A11

Screening of Fragile Mutations at Fraxa and Fraxe Lociin Mentally Retarded Males Using a Nonradioactive PCR Method

P.-W. Syu, M.-Y. Liu, M.-L. Chen, Y.-L. Lee, C.-J. Horng, K.-J. Hsiao and C.-H. Chen

Institute of Genetics, and Div. of Neuropsychiatry, School of Medicine, Yang Ming University;
Dept. of Psychiatry, and Med. Research, Veterans General Hospital-Taipei;
Div. of Psychiatry, Cheng Hsin General Hospital, Taipei, Taiwan

Fragile X syndrome is the most common inherited form of mental retardation in Caucasians. Abnormal expansion of a polymorphic CGG repeats at 5' end of FMR-I gene was found to be associated with FRAXA fragile site in mentally retarded patients. Recently, another CGG triplets expansion was found to be associated with another fragile site, FRAXE, which is 600 Kb distal to FRAXA site. Patients with abnormal CGG expansion at FRAXE site also have mental impairment.

To facilitate the genetic studies of fragile X syndrome, we adopted a convenient, non-radioactive duplex PCR method for screening fragile mutations at FRAXA and FRAXE loci simultaneously. This method can amplify DNAs containing CGG triplets of FRAXA and FRAXE from normal individuals, and visualize PCR products with ethidium bromide staining after electrophoresis in agarose gel, whereas abnormal expansions from patients can not be amplified by this method. In our preliminary screening of 200 male mentally retarded patients, 5 patients were positive for FRAXA mutation, and 4 patients were positive for mutations at FRAXE site. These patients need to be confirmed by southern blotting analysis, and the nature of these mutations remains to be characterized.

Our study demonstrates a convenient duplex PCR method for screening of FRAXA and FRAXE mutations in male patients with mental retardation. This method is potentially useful for large scale epidemiological survey, and for quick evaluation of referral patients with mental deficiency.

J. Biomed Lab Sci. 1996;8(2):A11

P161

SCREENING OF FRAGILE MUTATIONS AT FRAXA AND FRAXE LOCI IN MENTALLY RETARDED MALES USING A NON-RADIOACTIVE PCR METHOD

[1]P.-W. Shyu, [4]M.-Y. Liu, [3]M.-L. Chen, [1]Y.-L. Lee, [3]C.-J. Horng, [1,4]K.-J. Hsiao and [2,5]C.-H. Chen. [1]Institute of Genetics, and [2]Div. of Neuropsychiatry, School of Medicine, Yang Ming University; [3]Dept. of Psychiatry, and [4]Med.Research, Veterans General Hospital-Taipei; [5]Div.. of Psychiatry, Cheng Hsin General Hospital, Taiepi, Taiwan

Fragile X syndrome is the most common inherited form of mental retardation in Caucasians. Abnormal expansion of a polymorphic CGG repeats at 5' end of FMR-I gene was found to be associated with FRAXA fragile site in mentally retarded patients. Recently, another CGG triplets expansion was found to be associated with another fragile site, FRAXE, which is 600 Kb distal to FRAXA site. Patients with abnormal CGG expansion at FRAXE site also have mental impairment.

To facilitate the genetic studies of fragileX syndrome, we adopted a convenient,
non-radioactive, duplex PCR method for screening fragile mutations at FRAXA and FRAXE loci simultaneously. This method can amplify DNAs containing CGG triplets of FRAXA and FRAXE from normal individuals, and visualize PCR products with ethidium bromide staining after electrophoresis in agarose gel, whereas abnormal expansions from patients can not be amplified by this method. In our preliminary screening of 200 male mentally retarded patients, 5 patients were positive for FRAXA mutation, and 4 patients were positive for mutations at FRAXE site. These patients need to be confirmed by southern blotting analysis, and the nature of these mutations remains to be characterized.

Our study demonstrates a convenient duplex PCR meethod for screening of FRAXA and FRAXE mutations in male patients with mental retardation. This method is potentially useful for large scale epidemiological survey, and for quick evaluation of referral patients with mental deficiency.

