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Identification of FRAXA and FRAXE syndromes by using a non-radioactive duplex PCR and Southern blot analysis in Chinese mentally retarded males from Taiwan

C.-H. Chen[1,2], C.-J. Horng[4], P.-W. Shyu[3], M.-Y. Liu[5], S. Wang-Wuu[3], K.-D. Wuu[3], K.-J. Hsiao[3,5]. [1]Division of Psychiatry,Cheng Hsin Rehabilitation and Medical Center; [2]Division of Neuropsychiatry, School of Medicine and [3]Institute of Genetics, Yang Ming University; [4]Department of Psychiatry and [5]Medical Research, Veterans General Hospital-Taipei, Taiwan

Two folate-sensitive fragile sites, FRAXA and FRAXE, at the long arm of X chromosome are associated with mental retardation. Both syndromes have similar molecular pathogenesis, i.e. abnormal expansion of CGG trinucleotide repeats at FMR1 gene and FMR2 gene, respectively. FRAXA syndrome is the most common inherited form of mental retardation with the prevalence of 1/1,500-1/3,000 in male Caucasians, while FRAXE syndrome is rare with the estimated prevalence of about 1/50,000 in male Caucasians. To survey the prevalence of both FRAXA and FRAXE mental retardation in Chinese population, a non-radioactive duplex PCR method (Wang et al. J Med Genet 1995;32:170-173) and Southern blot analysis were used to study a cohort of Chinese mentally retarded males from Taiwan. The PCR method which detects normal sized CGG triplet alleles, but not abnormally expanded CGG triplet alleles, was used as an initial screening method. Positive cases were further analyzed by Southern blot analysis. Of 273 mentally retarded males studied, 4 cases were confirmed to have full mutation of CGG triplet expansion of FMR1 gene. One severely mentally retarded male was found to have full mutation of GCC expansion of FMR2 gene. Thus, the prevalence rate of FRAXA and FRAXE syndromes among Chinese mentally retarded males in the present study are 0.014 and 0.03, respectively. The FRAXE mentally retarded male identified in the present study, to our knowledge, is the first case reported in Chinese population.

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Fluorescent quantitative multiplex PCR for the screening of deletions in rBAT, one of the genes responsible for cystinuria M. J. Calonge[1,2], A. Zorzano[2], X. Estivill[1], M. Palacin[2], V. Nunes[1]. [1]Dept. Genetica Molecular. IRO. L'Hospitalet de Llobregat. Barcelona. Spain. [2]Dept. Biochemestry. Universitat de Barcelona. Spain.

Cystinuria is an autosomic recessive disease characterized by hyperexcretion of cystine, lys, arg and|orn. rBAT, a gene involved in the transport of these aminoacids through the apical membrane of the epithelial cells of the S3 segment of the nephron, has been found to be responsible for the subtype I of this disease. Up to now 22 mutations, found mainly by SSCP analysis, have been described. Most of them, 17, are missense, altough there are also frameshift and stop mutations. Two big deletions involving either the first exons or the last five exons were initially found by loss of heterozygosity. In the Spanish population only mutation M467T has been found, it accounts for 30% of the type I cystinuric chromosomes. In order to increase this percentage the promoter region of the gene could be studied. The existence of deletions could also account for these not yet characterized mutations. Classically, Southern analysis is the technique used to search for them. However it has the drawbacks of the need of large quantities of DNA in good conditions, and a lot of hands on work time. To overcome that and to speed up and simplify the analysis we have developed two quantitative multiplex PCRs to amplify the ten exons of the rBAT gene. Intronic primer pairs, amplifying complete exons, have been pooled in two groups together with external gene primer pairs used as controls for double dosis. The amount used of each primer pair is optimized for equal amplification. PCRs are only 20 cycles to ensure we are in the exponential phase of the reaction. For detection, a fluorescent nucleotide is added to the PCR and the fragments are analysed both for size and intensity using a 373A DNA sequencer and the Genescan software. Radioactive nucleotides, or radioactivelly labelled primers can also be used, using densitometry for fragment intensity analysis. This multiplex approach can also be used for rapid SSCP study of this gene in cystinuric patients. Three deletions have already been found using this method. Analysis of more patients could show deletions to be a frequent type of rBAT mutations.

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Novel Pax6 homeodomain mutations in congenital aniridia and identification of a new alternative splicing of Pax6 mRNA

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Aniridia is a congenital human panocular defect characterized by an iris hypoplasia. To date this condition is associated with more than 30 distinct Pax6 gene mutations. The Pax6 gene was screened for mutations in 8 families with aniridia, 2 familial forms and 2 sporadic cases of Peters' syndrome. The Pax6 exons were submitted to SSCP analysis. In aniridia patients, exons exhibiting variant migration patterns were sequenced. In Peters' syndrome cases, the entire coding region was sequenced. In aniridia patients, two novel mutations were found in Pax6 gene : a 15 bp deletion in exon 5 and a 2 bp deletion in exon 7. The former deprives the protein of the 1st α-helix, thus probably hampering the Pax6-protein DNA binding ability. The latter introduces premature stop codons leading to a truncated protein. In contrast, no mutations were found in the coding sequence of the Pax6 gene in patients with Peters' syndrome. In patients as well as in controls, an alternative splice donnor site was found in exon 6, skipping the major part of this exon (nt 574 to 774) from the mRNA. The predicted role of the shortened protein remains unclear. In conclusion, we report two novel Pax6 gene mutations in congenital aniridia while no mutations were found in the coding regions of Pax6 gene in 4 Peters' syndrome patients.

