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Identification of a Common 6-Pyruvoly-Tetrahydroptrin Synthase Mutation at Codon 87 in Chinese Phenylketonuria Caused by Tetrahydrobiopterin Synthesis Deficiency

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Phenylketonuria (PKU) may be caused by deficiency of phenylalanine hydroxylase or tetrahydrobiopterin (BH4), the essential cofactor for the aromatic amino acid hydroxylases. 6-pyruvoly-tetrahydropterin synthase (PTPS) deficiency is a major cause of BH4 deficiency PKU. About one third of southern Chinese PKU are caused by BH4 deficiency and this frequency is more prevalent than that of Caucasian PKU population. In this report we identify a single base transition of C to T at nucleotide 259 on PTPS gene, which have not been reported previously, in Chinese PTPS deficiency siblings by reverse trancription-polymerase chain reaction (RT-PCR). This nucleotide alteration results in amino acid change from proline to serine at codon 87. The Pro87 is an evolutionary conserved amino acid and locates at the edge of α-helices B of PTPS. Thus the Pro87Ser change is highly likely to disturb the a - helices B and causes the functional consequence. The C259T missense mutation can be identified by analysis of Bbv I restriction fragments of RT-PCR products and is found to account for about 42% (11/26) of 26 Chinese PTPS mutant alleles studied. But none of 100 normal chromosomes are found to have this C259T. The result indicates this C259T transition may be a common mutation in Chinese PTPS deficient patients.

J. Biomed. Lab. Sci. 1996;8(2):A12.

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variety of autosomal recessive conditions. These involve enzymic or DNA analysis of blood samples to identify asymptomatic heterozygotes, who can then be offered genetic counselling. Alternative specimens to peripheral blood have been utilised for genetic diagnosis, including buccal cells, hair roots, ufine, Guthrie cards, and mouthwash. To simplify the logistics of population screening, we have developed an optimised mouthwash specimen collection technique.

Specimens for genetic analysis were obtained by subjects rinsing their mouths with tap-water. DNA was isolated by simply boiling the centrifuged infranatant in NaOH. A single mouthwash yielded an average of 150 µg of DNA (equivalent to a 4mL peripheral blood collection). There was no observed degradation of genomic DNA by intrinsic nucleases in the specimen for up to two weeks between specimen collection and DNA isolation, even when specimens were stored at room temperature.

Genetic analysis by polymerase chain reaction amplified fragment length polymorphism (PCR-AFLP) encountered no inhibition of amplification in more than 120 random mouthwash specimens. There was also no inhibition of PCR amplification in mouthwash samples obtained from subjects following cigarette smoking, eating, brushing teeth with toothpaste, gargling with peroxide or inhaling salbutamol or beclomethasone asthma medication. Genetic analysis of mouthwash agreed with leukocyte DNA, and hair root DNA PCR-AFLP results.

We field-tested client acceptance of mouthwash samples at several metropolitan senior high schools as part of a Tay-Sachs disease community genetic screening programme. There was a 25% increase in the rate of client consent for testing when testing with mouthwash was offered as an alternative to venepuncture.

This simplified, optimised mouthwash collection technique appears to yield the ideal specimen for genetic screening. It can be easily and rapidly collected, has high client acceptance, requires no collection equipment, tolerates delay between collection and analysis and yields large amounts of DNA which is readily amplified with fidelity and without inhibition.

