Letters to the Editor

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Collation of RFLP Haplotypes at the Human Phenylalanine Hydroxylase (PAH) Locus

To the Editor:

Phenylketonuria (PKU) is an inborn error in amino acid metabolism that predisposes affected individuals to development of severe mental retardation (Folling 1934). The condition is secondary to a deficiency of the hepatic enzyme phenylalanine hydroxylase (PAH) (Gervis 1947). The disorder is transmitted as an autosomal recessive trait and has a prevalence of about 1/10,000 births among Caucasians (for review, see Scriver and Clow, 1980). Since the initial report on the construction of a rat PAH cDNA clone (Robson et al. 1982), a full-length human PAH cDNA clone has been obtained, from the nucleotide sequence of which the primary structure of the human enzyme has been deduced (Kwok et al. 1985). Functional PAH enzymatic activity can be obtained by transfection of the cDNA into cultured mammalian cells that normally do not synthesize PAH (Ledley et al. 1985), as well as in bacterial cells (Ledley et al. 1987). The data provided strong evidence that the human enzyme is a homopolymer encoded by a single locus that has been mapped to human chromosome 12q22-24.1 (Lidsky et al. 1984, 1985*c*).

The full-length human PAH cDNA was utilized to identify extensive RFLPs at the corresponding locus in the human genome (Lidsky et al. 1985*a*), which permit prenatal diagnosis of the disorder in most PKU families (Woo et al. 1983; Lidsky et al. 1985*b*; Daiger et al. 1986). The RFLPs defined distinct haplotypes at the PAH locus, and 12 such haplotypes have previously been observed in the Danish population (Chakraborty et al. 1987). It is interesting that 90% of all mutation alleles are associated with only four haplotypes in that population and that two of them have been fully characterized at the molecular levels. The mutant haplotype 3 allele is associated with a GT-to-AT transition at the canonical splice donor site of intron 12 of the PAH gene and causes the skipping of the preceding exon during RNA splicing (DiLella et al. 1986; Marvit et al. 1987). The mutant haplotype 2 allele is associated with a missense mutation involving an arginine-to-tryptophane substitution at residue number 408 of the enzyme (DiLella et al. 1987). Both mutant alleles are in linkage disequilibrium with the corresponding RFLP haplotypes throughout Europe (S. L. C. Woo, unpublished results). The data suggested two mutational events that occurred on background chromosomes of the two corresponding haplotypes, followed by spread and expansion of the two mutation alleles in the Caucasian population.

In collaboration with a number of laboratories and clinical centers throughout Eruope, haplotyping analysis at the PAH locus has been performed in several hundred PKU families. As a result, a number of novel RFLP haplotypes have been detected. At the present time, a total of 43 defined haplotypes have been observed in various European populations (fig. 1), some of which were first observed by independent investigators who kindly communicated their results to us prior to publication. Mutant alleles have been found in as-

