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Molecular Genetics of Phenylketonuria in East Asia

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Abstract Phenylketonuria is a highly heterogeneous disorder for which more than 150 different mutations have been identified world wide. These mutations exhibit an association with restriction fragment-length polymorphism haplotypes at the phenylalanine hydroxylase gene. A population genetic study for phenylketonuria revealed that each mutation may have originated in different populations, spreading in prehistoric and/or posthistoric times with the founder effect, genetic drift, and bottleneck effect, and arose and/or accumulated after Asian and Caucasian people diverged. Missense mutations have been examined by in vitro expression analysis, and a significant correlation has been observed between residual phenylalanine hydroxylase activity and clinical phenotypes.

Key Words:Phenylketonuria, Molecular genetics, Mutation

Introduction

Phenylketonuria (PKU) is an autosomal recessive disorder caused by a deficiency of hepatic phenylalanine hydroxylase (PAH). The incidence of PKU in Japan is 1/120,000, and is much less than that in Caucasians; 1/10,000, or Chinese 1/18,000. PKU is characterized by an accumulation of phenylalanine in the serum, and causes severe mental retardation unless the child is maintained on a strict low-phenylalanine diet. Newborn mass screening for early detection and treatment of PKU is performed, and has improved the prognosis of the disease dramatically. Its clinical phenotype varies widely from non-PKU hyperphenylalaninemia to classical PKU, and the phenotypic heterogeneity of this disease has been suggested to reflect the heterogeneity at a molecular level.

molecular diagnosis by polymorphism of genomic DNA

The study of PKU at a molecular level began with the detection of eight restriction-fragment-length polymorphisms (RFLP) at the PAH locus[1], and now more than 50 different RFLP haplotypes have been found. In northern Europe, RFLP haplotypes in PKU are evenly distributed from haplotype 1 to 4, and indicate that about 90% of all individuals are heterozygouse. These RFLP haplotypes segregate in a mendelian manner, and such analysis can be used in prenatal diagnosis for most families with a PKU patient[2].

The RFLP haplotype analysis among Asians has indicated that a distribution of haplotypes distinct from that of Caucasians, and that RFLP haplotype 4 is most common, accounting for more than 80% of both normal and mutant chromosomes in East Asians[3]. Due to the lack of heterogeneity among East Asians, RFLP haplotype analysis is of limited use for prenatal diagnosis of PKU in East Asians. Recently, a VNTR system and a short tandem repeat (STR) system which are polymorphisms of genomic DNA at PAH locus have been reported[4,5].

Especially, STR has heterozygosity of about 75% in Orientals (mainly Chinese) and about 80% in European Caucasian populations. In our kindred analysis of the STR system, 15 of 19 Japanese families with PKU indicated heterozygosity of STR, and the theoretical probability of heterozygosity in the Japanese was about 70% from our analysis (unpublished data). This STR system is also used for prenatal diagnosis and carrier screening in Japanese.

mutations and population genetics

The strong associations between some haplotypes and PKU chromosomes suggest that these haplotypes contain a single, predominant PAH mutation. The IVS12ntl splicing mutation on haplotype 3 and the R408W missense mutation on haplotype 2 have been identified by direct molecular analysis of PKU chromosomes[6,7]. Now, more than 150 different single base substitutions and deletions have been observed in the PAH gene of Caucasians by DNA sequencing and Southern blotting. The distribution and associations observed between certain PKU mutations and specific RFLP haplotypes in discrete European populatins suggests that the present distribution of mutant PAH chromosomes in Europe may by due to a founder effect and genetic drift. For example, the immigration of R408Q from Eastern to Western Europe and the immigration of IVS12nt-1 from Northern Europe to surrounding countries[8].

In East Asians, 25 PKU mutations have been identified, which are eighteen missense mutations, four nonsense mutations, three splicing mutations, and one deletion. Only five mutations, R111X, R261X, V388M, R408W and R408Q have been found in both Caucasians and East Asians with different haplotypes involving a C-to-T transition in the CpG dinucleotide, at a frequency of 42-fold that of random mutation. These five mutations probably occurred independently after racial divergence between Caucasians and East Asians. In other words, generally PKU mutations have occurred after racial divergence. We identified mutations of 60%, 70% and 45% of all the PKU alleles in the Japanese, Chinese, and Korean patients, respectively, by the population genetic study with 10 East Asian PKU mutations[9]. The spectrum of mutations in the Japanese PKU patients was similar to that in the Chinese patients, and was different from that in the Korean patients. The R413P mutation is found to be prevalent in northern China (13.8%) as well as in Japan (19.7%), and the R413P mutation might have occurred in the northern Mongoloid ancestor population, and then spread to northern China and Japan with the founder effect or genetic drift. The IVS4 nt-1 mutation is found to be prevalent in Korea (18.8%) and southern China (15.2%), and a founding population with the IVS4nt-1 mutation in the southern Mongoloid group may have immigrated to Korea, or Korea to southern China, where the frequency of the mutation increased through the founder effect and genetic drift.

correlation between biochemical phenotype and genotype

PKU genotype and in vitro PAH activity in expression analysis were found to be correlated with the clinical and biochemical phenotypes in Caucasians[10]. We also found correlation between clinical phenotype and genotype in East Asians[11]. The patients were classified into one of the 4 groups based on their in vitro PAH activity when at least one allele was involved in a mutation identified as one of the 9 East Asians mutations (Fig. 1). The pretreatment phenylalanine level of the patients in the severe mutation group was significantly higher than that in the patients with the R241C mutation ($p<0.01$) or the R243Q mutation ($p<0.02$). The others did not show any significant difference. From the data obtained on the Caucasians and East Asians, the PKU genotype regulates the in vivo PAH activity and the clinical phenotype of PKU. Both the genetic factor (gene dosage and negative alleleic complementation) and the environmental factor are considered not to have an important role for the ultimate manifestation of PKU phenotypes. The phenotypic heterogeneity in PKU and hyperphenylalaninemia patients reflects an underlying allelic heterogeneity, and the determina-

tion of genotypes is useful in the prediction of biochemical and clinical phenotypes in patients with PKU.

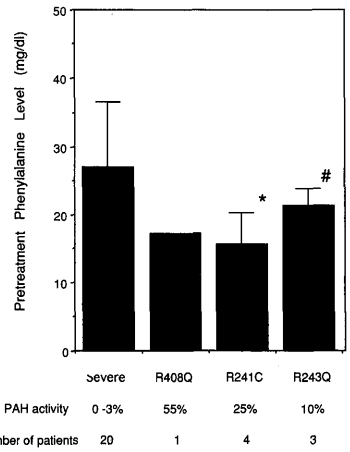

Fig. 1 Correlation between genotype and clinical phenotype. The severe mutations (0-3% group) are R111X, IVS4nt-1, IVS7nt-2, W356X, and R413P. Predicted PAH activity indicates average levels of PAH activity with each mutant enzyme in expression analysis. Unknown means mutation is not detected or any mutations. *: The severe mutation group was significantly different from R241C group ($p<0.01$) by Students' t test. #: The severe mutation group was significantly different from R243Q group ($p<0.05$) by Students't test.

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