Consensus Development Conference on Phenylketonuria (PKU): Screening and Management

October 16–18, 2000
William H. Natcher Conference Center
National Institutes of Health
Bethesda, Maryland

Sponsored by:

♦ National Institute of Child Health and Human Development ♦ NIH Office of Medical Applications of Research ♦

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Introduction

The National Institutes of Health (NIH) is sponsoring a Consensus Development Conference on Phenylketonuria (PKU): Screening and Management on October 16–18, 2000.

PKU is a rare, inherited metabolic disorder that, if untreated, causes mental retardation. Approximately one of every 10,000 infants in the United States is born with PKU, which usually results from a deficiency of a liver enzyme known as phenylalanine hydroxylase (PAH). This enzyme deficiency leads to elevated levels of the amino acid phenylalanine in the bloodstream.

All infants in this country undergo blood testing for PKU. The current treatment for this disorder involves dietary modification. When a very strict diet is begun early and maintained, children with PKU can expect normal development and a normal lifespan. The diet generally excludes all high protein foods, such as meat, milk, eggs, and nuts, since all protein contains phenylalanine.

Dietary noncompliance can result in a decline in mental and behavioral performance. Women with PKU must also maintain a strictly controlled diet before and during pregnancy to prevent fetal damage. Scientists are actively exploring nondietary treatments for PKU.

Research on PKU continues to broaden the knowledge base from which informed decisions regarding screening and treatment can be made. After a day and a half of expert presentations and public discussion of PKU epidemiology and genetics, screening strategies, and treatment regimens, an independent, non-Federal panel will weigh the scientific evidence and draft a statement that will be presented on the third day. The consensus development panel's statement will address the following questions:

- What are the incidence and prevalence of PKU and other forms of hyperphenylalaninemias, and what is known about the genetic and clinical variability?

- What newborn screening strategies are available for diagnosis, what is the effectiveness of these strategies, and what cost-savings are generated by screening and treatment?

- What treatment regimens are used to prevent the adverse consequences of PKU? What is known about the effectiveness of these treatment and management strategies overall and with respect to variables such as time of initiation of dietary management, levels of phenylalanine at various ages, methods for enhancing dietary compliance, duration of dietary management, and dietary regimens for women of childbearing age and other adults?

- Based on this information, what are the recommended strategies for optimal newborn screening and diagnosis and lifelong management and followup of PKU?

- What research is needed to gather information that will optimize the outcome for individuals with PKU and their families?

On the final daty of the meeting, the panel chairperson, Dr. Rodney Howell, will read the draft statement to the conference audience and invite comments and questions. A press conference will follow to allow the panel and chairpersons to respond to questions from media representatives.

Continuing Education Credit

The purpose of this Consensus Development Conference is to review the current state of knowledge regarding screening for and management of phenylketonuria (PKU) and to identify directions for future research.

The conference will (1) present in open, public sessions state-of-the-art information regarding screening for and management of phenylketonuria, (2) prepare a statement in response to the five specific questions, and (3) inform the biomedical research and clinical practice communities and the general public of the conclusions and recommendations of the panel.

The NIH/FAES is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to sponsor continuing medical education for physicians.

The NIH/FAES designates this educational activity for a maximum of 13.5 hours in category 1credit towards the AMA Physician's Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the educational activity.

General Information

Conference sessions will be held in the Natcher Conference Center (Building 45), National Institutes of Health, Bethesda, Maryland. Sessions will run from 8:30 a.m. to 5:30 p.m. on Monday, from 8:30 a.m. to 12:05 p.m. on Tuesday, and from 9 a.m. to 11 a.m. on Wednesday. The telephone number for the message center is (301) 496-9966; the fax number is (301) 480-5982.

Cafeteria

The cafeteria in the Natcher Conference Center is located one floor above the auditorium on the main floor of the building. It is open from 7 a.m. to 2 p.m., serving breakfast and lunch.

Sponsors

The primary sponsors of this meeting are the National Institute of Child Health and Human Development and the NIH Office of Medical Applications of Research. Cosponsors include the National Human Genome Research Institute, National Institute of Diabetes and Digestive and Kidney Diseases, National Institute of Neurological Disorders and Stroke, National Institute of Nursing Research, the Office of Rare Diseases of NIH, and the Maternal and Child Health Bureau of the Health Resources and Services Administration.

Statement of Interest

In accordance with ACCME requirements, each speaker presenting at this conference has been asked to submit documentation outlining all outside involvement pertaining to the subject area. Please refer to the chart in your participant packet for details.

Agenda

Monday, October 16, 2000

8:30 a.m. Opening Remarks
Duane Alexander, M.D., Director
National Institute of Child Health and Human Development

8:40 a.m. Charge to Panel
Susan Rossi, Ph.D., M.P.H., Deputy Director
Office of Medical Applications of Research

8:50 a.m. Conference Overview and Panel Activities
R. Rodney Howell, M.D., Conference and Panel Chairman
Professor and Chairman
Department of Pediatrics
University of Miami School of Medicine

I. Overview

9:00 a.m. Phenylketonuria: Paradigm for a Treatable Genetic Disease…?
Charles R. Scriver, M.D., Alva Professor of Human Genetics
McGill University

II. Epidemiology of PKU

9:30 a.m. Panel Overview
William B. Rizzo, M.D., Professor of Pediatrics and Human Genetics
Medical College of Virginia

9:40 a.m. The Use of Mutation Analysis To Anticipate Dietary Requirements in PKU
Flemming Güttler, M.D., Ph.D., Head
Departme nt of Inherited Metabolic Disease and Molecular Genetics
Associate Professor
University of Copenhagen

9:55 a.m. Human Pathophysiology
Friedrich K. Trefz, **M.D.,** Head
Children's Hospital Reutlingen
University of Tubingen School of Medicine

10:05 a.m. Discussion

Monday, October 16, 2000 (continued)

III. Screening for PKU

10:45 a.m. Panel Overview
Mark S. Kamlet, **Ph.D.,** Provost and Professor of Economics and Public Policy
Carnegie Mellon University

10:55 a.m. Screening Technologies: Types and Effectiveness
Harvey L. Levy, M.D., Senior Associate in Medicine and Genetics
Children's Hospital, Boston

11:15 a.m. Screening Procedures: Variations in Diagnostic Criteria and Followup
Michele A. Lloyd-Puryear, M.D., Ph.D., Chief, Genetic Services Branch
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11:35 a.m. Informed Consent for Newborn Screening and Future Uses of Tissue Samples
Mary Kay Z. Pelias, Ph.D., J.D., Professor of Genetics
Department of Biometry and Genetics
Louisiana State University Medical Center

11:50 a.m. Discussion

12:20 p.m. Lunch

1:20 p.m. Panel Overview
Jack M. Fletcher, Ph.D., Professor
Department of Pediatrics
University of Texas-Houston Medical School

1:30 p.m. Variations Among Programs in Time of Initiation of Diet Treatment, Level of Control, and Diet Relaxation/Discontinuation
Margretta R. Seashore, M.D., FAAP, FACMG, Director
Genetics Consultative Services
Professor of Genetics and Pediatrics
Yale University School of Medicine

IV. Treatment Regimens and Their Effectiveness

1:45 p.m. Effects of Dietary Treatment on Children With Classical Phenylketonuria (PKU): The United States PKU Collaborative Study, 1967–1984
Colleen Azen, M.S., Coordinator, Biostatistics
Maternal PKU Collaborative Study, Division of Medical Genetics
Children's Hospital Los Angeles

Monday, October 16, 2000 (continued)

IV. Treatment Regimens and Their Effectiveness (continued)

2:05 p.m. Dietary Treatment of PKU With Behavior Disorders
Harvey L. Levy, M.D., Senior Associate in Medicine and Genetics
Children's Hospital, Boston

2:15 p.m. Discussion

2:30 p.m. A 15-Year Followup Report on Participants in the Collaborative Study of Children Treated for PKU (PKUCS 1967–1984)
Richard Koch, M.D., Principal Investigator
Maternal PKU Collaborative Study, Division of Medical Genetics
Children's Hospital Los Angeles

2:50 p.m. **Dietary Control: An International Perspective**

A. Commentary on Recommendations in the United Kingdom for the Management of PKU
Forrester Cockburn, M.D., Emeritus Professor of Child Health
University of Glasgow
Royal Hospital for Sick Children

B. Recommendations of the German Working Group for Metabolic Diseases for Control of Phenylalanine in PKU
Peter Burgard, Ph.D., Department of General Pediatrics
University of Heidelberg

C. PKU Treatment in France
Véronique Abadie, M.D., Service de Pédiatrie Générale
Hôpital Necker-Enfants Malades

3:35 p.m. Treatment Regimens and Their Effectiveness: A Meta-Analytic Review of the Literature
Marilyn C. Welsh, Ph.D., Professor
Department of Psychology
University of Northern Colorado

4:05 p.m. Discussion of Effects of Age at Diet Initiation

4:30 p.m. Discussion of Effects of Level of Control: Ages 0–6 and Afterward

5:00 p.m. Discussion of Effects of Diet Relaxation/Discontinuation/Reinitiation at Various Ages

5:30 p.m. Adjournment

Tuesday, October 17, 2000

IV. Treatment Regimens and Their Effectiveness (continued)

8:30 a.m. Psychosocial Factors and Dietary Adherence
Susan E. Waisbren, Ph.D., Senior Psychologist
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Associate Professor of Psychology
Harvard Medical School

8:45 a.m. Maintaining Diet in Adolescence and Adulthood
Stephanie Stremer, Public Representative

8:55 a.m. Maternal PKU: Restarting and Monitoring Diet Before and During Pregnancy
Kimberlee Michals, Ph.D., R.D., L.D., Associate Professor of Nutrition
Department of Human Development and Consumer Sciences
College of Technology
University of Houston

9:10 a.m. Maternal PKU: Pregnancy and Child Outcomes in Relation to Dietary Control
Reuben Matalon, M.D., Ph.D., Director
Biochemical/Molecular Genetics
University of Texas Medical Branch at Galveston

9:30 a.m. Alternative Dietary Treatments
Joachim Pietz, M.D., Department of Pediatric Neurology
University of Heidelberg

9:45 a.m. Discussion

10:30 a.m. Perspectives on the Future of Gene Therapy
Savio L.C. Woo, Ph.D., Professor and Director
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Mount Sinai School of Medicine

10:45 a.m. Lifespan Perspectives
Keith F. Widaman, Ph.D., Professor
Department of Psychology
University of California, Davis

11:00 a.m. Discussion

Tuesday, October 17, 2000 (continued)

V. Public Presentations

11:20 a.m. The American College of Obstetricians and Gynecologists
The Association of Public Health Laboratories
Genetic Alliance
International Society of Nurses in Genetics
National Coalition of PKU and Allied Disorders
National Society of Genetic Counselors

12:20 p.m. Adjournment—Panel Meets in Executive Session

Wednesday, October 18, 2000

9:00 a.m. Presentation of Consensus Statement

9:30 a.m. Public Discussion

11:00 a.m. Panel Meets in Executive Session

1:00 p.m. Press Conference

2:00 p.m. Adjournment

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Abstracts

The following are abstracts of presentations to the NIH Consensus Development Conference on Phenylketonuria (PKU): Screening and Management. They are designed for the use of panelists and participants in the conference and as a reference document for anyone interested in the conference deliberations. We are grateful to the authors, who have summarized their presentations and made them available in a timely fashion.

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Phenylketonuria: Paradigm for a Treatable Genetic Disease…?

Charles R. Scriver, M.D.

Phenylketonuria (PKU) is a Mendelian recessive inborn error of metabolism in which phenotype can be modified by restriction of dietary phenylalanine (Phe) (Scriver, 1994; Scriver, Kaufman, 2000). PKU is the prototype for early diagnosis and treatment to prevent a major disease phenotype (impaired cognitive development). Early diagnosis of postnatal hyperphenylalaninemia, the metabolic hallmark of the trait, is critical, hence the importance of a newborn screening test to identify an affected person (incidence ~ 10^{-4}).

- The disease has "a cause." The ultimate cause is phenotype-modifying allelic variation in the phenylalanine hydroxylase gene (symbol *PAH*); the proximate cause is dietary intake of the essential amino acid L-Phe.

- Pathogenesis of PKU "disease" has both biochemical and physiologic components. The former involves extreme loss of Phe hydroxylation as served by hydroxylase enzyme function (Scriver, 1998). The catalytic reaction requires an intact homotetrameric enzyme, substrate (L-Phe), equimolar molecular oxygen, and catalytic amounts of tetrahydrobiopterin. Impaired synthesis or recycling of tetrahydrobiopterin cofactor impairs the aromatic hydroxylases (for Phe, tyrosine, and tryptophan) and nitric oxide synthase. The term "PKU" is reserved for primary dysfunction of PAH enzyme due to mutations in the *PAH* gene.

PAH enzyme disfunction results in metabolic dishomeostasis; Phe levels rise above the maximum normal level (0.125mM), and tyrosine levels may fall (because of impaired product formation). There is a subsequent imbalance in the distribution of many amino acids across cellular membranes and across the blood-brain barrier.

Historically, there were many other manifestations of PKU which today have little significance. The current focus of the applied knowledge is prevention of mental retardation.

The Gene. The human *PAH* locus is on chromosome region 12q24.1. The locus spans approximately 100kb. The gene comprises 13 exons, which take up no more than 3 percent of the total nucleotide sequence. Only the cDNA has been cloned (GenBank U49897), but a full genomic sequence is anticipated soon. The 5' regulatory region of the gene has been fully characterized (Konecki, Wang, Tretz, et al., 1992). A Web site (www.mcgill.ca/pahbd) provides a knowledge base, including a full description of the gene, its alleles, and a large range of annotations on every allele (Scriver, Waters, Sarkissian, et al., 2000). The *PAH* locus harbours well over 400 alleles, the majority of which are disease-causing mutations; 50 percent of these are missense mutations, the majority of which do not map to critical residues in the catalytic region of the protein. There are 28 "polymorphic" alleles in the *PAH* gene, of which 2 are multiallelic (STR and VNTR), 8 are RFLPs, and the remainder are so-called SNPs.

***PAH* Gene Expression.** The gene is both transcribed and translated into polypeptide in only two human tissues: liver (hepatocytes) and kidney (mainly proximal renal tubule epithelial cells) (Lichter-Konecki, Hipke, Konecki, 2000). The significance of *PAH* gene expression in kidney is not fully known, but it may contribute to net reabsorption of this essential amino acid by the process of metabolic run out; it also implies that renal transplantation might benefit an affected patient.

The Enzyme. PAH enzyme is a homotetramer; its crystal structure is known (Erlandsen, Stevens, 1999). The monomer contains 452 amino acids (~ 51kD mass) and has 3 domains: an N-terminal regulatory region, a central catalytic domain, and a C-terminal tetramerization domain. Mutations in any region can affect function. The enzyme functions in vivo as a homotetramer permitting allosteric modulation in the presence of substrate and cofactor; activity is modulated by phosphorylation of an N-terminal serine; the catalytic center requires an iron atom in each subunit. Recent work (Waters, Parniak, Akerman, et al., 2000) shows that missense mutations not mapping to key residues in the catalytic domain cause misfolding of the protein and diversion to proteolytic intracellular pathways. The study of missense *PAH* mutations suggests paradigms for the effect of missense mutations elsewhere in the human genome on other metabolic processes.

Metabolic Homeostasis. In the presence of normal dietary Phe intake, the obligatory response is accumulation of Phe in body fluids when PAH enzyme function is impaired. The "pathogenic" molecule is (apparently) L-Phe itself; in excess, it will disturb transport of other critical amino acids across the blood-brain barrier and across neuronal membranes themselves and will impair synthesis of neurotransmitters; the relevance of mediated Phe transport at the blood-brain barrier has been well documented (Pietz, Kries, Rupp, et al., 1999).

There was much interest earlier in the pathogenic significance of Phe metabolites (for example, phenylpyruvic acid and its derivatives). Studies in mutagenized mouse models of hyperphenylalaninemia/PKU (Sarkissian, Boulais, McDonald, et al., 2000) show that these derivative metabolites are not present at significant concentrations in brain to account for impaired cognitive development (Sarkissian, Scriver, Mamer, 2000).

Genotypes and Phenotypes

- *Genotypes and alleles.* Only one-quarter of the mutant human *PAH* genotypes identified so far are homoallelic. On a worldwide basis, about 10 different *PAH* mutations account for about two-thirds of the relative frequencies of mutations in human population; the remainder are rare, many even private. The distribution of mutations in human populations is nonrandom. It reflects an out-of-Africa hypothesis and independent distributions into Oriental and Caucasian populations; PKU alleles are also good markers of European range expansion over the past 500 years. It may be said that "the history of the population is (very frequently) the history of the PKU allele." Both the disease-causing and the polymorphic PAH alleles contribute to these conclusions (Scriver, Kaufman, 2000).

- *Phenotype correlations*. There was a hope that knowledge of *PAH* genotype would have predictive value for severity of phenotype. In broad terms, this is true (Kayaalp, Treacy, Waters, et al., 1997; Guldberg, Rey, Zschocke, 1998). Genotypes containing a pair of null alleles confer a severe form of hyperphenylalaninemia (HPA). Missense mutations conferring considerable residual activity (measured by in vitro expression analysis [see www.mcgill.ca/pahdb]) tend to confer non-PKU HPA when paired (homo- or heteroallelic) in a mutant genotype. A range of intermediate HPA phenotypes is associated with other mutant genotypes. The metabolic phenotype (HPA) is itself the result of many events other than Phe hydroxylation activity (Scriver, 1998; Scriver, Waters, 1999); hence, it is not surprising that discordance between genotype and metabolic phenotype is found.

There is also recognized discordance between metabolic and cognitive phenotypes; PKU does not always cause mental retardation (as recognized long ago by Penrose). One explanation involves events at the blood-brain barrier; siblings with identical mutant genotypes can have different cognitive phenotypes by virtue of differences in brain Phe levels that have been modulated by differences in Phe flux at the the blood-brain barrier.

A case has been made for looking at PKU and other Mendelian (metabolic) disorders as complex traits nested in Mendelian disorders (Scriver, Waters, 1999; Dipple, McCabe, 2000).

Treatment

PKU is an orphan disease. However, four decades of dietary therapy coupled with early postnatal diagnosis reveal that an approximation of mild HPA is compatible with near-normal cognitive development. The benefits of treatment have been seen in large cohorts of affected probands and also in smaller but convincing numbers of women with HPA who receive preconception and intrapartum treatment. Guidelines (Medical Research Council Working Party on Phenylketonuria, 1993; Recommendations on the dietary management of phenylketonuria, 1993) now recommend more aggressive treatment: earlier in onset, more stringent in restoring euphenylalaninemia, and longer in duration—perhaps for life. Premature termination of therapy is frequently associated with neurophysiologic and psychological dysfunction and perhaps a decline in cognitive function.

Compliance with the new guidelines will benefit from new approaches to therapy, for example, to improve the organoleptic properties of current diet treatment products, to develop new low-Phe proteins (perhaps by modifying milk proteins in lactating animals), and to provide enzyme substitution therapy (Sarkissian, Shao, Blain, et al., 1999). Each of these options is being investigated while PKU remains the flagship for the armada of treatable genetic disease.