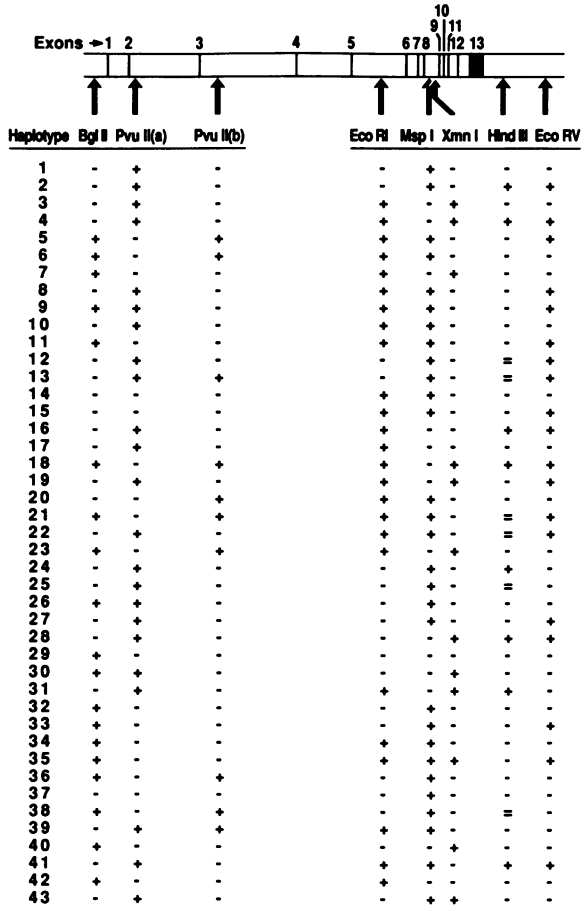

Figure 1 RFLP haplotypes at the human PAH locus. The molecular structure of the human PAH gene is shown schematically with its 13 axons encompassing about 90 kb of DNA. The heavy arrows correspond to the polymorphic restriction sites in and immediately flanking the gene. Plus (+) and minus (−) symbols designate the presence and absence of a polymorphic restriction site, respectively. An equals sign (=) designates a 4.4[kb *Hind*III allele. Contributing PKU centers in Europe include those in Denmark, Scotland, Switzerland, Germany, France, Italy, Hungary, and Czechoslovakia.

sociation with many of these haplotypes, suggesting that there may be a number of additional mutations in the PAH gene that cause PKU. It is hoped that the collated haplotype data will be adopted by the scientific community in order to avoid confusions in the literature with regard to the numerical designation of distinct RFLP haplotypes in the human PAH locus.

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References

Chakraborty, R., A. S. Lidsky, S. P. Daiger, F. Guttler, S. Sullivan, A. G. DiLella, and S. L. C. Woo. 1987. Polymorphic DNA haplotypes at the human phenylalanine hydroxylase locus and their relationship with phenylketonuria. Hum. Genet. **76**:40–46.

Daiger, S. P., A. S. Lidsky, R. Chakraborty, R. Koch, F. Guttler, and S. L. C. Woo. 1986. Effective use of polymorphic DNA haplotypes at the phenylalanine hydroxylase locus in prenatal diagnosis of phenylketonuria. Lancet **1**:229–231.

DiLella, A. G., J. Marvit, K. Brayton, and S. L. C. Woo. 1987. An amino acid substitution in phenylketonuria is in linkage disequilibrium with DNA haplotype-2. Nature **327**: 333–336.

DiLella, A. G., J. Marvit, A. S. Lidsky, F. Guttler, and S. L. C. Woo. 1986. Tight linkage between a splicing mutation and a DNA haplotype in phenylketonuria. Nature **322**:799–803.

Folling, A. 1934. Über Ausscheidung von Phenylbrenztraubensaure in den Harn als Stoffwechselanomalie in Verbindung mit Imbezillität. Z. Physiol. Chem. **227**:169–176.

Gervis, G. A. 1947. Studies in phenylpyruvic oligophrenia: the position of the metabolic error. J. Biol. Chem. **169**: 651–656.

Kwok, S. C. M., F. D. Ledley, A. G. DiLella, K. J. H. Robson, and S. L. C. Woo. 1985. Nucleotide sequence of a full-length cDNA clone and amino acid sequence of human phenylalanine hydroxylase. Biochemistry **24**:556–561.

Ledley, F. D., H. E. Grenett, A. G. DiLella, S. C. M. Kwok, and S. L. C. Woo. 1985. Gene transfer and expression of human phenylalanine hydroxylase. Science **228**:77–79.

Ledley, F. D., H. E. Grenett, and S. L. C. Woo. 1987. Biochemical characterization of recombinant human phenylalanine hydroxylase produced in *E. coli*. J. Biol. Chem. **262**:2228–2233.

Lidsky, A., F. D. Ledley, A. G. DiLella, S. C. M. Kwok, S. P. Daiger, K. J. H. Robson, and S. L. C. Woo. 1985*a*. Molecular genetics of phenylketonuria: extensive restriction site polymorphisms in the human phenylalanine hydroxylase locus. Am. J. Hum. Genet. **37**:619–634.