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Prenatal diagnosis of nephrogenic diabetes insipidus. SM Carter[1] HM Nitowsky[1], C Chazotte[1], L Shah[1], DG Bichet[2]. [1]Division of Reproductive Genetics, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY; [2]University of Montreal, Montreal, Quebec.

X-linked nephrogenic diabetes insipidus (NDI) is a rare disorder with defective renal and extrarenal arginine-vasopressin V2 receptor (AVPR) responses due to mutations in AVPR2 gene in Xq28. The urinary concentrating defect is associated with episodes of hypernatremia and severe dehydration that may lead to brain damage and mental retardation (MR). Prenatal diagnosis of the condition in a male fetus may help avert the deleterious effects of episodic dehydration postnatally by use of unrestricted water intake, a low osmolar diet, and diuretics.

A 35-year-old Puerto Rican G5P3014 woman presented for amniocentesis because of age-related risks and history of NDI in 3 sons, two of whom were MZ twins who had MR as a sequel of repeated episodes of dehydration. The affected hemizygous males and heterozygous mother carried the W296X nonsense mutation in the AVPR2 gene. Amniocentesis at 16 weeks revealed a normal male (46,XY) karyotype. DNA sequence analysis revealed the W296X mutation. The pregnancy was followed to term with a NSVD of a 3.38 kg male infant. The initial urine osmolality was low (162 mOsm/kg). Breast feeding was advised because of the lower osmolality of human milk. To date the infant has done well with unlimited water feedings q2h and thiazide diuretics. Prenatal diagnosis of NDI can optimize postnatal outcome and avoid the potentially serious sequelae of NDI.

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Sequence analysis of exon eight of MAO-A gene in alcoholics with antisocial personality and normal controls. S. Chakraverty, D. Tribune, C. R. Cloninger and A. Parsian, Washington University School of Medicine, St. Louis, MO, USA.

Brunner et al. 1993 (Science 262:592-93) reported a family in which several males were affected by a syndrome of borderline mental retardation and abnormal behavior. This syndrome was associated with a complete and selective deficiency of enzymatic activity of MAO-A. Sequencing of exon eight of the MAO-A gene revealed four base substitutions, three of which were neutral polymorphisms. However, the fourth mutation at position 936 (C→T), which results a termination codon, was associated with the syndrome in the family.

In order to determine the possible role of any mutation in exon eight of MAO-A gene in susceptibility to alcoholism, we sequenced genomic DNA from 50 alcoholics with antisocial personality and 50 normal controls. We detected only the point mutation at the position 941 (T→G). Additional samples of alcoholics with (n=44) and without (n=60) antisocial personality and normal controls (n=38) were screened for this mutation by PCR assay. The comparison between alcoholics with antisocial personality and normal controls for mutation frequency at the position 941 was significant ($p<0.05$). However, the differences between alcoholics without antisocial personality and normal controls for the same mutation was not significant. The difference between the mutation frequency in two types of alcoholics also was not significant. We are in the process of genotyping alcoholic families with the mutation at the position 941 of MAO-A gene to perform linkage analysis.

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Two folate-sensitive fragile sites, FRAXA and FRAXE, at the long arm of X chromosome are associated with mental retardation. Both syndromes have similar molecular pathogenesis, i.e. abnormal expansion of CGG trinucleotide repeats at FMR1 gene and FMR2 gene, respectively. FRAXA syndrome is the most common inherited form of mental retardation with the prevalence of 1/1,500-1/3,000 in male Caucasians, while FRAXE syndrome is rare with the estimated prevalence of about 1/50,000 in male Caucasians. To survey the prevalence of both FRAXA and FRAXE mental retardation in Chinese population, a non-radioactive duplex PCR method (Wang et al. J Med Genet 1995;32:170-173) and Southern blot analysis were used to study a cohort of Chinese mentally retarded males from Taiwan. The PCR method which detects normal sized CGG triplet alleles, but not abnormally expanded CGG triplet alleles, was used as an initial screening method. Positive cases were further analyzed by Southern blot analysis. Of 273 mentally retarded males studied, 4 cases were confirmed to have full mutation of CGG triplet expansion of FMR1 gene. One severely mentally retarded male was found to have full mutation of GCC expansion of FMR2 gene. Thus, the prevalence rate of FRAXA and FRAXE syndromes among Chinese mentally retarded males in the present study are 0.014 and 0.03, respectively. The FRAXE mentally retarded male identified in the present study, to our knowledge, is the first case reported in Chinese population.

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Detection of factor IX gene mutations in Korean hemophilia B patients. Y. H. Cho[1], B. Y. Kim[1], S. H. Shim[1] and G. Kong[2]. Department of Medical Genetics[1] and Pathology[2], College of Medicine, Hanyang University, Seoul 133-791, Korea (Intro. by: Si Houn Hahn).

Hemophilia B is an X-linked recessive disease resulting from sequence alterations of the coagulation factor IX gene. Hemophilia B is caused by a variety of mutations, which can be found in the whole coding sequences. Screening of mutations in factor IX gene of 8 Korean hemophilia B patients was performed. Eight exons, promoter region and their intron boundaries were amplified and the amplified products were analyzed by single strand conformation polymorphism(SSCP) with PhastSystem(Pharmacia) employing silver staining protocol. Six patients(75%) showed altered migration patterns on SSCP analysis in exon G or exon H. Altered migration patterns were further characterized with direct sequencing of amplified products. One G to A transition at nucleotide 30075(Gly208Ser), one G to T transversion at nucleotide 30098(Trp215Cys), two C to G transversion at nucleotide 31121(Ala334Gly) and two C to A transversion at nucleotide 31168(Phe349Leu) were detected. Of the four mutations, mutations at codon 208, 334 and 349 are the novel mutations of factor IX gene.