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Frequency, Association and Genetic Implications of Chromosomal Fragile Sites in Mental Retardation

K.R. Manjunatha, G.K. Chetan, R. Arathi, G.V. Bhaskara Rao, P. Latha, S. Padma, H.N. Venkatesh, S. Srinath*, S.R. Girimaji* and S. Sheshadri*.

****Departments of Human Genetics and PsychiatryNational Institute of Mental Health and Neurosciences, Bangalore 560 029, Karnataka, India***

KEY WORDS Mental retardation; chromosomal fragile sites; association; genetic implications.

ABSTRACT Population studies have shown that overall prevalence of mild to severe mental retardation ranges from 2.5 to 5 per thousand. Genetic contribution to this group accounts for 15-30%. The role of chromosomal fragile sites in cancer, recurrent abortions and fertility failure were reported in the literature, which prompted us to look in to the possible causative role of fragile sites with mental retardation. Herein, we discuss the frequency, association and genetic implications of chromosomal fragile sites with mental retardation.

INTRODUCTION

Mental retardation is defined as sub-average general intellectual functioning which originated during developmental period and is associated with impairment in adaptive behaviour (MR: mild, moderate, profound, severe). A classification based on the etiology of mental retardation has been detailed in the atlas of Holmes et al. (1972), they are viz.,

Metabolic and endocrine disease- where in the biochemical defects recognized are transmitted as autosomal dominant or recessive, X-linked dominant or recessive or polygenic traits.

Aquired conditions due to the birth trauma, intrauterine infections, maternal malnutrition, exposure to teratogens, and ionizing radiations during pregnancy and so on.

Chromosomal abnormalities- which includes the known syndromes of both autosomes and sex chromosomes.

Central nervous system malformations- including conditions such as microcephaly, macrocephaly, hydrocephaly and cerebral palsy.

Neurocutaneous syndromes, arising from single gene mutations, which affect systems of common embryonic origin.

Diseases of unknown causes includes Syndromes of multiple deformities in which etiologic factors remain unknown in a majority of the conditions. Genetic and environmental factors accounts for a majority of this group. Chromosomal aberrations and simple Mendelian traits account for about 20% of the MR and polygenic traits for 10 %. Atleast 5 % of the MR is solely due to environmental factors and etiology of remaining 65% is either controversial or unknown (Russel 1985).

Fragile sites were first discovered by Decaban in 1965. Active exploration of fragile sites began in late 70's, when fragile X was recognized to be associated with the heritable forms of MR. Fragile sites are heritable points on human chromosome with a tendency to break or show a chromatin gap of variable width at specific locations, which are inherited in a co-dominant manner.

In view of the recent reports on the specific role of fragile sites in the predisposition of pathological conditions such as cancer, recurrent abortion, fertility failure and congenital malformation (Hecht and Hecht 1984b; Yunis and Soreng 1984; Yunis et al. 1987), prompted us to look into the association and role of chromosomal fragile sites among MR group.

SUBJECT AND METHODOLOGY

Three hundred subjects consisting of 219 males and 81 females in the age group of 6 months and 39 yrs attending the MR clinic at National Institute of Mental Health and Neurosciences, Bangalore, India were selected, following exclusion and inclusion criteria (Table 1). These subjects had symptoms of MR, nonspecific MR and/or congenital malformations. Subjects with known biochemical disorders, symptoms suggestive of chromosomal trisomics or monosomics and commonly known genetic syndromes are excluded from the study group. One hundred intellectually and physically normal (sex and age matched)

Corresponding Author: Dr. K. R. Manjunatha, Additional Professor and Head Department of Human Genetics, NIMHANS, Bangalore 560 029, Karnataka, India *Fax:* 91-080-6564830/65622121
E-Mail: krmanjunatha@yahoo.com

Table 1: Details of subject group (n=300)

Groups	*Age group*	*Male*	*Female*	*Total*
Mild M.R	6 mts - 39 yrs	105	34	139
Moderate M.R	6 mts - 30 yrs	56	15	71
Severe M.R	1 yr - 30 yrs	22	04	26
Congenital anomalies	1 yr - 30 yrs	26	16	42
Syndromes	1 yr - 30 yrs	10	12	22
Total	6 mts - 39 yrs	219	81	300

individuals were subjected for control study.