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Identification of a common 6-pyruvoyl-tetrahydropterin synthase mutation at codon 87 in Chinese phenylketonuria caused by tetrahydrobiopterin synthesis 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 is the major cause of BH_4 deficient PKU. About one third of phenylketonuria in southern China is caused by BH_4 deficiency, which is much more prevalent than that found in Caucasian phenylketonuria. Recently, human PTPS cDNA has been cloned and thus facilitated molecular characterisation of PTPS deficiency. To investigate the molecular basis of PTPS deficient PKU in Chinese, total RNA isolated from fibroblasts was amplified by reverse transcription (RT)-polymerase chain reaction (PCR) and sequenced by a solid phase DNA sequencing technique. A single base transition of C to T at nucleotide 259 (C259T) on PTPS cDNA was identified in a non-consanguineous Chinese family with two PTPS deficient siblings. This nucleotide substitution has not been reported previously. The C259T alteration results in an amino acid change from proline to serine at codon 87. The Pro87 is an evolutionarily conserved amino acid located at the edge of α-helices B of PTPS. Thus the Pro87Ser change is highly likely to disturb the α-helices B of PTPS and cause the functional deficiency. The C259T missense mutation can be identified by analysis of the *Bbv* I restriction fragments of RT-PCR products and was found to account for about 42% (11/26) of 26 Chinese PTPS mutant alleles studied. None of 100 normal chromosomes was found to have this C259T transition. The results indicate that this C259T transition may be a common mutation in Chinese PTPS deficient patients. With the understanding of the PTPS gene mutation, molecular analysis may provide an effective aid for carrier detection as well as for prenatal diagnosis.

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Molecular pathology and prenatal diagnosis of haemoglobinopathies in Southern Turkey

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In this study, data on prenatal diagnosis of β-thalassemia and abnormal haemoglobin cases referred to our laboratory are presented. The prenatal diagnosis programme was started in Çukurova University Medical Faculty, Turkey, in 1992.

Blood samples from members of families at risk of haemoglobinopathies were taken into EDTA; chorionic villus sampling (CVS) was done by the gynaecologist after the 8th week of gestation. Haematological parameters were obtained from a Coulter Counter. Haemoglobin electrophoresis were done on cellulose acetate. HbF was determined by alkali denaturation and HbA_2 by column chromatography and HPLC using a cation exchange (Poly CAT-A) column. DNA was isolated from leucocytes and CVS by the methods of Poncz and Modell, respectively. The amplification refractory mutation system (ARMS™) technique was used for the identification of common mutations found in Çukurova. Sequence analysis was applied to cases that could not be determined by the ARMS™ method. HbS and HbD were verified by restriction enzyme analysis (DdeI and EcoRI). Variable number of tandem repeats (VNTR) analysis was applied to heterozygous cases to investigate possible contamination by maternal decidua. Up to the present, a total of 86 parents and CVSs have been analysed. The partners were AS, AE, A/βthal, AD, and SS in various combinations. The results of the CVSs were as follows: 48 were carriers of the above abnormalities, 16 were normal, 8 cases were SS and one was SE. Six of the CVSs could not be identified, two of them due to inadequate CVS, and four of the mutations could not be ascertained.

B441
Genetic heterogeneity of β-thalassaemia in Southern Turkey

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β-thalassaemia is the most common genetic abnormality causing a major health problem worldwide. Çukurova, the southern part of Turkey, being on the Mediterranean, is in the thalassaemic belt. Since there is no cure for the disease at present, the frequency of the mutation types of β-thalassaemia must first be identified to aid in clinical follow-up and prenatal diagnosis. For this purpose carriers identified during a screening survey and patients referred to our laboratory were studied. Haematological parameters were obtained from a Coulter Counter; Hb electrophoresis was performed on cellulose acetate. HbF and HbA_2 were determined by

Proceedings of the XVI International Congress of Clinical Chemistry

London, 1996.

Meeting of the International Federation of Clinical Chemistry

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  T Kishimoto Osaka Japan
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PUBLISHED BY
THE ASSOCIATION OF CLINICAL BIOCHEMISTS

ISSN 0959-9029

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THE ACTIVE SITE STUDIES OF VACUOLAR PYROPHOSPHATASE FROM HIGHER PLANT.