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The Use of Mutation Analysis To Anticipate Dietary Requirements in Phenylketonuria

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In a broad perspective, the identification of the enzyme defect, the development of effective methods for neonatal screening, and the implementation of dietary therapy regimens in phenylketonuria (PKU) are among the major achievements of medical science. Nevertheless, 40 years of experience with diagnosis and treatment of children with PKU have left us with a number of uncertainties regarding the classification of disease phenotypes and the effectiveness of treatment. This abstract reviews the novel suggestion of using molecular genetic analysis to directly assess the metabolic PKU phenotype and discusses how such data might be used to anticipate the treatment requirements for each individual patient.

The Different Phenotypes of PKU

The marker for neonatal detection of PKU is elevated serum levels (hyperphenylalaninemia) of phenylalanine (Phe) caused by reduced activity of the hepatic enzyme phenylalanine hydroxylase (PAH). The degree of enzyme impairment varies greatly among patients and is reflected in the broad continuum of metabolic phenotypes (Güttler, 1980; Scriver, Kaufman, Eisensmith, et al., 1995). Because direct determination of PAH activity through liver biopsies is not feasible, diagnosis and classification of PAH deficiency is done through indirect methods (Güttler, 1980; Scriver, Kaufman, Eisensmith, et al., 1995). One widely used approach is to determine the amount of dietary Phe tolerated while keeping blood Phe levels within the therapeutic range. On the basis of Phe tolerance data, patients can be assigned to one of four arbitrary phenotype categories: classic PKU, moderate PKU, mild PKU, and mild hyperphenylalaninemia (MHP) (Güttler, Guldberg, 1996).

Unfortunately, there are no international guidelines for classification parameters, including age at testing and therapeutic target Phe levels. This effectively hampers comparisons among patients treated at different centers. Meanwhile, however, a number of recent studies have demonstrated the usefulness of an alternative and more universal system for classification of PAH deficiency that is based on *PAH* mutation genotypes.

PAH Gene Mutations

Mutations in the gene encoding PAH are the ultimate cause of PAH deficiency (Scriver, Kaufman, Eisensmith, et al., 1995). A patient's *PAH* mutation genotype refers to the composition of inherited mutant genes (alleles) and is usually determined by identifying the two disease-causing mutations by means of molecular analysis. The ascertainment of *PAH* mutations is now nearly complete in many patient populations in Europe and the New World. The combined efforts of members of the *PAH* Mutation Analysis Consortium have raised the number of known *PAH* mutations to approximately 400. Each of these mutations has a quantitative effect on PAH

activity, which provides the molecular basis for the observed spectrum of metabolic phenotypes (Okano, Eisensmith, Güttler, et al., 1991). One further level of complexity is added by the enormous number of possible mutation combinations; the approximately 400 pathogenic PAH alleles known to date can form more than 80,000 heteroallelic genotypes. (A database of mutations by Scriver, Waters, Sarkissian, and colleagues is accessible on the Internet at http://www.mcgill.ca/pahdb.)

Genotype-Based Prediction of Metabolic Phenotype

Hitherto, the major constraints encountered during attempts to establish a correlation between individual mutations and a PAH-deficiency phenotype have been that patients with two identical mutations (homoallelic genotypes) were available for only a minority of mutations, and that patients with two different mutations (heteroallelic genotypes) were not informative. Some of these difficulties have now been circumvented by studying "functionally hemizygous" patients (Guldberg, Mikkelsen, Henriksen, et al., 1995). These patients carry on one chromosome one of several "null" mutations—that is, mutations that produce proteins with no enzyme activity in vivo. The functionally hemizygous constellation is equivalent to the homozygous constellation in the sense that only the enzyme encoded by the non-null allele contributes to the metabolism of Phe. A meta-analysis (Kayaalp, Treacy, Waters, et al., 1997) and a European multicenter study (Guldberg, Rey, Zschocke, et al., 1998) have identified approximately 35 null mutations and have assigned more than 100 different mutations to particular metabolic phenotypes.

In the European multicenter study, the degree of concordance between predicted versus observed phenotypes was tested in 651 patients for whom exact data on genotypes and metabolic phenotypes were available. The observed phenotype matched the predicted phenotype in 562 of the cases (86 percent), and in only 10 of the cases (1.5 percent) was the observed phenotype more than one phenotype category away from that expected. Notably, there was virtually complete association between genotype and phenotype in the group of individuals with MHP. This group is particularly informative because patients in the group are not being treated and thus can be classified solely on the basis of serum Phe values, with no interference from dietary therapy regimens. A substantial fraction of genotype-phenotype inconsistencies may be due to phenotype "misclassifications" related to differences in criteria and methods used for phenotype assessment (Guldberg, Rey, Zschocke, et al., 1998).

Genotype Related to Outcome and Dietary Requirements

The relationships among genotype, biochemical phenotype, and cognitive performance were studied in 199 PAH-deficient females enrolled in the Maternal PKU Collaborative Study (Güttler, Azen, Guldberg, et al., 1999). Most of them had been treated only for the first 6 years of life. Based on their *PAH* mutation genotype, the patients were assigned to one of the four classes of severity. Genotype severity was significantly related not only to untreated blood Phe levels but also to cognitive development in terms of intelligence quotient (IQ). Patients with genotypes indicating classic or moderate PKU showed IQ scores of 83 and 84, respectively, whereas the IQ score was 96 in females with a mild PKU genotype. Those who were treated for more than 6

years showed IQ scores 10 points above average for their group (Güttler, Azen, Guldberg, et al., 1999).

Preliminary data from a study of 108 Danish patients with PKU who have been on dietary therapy for between 10 and 14 years show that their median IQ is normal and not dependent on genotype. The results from these two studies demonstrate the importance of maintaining dietary therapy, at least until somewhere between the ages of 10 and 14 years.

Conclusion

DNA analysis may provide a new and powerful tool for refining PKU diagnosis and anticipating dietary requirements in PAH deficiency. *PAH* mutations may be determined by analysis of DNA extracted from blood deposited on a Guthrie card, and thus a genetic diagnosis can be made immediately after birth, with no further examination of the child. Although still not simple enough to be performed at all screening centers, methods for detection of *PAH* mutations are now at a stage where genetic diagnosis becomes feasible in the routine diagnosis and management of PKU. One of the most beneficial applications of mutation analysis may be the identification of patients for whom dietary treatment beyond 6 years of age is an absolute requirement for the prevention of mental retardation.

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Human Pathophysiology

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Although there has been great progress in the development of animal models of phenylketonuria (PKU), findings in humans with PKU are important in elucidating PKU pathophysiology because of fundamental differences between animals and humans in postnatal brain development. There is strong evidence that phenylalanine (Phe) itself is the *toxic agent* in patients with defects of the Phe hydroxylase enzyme, and not the increase in Phe metabolites. As shown by various groups (Kaufman, 1976), oxidative Phe metabolites do not reach sufficient intracerebral concentrations to interfere with the development of enzymes. Another argument against the possible effects of Phe degradation products is the existence of a rare inborn error described as "chemical PKU," which was found in two sisters who had no symptoms of untreated PKU even though they had elevated concentrations of phenylpyruvic, o-hydroxyphenylacetic, and phenyllactic acid in the blood and a low normal level of Phe in the urine (Wadman, Ketting, de Bree, et al., 1975; Trefz, Blau, Aulehla-Scholz, unpublished).

Reversible and Irreversible Effects

The target organ in untreated PKU is the brain, but it is necessary to differentiate between reversible and irreversible effects on brain function. Effects that are reversible may be more evident in severely retarded patients (Baumeister, Baumeister, 1998) or in infants whose diets are changed. In normally developed patients, neurophysiological tests must be used to detect brain dysfunctions, such as impaired attention or problems in information processing (Schmidt, Rupp, Burgard, et al., 1994). However, the long-term effects of high concentrations of Phe on the normal brain cannot be predicted and may be influenced by various life factors (Weglage, Oberwittler, Marquardt, et al., 2000).

The Hypotheses: Influence on Myelin, Protein, and Neurotransmitter Metabolism

This abstract is focused on the theory that high concentrations of Phe interfere with normal brain development and that these damaging effects may be caused by other genetic factors rather than by defects in the Phe hydroxylase gene. Recent studies of patients with PKU using magnetic resonance spectroscopy have confirmed high intracerebral concentration of Phe. This implies an imbalance of large neutral amino acids (LNAA) that can be compensated in part by LNAA supplementation (Pietz, Kreis, Rupp, et al., 1999).

This discussion of the pathophysiology of PKU is based on three hypotheses: (1) high concentrations of Phe interfere with *myelin metabolism*, which not only influences nerve function but also affects normal development of the brain; (2) high Phe or deficiencies in LNAA then create problems in *intracerebral protein synthesis* and (3) in the *synthesis of neurotransmitters*.

Neuropathological studies of untreated patients with PKU 50 years ago showed that they had decreased myelin content and decreased amounts of myelin components. It was later demonstrated that dendritic branching of oligodendrocytes decreases in untreated patients (Bauman, Kemper, 1982). Further studies in animals showed that decreased myelination is not caused by demyelination but by hypomyelination. More recent studies (Dyer, Kendler, Philibotte, et al., 1996; Dyer, Kendler, Jean-Guillaume, et al., 2000) using the genetic mouse model indicate that high Phe may interfere not only with myelin metabolism but also with cell differentiation of oligodendrocytes, which adopt a nonmyelinating phenotype. This hypothesis, transferred to humans, would explain hypomyelination and gliosis in untreated patients with PKU.

Since myelin is a proteolipid, hypomyelination and gliosis may also be due to a primary effect on cerebral protein synthesis (Kaufman, 1976). The problem, however, is not to show where high Phe interferes with various enzymatic developments but rather to show where it does not. Thus, the specific effect of amino acid imbalance on intracerebral protein synthesis, and in consequence on brain development, is difficult to quantify. Lowered levels of neurotransmitters have been found in the cerebral spinal fluid as well as in certain brain areas of humans with PKU (McKean, 1972). More recent studies in animals have shown that the functioning of the dopaminergic neurons in the prefrontal cortex, an area vulnerable to Phe/tyrosine imbalances (Diamond, 1996), is influenced by the availability of tyrosine (Tam, Roth, 1997). There are no studies, however, showing that neurotransmitter depletion in patients with PKU leads to irreversible changes in the developing brain. Thus, it is more likely that imbalances of LNAA in the brain leading to lowered neurotransmitters are responsible for reversible changes, especially those seen in the brain of untreated or late-treated patients.

None of these theories alone, however, explains the etiology of brain damage and the heterogeneity of the clinical picture of persons with untreated PKU. Since there is an absence of genotype/phenotype correlation in some patients, other factors (genes?) must be moderating the natural course of the disease. Between 5 and 10 percent of patients with classical PKU have normal intelligence (Hanley, Platt, Bachman, et al., 1999), even though they were never treated by being placed on a low Phe diet.

Normal Intelligence in Untreated PKU: The Possible Role of the Intracerebral LNAA Carrier

The best explanation for this may be the LNAA carrier in the brain and the gene(s) that control it. In vivo magnetic resonance spectroscopy has demonstrated different intracerebral concentrations of Phe after loading with Phe (Weglage, Möller, Wiederman, et al., 1998). Different plasma Phe to brain Phe ratios may explain different intellectual outcomes in patients whose PKU phenotype is otherwise comparable (Weglage, Wiedermann, Möller, et al., 1998; Moats, Koch, Moseley, et al., 2000). Möller and colleagues (1998) found blood levels of around 150 µmol/L in three women with classical PKU, compared with 400 to 730 µmol/L in most patients with PKU. The only explanation is that K^m variants of Phe in the cerebral LNAA carrier protected the three women from the devastating effects of high intracerebral Phe.

Further investigation of the role of LNAA and the gene(s) expressing this carrier may therefore be very useful. A fuller understanding of the carrier mechanism not only may provide better insight into the pathophysiology of PKU but also may be a key to alternative treatments. One of these might be optimizing supplementation of other amino acids, as shown by Pietz and colleagues (1999). Another might be the development of a pharmacologic modifier of this carrier that would prevent excess Phe from crossing the blood-brain barrier. A more clinical approach would be to test the effect of the natural cofactor tetrahydrobiopterin on elevated blood Phe in patients with classical or mild PKU. Kure and colleagues (1999) found that some enzyme mutations may be responsive to diet supplementation with tetrahydrobiopterin, as was found in one of our patients with mild PKU (Trefz, Blau, Aulehla-Scholz, et al., unpublished).

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Screening Technologies: Types and Effectiveness

Harvey L. Levy, M.D.

The foundation of newborn screening for phenylketonuria (PKU) was laid by Fölling, who identified PKU as a biochemical cause of mental retardation (Fölling, 1934), and 20 years later by Bickel, who developed a phenylalanine (Phe)-restricted diet that could prevent the mental retardation (Bickel, Gerrard, Hickmans, 1954). Less than 10 years after the diet became known, Guthrie developed a bacterial assay for Phe so that newborns with PKU could be identified for optimal dietary benefit (Guthrie, Susi, 1963). A collaboration between Guthrie and MacCready, a physician who directed the diagnostic laboratories of the Massachusetts Department of Public Health, resulted in the application of Guthrie's test to spots of blood routinely collected from the heel of newborn infants at nursery discharge and dried on filter paper. Screening in Massachusetts was quickly successful, producing the unexpectedly large number of 9 cases of PKU among the first 53,000 infants tested (MacCready, 1963). Very soon, States passed laws mandating screening for PKU, and today all infants in the United States are being screened (Levy, 1973).

Screening Organization

State laws requiring screening for PKU had the unfortunate consequence of leading to a separate screening program in each State rather than to a national program or regional programs (Levy, Albers, 2000). Currently, there are only two truly regional programs: (1) the Northwest Regional Program in which the Oregon Public Health Laboratory also screens specimens from Nevada, Alaska, Wyoming, and Montana, and (2) the New England Regional Program in which the Massachusetts Public Health Laboratory also screens specimens from Maine, Vermont, New Hampshire, and Rhode Island. Smaller pockets of "regionalization" also exist (e.g., Mississippi specimens are screened in Tennessee). For the most part, however, each State independently determines screening policies and conducts screening, leading to inefficiencies and substantial variations in quality (Levy, Albers, 2000).

The screening test is usually performed in a State laboratory within a health department. There are two notable exceptions. In California, the screening test is performed in eight medical center laboratories under contract to the State, and Pennsylvania contracts its screening to Neo Gen Screening, Inc., of Pittsburgh, a large private newborn screening laboratory (Chace, Naylor, 1999).

Screening Methodologies

Guthrie's bacterial assay remains the most frequently used PKU screening test in the United States (Dougherty, Levy, 1999). This assay is semiquantitative, interpreted by visually comparing the diameter of the bacterial growth zone around a disk from the newborn's blood specimen with the diameters of growth zones around standards that contain known amounts of

Phe (Kim, Levy, 1998). Thus, an infant's specimen surrounded by bacterial growth zone appearing to have the same diameter as the one around a standard disk containing 4 mg/dL of Phe is assigned the Phe value of 4 mg/dL. A specimen surrounded by a growth zone with slightly smaller or slightly larger diameter is said to have a Phe level of 2 to 4 mg/dL or 4 to 6 mg/dL, respectively. Aside from its lack of quantification, the bacterial assay can only be manually performed. These limitations led some programs to use a quantitative fluorometric assay, which provides a precise value for Phe (e.g., 3.8 mg/dL) and which can be automated (McCaman, Robins, 1962; Hill, Summer, Pender, et al., 1965). In California, this assay has been combined with a fluorometric assay for tyrosine in a two-channel approach that increases its specificity for PKU by providing a Phe-to-tyrosine ratio as well as a Phe level. Tandem mass spectrometry (MS/MS), a single quantitative and automated assay that covers more than 20 disorders, includes very specific and sensitive coverage for PKU (Chace, Millington, Terada, et al., 1993; Chace, Sherwin, Hillman, et al., 1998). Currently, several programs use a fluorometric assay instead of the bacterial assay, and two large programs (Neo Gen Screening, Inc., and New England Newborn Screening Program) use MS/MS.

Effectiveness

The effectiveness of screening for PKU incorporates sensitivity and specificity of the method as well as quality of the laboratory performance. In all of these areas, PKU screening has been quite effective. The vast majority of infants with PKU born in the United States have been detected by screening, confirmed, and treated with diet. Thus, although the fluorometric and MS/MS methods are somewhat more sensitive than bacterial assay, this does not appear to be a factor in the relative reliabilities of the methods (Bell, 2000). However, MS/MS displays an amino acid profile or pattern rather than only a Phe level, thus providing greater specificity (fewer false positive results) than either of the other two methods.

Despite a good record on effectiveness, there is much room for improvement. Lack of effectiveness, as determined by missed cases, has largely been the result of poor laboratory or program performance. The reasons have included failure to obtain the blood specimen or collecting an inadequate specimen, errors in the laboratory (e.g., misreading the bacterial assay), reporting abnormal results as normal, and failure to follow up an abnormal result (Holtzman, Slazyk, Cordero, et al., 1986). These missed cases have often occurred in relatively small newborn screening laboratories. This could be remedied by changing the structure of newborn screening from the present largely state-by-state approach to regionalization throughout the United States.

Stored Specimens and Ethical Concerns

Most (75 percent) of the screening programs store the newborn dried blood specimens, the length of time ranging from several months to as long as 25 years (McEwen, Reilly, 1994). These stored specimens have been very valuable in assessing the quality of newborn screening performance by allowing for retesting when a child not identified by screening is later found to have PKU. In virtually all of these instances, the retested result has been a marked increase in Phe rather than the normal result originally reported, demonstrating laboratory error (Levy,

Albers, 2000). Stored specimens have also been used for metabolic research and to assess new technologies (Levy, Albers, 2000).

Until recently, storage of the newborn specimens generated little concern. This has now dramatically changed because of their potential use for DNA testing. Since they represent entire populations of infants, these specimens have been called “DNA banks” (McEwen, Reilly, 1994). The concerns include use of the specimens without consent to identify children with untreatable diseases, particularly those that might not be clinically expressed until many years later. Research in which the identity of the infant is removed from the stored specimen (anonymous studies) has generated much less controversy. The current issue of stored specimens, therefore, centers on control of their use and whether they should be used with retention of identifiers.

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Screening Procedures: Variations in Diagnostic Criteria and Followup

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The term "hyperphenylalaninemia" refers to a wide range of disorders and is used to identify a biochemical phenotype characterized by persistently elevated serum phenylalanine (Phe) concentrations. Phenylketonuria (PKU) refers to a specific type of hyperphenylalaninemia, usually defined clinically with a plasma Phe value exceeding 16.5 mg/dL (1000 μM) and a low tolerance for dietary Phe. PKU is customarily caused by a deficiency of the phenylalanine hydroxylase apoenzyme (PAH), but other causes may include a deficiency of dihydropteridine reductase (DHPR) or reduced production of biopterin (BH_4).

Screening of newborns for PKU has been generally accepted as cost-effective and efficacious, especially when coupled with screening for congenital hypothyroidism (U.S. Congress Office of Technology Assessment, no date). Effective screening depends on smooth integration and appropriate timing of sample collection, laboratory testing, followup, diagnosis, treatment, and evaluation of outcome (American Academy of Pediatrics, 2000; Therrell, Panny, Davidson, et al., 1992). Universal screening systems are designed to respond to Federal and State mandates to provide all children with special needs, including those in traditionally underserved populations, with access to effective treatment.

