Journal of Autism and Developmental Disorders, Vol. 12, No. 3, 1982

Brief Note

Autism is Associated with the Fragile-X Syndrome

W. Ted Brown,[1] Edmund C. Jenkins, Erwin Friedman, Judith Brooks, Krystyna Wisniewski, Simhachalam Raguthu, and Joseph French
New York State Institute for Basic Research in Developmental Disabilities

INTRODUCTION

The fragile-X (fra(X)) syndrome has been recognized recently as a cause of nonspecific mental retardation (Giraud, Aymes, Mattei, Mattei, 1976; Harvey, Judge, & Wiener, 1977; Turner & Opitz, 1980). This syndrome is inherited in an X-linked recessive manner. Epidemiological studies give an incidence estimate of .92 per 1,000 males (Herbst & Miller, 1980). This frequency indicates that fra(X) may be second only to Down syndrome as an etiology of mental retardation in males. Fra(X) males have no specific physical abnormalities. They may have thin faces and long ears. Macroorchidism is often present in adults, and testicular volumes are frequently 2-6 times normal (Turner & Opitz, 1980; Brown, Mezzacappa, & Jenkins, 1981). Some fra(X) subjects have been reported to have a deficit of language (Jacobs, Glover, Mayer, Fox, Gerrard, Dunn, Herbst, 1980; Howard-Peebles, 1980). The range as well as the frequency of cognitive and behavioral manifestation in individual with fra(X) syndrome remains to be fully described.

Autistic individuals are usually males, and a 4:1 male to female ratio is common (Wing, Yeats, Brierly, & Gould, 1971). Since the fra(X) syndrome may be a common genetic syndrome associated with mental retardation in normal appearing males, we undertook to determine whether there is an association between autism and the marker X chromosome.

[1]Address all correspondence to Dr. W. Ted Brown, New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, New York.

0162-3257/82/0900-303$03.00/0

We have found the fra(X) syndrome to be present in four autistic males (Brown, Friedman, Jenkins, Brooks, Wisniewski, Raguthu, & French, 1982). We wish to report briefly here on our methods for establishing the presence or absence of the fra(X) chromosome and to discuss some of the clinical implications of these findings.

METHOD

The fra(X) syndrome is diagnosed by the presence of an abnormally appearing site, referred to as a fragile site, on the X chromosome at q27-28 following induction in a special medium (Harvey et al., 1977; Turner & Opitz, 1980). Fragile site induction can occur by growth in low folic acid media or by adding 5-fluorodeoxyuridine (FUdR) to short-term, phytohemagglutinin (PHA)-stimulated peripheral blood cultures during the last 24 hours of incubation (Glover, 1981; Tommerup, Poulsen, & Brondum-Nielsen, 1981). Our protocol for fra(X) identification (Brown et al., 1981) is as follows. A 5-ml sample of heparinized peripheral blood is required. Blood is drawn into a 5-ml syringe containing .05 ml heparin (Scientific Products) 1,000 U/ml. Then .1 to .2 ml of heparinized blood is added to 4 ml of cell culture medium that contains RPMI 1640 (GIBCO), 15% dialyzed fetal bovine serum (FEBS), 1 mM glutamine, 10,000 U (or μg/ml) of PEN-Strep (GIBCO), and .2 ml% PHA-P (Difco). The starting pH is 7.7, and a basic pH is maintained through 4 days of incubation at 36.5-37° C.

During the last 24 hours of culture, FUdR (Calbiochem) is added to replicate cultures at final concentrations of .05, .1, and .4 μM. Colchicine (Nutritional Biochemicals) is added during the last 2-2½ hours of culture so that the final concentration is .5 μM. Each culture is then exposed to 10 ml of .075 M KCl (hypotonic solution) for 7-10 minutes at 37° C and then fixed in 3:1 methanol:glacial acetic acid. After being washed several times in fresh fixative, the cellular suspension is dropped onto slides that are previously chilled in distilled water. A tabletop centrifuge (GLC-1, Sorvall) is used to wash the cells at a speed of 1,200 rpm. Except for the hypotonic solution, which requires a 10-min centrifugation time, all aliquots are about 5 ml, and centrifugation time is usually 5 minutes.

Air-dried slides are kept on the bench for about 2 days and are then incubated at 60° C for at least 3 hours or overnight. The slides are then G-banded according to a modification of the technique of Klinger (1972). They are exposed to a solution of trypsin-EDTA made from reconstituted 20-ml stock solution (GIBCO) that contains 5.0 gm trypsin (1:250) and 2.0 gm EDTA/L of normal saline. One ml of stock solution is allowed to stand for 30 min at room temperature and is then diluted with 50 ml of Hanks

balanced salt solution (HBSS) at a pH of 6.8. The slides are continuously agitated during immersion. After a few minutes (optimal times are determined for each culture), trypsin digestion is arrested by immersing the slides for 10 min into HBSS supplemented with 5% FBS. After a rinsing in HBSS, the slides are stained for a few minutes in a 2% solution of Giemsa (Gurr's). The Giemsa is made up by diluting stock Giemsa with Gurr's buffer, pH 6.8. The slides are then rinsed in two changes of buffer (1 min each) and allowed to air-dry. They are then coverslipped with mounting medium and scanned for fragile X chromosomes.

