PHENOTYPIC, GENETIC AND CYTOGENETIC STUDIES IN INFANTILE AUTISM

A THESIS

PRESENTED TO

THE DEPARTMENT OF HUMAN GENETICS

FACULTY OF MEDICINE

UNIVERSITY OF MANITOBA

In Partial Fulfillment of

the Requirements for the Degree

MASTER OF SCIENCE

by

PARUL JAYAKAR, M.B.B.S, D.C.H.

ISBN 0-315-86126-6

Canada

PHENOTYPIC, GENETIC AND CYTOGENETIC STUDIES IN INFANTILE AUTISM

BY

PARUL JAYAKAR

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

Acknowledgements.

I extend my sincere appreciation and gratitude to Dr. A.E.Chudley and Dr.M.Ray for their patient guidance and encouragement throughout this study. My special thanks to Dr.J.Evans for her constructive criticisms.

In addition , I would also like to thank the parents of the patients and the control subjects, for their willingness to participate in this study, without whose cooperation this study would not have been possible.

I am also grateful to Dr.R.Wand and Dr.J.Perlov for doing the psychological assessments, to Mary Cheng for her advice on statistical analysis, to Tranna Homenick for her secreterial assistance and to Joan Knoll for her help in chromosome analysis.

This study was supported by a grant from the Children's Hospital of Winnipeg Research Foundation,Inc.

Dedicated to

PRASANNA AND ANUJ

TABLE OF CONTENTS

Page

ABSTRACT

Twenty individuals with autism or related disorders underwent chromosome analysis and physical examinations with documentation of minor anomalies. There was a higher incidence of minor anomalies in the study patients. Chromosomal anomalies were identified in three, two had heritable folate sensitive fra(2)(q13) site and one had an inv(9)(p11q12). No heritable chromosomal variants or anomalies were seen in 20 age and sex-matched control individuals. When patients with the fra(2) were excluded from the analyses, there was no difference in the frequency of chromosome breaks and/or gaps between the study group and the control group. The results of this study suggest that heritable folate sensitive fragile sites and other chromosome variants may be more commonly seen in individuals with autism or related disorders in childhood than in the general population.

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

1.1 Nature and scope of the problem

Infantile autism (IA) is a recognizable but etiologically heterogeneous group of behavioural disorders. It is relatively uncommon and affects predominantly males (Spence et al.,1973; Tsai et al.,1981). Autism has been associated with a variety of etiologies including perinatal asphyxia (Folstein and Rutter,1977a; Knobloch and Pasamanick,1975), untreated phenylketonuria (PKU)(Friedman,1979) and infantile spasms (Taft and Cohen,1971).

This condition is very distressing for children and their families. The two most striking of the many difficulties which beset these children are social and educational difficulties.

A recently described and common form of X-linked mental retardation has been shown to be associated with a fragile site at the distal end of X chromosome in affected males and most carrier females (Turner and Opitz,1980). Recognition of the association between fragile X syndrome and infantile autism has renewed the interests of geneticists in the search for chromosomal markers in infantile autism and related disorders (Brown et al.,1982 a,b; Meryash et al.,1982; Levitas et al.,1983; Jorgensen et al.,1984; Watson et al.,1984; Blomquist et al.,1985; Fisch et al.,1985;

Gillberg and Wahlstrom,1985; Brown et al.,1986).

Though as far back as 1970 , Siva Sankar reported increased rate of chromosomal breakage in autistic children, the role of other chromosomal findings such as folate sensitive fragile sites in infantile autism has not yet been defined (Sutherland and Hecht,1985a). Gillberg and Wahlstrom (1985) reported other chromosomal abnormalities including fragile X in a population of autistic children. Hence this study was undertaken to determine the frequency of chromosomal variations including folate sensitive fragile sites in individuals diagnosed as having autism.

1.2 Purpose of the study

A: General Objective

To search for major genetic and/or chromosomal anomalies or variations in a population of children identified as having IA.

B: Specific Objectives

1) To obtain detailed histories, family pedigrees, physical and behavioural profiles on children with IA.

2) To compare the incidence of chromosomal anomalies in a population of autistic children with an age and sex matched control population.

3) To identify those children in which a major chromosomal anomaly or autosomal fragile site is found and attempt to

correlate the cytogenetic findings with family history and phenotypic abnormalities.

4) To identify those individuals with the fragile X or other fragile sites and provide appropriate genetic counselling to the families.

1.3 Definitions

Some of the terms which will be frequently used are defined below.

1.3.1 Autism

Autism is defined by DSM III (American Psychiatric Association , 1980) using the following criteria:

(1) Onset prior to 30 months of age.

(2) Pervasive lack of response to other people.

(3) Gross deficits in language development.

(4) If speech is present, peculiar speech problems such as immediate and delayed echolalia, metaphorical language and pronounimal reversal.

(5) Bizarre responses to the environment, eg., resistance to change, peculiar interest in, or attachment to, animate or inanimate objects.

(6) Absence of delusions, hallucinations, loosening of association and incoherence as in schizophrenia.

1.3.2 Residual state

This is a state in which the individual once had an illness

that met the criteria for IA and the current clinical picture no longer meets the full criteria for IA but signs of the illness ,such as oddities of communication and social awkwardness, have persisted to the present.

1.3.3 Childhood Psychosis

This term is used as an umbrella diagnosis for cases showing long standing (> 1 year) severe impairment of social relationships, characterized by failure to establish peer relations, signs of aloofness, speech and language impairment,in combination with behavioural problems dominated by stereotyped mannerisms more elaborate repetitive routines and / or insistance on sameness (Gillberg, 1984).

1.3.4 Asperger syndrome

Asperger (1944) published the first account of this syndrome. He referred to it as "autistic psychopathy". Burgoine and Wing (1983) reported identical triplets with Asperger syndrome. The major features of this syndrome include lack of empathy, naive, one-sided social interaction, inability to form friends, repetitive speech, poor nonverbal communication, limited imagination, poorly coordinated movements and odd posture. Prevalance is unknown and the male to female ratio is 9:1 (Wolff and Barlow ,1979). The cause of the condition could be organic, such as pre or postnatal trauma leading to cerebral damage.

1.3.5 Fragile sites

A fragile site is defined as follows:

(1) It is a non staining gap involving both chromatids.

(2) Site specific in any given individual or kindred.

(3) It is inherited in a Mendelian co-dominant fashion.

(4) Fragility is present in form of acentric fragments, deleted chromosomes or triradial figures.

1.3.6 Fragile X

Fragile X is one form of non-specific X-linked mental retardation (MR). This is the only form that can be diagnosed by cytogenetic analysis. The affected males and some carrier females have a constriction or a fragile site near the end of the long arm of the X chromosome on the band Xq27.3 which is referred to as the fragile X (Sutherland,1977). In the other forms of X linked MR carriers and affected males are not cytogenetically distinguishable from their noncarriers and unaffected counterparts. In this study we are concerned only with the fragile X form of X-linked MR.

2.0 REVIEW OF LITERATURE

2.1 Fragile Sites

2.1.1 History of Fragile sites

The existence of sites at which chromosomal breaks are liable to occur has been known for many years. These sites were regarded as enigmatic chromosomal variations seen from time to time in some laboratories. Fragile sites were first described by Dekaban in 1965 and their heritable nature was demonstrated by Lejeune et al.(1968). The term fragile site was coined in 1969 during the study of a family with a fragile 16 (Magenis et al.,1970).

Fragile sites are never seen in 100% of the cells examined and are presumably innocuous. Most of them are not associated with phenotypic abnormalities though Sutherland (1982a) found an incidence of 1:1000 in neonates and a ten fold increase in a population of MR. William and Howell (1976) suggested that a breakage at the fragile site could give rise to a variety of aneuploid cell lines , leading to abnormal development. The variety of phenotypic abnormalities associated with the sites suggests that they are without phenotypic effect in the heterozygote. However we might speculate that homozygosity for autosomal folate sensitive fragile sites could lead to an abnormal phenotype if the fragile site is responsible for the phenotype. However, contrary to this, a normal female homozygote with a

"satellited" chromosome 17 has been reported. In general the clinical significance of autosomal fragile sites (AFS) is as yet uncertain. Smith et al.(1985) have reported a male with two autosomal folate sensitive fragile sites 9p21 and 12q31, who had an offspring with abnormal chromosomal complement 46,XY/47,XY,+ fragment.

The discovery of fragile X chromosome in 1969 represents a major advance in the field of X-linked mental retardation. It was first described in retarded males and some carrier females of a family with X-linked MR (Lubs,1969). Such a family was thought to be rare as no others immediately prior to or immediately subsequent to this description were reported (Martin and Bell,1943; Losowsky,1961; Dunn et al.,1963; Opitz et al.,1965; Snyder et al., 1969; Turner et al.,1971,1972; Fried,1972;Steel and Chorazy, 1974; Yarbrough and Howard-Peebles,1976). As a result,the association between fragile X and MR was given little attention. Then in 1976-77 the fragile X was rediscovered. Giraud et al.(1976) reported on 6 unrelated individuals with fragile X linked MR and Harvey et al.(1977) reported on 8 families with the condition.

These reports prompted other investigators to reexamine families with fragile sites. One such investigator was Sutherland (1977). His unsuccessful attempt to identify the fragile site in a family that he had studied in 1972, stimulated an investigation and eventual discovery of the

effect of culture media on fragile site expression. He found that expression of fragile site required culture media such as TC199 which are low in folic acid and thymidine. The fragile sites expressed under such conditions are known as folate sensitive fragile sites. The 7 year span between the discovery and rediscovery of the fragile X has been recently outlined by Gerald (1980,1981). It seems that around the time of the discovery of the fragile X, laboratories were changing from culture media low in folic acid and thymidine to media enriched with them. As a result the expression of the fragile site was inhibited.

2.1.2 Classes of Fragile Sites

Fragile sites can be classified according to their mode of induction as shown in Table I.

2.1.3 Clinical significance

A) Significance of fragile X

This is the only chromosomal fragile site known to be of definite clinical significance. The classical feature is mental retardation which is also seen in 1/3 of carrier females. The other features are macroorchidism in males , large and protruding ears, short stature, prominent forehead, and prognathism. Hence a male patient with unexplained MR should have chromosomal analysis to exclude the fragile X (Michels ,1985). Affected males express the fragile X or other chromosomal anomalies in 4-56 % of their lymphocytes (Howard-Peebles, 1983).

Table I Classes of Fragile Sites.

Chr *	Class I FSFS **	Class II Dist.A +	ClassIII BrdU #	Class IV Common FS $	Class V Rare FS
1				1p22,1p32	1q32
				1p36,1q25	
2	2q13,2q11			2q31,2p13,2q23	2p11
3				3p14,3p24,3p27	
5				5q31	5q35
6	6p23			6q26	
7	7p11			7q23,7p13,7q22	
8	8p22			8q22	
9	9p11,9q32			9q32	
10	10q23		10q25		
11	11q13,11q23			11p13	
12	12q13				
14				14q24	
16	16p12	16q22		16q23	
17		17p12			
19	19p13				
20	20p11				
22				22q12	22q
X	Xq27			Xp22	Xq26
Y					Yq12

* Chromosome; ** Folate sensitive fragile site;

+ Distamycin A induced; # BrdU induced; $ aphidicolin induced.

B) Significance of autosomal fragile sites

The exact significance of autosomal fragile sites is not yet fully understood. They are inherited in a Mendelian fashion in normal families although they are often reported in families with high rates of congenital anomalies (Kubien and Kleczkowska,1977). The relationship of heritable fragile sites to malignant processes is speculative (Michels,1985). Some investigators believe that some fragile sites are the result of a persistant viral infection. Hence, these are seen in patients with immunodeficiency and certain nonheritable cancers (Shabtai et al.,1983). In some cases the virus may be necessary for interaction with the inherited site to produce the microscopically visible fragile site.

2.1.4 Population Data

A) Incidence of Fragile Sites

The sole newborn chromosomal survey to detect a fragile site in the prebanding era was that of Gerald and Walzer (1970). They found one child with a fragile site on a C group chromosome among 3643 normal infants, but they had examined only 2 metaphases per child.

Estimates of the frequencies of folate sensitive fragile sites vary between 1:200 and 1:700. Sutherland (1985) conducted population cytogenetic studies to determine folate sensitive and BrdU (bromodeoxyuridine) inducible fragile sites, in 2439 randomly selected neonates, special school

students, sheltered workshop employees and patients referred for chromosome analysis. The carrier frequency of folate sensitive fragile sites was 1:700 in the neonates and 1:100 in the mentally retarded group. The frequency of fragile X in the institutionalized male retardates was 1-2 % , whereas no fragile X was detected in the neonates when cord blood was used. As many women are given oral folic acid during pregnancy fragile site expression in cord blood may be more difficult to elicit than in blood from individuals not given folic acid. Fragile site 17p12 was found in 1:368 neonates but no fragile 16q12 was detected.

In virtually all other studies for fragile sites attention has been concentrated upon fragile X and data on other fragile sites is scarce. In Italy the carrier frequency for the Distamycin A inducible fragile site 16 q22 was 1:90, while in Adelaide no case was found in 408 individuals studied. The heterozygote incidence of the BrdU requiring fragile site 10 q25 was found to be 1:40 and had a gene frequency of 0.013 (Sutherland et al.,1980).

B) Ethnic Distribution

Fragile X is found in all racial groups. These include Europeans, Japanese, Filipinos, Polynesians ,Australian aboriginals (Turner and Jacobs,1983), South African Zulus (Venter et al.,1981) and American blacks (Howard-Peebles and Stoddard, 1980). Virtually nothing is known about the presence of other fragile sites in non European populations. The most interesting fragile site to study in other racial

groups would be 10q25 which is present in polymorphic frequency in the Australian white population.

C) Segregation Patterns

I. Fragile X

a) Males. The segregation ratio in males was found to be 0.406 which is a significant difference from the expected ratio of 0.5 for a fully penetrant X-linked gene ($p<0.028$)(Sutherland and Hecht, 1985b). This result, therefore, indicated that if the fragile X truly follows a Mendelian inheritance about 20 % of males who carry the fragile X 'gene' were not identified in the families studied. Some males with the fragile X might not be classified as mentally retarded due to the mild expression of the 'gene'. In some males the fragile X is not penetrant at either the phenotypic or cytogenetic level. These males do carry the fragile X and can transmit it to their daughters and have retarded grandsons.

b) Females. Many obligate fragile X carriers do not express the fragile site. Accurate segregation studies are not possible, until more precise tests can be found.