The chromosomal preparations were done using RPMI-1640 and TC 199 medium which is standardized in our center (Chetan et al. 2001). Specific inducers of fragile sites like MTX and FudR were added for the final 24 hrs of the culture (Table 2). A cut off point of 4% expression was taken as positive for each fragile site. The expression of a fragile sites were observed even in repeat cultures and confirmed by two different observers to minimize biased ascertainment.

RESULTS AND DISCUSSION

The karyotype analyses of 300 subjects showed normal chromosomal complement, however, 19 fragile sites were seen expressing in more than 4% of cells in 28 subjects (9.3%)from 23 families. 4 families had multiple sibs being affected for a particular fragile site (Table 3). Data on control population showed the presence of 9 fragile sites among 16 individuals in 1% of the cells (Table 4).

The search for genetic etiology among unspecified MR subjects remain unclear from the perusal of available literature. The association of fragile sites among MR place an important role in genetic implications in the absence of any other etiological factors as evidenced by accrued literature on human fragile sites (Hecht and Hecht 1984a).

Fragile sites exhibit fragility under appropriate conditions of induction as evidenced cyto-genetically by the presence of acentric fragments, deleted chromosomes, chromatin gaps and triradial figures. They are usually expressed in a lower proportion of metaphases (Soudek and McGregor 1981), but some reports suggests 100% expression (Anneren and Gustavary 1981). Variations in the percentage of expression is also seen among members of the same family for the same heritable fragile sites. Most of the folate sensitive fragile sites and others are seen in heterozygous conditions.

Fragile sites has been recorded from many workers in various conditions (Table 5A,B,C,D). In the present study, the cytogenetic studies revealed normal karyotype among the subject and control group. However, 28 patients who were seen to be positive for various autosomal fragile sites. One patient had Fra 1p36, which was expressed in 10% of the metaphases. Fra 1p 36 was first reported by Hecht and Hecht (1984a). They had reported this common fragile site in their study on amniocenteses, abortion, still births and live births. Fra 2q 31 was seen in four patients, 2q 31 is a common fragile, site which was first reported by Sutherland et al. (1985), from their study on patients referred for chromosome study, neonates, blood donors and laboratory staff. They presume it to be heritable but family studies were not conducted for confirmation. In this present study, one of the families with multiple sibs show the same site suggesting it to be heritable in nature. Fra 2q 33 is a common fragile site first reported by Hecht and Hecht (1984b). In our study it was seen in one patient who had developmental delay. Fra 3q 27 was seen in 1 patient in 10% of cells. This is a common fragile site first reported by Sutherland et al. (1985). Fra 4q 31 was seen in 4 patients from two families.

Table 2: Culture protocol used for the induction of various fragile sites

Medium	*FBS (ml) %*	*pH*	*Duration (hrs)*	*Inducer*	*Final conc. of the inducer*	*Duration of the inducer*
RPMI-1640	10-15	7.0-7.2	72	-	-	-
TC-199	6-8	7.4-7.6	72	-	-	-
RPMI-1640	10-15	7.2-7.4	96	MTX	0.01mg/ml	Final 24 hrs
RPM-1640	10-15	7.2-7.4	96	FudR	10^{-7}M	Final 24 hrs

Table 3: Details of fragile sites seen in more than 4% among subject group(n=28)

