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THE MUTATIONS FOUND IN 6-PYRUVOYL-TETRAHYDROPTERIN SYNTHASE DEFICIENT PHENYLKETONURIA

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ABSTRACT. 6-Pyruvoyl-tetrahydropterin synthase (PTPS) deficiency (MIM 261640) is the major cause of tetrahydrobiopterin (BH_4) deficient phenylketonuria (PKU). Five single base mutations at nucleotides 155 (A→G), 166 (G→A), 259 (C→T), 286 (G→A), and 317(C→T) on PTPS cDNA were detected in Chinese PTPS deficient PKU by reverse transcription (RT)-polymerase chain reaction (PCR) and solid phase DNA sequencing. These nucleotide alternations result in Asn→Ser, Val→Met, Pro→Ser, Asp→Asn, and Thr→Met amino acid changes at codon 52, 56, 87, 96, and 106, respectively. The allele frequency of these mutations in Chinese PTPS deficient PKU were determined to be around 32% (A155G), 7%(G166A), 39% (C259T), 11% (G286A), and 4% (C317T) respectively, by analysis of 17 PTPS-deficient patients from 14 unrelated Chinese PTPS deficient PKU families. The ΔT^{164}-G^{186} (Lys54stop) mutation, previously identified in Caucasian PTPS deficiency, was also detected in normal Chinese lymphoblast and to less extent in normal fibroblast and hepatoma cell lines, which indicates that the ΔT^{164}-G^{186} alternation in PTPS cDNA may be a result of alternative RNA splicing instead of a mutation causing PTPS deficiency.

INTRODUCTION. Phenylketonuria (PKU) may be caused by deficiency of phenylalanine hydroxylase or tetrahydrobiopterin (BH_4), the essential cofactor for the aromatic amino acid hydroxylases. 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency (MIM 261640) is the major cause of BH_4 deficient PKU [1]. Although the incidence of PKU in southern Chinese was found to be around 1/33, 000 by neonatal screening in Taiwan. About one third of the southern Chinese PKU is caused by BH_4 deficiency, which is much more prevalent than that found in Caucasian PKU [2]. In this report, five single base mutations at nucleotides 155 (A→G), 166 (G→A), 259 (C→T), 286 (G→A), and 317(C→T) on PTPS cDNA were identified in 14 Chinese PTPS-deficient families.

MATERIALS AND METHODS. The PTPS-deficient families were not related and were described elsewhere [3]. Fifty apparently normal Chinese for mutation survey were not related. The methods for mutational analysis by reverse transcription-polymerase chain reaction (RT-PCR) were performed as described [3]. The RT-PCR products of 50 normal Chinese were subjected to *Fok* I and *Bsi*HKA I (New England Biolabs Inc., Beverly, Massachusetts) digestion to detected the G286A and C317T substitutions, respectively. To survey whether the G166A substitution was polymorphism or not, the PCR product of the genomic DNA of 50 individuals were subjected to solid phase DNA sequencing.

RESULTS. In addition to the previously reported A155G and C259T mutations [3], three single base substitutions, namely G166A, G286A, and C317T, were identified in the PTPS cDNA of 14 PTPS-deficient families. These G166A, G286A, and C317T permit amino acid changes at codon 56, 96,106 as Val→Met, Asp→Asn, and Thr→Met, respectively. None of the 50 normal Chinese revealed these three nucleotide substitutions. By analysis of 17 PTPS-deficient patients from 14 unrelated Chinese PTPS deficient families, the allele frequencies of A155G, G166A, C259T, G286A, and C317T were determined to be around 32%, 7%, 39%, 11%, and 4%, respectively. In this study, a 23-bp deletion extended from nt 164T to 186G (ΔT^{164}-G^{186}) at PTPS cDNA was found in both normal and mutant lymphoblasts and to less extent in normal fibroblast and hepatoma cell lines. This deletion resulted in a frameshift after codon 54 followed by premature termination (Lys54stop).

DISCUSSION. In this communication, three additional mutations, G166A, G286A, and C317T, were identified from 28 Chinese mutant alleles. The data that none of the 100 apparently normal PTPS alleles shown these mutations, strongly suggest that G166A, G286A, and C317T alteration are not polymorphisms but mutations that cause PTPS deficiency. The allele frequency of these mutations in Chinese PTPS deficient PKU were determined to be around 32% (A155G), 7%(G166A), 39% (C259T), 11% (G286A), and 4% (C317T) respectively. The A155G and C259T mutations account for 71% of mutant alleles indicated that these two mutations are common mutations for Chinese PTPS-deficient PKU. Current prenatal diagnosis of PTPS deficient phenylketonuria is performed by determining PTPS activity in fetal blood and analysis of neopterin/biopterin quantity in amniotic fluid [4, 5]. With the understanding of the mutations on PTPS gene, molecular analysis may provide as an effective aid to prenatal diagnosis by amniocentesis and may be applied to prenatal diagnosis at early pregnancy by chorionic villi sampling. Previously, the ΔT^{164}-G^{186} deletion was identified as Lys54stop mutation in Caucasian PTPS deficiency. In this study, the ΔT^{164}-G^{186} deletion could be detected in both normal and mutant Chinese lymphoblast and to less extent in normal fibroblast and hepatoma cell lines. This finding might indicates the ΔT^{164}-G^{186} alternation in PTPS cDNA may be a result of alternative RNA splicing instead of a mutation causing PTPS deficiency.

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Phenylketonuria (PKU) may be caused by deficiency of phenylalanine hydroxylase or tetrahydrobiopterin (BH_4), the essential cofactor for the aromatic amino acid hydroxylases. 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency (MIM 261640) is the major cause of BH_4 deficient PKU. Although the incidence of PKU in southern Chinese was found to be around 1/33, 000 by neonatal screening in Taiwan. About one third of the southern Chinese PKU is caused by BH_4 deficiency, which is much more prevalent than that found in Caucasian PKU.

To investigate the molecular basis of PTPS deficient PKU in Chinese, total RNA isolated from fibroblasts and lymphoblasts was amplified by reverse transcription (RT)-polymerase chain reaction (PCR) and sequenced by solid phase DNA sequencing technique. Five single base mutations at nucleotides 155 (A→G), 166 (G→A), 259 (C→T), 286 (G→A), and 317(C→T) on PTPS cDNA were detected in Chinese PTPS deficient PKU. These nucleotide alternations result in Asn→Ser, Val→Met, Pro→Ser, Asp→Asn, and Thr→Met amino acid changes at codon 52, 56, 87, 96, and 106, respectively. The G166A, A155G, and C317T were novel mutations found in PTPS gene. The allele frequency of these mutations in Chinese PTPS deficient PKU were determined around 29% (A155G), 7%(G166A), 39% (C259T), 11% (G286A), and 4% (C317T) respectively, by analysis of 28 PTPS mutant alleles from 14 unrelated Chinese PTPS deficient PKU families. The ΔT^{164}-G^{186} (Lys54stop) mutation, previously identified in Caucasian PTPS deficiency, was also detected in normal Chinese lymphoblast and to less extant in normal fibroblast and hepatoma cell lines, which indicates that the ΔT^{164}-G^{186} alternation in PTPS cDNA may be a result of alternative RNA splicing instead of a mutation causing PTPS deficiency. With the understanding of the mutations on PTPS gene, molecular analysis may provide as an effective aid for carrier detection as well as prenatal diagnosis.