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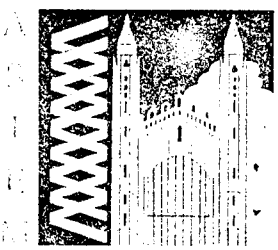

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ABSTRACTS

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317-O

MOLECULAR CHARACTERIZATION OF GALACTOKINASE DEFICIENCY IN JAPANESE

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Galactokinase (GALK) deficiency is an autosomal recessive disorder and causes cataract formation. Using DGGE and sequence analysis, we found 13 missense mutations (M1I, S33P, N39S, E43K, G137R, A198V, R256W, R277Q, T288M, M307R, S313L, T344M, G349S), a nonsense mutation (E245X), two deletions (410delG, 509-510delGT) from 16 Japanese patients with GALK deficiency. Using the COS cell expression analysis, in vitro GALK activities of missense mutations were remarkably reduced, and corresponded with those in patients' RBC. The immuno-reactivity of mutant constructs indicated qualitative and quantitative abnormalities of GALK proteins. We found the new variant "Osaka" in Japanese, which was associated with A198V. The GALK activities in the patients' RBC with null and A198V were 9.0 - 13.9%, consistent with 18.5% in expression analysis. The population genetics study of A198V revealed 24/582 (4.1%) in Japanese, 8/288 (2.8%) in Koreans, 1/264 (0.4%) in Taiwanese, and 0/188 (0%) in Caucasians. The Japanese patients with bilateral cataracts had a relatively high frequency of A198V (15/200, 7.5%, no significance). This new "Osaka variant" is prevalent in Koreans and Japanese populations, and may be possible to be one of the factors for presenile cataracts.

318-P

MEASUREMENT OF BLOOD GALACTOSE ON FILTER PAPER FOLLOWING DERIVATIZATION WITH 8-AMINO-2-NAPHTHALENSULFONIC ACID BY REVERSED PHASE HPLC IN GALACTOSEMIA

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Deficiency of galactose -1-phosphate uridyl transferase(GALT) is a polymorphic enzyme and Duarte(D) is the most common enzyme variant. A new reversed-phase HPLC method has been developed for the measurement of galactose in blood (50μL) on Guthrie filter paper using 8-amino-2-naphthalenesulfonic acid (8,2-ANS) as derivatization reagent for the diagnosis of Galactosemia. Galactose was extracted from blood spot on filter paper and derivatized with 8,2-ANS to produce Schiff bases, and reduced under sodiu m cyanoborohydride. The linear range was between 7.2 μ g/dL and 29 mg/dL and the limit of detection (S/N=3) of this method was 90 ng/dL. The mean recovery of galactose was 104.29 % with a S.D. of 3.62 % with correlation coefficiency of 0.9999. Control range of blood galactose in Korean newborn was below 6 mg/dL for male and female without any gender difference (n=5). We applied 11 anonymous blood spots in which diagnosis has already been made by enzyme assay as one of the GALT or epimerase deficiencies. All the patients blood spots showed abnormal elevation of galactose. These results suggest that the developed method could be a practical tool for the diagnosis of Galctosemia with high sensitivity.

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DIAGNOSIS OF DOPA-RESPONSIVE DYSTONIA (DRD) AND OTHER TETRAHYDROBIOPTERIN (BH4) DEFECTS BY STUDY OF PTERIN METABOLISM IN CYTOKINE STIMULATED FIBROBLASTS

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Autosomal recessive BH4 deficiencies (GTP cyclohydrolase I, GTPCH; 6-pyruvoyl-tetrahydropterin synthase, PTPS; dihydropteridine reductase, DHPR) are usually diagnosed by the presence of hyperphenylalaninemia, analysis of urinary and CSF pterins, followed by enzyme assays and mutation analysis. DRD, the autosomal dominantly inherited GTPCH deficiency, is diagnosed mainly by clinical criteria and CSF examination. In order to establish a diagnostic tool for these disorders, neopterin (N) and biopterin (B) production in cytokine (IFNγ and TNFα)-stimulated fibroblasts as well as enzyme activities were measured in 22 patients with different BH4 disorders and 38 controls. Reference ranges for N and B production and enzyme activities were age-dependent. GTPCH-deficient cells showed very low N and B levels; enzyme activity was significantly decreased, being lower in DRD. In PTPS-deficient cells very high levels of N and no B were produced. DHPR-deficient cells exhibited intracellular pterins levels in the normal range, and no detectable enzyme activity. In conclusion, cultured skin fibroblasts are a useful system for the diagnosis of all forms of BH4 deficiency. The same method was used successfully in cultured amniocytes for prenatal diagnosis of PTPS and DHPR deficiencies. Fibroblasts from DRD patients showed typical pterin production patterns and low residual enzyme activity; both are essential for the diagnosis of this disease.

086-P

THE STUDY OF TETRAHYDROBIOPTERIN DEFICIENCY IN NORTHERN CHINESE WITH HYPERPHENYLALANINEMIA

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A screening program for tetrahydrobiopterin (BH_4) deficiency in hyperphenylalaninemia (HPA) from northern Chinese has been performed at China–Japan Friendship Hospital since 1993. The following tests were done in 280 patients with HPA: (i) analysis of pterins in urine; (ii) measurement of dihydropteridine reductase activity in erythrocytes from Guthrie cards. Of the 280 HPA patients, 10 patients with BH_4 deficiencies have been recognized as a result of screening carried out. All of these 10 BH_4 deficient patients suffered from 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency. The frequency of BH_4 deficiency is 3.6% in northern Chinese HPA. Four single base mutations at nucleotides 166(G>A), 209 (T>A), 259(C>T), and 286(G>A) on PTPS cDNA were detected in northern Chinese PTPS-deficient HPA by polymerase chain reaction and solid phase DNA sequencing. By analysis of 18 PTPS mutant alleles from 9 unrelated northern Chinese PTPS-deficient HPA families, the allele frequencies of these mutations were determined to be 5.6% (166G>A), 5.6% (209T>A), 61.0% (259C>T) and 27.8% (286G>A), respectively. The latter two mutations are common mutations in northern Chinese PTPS-deficient HPA. Eight patients suffering from PTPS deficient HPA were treated with neurotransmitter precursors as well as with tetrahydrobiopterin, and were followed up by health workers. Five patients were treated when the infants were less than 6 months old, the mental and physical developments of them were normal. Three patients were treated at the age of 1–2 years, although their neurological symptoms were remarkably improved, they still have various degrees of mental retardation. These results again suggest that early differential diagnosis of HPA is essential for the treatment and outcome of HPA patients.

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