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Commentary

Phenylketonuria: Old disease, new approach to treatment

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If one were to construct a fantasy about a human genetic disease for which all is known and a cure available, phenylketonuria (PKU) is likely to come to mind. In what other genetic disorder have the following been accomplished: characterization and mapping of the relevant gene (1, 2); identification of the mutations (3); determination of enzyme structure and functional sites (4); identification of the clinical (5) and biochemical characteristics (6); correlations of genotype with phenotype (7); prenatal diagnosis (8); recognition of the teratogenic risks in the maternal condition (9); development of treatment that prevents mental retardation (10); production of an animal model that mimics the biochemical phenotype and expresses much of the clinical phenotype (11); and, as if these were not enough, establishment of newborn screening for the disease so that virtually all affected individuals in the developed world receive preventive treatment (12)?

It is an astounding story, the standard to which all genetic disease is held. Nevertheless, there is another side to the story. To prevent mental retardation, affected individuals must maintain a strict and difficult diet, probably for life (13). This requirement can so alter the normal lifestyle that dietary compliance and its concomitant metabolic control too often suffer, the result being clinical complications such as reduced cognitive abilities (14), neuropsychological dysfunction (15), and emotional disturbance (16, 17). Less than optimal metabolic control during pregnancy produces offspring with birth defects (18). Consequently, less onerous treatment for PKU is sorely needed, and the paper by Sarkissian *et al.* published in this issue of the *Proceedings* (19) suggests that this might not be far away. They show that the blood phenylalanine concentration in the phenylalanine hydroxylase $(PAH)^{enu2}$ mouse model of human PKU can be reduced substantially by the administration of phenylalanine ammonia lyase (PAL), a nonmammalian enzyme that degrades phenylalanine. Applied to humans with PKU, this type of substitute enzyme therapy could provide relief for the rigors of the diet and improve compliance, metabolic control, and clinical outcome in this relatively frequent genetic disease.

PKU is a biochemical genetic disorder, but, as pointed out by Scriver *et al.* (3), an environmental disease. The disorder is caused by a mutant allele at the phenylalanine hydroxylase (PAH) locus that results in lack of functional PAH, the liver enzyme required for hydroxylation of phenylalanine. The disease is environmental in that the clinical phenotype is produced by the presence in food of L-phenylalanine, an essential amino acid. Thus, combining dietary phenylalanine with lack of phenylalanine catabolism produces hyperphenylalaninemia, and this leads to mental retardation. Successful treatment requires control of the hyperphenylalaninemia.

The relationship between hyperphenylalaninemia and brain dysfunction in PKU is indisputable, even though the proximate cause of the brain damage is unknown (6). Individuals with untreated classic PKU, in which the blood phenylalanine level is increased >20-fold, almost invariably become mentally retarded, often severely so (3); those with mild PKU, having a 10- to 15-fold increase in the concentration of blood phenylalanine, also suffer cognitive loss when untreated, but the loss is less severe than in those with classic PKU (20); and those with non-PKU mild hyperphenylalaninemia, with blood phenylalanine concentrations increased 2- to 8-fold, appear to remain cognitively normal and may not require treatment (21, 22). The best evidence for a relationship between hyperphenylalaninemia and brain effect, however, is from the experience with treatment. Children born with even the most severe form of PKU can have normal cognitive development when dietary treatment begins in early infancy and the blood phenylalanine concentration is maintained at near normal or normal levels (23).

Woolf and Vulliamy in 1951 (24) were the first to suggest that the levels of phenylalanine and the biochemical byproducts of hyperphenylalaninemia could be reduced by restricting the dietary intake of phenylalanine. Two years later, Bickel *et al.* (10) followed this suggestion by constructing a phenylalanine-restricted diet and using it to treat a child with PKU who was mentally retarded. They demonstrated that the diet produced both a marked reduction in the blood phenylalanine concentration and improvement in behavior. During the next few years, a number of infants known to be at risk for PKU were diagnosed and begun on the diet soon after birth. Follow-up studies of these infants indicated that, under these conditions, the diet could prevent mental retardation (25). This led to newborn screening for PKU which, by the mid-1960s, was routine throughout the United States and, by the early 1970s, was routine throughout most of the developed world (26). Over 150 million infants now have been screened, and over 10,000 have been detected with PKU and have been treated with diet.

