Molecular Genetics of PKU in Eastern Europe: A Nonsense Mutation Associated with Haplotype 4 of the Phenylalanine Hydroxylase Gene

Tao Wang, Yoshiyuki Okano, Randy C. Eisensmith, Gyorgy Fekete, Dezso Schuler, Gyorgy Berencsi, Istvan Nasz, and Savio L.C. Woo

Howard Hughes Medical Institute, Department of Cell Biology and Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030; and Departments of Microbiology and Pediatrics II, Semmelweis University of Medicine, Budapest, Hungary

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Abstract—Phenylketonuria (PKU) is a genetic disorder secondary to a deficiency of hepatic phenylalanine hydroxylase (PAH). Several mutations in the PAH gene have recently been reported, and linkage disequilibrium was observed between RFLP haplotypes and specific mutations. A new molecular lesion has been identified in exon 7 of the PAH gene in a Hungarian PKU patient by direct sequencing of PCR-amplified DNA. The C-to-T transition causes the substitution of Arg 243 to a termination codon, and the mutant allele is associated with haplotype 4 of the PAH gene. The mutation is present in two of nine mutant haplotype 4 alleles among Eastern Europeans and is not present among Western Europeans and Asians. The rarity of this mutant allele and its restricted geographic distribution suggest that the mutational event occurred recently on a normal haplotype 4 background in Eastern Europe.

INTRODUCTION

Classical phenylketonuria (PKU), the most common of inherited metabolic disorders, is caused by a deficiency in hepatic phenylalanine hydroxylase (PAH). The decrease in PAH activity results in the accumulation of phenylalanine in serum and irreversible mental retardation in untreated PKU patients (1). The disease is prevalent among Caucasians and 1 in 50 individuals is a carrier of the trait (2, 3).

The human PAH locus has been studied extensively since the isolation of a full-length human PAH cDNA (4–7). RFLP haplotype analysis at the PAH locus revealed the presence of at least 47 haplotypes among different ethnic groups (8–10). Four haplotypes (1, 2, 3, and 4) comprised 80% of all mutant alleles in the Northern European population (11) and were shown to be prevalent throughout the European continent as well (9). Further analyses of the PAH gene in PKU patients demonstrate that PKU is caused by multiple mutations, which contribute to the heterogenous clinical course. Interestingly, all PAH mutations characterized in Caucasians so far are point mutations and are in obvious linkage disequilibrium with the respective RFLP haplotypes (12–16).

Although mutant haplotypes 1, 2, 3, and 4 are prevalent throughout the European continent, mutant haplotypes 1 and 3 are much less common in Eastern Europe than in Western Europe. As the result, haplotypes 2 and 4 become the predominant mutant haplo-
types in this area, accounting for 62% and 17% of the total mutant alleles, respectively (9). This haplotype distribution suggests that there may be some differences in the genetic background of PKU between the two populations. The mutation associated with haplotype 2 has already been identified, and linkage disequilibrium between mutation and haplotype is almost absolute. This mutation, an amino acid substitution (Arg^{408}-Try^{408}) of PAH, produces no PAH activity and therefore a severe clinical course (13). Another mutation, identified recently in Caucasians as a single amino acid substitution (Arg^{158}-Gln^{158}), produces less than 10% of normal PAH activity and may be responsible for a mild PKU phenotype (16). Interestingly, the mutation shows an obvious exclusive but not inclusive linkage disequilibrium with mutant haplotype 4. This finding suggests that there must be other mutations on haplotype 4 background of the PAH gene that are responsible for PKU in this population. In this paper, we report the identification of a nonsense mutation in the PAH gene that is in linkage disequilibrium with mutant haplotype 4 alleles in Eastern Europe.

MATERIALS AND METHODS

Patients. The pedigrees selected for population studies are nuclear families with at least one PKU child. All patients were diagnosed and evaluated at their institutions with standardized clinical criteria (3, 9, 17). The proband for gene analysis was a classical PKU child from Hungary who is a haplotype 4 and 8 compound heterozygote (9).

PCR Amplification of Exonic Regions in PAH Gene of the Patient. Primers were designed for PCR amplification of exonic regions of the human PAH gene and were synthesized by Genetic Designs Inc., Houston, Texas. Individual exons with flanking intronic sequence of the PAH gene were amplified independently as the following: a 100-μl reaction volume containing 0.5 μg of genomic DNA; 0.2 mM each of dATP, dGTP, dCTP and dTTP; 10 mM Tris HCl (pH 8.3); 50 mM KCl; 1.5 mM MgCl₂; 0.01% (w/v) gelatin; and 0.5 μM each of an amplification primer pair. Samples were denatured at 97°C for 7 min and annealed at 50°C for 1 min. Two units of Taq DNA polymerase (Perkin Elmer Cetus) were added and samples were incubated at 72°C for 1 min. The following cycles consisted of DNA denaturation at 92°C for 10 sec, annealing at 50°C for 10 sec, and primer extension at 72°C for 40 sec. A total of 35 cycles were performed using the Perkin Elmer Thermal Cycler with final extension at 72°C for 7 min. PCR products were purified using 4% Nusieve agarose gels and recovered using Gene Clean Kit (Bio-101) according to manufacturer’s instruction.

Direct Sequencing of PCR-Amplified Products. Direct sequencing was performed using PCR-amplified DNA according to a modified procedure of that reported by Winship (18): 150 ng of purified template (200–300 bp in length) was mixed with 150 ng of sequencing primer (17 mer) in 6 μl of 40 mM Tris HCl (pH 7.5), 25 mM MgCl₂, 50 mM NaCl, 10% DMSO. The samples were denatured at 97°C for 3 min and put on dry ice immediately. Then 4 μl of labelling mixture containing 50 mM DTT, 10 μCi [35S]dATP (>1000 Ci/mmol, NEN), and 2 units of Sequenase (USB) was added. The resulting 10-μl mixture was divided equally into four tubes, each containing 2 μl of 80 μM dATP, dTTP, dCTP; 50 mM NaCl; 10% DMSO; and 8 μM ddGTP, 0.08 μM ddATP, 8 μM ddTTP, or 8 μM ddCTP. The samples were incubated at 37°C for 5 min, 2 μl of chase solution containing 50 mM NaCl, 10% DMSO, 0.25 mM dGTP, dATP, dTTP, and dCTP were then added and the reaction was kept at 37°C for another 5 min. Then 4 μl of stop solution was added to each tube. The samples were heated at 75°C for 2 min immediately before electrophoresis.

Allele-Specific Oligonucleotide Hybridization and Population Screening. A pair of oligonucleotides (17-mers) was synthesized according to the determined sequence for the