

Abstract 74

SELECTIVE IDENTIFICATION OF LYMPHOBLASTOID EXTRACTS FROM FRAGILE-X OR CONTROL SUBJECTS UTILIZING MONOCLONAL ANTIBODIES TO FMR1 (FMRP)

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Fragile X syndrome is one of the most common forms of inherited childhood developmental disabilities. This syndrome results from a triplet repeat mutation that generates a fragile site on the X chromosome and is characterized by cognitive and behavioral abnormalities. Presently, Fragile-X diagnosis is confirmed by identification of increased trinucleotide repeats and DNA methylation changes in the FMRP gene. Such DNA testing is costly and time-consuming. Our goal has been to develop an inexpensive protein based diagnostic assay that could be used for population screening. Our group has previously reported on the generation of a large array of monoclonal antibodies to FMRP using recombinant mouse or human baculovirus expressed immunogens. Antibody specificity was confirmed by comparing reactivity to FMRP (FMR1) relative to FXR1 or FXR2 proteins. In the present study, immunoassays were developed utilizing these monoclonal antibodies to examine FMR1 expression in lymphocytes isolated from Fragile X affected individuals and age-matched controls. FMRP expression is drastically reduced or absent from cell isolates from individuals with Fragile X syndrome and, therefore, the absence of FMRP protein is diagnostic. The current studies were performed using EB-transformed human B lymphocyte isolated from the blood of our test subjects. Various extraction protocols were examined to optimize FMRP solubilization and identification. Proteins extracted from such cell lines were examined by ELISA, Luminex and Western blot immunoassays using our array of monoclonal antibodies to FMRP. This report will detail these studies and the resultant rapid non-invasive and highly specific approach for the diagnosis of Fragile X syndrome. Our goal is to extend these studies for application to primary B lymphocytes obtained from suspected Fragile X individuals in this protein based diagnostic paradigm.

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