II. Autosomal Fragile Sites

Segregation analysis indicates that autosomal folate sensitive fragile sites follow a Mendelian codominant inheritance. Segregation analysis confirmed that fragile site 10 q25 follows a codominant inheritance. This fragile site and its nonfragile allelomorph is considered to constitute the first true chromosomal polymorphism to be

described in man (Sutherland,1982b). Sherman and Sutherland (1986) performed segregation analysis on pedigrees with rare autosomal fragile sites and concluded that the gene was 50 % penetrant when transmitted by the carrier father and fully penetrant when transmitted by the carrier mother.

2.1.5 Prenatal Diagnosis

The association of the fragile site on the Xq with a form of mental retardation could provide a means of prenatal diagnosis (Sutherland,1977). Certain fragile sites have selective disadvantage and may predispose to spontaneous abortions or nonlethal damage in homozygotes . Jenkins et al. (1986) attempted prenatal detection of fragile X in amniocytes and concluded that positive results are reliable but negative or low frequency positive results should undergo further studies. There is no indication for prenatal diagnosis of autosomal fragile sites as it is yet unknown if they are associated with any specific phenotypic abnormality (Hecht and Hecht,1984).

2.1.6 Culture Conditions

A) Tissue culture media

Sutherland (1977) observed that fragile sites at 2q13,10q23, 20p11,Xq27 were only expressed when lymphocytes were cultured in medium TC 199 as the medium was deficient in folic acid and thymidine. Fragile sites expressed under such conditions became known as folate sensitive fragile sites. Once the essential conditions for the expression of

folate sensitive fragile sites became known , various other ways of inducing them were discovered, eg, adding Methotrexate (MTX) an inhibitor of folate metabolism or adding FUdR (fluorodeoxy uridine) which inhibits the enzyme thymidine synthetase.

B) Some of the factors affecting expression of folate sensitive fragile sites are discussed below.

I. Chemical Factors

Sutherland (1979) observed that by supplementing medium TC 199 with folic acid, expression was almost totally inhibited. The time of action of folic acid was probably late in the S or the early G2 phase of the cell cycle.

Mattei et al. (1981) used MTX for fragile X induction in lymphocytes and fibroblasts. MTX is supposed to act in the late S or early G2 phase. Other inhibitors of folate are some antibiotics like trimethoprim and pyrimethamine.

The ability of thymidine to inhibit the expression of folate sensitve fragile sites was discovered shortly after folic acid was found to do so. It acts in the late S2 or early G2 phase. The inhibitory effects of low levels of thymidine and folic acid are additive (Sutherland , 1979).

BrdU an analog of thymidine, inhibits the expression of folate sensitive fragile sites. BrdC (bromodeoxycytidine) has the same effect but is less toxic and can be used at higher concentrations (Sutherland et al.,1984).

Methionine is essential for expression of fragile X even under conditions of folic acid and thymidine deprivation. Attempts to confirm this need for Methionine were only partly successful, but some individuals with fragile sites at 10q23, 11q13, 12q13, Xq27 did have reduced frequency of expression in absence of methionine (Howard-Peebles et al.,1980).

Jacky and Dill (1983) examined effects of ethidium bromide, Hoechst 33258 and actinomycin D on the expression of fragile X. No effect was found for any of these substances on folate sensitive fragile sites. However they act as inducing agents for some folate insensitive fragile sites.

Foetal bovine serum is most commonly used. The amount added varies from 2-30 % . Sutherland (1979) used 5 % which gave good cell growth. Howard-Peebles and Pryor (1979,1981) claimed that high serum concentration inhibits fragile X expression, though this was not a detailed study of serum concentration versus frequency of expression.

Aphidicolin, an inhibitor of DNA polymerase alpha,induces nonrandom chromosomal gaps and breaks in cultured human lymphocytes. The bands most frequently damaged are 2q31,3p14,6q26,7q32,16q23, and Xp22. The sites most sensitive to aphidicolin damage include the 'hot spots' seen under conditions of thymidylate stress. However, fragile X which can also be induced by thymidylate stress was not

induced by aphidicolin. The hot spots induced by aphidicolin represent a new class of fragile sites called the common fragile sites (Glover et al.,1984)(Table I).

II. Physical factors

Sutherland (1979) studied the addition of folic acid to TC199 to show that it inhibited fragile site expression. The folic acid was dissolved in a bicarbonate solution. Addition of extra bicarbonate resulted in a rise of medium pH . A control experiment was performed to ensure that the addition of bicarbonate alone was not responsible for inhibition of fragile site expression. A positive correlation between increasing pH and fragile site expression was found for sites at 2q13, 20p11, Xq27, but not for those at 10q25, 11q13, 16p12 and 12q13 (Sutherland and Hinton,1981). Branda and Nelson (1982) showed that the rate of uptake of folate by cells was inversely proportional to pH and at lower pH, there were higher intracellular levels of folate in a steady state situation.

Jacobs et al. (1980), Jennings et al. (1980) and Howard-Peebles and Pryor (1981) showed that increasing the duration of lymphocyte culture from 72 hours to 96 hours , increases the fragile site expression, while Gustavson et al. (1981) found no difference. The frequency of expression reaches a maximum around 4 days and then decreases along with the quality of the chromosomal preparation. The reasons for this temporal effect are unclear but depletion of media components that inhibit the expression may account for it.

Brookwell et al. (1982) claimed that blood which has been delayed in transit, yielded lower frequencies of expression of fragile X than fresh blood , when cultured under conditions of folate deprivation, but this could be overcome by using FUdR induction. Jacky and Sutherland (1983) found no consistent decrease in fragile site expression on storage, or differences in the frequencies of expression between folate free conditions of culture and FUdR induction. Mattei et al. (1981) found a decrease in frequency of expression with storage time ; they reported a decline in metaphase quality which affected fragile site frequency.

Jacky and Dill (1980) found a higher frequency of fragile X in fibroblasts when sodium citrate was used as hypotonic instead of KCl. Howard-Peebles and Pryor (1981) reported a higher frequency of fragile X expression if slides were air dried rather than flame dried. Jacky and Dill (1983) reported that the frequency of expression increased with the length of the metaphase chromosome. Zankl and Eberle (1982) found that Giemsa staining of the chromosome along with normal bright field microscopy was least satisfactory . The frequency could be doubled by use of phase contrast and further increased if Orcein stain was used.

Eberle et al.(1982) cocultivated male fragile X lymphocytes with lymphocytes from normal males. They showed a decrease in frequency of expression of fragile sites ,

suggesting that normal cells in culture produce a soluble factor which inhibits fragile site expression. Cocultivation of leucocytes from known carrier females and affected males also decreased the expression of fragile site.

2.1.7 Cell Types Other Than Lymphocytes

Early attempts to elicit fragile site expression in fibroblasts were unsuccessful (Magenis et al.,1970). Ferguson-Smith (1973) found fragile 2q13 in fibroblasts. at a lower frequency of expression than in lymphocytes. Sutherland (1979) studied fibroblasts from carriers of fragile sites at 2q13, 10q23, 11q13, 16q22, 20p11 and Xq27 under conditions of folate deprivation . They saw only some of the autosomal fragile sites in up to 4 % of metaphases and no fragile X. The first reported demonstration of fragile X in fibroblasts was by Jacky and Dill (1980) who used rigorous folate restriction. Barbi et al. (1984) described a method for studying X activation in fragile X fibroblasts culture. It involved cell synchronization with 10 M FUdR and addition of MTX and BrdU. This method resulted in a reduced frequency of fragile X expression compared to cultures without BrdU. Fibroblasts are the only cell types other than lymphocytes in which the classes 2,3 and 4 fragile sites have been studied (Table I). Sutherland et al.(1984) reported the induction of fragile 10q25 with BrdU in fibroblasts. The frequency of expression was not as high as seen in lymphocytes and the method was unreliable

since attempts at induction were not always successful.

Lymphoblastoid cell lines (LCL) from individuals with fragile 2q13 and Xq27 were examined by Sutherland (1979) under conditions of folic acid and thymidine deprivation and were found not to express the fragile sites. Jacobs et al. (1982) showed that fragile X in LCL is not expressed under such conditions but can be induced by FUdR.

Bone marrow from a male with fragile Xq27 did not show a single fragile site in 200 metaphases (Sutherland,1979). Blood lymphocyte cultures at the same time expressed the site in 10 % . Similarly bone marrow from a carrier of 16p12 showed no evidence but the fragile sites were present in 46 % in lymphocytes.

The only fragile site which has been sought in amniotic fluid is fragile X. The preferred method of prenatal diagnosis is foetal blood sampling and lymphocyte culture (Webb et al.,1983), if fetoscopy is available and if its inherent risks are acceptable. Numerous groups have reported the prenatal diagnosis of fragile X by exposing cultured amniotic fluid cells to various conditions designed to induce this fragile site.

Very little data on hybrid cells has been published. Bryant et al. (1983) found fragile X expression depressed to 4-7 % (from 6-12 %) when fragile X fibroblasts and normal fibroblasts were fused and aminopterin was used to induce

expression. Wegner et al. (1982) were not able to induce fragile X expression in human mouse hybrids with MTX, aminopterin, or FUdR. Nussbaum et al. (1983) reported induction of the fragile site on the X chromosome using FUdR and MTX in a human/ hamster hybrid in which the fragile X was the only human chromosome present. Warren and Davidson (1984) also found fragile X in a human/hamster hybrid.

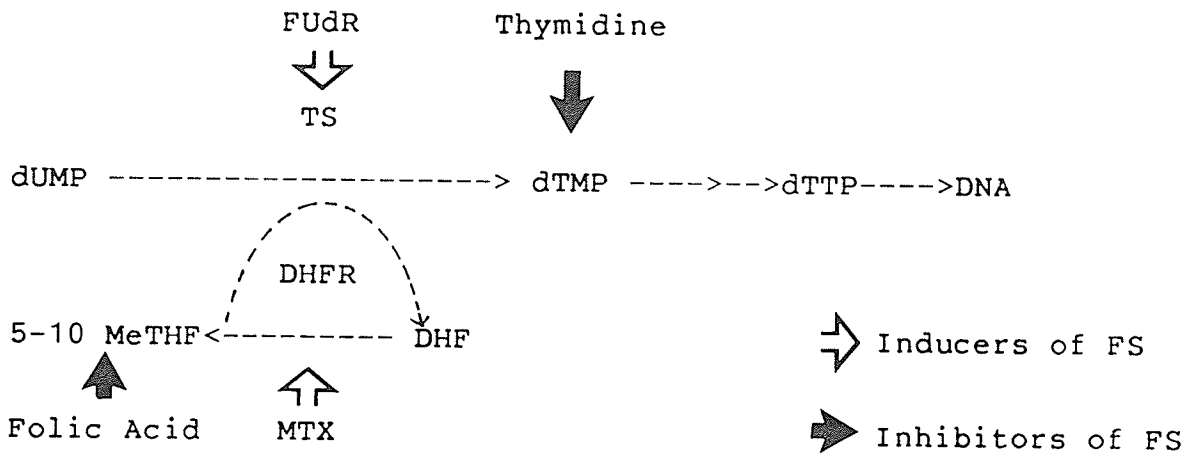
Because of the difficulties in inducing fragile sites in some cell types , reports of failure to induce them should be treated with caution. It is not inconceivable that in some forms of hybrids fragile site expression will be inhibited whereas, in others it will be easily inducible.

2.1.8 Mechanisms of Production of Fragile Sites

Sutherland (1979) proposed the possible explanations for the structural nature of the fragile sites. Chaudhari (1972) examined the nature of chromatid gaps and concluded that gaps result from extreme despiralization of the DNA due to failure of compact folding in the metaphase chromosome. Four factors were involved in the despiralization : the DNA itself , histones , non histone proteins and divalent cations. They could also be due to process operating during DNA synthesis, specifically the production of thymidine monophosphate (dTMP). The area of metabolism believed to be involved is the conversion of uridine monophosphate (dUMP) to dTMP and 5,10 methylene tetrahydrofolate (5,10-meTHF) to dihydrofolate (DHF). Both the reactions are catalyzed by

thymidylate synthetase (Sutherland 1979). Folic acid results in an increase in 5,10-meTHF which in turn leads to an increase in dTMP production whereas, thymidine is directly phosphorylated to dTMP (Sutherland,1979) (fig 1a). They thus act as inhibitors of fragile site expression by increasing the pool of dTMP available for incorporation into the DNA. Inducers of fragile sites such as MTX, FUdR, folic acid deficiency and thymidine deficiency limit the pool of dTMP available for DNA synthesis. MTX blocks the conversion of DHF back to THF by inhibiting dihydro folate reductase (Sutherland,1979) and FUdR inhibits conversion of dUMP to dTMP (Glover,1981; Tommerup et al.,1981) by inhibiting thymidylate synthetase. In the presence of sufficient thymidine supply the DNA synthesis will be completed and the fragile site will not be produced. But on thymidine deprivation, DNA synthesis will not be completed and fragile site will result. Sutherland et al. (1985) and Sutherland and Baker (1986) proposed a model for the DNA at the fragile site, composed of alternating repeating polypurine and polypyrimidine sequences. He found that folate sensitive fragile sites on human chromosomes can also be induced by high levels of thymidine but not by high levels of BrdU. High levels of thymidine lead to elevated levels of thymidine triphosphate (dTTP) which inhibits the ribonucleotide reductase catalyzed reduction of cytidine diphosphate to deoxycytidine diphosphate (Reichard et al.,1961). This results in deficiency of deoxycytidine

1a.



1b.

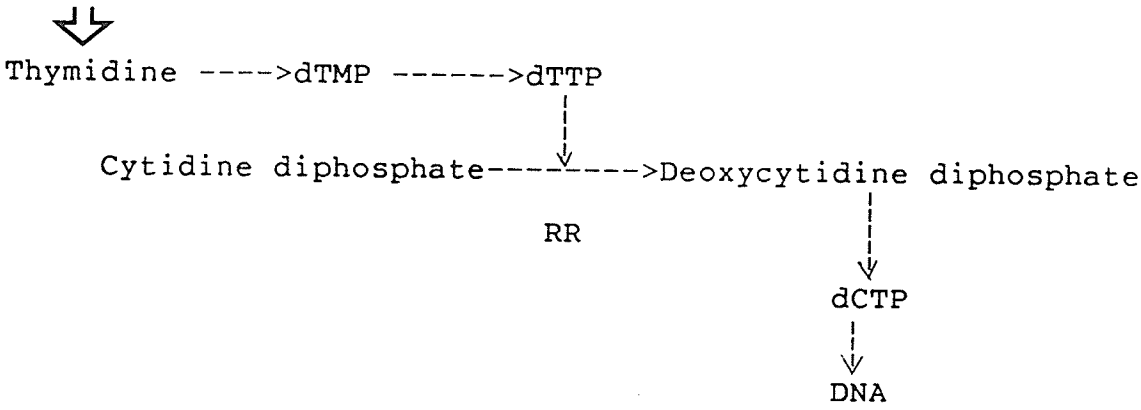


Fig 1. Mechanism of production of fragile sites (FS)(see text)

a. By thymidylate synthetase stress.

b. By excess of thymidine.