Screening is universal across U.S. jurisdictions. Screening systems, however, vary in breadth and depth (CORN, 1996; Pass, Lane, Fernhoff, et al., in press; Therrell, Panny, Davidson, et al., 1992). Procedures for establishing screening policy, providing laboratory services, and determining program rules and regulations vary. This variability particularly affects three aspects of screening systems: (1) the criteria used in screening laboratories to diagnose infants with PKU, (2) followup procedures to confirm and treat infants presumed to have PKU, and (3) financing for screening (including education, testing, and followup) and treatment (including infant formula and food).

All screening and diagnostic laboratory testing must meet the standards of the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88), which include requirements for laboratory directors and technical supervisors (Public Law 100-578, 1988). There is no CLIA-approved proficiency testing program for bloodspot screening, however, and the Centers for Disease Control and Prevention (CDC) is filling this role. States vary in their laboratory testing procedures and in the Phe levels above which newborns are considered at risk for PKU. Currently, 15 of the 60 U.S. laboratories participating in the CDC Newborn Screening Quality Assurance Program hold that followup should begin at levels above 4 mg/dL (250 μM), 43 begin followup at values between 2 mg/dL (125 μM) and 3.5 mg/dL (~220 μM), and 1 begins followup at 6 mg/dL (375 μM) (CDC, no date). Some States suggest taking a repeat filter paper specimen on certain lower elevated levels, and others suggest that all elevations be referred for serum followup because false positives are infrequent and delays in diagnosis and treatment can affect outcome.

Certain serum diagnostic tests have been recommended for confirmatory testing (Pass, Lane, Fernhoff, et al., in press), including plasma Phe and tyrosine concentrations. If Phe is elevated, a test of erythrocyte DHPR activity and a urine pteridine profile are recommended. Confirmatory laboratories should have the capability to perform ion exchange chromatography (or another accepted method for measuring plasma Phe and tyrosine), erythrocyte DHPR analysis, and urinary pteridine testing (California Department of Health Services, 1997). Biopterin studies are also suggested by most screening programs, along with supplemental tests that may help in further diagnosis and nutritional management. These include a complete amino acid profile, urine organic acid analysis, and DNA analysis for specific mutations in PAH. Evaluation of parental PAH genotypes by biochemical or molecular methods may also be useful in diagnosis, prognosis, and genetic counseling. Core and supplemental tests and procedures for the diagnosis of hyperphenylalaninemia and PKU are summarized in Table 1 (California Department of Health Services, 1997).

Further management of PKU depends on the outcome of diagnostic testing and clinical evaluation. Management is urgent because the cognitive ability of children with PKU is related to the age at which blood Phe is reduced to therapeutic levels. Once the diagnosis of PKU is confirmed, metabolic control should be achieved as rapidly as possible, ideally within the first 2 to 3 weeks of life; all screening programs report compliance with this recommendation.

The financing mechanisms of newborn screening programs vary (American Academy of Pediatrics, 2000; CORN, 1996). In 1997, 11 U.S. programs did not charge a fee for newborn screening (Simmank, Therrell, 1997); this number decreased to 9 in 1999 (Therrell, personal communication, 2000). Of the States charging a fee, only 12 reported using the money to cover the costs of treatment, including formula (CORN, 1996). Federal funding allows compensation for supplemental foods and formula as part of the Women, Infants, and Children program, or from Medicaid if the individual qualifies. Those ineligible for Federal funding do not always have access to third-party payers of the costs of food and formula required in all States. State laws mandating third-party coverage vary. In self-employment insurance programs, payment decisions may rest with the employer (California Department of Health Services, 1997).

Table 1. State laboratory fees and methods

State/Jurisdiction	PKU Laboratory	PKU Method	Abnormal (mg/dL)	Comprehensive NBS Fee
1. Alabama	State	BIA	≥2.0 if < 24 hr old ≥4.0 if < 48 hr old ≥6.0 if > 48 hr old	$34
2. Alaska	Oregon	BIA	≥4.0	$24
3. Arizona	State (Contract)	Fluorometric 1	≥2.1	$20 (1st) $15 (2nd)
4. Arkansas	State	BIA	≥3.5	$14.83
5. California	8 Contract Labs	Fluorometric 2	Phe/Tyr > 1.5	$42
6. Colorado	State	Fluorometric 1	≥2.1	$33.50 (2 samples)
7. Connecticut	State	BIA	≥4.0	$18
8. Delaware	State	BIA	≥2.7	$42
9. District of Columbia	Neo Gen (Contract)	MS/MS	≥2.0	$14.52
10. Florida	State	BIA	≥2.5	$20
11. Georgia	State	BIA	≥4.0	$32
12. Hawaii	Oregon	BIA	≥4.0	$27
13. Idaho	Oregon	BIA	≥4.0	No fee
14. Illinois	State	Fluorometric 2	≥4.0	$32
15. Indiana	Indiana Univ. (Contract)	Fluorometric 1	≥2.3	$28.50
16. Iowa	State	Fluorometric 1	≥3.1	$31
17. Kansas	State	BIA	≥4.0	No fee
18. Kentucky	State	BIA	≥2.0	$14.50
19. Louisiana	State	BIA	≥3.0	$12
20. Maine	U. Mass. (Contract)	MS/MS	≥2.3	$26.75
21. Maryland	State	BIA	≥2.0	$15.75 (2nd free)
22. Massachusetts	U. Mass. (Contract)	MS/MS	≥2.3	$49
23. Michigan	State	Fluorometric 1	≥2.0	$39
24. Minnesota	State	BIA	≥2.0 if <24 hrs old ≥4.0 if >24 hrs old	$21
25. Mississippi	Tennessee (Contract)	Fluorometric 2	≥4.0	$25
26. Missouri	State	Fluorometric 1	≥3.0	$13
27. Montana	State	Fluorometric 1	>3.0	$10.19
28. Nebraska	2 Contract	Fluorometric 1	≥3.4	$53 / $54.60 (2 labs)
29. Nevada	Oregon (Contract)	BIA	≥4.0	$32
30. New Hampshire	U. Mass (Contract)	MS/MS	≥2.3	$18
31. New Jersey	State	Fluorometric 1	≥2.1	$34
32. New Mexico	State	Fluorometric 1	≥3.0	$20
33. New York	State	BIA	≥3.0	No fee
34. North Carolina	State	MS/MS	≥2.5; Phe/Tyr ≥3	
35. North Dakota	Iowa (Contract)	Fluorometric 1	≥3.1	$16
36. Ohio	State	Fluorometric 1	≥3.5	$27
37. Oklahoma	State	Enzyme (Accuwell)	>3.5	$10.50
38. Oregon	State	BIA	≥4.0	$32
39. Pennsylvania	Neo Gen (Contract)	MS/MS	≥2.0	No fee
40. Rhode Island	U. Mass. (Contract)	MS/MS	≥2.3	$59
41. South Carolina	State	Fluorometric	≥4.0	$21
42. South Dakota	Clin. Labs of Midwest (Contract)	Quantase	≥4.0	$12.28
43. Tennessee	State	Fluorometric 2	≥4.0	$10
44. Texas	State	BIA	≥4.0	$13.75 each sample
45. Utah	State	Fluorometric 1	≥2.1	$27.00 (2nd at no chrge.)
46. Vermont	U. Mass (Contract)	MS/MS	≥2.3	$27
47. Virginia	State	BIA	≥4.0	$16
48. Washington	State	BIA	≥3.0; phe/tyr ≥2 if <24 hrs old ≥4.0 if >24 hrs old	$35.75
49. West Virginia	State	Fluorometric 2	≥4.0	No fee
50. Wisconsin	State	Fluorometric 1	≥2.1	$55.50
51. Wyoming	Colorado (Contract)	Fluorometric 1	≥2.1	No fee

Fluorometric 1 = Perkin Elmer Wallac Kit
Fluorometric 2 = Astoria Pacific Kit

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Informed Consent for Newborn Screening and Future Uses of Tissue Samples

Mary Kay Z. Pelias, Ph.D., J.D.

Over the past century, the world of biomedical science has enjoyed an exponential growth of technologies that now permit the molecular dissection of the human genome and the treatment of an expanding array of devastating genetic diseases. As the technological powers of health care have generated potential benefits, practitioners in medical genetics and genetics research have studied a plethora of ethical, legal, and social issues related to the applications and implications of new knowledge about hereditary health problems. At the interface of technology and its applications and implications are questions derived from concepts of personal autonomy, personal privacy, and confidentiality of genetic information. Also at this interface are concerns about making the new benefits available across the human population as a matter of public health. Out of these broad concerns have developed newborn screening programs that confer immense benefits to individuals, to families, and to society.

The ethical principles of personal autonomy, personal privacy, and confidentiality of personal information now constitute the foundation of the practice of medicine. The principle of personal autonomy is understood as decisional privacy, or the right of an individual to make his or her own decisions, without duress or coercion, about which medical or other options to pursue. The concept of personal privacy protects individuals within the private sphere of the body so that others are constrained from touching or viewing the person inappropriately. Privacy of information includes respect for confidentiality of personal information that is exchanged within the professional-patient relationship, thus assuring candor in communication and resulting in accrual of benefits to the individual. Respect for these three facets of privacy is the basis of principles that govern the practice of medical genetics and genetic counseling.

The doctrine of informed consent developed from principles of personal autonomy and personal privacy. One branch provided standards of conduct in the practice of medicine and surgery, and the other branch developed the principles of conduct in biomedical research. The principle of informed consent in the professional-patient relationship grew out of an expanded array of options that became available in medicine and surgery early in the 20th century. As patients became more aware of their options, they became quick to allege negligence when professionals failed to present all options that a patient might choose to pursue. Over a series of medical malpractice cases in the common law, patients acquired the right to hear all “material” information before deciding which course of treatments, if any, to pursue, with “material” defined as any information that could cause the patient to choose another course. The principle of informed consent in biomedical research grew out of numerous experimental regimes, carried out over the course of the 20th century, both in the United States and elsewhere, without the knowledge or consent of the individual subjects. The rules for informed consent in biomedical research were first promulgated in the “Nuremberg Code” and later in the “Declaration of Helsinki” and the “Belmont Report,” and they were ultimately codified in the “Code of Federal Regulations.” Informed consent in biomedical research is valid only if the subject is competent

and understands information about the research, its risks, benefits, and alternatives, with assurances about confidentiality and the subject's right to withdraw. Federal regulations also include special provisions for protecting vulnerable populations, including pregnant women, children, and prisoners. These principles now influence every aspect of the practice of medical genetics and genetic counseling and all phases of research in human and medical genetics.

The advent of newborn screening for phenylketonuria in the 1960s ushered in the era of treatment for some infants who are born with devastating genetic diseases. Simple, reliable, inexpensive tests permit the early identification of serious genetic diseases that can then be managed for the benefit of the infant, in terms of normal, or nearly normal, development or health, and for the benefit of society, in terms of the public fisc. Several drops of blood samples are collected shortly after birth, blotted and dried on personally identified cards, and sent to screening laboratories for testing. Most States have legislated mandatory screening so that these samples can be gathered and tested without formal parental consent. Cards with surplus blood spots are retained, with identifiers, in laboratories for varying periods defined by State law. Debate over the past four decades about mandatory screening has weighed the negative invasion of privacy and parental autonomy against the positive benefits of screening, and all but two States have favored mandatory screening because of the immense benefits that are realized when infants are detected and treated early in life. Only two States require parental consent before samples are collected from the infant. Most States have loose provisions for telling parents about screening and for permitting parents to opt out for religious reasons. However, mandatory screening is so rigid in two other States that parents who refuse testing are subject to criminal penalties. The immense success of newborn screening programs reinforces and supports legislative decisions to screen for certain diseases without parental consent.

Over the past decade, technical developments in molecular genetics have opened the door for vastly expanded research into the structure and function of countless genes. As the new technologies expand the power of research, genetics professionals have inquired about the possibility of using minute bits of dried blood from newborn screening samples to conduct research in medical and molecular genetics. The push to gain access to newborn screening samples has generated new debate about the appropriate use of samples that are collected without parental consent. Some professionals have noted that samples collected in the past were legally separated from the donors at the time of collection and should therefore be available to researchers on request. Others have carefully argued that these samples may be used for future research only after parents are recontacted for specific consent for specific research projects. Others have argued that these samples should be available to any researchers provided the samples are stripped of identifying information.

The middle ground in using new collections of newborn screening tissue samples for present and future research may lie in establishing a protocol for mandatory screening, followed by a request for parental consent for future use of samples if the samples are likely to be sought for genetics research. The undeniable benefits of newborn screening justify continued mandatory programs so that parental consent for screening is not a critical factor. Decisions about future use in unspecified genetic research and tests should, however, rest with the donors of the samples or their parents. A short protocol of six simple, dichotomous questions could be presented to parents. These questions address the use of samples, with or without identifiers, with or without the option for being notified about the development of significant new information. The protocol

could provide for reciprocity or responsibility in following up on any new developments. This protocol protects families as the persons who may have a significant interest in future developments, and it also protects the researcher without imposing an undue burden.

New technologies are expanding the interests of individuals, families, and genetics professionals. Achieving an acceptable balance among ethical principles and professional activities is a challenge that can be resolved by acknowledging that everyone has a stake in genetic information that is developed now and in the future.

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Variations Among Programs in Time of Initiation of Diet Treatment, Level of Control, and Diet Relaxation/Discontinuation

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A survey was sent to the directors of 111 clinics in the United States that treat patients with phenylketonuria (PKU), and we received 87 responses. These 87 clinics were treating a total of 4,669 patients. Approximately one-half of the patients were younger than 12 years of age, and 93 percent of these were on a phenylalanine (Phe)-restricted diet. Of those patients 12 years of age and older, 54 percent were on a Phe-restricted diet. The survey instrument included questions about diagnosis, initiation of treatment, assessment of biochemical control, and continuation of dietary restriction.

Blood Phe Concentrations That Initiate Treatment

The clinic directors were asked what concentration of Phe caused them to make a presumptive diagnosis of PKU and begin dietary restriction of Phe. For 70 of the 87 clinics (80 percent), a Phe concentration of 10 mg/dL or less (600 µM) was considered the appropriate level to begin dietary restrictions. At 10 of the 87 (11 percent), treatment began at a concentration of between 10 and 15 mg/dL (600 to 900 µM). Five clinics (6 percent) considered a concentration of more than 15 mg/dL (900 µM) the appropriate level to initiate treatment.

Current Practice Regarding Diet Maintenance

The clinic directors were asked about their approach to long-term treatment of PKU. Some 99 percent of the clinics prescribed a restricted diet for life for males; 85 percent prescribed a restricted diet for life for females. These practices had been in place for more than 7 years at 54 of the 87 clinics (62 percent), and for more than 3 years at 10 of the 87 (11 percent).

Blood Phe Levels During Treatment

The most commonly advocated levels were 2 to 6 mg/dL (120–360 µM) for patients up to 12 years of age, and 2 to 10 mg/dL (120–600 µM) for patients older than 12 years of age.

Monitoring Intervals After Instituting a Phe-Restricted Diet

Monitoring consisted of measurement of Phe concentration in a blood sample, collected at most centers from patients after they had fasted overnight. Monitoring intervals varied according to the age of patients. At less than 1 year of age, the mean frequency was 3.6 times per month (the range was 1 to 8 times per month). Between the ages of 1 and 3 years, the mean

frequency was 1.9 times per month (the range was 0.33 to 4 times per month). The frequency of monitoring decreased with increasing age. By 18 years of age, the mean frequency was 1.0 times per month (the range was 0 to 4 times per month).

Laboratory Technique Used

The laboratory technique used for measuring serum or plasma Phe varied from the semiquantitative Guthrie method used in 19 of the 87 clinics (22 percent) to highly quantitative plasma amino acid column chromatography used by 19 (22 percent) and HPLC by 4 (5 percent). A majority of the clinics (41 of the 87, or 47 percent) used the McCaman-Robins fluorometric method.

Time Between Testing and Notification

The amount of time between obtaining blood from patients and reporting the results to the family varied from 1 to 10 days. Most clinics reported the results within 1 to 3 days (52 of the 87, or 60 percent). Five clinics (6 percent) needed 8 to 10 days.

Need for Further Research

Deficiencies in our knowledge still affect our ability to treat patients with PKU optimally. Some involve the pathophysiology of central nervous system injury in PKU, such as the relationship of brain Phe concentration to outcome, the existence of modifying genetic factors, and genotype-phenotype correlation between phenylalanine hydroxylase (PAH) mutations and the clinical phenotype. Other deficiencies involve factors related to compliance with a restricted diet, such as how to design a more palatable diet. We also need a better definition of who should resume the diet. Ideally, a diet treatment that addressed abnormal PAH protein or the gene mutation in PAH may have the best chance of producing a normal outcome.

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Effects of Dietary Treatment on Children With Classical Phenylketonuria (PKU): The United States PKU Collaborative Study, 1967–1984

Eva Gross Friedman, Colleen G. Azen, M.S., Richard Koch, M.D., and the Writing Committee for the PKU Collaborative Study

This randomized study was conducted in two phases: phase A compared randomly assigned strict (TG1: 1.5–5.4 mg/dL) versus moderate (TG2: 5.4–9.9 mg/dL) restriction of phenylalanine (Phe) intake during the first 6 years of life. Phase B investigated diet continuation versus discontinuation after the age of 6 years. Non-PKU full natural siblings of the PKU index cases constituted the control group. The study designs for both investigations have been published (Williamson, Dobson, Koch, 1977; Koch, Azen, Friedman, et al., 1982). Subjects with PKU were identified by newborn screening, treated within the first 120 days of birth, and underwent confirmatory challenge by 1 year.

The Phe-restricted diet was based on Lofenelac, a casein hydrolysate, and was designed to provide a balance of nutrients with adequate protein, calories, and other essential amino nutrients. Good growth of those in both TG1 and TG2 was evidence of the adequacy of nutrient intake (Acosta, Trahms, Wellman, et al., 1983).

Blood Phe levels were measured weekly during the first year and monthly thereafter. These serial Phe levels were then summarized into Indices of Dietary Control (IDC). IDC-1 was the median of Phe levels during specific periods, and IDC-2 was the area under the curve of Phe over time. Other summary measures included rate of rise in blood Phe levels above 900 μmol/L through 6 years of age (Williamson, Koch, Azen, et al., 1981), total exposure to Phe levels above 900 μmol/L, and age after which Phe levels consistently exceeded a specified value—i.e., 900 μmol/L (Holtzman, Kronmal, van Doorninck, 1986).

Results

Of the 167 confirmed infants with PKU who were initially enrolled in the collaborative study, 133 completed phase A, and 118 completed phase B through age 10. When the study concluded, 96 had completed 12-year evaluations. The subjects were predominantly Caucasian (96 percent); 43 percent were first-born, and 57 percent were male. Of the mothers, 16 percent were younger than 20 years of age at the birth of the child. The mean parent Weschler Adult Intelligence Scale (WAIS) score was 109 (SD=13) for 109 fathers, and 106 (SD=12) for 120 mothers.

