Levy published their epic article concerning the poor outcome of >500 pregnancies of women with untreated phenylketonuria (PKU). As a result of that publication, Eva Friedman, Malcolm Williamson, and Richard Koch prepared an application to the National Institutes of Health (NIH) to fund a study of pregnancy outcome in women with treated PKU. The application was not approved. The primary critique was that the study was too large and that a smaller pilot study should be done in preparation for a subsequent larger study. Therefore, in 1982, we submitted a smaller proposal, which also was rejected. Early in 1983, because the National Institute of Child Health and Development (NICHD) had funded a previous collaborative study of children who were treated for PKU, I expressed my frustration to Ted Tjossem, PhD, whom I had known for a number of years and was then the chief of the Mental Retardation and Developmental Disabilities Branch of NICHD. Shortly thereafter, I received a call from Dr Felix de la Cruz. He stated that NICHD was considering what research questions were important to solve for maternal PKU and how a study should be organized if they were to respond to the need for more research on the topic. Subsequently, NICHD offered a request for proposals to develop a coordinating center for data collection and 4 regional centers to locate and enroll potential women who had PKU and were already pregnant or were planning a pregnancy. The initial research questions were as follows:

1. Does the phenylalanine (Phe)-restricted diet reduce the frequency of mental retardation, spontaneous abortion, low birth weight, congenital malformation, and neurologic and behavioral impairment reported in pregnancies of mothers who had hyperphenylalaninemia (HPA) and were on unrestricted Phe intake during pregnancy?
2. Is pregnancy outcome in women who have HPA and restricted Phe intake during pregnancy comparable to that of women without HPA?
3. Is pregnancy outcome in women with HPA related to maternal Phe levels during pregnancy?
4. Is gestational age at the onset of intervention predictive of fetal outcome?
5. Are there beneficial effects of starting the diet before conception?
6. What are the levels of tyrosine and trace metals during pregnancy, and what are the effects on pregnancy outcome of supplementation if levels are found to be reduced?

Subsequently, an NICHD contract was awarded, which called for a 7-year study period, during which 200 mother–infant pairs were to be studied. de la Cruz was designated as the NIH Project Officer, and Harvey Shifrin was the NIH Contracting Officer. Their primary role was to guide the project development for NICHD. A Coordinating Center and a Western Contributing Center were located at Children’s Hospital Los Angeles. Three other contributing centers were located at Boston Children’s Hospital, the University of Illinois at Chicago, and the University of Texas Medical Branch in Galveston. Each Contributing center was to relate to a specific geographical region of the United States.

It had been anticipated that the goal of studying 200 pregnancies would be easily met during the initial 7-year period of the research project, but locating and enrolling pregnant women with PKU proved to be more difficult than we had anticipated. There were 2 major reasons for this. The most important one was related to the clinical impression that fetal outcome was so poor that pregnancy was actively discouraged in these women, and some of them had already been sterilized and advised to adopt children. The second reason related to the intelligence of the women who were born before newborn screening, diagnosed late, and thus were mentally limited. Newborn screening for PKU began early in 1963 in a few states such as Massachusetts, but other states lagged behind and did not start until 1970. Thus, the identification of women with normal intelligence did not become available for study until the mid-1980s. Canada was invited to join the study in 1985. The number of offspring born to enrolled PKU women as of October 1991 was 185, only 23 of whom women were in control before pregnancy (Table 1). Furthermore the mean IQ of this initial cohort was only 83 ± 12.

In 1992, the NICHD planned a site visit with a group of outstanding individuals, who reviewed the project carefully and recommended a 4-year extension. Two members of the site review team became part of the Steering Committee, whose meetings were held biannually. For improving the number of pregnancies treated before conception, Germany was invited to join the study in 1992. Subsequently, Canada enrolled 47 pregnancies, and Germany enrolled 48. These pregnancies had higher percentages on diet before conception (43% and 62%, respectively) than those enrolled in the United States (24%).

The Site Committee added 2 new research questions to the original 6 questions:

7. Does maternal genotype, as determined by DNA mutational analysis, correlate with offspring phenotype?
8. Does detailed long-term neuropsychological evaluation of offspring correlate with efficacy of maternal dietary treatment?