Key:

dUMP:Uridine monophosphate; dTTP:Thymidine triphosphate;
dTMP:Thymidine monophosphate; TS:Thymidylate synthetase;
DHF:Dihydrofolate; dCTP:Deoxycytidine triphosphate;
MTX:Methotrexate; meTHF:Methylene tetrahydrofolate;
RR:Ribonucleotide reductase; DHFR:Dihydrofolate reductase.

triphosphate (dCTP) available for DNA synthesis (fig 1b). An increase in dCTP level is seen in low levels of thymidine (Meuth et al.,1979). This implies that folate sensitive fragile sites are expressed when either dCTP or dTTP is depleted and that dCTP/dTTP ratio must be maintained within a certain range to inhibit fragile site expression.

Fragile sites may also be sites of viral DNA modification. The virus may only be able to modify specific DNA sequences. Adenovirus 12 causes specific lesions of chromosomes 1 and 17 which resemble the lesions seen at fragile sites. The same virus may have one or more modification sites and the sites may differ in different individuals according to the genetic constitution (Shabtai et al.,1983).

Another hypothesis put forth by Soudek et al. (1981), suggested that a transmissable agent could damage a chromosome and result in fragile sites. Lymphocytes from a patient with fragile X-linked MR were cultured with cells from a patient who was negative for this marker. In another experiment the serum from a patient with this marker was added to the lymphocyte culture of a healthy man. In both experiments the marker was never induced in the fragile Xq27 negative cells. Diseases like Alzheimers and Kuru are thought to be due to transmissable agents but no fragile sites were observed. These negative results do not support the hypothesis that fragile sites originate from

transmissable agents.

2.2 Autism

2.2.1 History of Autism

Kanner in 1942 first described the syndrome of autism and in 1943 published the features of early autism which consisted of; (i) profound withdrawal from contact with people existing from the begining of life ,(ii) an obsessive desire for the preservation of sameness,(iii) a skillful and affectionate relation to objects,and (iv) the retention of an intelligent and pensive physiognomy and either mutism or noncommunicative speech (Kanner, 1943,1944,1949). This description did not enter the official diagnostic nomenclature until 1980, when the 3rd edition of the American Psychiatric Association Diagnostic and Statistical Manual -DSM III was published.

Over the four intervening decades investigators reached a consensus that the symptoms indicative of autism are due to underlying neuropathology of diverse etiologies (Ornitz and Ritvo , 1976).

2.2.2 Epidemiological aspects of autism and other childhood psychosis

Autism is not a disease entity but a symptom complex of many diseases. It occurs in all social classes and is 3 to 4 times more frequent in males. Longevity is normal. Two to

six % of autistic probands have autistic siblings (Demeyer et al.,1981). Siblings of autistic children have a 50 times greater chance of being autistic than siblings of non autistics. The higher rate of concordance in monozygotic twins in autism and other cognitive abnormalities indicates the importance of genetic factors in the etiology of autism (Folstein and Rutter,1977b). Ritvo et al.(1985) found a concordance of 95 % in monozygotes and 23.5 % in dizygotes.

Etiology is nonspecific, prenatal or postnatal insults to the brain (Torrey et al., 1975; Gillberg and Gillberg,1983) being some of the major factors. It can thus result from CNS infections such as rubella (Chess,1971), infantile spasms (Taft and Cohen,1971; Rii Konen and Amnell, 1981), tuberous sclerosis (Wing, 1980), neurofibromatosis (Gillberg and Forsell,1984) and PKU (Friedman, 1979).

2.2.3 Incidence

The incidence of Autism is 4.5/10,000 with a male to female ratio of 4:1 (DeMeyers et al.,1981).

2.2.4 Association of Fragile X with Autism

Although early descriptions of the behaviour of fragile X males have mentioned autism (Martin and Bell,1943; Turner et al.,1980; Jacobs et al.,1980). Brown et al.(1982b) reported specifically on this association by describing 4 fragile X males with autism. Siva Sankar (1970) had earlier reported an increased rate of chromosomal breaks in leucocyte culture from autistic children. Brown et al.(1986) found that 12.3

% of Fragile X males had autism and an overall frequency of 7.7 % Fra(X) amongst autistic males. Hence they concluded that there is likely to be a significant positive association of Fra(X) with autism. The association of fra(X) syndrome and autism in females has not been well described. Hagerman et al. (1986) have described 2 affected heterozygous fra(X) females with autism. Both women had high frequency fra(X) expression and severe behavioural problems.

3.0 MATERIALS AND METHODS

3.1 Materials

The present study was carried out on autistic patients.

3.1.1 Patient selection

We studied 20 patients (19 males and 1 female) who had been diagnosed as having infantile autism (IA) based on the DSM III and C.A.R.S (Childhood Autism Rating Scale). We excluded patients with an organic cause of autism such as rubella embryopathy, PKU, Down syndrome and birth asphyxia. The patients were referred by pediatricians, psychiatrists, or were identified through advertisements in a local autism association newsletter. Consent for this study was obtained from each individual or from their legal gaurdian when the individual was under the legal age. The age range of the patients was from 5 to 24 years, with a mean of 11.5 years and a median of 10 years (Table II).

3.1.2 Cytogenetic control selction

Twenty age and sex matched controls with no obvious physical or neurological impairment were also included in the study. Control subjects were patients coming in for minor surgical procedures at the day surgery clinic at the children's hospital. Verbal consent was taken from the parents or legal gaurdians. The ages ranged from 6 to 22 years with a mean of 11.5 and median of 10 years (Table II). Only blood

Table II Study Patients and Controls

	Study Patients N=20; 19M:1F	Cytogenetic Controls N=20; 19M:1F	Minor Anomalies Controls N=76; 48M:28F
Age Range	5-24 yrs	6-22 yrs	1 mo - 13 yrs
Mean Age	11.5 yrs	11.5 yrs	5.3 yrs

specimens were obtained from control subjects.

3.1.3 Minor anomalies in a control population

The data for the minor anomalies in a control group was taken from Evans et al. (1978). The age range varied from 1 month to 13 years with a mean age of 5.3 years (Table II). The sex ratios were different in this control group (48 males and 28 females) as compared to our patient group (19 males and 1 female). We recognize the fact that this control group was not ideal because of sex ratio and mean age differences, but it was not possible to do the physical examination of cytogenetic controls. It would have been also inappropriate to use other published control data for the incidence of minor anomalies because of lack of standardization in measurements. The patients in Evans et al. (1978) study and our own were subjected to the same standardized examination to reduce the interobserver variability. Also the physical anomalies recorded would not be expected to be influenced by the sex of the child with the exception of genital anomalies. Furthermore the appropriate measurements were age corrected and were derived from the same population base.

3.2 Methods

3.2.1 Patient Evaluation

A). Psychological evaluation

Each patient was observed simultaneously by 2 psychiatrists

through a one-way mirror and assessed according to the DSM III criteria for IA (APA 1980) and scored according to the childhood autism rating scale - C.A.R.S. (Schopler et al.,1980).

B). Clinical evaluation

Complete family, prenatal, birth and medical histories were obtained on each patient. A detailed physical examination including documentation of anthropometric measurements and physical traits was performed by two pediatricians. Blood for cytogenetic analysis was obtained from all 20 patients.

3.2.2 Cytogenetic Methods

10 ml. of blood was obtained by venipuncture from the autistic patients and from the 20 age and sex matched healthy cytogenetic control subjects. All samples were collected in heparinized vacucontainers. The blood samples from the patients and controls were usually set up simultaneously in medium TC199. 5 of the control samples could not be obtained at the same time as the control patients. All the samples were coded before culturing. Thus all observations were made without knowledge of the individual status.

The blood (0.2 ml / 5 ml medium) was cultured in medium 199 (GIBCO # 320-1150, Grand Island ,N.Y.) supplemented with 5 % fetal bovine serum and gentamycin (5 ug /ml of the medium). The medium was adjusted to a pH of 7.6 at room temperature. Phytohemagglutinin P (PHA) (Bacto- DIF Co,

Detroit,Michigan) was the mitogen added to the blood culture. All cultures were incubated for 96 hours at 37 C. Colcimid (0.05 ug /ml) was added in the final 2 hours of the culturing. All cultures were set up in 4 tubes. KCl of 0.075 M and 3:1 methanol:acetic acid were used for hypotonic treatment and fixation respectively. Cells were fixed 3 times and the cell suspension was dropped onto slides and allowed to dry on a hot plate. Slides (5 days to 5 weeks old) were stained conventionally with Giemsa and then 50 good metaphases were analysed for evidence of autosomal fragile sites, fragile X, breaks and gaps and other anomalies. Sequential G or Q banding was done to confirm the site and location of the chromosomal fragile site. The methods were as follows:

A). G-banding with trypsin (modified method of Seabright 1971). 7 day old slides were used. One ml. Bacto trypsin Difco Cat. # 0153-59 was diluted with 24 ml of 0.85 % isotonic saline. The slides were dipped in the above solution for 5-6 seconds. The temperature of trypsin was maintained at 17 C. The slides were then rinsed in isotonic saline to remove the excess of trypsin. They were then stained with 4 % Giemsa stain for 5 to 6 minutes. Finally they were washed in buffer and air dried.

B). Q-banding (modified from Casperson et al.,1971). The 7 day old slides were treated in various changes of ethanol and McIlvaines buffer (pH 4.5) . The stain used was Atebrine (Quinacrine dihydrochlride). The slides were

placed in 0.5 % staining solution for 30 minutes. The differentiation was done using McIlvaines buffer 3 times for 2 minutes ,2 minutes and 10 minutes respectively. The slides were removed from the buffer and allowed to drain but not to dry and were mounted and sealed.

C). C-banding (Arrighi and Hsu,1971).It was done to document heterochromatin variants. 7 to 9 days old slides were placed in 0.2 N HcL for 15 to 17 minutes. They were then washed with distilled water twice. They were then dipped in a solution of alkaline SSC (1 portion of 0.07N NaOH : 6 portions of 2 x SSC) for approximately 2 minutes. They were then rinsed in 2 x SSC, twice in 70 % ethyl alcohol and twice in 95 % ethanol and then allowed to air dry. They were then placed in a moist chamber the bottom of which was flooded with 2 x SSC. The slides were covered with a coverslip and incubated overnight in an oven at 60 C. The slides were then rinsed with ethanol, air dried and stained with 4 % Giemsa for 7 to 10 minutes.

The conventionally stained G- and C-banded slides were viewed under the microscope. Metaphases with fragile sites, breaks, gaps or other anomalies were photographed on Kodak Technical film with a green interference filter. Sequential Q-banding slides were examined under the fluorescent microscope and the fragile sites were photographed. Five C-banded and G-banded metaphases were photographed and the pictures analysed.

3.2.3 Statistical Methods

The mean frequency of breaks per cell in the study and control subjects were calculated using the following statistical tests:

(1). Wilcoxon matched pair test was used because the data did not have a normal distribution.

(2). Kolmogorov Smirnov test was used to compare the distribution of frequency of breaks in four categories; 0,1, 2 to 3 and >3 .

4.0 RESULTS

4.1 Clinical Findings

Sixteen of the twenty patients fulfilled the criteria of IA by both DSM III and C.A.R.S. (Table III). The other 4 were believed to have either residual state IA or a form of childhood psychosis.

4.2 Cytogenetic Findings

4.2.1 Autosomal Aberrations

Chromosomal analysis showed variants in three individuals, all of whom were study patients. No heritable chromosomal anomalies or variations were identified in the control subjects. Two study patients AG and LS demonstrated the fra(2)(q13). In AG this was present in 36 % of his metaphases examined and a similar proportion was also found in his healthy mother. In LS the fragile site was present in 6 % of metaphases and was found to be inherited from his healthy father at an identical frequency. A third study patient BL had a small paternally derived inversion (9)(p11q12). Five of the 20 (25 %) chidren in the IA group showed fragile sites at 16q23 location as compared to two out of twenty in the controls. Two of the study patients had other nonrandom breaks in addition to a heritable folate

Table III Behavioral Diagnosis

Study Patient	Sex	Age	C.A.R.S	DSM III criteria	Diagnosis
MRW	M	12	37.5	+	IA
IW	M	11	30.5	+	IA
CK	M	6	26.0	±	? IA
AG	M	8	44.0	+	IA
KM	M	6	41.5	+	IA
JK	M	9	18.5	-	CP.? As
JG	M	9	54.0	+	IA
SC	M	12	50.5	+	IA
MC	M	10	39.5	+	IA
CB	M	6	49.5	+	IA
EA	F	16	60.0	+	IA
MM	M	24	39.0	+	IA
BL	M	15	30.5	+	IA
BW	M	10	36.5	+	IA
JV	M	16	34.0	+	IA
LS	M	21	21.0	±	? Residual IA , ? As
DR	M	5	48.5	+	IA
MR	M	12	36.5	+	IA
BP	M	9	18.5	±	? As

IA: Infantile Autism
CP: Childhood Psychosis
As: Asperger Syndrome

sensitive fragile site on the long arm of chromosome 2. One boy in the study group had heritable fragile site on the short arm of chromosome 19 at 19p13 in 2 % of the metaphases. The 7 boys in the autistic group had fragile 6q26; this also included the two patients with fragile 2q13 (fig 2).

4.2.2 Sex Chromosome Aberrations

One boy in the cytogenetic control group had a fragile site in 2 % of his cells at Xp22. No fragile Xq27, sex chromosome aneuploidy or other major heterochromatic variations such as Yqh+ were seen in the study or the control patients.

4.3 Statistical Findings

The results of the mean frequency of breaks per cell in cases and controls are given in Table IV.

Comparison of the distribution of the mean frequency of breaks per cell in study and control subjects indicated that they were not normally distributed ($p<0.025$). Therefore a Wilcoxon matched pair test was used to compare the two groups. No statistical difference was found in the number of breaks ($p=0.65$). Table V shows the distribution of breaks by specific classes in study and control subjects. More than 3 breaks per cell were seen in 5 study patients and 2 control subjects.