For both groups, the mean 6-month IDC-1 values rose during phase A. Although TG1 values were generally below those of TG2, mean values exceeded the target range after 6 months of age for TG1 and after 36 months of age for TG2. The average rate of increase in Phe levels per year was 67.9 μmol/L for TG1 and 57.1 μmol/L for TG2. Twenty percent of the children in

TG1 and 30 percent of those in TG2 had exceeded 900 μmol/L by the age of 6 years. Furthermore, many children who were "on diet" had blood Phe levels well above the target ranges, even during phase A. Holtzman and colleagues defined loss of dietary control to have occurred when IDC-1 rose above 900 μmol/L and remained there for three consecutive 6-month periods (Holtzman, Kronmal, van Doorninck, 1986).

The children in this sample exhibited an overall prevalence of congenital anomalies (9.3 percent) that was not significantly different from the figure for the general population (Johnson, Koch, Peterson, et al., 1978). Nineteen of the 161 children with PKU had abnormal electroencephalograms (EEG) shortly after birth that became normal by 1 year of age in all but 2 children (Blaskovics, Engel, Podosin, et al., 1981).

Annual assessments of height, weight, and head circumference through 4 years of age compared favorably with national norms (Holm, Kronmal, Williamson, et al., 1979). No significant differences were noted at any age between the PKU sample and a cohort of 184 normal children followed by the Fels Research Institute. However, the girls with PKU had a significantly greater increase in weight at later ages than did girls in the Fels group. This trend was still apparent at 8 years of age. Higher serum Phe levels were found to be positively correlated with weight, particularly for girls ($p<.001$), perhaps reflecting poorer diet adherence in those with more rapid weight gain (McBurnie, Kronmal, Schuett, et al., 1991).

Cognitive Development and Academic Achievement

At age 6 the average intelligence quotient (IQ) score was 98 (SD=16). Significant predictors of IQ scores were mother's IQ score, age at initiation of treatment, and rate of increase in IDC, which together accounted for 36 percent of the total variation in IQ score (Williamson, Koch, Azen, et al., 1981). By the time subjects were 10 years of age, Holtzman and colleagues found that the age at which control of Phe was lost was the best, and often the only, significant predictor of the deficit in Wechsler Intelligence Scale for Children (WISC) IQ scores, Wide Range Achievement Test (WRAT) achievement, and behavior test scores between the child with PKU and other family members. A followup report on the subjects confirmed these findings at 12 years of age (Azen, Koch, Friedman, et al., 1991). Table 1 depicts mean IQ score from ages 6 through 12 years for children with PKU grouped by age at loss of dietary control.

The greatest discrepancies between the scores of the children with PKU and other family members occurred in those who lost dietary control before age 6, whereas those who lost control between 6 and 10 years of age had the greatest change in scores from age 6. Those still in control maintained their scores at 6 years of age in all areas except arithmetic. Arithmetic scores declined more than 10 points in all groups.

Table 1. Mean IQ at ages 6 to 12 years for children with PKU grouped by age at which control of blood Phe below 900 μmol/L was lost

Age Lost Control	Age/Test	N	Mean	SD
Before age 6	6-year S-B*	22	91	15
	7-year S-B	19	93	12
	8-year WISC†	18	91	13
	10-year WISC	20	93	14
	12-year WISC-R‡	22	88	15
Between 6 and 10	6-year S-B	42	100	14
	7-year S-B	42	98	13
	8-year WISC	41	99	14
	10-year WISC	39	99	13
	12-year WISC-R	42	95	14
After age 10	6-year S-B	30	102	14
	7-year S-B	30	103	14
	8-year WISC	29	103	12
	10-year WISC	25	105	12
	12-year WISC-R	30	101	10
After age 10 and low Phe	6-year S-B	6	113	17
	7-year S-B	6	117	21
	8-year WSC	5	111	15
	10-year WISC	2	113	22
	12-year WISC-R	4	106	14

*S-B: Stanford Binet, 1972 Norms.
†WISC: Wechsler Intelligence Scale for Children
‡WISC-R: Wechsler Intelligence Scale for Children (Revised).

In addition to IQ and WRAT scores, the collaborative study evaluated perception and language development using the Bender Gestalt Test and the Illinois Test of Psycholinguistic Abilities (ITPA) at various ages. Those who continued on the diet and maintained control of their blood Phe levels had better mean scores than those who discontinued, but both groups had declining scores, similar to the pattern observed for arithmetic scores (Fishler, Azen, Henderson, et al., 1987). Among 112 children with PKU for whom school history data were collected, 32 percent were having mild to severe problems by the first grade, including repeating a grade or spending at least half of each day in special classes. By the fourth grade, 34 percent were at least 1 year below modal grade.

Comparison With Siblings

The non-PKU control group comprised 163 children from 91 families, allowing for within-family paired comparisons on cognitive tests. Table 2 presents these results at the ages at which siblings were tested.

Table 2. Paired comparisons between children with PKU and their unaffected siblings on psychological test scores at various ages

Psychological Test	Age at Test	Number of Pairs	Mean Scores		Sib-PKU Difference	*p*-Value
			PKU	Sib		
Stanford-Binet, 1972 norms	4 years	36	94	99	5	<0.02
Frostig Perceptual Quotient	5 years	51	94	103	9	<0.001
Bender Perceptual Maturity*	7 years	40	79	84	5	<0.05
ITPA Psycholinguistic Quotient	7 years	42	97	101	4	NS
WISC verbal scale	8 years	57	101	105	5	0.034
WISC performance scale	8 years	58	101	108	7	<0.001
WISC full scale	8 years	57	101	107	6	0.005
WRAT reading	8 years	54	101	107	6	0.006
WRAT spelling	8 years	54	100	104	4	NS
WRAT arithmetic	8 years	54	96	100	4	0.007

*Bender Perceptual Maturity scores are month equivalents.

Children with PKU scored significantly below their siblings on all tests except WRAT spelling at 8 years and the ITPA at 7 years (Fishler, Azen, Henderson, et al., 1987). The sibling-PKU differences were correlated with IDC at the time of the test and/or the age at loss of dietary control, except for the Frostig and ITPA tests. Children who maintained control of their blood Phe levels at least through 8 years of age had scores comparable with those of their siblings on all tests (Holtzman, Kronmal, van Doorninck, 1986).

Summary

The U.S. PKU Collaborative Study provided answers to several basic questions regarding treatment for PKU, namely, that the Phe-restricted diet is a safe and effective method of treatment, that continuation of the diet throughout childhood is recommended, and that early initiation of treatment and maintenance of good control of Phe levels in the early years of life are crucial.

Although IQ scores remain relatively stable after diet discontinuation, other areas of cognitive functioning (such as reading and spelling) may be more sensitive to rising Phe levels. Other areas, such as arithmetic, language, and perception, appear to be problematic for all children with PKU, at least in the ranges of Phe control seen in these children. Too few children in the study maintained Phe levels in the ranges of 120 to 360, or even 600 μmol/L, to address whether stricter control would have prevented even these subtle deficits.

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Dietary Treatment of Phenylketonuria With Behavior Disorders

Alfred Baumeister, Ph.D.

Common comorbidities associated with phenylketonuria (PKU) are highly elevated rates of aberrant behavior, ranging from relatively mild psychiatric disorders to extreme—even life-threatening—behavioral disturbances, such as aggression, disruption, pica, and self-injury. Typically, these clinically challenging problems have been treated through pharmacologic therapy, behavior modification, physical restraint, or some combination of the three. Reviews of the literature show that these interventions generally do not produce enduring and functionally meaningful effects.

Although behavior problems are common among persons with mental retardation, especially the more severe cases, examination of the literature indicates that disease etiology is usually not a major consideration in the development of clinical interventions. Nevertheless, the hyperphenylalaninemias present an excellent opportunity for designing treatments with high degrees of biological plausibility and etiological specificity. Not only may it be possible to treat such problems in persons with PKU, but these and other inborn errors of metabolism should provide models for designing therapies based on an understanding of specific disease pathogenesis.

Over the years, controlled experiments and case studies have found that the disordered behavior exhibited by persons with elevated blood phenylalanine (Phe) may be effectively managed by placing these individuals on a Phe-restricted diet. Such therapy may also be useful for those who have never been on a Phe-restricted diet, those with PKU who were identified early but removed from the diet in childhood, those treated late (Brown, Guest, 1998), and those with atypical or mild hyperphenylalaninemia. This literature, along with a great deal of corroborating evidence (dietary challenge, magnetic resonance imaging [MRI] results, free diet supplementation with amino acids, neurotransmitter regulation), was reviewed by Baumeister and Baumeister (1998). All the evidence, taken together, suggests that restricted-Phe dietary treatment and, perhaps, supplementation with other amino acids may be useful for treating behavioral disturbances in hyperphenylalaninemia.

As reported by Baumeister and Baumeister (1998), there appears to be a fairly close correlation between blood Phe concentrations and the occurrence of aberrant behavior. In the case of three patients, the investigators found that the correlations ranged from .64 (tantrum) to .71 (assault) and .96 (tantrum). For a fourth subject the correlation was also positive (face-slapping) but not statistically significant. In addition, when positive changes are observed, they are related to the length of time necessary for Phe blood levels to drop substantially. It is important to note that observed benefits are not necessarily conditional on attaining normal Phe levels. The range of blood Phe typically observed in studies with positive outcomes runs from 3 to 10 mg/dL.

Data from MRI studies also indicate that abnormalities (in white cerebral matter) are related to Phe concentration and that it takes at least 2 months of good dietary control to resolve such abnormalities. Nevertheless, it must be emphasized that the connection between changes in MRI results and behavioral disturbance is still unclear.

Although case studies and controlled experiments indicate that behavioral disturbances among patients with hyperphenylalaninemia often respond positively to the medical diet, there remains considerable variability both between and within individuals. In some cases, no improvement has been observed; in a few, symptoms have become more severe. In still other cases, the initial response is good, but then there is a relapse. The sources of this variability are uncertain, but degree of control over serum Phe is undoubtedly implicated. In addition, hyperphenylalaninemias are heterogeneous diseases, both phenotypically and genetically (Güttler, Azen, Guldberg, et al., 1999). Thus, mutational severity may be a critical determinant.

Recent studies (for example, Moats, Koch, Moseley, et al., 2000) indicate that brain Phe concentration is a more critical variable in management of patients with PKU than blood Phe. The correlation between blood and brain concentrations of Phe is, in general, not very high. All studies of dietary intervention in which behavioral and neurologic abnormalities have been targeted have measured blood levels of Phe. It could be that a better understanding of the effects of dietary management of behavioral and neurologic abnormalities would be achieved by measuring concentrations of Phe in the brain.

Virtually all intervention studies have focused on individuals with classical PKU. Nevertheless, as with other toxic agents, there is probably a dose-gradient relationship. A recent unpublished study has shown that two individuals with atypical PKU (Phe <12 mg/dL) who displayed disruptive behavior responded positively to dietary intervention to reduce Phe or dietary supplementation with the neurotransmitter precursors tryptophan and tyrosine.

Despite accumulating evidence that aberrant behavior among patients with hyperphenylalaninemia may be effectively treated with medical diet, several important considerations remain:

- The level of Phe restriction required to produce positive results.
- The extent to which free diet supplementation with amino acids can have positive effects.
- The relation of brain versus blood concentrations of Phe to clinical outcome.
- The generality of dietary effects across time, situation, and other indicators (e.g., seizures, motor control, socialization).
- The relation between changes in myelination and abnormalities in MRI and electroencephalographic findings.
- The applicability of Phe restrictions to atypical PKU.

- The serotonin and dopamine regulation of patients with PKU.
- The timing and degree of relaxation of diet.
- The advisability of Phe screening of adults with behavioral disorders who are not known to have PKU.
- The relative importance of the factors involved in dietary management (e.g., nutritional needs, family support, costs, variability of control).
- The relative importance of laboratory measures (e.g., plasma amino acids, urine pterins, albumin, CBC, ferritin, folate, and RBC indices).
- The relationship between phenylalanine hydroxylase (PAH) genotype, biochemical control, and outcome.
- The handling of methodological problems in most reported studies.

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A 15-Year Followup Report on Participants in the Collaborative Study of Children Treated for Phenylketonuria (PKUCS 1967–1984)

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The study design, methods, and results of the 1967–1984 study were published and strongly supported a recommendation that a phenylalanine (Phe)-restricted diet should be maintained throughout adolescence (Holtzman, Kronmal, van Doorninck, et al., 1986; Azen, Koch, Friedman, et al., 1991). This recommendation was based on an evaluation of the Wechsler Intelligence Scale for Children-Revised (WISC-R) and the Wide Range Achievement Test (WRAT) standard scores at the age of 12 years for 95 children who had been diagnosed by newborn screening and had begun dietary treatment during the neonatal period. Dietary control of blood Phe <900 μmol/L was maintained in 23 of these children beyond 10 years of age; in 72 others, the blood Phe level was persistently >900 μmol/L at varying ages. Test scores were negatively correlated with age at initiation of diet and with blood Phe levels from ages 4 to 10 years, and were positively correlated with parent intelligence quotient (IQ) scores and age when dietary control was lost.

The findings of that study and others have gradually resulted in a philosophy of a "Diet for Life" at most clinics in the United States. In preparation for the Consensus Development Conference on Phenylketonuria (PKU), the National Institute of Child Health and Human Development (NICHD) authorized funding to conduct a followup study on the enrollees in the study, who are now 27 to 32 years old, and to compare the findings with the data collected on the subjects during childhood. The followup effort is still under way, and this abstract presents an initial summary of the results to date.

Sample

After a 15-year interval between the 2 studies, 59 of the 129 enrollees who were still active at the end of the original study were located and evaluated. There were no significant differences between those who were and those who were not evaluated as adults on diagnostic, treatment, demographic, or childhood psychometric variables. Seven of the 59 never discontinued diet, whereas 30 discontinued before 6.5 years of age, 12 between 6.5 years and 12 years of age, and 10 between 12 and 20 years of age. Among discontinuers, nine had 3 or more additional years of treatment.

Results

Medical findings as reported by questionnaire and current medical evaluation are summarized in Table 1. Eczema was reported in 26 percent, recurrent headache in 28 percent, neurological signs in 26 percent, hyperactivity in 15 percent, and lethargy in 20 percent of the subjects off diet, and in none of those who remained on a Phe-restricted diet. Furthermore, these symptoms were more common in those who discontinued dietary treatment before 6.5 years of age. In those off diet, depression was reported in 24 percent, phobias in 24 percent, and unspecified mental disorders in 15 percent. In comparison, the on-diet group had only one reported episode of transient depression, and that was related to a medical product change. Intellectual development and academic achievement were measured by the Wechsler Adult Intelligence Scale-Revised (WAIS-R) and the WRAT-3, respectively. The mean standard scores have remained fairly stable since age 12, as shown in Figures 1 and 2. One interesting phenomenon is that arithmetic scores on the WRAT-3 are much improved in adulthood when compared with scores at age 12. Because extensive neuropsychological testing on a subset of 20 subjects is still in process, however, our followup study is incomplete at this time.

Table 1. Medical findings in 52 PKUCS adult subjects

	<6.5 yr		6.5–12.5 yr		>12.5 yr		Never		Total Sample	
Findings	n	%	n	%	n	%	n	%	n	%
Convulsions	1	4	0	0	0	0	0	0	1	2
Eczema	10	43	1	9	1	8	0	0	12	23
Hyperactivity	4	17	2	18	1	8	0	0	7	13
Hypoactivity/lethargy	8	35	0	0	1	8	0	0	9	18
Autistic behavior	0	0	0	0	0	0	0	0	0	0
Phobias	7	30	1	9	3	25	1	17	12	23
Depression	8	35	1	9	2	17	1	17	12	23
Headaches	7	30	2	18	4	33	0	0	13	25
Mental disorders	5	22	1	9	1	8	0	0	7	13
Surgery	15	62	3	27	5	42	1	17	24	46
Heart disease	1	4	0	0	0	0	0	0	1	2
Hypertension	1	4	2	18	0	0	2	33	5	10
Cancer	0	0	0	0	0	0	0	0	0	0
Asthma	2	8	2	18	0	0	0	0	4	8
Obesity (BMI≥30)	4	16	3	27	0	0	2	29	9	16
Neurological Signs	4	22	4	40	2	18	0	0	10	22

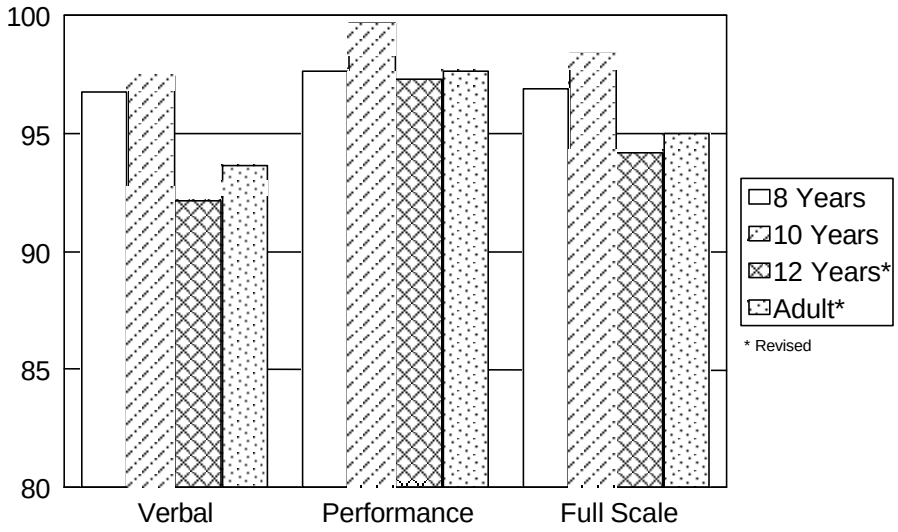

Figure 1. Wechsler IQ test scores, PKUCS adult followup study**

**Not corrected for mutation severity, parental IQ, brain Phe level, or age of diagnosis.

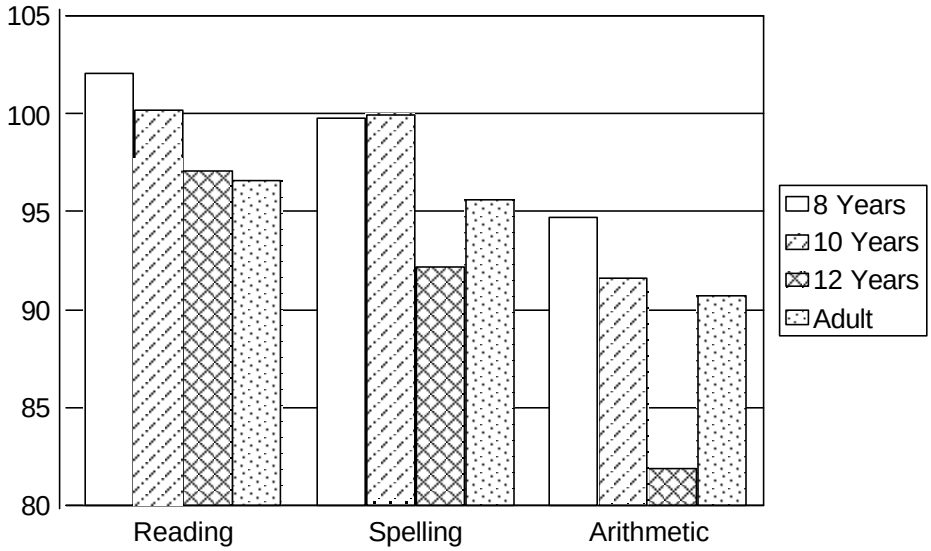

Figure 2. Wide Range Achievement Test scores, PKUCS adult followup study

Magnetic Resonance Imaging and Magnetic Resonance Spectroscopy (MRI/MRS) Findings

Fifteen subjects have completed this part of the study. Brain Phe levels were measured with simultaneous blood samples. (See Table 2.) Blood Phe did not predict brain Phe levels, although three of the six persons with low brain Phe levels exhibited blood Phe levels of 900, 255, and 151 µmol/L. The other three persons with low brain Phe levels, exhibited blood Phe levels of 1,965, 1,591, and 1,560 µmol/L. Mean IQ in persons with the six lowest brain Phe levels was 109, and in those with the nine highest brain Phe levels it was 104. The sample size, however, is too small for meaningful statistical comparison.