Fragile X chromosomes are scored using bright-field microscopy with a 100X oil immersion objective. Other autosomal fragile sites are not uncommon and can be used as an indication that the FUdR fragile site induction system is effective, whether or not the individual is fragile X positive. Therefore, a procedural internal control is present in the system. Initial analysis entails the examination of 20 cells. An individual is considered fragile positive if he presents with 3 or more positive cells in a total of up to 100 cells ($\geq$ 3%).

RESULTS

Currently, we have identified five males with the fra(X) syndrome who had a previously established clinical diagnosis of autism. They were referred to us for diagnostic evaluation, and fra(X) chromosome testing was effected. The first patient, age 17, had a family history of mental retardation. A brother and two male maternal first cousins were known to be retarded but not autistic. Macroorchidism was present and 38% (10/26) of the cells were fra(X) positive. The second autistic child was unrelated. At age 16, he was evaluated for fra(X) after enlarged tests were noted on a physical examination. The frequency of fra(X) after enlarged testes were noted on a physical examination. The frequency of fra(X) positive cells was 32% (10/31). The third and fourth autistic subjects were brothers aged 12 and 6. The older brother had 25% (5/20) and the younger brother had 24% (4/17) fra(X) positive cells. A fifth autistic child, age 11, presented for testing because his mother wished a prenatal evaluation for fra(X). He had 4% (3/75) present on initial testing. All five cases met the general diagnostic criteria for autism—namely, impaired social relationships, delayed and deviant language development, stereotyped or ritualistic behavior, and onset before the age of 30 months (Rutter, 1977). Family studies now in progress will be presented in detail later.

Currently, we have studied a total of 16 fra(X) positive families. In 12 families, the affected males were not autistic. The 22 fra(X) positive males we have identified have uniformly been retarded. Their I.Q.s range from

10 to 60. The average frequency of fra(X)| positive cells in 22 affected males was 32%, with a range of 4-70%. In 15 of the 22 individuals, a frequency greater than 20% was obtained by examination of only 20-30 cells.

DISCUSSION

Genetic causes for autism are suggested by the significant concordance for autism in identical twins (Folstein & Rutter, 1977) and sex-linked genetic factors by the high male-to-female ratio of about 4:1 (Wing, Yeates, Brierley, & Gould, 1971). Although no primary genetic cause has been found, it has been noted that some genetic syndromes and a number of neurological conditions have been associated with some cases of autism. Thus, a significant number of cases of phenylketonuria (PKU) are associated with autism (Friedman, 1969). Chess (1971) found that 8% of cases of congenital rubella manifested autism. In a study of 64 patients with a diagnosis of autism selected from 1,900 cases evaluated for developmental delay (Knobloch & Pasamanick, 1975), 14 were found to have PKU. Among the remaining 50, 24 had no diagnosis while 26 had a specific condition. Nine had cerebral palsy, and 17 had a variety of genetic and neurologic syndromes that included trisomy 21, Hurler's disease, Schilders acute disseminated sclerosis, congenital hypothyroidism, congenital rubella, congenital syphilis, Dandy Walker syndrome, craniostenosis, Cornelia de Lange syndrome, and a familial metabolic defect of undetermined cause. In a study of fra(X)| families by Turner, Daniel, and Frost (1980), one fragile X positive male had been reported to be autistic.

We have found that macroorchidism is useful as a screening procedure to identify postpubescent males likely to have fra(X) (Brown et al., 1981). This simple test may be a useful way to identify autistic males likely to have the fra(X) syndrome. The testicular volume can be determined by measuring the width and length, then multiplying the width times the width times the length times .52 (Π/6). A volume greater than 25 cc is considered to indicate macroorchidism. By this criterion, autistic males with macroorchidism would be candidates for X chromosome testing.

Families with two or more autistic or retarded males in which there is apparent X-linked inheritance will presumably have the highest likelihood of being fra(X) positive. However, by Haldane's formula (Vogel & Motulsky, 1979), one-third of cases might be expected to be new mutations, since the condition is usually an X-linked genetic lethal. Therefore, screening of all autistic males of undiagnosed etiology should be considered in spite of an apparent lack of familial incidence. At the present time, this might overwhelm most cytogenetic laboratories.

Information about the diagnosis of fra(X) syndrome will allow genetic counseling and recurrence risks to be given to the affected families. We have demonstrated the feasibility of prenatal fra(X) chromosome diagnosis using amniotic fluid cells (Jenkins, Brown, Duncan, Brooks, Ben-Yishay, Giordano, & Nitowsky, 1981). This approach has been applied in several cases (Webb, Butler, Insley, Weaver, Green, & Rodeck, 1981; Shapiro, Wilmont, Brenholz, Leff, Martino, Mahoney, & Hobbins, 1982). The procedure makes primary prevention and early intervention feasible. In addition, the possible treatment of fra(X) syndrome by folic acid administration has been suggested by Lejeune (1982). The efficacy of this treatment must await controlled trials.