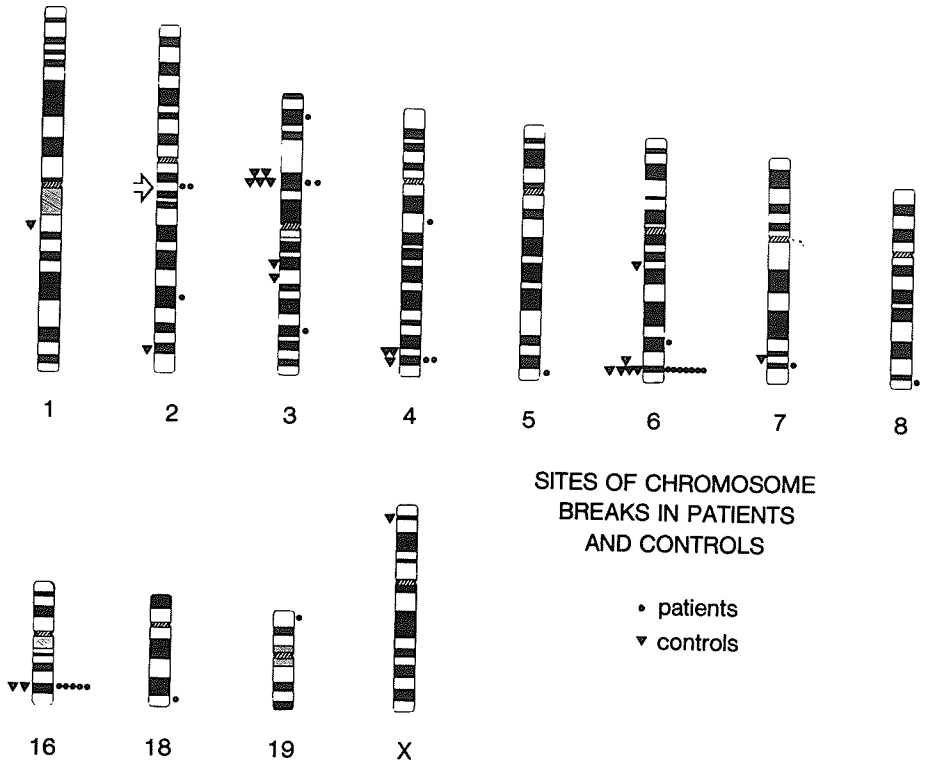


Fig 2. Ideogram showing the sites of chromosome breaks in patients and controls.

Table IV Mean Frequency of Breaks per Cell

Subjects	Mean	± SD
Patients (N=20)	2.90	4.54
Patients without fra(2)(q13) (N=18)	1.61	2.33
Control subjects (N=20)	1.55	1.76

Table V Frequency of Breaks in Patients and Controls

Number of subjects	Number of breaks per cell			
	0	1	2-3	>3
Patients: 20(*)	8(40%)	5(25%)	2(10%)	5(25%)
Control subjects: 20	6(30%)	7(35%)	5(25%)	2(10%)

* includes the 2 patients with fra(2)(q13).

(p=0.20).

However when the two distributions are compared using the Kolmogorov Smirnov test they do not differ significantly (p=0.20).

4.4 Key Minor Anomalies

4.4.1 Frequency and distribution of minor anomalies in study patients and normal controls:

A higher incidence of anomalies including epicanthus, high arched palate and brachycephaly was identified in the study patients as compared to the normal control population (Table VI).

4.4.2 Frequency of minor anomalies seen in at least two patients (Table VI).

Anomalies seen in at least two autistic children were considered as key anomalies. The number of key anomalies in patients varied from 0 to 8. It should be noted however that the two patients with autosomal fragile sites on chromosome 2 had a relatively low frequency of these key anomalies. One way to evaluate the significance of minor dysmorphic findings is to use a weighted score ,where the more frequent anomalies receive a higher score (Cantor et al., 1981). When these key anomalies were weighted in this fashion and compared with the frequency of chromosome breaks,it was found that there was no linear correlation between the total number of breaks and the weighted scores. The four patients

TABLE VI

Frequency and Distribution of Minor Anomalies In Study Patients and Normal Controls

Anomalies	Study Patients																				Weighted Score	% Study Patients With Anomalies	% Normal Controls With Anomalies
	MR	IW	CK	AG'	KM	JK	JG	SC	MC	CB	EA	MM	BL	BW	JV	DS	LS'	DR	MR	BP			
Epicanthus	+	+			+	+		+			+		+				+	+			3	45	3.4
High Arched Palate	+	+					+					+	+	+	+				+		3	40	0.0
Brachycephaly					+			+	+			+	+								3	25	0.0
Prom. Supraorbital Area	+	+												+							2	15	N/A*
Down-slanting Fissures		+						+					+								2	15	0.0
Pouting Lips						+		+	+												2	15	N/A*
Axial Triradii t'/t*	+						+		+												2	15	3.4
Scoliosis/Lordosis		+						+							+						2	15	N/A*
Upslanting Fissures						+											+				2	15	1.7
Broad Forhead	+	+								+				+				+			1	10	12.1
Medial Flare (Eyebrows)																+					1	10	20.7
Widow's Peak																			+		1	10	0.0
Scaphocephaly															+						1	10	1.7
Small Ears											+								+		1	10	0.0
Prognathism				+								+		+							1	10	0.0
Short Philtrum									+												1	10	0.0
Facial Asymmetry	+											+									1	10	N/A*
Hypothenar Loops								+	+												1	10	N/A*
Cubitus Valgus	+															+					1	10	5.3
Abnormal Feet	+	+																			1	10	0.0
Cryptorchidism														+					+		1	10	0.0
Hypotonia															+					+	1	10	N/A*
Lax Joints		+											+								1	10	N/A*
Strabismus					+					+											1	10	0.0
Character Number Score	8	8	0	1	3	3	2	6	5	2	2	4	5	5	4	2	2	2	4	1			
Total Weighted Score	14	15	0	1	7	5	5	13	9	2	4	8	12	8	7	2	4	4	6	1			

' Study Patients with fra(2)(q13)
* Not Available

with the highest weighted scores had in fact fewer breaks. This suggests heterogeneity in both cytogenetic and dysmorphological findings within the autistic population studied (fig 3).

4.5 Case Histories

A). Family G (fig 4).

The proband AG is a 9 year old male, the only child of a 20 year old unmarried mother and a 17 year old father. The pregnancy was unplanned but there was no history of exposure to any known teratogens. Prenatal and natal periods were uncomplicated. The birth weight was 3.8 kg. Gross motor development was normal till 1 year but speech was delayed. There was no serious medical illness. At 2 years of age, AG demonstrated hand flapping,excessive rocking , eye aversion and tactile defensiveness. Because of his behavioural and learning difficulties he was placed in a special educational program. He was diagnosed as autistic in 1982 and met both the DSM III and CARS criteria. Cytogenetic analysis showed a fragile site at 2q13 in 36 % of his metaphases (fig 5).

The proband's father was described as a slow learner, was lazy and had violent fits of rage. He studied till grade 10 with great difficulty in mathematics and reading. At present he works as a mechanic. The mother is a university student and has average intelligence.

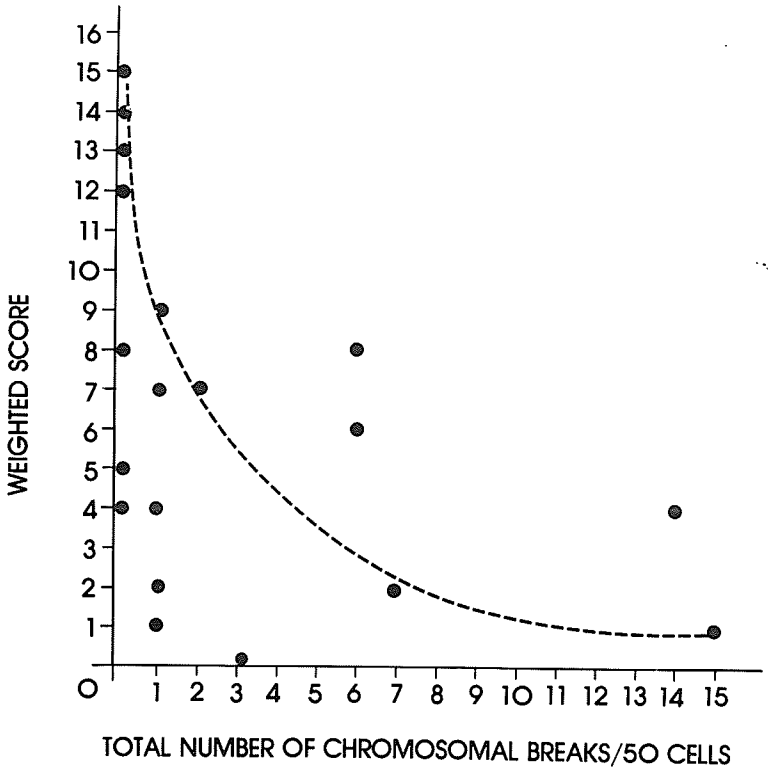


Fig 3. Scatter diagram showing non-linear relationship between the chromosomal breaks and the weighted scores.

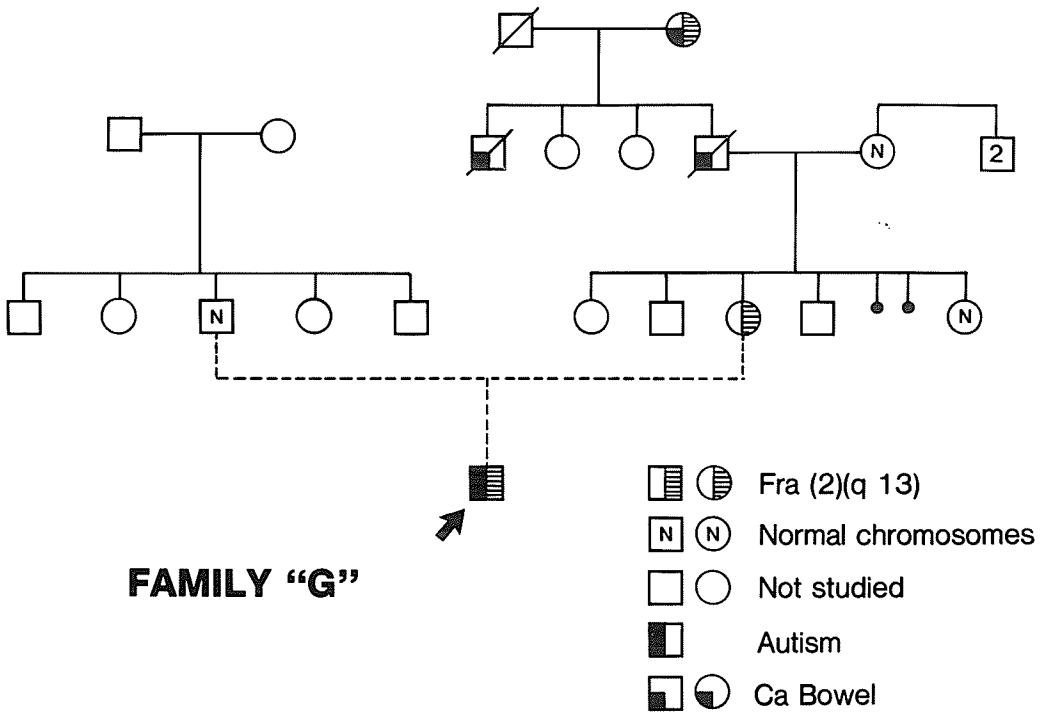


Fig 4. Pedigree of family 'G'.

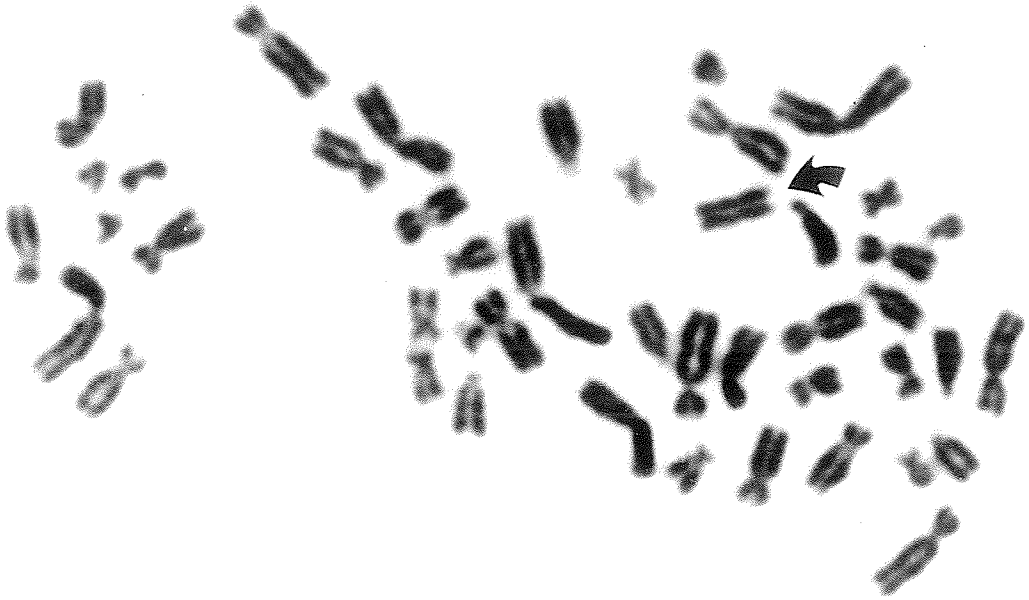


Fig 5. Conventionally stained metaphase spread from proband AG showing the fragile site on chromosome 2 at the break point q13 (arrow).

Her cytogenetic analysis showed the same fragile site (fig 6).

There was no evidence of autism in other family members on either the maternal or paternal side. However the maternal uncle has some behavioural problems ,but is intellectually above normal. The 2 sisters of the maternal grandfather each have a son with learning difficulties.

There is also evidence of cancer of the bowel on the maternal side. The maternal grandfather died of cancer (Ca) of the bowel at the age of 53. A maternal grandaunt also died of Ca bowel. The maternal great grandmother is alive at the age of 82 after being operated for Ca bowel. She also carried the same fragile 2q13 as the proband (fig 7). Thus the fragile site in this case was maternally inherited.

B). Family L (fig 8).