Lidsky, A., K. Robson, T. Chandra, P. Barker, F. Ruddle, and S. L. C. Woo. 1984. The PKU locus in man on chromosome 12. Am. J. Hum. Genet. **36**:527–533.

Lidsky, A. S., F. Guttler, and S. L. C. Woo. 1985*b*. Prenatal diagnosis of classic phenylketonuria by DNA analysis. Lancet **1**:549–551.

Lidsky, A. S., M. L. Law, H. G. Morse, F. T. Kao, M. Raben, F. H. Ruddle, and S. L. C. Woo. 1985*c*. Regional mapping of the human phenylalanine hydroxylase gene and the PKU locus on chromosome 12. Proc. Natl. Acad. Sci. USA

82:6221–6225.
Marvit, J. A. G. DiLella, F. D. Ledley, and S. L. C. Woo. 1987. GT to AT transition in a splice donor site causes skipping of the preceding exon in phenylketonuria. Nucleic Acids Res. **15**:5613–5628.
Robson, K., T. Chandra, R. MacGillivray, and S. L. C. Woo. 1983. Polysome immunoprecipitation of phenylalanine hydroxylase mRNA from rat liver and cloning of its cDNA. Proc. Natl. Acad. Sci. USA **79**:4701–4705.
Scriver, C. R., and C. L. Clow. 1980. Phenylketonuria and other phenylalanine hydroxylation mutants in man. Annu. Rev. Genet. **14**:179–239.
Woo, S. L. C., A. Lidsky, T. Chandra, F. Gattler, and K. Robson. 1983. Cloned human phenylalanine hydroxylase gene allows prenatal diagnosis and carrier detection of classical phenylketonuria. Nature **306**:151–155.

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Polygenes versus One Gene Plus Chance: What Are the Real Differences?

To the Editor:

In their fascinating paper, Kurnit et al. (1987) suggest that they have demonstrated that the multifactorial model of inheritance may be explained by a model involving a single major gene plus chance, as well as by a polygenic model. Yet they also note that, to explain the steep falling off of recurrence risk with diminishing relatedness, "selection" effects or an oligogenic model involving a few major loci must also be introduced to the single gene model. Doesn't this concession vitiate what I understand to be the main conclusion of the authors' papers, namely, that multifactorial inheritance can be *entirely* simulated by a "single gene plus chance" model? "Chance" by this definition excludes genetic *or* environmental factors. But selection is mediated by environmental factors, and the boundary between "oligogenic" and "polygenic" is purely arbitrary. It would appear that "environment" *or* polygenes must be introduced *over and above* "chance plus a single gene" to simulate multifactorial determination.

Second, even if my understanding on this point is incorrect, and these are really distinct hypotheses, would the authors comment on what *practical* difference it makes, at present, as to which of the competing hypotheses is correct? As I understand their claim, the predictions of the polygenic or their single gene model cannot be distinguished in any event. *If* this is the case, then, for heuristic purposes, why may one not just assume the simpler, more elegant or more familiar model, in, for example, the context of genetic counseling? For most readers, this model would be the polygenic one. Certainly one might argue that the possibility of a "single gene plus chance" model might stir the search for the major allele responsible for a trait hitherto held to be polygenic. Discovery of a probe might well help identify a subset of the population at high risk. But until such probes are available, if ever, what difference does the alternative model make to the usual approach to such conditions?

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References

Kurnit, D. M., W. M. Layton, and S. Matthysse. 1987. Genetics, chance, and morphogenesis. Am. J. Hum. Genet. **41**:979–995.

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Reply to Letter from Dr. Ernest Hook

To the Editor:

Dr. Hook is almost certainly right in suggesting that environmental, oligogenic, or even polygenic factors have to be included to get a complete picture of inheritance. Models always simplify—and indeed are only useful because they simplify. The way to read our conclusions is that "a simple major locus model which allows for the effects of chance accounts for common patterns of inheritance surprisingly well." We did not mean