Molecular Genetic Analysis of Korean Patients with Fragile X Syndrome

Young-Min Choi, M.D., Do-Young Hwang, M.D., Jin Choe, M.D.,
Sun-Hee Park, M.D., Seok-Hyun Kim, M.D., Sei-Won Yang, M.D.,
Soo-Cheol Cho, M.D., Shin-Yong Moon, M.D., Jin-Yong Lee, M.D.
Dept. of Obstetrics and Gynecology, Dept. of Pediatrics, Dept. of Psychiatry,
College of Medicine, Seoul National University,
Seoul, Korea

Fragile X syndrome is the most common cause of inherited mental retardation. The diagnosis of fragile X syndrome was originally based on the expression of a folate-sensitive fragile site at X chromosome (Xq27.3) in cell culture under conditions of folate deprivation. The cytogenetic study test has limitations, especially in testing for carrier status, and it exhibits a high degree of variability between individuals and laboratories. In 1991, the fragile X gene (FMR-1) was characterized and the nature of the fragile X mutation has been elucidated. At present in developed countries, DNA testing has been utilized in the diagnosis of fragile X syndrome and its carriers.

This study was undertaken to analysis the FMR-1 gene in Korean patients with fragile X syndrome and its carriers and to establish the methodology for molecular genetic diagnosis of fragile X syndrome and its carrier. Three male patients with fragile X syndrome previously diagnosed by cytogenetic analysis and their family members, as well as 16 persons proven cytogenetically normal were analysed by direct genomic Southern blot analysis and polymerase chain reaction (PCR) for the status of FMR-1 gene. Southern blot analysis consisted of double DNA digestion with EcoRI and EcoRI and the hybridization with StBr 12.3 probe. Two kinds of PCR methods were used. The one method involved the addition
of radioactive dNTP in PCR reaction, and the other involved Southern transfer of amplified PCR products and the hybridization analysis with synthetic oligonucleotide probe.

In normal females and males, direct genomic Southern blot analysis revealed the patterns as follows: 2.8 kb band in normal male, and 2.8 kb band with 5.8 kb band in normal female. And the patients(male) with fragile X syndrome showed the band sized more than 5.8 kb, indicating the expansion of FMR-1 gene and loss of EcoR1 restriction site within the gene. The mothers of patients showed the carrier pattern: 2.8 kb with 2.8 + 5 kb band, and 5.2 kb with 5.2 + 1 kb band. The father of a patient with fragile X syndrome showed the normal pattern.

PCR analysis of the cytogenetically normal males and females revealed the normal patterns, namely the bands of sizes expected from the primers used. However, either PCR method failed to reveal full mutation alleles in patients with fragile X syndrome. And pre-mutation alleles could be detected in two mothers of patients by PCR method. The father of a patient with fragile X syndrome showed the normal pattern by either PCR method.

These data suggest that Southern blot analysis can be used in the accurate diagnosis of fragile X syndrome and its carrier and that PCR method can be utilized to exclude the diagnosis of fragile X syndrome in unaffected males.

**Key Words**: Fragile X syndrome, Mental Retardation, Southern blot analysis, PCR.