The success of dietary treatment, however, has come at high personal cost to affected individuals and their families. The restriction in protein requires exclusion of natural foods such as meat, fish, milk, cheese, bread, cake, nuts, and many other common food items. Even the intake of vegetables is limited. Children on this diet cannot eat hamburgers, pizza, ice cream, and many other foods that are popular and socially pleasurable. To avoid nutritional deficiencies, they are required to ingest a synthetic dietary supplement consisting of a phenylalanine-free amino acid mixture, a source of calories, minerals, and vitamins (27). The supplement is offensive in odor and taste (28) and probably must be continued for life (13). Dietary adherence is especially important during pregnancy if the teratogenic effects of maternal PKU are to be avoided (29). Emotional stress in these families is often high, probably in large part because of the stringency of the diet (30). The diet also produces biological side effects. Reduced blood levels of several nutrients are frequent and might be important to ultimate outcome (3).

Any method to alleviate the strain and biochemical abnormalities imposed by the diet would be welcomed by patients with PKU, their families, and those who provide them with

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health care. Consequently, there is great interest in approaches that address the biochemical genetic basis of the disorder. Unfortunately, none of the efforts devoted to such approaches have yet been successful. The idea of PAH enzyme therapy has bogged down over lack of a method to protect the enzyme and its catalytic property (31). Somatic gene therapy is yet inapplicable. Infusions of the recombinant adenoviral vector containing human PAH cDNA in the PAH^{enu2} mouse model reduce the markedly increased blood phenylalanine concentration to normal, but the effect is transient and, because of an immune response against the adenoviral vector, is not reproducible (32). Liver transplantation corrects the molecular disorder but is too complicated and hazardous to be justifiable as an appropriate substitute for the diet (33). Failure of these approaches to provide the possibility of treating PKU at a mechanistic level has returned the focus of treatment to better control of the enteric source of phenylalanine.

Adding PAL to the diet could be one of these ways. PAL degrades phenylalanine to ammonia and transcinnamic acid. The latter is a harmless product that is degraded further to benzoic acid, which in turn is converted to rapidly excreted hippuric acid (34). The ammonia does not accumulate in sufficient quantity to pose a threat of hyperammonemia. Use of PAL to reduce the hyperphenylalaninemia of PKU first was proposed not as a dietary agent but to attack the phenylalanine in the blood (35). Yeast PAL was immobilized in the wall of hollow fibers through which blood was perfused by extracorporeal shunting. Used in this manner, PAL reduced artificially induced hyperphenylalaninemia in animals (36) and produced a modest reduction in the hyperphenylalaninemia of an adult with PKU (37). However, it was clear that a method requiring extracorporeal shunting could have no practical role in the treatment of PKU. Investigators in England did find that administering PAL in enteric-coated capsules could reduce the blood phenylalanine concentration in PKU by 25% (34). Although this degree of reduction in the phenylalanine level is insufficient for adequate treatment of PKU, the results indicated that enterically administered PAL was safe and that the enzyme might provide relief for the stringency of the diet. Ultimately, the prohibitive cost of the required amount of PAL led to discontinuation of this investigation.

The Montreal group has picked up the slack and for over a decade has been examining methods for removing enteric phenylalanine with PAL (38–40). Their work is based on two assumptions: that enterically placed PAL will metabolize dietary phenylalanine before it is absorbed and that phenylalanine that recirculates into the intestinal lumen from body pools also can be degraded by PAL (38). To protect intestinal PAL from proteolysis by intestinal digestive enzymes yet allow access to phenylalanine, they have immobilized PAL in semipermeable microcapsules (39, 40). Feeding these microcapsules to the PAH^{enu2} mouse model resulted in a 50% reduction in the blood phenylalanine concentration (40).