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Plant vacuoles possess a novel H^+-translocating pyrophosphatase (H^+-PPase), which catalyzes inward electrogenic H^+-translocation. In our studies, the tonoplast H^+-PPase was purified from etiolated mung bean (*Vigna radiata* L.), and the molecular mass of the single polypeptide on SDS-PAGE was 73 kDa. Size exclusion gel filtration chromatography yielded a molecular mass of 145 kDa for the native enzyme, suggesting that the tonoplast H^+-PPase of mung bean is a homodimer. We investigated the structure and function of the active site of this H^+-translocating enzyme by using the group specific chemical probes to identify the essential amino acid residues in the active site. All chemicals employed caused marked inactivation of enzymatic activities and its associated H^+-translocation. The kinetic analysis and protection study indicated that at least one essential carboxylate residue, one essential tyrosine residue, and one essential lysine residue are involved in the enzymatic activity, probably at the catalytic site of H^+-PPase. The exactly site of these essential amino acid residues will be identified and confirmed with peptide mapping.

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CHARACTERIZATION OF STARCH AND ENDOAMYLASES OF SWEET POTATO

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Among the sweet potato tuberous root enzymes that cleave α-1,4-glucosidic bonds of starch, we have studied extensively the exoamylolytic phosphorylase and β-amylase but not the endoamylolytic α-amylase (AA). We have experienced great difficulties in proving the presence of AA in growing and resting roots, and never attempted to prove the presence of another endoamylolytic activity exhibited by a debranching enzyme (DE) that hydrolyzes α-1,6-glucosidic bonds.

In order to study the starch metabolizing enzymes of sweet potato, use of starch substrates isolated from the plant is desirable. So, we first purified amylose and amylopectin from sweet potato starch by established methods; they were obtained in 99.3 and 63.9% purity, respectively. By using these preparations as standards, the amylose/amylopectin ratio of sweet potato starch was estimated by the spectrophotometric measurement of starch-iodine complex. The average chain length of amylopectin was determined by the periodate oxidation method.

By using dextran and β-limit dextrin as substrates and a crude extract of sweet potato tuberous root as enzyme, the hydrolytic products were characterized by changes in iodine coloration and reducing sugar analysis. The results inferred the presence of both AA and DE. These results will be confirmed by using the isolated sweet potato starch samples as substrates and analyzing the hydrolytic products by a Dionex HPLC dedicated for carbohydrate analysis.

Acknowledgment: The authors thank the National Science Council for providing an undergraduate training grant in support of this project.

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Purification and characterization of a glycerol oxidase from *Penicillium* sp. TS-622

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A novel extracellular glycerol oxidase was purified 39-fold from wheat bran culture of a soil-isolated *Penicillium* strain TS-622 with an overall yield of 3%. The addition of Triton X-100 into the extraction buffer improved the extraction yield by 90 times, indicating that the enzyme is bound to the cell surface. The molecular weight of this enzyme was 400,000 as determined by by size exclusion high-performance liquid chromatography. The optimum pH was from 6 to 7 and the optimum temperature was 45℃. This enzyme showed high specificity toward dihydroxyacetone and glycerol. It was inhibited by KCN, NaN_3 and hydroxylamine.

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IDENTIFICATION OF A COMMON 6-PYRUVOYL-TETRAHYDROPTERIN SYNTHASE MUTATION AT CODON 87 IN CHINESE PHENYLKETONURIA CAUSED BY TETRAHYDROBIOPTERIN SYNTHESIS 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 is a major cause of BH_4 deficient PKU. About one third of southern Chinese PKU are caused by BH_4 deficiency and this frequency is more prevalent than that of Caucasian PKU population. In this report we identify a single base transition of C to T at nucleotide 259 on PTPS gene, which have not been reported previously, in Chinese PTPS deficient siblings by reverse transcription-polymerase chain reaction (RT-PCR). This nucleotide alteration results in amino acid change from proline to serine at codon 87. The Pro87 is an evolutionary conserved amino acid and locates at the edge of α-helices B of PTPS. Thus the Pro87Ser change is highly likely to disturb the α-helices B and causes the functional consequence. The C259T missense mutation can be identified by analysis of the *Bbv* I restriction fragments of RT-PCR products and is found to account for about 42% (11/26) of 26 Chinese PTPS mutant alleles studied. But none of 100 normal chromosomes are found to have this C259T. The result indicates This C259T transition may be a common mutation in Chinese PTPS deficient patients.