For answering research question 7, Flemming Gütler of Denmark was contracted to perform mutation studies on the phenylalanine hydroxylase gene on the mothers and offspring in the Maternal PKU Collaborative Study (MPKUCS). For addressing research question 8, an extensive offspring follow-up battery of psychological tests was introduced and the follow-up period was extended to age 8 and beyond.

For improving the quantity and quality of the psychological data, a network of local psychologists was developed by Susan Waisbren, to assist in testing, as needed, and a procedure for double scoring all tests in Boston was implemented. The additional 4 years of sample acquisition greatly increased the total number of offspring, as well as the number old enough for psychological testing beyond the Bayley at 2 years of age.

Although the sample of maternal PKU/HPA pregnancies increased the numbers in key study groups who were old enough for psychological testing, the sample was still too small to provide adequate statistical power for meaningful hypothesis testing involving endpoints beyond birth. Psychological data collection rates improved to >80%, when enough time was allowed to arrange for testing and data acquisition in the 75% of children who were followed by the participating clinics, rather than at 1 of the 6 contributing centers. Evaluation of psychological data continued to show a strong relationship to maternal Phe levels during pregnancy, even after controlling for environmental variables, and that offspring of women who established control between 10 and 20 weeks were scoring higher than expected on developmental tests, although the number was still small.

A second extension of the contract was awarded in 1996 to allow all study offspring to reach 4 years of age and undergo intelligence testing with the McCarthy Scales of Children’s Abilities (see Waisbren and Azen in this supplement for details). The research questions were extended to focus on those...
aspects of the original and revised questions that still could not be answered with certainty. Additional research questions included the following:

9. Are offspring of treated PKU/HPA women with Phe levels 120 to 360 μmol/L from early in pregnancy different from those of women with Phe between 360 and 600 μmol/L on variables assessing medical status, physical growth, and psychological development at 4 and 6 years of age?

10. Are offspring of treated PKU/HPA women with Phe levels 120 to 360 μmol/L different from those with levels 120 to 600 μmol/L? If there is no difference in these 2 groups, then are there differences in those who started dietary treatment before conception from those who established the same degree of control by 10 weeks’ gestation on outcome variables assessing medical status, physical growth, and psychological development at 4 and 6 years of age?

11. Are offspring of treated PKU/HPA women with “optimal” Phe control, by degree of timing, different from control offspring on variables assessing medical status, physical growth, and psychological development at 4 and 6 years of age?

12. What is the developmental outlook for offspring of women who established control between 10 and 20 weeks’ gestation?

13. Are offspring of treated PKU/HPA women with “optimal” Phe control, by degree and timing, different from those of untreated mild HPA with Phe in the same range on an unrestricted diet, on outcome variables assessing medical status, physical growth, and psychological development at 4 and 6 years of age?

SUBJECTS

The MPKUCS attempted to enroll and follow all pregnancies that occurred in women with HPA during the recruitment period. Eligibility criteria included having an off-diet blood Phe level of 240 μmol/L or higher, living in the community, and having sufficient intellectual capacity to follow treatment recommendations and sign an informed consent. A control sample of pregnancies in normal women matched as closely as possible to the HPA sample of age, race, parity, and weeks gestation at enrollment was also enrolled and followed prospectively.

TREATMENT

The treatment plan consisted of 4 components:

1. Provision of adequate nutrition during pregnancy.
2. Offering dietary restriction of Phe to women with HPA with blood Phe concentrations ≥600 μmol/L.
3. Maintaining blood Phe levels between 120 and 600 μmol/L (later reduced to 120–360 μmol/L) and obtaining phenylalanine hydroxylase mutation data on mother and child.
4. Supplementation with tyrosine and trace elements as indicated.

Ideally, pregnancies would be planned and dietary therapy would be initiated before conception. Adequacy of and compliance with the treatment plan were monitored by obtaining weekly blood Phe levels and monthly 3-day diet records, plasma amino acids, trace metals, and other laboratory assessments.

