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Invited Editorial: Molecular Genetics of Phenylketonuria and Its Implications

Harvey L. Levy

Joseph P. Kennedy, Jr. Laboratories of the Neurology Service, Massachusetts General Hospital, and the Department of Neurology, Harvard Medical School, Boston

The advent of routine newborn screening seemed to bring the story of phenylketonuria (PKU) to a close. With the Guthrie test (Guthrie and Susi 1963), every baby with PKU would be identified during the first days of life and begun on the phenylalanine-restricted diet. Mental retardation from PKU would be eliminated. It was to be a perfect ending to a remarkable sequence of events that began with the discovery of PKU as a biochemical cause of mental retardation (Fölling 1934) and proceeded to studies that identified the autosomal recessive inheritance pattern (Penrose 1935), the major biochemical aberrations (Jervis 1938; Jervis et al. 1940), and the enzymatic defect (Jervis 1953) and that resulted in a diet for control of the biochemical abnormalities (Bickel et al. 1954).

Newborn screening has been a major medical achievement and has brought great benefit to thousands of children with PKU and to their families. It has essentially ended mental retardation as a complication of PKU. But it did not end the story of PKU. To the contrary, it began not only a new chapter in the story but a new era in the history of PKU, an era in which the focus has shifted from mental retardation to other problems that have arisen or have become evident because of early treatment. First came the recognition that an occasional child with what seemed to be PKU became retarded and neurologically impaired despite early dietary therapy (Smith et al. 1975). Kaufman and his colleagues, with detailed knowledge about phenylalanine hydroxylase (PAH) derived from their studies (Kaufman 1971), discovered that these children suffered from defects in the production of the tetrahydrobiopterin cofactor required for hydroxylase activity rather than from PKU (Kaufman et al. 1975, 1978). Then came the realization that maternal PKU could nullify all of the gains in preventing mental retardation from PKU that had been brought about by newborn screening and early treatment (Kirkman 1982). Women with PKU, now mentally normal, would bear children with birth defects who would later become mentally retarded (Lenke and Levy 1980). Now it is clear that, despite early treatment, most children with PKU have lower IQ scores than expected (Dobson et al. 1977), many have learning disabilities (Berry et al. 1979), and a number develop psychological and emotional difficulties in adolescence and early adulthood (Levy and Waisbren 1987). To these problems has been added the need to continue the special diet well beyond childhood and perhaps indefinitely rather than reverting to a normal diet in middle childhood (Schuett and Brown 1984), thus prolonging the very substantial difficulties of dietary treatment.

Finding solutions to most of these problems will require far more knowledge about PKU than we now have, as impressive as is the current amount of information. We will need to understand the pathogenesis of the brain damage if totally preventive therapies are to be designed. Animal models for PKU, not yet available, will be crucial for this type of research. Somatic gene therapy might not only provide maximum benefit but might also eliminate the serious difficulties of maintaining the diet. Before even considering such therapy, we need to know a great deal about the PAH gene and its function. Prevention also plays a role. Reliable carrier detection, especially if conducted on a mass basis, could identify all couples at risk for bearing a phenylketonuric child and allow them to knowledgeably choose the reproductive option most appropriate for them. Prenatal diagnosis for PKU would be critical for many of these couples. These options cannot become available to all until the mutations in the PAH gene that cause PKU are known and readily detectable.

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Address for correspondence and reprints: Harvey L. Levy, M.D., Amino Acid Laboratory, Massachusetts General Hospital-East, 149 East 13th Street, Charlestown, MA 02129.

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Fortunately, Savio Woo and his colleagues are obtaining information about the PAH gene. Their continuing series of classical studies began 4 years ago when they isolated a human PAH cDNA clone (Kwok et al. 1985) and characterized the human gene (Ledley et al. 1985; Lidsky et al. 1985*b*). By restriction enzyme analysis of normal and PKU-linked mutant chromosomes, they have found extensive polymorphism at the PAH locus. European populations have at least 46 distinct RFLP haplotypes (Chakraborty et al. 1987; Woo 1988; Daiger et al. 1989*a*; Sullivan et al. 1989). Asian (Chinese and Japanese) families have at least 10 haplotypes, all of which are among the 46 observed in Caucasians (Daiger et al. 1989*b*). As expected, since mutations arise on a chromosome background defined by a haplotype, some haplotypes are considerably more likely to occur in PKU-linked mutant chromosomes than in normal chromosomes. For instance, four of the 46 haplotypes (haplotypes 1–4) account for more than 80% of PKU-linked chromosomes in European populations (Daiger et al. 1989*a*). On the other hand, only haplotype 38 is unique to a mutant chromosome (Rey et al. 1988; Lyonnet et al. 1989). Among Chinese and Japanese one haplotype (haplotype 4) is extraordinarily predominant and accounts for approximately 80% of both normal and PKU-linked chromosomes (Daiger et al. 1989*b*).

RFLP haplotype analysis is valuable in following the mutant allele within certain families. Thus it can provide prenatal diagnosis of PKU (or the exclusion of PKU) when there is a proband in the family and when the carrier parents are heterozygous for haplotypes (so that the normal and PKU-linked chromosomes can be separately identified) (Lidsky et al. 1985*a*). Carriers of the PKU gene can also be identified in these families. However, RFLP haplotype analysis cannot provide either prenatal diagnosis or carrier detection for PKU when there is no proband or when the couple do not have haplotypic heterozygosity. For instance, only 35%–40% of Chinese and Japanese carriers are haplotypically heterozygous (informative) at the PAH locus (Daiger et al. 1989*b*).