Sarkissian *et al*. describe their new work (19). To address the problem of PAL availability, they have produced recombinant PAL from the yeast gene. They show that this PAL administered i.p. to the PAH^{enu2} mouse produces a dose–response reduction in the blood phenylalanine level. More importantly, they demonstrate a 30–40% reduction in the blood phenylalanine concentration when the recombinant PAL encased in its original *Escherichia coli* expression cells is fed to the PAH^{enu2} mice and a 50% reduction in the phenylalanine concentration when naked recombinant PAL and a protease inhibitor are fed together.

Could enteric PAL substitute for diet in the treatment of human PKU? Probably not. The treatment of PKU requires reductions of 70–80% in the blood phenylalanine concentration, about twice the reductions seen in the mice. Moreover, PAL does not lead to the production of tyrosine. If tyrosine deficiency is a factor in the brain effect of PKU, as some believe (41, 42), this would require attention. Nevertheless, PAL therapy could substantially increase dietary protein tolerance. If so, this could lift much of the burden of the PKU diet and would be welcomed warmly not only by the health care community but especially by those with PKU and by their families.

1. Kwok, S. C. M., Ledley, F. D., DiLella, A. G., Robson, K. J. H. & Woo, S. L. C. (1985) *Biochemistry* **24,** 556–561.
2. Lidsky, A. S., Robson, K. J. H., Thirumalachary, C., Barker, P. E., Ruddle, F. H. & Woo, S. L. C. (1984) *Am. J. Hum. Genet.* **36,** 527–533.
3. Scriver, C. R., Kaufman, S., Eisensmith, R. C. & Woo, S. L. C. (1995) in *The Metabolic and Molecular Bases of Inherited Disease*, eds. Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D. (McGraw–Hill, New York), pp. 1015–1075.
4. Fusetti, F., Erlandsen, H., Flatmark, T. & Stevens, R. C. (1998) *J. Biol. Chem.* **273,** 16962–16967.
5. Jervis, G. A. (1937) *Arch. Neurol. Psychiatry* **38,** 944–963.
6. Kaufman, S. (1976) in *Advances in Neurochemistry*, eds. Agranoff, B. W. & Aprison, M. H. (Plenum, New York), Vol. 2, pp. 1–132.
7. Guldberg, P., Rey, F., Zschocke, J., Romano, V., Francois, B., Michiels, L., Ullrich, K., Hoffman, G. F., Burgard, P., Schmidt, H., *et al.* (1998) *Am. J. Hum. Genet.* **63,** 71–79.
8. Woo, S. L. C. (1984) *Pediatrics* **74,** 412–423.
9. Lenke, R. R. & Levy, H. L. (1980) *N. Engl. J. Med.* **303,** 1202–1208.
10. Bickel, H., Gerrard, J. & Hickmans, E. M. (1953) *Lancet* **ii,** 812–813.
11. Shedlovsky, A., McDonald, J. D., Symula, D. & Dove, W. (1993) *Genetics* **134,** 1205–1210.
12. Guthrie, R. & Susi, A. (1963) *Pediatrics* **32,** 338–343.
13. Medical Research Council Working Party on Phenylketonuria (1993) *Arch. Dis. Child.* **68,** 426–427.
14. Azen, C., Koch, R., Friedman, E. G., Berlow, S., Coldwell, J., Krause, W., Matalon, R., McCabe, E., O'Flynn, M., Peterson, R., *et al.* (1991) *Am. J. Dis. Child.* **145,** 35–39.
15. Waisbren, S. E., Brown, M. J., de Sonneville, L. M. J. & Levy, H. L. (1994) *Acta Paediatr. Scand.* **407,** 98–103.
16. Smith I., Beasley, M. G., Wolff, O. H. & Ades, A. E. (1988) *J. Pediatr.* **112,** 403–408.
17. Hendrikx, M. M. T., vander Schot, L. W. A., Slijper, F. M. E., Huisman, J. & Kalverboer, A. F. (1994) *Eur. J. Pediatr.* **153,** 832–835.
18. Koch, R., Levy, H. L., Matalon, R., Rouse, B., Hanley, W. & Azen, C. (1993) *Am. J. Dis. Child.* **147,** 1224–1230.
19. Sarkissian, C. N., Shao, Z., Blain, F., Peevers, R., Su, H., Heft, R., Chang, T. M. S. & Scriver, C. R. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 2339–2344.
20. Waisbren, S. E., Schnell, R. & Levy, H. L. (1984) *J. Pediatr.* **105,** 955–8.
21. Levy, H. L., Shih, V. E., Karolkewicz, V., French, W. A., Carr, J. R., Cass, V., Kennedy, J. L. & MacCready, R. A. (1971) *N. Engl. J. Med.* **285,** 424–429.
22. Weglage, J., Ullrich, K., Pietsch, M., Funders, B., Guttler, F. & Harms, E. (1997) *Pediatr. Res.* **42,** 378–384.
23. Waisbren, S. E., Mahon, B. E., Schnell, R. R. & Levy, H. L. (1987) *Pediatrics* **79,** 351–355.
24. Woolf, L. I. & Vulliamy, D. G. (1951) *Arch. Dis. Child.* **26,** 487–494.
25. Knox, W. E. (1960) *Pediatrics* **26,** 1–11.
26. Levy, H. L. (1973) in *Advances in Human Genetics*, eds. Harris, H. & Hirschhorn, K. (Plenum, New York), Vol. 4, pp. 1–104.
27. Elsas, L. J. & Acosta, P. B. (1988) in *Modern Nutrition in Health and Disease*, eds. Shils, M. E. & Young, V. R. (Lea & Febiger, Philadelphia), pp. 1337–1379.
28. Prince, A. P., McMurry, M. P. & Buist, N. R. M. (1997) *J. Inherited Metab. Dis.* **20,** 486–498.
29. Levy, H. L. & Ghavami, M. (1996) *Teratology* **53,** 176–184.
30. Awiszus, D. & Unger, I. (1990) *Eur. J. Pediatr.* **149,** Suppl. 1, S45–S51.
31. Weiss, B., Hui, M. & Lajtha, A. (1977) *Biochem. Med.* **18,** 330–343.