Case No.	*Fragile site*	*Sex*	*Age*	*Nature of fragile site*	*Frequency (%)*	*Clinical status/diagnosis*
1	2q 31	M	12 yrs	Common	8	Mild M.R.
2	2q 33	M	8 mts	Common	10	Development delay
3	3q 27	M	6 yrs	Common	10	Moderate M.R.
4	4q 31	M	14 yrs	Common	8	Moderate M.R.
	2q 31			Common	4	
5	4q 31	M	12 yrs	Common	4	Mild M.R.
	2q 31			Common	4	
6	4q 31	M	9 yrs	Common	12	Severe M.R.
	2q 31			Common	4	
7	4q 31	F	1 yr	Common	8	Moderate M.R.
8	5q 21	F	10 yrs	Common	30	Mild M.R., Myasthenia
	Xq 26			Possible rare	6	
	14q 32			Common	5	
9	5q 31	F	4 yrs	Common	8	Mild M.R./MCA
10	5q 31	M	8 yrs	Heritable Common	10	Mild M.R.
11	5q 31	M	5 yrs	Heritable Common	30	Moderate M.R.
	1q 36			Common	10	
12	5q 31	M	25 yrs	Heritable Common	6	M.R.Schizophrenia
13	5q 35	M	1½ yrs	Other possible rare	7	Mild M.R./MCA
14	6q 21	M	10 yrs	Common	12	Severe M.R.
15	9q 32	M	4½ yrs	Common	6	MCA
16	10q 21	M	3 yrs	Folate sensitive common	8	Mild M.R.
17	10q 25	M	15 yrs	BrdU requiring	6	Moderate M.R.
	3p14			Folate sensitive common	4	
18	10q 26	M	16 yrs	Common	12	Mild, Mood disorder
19	13q 21	F	15 yrs	Common	50	Moderate M.R.
20	13q 21	F	12 yrs	Common	60	Moderate M.R.
21	13q 21	M	17 yrs	Common	10	Moderate M.R.
22	13q 21	F	10 yrs	Common	8	Severe M.R./MCA
23	13q 21	F	10 yrs	Common	6	Severe M.R./MCA
24	14q 32	F	1 yr	Common	9	Moderate M.R./MCA
25	16q 23	M	3 yrs	Common	7	Moderate M.R./MCA
26	22q 13	M	11 yrs	Folate sensitive rare	8	Moderate M.R.
27	22q 13	M	9 yrs	Folate sensitive rare	8	Moderate M.R.
28	Xq26	M	4½ yrs	Other possible rare	9	Mild M.R./MCA

Table 4: Chromosomal fragile sites (1%) observed in control population (n=100)

S. No.	*Fragile site*	*No. of subjects*	*Nature*
1	1p 32	2	Common
2	1p 36	2	Common
3	2q 11	1	Folate sensitive
4	3p 14	3	Folate sensitive
5	6q 26	1	Common
6	5q 35	2	Rare
7	6p 21	2	Folate sensitive
8	16q 23	1	Common
9	Xp 22	2	Common

Three affected sibs and the mother from a single family showed this fragile site. 4q 31 is a common fragile site reported by Holmes et al. (1987) and Yunis (1987) from their study on cancer. From our study it is seen to be associated with mental retardation, as all the affected sibs of the family have the same fragile site and their normal brother does not have the said fragile site. Fra 5q 21 was seen in a patient of the study group. This fragile site was first reported by Yunis (1987). No literature is available citing its association with mental retardation except our study. The other fragile site seen is 5q 31. This is a common fragile

Table 5A: Review of literature on the Fragile sites and breakpoints from abortions, still births and live births (reproduced from Hecht and Hecht 1984b)

Fragile sites	*Class*	*Fragile sites*	*Class*
1p 36	Common	7p 11	Folate sensitive
1p 32	Common	7q 22	Common
1p 22	Common	7q 32	Common
1q 25	Common	8q 22	Common Folate sensitive
1q 32	Other possible rare	9q 21	Folate sensitive
2p 24	Common	10q 23	Folate sensitive
2p 13	Common	10q 25	BrdU requiring
2p 11	Other possible rare	11p 13	Common
2q 13	Folate sensitive	11q 13	Foalte sensitive
2q 31	Common	11q 23	Folate sensitive
2q 33	Common	12q 13	Folate sensitive
3p 14	Common	14q 24	Common
3q 27	Common	16q 22	Distamycin A inducible
5q 31	Common	17p 12	Distamycin A inducible
5q 35	Other possible rare	20p 11	Common
6p 23	Folate sensitive	22q 12	Common
6q 21	Common	Xp 22	Common
7p 13	Common	Xp 22	Common