MRI codes ranged from 0 to 5, where 0 indicates complete normality and 5 indicates severe abnormality. One subject was rated as 0, six as 1, five as 2, and two as 3. There were no subjects in categories 4 and 5. By grouping ratings 0 and 1, the mean IQ of seven subjects was 112; by combining 2 and 3, the mean IQ in seven subjects was 102. Again, the sample is too small for meaningful comparisons. Five subjects remain to be studied, and these studies will be completed by September 2000.

Table 2. MRI/MRS findings to date

Subject ID	Diet Status*	Age Diet Discontinued	WAIS-R IQ	MRI Code	MRS Brain Phe mm/L	Blood Phe µmol/L
L36		14	102	2	0.55	1500
L30	C	-	103	1	0.34	1111
I17	C	12	124	1	0.19	1965
L22	C	-	113	2	0.22	900
L18	C	-	123	3	0.41	1371
Q04	C	-	108	1	0.18	0255
A08	C	-	112	1	0.16	0151
L01	D	6	116	2	0.92	1440
L07	D	6	126	1	0.35	1185
L27	D	5	75	3	0.45	1620
L13	D	6	105	Not done	Agoraphobic	1500
L32	D	6	101	2	0.35	1080
S07	D	7	102	1	0.31	1137
L10	D	8	114	0	0.16	1591
S01	D	6	83	2	0.22	1560

* At end of PKU study in 1984.
\+ Plan to repeat MRI/MRS. Subject data were accidentally erased.

C Continuer of Phe-restricted diet.
Mean IQ = 112.0.

D Discontinuer of Phe-restricted diet.
Mean IQ = 103.

Adult Test Scores for Subjects Grouped by Age at Which Diet Was Discontinued

Continuers on diet had a higher mean full-scale IQ score of 108, which represents a significant increase over their 12-year mean WISC-R IQ (p=0.03) score. (See Table 3.) Subjects who discontinued dietary treatment before 6.5 years of age had a mean IQ of 90, whereas those who discontinued dietary treatment between 6.5 and 12.5 years of age scored a mean IQ of 95. Those who stayed on diet longer than 12 years scored a mean IQ of 99. None of the mean scores of those off diet differed from their mean scores at age 12. WRAT-3 achievement test scores appear to be higher in those who remained on diet, although the sample size was too small for statistical testing.

Table 3. Adult test scores for PKUCS subjects grouped by age at diet discontinuation (values tabulated are mean + s.d.)

Age Diet Discontinued	WAIS-R				WRAT 3			
	n	Verb.	Perf.	Full	n	Read.	Spell.	Arith
Before 6.5 yrs								
Parent IQ	23				104±10			
Adult score	24	88±11	94±16	89±12	90±13	20	94±12	92±14
Change*	20	-1	+2	+9	0	16	0	+3
p†		0.58	0.49	0.001	0.98		0.83	0.29
6.5–12.5 yrs								
Parent IQ	12				110±8			
Adult score	13	93±15	97±22	91±6	95±18	12	98±18	94±17
Change*	5	0	-2	+10	-1	8	+1	+2
p†		1.00	0.70	0.03	0.73		0.62	0.43
12.5–20 yrs.								
Parent IQ	8				102±15			
Adult score	8	97±16	102±20	89±31	99±19	4	91±32	91±31
Change*	8	+5	+3	+9	+4	3	-3	-9
p†		0.18	0.43	0.08	0.35		0.38	0.28
Never Off Diet								
Parent IQ	7				111±7			
Adult score	7	110±9	105±12	98±5	108±8	5	109±5	110±6
Change*	6	+12	+6	+16	+10	3	+2	+8
P†		0.01	0.29	0.01	0.03		0.51	0.06

*Change from 12-year WISC-R or WRAT score.
† p-values are for illustration only, due to small sample sizes.

Adult Test Scores for Subjects Grouped by Current Phe Levels

The importance of blood Phe is shown by the progressive decline in adult mean IQ scores with increasing blood Phe. (See Table 4.) In 21 adults with blood levels <1,200 μmol/L (range 230 to 1,156), the mean IQ was 103. In 29 adults with blood levels >1,200 μmol/L, the mean IQ was 89. The difference of 14 points in IQ between the two groups is remarkable when one takes into account that the parental mean IQ of the two groups differed by only 2 points.

Table 4. Adult test scores for PKUCS subjects grouped by usual adult Phe level (values tabulated are mean + s.d.)

Usual Adult Phe Level	WAIS-R				WRAT 3			
	n	Verb.	Perf.	Full	n	Read.	Spell.	Arith
<15.9 mg/dL								
Parent IQ	9			115±6				
Adult score	9	109±10	113±14	112±11	8	108±5	108±6	103±7
Change*	9	+6	+7	+8	7	+1	+5	+16
p†		0.20	0.10	0.08		0.71	0.25	0.002
16.0–19.9 mg/dL								
Parent IQ	12			102±8				
Adult score	12	95±12	101±13	97±13	8	101±8	100±6	95±7
Change*	11	+3	+3	+2	7	+3	+3	+12
p†		0.28	0.40	0.37		0.21	0.47	0.008
20.0–23.9 mg/dL								
Parent IQ	11			108±9				
Adult score	12	93±16	92±18	92±15	9	95±12	96±14	89±8
Change*	8	+5	+5	+5	6	-2	+4	+10
p†		0.10	0.49	0.18		0.67	0.47	0.01
24.0–27.9 mg/dL								
Parent IQ	8			100±13				
Adult score	8	85±15	89±13	85±14	6	82±27	79±26	78±25
Change*	6	-1	-4	-3	4	-2	-1	+8
p†		0.76	0.32	0.39		0.63	0.82	0.14
=>28.0 mg/dL								
Parent IQ	9			106±12				
Adult score	9	88±12	95±20	90±16	9	94±18	92±17	86±16
Change*	6	-2	0	-2	7	-1	-2	+3
p†		0.37	0.90	0.31		0.85	0.57	0.39

*Change from 12-year WISC-R or WRAT score.
† *p*-values are for illustration only, due to small sample sizes.

Educational and Occupational Status

In the group of adults with PKU, 32 percent were college graduates, 21 percent had professional- level occupations, and 20 percent were in the highest two Hollingshead socioeconomic classes. Although the percentages are higher with increased years of dietary treatment and lower Phe levels, even among those who discontinued diet before age 12.5 years, 21 percent were college graduates and 14 percent reported professional occupations. The present study also collected educational and occupational data on siblings. In comparisons between 24 pairs of adults with PKU and their adult non-PKU siblings, the percentages who were college graduates were identical (37.5 percent), as were the percentages in professional occupations (25 percent).

Mutation Studies of the Phenylalanine Hydroxylase Enzyme

Mutation studies were performed by a group in Glostrup, Denmark, headed by Flemming Güttler. A discussion of these is beyond the scope of this report, but it may suffice to say that only four subjects exhibited one mild mutation on chromosome number 12. Thus, the diagnosis of classical PKU was confirmed by the mutation studies.

Conclusion

In summary, the data collected so far favor diet continuation through adulthood. Both the higher frequency of reported medical and mental disorders and the lower cognitive test scores among discontinuers support this practice. In addition, the newer medical food products now available and the possibility, based on knowledge gained from MRI/MRS studies, of therapy with large neutral amino acids as an adjunct to dietary restriction of Phe, provide encouragement that care of individuals with PKU will be better in the future.

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Commentary on Recommendations in the United Kingdom for the Management of Phenylketonuria

Forrester Cockburn, M.D.

Phenylketonuria (PKU—meaning persistent hyperphenylalaninemia >240 µmol/L, a relative tyrosine deficiency, and excretion of an excess of phenylketones) occurs in approximately 1 in 10,000 births in the United Kingdom (Smith, Cook, Beasley, 1991; Smith, 1985). Except for the 1 to 2 percent with defective metabolism of tetrahydrobiopterin, infants with PKU have a recessively inherited deficiency in the hepatic enzyme phenylalanine hydroxylase (PAH) due to a large number of sequence variations at the *PAH* gene (Tyfield, Stephenson, Cockburn, et al., 1997).

Screening of neonates using capillary blood obtained between 5 and 21 days after birth and early treatment with a diet low in phenylalanine (Phe) produced the virtual disappearance of children with a mental handicap because of PKU in the United Kingdom between 1964 and 1970. In 1993, a working group convened by the Medical Research Council (MRC) of the United Kingdom reviewed current knowledge on PAH deficiency (Medical Research Council, 1993), and a set of recommendations on the dietary management of PKU evolved from that review (Recommendations, 1993). The MRC report noted that the intellectual status of early-treated subjects was not as good as had been expected. Subtle but global intellectual impairments were to a substantial degree occurring in the preschool years and were closely linked with the character of Phe control in the preschool years and to a lesser extent in the pre-adolescent years. In addition, the performance of executive tasks appeared to depend on control of Phe. The appearance of neurological deterioration in older individuals with PKU and changes in myelin structure shown by magnetic resonance imaging (MRI) emphasized the need to appraise existing treatment protocols for PKU (Smith, Beasley, Ades, 1990; Smith, Beasley, Wolff, et al., 1988; Welsh, Pennington, Ozonoff, et al., 1990; Villasana, Butler, Williams, et al., 1989; Thompson, Smith, Brenton, et al., 1990; Thompson, Smith, Kendall, et al., 1991; Bick, Fahrendorf, Ludolph, et al., 1991).

Levels of Blood Phe at Which Treatment Is Initiated and Maintenance Levels During Treatment

Screening of neonates for PKU must be conducted soon after birth so that diagnosis and treatment can begin with a minimum of delay—certainly by 20 days of age. Diagnostic investigation should include an assessment of protein intake, quantitative measurement of plasma amino acids, and separation of infants with defective biopterin metabolism. All infants whose blood Phe concentrations exceed 600 µmol/L, and who have a normal or low plasma tyrosine and an otherwise normal plasma amino acid profile while receiving a normal protein intake (2 to 3g/kg/day), should receive a low Phe diet immediately. Infants whose blood Phe concentrations remain between 400 and 600 µmol/L for more than a few days should also be given dietary treatment. The diet should contain a protein substitute which is Phe-free (or at least very low in Phe) and otherwise nutritionally complete, with a composition sufficient to provide 100 to 120

mg/kg/day of tyrosine and an amino acid intake of at least 3g/kg/day in children younger than 2 years of age. In children 2 years of age and older, the intake of amino acids should be maintained at a level of 2g/kg/day. The protein substitute should be given as evenly as possible over a 24-hour period (Cockburn, Clark, 1996). If Phe concentrations exceed 900 µmol/L at the time of diagnosis, natural forms of milk should be excluded long enough to ensure a rapid fall in blood Phe concentration to below 600 µmol/L. Concentrations of Phe are likely to fall at a rate of 300 to 600 µmol/L/day. Daily monitoring of blood Phe should be conducted in order to ascertain individual protein requirements (usually between 60 and 110 mg/kg/day) and to prevent Phe deficiency. Phe readings made at a standard time (ideally, early morning, when concentrations are likely to be at their peak) should be conducted at least weekly. The aim is to keep Phe concentrations between 120 and 360 µmol/L. Biochemical monitoring should continue on a weekly basis up to at least 4 years of age. After 4 years of age, monitoring can be done every 2 weeks. Phe intake should be adjusted to produce therapeutic blood Phe levels.

The overall nutrient intake, body growth, feeding pattern, and general health of patients with PKU should be reviewed every 2 to 3 months in infancy, every 3 to 4 months up to school age, and every 6 months thereafter. In subjects with mild PKU, treatment should be withdrawn only if intake of natural protein reaches optimum requirements for their age while blood Phe concentrations remain below 400 µmol/L. Low protein diets and protein low tests are not recommended. Feeding strategies should aim to have children responsible for their own diet and the consequent blood test results by school age. The aim should be to maintain strict control of Phe levels as long as possible.

Age at Dietary Relaxation and Discontinuation

An upper limit of 480 µmol/L may be acceptable in children of school age. It becomes increasingly difficult to maintain strict control of Phe blood levels in older children, but every effort should be made to hold Phe concentrations no higher than 700 µmol/L. Adolescent and young adult patients should be made aware of the evidence that, even at that level of Phe concentration, the performance of decision-making tasks may improve if Phe levels are reduced.

It is currently recommended in the United Kingdom that treatment be for life. Adults and adolescents with PKU require continued delivery of services in an appropriate setting. This requires the involvement of physicians with a special interest in metabolic disease who are linked to regional pediatric services; adult services should include frequent monitoring and dietetic advice (by mail and telephone) and the availability of specialists for outpatient followup and inpatient care.

Female subjects require counselling about the need for very strict dietary control before conception. Those who conceive when Phe concentrations are 900 µmol/L or more should be offered termination of pregnancy because of the high risk of infant malformation. Hyperphenylalanine poses some risk to brain growth and intellectual development even at levels below 900, and offers of detailed fetal ultrasound assessment and possible termination should be extended to patients with concentrations of 700 µmol/L or more. Because of positive amino acid gradients across the placenta, the fetus is exposed to higher concentrations of Phe than the mother is (Cockburn, Farquhar, Forfar, et al., 1972). Monitoring should be undertaken twice

weekly, both in the period before conception and during pregnancy, aiming at values of 60 to 250 µmol/L. Effective contraception should be practiced until such control has been achieved.

Outcome of Long-Term Followup After Dietary Relaxation

Data from the German and U.S. collaborative studies, like United Kingdom studies of PKU, show that maintenance of lower plasma and blood Phe concentrations affects intelligence quotient (IQ) up to the ages of 8 to 10 years but probably not thereafter (Schmidt, Mahle, Michel, et al., 1987; Burgard, Bremer, Bhhrdel, et al., 1999; Holtzman, Welcher, Mellits, 1975; Smith, Beasley, Ades, 1991). Waisbren and colleagues have reviewed the neuropsychological functioning of treated phenylketonuric patients and found that impaired choice reaction times appear to be the only consistent finding in patients with greater concentrations of Phe in their blood (Waisbren, Brown, de Sonneville, et al., 1994). Specific executive function deficits can be demonstrated in younger patients with PKU (Welsh, Pennington, Ozonoff, et al., 1990; Diamond, 1994). In psychometric assessment of older treated patients with PKU, the results are in many ways ambiguous, but patients regularly give subjective reports of poor functioning during periods when their Phe concentrations are elevated (Griffiths, Paterson, Harvie, 1995; Weglage, Pietsch, Fünders, et al., 1996).

MRI scans have on occasion demonstrated the partial reversibility of an increase in Phe through the introduction of stricter dietary control (Cleary, Walter, Jenkins, et al., 1994). There is recent evidence that the genotypes of affected individuals might be useful in predicting the likelihood of intellectual changes in patients with hyperphenylalaninemia and PKU whose diet is relaxed after the age of 8 years (Greeves, Patterson, Carson, et al., 2000).

Deficiencies in Knowledge That Require Further Research

During the first 2 years after birth, the infant brain increases in weight from 350g to 1,200g. This growth is not caused by an increase in cell numbers but by formation of dendritic communication channels and myelination of neuronal axones. Inhibition of these processes, which are essential for early learning, can produce permanent deficits in adult brain function and could predispose to later degenerative disorders (Cockburn, 1999).

Feeding synthetic diets to normal infants during this critical period of brain growth increases the risk of an imbalanced supply of essential nutrients, and in infants with metabolic disorders like PKU, the risk is increased. There is evidence that “well-managed” children and infants with PKU have deficiencies of long chain polyunsaturated docosahexaenoic acid (DHA) (Galli, Agostoni, Masconi, et al., 1991; Sanjunjo, Perteagudo, Soriano, et al., 1994; Cockburn, Clark, Caine, et al., 1996). Deficient intake of DHA in the first weeks of life alters the fatty acid composition of the infant neuronal membrane phospholipids and, later, the visual and intellectual functions (Farquharson, Jamieson, Abbasi, et al., 1995; Birch, Garfield, Hoffman, et al., 2000).

Further research on the provision of a balanced nutrient intake during the critical early months is required not only for the fatty acids but also for other nutrients essential for neuronal growth and development. Growth of the fetal brain involves increases of cell numbers as well as cell and cell process migration, so maintenance of metabolic homeostasis and optimal nutrition

during pregnancy are essential. In women with PKU during pre-pregnancy and pregnancy, strict control of Phe and tyrosine values reduces the risk of intellectual impairment in the infant (Smith, Glossop, Beasley, 1990).

A low Phe diet cannot substitute for the fine-tuning of Phe turnover normally exerted by hepatic PAH. It is particularly difficult to maintain Phe control in subjects with severe enzyme deficiency, in whom even a minor illness or a fall in energy intake may lead to an increase in Phe concentrations. Aiming at “normal” concentrations runs the risk of inducing Phe and tyrosine deficiency, which several lines of evidence suggest is harmful to both growth and brain development. It is clear that better therapeutic strategies may be needed if we are substantially to improve outcomes in subjects with PKU. Given the difficulties in implementing treatment, the human and financial costs, and concerns about neurologic progress and fetal outcome, PKU is a potential candidate for gene therapy. But even if molecular genetics ultimately provides a better form of treatment, we still need to evaluate and, where possible, improve our present dietary management strategies.

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Recommendations of the German Working Group for Metabolic Diseases for Control of Phenylalanine in Phenylketonuria

Peter Burgard, Ph.D.

A working party of the German Working Group for Metabolic Diseases has evaluated patients with phenylketonuria (PKU) in terms of their intelligence quotient (IQ), education, neuropsychology, electroencephalography (EEG) results, magnetic resonance imaging (MRI) results, neurology, and behavior. All members of the working party were experienced in research on PKU and treatment of patients with PKU (Burgard, Bremer, Bührdel, et al., 1999).

Level of blood phenylalanine (Phe). Patients with Phe blood levels ≥600 µmol/L (10 mg/dL) on a normal diet must be treated with a low Phe diet, including a Phe-free protein or amino acid supplement. Phe blood levels should remain between 40 and 240 µmol/L (0.7–4 mg/dL) until the age of 10, not exceed 900 µmol/L (15 mg/dL) between ages 10 and 15, and not exceed 1200 µmol/L (20 mg/dL) after age 15.

Age at dietary relaxation. Patients ages 15 years and older with Phe levels ≤1200 µmol/L (20 mg/dL) may be allowed to discontinue a restricted diet. Patients who show overt impairments or symptoms related to PKU should continue to follow a strict dietary regimen. Diet should be monitored by regular examination at least once a year.