From our study, we conclude the fra(X) syndrome is associated with some cases of autism. Further, our finding of five autistic males suggests that the association may show a significant frequency. The actual frequency has yet to be determined by a large-scale screening of autistic male individuals. At this time, we suggest that fra(X) chromosome screening of any autistic males with undiagnosed etiology should be considered.

REFERENCES

Brown, W. T., Friedman, E., Jenkins, E. C., Brooks, J., Wisniewski, K., Raguthu, S., & French, J. H. Association of fragile X syndrome with autism. *Lancet,* 1982, *I,* 100.

Brown, W. T., Mezzacappa, P., & Jenkins, E. C. Screening for fragile X syndrome by testicular size measurement. *Lancet,* 1981, *II,* 1055.

Chess, S. Autism in children with congenital rubella. *Journal of Autism and Childhood Schizophrenia,* 1971, *1,* 33-47.

Folstein, S., & Rutter, M. Infantile autism, a genetic study of 21 twin pairs. *Journal of Child Psychology and Psychiatry,* 1977, *18,* 297-321.

Friedman, E. The autistic syndrome and phenylketonuria. *Schizophrenia,* 1969, *1,* 249-261.

Giraud, F., Aymes, S., Mattei, J. F., Mattei, M. G. Constitutional chromosomal breakage. *Human Genetics,* 1976, *34,* 125-136.

Glover, T. W. FUdR induction of the X chromosome fragile site: Evidence for the mechanism of folic acid and thymidine inhibition. *American Journal of Human Genetics,* 1981, *33,* 234-242.

Harvey, J., Judge, C., & Wiener, S. Familial X-linked mental retardation with an X chromosome abnormality. *Journal of Medical Genetics,* 1977, *14,* 46-50.

Herbst, D. S., & Miller, J. R. Nonspecific X-linked mental retardation II: The frequency in British Columbia. *American Journal of Medical Genetics,* 1980, *7,* 461-469.

Howard-Peebles, P. N. Fragile sites in human chromosomes II: Demonstration of the fragile site Xq27 in carriers of X-linked mental retardation. *American Journal of Medical Genetics,* 1980, *7,* 497-501.

Jacobs, P. A., Glover, T. W., Mayer, M. Fox, P., Gerrard, J. W., Dunn, H. G., Herbst, G. S. X-linked mental retardation: A study of 7 families. *American Journal of Medical Genetics,* 1980, *7,* 497-489.

Jenkins, E. C., Brown, W. T., Duncan, C., Brooks, J., Ben-Yishay, M., Giordano, F. M., & Nitowsky, H. M. Feasibility of fragile X chromosome prenatal diagnosis demonstrated. *Lancet,* 1981, *II,* 1292.

Klinger, H. P. Rapid processing of primary embryonic tissue for chromosome banding pattern analysis. *Cytogenetics,* 1972, *11,* 424-435.

Knobloch, H., & Pasamanick, B. Some etiologic and prognostic factors in early infantile autism and psychosis. *Pediatrics,* 1975, *55,* 182-191.

Lejeune, J. Is the fragile X syndrome amenable to treatment? *Lancet,* 1982, *I,* 273.

Rutter, M. Infantile autism and other childhood psychosis. In M. Rutter & L. Heuson (Eds.), *Child psychiatry: Modern approach.* Oxford: Blackwell, 1977.

Shapiro, L. R., Wilmot, P. L., Brenholz, P., Leff, A., Martino, M., Mahoney, M. J., & Hobbins, J. C. Prenatal diagnosis of fragile X chromosome. *Lancet,* 1982, *I,* 99-100.

Tommerup, N., Poulsen, H., & Brondum-Nielsen, K. 5-fluoro-2'-deoxyuridine induction of the fragile site on X28 associated with X linked mental retardation. *Journal of Medical Genetics,* 1981, *18,* 374-376.

Turner, G., Daniel, A., & Frost, M. X-linked mental retardation, macroorchidism, and the Xq27 fragile site. *Pediatrics,* 1980, *96,* 837-841.

Turner, G., & Opitz, J. M. Editorial comment: X-linked mental retardation (Special issue on Fra(X)). *American Journal of Medical Genetics,* 1980, *7,* 407-415.

Vogel, F., & Motulsky, A. G. Haldane's indirect method for mutation rate estimation. In F. Vogel & A. G. Motulsky (Eds.), *Human genetics: Problems and approaches.* Berlin-Heidelberg: Springer-Verlag, 1979.

Webb, T., Butler, D., Insley, J., Weaver, J. B., Green, S., & Rodeck, C. Prenatal diagnosis of Martin-Bell syndrome associated with fragile site at Xq27-28. *Lancet,* 1981, *II,* 1423.

Wing, L., Yeates, S. B., Brierley, L. M., & Gould, J. The prevalence of early childhood autism: Comparison of administrative and epidemiological studies. *Psychological Medicine,* 1971, *46,* 89-100.