The proband,BL, a 9 year old male with inv (9)(p11q12) was diagnosed as having IA. He is the only child of 35 year old nonconsanguinous parents. BL was born at term after an uncomplicated pregnancy. He was delivered vaginally with outlet forceps and required oxygen. His birth weight was 4.2 kg . Apgar scores were not available. A congenital torticollis of the neck was corrected at 16 months of age. He was hypotonic for the first several months. He was described as a very

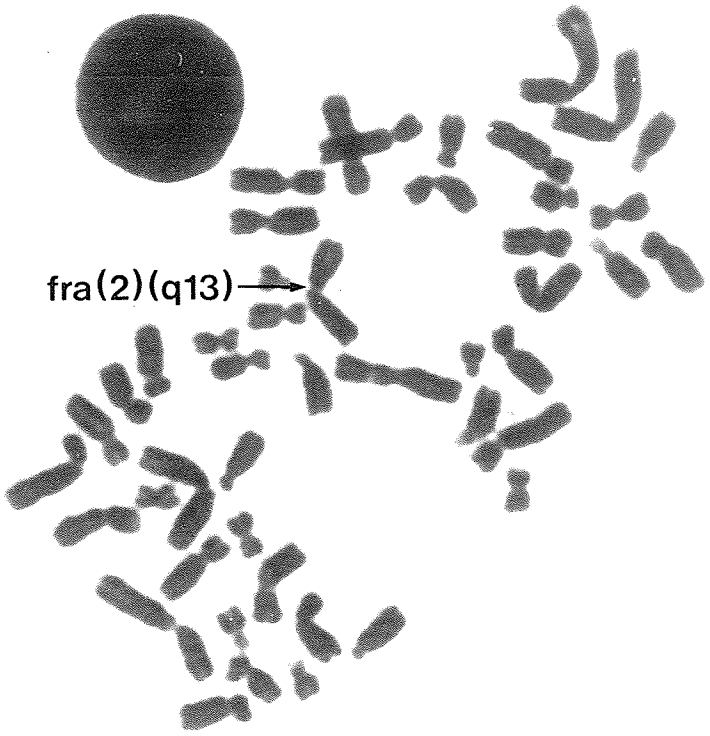


Fig 6. Conventionally stained metaphase spread from mother of proband AG showing the fragile site 2q13.

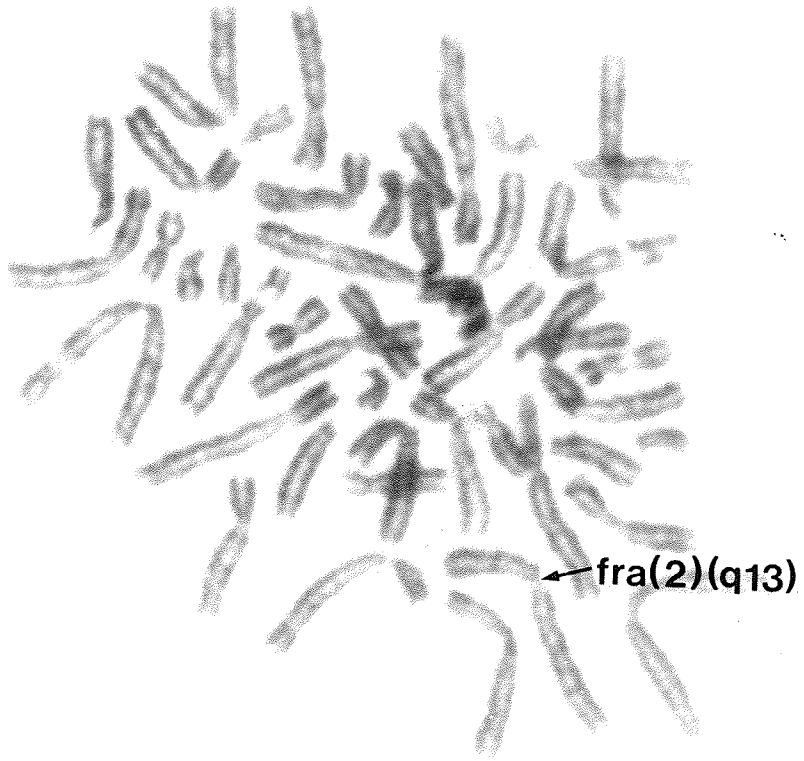


Fig 7. Conventionally stained metaphase spread from maternal great grand-mother of the proband AG showing the fragile site 2q13.

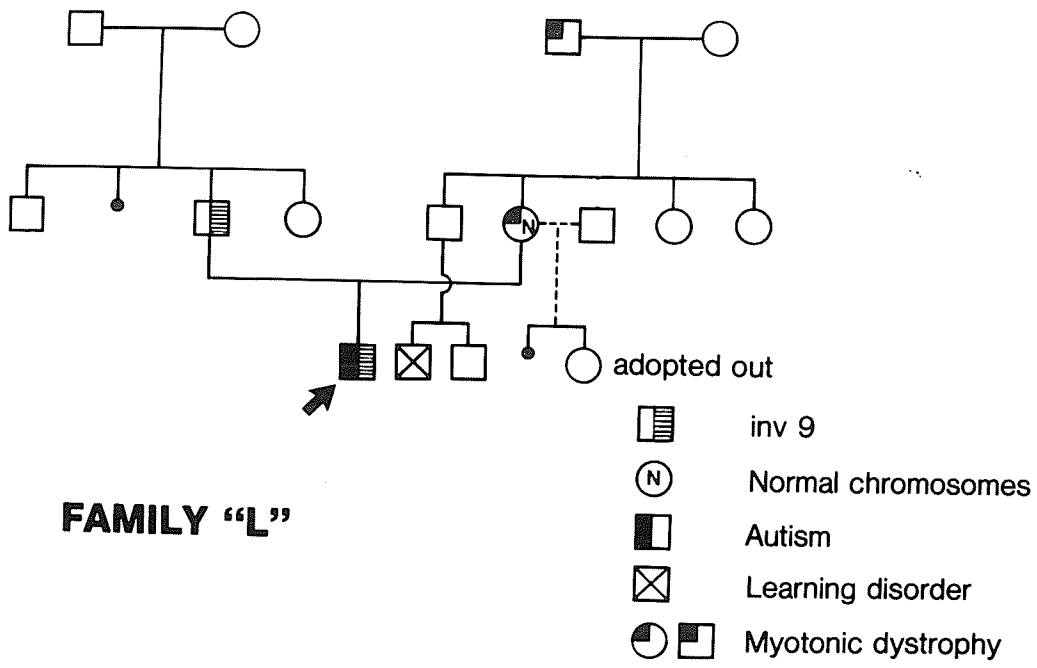


Fig 8. Pedigree of family 'L'.

affectionate and distractable child. He did not respond to voices or to noise. At around 6 months he did not want any one to hold him. Gross motor development was normal but speech was delayed. He spoke monosyllables with speech therapy. He had a number of mannerisms characteristic of autism and is presently in a special day care center for autistic children.

The family history is significant in that the proband's maternal grandfather has pain and cramps while driving and inability to release the grip after driving for long. There is also a history suggestive of myotonic dystrophy in the mother but her EMG results were normal.

On examination , the proband's height and weight were normal. He had mild brachycephaly, epicanthic folds with down slanting palpebral fissures and a high arched palate. There was no evidence of myotonia but he was mildly hypotonic and had hyperextensible joints. The deep tendon reflexes were normal.

The proband demonstrated an inv(9)(p11q12) which was paternally inherited (fig 9 and fig 10).

C). Family S (fig 11).

The proband is a 21 year old man with fragile site on chromosome 2 at break point q13. He is the last born of nonconsanguineous parents. The 2 older sibs are normal. There is history of schizophrenia in a paternal aunt who

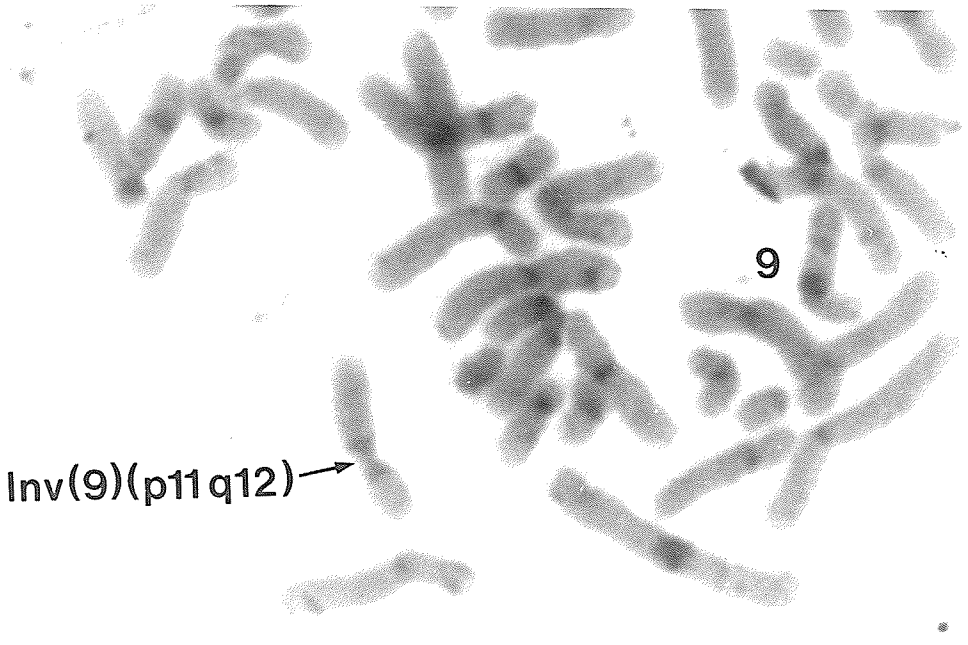


Fig 9. C-banded metaphase spread from the proband BL showing the pericentric inv(9)(p11q12).

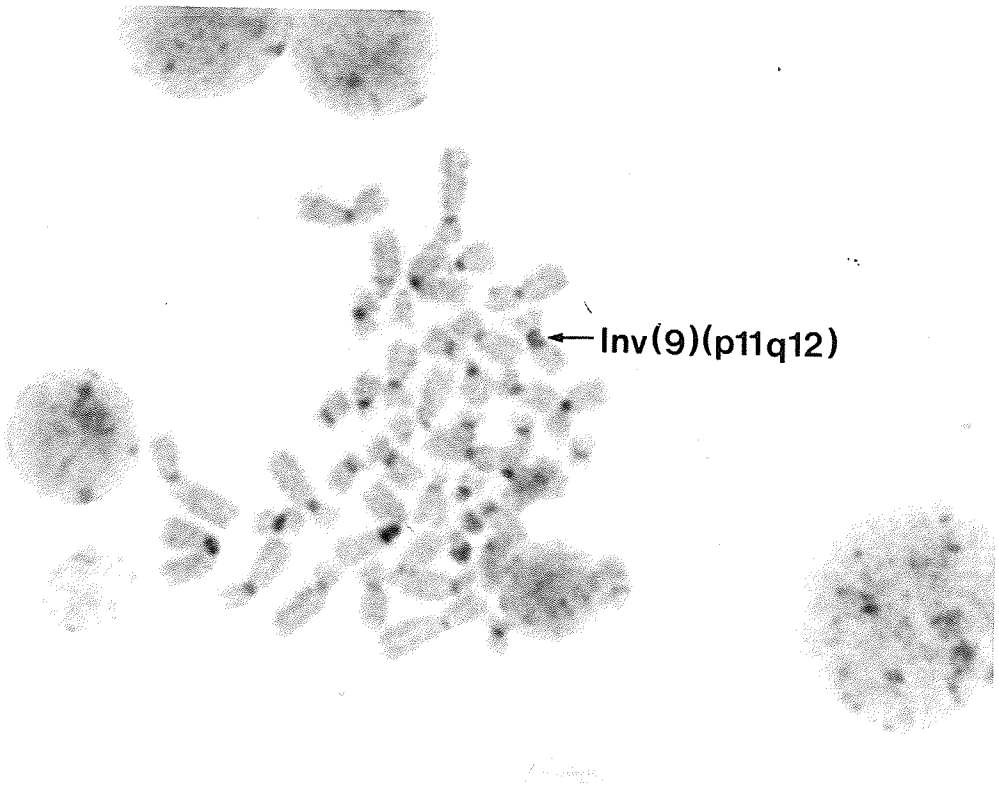


Fig 10. C-banded metaphase spread from the father of proband BL showing pericentric inv(9)(p11q12).

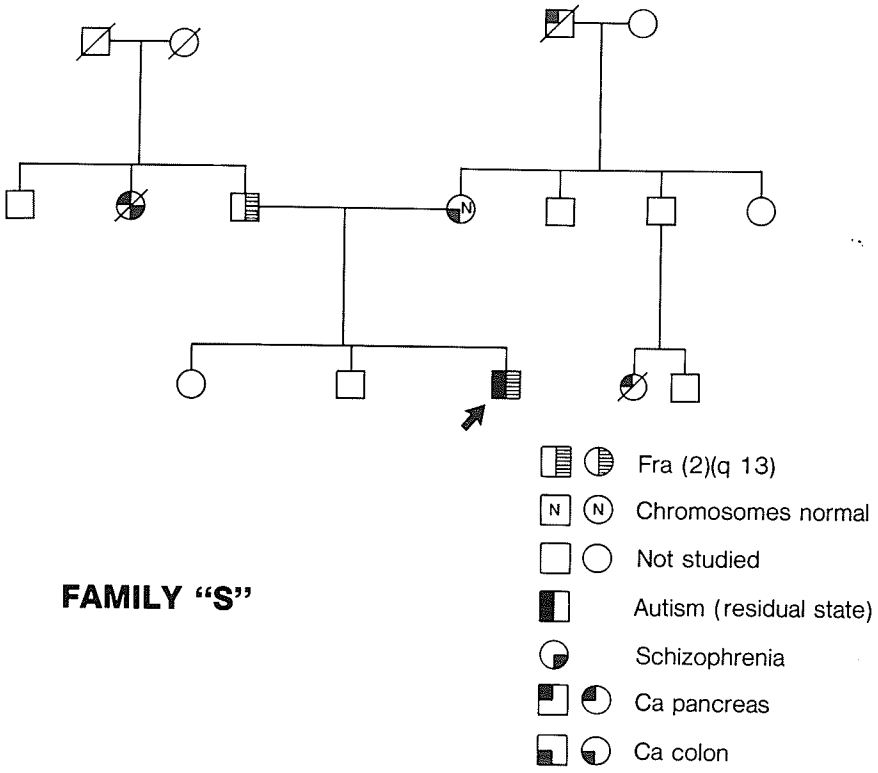


Fig 11. Pedigree of family 'S'.

died of Ca pancreas. There is also a history of Ca rectum in the mother and a maternal uncle. The 2 children of this uncle had communication problems, were introverts and loners,one of whom died from Ca pancreas.

The patient was born after a normal pregnancy at term and had a birth weight of 3.5 kg. There was no evidence of birth asphyxia. Gross motor development was normal. At an early age he demonstrated unusual behaviour,rejected physical contact, was thought to be in a world by himself and enjoyed prolonged periods of rocking. At 15 months a diagnosis of autism was suggested. In school he completed grade XII but had problems in establishing close relations with his peers. He is a pleasant , intelligent man who interacts in rather a mechanical fashion and speaks in a monotonous voice. Physical examination showed anti-mongoloid slant of the eyes, epicanthus, and unusual dermatoglyphics and facial appearance. Growth parameters were within normal limits. The diagnosis of IA mild residual state was made. The proband shows the fra(2)(q13) in 6 % of his metaphases and this was inherited from his normal healthy father who expressed the fragile site at an identical frequency (fig 12).