Thus RFLP haplotyping does not allow the PKU allele(s) to be precisely followed in populations or even in all families. This requires direct identification of the mutant PKU allele, now a distinct possibility. Already seven mutations linked to PKU have been found in the PAH gene (DiLella et al. 1986, 1987; Avigad et al. 1987; Lichter-Konecki et al. 1988; Lyonnet et al. 1989; John et al. 1989; Wang et al. 1989). These are listed in table 1. In this issue of the *Journal* the Baylor group describes the only one identified so far in Orientals. It is a nonsense mutation in exon 3 in Chinese families with PKU (Wang et al. 1989). The C-to-T transition in codon 111 substitutes a termination for an arg codon. This is only one of what must be at least several mutations producing PKU in the Chinese, since it was found in only 10% of the Chinese mutant alleles analyzed. All except one of the mutations described so far have been associated with classic PKU. The single exception is the missense mutation in exon 7 uniquely linked to haplotype 38 which is present in North African and French families who have an intermediate ("atypical") form of PKU (Lyonnet et al. 1989).

When the major mutations causing PKU have been defined and when the corresponding mutant-specific oligonucleotides have been synthesized, reliable carrier detection and prenatal diagnosis for PKU will become available to almost all families. The allele-specific oligonucleotide (ASO) hybridization technique used in the studies reported in this issue of the *Journal* (Wang et al. 1989), especially with polymerase chain reaction (PCR) amplification of the gene, has greatly simplified

Table 1

Mutations in the PAH Gene That Produce PKU

Exon	Intron	Mutation	Codon Change	Haplotype[a]	Reference
	12	G→A		3	DiLella et al. 1986
12		C→T	arg^{408}→try^{408}	2	DiLella et al. 1987
3		Deletion			Avigad et al. 1987
9		T→C	leu^{311}→pro^{311}	*Msp*I polymorphism	Lichter-Konecki et al. 1988
7		G→A	glu^{280}→lys^{280}	38	Lyonnet et al. 1989
3		C→T	arg^{111}→ter^{111}	4	Wang et al. 1989
		A→G	met^{1}→val^{1} [b]	2	John et al. 1989

[a] Haplotype with which the mutation is in linkage disequilibrium.
[b] Initiation codon.

the process of detecting mutant alleles in human DNA. This is possible even with a filter-paper blood specimen such as the "Guthrie" specimen collected for newborn screening (Lyonnet et al. 1989). Consequently, population screening such as for carriers of mutant PAH alleles could soon become a reality. Does this mean that newborn screening for PKU will be performed by molecular genetic techniques and that the affected infant will be identified on the basis of his or her mutation(s) rather than on the basis of an increased level of phenylalanine? No, newborn screening for PKU will depend on the phenylalanine marker for many years to come. Molecular genetic technology is not ready to replace the simple and inexpensive assays currently used in routine newborn screening. Perhaps more important, the principle of specific mutation identification is contrary to that of an optimal screening approach, which is to net the largest number of affected infants possible by the primary test and then to sort out these infants with secondary diagnostic tests. The use of ASOs, however, may become the most valuable of these secondary diagnostic tests. Since mutations usually predict phenotype (Guttler et al. 1987; Lyonnet et al. 1989), a specific mutational diagnosis using the newborn blood specimen could rapidly determine whether the newborn infant is likely to have classic PKU or a less severe variant. Not only would this provide valuable information for the medical providers and family when the infant is evaluated, but it could also reduce the number of requested repeat specimens for "false-positive" screening results.

It seems quite certain that within the next few years many more mutations associated with PKU will be identified, analogous to the numerous mutations that cause β-thalassemia (Orkin 1987). The presence of different mutations producing PKU idicates that there has not been a single focus wherein a PKU mutation arose and from there spread outward. Rather, multiple independent mutations have produced PKU throughout the world. Furthermore, each of these mutations is in linkage disequilibrium with a haplotype (table 1). Accordingly, while the haplotypes are ancient, the "PKU mutations" must be of relatively recent origin and must have remained with the haplotypes upon which they arose. Given sufficient evolutionary time, recombination should produce linkage equilibrium between mutations and haplotypes. Again, this is very similar to the thalassemia story (Orkin 1987) and is likely to be repeated for most, if not all, of the genes associated with genetic disorders.

The association between mutations and haplotypes also provides evidence in an ongoing discussion about why the frequency of PKU remains high. Since PKU has been essentially a genetic lethal (i.e., until the present generation, phenylketonuric individuals rarely reproduced), the frequency of PKU should have decreased, but this seems not to have occurred (Woolf 1976). One possible explanation is that new mutations occur with sufficient frequency to replace the mutations lost to reproductive unfitness. This is clearly not so; otherwise, mutations would generally be in linkage equilibrium with haplotypes. Consequently, another possibility, selective advantage of the heterozygote, seems more likely (Kidd 1987). What this selective advantage could be is yet unknown, although Woolf (1976) has suggested that heterozygotic women have a lower spontaneous abortion rate than do other women.

In so many ways, PKU encompasses the questions raised—and the answers sought—for all inborn errors of metabolism. This is why the molecular findings in PKU are particularly exciting. As Scriver and Clow (1980) stated at the beginning of this decade, PKU is the epitome of human biochemical genetics.

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