DATA COLLECTION

Data collected on the mothers at enrollment included details of her PKU diagnosis and treatment history, family pedigree, socioeconomic information (Hollingshead Scale) and intellectual status (Wechsler Adult Intelligence Scale–Revised), pregnancy history, and a baseline medical and obstetric evaluation. Data collected during pregnancy, in addition to blood Phe, dietary, and laboratory information, included monthly obstetric evaluation (weight, blood pressure, complications) and ultrasound at 8, 20, and 28 to 34 weeks.

The research questions were to be evaluated using a variety of outcome variables, measured during pregnancy, at birth and during early childhood. Specifically, offspring outcome was assessed at birth (gestational age, birth length, weight, and head circumference; and overall assessment by a neonatologist; and an echocardiogram if indicated); at 6, 12, and 24 months with the Bayley Scales of Infant Development; at 3, 4, to 5 years with the McCarthy; and at 6 to 7 years with the WISC-R, and additional evaluations of behavior, perception (Vineland, PPVT, etc). Annual medical and physical assessments, including measurements of height, weight, and head circumference, and a dysmorphology evaluation during the first year of life were also specified.

STUDY EXTENSION AND MODIFICATIONS TO THE RESEARCH DESIGN

In April 1992, a 4-year extension of the contract was awarded, with certain modifications recommended by a site visit committee, to address the problems encountered in the first 7-year phase of the MPKUCS. The 2 research questions added to the original 6 were designed to assess the relationship of maternal genotype to offspring phenotype and to provide a more extensive developmental profile of the offspring.

The additional 4 years of sample acquisition greatly increased the total number of offspring, particularly of well-treated pregnancies, as well as the number old enough for psychological testing beyond the Bayley at age 2. The sample now included 413 offspring of maternal PKU/HPA pregnancies and 100 control offspring. However, the numbers in key study groups who were old enough for psychological testing were still too small to provide adequate statistical power for meaningful hypothesis testing involving endpoints beyond birth. Psychological data at this time continued to show a strong relationship to maternal Phe during pregnancy, even after controlling for environmental variables. Furthermore, offspring of women who established control between conception and 10 weeks’ gestation were scoring higher than expected on developmental tests,
although the sample size was inadequate do draw firm conclusions.

A second extension to the contract was awarded in 1996 to allow all study offspring to reach 4 years of age and a maximum number to undergo IQ testing with the WISC-R at age 6. For this phase, the research questions were reworded to focus on those aspects of the original and revised questions that still could not be answered with certainty: is there a “safe” level of maternal Phe during pregnancy, and, if so, is it necessary to establish this level of control before conception? What happens when control is not achieved until later in pregnancy? Do women with mild HPA need to be treated?

When data collection ended on October 31, 2001, all but 12 offspring were old enough for a WISC-R IQ evaluation. During the course of the study, 9 offspring died, 6 from congenital heart disease. Another 7 withdrew when their families refused further participation. The overall data collection rate for key offspring follow-up variables approached 80%, a remarkable achievement, considering the duration of the project and the organizational complexity of the study.

As a result of the contract extensions provided, we have been able to collect psychological data on 6- to 7-year-old offspring of women with PKU. In the future, women with PKU can thank Drs Duane Alexander and Felix de la Cruz for their foresight in discerning the need for factual information that will guide health professionals and women with PKU in making informed decisions about pregnancy.

The sample consisted of 95% white, 2% black, 2% Native American, and 1% Asian-Pacific Islander. Educational attainment of the sample revealed that 43% graduated from high school and another 32% attended college. The group that did not graduate from high school primarily had mental disabilities. Social class revealed that 74% were represented by the 2 lowest classes and only 8% were in the highest classes, whereas the middle class represented 15%.

By the end of the study, significant changes in the enrolled population had occurred. In the beginning, there were few pregnancies in women who were treated before conception and were in control with blood Phe levels of 120 to 360 μmol/L, but during the last years, more were in this category. In addition, the mean IQ of the mothers enrolled in the study had also risen to 88 ± 14.

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Richard Koch, Colleen Azen, Eva Friedman, William Hanley, Harvey Levy, Reuben Matalon, Bobbye Rouse, Friedrich Trefz, Jiaping Ning and Felix de la Cruz

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