In the pedigrees discussed above all the cases were isolated cases, with no evidence of autism in any other family member.

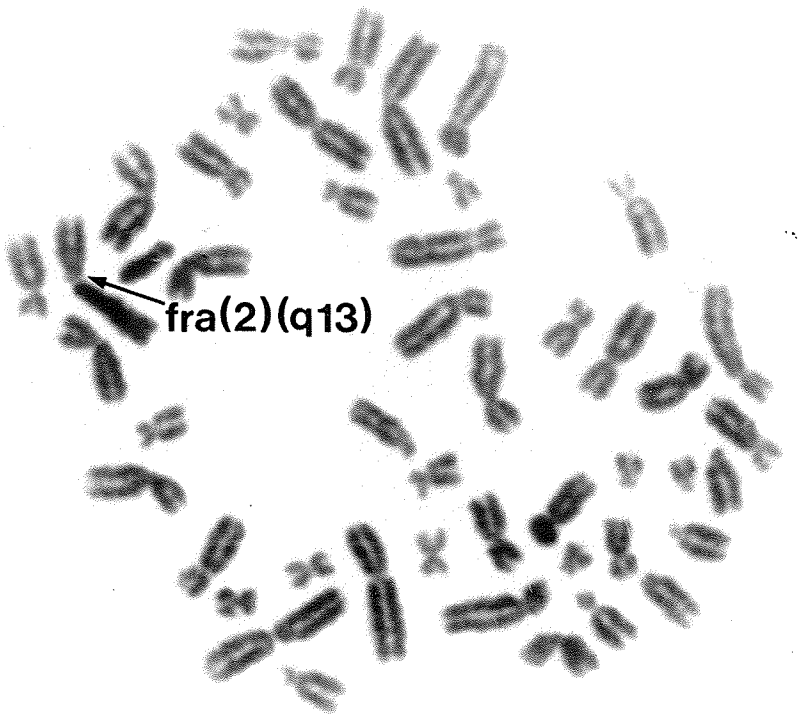


Fig 12. Conventionally stained metaphase spread from the father of proband LS showing fragile site 2q13.

5.0 DISCUSSION.

In this study we have investigated the occurence of fragile sites and chromosomal breakage in children diagnosed as having infantile autism (IA) or other related conditions. Most of the fragile sites are not associated with phenotypic abnormalities and are presumably innocuous. They are never seen in 100 % of the cells examined (Kaiser-McCaw and Hecht, 1980). How does one then consider the potential association between fragile sites and autism or other phenotypic abnormalities seen in our investigation ?

5.1: Phenotypic findings.

Analysis of the individual dysmorphic findings indicated that the frequency of occurrence of almost every anomaly was greater amongst autistics as compared to the controls. The anomaly showing the highest frequency was epicanthus in contrast to a much lower incidence in normals. There was also a higher incidence of minor anomalies such as high arched palate and brachycephaly in autistic patients as compared to the normal controls. In support of this finding Mehes (1983) also reported an increased occurrence of minor anomalies in children with mental retardation, intra-uterine growth retardation, hyperactivity and autism. Therefore, do autistics have a higher incidence of minor dysmorphic findings per se or

is the excess of minor anomalies seen in these patients due to early prenatal insults or genetic predisposition ? Most of the findings were subjective (qualitative) and not objective (quantitative). Therefore clarification of the importance of familial traits would be possible if parents and or siblings are similarly examined. In his study on anomalies in children with malignancies in children and their normal sibs and controls, Mehes et al.(1985) found that patients and their normal siblings have a significantly higher prevalence of minor anomalies. Therefore it may well be important to know more about the phenotype of other family members. All of the autistic patients in this study were isolated cases with no family history of autism. We did not examine the parents and sibs. Hence the significance of the higher incidence of anomalies in our autistic population or of specific dysmorphic findings is difficult to assess. The autistic children did not show a distinctive phenotype of minor anomalies. However the higher incidence of anomalies suggests that probably early prenatal factors may well be associated with the development of autism.

An attempt was made to document the frequency and distribution of minor anomalies in the study group and determine if any correlations could be established between chromosome breakage and minor anomalies. No clear linear relationship exists and , surprisingly , the 2 patients with the fragile (2) had the least number of

abnormal findings on physical examination. In conclusion, the overall frequency of minor anomalies was higher than in normal control population, which is in keeping with previous reports (Coleman,1976). This finding further acknowledges the heterogeneous nature of infantile autism and related disorders.

5.2 Cytogenetic Findings

5.2.1 Fragile X

The fragile site on the long arm of chromosome X is now recognized as the 2nd commonest chromosomally determined cause of mental retardation in males after Down syndrome (Gerald,1980) and may well be a significant cause of mental handicap in females (Turner and Opitz,1980; Hagerman et al., 1986). The frequency of autism among males with fragile X is 12.3 % (Brown et al., 1986). Conversely, the incidence of fragile X in autistic males is between 5.3 and 17 % (Watson et al.,1984; Gillberg and Wahlstrom ,1985). Considering the size of our study it is not surprising that no cases of fragile X were seen. However, Brown et al. (1986) point out that since the genetic disorder constitutes a major heritable cause of autism all patients with IA should be screened and their families examined.

5.2.2 Autosomal fragile sites

The finding of fra (2)(q13) in 2 patients was unexpected.

This heritable folate sensitive fragile site has been seen in children with mental retardation and multiple congenital anomalies (Giraud et al.,1976), but a causal link is yet to be proved, since these fragile sites are found in phenotypically normal first degree relatives of affected individuals, as in the families of this study. Sutherland (1985) carried out parental studies. In 28 of the autosomal fragile site ascertainments, the fragile site was maternally inherited whereas in only one was it paternally inherited. In our study of twenty patients, two autosomal fragile sites were ascertained of which one was maternally inherited and the other paternally inherited. There is no evidence that detection of fragile sites in males is more difficult than in females. In family G, the fragile site was transmitted to the proband from the great-grand mother via the grand father and the mother. However the sibs of the mother did not carry the fragile site. In family L the autosomal fragile site was transmitted through the father to the proband though the two sibs of the proband did not carry the fragile site. In a study of 15 autistic patients Chudley (personal communication) found 4 cases with maternally derived chromosomal anomalies, one of which was fra(2). This finding is in keeping with the segregation analysis done by Sherman and Sutherland (1986) , in which 25 % of the children of carrier fathers expressed the fragile site while 50 % of the children of

carrier mothers expressed the fragile site. Gustavson et al.(1981) reported 5 cases of fra (2) with considerable phenotypic variations. The heritable fragile site on 19p13 was found only in one metaphase out of 50 examined. This could have been an artifact or a random break. No further family studies were carried out. The incidence of the fra(2) and other folate sensitive fragile sites in the normal population is not known, but based on the surveys in a population of newborn infants, the overall incidence is approximately 1:700 as compared to an incidence of 1:100 in a population of mentally retarded individuals (Sutherland,1985). As pointed out earlier Sutherland's study utilized cord blood for analysis, which is known to have a higher level of folic acid . Therefore the study may have underestimated the incidence of these fragile sites in the general population. Clearly further studies are required to determine the relevance of autosomal heritable folate sensitive fragile sites to psychiatric disorders, mental retardation and birth defects.

5.2.3 Fragile Sites and Cancer

The possibility that autosomal fragile sites may lead to cancer was discussed at the annual meeting of the human genetic society of Australia in 1982. We found a history of cancer of the bowel in the families of two of the patients with fra (2). The cancer affected several members on the maternal side in one proband. There is

distortion in the other family where the cancer is not segregating with the fragile site. A similar association between familial polyposis and the deletion of chromosome 2 in the region 2q(14.3-21.3) was reported by Gardner et al. (1982). Furthermore , chromosome 2 has been shown to be involved in rearrangements or numerical changes in malignant cells. Some basic questions remain to be answered:

a). Do fragile sites act as predisposing factors for chromosomal rearrangements in human neoplasms ?

b). Do they carry genes whose functions are related to malignant transformations (LeBeau and Rowley,1984) ?

The answers to these questions are essential in order to establish clinical criteria to identify potentially afflicted individuals at an early stage of cancer. Our data do not necessarily support or refute a relationship of fragile sites and cancer.

5.2.4 Chromosomal Anomalies and Other Fragile Sites

Heritable folate sensitive fragile sites were not seen in our control subjects. However , the common non-heritable fragile sites 3p14,6q26, and 16q23 were seen in individuals from both the study and control groups. In a report by Gillberg and Wahlstrom (1985) , 13 % of the children with autism or other psychosis showed the 16q23 in low frquency. They raised the possibility that this fragile site may be an important chromosomal marker reflecting an underlying predisposition to childhood

psychosis. Unfortunately their study did not include controls and the chromosomes were not examined in a blind fashion as in this study. Fra(16)(q23) is a common fragile site and was seen in 5 of the study patients (2 of whom also had fra(2)),and was present in 2 control individuals. This fragile site was present in patients as well as normal controls in our study. Therefore , this fragile site is not a useful marker for autism. Gillberg et al.(1984) had also reported fra(X)(p22) in 12% of their autistic /psychotic study group and considered the possibility of that being a marker for psychiatric abnormality.

We found one boy in the cytogenetic control group who had the Xp22 fragile site in 2 % of his cells. Our finding is in keeping with the conclusions of Fitchett (1984) and Jenkins et al. (1984) that fra(X)(p22) is neither specific nor predisposes to any developmental disabilty or to psychosis.

We were unable to confirm a relationship between Yqh+ and childhood psychosis as previously suggested (Christensen and Nielsen, 1974; Akesson and Wahlstrom,1977; Gillberg and Wahlstrom,1985). One study patient had a small pericentric inversion of chromosome 9 which was paternally inherited. Chudley (personal communication) in a study of 15 autistic patients found a pericentric inv(9). This inversion is of questionable

clinical significance though Nielson et al. (1974) found 9qh+ variant more frequently in parents and relatives of individuals with chromosomal aberration. This inversion is also the commonest heterochromatin variant seen. Its association with Down syndrome is controversial (Lopetegui, 1980). The mother of our patient with inv (9) clinically seems to have myotonic dystrophy but the EMG studies on her have been negative. This finding, in addition to the history of mild asphyxia at birth , emphasizes the need to obtain detailed family and medical history and to carefully examine the parents and sibs of autistic children. The report by Siva Sankar (1970) of excessive chromosomal breaks in infantile autism is of interest. Some of his patients may in fact have had the fragile X or other fragile sites, in which case an excess number of breaks or gaps would be expected. No differences in the frequency of chromosome breaks or gaps between the two groups was identified in our study after excluding the patients with fra(2).

6.0 CONCLUSIONS

In this study the relationship of autism to other autosomal folate sensitive fragile sites besides fra(X) has been studied using age and sex matched controls. Two of the twenty study patients demonstrated the same heritable fragile site (2)(q13) while none of the controls did so. This finding of fra(2)(q13) in two patients was unexpected. This heritable folate sensitive fragile site has been seen in children with mental retardation and multiple congenital anomalies. However it has also been reported in phenotypically normal first degree relatives of the affected individuals as in the families discussed in the text. Hence a causal link is yet to be proved. The incidence of fra(2) and other heritable folate sensitive fragile sites in the normal population is not known but surveys in the newborn population showed a collective incidence of 1 in 770 as compared to 1 in 100 in a population of mentally retarded individuals. There was also no correlation between the number of breaks, gaps, fragile sites and minor anomalies. The relevance of these fragile sites to behavioral disorders has yet to be defined and further studies will be required to determine the relevance of autosomal folate sensitive fragile sites to psychiatric disorders, mental retardation and birth defects. Hence it is recommended that children with pervasive

developmental disorders or autism should have chromosomal analysis.

7.0. REFERENCES

Akesson H, Wahlstrom J (1977). The length of the Y chromosome in men examined by forensic psychiatrists. Hum Genet 39:1-5.

Arrighi FE, Hsu TC (1971). Localization of heterochromatin in human chromosomes. Cytogenetics 10:81-86.

Asperger H (1944). Die autistischen psychopathen' imkindesalter. Archiv fur Psychiatrve und Nervenkran Kheiten 117:76-136.

Barbi G, Steinbach P, Weidenmann A, Vogel W (1984). Manifestation of the fragile Xq27 in fibroblasts. III. A method to demonstrate R type replication patterns and the fragile site. Hum Genet 65:76-78.

Blomquist H K ,Bohman M, Edvinsson S O , Gillberg C, Gustavson K , Holmgren G, Wahlstrom J (1985). Frequency of the fragile X syndrome in infantile autism. A swedish multicenter study. Clin Genet 27:113-117.

Branda R, Nelson L (1982). Effects of pH on 5-methyltetrahydro folic acid transport in human erythrocytes. Biochem Pharmacol 31:2300-2302.

Brookwell R, Daniel A , Turner G, Fishburn J (1982). The fragile X(q27) form of X-linked mental retardation. FUdR as an inducing agent for fragile(X)(q27) expression in

lymphocytes, fibroblasts and amniocytes. AM J Med Genet 13:139-148.

Brown W T, Friedman E, Jenkins E ,Broooks J, Wisniewski K, Raguthu S, French J (1982 a). Association of fragile X syndrome with autism. Lancet 1:100.

Brown WT, Friedman E, Jenkins E, Brooks J, Raguthu S, French J (1982b). Autism is associated with the fragile X syndrome. J Aut Dev Disord 12:303-308.

Brown WT, Jenkins EC, Cohen IL, Fisch GS, Wolf-Schein EG, Gross A, Ritvo E, Bently W, Waterhouse L, Fein D, Mason A, Ruttenberg B, Castells S (1986). Fragile X and Autism: Multicenter survey. Am J Med Genet 23:341-352.

Bryant E, Martin G , Hoehn H (1983). Fragile X expression studied by clonal analysis and somatic cell hybridization. Cytogenet Cell Genet 35:223-225.

Burgoine E, Wing L (1983). Identical triplets with Asperger syndrome. Brit J Psychiatr 143:261-265.

Cantor S, Pearce J, Pezzot-Pearce T, Evans J (1981). The group of hypotonic schizophrenics. Schizophrenia Bull 7:1-11.

Caspersson T, Lonakka G, Zech L (1971). The 24 fluorescence patterns of the human metaphase chromosome: Distinguishing characters and variability. Hereditas 67:89-102.

Chaudhari J (1972).On the origin and nature of achromatic lesions. Chromosome today 3:147-151.

Chess S (1971).Autism in children with congenital rubella. J autism and childhood schizophrenia 1:33-47.