Psychometric intelligence and educational progress. Longitudinal data from the German and U.S. collaborative studies of PKU (CSPKU) and the British PKU Register reveal that high levels of Phe adversely influence IQ until age 10, but not later (Burgard, in press). The mean IQ of German patients with average levels of 270 µmol/L during the first 5 years of life and of 300 µmol/L (5 mg/dL) between ages 5 and 9 was no different from the mean IQ of a healthy control group matched for age, sex, and socioeconomic status. Patients with higher levels during their first 5 years showed lower IQ scores in their first IQ test at the age of 5, but showed no further deterioration. At the age of 12, type of schooling and number of repeated classes were normal for both groups. In a sample of 51 young adult patients with mean annual Phe levels of 250 µmol/L (4.1 mg/dL) up to the age of 10, IQ remained stable between adolescence and adulthood as levels increased to an average of 900 µmol (15 mg/dL) at the age of 20. School grades of patients were higher than those of their fathers (secular trend), and grade distribution was not significantly different from that of the entire German population (Schmidt, Burgard, Pietz, et al., 1996).

Neuropsychological results. Impaired choice reaction-time appeared to be the only consistent result in a review of 21 neuropsychologic studies (Waisbren, Brown, de Sonneville, et al., 1994). Reanalysis of studies with repeated measurements of low and high Phe levels revealed a mean change in reaction time of 111 milliseconds (ms) for mean level change of 966 µmol/L (Schmidt, Rupp, Burgard, et al., 1994). Phe level effects were reversible regardless of patient's age and amount of time without dietary treatment, and could be observed 1 week after

changing Phe blood levels. Compared with the reaction times of healthy controls, mean reaction times increased by 194 ms for children ages 10 years and younger (mean concurrent Phe level 558 µmol/L [9.2 mg/dL]), 82 ms for adolescents between ages 11 and 18 (mean concurrent Phe level 942 µmol/L [15.6 mg/dL]), and 159 ms for young adults between ages 17 and 25 (mean concurrent Phe level 1146 µmol/L [18.9 mg/dL]). After 5 to 10 years of strict treatment to maintain Phe levels below 400 µmol/L, diet relaxation does not result in long-term deterioration of reaction times (Burgard, Rey, Rupp, et al., 1997; Griffiths, Paterson, Harvie, 1995). The hypothesis of impaired executive functions presumably due to dopamine deficiency in the frontal lobes was investigated with children younger than age 6, adolescents, and young adults. Results for children were in line with IQ data and confirm the recommendation of therapeutic Phe levels below 360 µmol/L (6 mg/dL) during the first 5 years (Diamond, 1994; Welsh, Pennington, Oznoff, et al., 1990). Results for adolescents and adults were patchy (Griffiths, Paterson, Harvie, 1995; Weglage, Pietsch, Fünders, et al., 1996). A policy of minimizing risks may be justified by the fact that synaptic density in the frontal cortex reaches maturity at approximately age 16 (Huttenlocher, 1979).

Results of EEG, visually evoked potentials (VEPs), MRI, MR spectroscopy, and neurological tests. Abnormal EEG findings (general slowing, generalized paroxysmal activity with and without spikes) increase with age but were not regarded as crucial for decisions about treatment (Pietz, Benninger, Schmidt, et al., 1988). VEPs, significantly prolonged in about 30 percent of patients, did not correlate with age at start of treatment, parameters of biochemical control (Bick, Ullrich, Stöber, et al., 1993; Ludolph, Vetter, Ullrich, 1996; Pietz, Kreis, Schmidt, 1996), MRI results, or clinical abnormalities. Auditory-evoked potentials in early-treated patients were normal (Ludolph, Ullrich, Nedjat, et al., 1992). Abnormal MRI scans show partial reversibility after 2 to 3 months of continuous reduction of Phe below 360 to 900 µmol/L (6–15 mg/dL), regardless of the amount of time without treatment preceding the scan (Bick, Ullrich, Stöber, et al., 1993; Cleary, Walter, Jenkins, et al., 1994). MRI changes were not correlated with age at diet initiation or results of MR examination, nor with electrophysiological results, neurological deficits, psychiatric problems, or IQ. These changes probably reflect a reversible structural defect of myelin, rather than permanent demyelination. Overt neuropathology (para- and quadriparesis, tremor, ataxia, epilepsy) was found in a few young adults (Thompson, Smith, Brenton, et al., 1990). Most of these patients were treated late or lacked dietary control in infancy. Clinical examinations of 51 early and strictly treated adolescents and young adults showed no neurological abnormalities except for discrete resting tremor and slightly brisk tendon reflexes of the lower limbs, not associated with MRI grading or biochemical control (Pietz, Kreis, Schmidt, et al., 1996).

Behavioral problems. Psychiatric interviews of 60 patients in the German CSPKU at the age of 13 (mean Phe level from infancy to 13 years is 500 µmol/L [8.3 mg/dL]) showed an increase of mild behavioral or emotional disturbances (without need for psychiatric treatment) by a factor of 1.5 compared with the normative sample (Burgard, Armbruster, Schmidt, et al., 1994). Symptoms were not correlated with Phe levels, and no PKU-specific behavioral pattern could be delineated. Similar results were found in a study of adult PKU patients (Pietz, Fätkenheuer, Burgard, et al., 1997).

Non-PKU hyperphenylalaninemia. Untreated patients with Phe levels <600 µmol/L (10 mg/dL) were no different from their unaffected siblings with regard to MRI and IQ results, educational and professional progress, fine motor performance, and neuropsychological variables (Weglage, Schmidt, Fünders, et al., 1996; Weglage, Ullrich, Pietsch, et al., 1996).

Need for further research. Our present knowledge suggests that infancy and early childhood are the most vulnerable periods for most patients with PKU and therefore the most important periods for strict treatment to reduce abnormal Phe levels. However, the developmental sciences also regard old age as a stage of human development relying heavily on biological variables. It is not possible to know whether adverse effects in late adulthood and old age can be successfully prevented by treatment in childhood. Nor do we know whether statistically significant neuropsychological data on children are of clinical relevance—for example, as markers of late effects. Therefore, careful longitudinal followup is necessary to evaluate current as well as future treatment policies. Not all patients with the same genotype or Phe level have the same vulnerability to PKU and need the same kind of treatment (Weglage, Wiedermann, Möller, et al., 1998). The pathogenesis of PKU is still not very well understood, and a three-step model from genotype to Phe blood level to outcome is too simple (Pietz, Kreis, Schmidt, et al., 1996; Scriver, Waters, 1999).

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PKU Treatment in France

Véronique Abadie, M.D.

In France, screening for phenylketonuria (PKU) started in 1966. In 1978, the Association FranHaise pour le Dépistage et la Prévention des Handicaps de l'Enfant (AFDPHE) began systematic and coordinated screening and management of patients with phenylalanine hydroxylase (PAH) deficits or hypothyroidism. The association later added congenital adrenal hyperplasia and sickle cell disease to the list. The AFDPHE pays the financial costs of screening and of diet products for patients with PKU, handles technical questions regarding screening, and keeps tabs on children with PKU by means of a yearly questionnaire sent to physicians. The AFDPHE has a branch in each of the country's 20 administrative regions that is responsible for operation of a PKU screening laboratory.

Initially, neonatal screening was done by the Guthrie method. In 1996 we began using an enzymatic test (Quantase^{TM}) for screening, along with fluorimetry, to uncover patients with phenylalanine (Phe) levels exceeding 2.5 mg/dl (150 µmol/L). The PAH deficit phenotype is defined by the Phe level found in patients after they have received 500 mg a day of Phe for 4 days, with a tolerance for Phe levels of between 2 and 5 mg/dl (120-300 µmol/L). In the Paris region, all screened children are genotyped.

The PKU screening program expanded over the years, and by 1979 the program covered all infants born in France. Over the past 20 years the incidence of PKU has been quite stable, at 1 case for every 17,124 living births (44 new cases per year). Since PKU screening started, 11 children have been detected with a false negative test (sensitivity 99.1 percent), 1.7 percent had a cofactors deficiency, and 17 percent a non-PKU hyperphenylalaninemia (HPA) (<10 mg/dl, or 600 µmol/L on a normal diet). The proportion of moderate phenotypes varies from one region to another, being higher in the south than in the north of France. A precise proportion of moderate phenotypes is not available in the national database because phenotypic definition has varied over time. The age at which infant patients with PKU are placed on a diet has regularly decreased during the last 25 years, reaching a median of 14 days in 1996.

PKU Management During the First Years

Only children on a normal diet with a spontaneous Phe level of more than 10 mg/l are treated in our group. In a group of 202 children, 19 percent had a moderate phenotype (10 to 20 mg/dl on a normal diet and Phe tolerance >350 mg/J). The objective is to reduce the Phe plasma level to between 2 and 5 mg/dl. Testing for Phe levels is done weekly during the first 2 years, then fortnightly to the age of 5, then monthly until dietary restrictions are relaxed. Visits with a doctor, a dietician, and a psychologist occur monthly during the first year, then on a variable schedule, depending on Phe levels and family cooperation. Three times a year is the average during the strict diet period. Intellectual development is tested at 3 years of age (DQ, Brunet-Lésine), 6 years (WPPSI), 10 and 14 years (WISC), and 18 years (WAIS). One sibling undergoes an IQ test between 10 and 14 years of age. A nutritional assessment is performed every 2 years.

Age at Which Diet is Relaxed

Up until 1990, the standard approach was to relax the strict diet of patients with PKU at the age of 6. Since then, however, the standard has risen. The trend now is to leave children with PKU on a strict diet until they reach the age of 10. This change was based on international practice, especially in the United Kingdom (Smith, Beasley, Ades, 1991) and Germany (Schmidt, Mahle, Michel, et al., 1987). It was also based on national data showing that patients with PKU in France entered primary school at the same age (6 years) as the general population but entered secondary school at a later age (if born before 1979), compared with both children in the general population and children with PKU born after 1979. Children with PKU who entered secondary school without delay had been placed on a restricted diet at an earlier age (23.5 vs. 28.5 days) and had a higher age of diet discontinuation (8 vs. 6 years) compared with children with PKU who entered secondary school a year or more later than their peers in the general population.

Views on dietary treatment of adolescents and adults with PKU lack a consensus, but the majority of French investigators continue to think that there are more benefits than disadvantages to diet relaxation after 10 years of age. Phe levels are maintained at 20±2 mg/dl, respecting the RDA for protein intakes (1 g/kg/day for children from 10 to 18 years old, and 0.75 g/kg/day thereafter). This allows children with moderate PKU to have a normal diet and allows children with classical PKU to increase their natural protein intake in order to have a normal lunch at school, except for meat and fish. Hypoprotein products and amino acid substitutes are still taken by the majority of patients with classical PKU, depending on their Phe tolerance, age, protein requirements, and appetite.

Children with PKU are clinically investigated at least once a year, especially females, in part to provide them with warnings about the problems caused by maternal PKU. Phe levels are measured two to four times a year, and school performance, social behavior, neurological health, and IQ are watched so that a stricter diet can be reinstituted, if necessary.

Studies of the adverse effects of diet relaxation on IQ have failed to show any effect after the ages of 8 to 10 (Burgard, Bremer, Bührdel, et al., 1999). Moreover, in most studies arguing for prolongation of a restricted diet on the basis of IQ data, children who discontinue a strict diet early are also those who had the worst index of dietary control during the first years and those who had the lowest family IQ (Holtzman, Welcher, Mellits, 1975). In the Paris group, 31 children with classical PKU (who were treated for 5 years) had stable intellectual performance between 7 years of age and adulthood, either in mean or by pairs (IQ=102.6±16.2, 104.8±16 [WISC], and 101.8±14 [WAIS] at 7 to 8 years, 11 to 13 years, and 17 to 18 years, respectively).

The long-term effects of high Phe plasma levels are unknown. However, a comparison between French (relaxed diet) and German (strict diet) children showed that adverse effects of high Phe levels (20 mg) on neuropsychologic functions do not increase with time (Burgard, Rey, Rupp, et al., 1997). This study found lower reaction time in French children but decreasing differences with age between the two groups and no significant difference in reaction time in the adult groups. Similarly, an increase in the Phe level in children older than 10 years who were previously treated showed no midterm effects on neuropsychological tests, either after 3 months

(Griffiths, Ward, Harvie, et al., 1998) or 3 years (Weglage, Pietsch, Denecke, et al., 1999). The educational and professional levels achieved by French adults with PKU who were treated for 8 years are similar to those of the general population and are consonant with their family socioeconomic levels (Farriaux, Dhondt, Paux, et al., 1997). An inquiry is in progress comparing the educational background, quality of social integration, and personal autonomy of adults with PKU whose diet was relaxed early with those of matched friends.

The French view regarding management of adolescents with PKU differs from other views, for two reasons. First, for the French, meals have great social value—thus, to be on a diet is felt as quite regrettable. Second, it is socially less easy in France than in the United States for people to accept those with handicaps. Our aim in PKU treatment is to reach the best balance between long-term protection of the intellectual abilities of patients and giving them the feeling that they can lead a normal life.

In France, the cost of one Guthrie test is 10 francs or US$2. Thus, it costs around 150,000 francs (US$30,000) to discover one infant with PKU, if we consider that screening uncovers 50 children out of 750,000 births per year. The annual cost of the PKU diet for one child from 10 to 14 years of age and from 15 to 21 years of age is 200,000 francs (US$40,000) and 250,000 francs (US$50,000), respectively. In other words, 10 additional years of treatment for one PKU child costs the same price as screening to find 15 children. Or, to put it another way, screening all of the country's infants costs about the same as 10 more years of strict diet for fewer than four children with PKU.

In France, as in most developed countries, screening and management of children with PKU is a success. Ten years of close medical followup and strict diet allow satisfactory results. The long-term adverse effects of high Phe levels on cerebral functioning in adolescence and adulthood have to be evaluated more carefully and should be compared with the adverse nutritional and psychological effects of a prolonged strict diet. Our method of PKU treatment moderates the efficient but very constraining dietary treatment used in some other countries.

Other methods may be proposed in order to discover which adolescents with PKU are able to tolerate a higher-than-normal Phe plasma level. Measuring brain concentration of Phe may be a good tool for that purpose.

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Treatment Regimens and Their Effectiveness: A Meta-Analytic Review of the Literature

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Phenylketonuria (PKU) is a well-known genetic cause of mental retardation, affecting one in 10,000 to 20,000 live births (Stanbury, Wyndgaarden, Friedrickson, 1983). The disorder is the consequence of mutations in the gene that codes for the enzyme phenylalanine hydroxylase (PAH). This enzyme is essential for hydroxylation of dietary phenylalanine (Phe) to tyrosine in the liver (Güttler, Lou, 1986). Given normal intake of Phe, severe mental handicap results (Jervis, 1939; cited in Knox, 1972). For the past four decades, however, newborn screening programs have identified individuals with PKU (Knox, 1972) so that a diet low in Phe can be initiated early in life. If dietary treatment is begun early and controlled consistently during childhood, mental retardation is averted.

Even with early dietary treatment of PKU, research suggests that impairments in the intellectual, cognitive, and behavioral domains occur. For example, whereas early treatment appears to result in intellectual levels in the normal range, the intelligence quotient (IQ) scores of children with PKU often do not achieve the levels predicted from the IQ scores of unaffected parents and siblings (Williamson, Dobson, Koch, 1977). In addition, studies that have found that IQ declines with age during the school years suggest that such declines can be linked to elevated Phe caused by poor or absent dietary control (Waisbren, Schnell, Levy, 1980). Research in the 1970s and 1980s on the cognitive skills of early-treated children with PKU indicated a pattern of cognitive function characterized by intact speech and language skills but impaired visual-spatial, perceptual-motor, and problem-solving abilities (Welsh, Pennington, 2000). With regard to behavior, early-treated children do not present a consistent clinical profile; however, behavioral problems tend to cluster in the areas of hyperactivity, impulsivity, poor planning, and less task persistence (Welsh, Pennington, 2000).

Although the specific neuropathology of PKU has not been explicated fully, one hypothesis proposes that PKU produces a prefrontal cortex dysfunction (Chamove, Molinaro, 1978; Pennington, van Doorninck, McCabe, et al., 1985; Welsh, 1996), and several lines of evidence are consistent with this hypothesis. First, the biochemical alterations resulting from genetic mutation cause a disruption in catecholamine biosynthesis. One of these catecholamines, dopamine, is essential for prefrontal cortical function. There is empirical evidence of lower levels of central dopamine (McKean, 1972) and an inverse association between Phe levels and dopamine levels in individuals with PKU (Krause, Halminski, McDonald, et al., 1985). Impaired prefrontal cortical function due to diminished levels of dopamine is consistent with decades of cognitive and behavioral research demonstrating a particular profile of deficits in early-treated PKU. The profile includes lower nonverbal intelligence, impairments in novel problem-solving, and impulsive behavior lacking in planning and goal orientation. Moreover, studies designed to explore the prefrontal dysfunction model of PKU have found evidence of specific impairments in

a cognitive domain (executive function) that has been linked to this brain region (Diamond, Prevor, Callender, et al., 1997; Welsh, Pennington, Ozonoff, et al., 1990).

Our current understanding of the intellectual, cognitive, and behavioral characteristics of early-treated PKU, as well as the efficacy of diverse dietary regimens with regard to these characteristics, is the product of several qualitative reviews (e.g., Waisbren, Schnell, Levy, 1980; Welsh, Pennington, 2000). In what follows, an alternative systematic and quantitative approach to reviewing the empirical research on PKU will be described, and its potential value for making sense of the vast literature on early-treated PKU will be discussed.

The Meta-Analysis Approach

Glass (1976) developed and advocated an approach to research integration referred to as "meta-analysis." According to Glass, McGraw, and Smith (1981), "it is nothing more than the attitude of data analysis applied to quantitative summaries of individual experiments. By recording the properties of studies and their findings in quantitative terms, the meta-analysis of research invites one who would integrate numerous and diverse findings to apply the full power of statistical methods to the task. Thus, it is not a technique; rather, it is a perspective that uses many techniques of measurement and statistical analysis." Meta-analysis can also be viewed "as a more explicit approach to literature review, complementary to narrative review, not in opposition to it" (L'Abbe, Detsky, O'Rourke, 1987). Thus, meta-analysis does not exclude the need for traditional reviews. Rather, it should be viewed as a statistical tool to summarize research findings.

At a time of great proliferation in published scientific research, meta-analyses are a vital necessity. It has been estimated that general practice physicians would need to read 19 journal articles a day, 365 days a year, just to keep their knowledge current (Anonymous, 1995). Given this large volume of research to be assimilated, the narrative method of research review—that is, studies chronologically and/or categorically arranged and described—is inadequate by itself to summarize this accumulated research.

Meta-Analysis of PKU Outcome Research

In other words, qualitative and quantitative reviews of the literature are complementary approaches to addressing research questions in a comprehensive and coherent way. The following characteristics of the empirical literature on early-treated PKU provide a particularly compelling argument for the use of meta-analysis:

- **Number of articles.** Thousands of articles have been published in the past few decades on neuropsychological sequelae and molecular genetics. Typically, these studies present small sample sizes, heterogeneous samples, small effect sizes, and large variability in outcome. Each of these characteristics reduces the statistical power of an individual study (i.e., reduces the possibility of statistically significant results). However, when many "low power" studies are combined and incorporated into a single analysis or small set of analyses, statistical power is substantially increased.

- **Operational definitions.** There is a wide variety of operational definitions of both the "independent variable" (i.e., treatment) and the dependent variables (e.g., intelligence, executive function, behavior). Meta-analysis requires the analyst to carefully and explicitly code each study with regard to the operational definitions of these variables. Studies then can be appropriately grouped for statistical analysis.