P162

DEVELOPMENT OF TWO ONE-STEP IMMUNOASSAYS FOR DETECTION OF METHAMPHETAMINE AND MORPHINE IN URINE

Ching-Ying Chen*, Mei-Chun Yang, Jiuan J. Liu, Tong H. Chang. Cell Biology and Immunology Division, Development Center for Biotechnology, Taipei, R.O.C.

Methamphetamine (MA) and heroin abuse are the common abused grugs in Taiwan. The official cut-off for MA is 500 ng/mL, and for Morphine is 300 ng/mL. We have developed two one-step immunochromatographic assays for detecting MA and Mor, respectively, in urine. One-step immunoassay is based on the principle of competition. Drug in specimens competes with the drug-carrier to bind to the antibody-colored latex conjugates. The sensitivity and specificity of the MA one-step immunoaassay was greater than 99% as compared to the result by GC/MS (gas chromatography/mass spectron) analysis. For the sensitivity and specificity of the Mor one-step immunoaassay, the results are same as MA assay. This method is convenient and easy to perform within 5 minutes and can screen a lot of urine samples. Therefore these two kits can substitute for the imported products.

P163

DEVELOPMENT OF A DIRECT COMPETITIVE ENZYME-LINKED IMMUNOSORBENT ASSAY KIT FOR DETECTION OF MORPHINE (MOR) IN URINE

Jia-Chang Hung*,Kuo-Lan Wen,Jiuan J. Liu, Tong H. Chang. Cell Biology and Immunology Division, Development Center for Biotechnology, Taipei, R.O.C.

Heroin is a common abused drug in Taiwan and the trend of addicts is increased. Morphine (Mor) is the major metabolite of heroin. Therefore,it is a need to develop a diagnostic kit for Morphine. The official cut-off for Mor is 300 ng/ml. A direct competitive ELISA was designed to detect Mor in urine. In this assay, Mor in urine was competed with the Mor-Enzyme conjugate for binding to a limited amount of anti-Mor monoclonal antibodies immobilized on microtiter wells. The presence of Mor in urine above 10 ng/ml can be detected in this assay. The precision of this assay as determined by inter and intra-assay variation was below 10 %. The sensitivity is 97.8 % and specificity is 96.9 % compared to GC/MS result. Urine samples can be analyzed within 30 minutes. This assay is sensitive and easy to perform. This ELISA kit has a good potential for quantitative screening of the presence of Mor in urine.

P164

DEVELOPMENT OF AN ENZYME IMMUNOASSAY FOR ENDOGENOUS OUABAIN-LIKE FACTOR IN HUMAN

Y.W. Chin, M.H. Lin, K.J. Tsai, D.T.Y. Chiu, T.Z. Liu and J.S. Wei#
School of Medical Technology, Chang Gung College of Medicine and Technology, Kweishan, Taoyuan, Taiwan

Previous reports suggest that natural ligands of the digitalis receptor of the sodium, potassium adenosine triphosphatase may exist in the mammalian body. In this report, we describe the development of an enzyme immunoassay (EIA) for ouabain since it was suggested as one of the endogenous digitalis-like factors in human.

For the production of rabbit antiserum, three ouabain-protein conjugates, namely, ouabain-C_6 spacer-bovine serum albumin, ouabain-C_6-ovalbumin, and ouabain-succinyl dihydrazide spacer-ovalbumin were prepared for use as immunogens. Antibodies against the above-mentioned ouabain-carrier protein conjugates were obtained and immobilized on polystyrene beads for antigen capture EIA. Ouabain-horseradish peroxidase conjugate was prepared similarly via reductive amination between the oxidized ouabain and the enzyme tag. Standard curve of this EIA consisting ouabain concentration ranged from 10 to 75 pg/mL was achievable. The mean ± S.E.M of ouabain-like factor in sera of ten students was determined by this EIA as 53 ± 5 pg/mL.