Christensen KR, Nielsen J (1974). Incidence of chromosome aberration in a child psychiatry hospital. Clin Genet 5:205-210.

Coleman M (1976). The Autistic Syndromes. New York. American Elsevier.

Dekaban A (1965). Persisting clone of cells with an abnormal chromosome in a woman previously irradiated. J Nucl Med 6:740-746.

Demeyer MK, Hingtgen J, Jackson RK (1981). Infantile Autism reviewed : A decade of research. Schizophrenia Bulletin 7:388-451.

Diagnostic and Statistical Manual of Mental Disorders (DSMIII)(1980) (3rd ed.). Washington D.C.: American Psychiatric Association.

Dunn HG, Renpenning H, Gerrard JW, Miller JR,Tabata T, Federoff S (1963). Mental retardation as a sex linked defect. Am J Ment Defic 67:827-848.

Eberle G, Zankl H, Zankl M (1982). Cocultivation studies with cells of patients bearing fragile X chromosomes.

Hum Genet 61:163-164.

Evans JA, Canning N, Hunter AGW, Martsolf JT, Ray M, Thompson DR, Hamerton JL (1978). A cytogenetic survey of 14,069 newborn infants. III. An analysis of the significance and cytogenetic behaviour of the Robertsonian and reciprocal translocation. Cytogenet and Cell Genet 20:96-123.

Ferguson-Smith MA (1973).Inherited constriction fragility of chromosome 2. Ann Genet 16:29-34.

Fisch GS, Cohen IL, Wolf EG, Brown WT, Jenkins EC, Gross A, (1985). Autism and fragile X chromosome. Submitted to Am J Psychiatry.

Fitchett M (1984). Fra(X)(p22) not associated with infantile autism. Lancet 2:1397.

Folstein S, Rutter M (1977a) . Genetic influences and infantile autism. Nature 265:726-728.

Folstein S, Rutter M (1977b). Infantile Autism: A genetic study of 21 twin pairs. J Child Psychol Psychiat 18:297-321.

Fried K (1972). X-linked mental retardation and/or hydrocephalus. Clin Genet 3:258-263.

Friedman E (1979). The autistic syndrome and phenylketonuria. Schizophrenia I:249-261.

Gardner EJ, Rogers SW, Woodward S (1982). Numerical and structural chromosomal aberrations in cultured lymphocytes and cutaneous fibroblasts of patients with multiple adenomas of the colorectum. Cancer 49:1413-1419.

Gerald PS, (1980). X-linked mental retardation and an X-chromosome marker. New Engl J med 303:696-697.

Gerald PS (1981). X-linked mental retardation and the fragile X syndrome. Pediatrics 68:594-595.

Gerald PS, Walzer S (1970). Chromosome studies of normal newborn infants. In Human Population Cytogenetics. PA Jacobs, WH Price (eds.) Edinburgh University Press , pp 143-151.

Gillberg C (1984). Infantile autism and other childhood psychosis in a swedish urban region, epidemiological aspects. J Child Psychiat 25:35-43.

Gillberg C, Forsell C (1984). Childhood Psychosis and Neurofibromatosis more than a coincidence. J Autism and Develop Disorders 14:1-8.

Gillberg C, Gillberg IC (1983). Infantile Autism :A total population study of reduced optimality in the pre peri and neonatal period. J Autism Develop Disorders 13:153-166.

Gillberg C, Wahlstrom J, Hagberg B (1984). Infantile autism and Retts syndrome: Common denominator. Lancet

2:1094-1095.

Gillberg C, Wahlstrom J (1985). Chromosomal abnormalities in infantile autism and other childhood psychosis: A population study of 66 cases. Dev Med Child Neurol 27:293-304.

Giraud F, Ayme S, Mattei JF, Mattei MG (1976). Constitutional chromosomal breakage. Hum Genet 34:125-136.

Glover TW (1981). FudR induction of the X chromosome fragile sites: Evidence for the mechanism of folic acid and thymidine inhibition. Am J Hum Genet 33:234-242.

Glover TW, Berger C, Coyle J, Echo B (1984). DNA polymerase alpha inhibition by aphidicolin induces gaps and breaks at common fragile sites in human chromosomes. Hum Genet 67:136-142.

Gustavson KH, Holmgren G, Blomquist HK, Mikkelson M, Nordenson I, Poulsen H, Tommerup N (1981). Familial X-linked mental retardation and Fragile X chromosome in two Swedish families. Clin Genet 19:101-110.

Hagerman RJ, Chudley AE, Knoll JH, Jackson AW III, Kemper M, Ahmad R (1986). Autism in fragile X females. Am J Med Genet 23:375-380.

Harvey J, Judge C, Wiener S (1977). Famailial X-linked mental retardation with an X-chromosome abnormality. J

Med Genet 14:46-50.

Hecht F, Hecht BK (1984). Fragile sites and chromosomal break points in constitutional rearrangements. I. Amniocentesis. Clin Genet 26:174-177.

Howard-Peebles PN (1983). Cytogenetics of the mammalian X chromosome, part B:X chromosome anomalies and their clinical manifestations. pp 431-443.

Howard-Peebles PN, Pryor JC (1979). Marker X chromosomes and tissue culture conditions. New Engl J Med 301:166.

Howard-Peebles PN,Pryor JC (1981). Fragile sites on human chromosomes.I. The effect of methionine on Xq fragile site. Clin Genet 19:228-232.

Howard-Peebles PN, Stoddard GR (1980). Race distribution in X-linked mental retardation with macroorchidism and fragile site in Xq. Am J Hum Genet 32:629-630.

Howard-Peebles PN,Stoddard GR , Pryor JC (1980). X-linked mental retardation the fragile site in Xq and the role of methionine. Am J Hum Genet 32:73A.

Jacky PB, Dill FJ (1980). Expression in fibroblasts culture of satellite-X chromosome associated with familial sex linked mental retardation. Hum Genet 53:267-269.

Jacky PB, Dill FJ (1983). Fragile X chromosome and

chromosome condensation. Ann Genet 26:171-173.

Jacky PB, Sutherland GR (1983). Thymidylate synthetase inhibition and fragile site expression in lymphocytes. Am J Hum Genet 35:1276-1283.

Jacobs PA, Glover TW, Mayer M, Fox P, Gerrard JW, Dunn HG, Herbst DS (1980). X-linked mental retardation: A study of 7 families . Am J Med Genet 7:471-489.

Jacobs PA, Hunt PA, Mayer M, Wang J, Boss GR, Erbe RW (1982). Expression of the marker (X)(q28) in lymphoblastoid cell lines. Am J Hum Genet 34:552-557.

Jenkins EC, Brown TW, Krawczun MS, Duncan FJ, Brooks J, Gross A, Sanz MM, Lele K, Masva A, Nolin SL, Sanz MM, Cohen I (1984). Fra(X)(p22) not associated with infantile autism. Lancet 2:1397.

Jenkins EC, Brown TW, Wison MJ, Lin MS, Alfi OS, Wassman RE, Brooks J, Duncan CJ, Masla A, Krawczun MS (1986). The prenatal detection of the fragile X chromosome: Review of recent experience. Am J Med Genet 23:297-311.

Jennings M, Hall JG, Hoehn H (1980). Significance of phenotypic and chromosomal abnormalities in X-linked mental retardation. Am J Med Genet 7:417-432.

Jorgensen OS, Brondum-Nielsen K, Isager T, Mouridsen SE (1984). Fragile X chromosome among child psychiatry patients with disturbance of language and social

relationships. Acta Psychiatr Scand 70:510-514.

Kaiser-McCaw B, Hecht F (1980). Letter to the editor: Fragile X-linked mental retardation. Am J Med Genet 7:503.

Kanner L (1943). Autistic disturbance of affective contact. Nerv Child 2:217-250.

Kanner L (1944). Early infantile autism. J Pediatr 25:211-217.

Kanner L (1949). Problems of nosology and psychodynamics of early infantile autism. Am J Orthopsychiatr 19:416-426.

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

Kubien E, Kleczkowska A (1977). Familial occurrence of chromosome variant 17ph+. Clin Genet 12:39-42.

LeBeau MM, Rowley J (1984). Heritable fragile sites in cancer. Nature 308:607.

Lejeune J, Dutrillaux B, Lafourcade J, Berger R, Abonyi D, Rethore MO (1968). Endoreduplication selective du bras long du chromosome 2 chez une femme et sa fille. C.R. Acad Sci Paris 266:24-26.

Levitas A, Hagerman RJ, Braden M, Rimland B, Mcbogg P,

(1983). Autism and fragile X syndrome. J Dev Behav Pediatr 4:151-158.

Lopetegui PH (1980). 1, 9 and 16 C-band heteromorphisms in parents of Down syndrome patients: Distribution and etiological significance. Jpn J Hum Genet 25:29-37.

Losowsky MS, (1961). Hereditary mental defect showing the pattern of sex influence. J Ment Defic Res 5:60-62.

Lubs HA (1969). A marker X chromosome . Am J Hum Genet 21:231-244.

Magenis RE, Hecht F, Lovrien EW (1970). Heritable fragile site on chromosome 16: probable localization of haptoglobin locus in man. Science 170:85-87.

Martin JP, Bell J (1943). A pedigree of mental defect showing sex linkage. J Neurol Psychiatr 6;154-157.

Mattei MG, Mattei JF, Vidal I, Giraud F (1981). Expression in lymphocytes and fibroblast culture of the fragile X chromosome : A new technical approach. Hum Genet 59:166-169.

Mehes K (1983). Minor malformations in neonates. Akademiai Kiado Budapest.

Mehes K, Signer E, Piuss HJ, Muller HJ, Stalder G (1985). Increased prevalence of minor anomalies in childhood malignancies. Eur J Pediatr 144:243-249.

Meryash DL, Szymanski LS, Garald PS (1982). Infantile autism associated with the fragile X chromosome. J Aut Dev Disord 12:295 -301.

Meuth M, Trudel M, Siminovitch L (1979). Selection of chinese hamster cells auxotrophic for thymidine by 1-B-D-arabinofuranozyl cytosine. Somat Cell Genet 5:303-318.

Michels V (1985). Fragile sites on human chromosomes: Description and clinical significance. Mayo Clin Proc Vol 60.

Nielsen J, Friedrich V, Hreidarsson AB, Zeuthen E (1974). Frequency of 9qh+ and risk of chromosomal aberrations in the progeny of individual with 9qh+. Hum Genetik 21:211-216.

Nussbaum RL, Airhart SD, Ledbetter DH (1983). Expression of the fragile (X) chromosome in an interspecific somatic cell hybrid. Hum Genet 64:148-150.

Opitz JM, Segal AT, Klove H, Mathews C, Lehrke RG (1965). X-linked mental retardation : study of a large kindred with 20 affected members . J Pediatr 67:713-714.

Ornitz EM, Ritvo ER (1976). The syndrome of autism: a critical review . Am J Psychiatr 133:609-621.

Reichard P, Canellakis ZN, Canellakis ES (1961). Studies on possible regulatory mechanism for the biosynthesis of deoxyribonucleic acids. J Biol Chem 236:2514-2519.

Riikonen R, Amnell G (1981). Psychiatric disorders with earlier infantile spasms. Dev Med Child Neurol 23:747-760.

Ritvo ER, Freeman BJ, Mason-Brothers A, Mo A, Ritvo M (1985). Concordance for the syndrome of autism in 40 affected pairs of twins. Am J Psychiatr 142:74-77.

Schopler E, Reichler RJ, DeVellis RF, Daly K (1980). Toward objective classification of childhood autism: Childhood Autism Rating Scale (CARS). J Aut Dev Dis 10:91-103.

Seabright M (1971). Rapid banding techniques for human chromosomes. Lancet 2:971-972.

Shabtai F, Klar D, Bichacho S, Hart J, Halbrecht I (1983). Familial fragility on chromosome 16 [fra16q22] enhanced by both interferon and distamycin A . Hum Genet 63:341-344.

Sherman SL, Sutherland GR (1986). Segregation analysis of rare autosomal fragile sites. Hum Genet 72:123-128.

Siva Sankar DV (1970). Chromosome breakage in infantile autism. Dev Med Child Neurol 12:572-575.

Smith A, Manuel A, Dendulk G, Lawrence R (1985). A male with two different familial autosomal fragile sites and cytogenetically abnormal offsprings. Ann Genet 28:245-247.

Snyder RD, Robinson A (1969). Recessive sex linked mental retardation in the absence of other recognizable abnormalities. Clin Pediatr 8:669-674.

Soudek D, Partington MW, McGregor T (1981). Cytogenetic variations between families with fragile X disease. Abstract, presented at the 6th International Congress of Human Genetics, Israel.

Spence MA, Simmons JQ, Brow A, Wikler L (1973). Sex ratios in families of autistic children. Am J Met Defic 77:405-407.

Steele MW, Chorazy AL (1974). Renpenning's syndrome. Lancet 1:752-753.

Sutherland GR (1977). Fragile sites on human chromosomes: Demonstration of their dependence on the type of tissue culture medium. Science 197:265-266.

Sutherland GR (1979). Heritable fragile sites on human chromosomes. I. Factors affecting expression in lymphocyte culture. Am J Hum Genet 31:136-148.

Sutherland GR (1982a). Heritable fragile sites on human chromosomes. VIII. Preliminary population cytogenetic data on the folic acid sensitive fragile sites. Am J Hum Genet 34:452-458.

Sutherland GR (1982b). Heritable fragile sites on human chromosomes. IX. Population cytogenetics and

seggregation analysis of the BrdU requiring fragile site at 10q25. Am J Hum Genet 34:753-756.

Sutherland GR (1985). Heritable fragile sites on human chromosomes. XII. Population cytogenetics. Ann Hum Genet 49:153-161.

Sutherland GR, Baker E (1986). Effects of neucleotides on expression of the folate sensitive fragile sites. Am J Med Genet 23:409-417.

Sutherland GR, Hecht F (1985a). Fragile sites on human chromosomes. New York: Oxford University Press pp 150-155.

Sutherland GR, Hecht F (1985b). Fragile sites on human chromosomes. New York: Oxford University Press pp 163-178.

Sutherland GR, Hinton L (1981). Heritable fragile sites on human chromosomes . VI. Characterization of the fragile site at 12q13. Hum Genet 57:217-219.

Sutherland GR, Baker E, Sheshadri RS (1980). Heritable fragile sites on human chromosomes . V. A new class of fragile site requiring BrdU for expression. Am J Hum Genet 32:542-548.

Sutherland GR, Baker E, Jacky PB (1984). Heritable fragile sites on human chromosomes. XI. Factors affecting expression of fragile sites at 10q25, 16q22 and 17p12. Am

J Hum Genet 36:110-122.