The meta-analytic approach to the literature typically involves five stages: (1) formulation of the questions to be answered, or specific aims; (2) execution of a complete literature search; (3) collection, classification, and coding of studies meeting inclusion criteria; (4) application of statistical techniques for pooling and analyzing the compiled data; and (5) evaluation and interpretation of the results in a written report.

The meta-analytic approach to the literature on PKU for the Consensus Development Conference on PKU has two stages. Regarding stage one, the specific questions to be addressed are as follows:

- What is the effect of treatment on cognitive and behavioral outcomes?
- What is known about the effects of age at time of diet discontinuation on outcome?
- What is known about the reversibility of clinical symptoms upon reinstitution of treatment at different ages?
- What is known about the relationship of blood levels of Phe and tyrosine to cognition and behavioral outcomes at different ages?

For the purposes of this report, we have restricted our analysis to the following:

- A bibliography compiled by the National Library of Medicine (NLM).
- Studies of IQ, cognitive processes, neuropsychological sequelae, executive function, and behavior.
- Studies that report sufficient data for the dependent variable so that a standardized difference effect size can be calculated (means, standard deviations, correlations, exact *p* values, etc.).
- Studies that report a correlation between some index of treatment (e.g., Phe level) and outcome (e.g., IQ).

Screening of the 2,346 abstracts listed in the NLM bibliography is under way. It is estimated that approximately 200 of them will meet inclusion criteria. Our objective is to apply meta-analytic methodology to summarize what is known and, when possible, to arrive at clear, definitive answers to issues of intellectual, cognitive, and neuropsychological performance in individuals with early-treated PKU. The relation of Phe level, dietary control, dietary termination, and dietary reinstitution to behavioral outcomes will also be evaluated.

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Psychosocial Factors and Dietary Adherence

Susan E. Waisbren, Ph.D.

Adherence to medical recommendations in maternal phenylketonuria (PKU) ought to be a straightforward process. Women considering pregnancy need only initiate the diet, monitor their blood phenylalanine (Phe) levels, adjust their Phe intake accordingly, and when their metabolic control is adequate, stop birth control and conceive. In reality, the level of adherence to medical recommendations in maternal PKU defies our worst predictions and fears. Naïve assumptions about human behavior lead us to believe that people, if given proper information, will do what needs to be done in order to protect their future children.

In maternal PKU, and probably in PKU as well, models for understanding acceptance or avoidance of positive health behaviors apply. The Theory of Reasoned Action (Ajzen, Fishbein, 1980) postulates that intentions to act are a function of "subjective norms" (expectations by important others) and "attitudes toward the behavior." An extension of this theory, the Theory of Planned Behavior, adds a third dimension, "manageability" (Ajzen, 1988; Ajzen, 1991). Accordingly, individuals with PKU will be more likely to adhere to medical recommendations and follow the Phe-restricted diet if (1) there is social support for them to adhere to medical recommendations and follow the diet; (2) they have positive attitudes about the diet and believe in its efficacy; and (3) the treatment is manageable in their daily lives.

Fifteen years ago, we initiated a psychosocial study of maternal PKU. We developed a model of psychosocial development in which each stage in the maternal PKU life cycle is associated with a particular treatment goal. The stages include (1) consistent birth control use to prevent unplanned pregnancies, (2) formation of a reproductive decision, (3) diet initiation for pregnancy, and (4) diet continuation during pregnancy. Sixty-nine women with PKU, 68 of their acquaintances, and 69 women with diabetes were interviewed annually for 5 years. For the women with PKU, social support and attitudes were significantly associated with adherence to medical recommendations at the stages of prevention of unplanned pregnancy, treatment initiation, and diet continuation throughout pregnancy (Waisbren, Shiloh, St. James, et al., 1991; Waisbren, Hamilton, St. James, et al., 1995). Interventions that focus on improving social support networks and attitudes toward treatment may increase adherence to medical recommendations.

Thereafter, we established the PKU Community Outreach Resource Programs (PKU CORPS) to develop specific interventions for women with PKU. For 5 years, a maternal PKU camp provided education and social support for young women with PKU. The curriculum combined educational and recreational activities aimed at instilling social support and enhancing positive attitudes regarding treatment for maternal PKU. Subjects for a study conducted during the fifth camp year included 25 young women, ages 11 to 32 years. The mean blood Phe concentrations of the subjects were significantly reduced by 37 percent by the end of camp, with 96 percent (24 of 25) of the campers lowering their levels. Forty-eight percent of the campers had concentrations below 10 mg/dL at the end of camp, compared with 12 percent on the first day of camp. There were significant increases in scores on tests of knowledge of maternal PKU

and the maternal PKU diet. There was a significant increase in the campers' ratings of the degree to which they perceived other people wanting them to use birth control if they were sexually active. Returning campers had a significantly greater increase in their social support networks after camp than did new campers. On followup, the most recent blood Phe determinations reflected a 20 percent reduction in precamp levels. One woman attained metabolic control before becoming pregnant following the end of the camp. As much as 96 percent of the campers were still in contact with at least one other camper, with the mean number of contacts being 7.84 (Waisbren, Rokni, Bailey, et al., 1997).

The Maternal PKU Resource Mothers Program was a second social support program sponsored by the PKU CORPS. Resource Mothers are mothers of children with PKU. Since these women have children with PKU, they are familiar with the diet, the methods of calculating Phe in food, and the stresses and strains such restrictions produce on a family. The Resource Mothers explain the issues of maternal PKU, help pregnant women obtain prenatal care, purchase appropriate foods or pregnancy-related items, plan and cook meals, and arrange transportation to doctor appointments. They provide emotional support and maintain communication with the metabolic center. They are paid a stipend for time spent in training and supervision and for their visits to the young women.

A recently completed nonrandomized study of the Resource Mothers Program (St. James, Shapiro, Waisbren, 1999) compared 19 pregnancies in women with PKU who received the services of a Resource Mother with pregnancies in 66 women with PKU, enrolled in the Maternal PKU Collaborative Study (MPKUCS), who did not have a Resource Mother. The results indicated that the pregnant women who had Resource Mothers achieved metabolic control at a mean of 8.5 weeks, compared with a mean of 16.1 weeks for those in the comparison group. At 1 year of age, infants whose mothers had a Resource Mother had a mean developmental quotient (DQ) of 108, and the mean DQ of the control group was 95.

Although the study suggests promising results from this type of home visitation program, a number of factors may have confounded the results. Only the women in the Resource Mothers group had been enrolled in a previous study in which they had annual visits from professionals, and many had also attended the maternal PKU camp. Data from the comparison group were retrospective and had been obtained 5 to 10 years previously, when recommendations for dietary control were not as strict. Finally, assignment to groups was not randomized.

Currently, a randomized clinical trial of the Resource Mothers Program is under way in six metabolic centers. More than 100 pregnancies will be randomly assigned to the group with or without Resource Mothers. The most recent MPKUCS analyses document the continued problem of late-treated pregnancies. The MPKUCS also found, however, that a stimulating home environment during the first year of the offspring's life may attenuate the adverse effects of maternal PKU (Waisbren, Chang, Levy, et al., 1998). Thus, a home visitation program only during pregnancy, the original plan for the Resource Mothers Program, is unlikely to be optimally effective. Accordingly, the current project now includes outreach before pregnancy to all adolescent girls and young women tracked by the metabolic centers and to all families after a baby is born. Only those randomized to the treatment group receive the services of a Resource Mother during pregnancy. The research question is whether the Resource Mothers Program

yields benefits beyond what are provided by basic metabolic services, as well as outreach before and after pregnancy.

Adolescent females will be targeted for health education outreach programs. Information about maternal PKU will be provided to the patients tracked by each clinic and to their primary care physicians. School-based health centers will be contacted about the needs of young women with PKU and the risks of maternal PKU. A Web site is being established. Public discussions are under way with officials at the Department of Public Health in Massachusetts regarding the possibility of having maternal PKU listed as a risk factor on the confidential section of birth certificates. This would trigger followup from the Department of Public Health and automatically qualify the child for early intervention.

Adherence to medical recommendations in maternal PKU no longer appears to be a straightforward process. A comprehensive system of care that includes programs to enhance social support, engender positive attitudes, and increase the manageability of treatment requires integrated services and adequate financial support. At this time, with the conclusion of the MPKUCS, the infrastructure for maternal PKU treatment is at best precarious, and the will to set aside funds for broadly focused programs may be lacking. Research must focus on continuity of care, because no single program or intervention will address the multitude of challenges facing women with PKU.

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Maternal PKU: Restarting and Monitoring Diet Before and During Pregnancy

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Discontinuation of the phenylalanine (Phe)-restricted diet at the age of 6 was standard policy for patients with phenylketonuria (PKU) for many years before the National Collaborative Study for PKU began an investigation of whether that was the right approach (Williamson, Dobson, Koch, 1977). A summary report of the investigation (Holtzman, Kronmal, van Doornick, et al., 1986) then indicated that high blood Phe levels had negative effects on intelligence quotient (IQ) and learning and that the restricted diet should be continued beyond age 6. There was no specific recommendation, however, on how long the restricted diet should be continued.

A confounding issue was that when females with PKU enter the reproductive age, the fetuses become vulnerable to the effects of high blood Phe concentrations (Lenke, Levy, 1980). Reports of improved pregnancy outcomes with a low Phe diet led to new studies by Michals, Dominik, Schuett, and colleagues (1985) and Schuett and colleagues (1985) on the benefits of diet therapy. These studies were undertaken because of reports of declines in IQ and academic performance, behavior disorders, electroencephalograph (EEG) abnormalities, tremors, and eczema among children of mothers with PKU. In one study, less than half of the patients (18 of 44) were able to achieve a mean reduction of blood Phe from 29.3 mg/dL to 15 mg/dL through diet control. The studies highlighted the difficulties of patients with PKU in resuming a Phe-restricted diet and reaching acceptable levels of blood Phe (2 to 6 mg/dL).

The International Collaborative Study of Maternal PKU (MPKU) was started in 1984 to test whether restrictions on Phe intake and lowering of blood Phe would result in better pregnancy outcomes (Koch, Hanley, Levy, et al., in press). Most of the women enrolled in the study had previously discontinued the Phe-restricted diet. There were 574 pregnancies in 382 women with hyperphenylalaninemia (HPA) and 412 live births. Diet aimed at lowering blood Phe levels to <6 mg/dL was initiated before conception in 35.6 percent of the pregnancies, but only 15.9 percent of the women achieved blood Phe levels of <10 mg/dL by 10 weeks gestation. The restricted diet was initiated in the first trimester in 50 percent of the pregnancies, but only 18.4 percent of these women had blood Phe levels of <10 mg/dL by 10 weeks gestation. The remaining women started diet control in the second or third trimesters of pregnancy. The majority of subjects in this study attained diet control of blood Phe <10 mg/dL after 10 weeks gestation. Therefore, 50 percent of the fetuses were exposed to high blood Phe levels during the critical period of organ development, especially the heart.

Dietary intake of protein and maternal weight gain were found to be positively related to offspring birth measurements (Acosta, Michals-Matalon, Austin, et al., 1997; Michals, Acosta, Austin, et al., 1996; Rohr, Matalon, Acosta, et al., 1997). Furthermore, birth measurements were found to be related to the length of time required to lower the mother's blood Phe concentration to <10 mg/dL. The longer the Phe was elevated, the smaller the birth measurements. Hence, treatment guidelines were changed to favor rapid reduction of blood Phe concentrations during

pregnancy (Acosta, Austin, Castiglioni, et al., 1992; Michals-Matalon, Acosta, Castiglioni, et al., 1998).

Other investigators have also reported on the importance of dietary protein in the treatment of PKU. Pietz and colleagues (1999) studied the role of amino acids in the PKU diet and found that it was important for the diet to have adequate amounts of large neutral amino acids (LNAA), which share the same transporter to the brain as Phe.

Women in the MPKU study who had high levels of blood Phe and lower rates of congenital heart disease were found to have improved dietary intake of nutrients, especially B_{12} and protein (Michals-Matalon, Matalon, Acosta, et al., 1999). Poor weight gain and inadequate intake of dietary fat were found to be predictive of microcephaly. In studies unrelated to PKU, intake of multivitamins before pregnancy has been associated with reduced risk for conotruncal heart defects (Botto, Khoury, Mulinare, et al., 1996; Czeizel, 1993), and reduced dietary intake of folate has been related to increased congenital heart disease (Scanlon, Ferencz, Loffredo, et al., 1998). Thus, adequate intake of vitamins and other nutrients needs to be considered in treating PKU during pregnancy.

The Question of Breastfeeding

Health professionals and women with PKU frequently ask whether women with PKU should nurse their babies. Although breast milk is the most appropriate food for infants, data regarding Phe content of breast milk in women with PKU and the effect on offspring blood Phe levels is limited (Fisch, Jenness, Doeden, et al., 1967). The amino acid content in breast milk is fixed. However, the amount of free amino acids present in breast milk can vary, depending on the mother's blood Phe level. We studied free amino acids in the breast milk of two women with PKU and found differences in breast milk Phe content that reflected differences in blood Phe levels (Matalon, Michals, Gleason, et al., 1986; Sullivan, Goss, McMaster, et al., 1992). Fox-Bacon and colleagues (1997) measured total Phe in the breast milk of two sisters with PKU and elevated blood Phe levels, and compared them with blood Phe levels in controls and offspring. Although the Phe content of the breast milk was greater for the sisters than the controls, there was no significant effect on the infants' blood Phe levels. The limited data seem to indicate that breastfeeding is safe for the infants of women with PKU.

The data from the MPKU study indicate that continued followup and treatment of PKU is needed. Long-term dietary treatment of PKU should lead to better control of blood Phe and safeguard against deficiency of nutrients.

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Maternal PKU: Pregnancy and Child Outcomes in Relation to Dietary Control

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The effects of blood phenylalanine (Phe) on the offspring of women with phenylketonuria (PKU) were recognized in the 1960s (Mabry, Denniston, Nelson, et al., 1963; Richards, 1964; Denniston, 1963; Mabry, Denniston, Coldwell, 1966; Fisch, Doeden, Lansky, et al., 1969; Stevenson, Huntley, 1967). These effects include mental retardation, cardiac defects, low birthweight, and spontaneous abortions. Lipson and colleagues (1984) showed that dysmorphic facial features were associated with the maternal PKU syndrome. Interest in the prevention of the effects of high blood Phe on offspring was prompted by the survey of Lenke and Levy (1980). The effect of high blood Phe during pregnancy raised the possibility of rebound frequency of mental retardation from PKU (Kirkman, 1982). Therefore, most metabolic clinics initiated low Phe treatment in pregnant women with PKU (Fisch, Matalon, Weisberg, et al., 1997). Treatment regimens, however, did not follow uniform guidelines about diet composition and blood levels of Phe.

Maternal PKU Collaborative Study

In 1984, a national Maternal PKU Collaborative Study was started in this country, with Canada and Germany joining subsequently. There were 574 pregnancies among 382 women with hyperphenylalaninemia (HPA), resulting in 412 live births (Koch, Hanley, Levy, et al., in press). The 350 pregnancies treated with a low Phe diet included 255 women with classical PKU, 62 with atypical PKU, and 33 with natural blood Phe of 6 to 10 mg/dL. There were 62 pregnancies with natural blood Phe of 2 to 6 mg/dL.

Offspring growth measurements were related to the length of time required to lower the blood Phe concentration to <10 mg/dL (Matalon, Michals, Azen, et al., 1991; Matalon, Michals, 1991; Matalon, Michals, Azen, et al., 1994). The longer the blood Phe was elevated, the smaller the birth measurements (Koch, Levy, Matalon, et al., 1993; Koch, Hanley, Levy, et al., in press). Other factors related to blood Phe and pregnancy outcome were the time when diet treatment began, the time when blood Phe control was achieved, mother's intelligence quotient (IQ), weight gain, protein level, and energy intake (Matalon, Michals, Azen, et al., 1991; Michals, Acosta, Austin, et al., 1996; Waisbren, Hanley, Levy, et al., 2000).

Microcephaly and Developmental Outcomes

Growth parameters and psychological functioning were studied in a subgroup of 275 offspring from the Maternal PKU Collaborative Study. Growth measurements were obtained at birth and 2 years of age, and each child had Bayley developmental testing. The subjects were grouped by the time when blood Phe reached <10 mg/dL; 45 women achieved diet control before conception, 50 before 10 weeks gestation, 72 by 10 to 20 weeks, and 108 after 20 weeks or

never. In addition, 45 offspring were born to women with mild untreated HPA, and 61 offspring were born to control women.

Of the 275 treated offspring, 53 (19 percent) were microcephalic at birth. The birth head circumference (HC) Z-score was positively correlated to the Bayley mental developmental index (MDI) and psychomotor developmental index (PDI) (p=0.001). At 2 years of age, there was an increase in the number of microcephalic infants to 101 (37 percent). In the untreated HPA and control groups, there were three (2.8 percent) microcephalic infants at birth and five (4.7 percent) at 2 years of age. The McNemars test showed a significant increase in microcephaly at 2 years of age in groups where blood Phe control was reached before 10 weeks gestation (p<0.05), 10 to 20 weeks (p<0.001), and more than 20 weeks (p<0.001). The group that did not have microcephaly at birth or at 2 years of age (n=177) had the highest mean Bayley score, 104$\pm$20, compared with the other groups. The group that was microcephalic at birth and remained microcephalic at 2 years of age (n=35) had the lowest mean Bayley MDI, 71$\pm$22 (p=0.0001). Ten children who were microcephalic at birth but had normal HC at 2 years of age had an MDI of 93$\pm$29 (p=0.0036). The group with a normal HC at birth who became microcephalic at 2 years of age (n=48) had a Bayley MDI of 88$\pm$21 (p=0.0001).

The fact that there were an increased number of offspring with microcephaly at 2 years of age suggests that the effect of Phe on brain growth is carried over even after birth (Matalon, Michals, Azen, et al., 1994; Matalon, Michals-Matalon, Azen, 1997; Matalon, Michals-Matalon, Azen, et al., 1998). Repeated growth measurements are important. The size of the head and the Bayley development scores were related. Head growth is an important criterion that is affected by high blood Phe levels.

In addition to head size, blood Phe levels during pregnancy affect the facial features of the developing fetus. These facial features, which are similar to the dysmorphology seen in fetal alcohol syndrome, are usually associated with microcephaly (Rouse, Lockhart, Matalon, et al., 1990). The higher the Phe levels during the pregnancy, the more pronounced the dysmorphic features.

WISC IQ and Blood Phe Levels

The scores of offspring on the Wechsler Intelligence Scale for Children-Revised (WISC-R) were compared between mothers who had blood Phe control before conception and those with control at 0 to10 weeks of gestation. The level of control was grouped as blood Phe <6 mg/dL or 6 to 10 mg/dL. There were 13 children with WISC-R full-scale (FS) IQ of 109 where blood Phe control was <6 mg/dL, and 11 children with IQ of 95 where blood Phe control was 6 to 10 mg/dL. In six children where blood Phe control of <6 mg/dL was achieved between 1 to 10 weeks gestation, the IQ was 99. In 15 children where blood Phe control was 6 to 10 mg/dL, the IQ was 95. In 16 children of women with untreated HPA with blood Phe control of <6 mg/dL, the IQ was 97. In four children where blood Phe control was 6 to10 mg/dL, the IQ was 107. In 67 control children, the IQ was 109. These data suggest that the level of blood Phe that affects brain development and head size is reflected in IQ (Koch, Hanley, Levy, et al., in press).