32. Fang, B., Eisensmith, R. C., Li, X. H. C., Finegold, M. J., Shedlovsky, A., Dove, W. & Woo, S. L. C. (1994) *Gene Ther.* **1,** 247–254.
33. Vajro, P., Strisciuglio, P., Houssin, D., Huault, G., Laurent, J., Alvarez, F. & Bernard, O. (1993) *N. Engl. J. Med.* **329,** 363.
34. Hoskins, J., Jack, G., Peiris, R. J. D., Starr, D. J. T., Wade, H. E., Wright, E. C. & Stern, J. (1980) *Lancet* **i,** 392–394.
35. Ambrus, C. M., Ambrus, J. L., Horvath, C., Pedersen, H., Sharma, S., Kant, C., Mirand, E., Guthrie, R. & Paul, T. (1978) *Science* **201,** 837–839.
36. Ambrus, C. M., Sharma, S. D., Horvath, C. S., Kalghatgi, K., Anthone, S., Ambrus, J. L., Cooley, C. & Mirand, E. A. (1983) *J. Pharmacol. Ther.* **224,** 598–602.
37. Ambrus, C. M., Anthone, S., Horvath, C., Kalghatgi, K., Lele, A. S., Eapen, G., Ambrus J. L., Ryan, A. J. & Li, P. (1987) *Ann. Intern. Med.* **106,** 531–537.
38. Chang, T. M. S., Bourget, L. & Lister, C. (1995) *Artif. Cells Blood Substit. Immobil. Biotechnol.* **23,** 1–21.
39. Bourget, L. & Chang, T. M. S. (1986) *Biochim. Biophys. Acta.* **883,** 432–438.
40. Safos, S. & Chang, T. M. S. (1995) *Artif. Cells Blood Substit. Immobil. Biotechnol.* **23,** 681–692.
41. Lou, H. C., Lykkelund, C., Gerdes, A.-M., Udesen, H. & Bruhn, P. (1987) *Acta Paediatr. Scand.* **76,** 560–565.
42. Diamond, A. (1994) *Acta Paediatr. Scand.* **83,** Suppl. 407, 89–91.