Sutherland GR, Baker E, Fratini A (1985). Excess thymidine induces folate sensitive fragile sites. Am J Med Genet 22:433-443.

Taft LT, Cohen HJ (1971). Hypsarrhythmia and infantile autism: A clinical report. J Autism Child Schizophrenia I:327-336.

Tommerup N, Poulsen H, Brondum-Nielson K (1981). 5-Fluoro-2-deoxyuridine induction of fragile site on Xq28 associated with X-linked mental retardation. J Med Genet 18:374-376.

Torrey EF, Hersh SP, McCabe KK (1975). Early childhood psychosis and bleeding during pregnancy. A prospective study of gravid women and their offspring. J Autism Child Schizophrenia 5:287-297.

Tsai L, Stewart MA, August GT (1981). Implication of sex differences in the familial transmission of infantile autism. J Autism Dev Disord 1:165-173.

Turner G, Jacobs PA (1983). Marker X-linked mental retardation. Adv Hum Genet 13:83-112.

Turner G, Opitz JM (1980). X-linked mental retardation. Am J Med Genet 7:407-415.

Turner G, Turner B, Collins E (1971). X-linked mental

retardation without physical abnormality. Dev Med Child Neurol 13:71-78.

Turner G, Engisch B, Lindsay DG, Turner B (1972). X-linked mental retardation without physical abnormality (Renpenning syndrome) in sibs in an institution. J Med Genet 9:324-330.

Turner G, Daniel A, Frost M (1980). X-linked mental retardation ,macroorchidism and the Xq27 fragile site. J pediatr 96:837-841.

Venter PA, Gericke GS, Dawson B, Op't Hof J (1981). A marker X chromosome associated with nonspecific male mental retardation. The first South African cases. Afr Med J 60:807-811.

Warren ST, Davidson RL (1984). Expression of the fragile X chromosome in human-rodent somatic cell hybrids. Somatic cell Molec genet (in press).

Watson MS, Leckman JF, Annex B, Breg WR, Boles D, Volkmar FR, Cohen DG (1984). Fragile X in a survey of 75 autistic males (letter). N Engl J Med 310:1462.

Webb T, Gosden CM, Rodeck CH, Hamill MA, Eason PE (1983). Prenatal diagnosis of X-linked mental retardation with fragile (X) using fetoscopy and fetal blood sampling. Prenat Diag 3:131-137.

Wegner R, Geisler B, Sperling K (1982). Expression of

fra(X)(q28) is suppressed in man-mouse hybrid cells. Hum Genet 62:353-354.

William AJ, Howell RT (1976). A fragile secondary constriction on chromosome 2 in a severely mentally retarded patient. J Mental Defic Res 21:227-230.

Wing L (1980). Early childhood autism. 2nd edition.Oxford:Pergamon.

Wolff S, Barlow A (1979). Schizoid personality in childhood: A comparative study of schizoid autistic and normal children. J Child Psycho Psychiatr 20:29-46.

Yarbrough KM, Howard-Peebles PN (1976). X-linked nonspecific mental retardation. Report of a large kindred. Clin genet 9:125-130.

Zankl H, Eberle G (1982). Methods of increasing the visibility of fragile X chromosomes. Hum genet 60:80-81.

8.0 APPENDIX

A). Letter to the parents.

B). Letter to the physicians.

C). Statement to the participants.

D). Consent form.

E). Appointment letter to the parents.

F). Physical examination form.

G). Childhood autism rating scale scoring sheet.

H). Global impression scale.

Health Sciences Centre

CHILDREN'S HOSPITAL
678 William Avenue
Winnipeg, Manitoba R3E 0W1
Dial Direct (204) -

Dear

We are writing to you because of your interest in participating in the genetic study of families who have children with autism. The study is supported by the Children's Hospital of Winnipeg Research Foundation, Inc. The study will include an assessment of your child by Dr. J. Perlov or Dr. R. Wand, Child Psychiatrists. Following this assessment, which will take approximately one hour, Dr. Jayakar and Dr. Chudley will meet with you and your child for a review of the family history and a complete examination of the child. Following this examination, we will obtain a small blood specimen from the child for chromosome studies.

If you agree to participate, please contact us directly at 787-2494 or notify us by letter at the above address. We will then contact you at a later date regarding the time and place of the assessments.

If you have any further questions or concerns, please feel free to contact us.

Yours sincerely,

Parul P. Jayakar, MB, Bs, DCH
Research Associate

Jack Perlov, MD, FRCP(C)

Albert E. Chudley, MD, FRCP(C), FCCMG

Rox Wand, MD, FRCP(C)

/tmh
Enc. Statement to Participants

Health Sciences Centre

CHILDREN'S HOSPITAL
678 William Avenue
Winnipeg, Manitoba R3E 0W1
Dial Direct (204) -

As you may be aware, we are initiating an investigation of infantile autism, which will include a comprensive study of familial, behavioural, phenotypic and cytogenetic aspects of this disorder. We would appreciate your help in bringing this study to the attention of parents of autistic children who are in your practice. The age ranges from four to eighteen years, although older individuals will be considered. The thrust of the study is to search for major cytogenetic abnormalities, including the fragile X in this broadly defined group of children. Parents who wish to participate should be advised to contact us directly at the Children's Hospital, either by phone or letter. This study has been approved by the Faculty Committee on the Use of Human Subjects in Research and is supported by the Children's Hospital of Winnipeg Research Foundation, Inc. A copy of the research proposal is enclosed for your records.

We appreciate your interest and help in the study. Best wishes.

Sincerely,

Parul P. Jayakar, MB, BS, DCH
Research Associate

Albert E. Chudley, MD, FRCP(C), FCCMG

/tmh
Enc.

STATEMENT TO PARTICIPANTS ABOUT THE STUDY

We plan to study a group of approximately forty (40) individuals between the ages of 4 to 18 year old individuals diagnosed as having infantile autism of unknown cause. Many studies suggest that, in some families, genetic factors may play an important role in the cause of autism, while in other families, genetic factors are not important. We would like to study children with autism by doing a review of the family tree, pregnancy, birth and developmental history. We will observe the child and carefully undertake a physical examination for signs of minor anomalies, (variations in appearance or minor birth defects). We would also like to do a blood test on the affected child to look at the carrier of the genes (chromosomes). Variations in the chromosomes may give us some clues to the genetic contributing factors in infantile autism. We would like to see if we can predict possible chromosome abnormalities based on the findings on the physical examination and type of behaviour exhibited. In a very few instances only, based on the finding of a chromosome abnormality we call the fragile X, is there a possible benefit of a vitamin treatment for boys with autism who have the fragile X. We will be pleased to let you know the results at the conclusion of the project. If a chromosome variation is found, this will be explained to you to the fullest.

Health Sciences Centre

700 William Avenue
Winnipeg, Manitoba R3E 0Z3
Dial Direct (204) -

Consent Form

Children's Hospital of Winnipeg

AUTISM - GENETICS STUDY

I, _____, _____ of
(parent or guardian) (relationship)
_____ give permission to Dr. Chudley or his
(name of patient)
associates to involve _____ my (son/daughter/other)
(name)
and family in an investigation which will attempt to establish the possible causes and possible role of genetic factors in autism. The study will involve a family interview and an observation study and physical examination of the child. A blood specimen (5 cc's) for chromosome study will be obtained from the child and at a later time, other family members may be requested to donate a blood specimen. This is a voluntary study and I may withdraw from this project at any time without prejudice.

_____ _____
(Witness) (Signed)

_____
(Dated)

Health Sciences Centre

Section of Clinical Genetics
CHILDREN'S HOSPITAL
678 William Avenue
Winnipeg, Manitoba R3E OW1
Dial Direct (204) - 787-2494

A.E. Chudley, MD, FRCP(C), FCCMG (Head of Section)
J.A. Evans, PhD, FCCMG
C.R. Greenberg, MD, CM, FRCP(C), FCCMG
J.L. Hamerton, DSc, FCCMG
P.J. McAlpine, PhD, FCCMG
M. Ray, PhD, Assoc IARI, FCCMG
H. Wang, PhD

Dear _____:

We have arranged for _____ to be assessed in the Genetics-Autism Study Clinic on Monday, _____ at 9:30 a.m. Please report to the Clinical Genetics Office, Room FE231, in the Community Services Building at 685 William Avenue across from Children's Hospital. Please anticipate the assessments to take at most 2 hours. We are enclosing 2 questionnaires which we ask you to fill in and return to us prior to or at the time of the assessment. Please do not get overwhelmed by the number of questions, they will not take long to answer. The questions are a most important part of the study and will complement other information we obtain.

If for some reason you cannot attend, please call us well in advance of the appointment at 787-2494 so that we will have ample time to make other arrangements. If you have any other questions please do not hesitate to call us.

Sincerely,

Parul P. Jayakar, MB, BS, DCH
Research Associate

Albert E. Chudley, MD, FRCP(C), FCCMG

/tmh
Enc.

Children's Hospital of Winnipeg

AUTISM - GENETICS STUDY

Physical Examination Form

Name: _____

Height: _____ Weight: _____

A1

Head Circumference: _____ (%ile)

Head Shape: _____

Head Colour: _____ Hair Texture: _____

Hair Patterning: Whorl _____ L _____ M _____ R _____ Ant. _____ Bilat. _____

Widow's Peak _____ Upsweep _____

Posterior nuchal hair from T1: Mid _____ Upsweep _____

Alopecia of scalp (cutis aplasia): _____

Forehead Area: _____

_____

_____

A2

Eyebrows: _____

_____

Eyelashes: _____

Eyes: Up _____ Nil ___

Palpebral Fissures: Horizontal _____ Epicanthus + - Brushfield: Complete ___

Down _____ Imcomplete ___

Heterochromia: _____ Intra: _____ Inter: _____

Intercanthal: _____ Outercanthal: _____ Diff/2 ___

Strabismus: _____ Lens: _____

Coloboma: _____

Funduscopic: _____

Ptosis: _____ Lacrimal Ducts: _____

A3

Nose: Bridge: _____ Anteverted: _____

Hypoplastic Alae: _____ Small Nares: _____

Anterior Septum: _____ Philtrum: _____

A4

Mouth: Palate: _____

Dental: _____

_____

Tongue: _____

Frenula: _____

Alveolar Bridges: _____

Mandible: _____

Maxilla: _____

Shape: _____

Mouth Width: _____

A5

Ears: General Shape: _____

_____

U/L Segment: R: _____ L: _____

Rotation: R: _____ L: _____

Canal: _____

Tags: R: _____ L: _____

A6

Neck: Webbing: _____ Branchial Cleft: _____

Length: _____ Circumference: _____

B

Limbs: _____

_____

_____

B1

Upper Limbs and Hands:

Dimples: _____

Cubitus Valgus: _____ Extension: _____

Length: _____

Iliac Spine - Tip of Index Finger/Ant. iliac spine - tibial tuberosity: _____

_____

Midfinger/palmar: _____

Fingers: L: I II III IV V R: I II III IV V

_____

_____

_____

Triradii: L: A B C D R: A B C D

Axial Triradii: WT/WB: _____

Single Crease (Simian): _____

Nails: _____

Proximal Thumbs: _____

Joints: _____

B2

Lower Limb and Foot:

Patella: _____ Lower Segemnt: _____

Digits: _____

_____

Hallucal Pattern: _____

Nails: _____

Foot Length: _____

Others: _____

C

Chest and Abdomen:

Chest circumference: _____ Internipple Distance: _____

Accessory Nipples: _____

Tanner Breast Development: _____

Sternum: Excavatum: _____ Carinatum: _____

Length: _____ Scoliosis: _____

Kyphosis: _____

Heart: _____

Hernias: _____

Liver: _____ Spleen: _____

Diastasis Recti: _____ Abdominal Reflexes: _____

D

Genitourinary:

Hypospadius: _____

Testes: Right: _____ Left: _____

Penis: _____ Ventral Folds: _____

Sacral Dimple: _____

E

Dermatoglyphics: _____

Capillary Hemangioma: _____

Hairy Nevus - Back Midline Deep Sacral Dimple, Limpoma - Midline of Back: _____

_____

Cafe au Lait Spots: _____

Pigmented Nevi: _____

Small: (> 14 only)

Large: (> 1.5 cm)

Mongoloid spots in caucasians: _____

Depigmented Spot: _____

Hirsutism: _____

F1

Neuromuscular:

Muscles: _____

Atrophy: _____

Contractures: _____

Myotonia: _____

Fasciculations: _____

Others: _____

F2

Neurologic Behaviour: _____

_____

Strength: _____ Tone: _____

Reflexes: _____

Ataxia: _____ Nystagmus: _____

Gait: _____

Involuntary Movements: _____

_____

Right-Handed: _____ Left-Handed: _____

Focal Neurologic Signs: _____

_____

_____

Summation:

Minor Anomalies: _____

_____

_____

_____

_____

Major Anomalies: _____

_____

_____

_____

_____

Laboratory: _____

_____

_____

_____

_____

_____

CHILDHOOD AUTISM RATING SCALE
Scoring Sheet

Name _____
Date _____
Rater _____

/2
/7

1. Relationship with people

1 1½ 2 2½ 3 3½ 4 /8

2. Imitation-verbal and motoric

1 1½ 2 2½ 3 3½ 4 /9

3. Affect

1 1½ 2 2½ 3 3½ 4 /10

4. Body Awareness

1 1½ 2 2½ 3 3½ 4 /11

5. Relation to Nonhuman Objects

1 1½ 2 2½ 3 3½ 4 /12

6. Adaptation to change

1 1½ 2 2½ 3 3½ 4 /13

7. Visual responsiveness

1 1½ 2 2½ 3 3½ 4 /14

8. Auditory responsiveness

1 1½ 2 2½ 3 3½ 4 /15

9. Near receptor responsiveness

1 1½ 2 2½ 3 3½ 4 /16

10. Anxiety reaction

1 1½ 2 2½ 3 3½ 4 /17

11. Verbal communication

1 1½ 2 2½ 3 3½ 4 /18

12. Nonverbal communication

1 1½ 2 2½ 3 3½ 4 /19

13. Activity level

1 1½ 2 2½ 3 3½ 4 /20

14. Intellectual functioning

1 1½ 2 2½ 3 3½ 4 /21

15. General impressions

1 1½ 2 2½ 3 3½ 4 /22

TOTAL SCORE /24

Number of items scored 3 or higher /26

Diagnostic Category--

_____ Not Autistic

_____ Mild-Moderately Autistic

_____ Severely Autistic

GLOBAL IMPRESSION SCALE

Date_____ Name_____

/ / /2
/ / / / / /7

Diagnosis

Autistic /___/8

High Functioning /___/9

Low Functioning /___/10

Non Autistic /___/11

Probable Autism /___/12

*High Functioning - Good interpersonal and verbal skills according to age