Congenital Heart Disease (CHD)

The Maternal PKU Collaborative Study found 31 (7.5 percent) infants with CHD (Rouse, Matalon, Koch, et al., 2000). There was no CHD in the group of women who achieved blood Phe control of 2 to 6 mg/dL by 8 weeks gestation. Heart development is usually completed by 10 to 12 weeks of pregnancy. The most likely etiology is related to the elevated blood Phe. However, since not all offspring develop CHD due to a certain increase in blood Phe, other factors may be involved.

Michals-Matalon and colleagues (1999) have found that nutritional components other than high blood Phe are associated with CHD. All of the women who had infants with CHD had blood Phe concentrations $\geq$10 mg/dL during 0 to 8 weeks gestation, a critical time for heart development. A significantly greater percentage of offspring (30.5 percent) of women who had low protein intake (<50 percent RDA) had CHD, compared with women who had higher protein intake (8.3 percent) (p<0.0013). The low protein intake is also related to low intake of vitamins folate and B_{12}.

Summary

The effect of high blood Phe levels on brain growth, CHD, and IQ are still challenging problems. The data indicate that blood Phe levels need to be below 6 mg/dL for optimal pregnancy outcome. This level is not easily achieved, and education toward this goal needs to be a continued process in the treatment of PKU. Continued dietary treatment for individuals with PKU should improve the level of compliance and pregnancy outcome.

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Alternative Dietary Treatments

Joachim Pietz, M.D.

Dietary treatment of phenylketonuria (PKU) places stringent demands on patients. Some patients are unable to meet these demands early in life and subsequently exhibit poor outcomes. High levels of cerebral phenylalanine (Phe) can also cause acute but most likely reversible brain dysfunction in older and normally developed patients with PKU (Krause, Epstein, Averbook, et al., 1986; Krause, Halminski, McDonald, et al., 1985; Leuzzi, Fois, Carducci, et al., 1997). Consequently, attempts have been made to find alternative treatments for young noncompliant patients and for adolescents and adults unable to adhere to a strict diet. Three approaches have been used: (1) to overcome suspected depletion of cerebral amine neurotransmitters, patients with PKU off diet have been treated with tyrosine (Tyr), L-dopa, and tryptophan (Trp), both individually and in combination; (2) the branched-chain amino acids valine, isoleucine, and leucine (VIL), and a mixture of all large neutral amino acids (LNAA), have been administered to reduce Phe influx into the brain; and (3) ammonia lyase capsules have been developed to reduce uptake of Phe from the gastrointestinal tract.

Use of neurotransmitter precursors. In vitro and animal studies, as well as investigation of specimens (plasma, urine, and cerebrospinal fluid [CSF]) from patients with PKU, have shown that increased Phe interferes with amine neurotransmitter synthesis. Because an increase in Phe has led to decreases in plasma L-dopa in older patients with PKU, it was speculated that Phe-related brain dysfunction may be caused by disturbed dopamine synthesis (Krause, Epstein, Averbrook, et al., 1986; Krause, Halminski, McDonald, et al., 1985). Dopamine depletion and the resulting dysfunction of the prefrontal cortex then became a focus of research. Lou (1985) first reported that supplementation of a free diet with high doses of both Tyr and Trp caused normalization of amine neurotransmitter metabolites in CSF and improvement in performance of a reaction-time task in two patients with PKU. Tyr alone was used in two studies and also seemed to improve cognitive function (Lou, Lykkelund, Gerdes, et al., 1987; Lykkelund, Nielson, Lou, et al., 1988). Trp supplementation alone normalized serotonin metabolites in CSF, but cognitive function was not investigated (Lykkelund, Nielson, Lou, et al., 1988).

These results were said to confirm the theoretical framework of disturbed neurotransmitter synthesis and its effects on brain function in PKU. However, two placebo-controlled studies failed to confirm the results (Pietz, Landwehr, Kutscha, et al., 1995; Smith, Hanley, Clarke, et al., 1998). Although both of the latter studies demonstrated impairment of brain function in adult patients with PKU off a strict diet, no change was observed during high-dose Tyr treatment. In another study, L-dopa was used and failed to demonstrate beneficial effects on VEP (visually evoked potentials) changes (Ullrich, Weglage, Oberwittler, et al., 1994).

Several points must be made about these various studies. First, the two studies with positive results apparently included the same patients and therefore do not confirm each other. Second, the samples were heterogeneous, with approximately 50 percent neurologically impaired and late-treated patients. Third, improvement in brain function was confirmed for only one

parameter, the 90 percentile values from a reaction-time task. The clinical relevance of this parameter remains unclear.

Other explanations for the discrepant results of the studies should be mentioned. First, low concentrations of neurotransmitters in plasma, urine, and CSF may be misleading indicators of intracellular concentrations in the central nervous system (CNS) neuron. Second, low neurotransmitters may be clinically insignificant and show only a relative depletion that may be counterbalanced by autoregulative processes—for example, up-regulation of receptor density or activity. Third, it may be that blockage of the rate-limiting enzyme Tyr hydroxylase (Tyr to L-dopa) is the relevant mechanism, and elevation of substrate Tyr could therefore have no effect. This hypothesis, however, is contradicted by confirmation of increased CSF neurotransmitters during Tyr treatment. An early attempt at high-dose Tyr supplementation in a young child failed to prevent brain damage (Batshaw, Valle, Bessman, 1981). It therefore is not likely that dopamine depletion plays a significant role in the etiology of permanent brain damage in untreated PKU.

In summary, the clinical relevance of impaired neurotransmitter synthesis in PKU is not clear. Although treatment with Tyr and Trp has been shown to influence neurotransmitter synthesis, clinically relevant improvements of brain function following Tyr, Trp, or L-dopa treatment have not been confirmed, and high-dose Tyr, Trp, and L-dopa treatment cannot be recommended.

Attempts to block Phe influx through the blood-brain barrier. LNAAs, including Phe, compete for transport across the blood-brain barrier via the L-type amino acid carrier. Net uptake through the blood-brain barrier is determined by their ratio in plasma and differing affinity to the carrier system. Carrier saturation and inhibition of blood-brain barrier transport can be expected at the levels of supraphysiological plasma Phe usually found in PKU and may even be present at concentrations in the range of 200-500 mol/L (Pardridge, 1998). The direct effects of elevated brain Phe and depleted LNAAs are probably major causes of disturbed brain development and function in PKU. Competition for the carrier therefore might be put to use to lower the influx of Phe by increasing plasma concentrations of other LNAAs.

Branched-chain amino acids: valine, leucine, isoleucin (VIL). Oral VIL treatment was designed to inhibit influx of Phe into the brain. Although plasma Phe remained unchanged during VIL intake, a decrease of approximately 20 percent in concentration in CSF confirmed the hypothesis (Berry, Bofinger, Hunt, et al., 1982), which was corroborated by concomitant, moderate improvements in neuropsychological performance (Berry, Brunner, Hunt, et al., 1990). CSF Tyr concentrations were lowered during VIL treatment, and administration of VIL did not produce any relevant side effects. Leuzzi and colleagues (1997) recently reported beneficial effects of VIL treatment on neuropsychological performance during high Phe intake, with no MRI-visible changes in white matter. It remains unclear, however, why these three branched-chain amino acids were chosen and other LNAAs were neglected. The rate of protein synthesis depends on a supply of all essential amino acids, and an increase in only three (i.e., VIL) may accentuate disturbances of neurotransmitter or protein synthesis.

In summary, VIL treatment was able to lower CSF concentrations of Phe, and effects on brain function were moderate, but VIL therapy cannot be recommended on the basis of the results of published studies.

Administration of LNAAs. Since earlier studies showed that cerebral Phe can be measured noninvasively by proton MR spectroscopy (^{1}H-MRS), ^{1}H-MRS was used in a recent study which investigated Phe transport through the blood-brain barrier in patients with PKU while blood concentrations of Phe and LNAAs (valine, methionine, isoleucine, leucine, Tyr, histidine, and Trp) were manupulated during two series of amino acid loading experiments (Pietz, Kreis, Rupp, et al., 1999). Brain activity was monitored by EEG spectral analysis. Baseline plasma Phe was approximately 1,000 µmol/L, and brain Phe was approximately 250 µmol/L in both series. Without LNAA supplementation, brain Phe increased to approximately 400 µmol/L after oral Phe loading, and EEG spectral analysis revealed acute disturbances in brain activity. With concurrent LNAA supplementation, Phe influx from blood into brain tissue was completely blocked, and there was no slowing of brain activity. These results could become significant for treatment of patients with PKU. Further studies are warranted to find out whether brain Phe concentration can be lowered during steady state and brain function improved by long-term LNAA supplementation.

Use of ammonia lyase. Phenylalanine ammonia lyase (EC 4.3.1.5) can now be produced through a recombinant approach that degrades Phe to a harmless metabolite (Sarkissian, Shao, Blain, et al., 1999). Attempts have been made to use this enzyme as a substitute for phenylalanine hydroxylase (PAH). Tests in animal models of PKU have shown that ammonia lyase lowered blood levels of Phe when the enzyme was injected or administered orally in order to degrade Phe in the gastrointestinal tract. In the only report of a test using humans (Ambrus, Anthone, Horvath, et al., 1987), ammonia lyase was used to lower the Phe blood level in one patient with PKU by means of a hemodialysis-like procedure. Because of its high invasiveness, this procedure cannot be recommended as a routine treatment for PKU.

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Perspectives on the Future of Gene Therapy

Savio L.C. Woo, Ph.D.

Gene therapy is the use of genes as medicines for the purpose of preventing the occurrence of disease or for altering the clinical course of an existing disease. Over the past decade, dramatic progress has been made by many investigators in the field to develop and refine technologies used to deliver genes into various cells and organs in living animals, including humans. In several instances, significant treatment benefits achieved in laboratory animal models of human disease have been observed in recent clinical studies as well. An example is hemophilia B, which is caused by a deficiency of clotting factor IX in the blood. Normal blood clotting times have been restored for extended periods of time after a single application of the gene in genetically affected mice and dogs. Very encouraging results have also been reported in patients during early-phase clinical studies after intramuscular delivery of a recombinant adeno-associated virus expressing the human factor IX gene. Another example is X-linked severe combined immunodeficiency syndrome secondary to a deficiency of the gamma chain of cytokine receptors on T-cells. Autologous transplantation of CD34+ cells transduced with a recombinant retroviral vector expressing the normal human gene has resulted in the reconstitution of T-cell counts and immune functions in several affected children for up to 1 year. These achievements resulted from recent technological advancements and will lead not only to extensive application in the treatment of patients affected with relatively rare inherited disorders, such as phenylketonuria (PKU), but also to the future treatment of complex and acquired disorders, such as cardiovascular diseases, cancers, diabetes, obesity, infectious diseases, and neurodegenerative disorders, which represent the leading causes of mortality and morbidity in developed countries. The most notable recent accomplishments in these areas include, but are not limited to, the treatment of patients with ischemic limbs by the administration of an angiogenic gene that stimulates blood vessel growth, and the destruction of tumors in patients by the administration of suicide and immunomodulatory genes that specifically destroy cancer cells. Although the gene treatments for these complex disease targets are only partially effective at present, future advancements in technologies for the delivery of novel genetic medicines promise to result in much-improved clinical benefits for these and other human diseases. It is anticipated that the scientific principles of gene therapy as a new biomedical discipline will be further validated in the coming years and decades. The future widespread application of gene therapy in the treatment of various human diseases will have a major impact on the practice of medicine, health, and health care delivery in this century.

Lifespan Perspectives

Keith F. Widaman, Ph.D.

The International Collaborative Study of Maternal Phenylketonuria (ICSMPKU) was designed to monitor and treat the pregnancies of women with phenylketonuria (PKU). Early research on untreated pregnancies of women with PKU documented an array of negative fetal outcomes, including mental retardation, microcephaly, congenital heart disease, low birthweight, and spontaneous abortion (Lenke, Levy, 1980). Each of these outcomes tended to show a dose-response curve, with higher levels of maternal phenylalanine (Phe) when on a regular diet associated with higher levels of negative outcomes for offspring. For example, 73 percent of the offspring of mothers with classical PKU (Phe $\leq$1200 µmol/L) had microcephaly, whereas 68 percent of offspring of mothers with moderate PKU (1200 µmol/L > Phe $\leq$900 µmol/L), 35 percent of offspring of mothers with mild PKU (900 µmol/L > Phe $\leq$600 µmol/L), and 24 percent of offspring of mothers with mild hyperphenylalaninemia (600 µmol/L > Phe $\leq$180 µmol/L) had microcephaly.

A primary goal of the ICSMPKU was to encourage pregnant women with PKU to remain on a low Phe diet and thereby to maintain low Phe levels during pregnancy. This effort was, as an overall intervention, highly successful. In these treated pregnancies, only 23 percent of offspring of mothers with classical PKU, 8 percent of offspring of mothers with moderate PKU, 10 percent of offspring of mothers with mild PKU, and 5 percent of offspring of mothers with mild hyperphenylalaninemia had microcephaly (Koch, Friedman, Azen, et al., 1999).

However, large differences in average Phe levels during pregnancy were still apparent in the ICSMPKU study sample. Some mothers were able to maintain average Phe levels of less than 180 µmol/L throughout pregnancy, whereas other mothers had average Phe levels of more than 1200 µmol/L. The potential reasons for this large range in average Phe levels during pregnancy are numerous, including the poor taste of low-Phe diet food and the failure of mothers to comprehend the potential danger to the fetus of high maternal Phe levels. Another major factor was the mother's Phe level when on a regular diet. Mothers with classical PKU found it much more difficult to maintain low Phe levels than did mothers with less severe forms of PKU.

The large range of individual differences in average Phe levels during pregnancy presented us with an opportunity to explore the causes and consequences of average Phe levels. The results presented here have not yet appeared in a peer-reviewed journal, but they are key for describing (1) the amount and extent of Phe exposure necessary to produce negative effects on offspring; (2) the causes of, or the variables that influence, maternal Phe levels during pregnancy; (3) the consequences of Phe level during pregnancy; and (4) what remains unknown about the effects of prenatal exposure to high levels of Phe on developmental outcomes in offspring.

Prior research was equivocal about the level of Phe that could be tolerated by mothers with no ill effects on the fetus, so several important outcomes in offspring were measured in the ICSMPKU, including congenital heart disease and intelligence quotient (IQ). A consistent

finding of current analyses is that average Phe exposure of up to approximately 360 µmol/L has no effect on offspring IQ. But for exposure above that level, infant IQ showed a 3- to 3.5-point drop for every additional 60 µmol/L. Thus, infants who were exposed during pregnancy to an average Phe of 600 µmol/L (the upper limit defining hyperphenylalaninemia) had a mean infant IQ between 12 and 14 points lower than did infants exposed to average Phe levels of 360 µmol/L or less.

The timing of Phe exposure during pregnancy is also a crucial variable. Average Phe levels during the first 2 months of gestation had rather low levels of correlation with infant and child IQ (below .20). But from the third month of gestation on, the correlations between average Phe levels and infant and child IQ rose to the .30 to .40 range. These results are consistent with the hypothesis that Phe exposure has damaging effects on the fetal systems undergoing greatest growth during that period. Because the greatest amount of cortical development occurs during the latter two trimesters of pregnancy, average Phe levels during those periods correlate most strongly with intelligence, which depends on adequate development of cortical areas.

However, for other outcomes, such as congenital heart problems, average Phe levels during the first 2 months of gestation appear to be crucial, since the heart is undergoing rapid development during this period. These findings lead to a general conclusion: The amount and timing of Phe exposure during gestation that lead to negative effects are likely to differ across type of outcome. That is, certain outcomes may be affected by high Phe exposure during early pregnancy, whereas other outcomes are affected only by high Phe exposure during later pregnancy. In addition, the amount of Phe exposure necessary for negative outcomes may vary; therefore, determining the levels of Phe exposure that lead to teratogenic effects is an important research endeavor.

Given our results showing that average Phe levels during pregnancy were related in a nonlinear fashion to infant and child intelligence, we turned our attention to variables that influence Phe levels during pregnancy. In work to date, we have documented the influences of maternal education, intelligence, and socioeconomic status on average Phe levels during pregnancy. Of these three variables, maternal intelligence is the strongest ($\leq$ = -.47), whereas maternal education and socioeconomic status are moderate in their effects ($\leq$s around -.30). All of these effects are in the predicted direction: Higher levels of maternal intelligence, education, and socioeconomic status lead to lower levels of average Phe during pregnancy. These results suggest that practitioners should concentrate their efforts to ensure compliance with the low-Phe diet regimen in pregnant women with lower levels of IQ and education.

The consequences of high Phe levels during pregnancy for infant and child intelligence are quite strong. To assess offspring outcomes in the domain of mental abilities, the ICSMPKU used the Bayley Scales of Infant Development at 1 and 2 years of age, the McCarthy Scales of Children's Abilities at 4 years of age, and the Wechsler Intelligence Scale for Children–III (WISC-III) at 7 years of age. In predicting the Bayley Mental Development Index (MDI) at 1 year of age, average Phe exposure was the strongest predictor ($\leq$ = -.39); other predictors had smaller regression weights ($\leq$s < |.20|). Using the Bayley MDI at 2 years as the criterion, average Phe exposure was again the strongest predictor ($\leq$= -.46); other predictors had smaller regression weights ($\leq$s < |.22|).

A similar trend held for the McCarthy Scale General Cognitive Index (GCI) and the WISC-III Full Scale IQ (FIQ). Average Phe exposure during gestation was the strongest predictor, whereas other predictors had rather small regression weights. The relationship between average Phe exposure during gestation and intelligence got stronger as age increased. For example, the effect of average Phe exposure on the GCI at 4 years was $\underline{\leq}$ = -.53, and on the FIQ at 7 years was $\underline{\leq}$ = -.59. These results run counter to those found in most developmental studies, where the strength of effect of a predictor decreases as the length of time between measurements increases.

Much remains unknown about the effects of prenatal exposure to high levels of Phe on later developmental outcomes. Analyses of the outcomes at 1, 2, 4, and 7 years converged on the estimate of average Phe exposure of 360 µmol/L as the point at which teratogenic effects on offspring begin to appear. We currently have no firm estimates of the amount of Phe exposure that can be tolerated before teratogenic effects appear in other key outcomes. It appears that exposure to high levels of Phe in the first 2 months of gestation may contribute to congenital heart problems, and that exposure to high levels of Phe in the latter two trimesters of gestation affects intelligence, but the timing of Phe exposure for other developmental outcomes is little understood. Furthermore, we can only speculate on the effects of prenatal exposure to high levels of Phe for intellectual development during adolescence and adulthood. The most prudent guess would be that children exposed to high Phe levels during gestation will continue to show pronounced deficits during adolescence and adulthood, especially given the trend of stronger effects of prenatal Phe exposure on intellectual measures at later ages. Whether behavioral or biomedical treatments can alleviate the negative effects of exposure to high Phe levels during gestation is a topic to be pursued in future research. We can only supply a motivation for such research by documenting the pervasive negative effects of prenatal exposure to high levels of Phe.

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