Table 5B: Data on individuals with autosomal folate sensitive fragile sites among the mentally retarded subjects (reproduced from Sutherland 1985)

Fragile site	*Sex*	*Max Freq of fragile site*	*Parental origin*	*Clinical status*
16p 12	M	20%	Not studied	Dysmorphic feature
6p 23	M	44%	Maternal	Developmental delay
10q 23	F	28%	Maternal	Mildly retarded, Multiple malformation
9q 32	M	34%	Maternal	Mildly retarded, Multiple malformation
11q 13	M	22%	Maternal	Mildly retarded, Multiple malformation
2q 11	F	31%	Not studied	H/O Recurrent abortion & MR
9p 21	F	18%	Not studied	H/O Recurrent abortion & MR
12q 13	M	36%	Maternal	Tuberous sclerosis, retarded
7p 11	F	36%	Not studied	Mildly retarded

site, which was first reported by Hecht and Hecht (1984a). Later Shobha Rani and Ahuja (1986) have shown it to be heritable. In the present study fragile site 5q 31 was seen in 4 patients with an expression rate of 6-30% of cells and all had mental retardation. Only one patient showed a fragile site on chromosome 6. The fragile site was 6q 21, which is a common fragile site reported by Yunis (1987). No reference of its association with mental retardation was available. Fragile site on 9q 32 is seen in one subject in 6% of cells. 9q 32 is a possible common fragile site not induced by Aphidicolin. 9q 32 is first observed by Kanata et al. (1987). In the present study 3

Table 5C: Literature on autosomal fragile sites detected in non-specific M.R patient population (Reproduced from Pettit et al. 1986)

Fragile site	*Freq of expression*
2q 11	2%
3p 14	4%
6q 26	2%
9q 32	3%
10q 23	3%
16q 22	4%

Table 5D: Cytogenetic survey of mentally retarded school age population showing various autosomal fragile site of associa-tion. (Reproduced from Webb et al. 1987)

Fragile site	*Nature*
2q 31	Common
3p 14	Common
6q 26	Common
16q 23	Common
Xp 22	Common
2q 11	Rare Autosomal
12q 13	Rare Autosomal
22q 13	Rare Autosomal

different fragile sites were seen on chromosome 10, they being fragile 10 q 21 which was reported as a common site by Kanata et al. (1987). It is a folate sensitive common fragile site. Kanata et al. (1987) had seen fra 10q 21 in a patient with Prader-willi syndrome. The second fragile site seen at 10q 25 which was independently reported by Sutherland (1985) and Scheres and Hustinx (1980). Fra 10q 25 is a BrdU requiring fragile site and is seen as a polymorphic feature among Australian Cauca-sian population. The third fragile site obtained on chromosome 10 was fra 10q 26, a common fragile site reported by Yunis (1987). In our study it was seen to be associated with mental retardation and mood disorder. Several authors have reported a variety of neuro developmental abnormalities and mental retardation in individuals with fragile sites on chromosome 10 (See Table 5A,B,C). Five patients of the study group had fragile sites on chromosome 13. All these patients had the same site fra 13q 21, which is a common site and has been reported by Yunis (1987) from his study on cancer. Of the five patients, two were sibs with same degree of mental retardation and their other phenotypic features were similar. The rate of expression in these two sibs was as high as 50 and 60%. The other patient with mental retardation had an expression of 10% in the cells analysed. 2 twin sisters from a family showed the presence of 13q 21 in 6-10% of expression and they had severe MR with MCA. 14q 32 was seen only in two patients. Fra 14q 32 was first reported by Yunis (1987). Recent studies shows that the mutation of the gene at this locus leads to Muscular Dystonia. One patient in our study, who had fra 14q 32 also had Muscular Dystonia, in addition to mental retardation. 16q 23 was seen in one male subject from our study, in 7% of cells, which was also observed by Sutherland (1985). Fra 22q 13 was seen in two patients, both were sibs and had the same phenotypic features. Chromosomal analysis was done on the parents of these sibs, they had a normal karyotype, suggesting de-novo condition. The fragile site on the X chromosome, at the band 26, fra Xq 26 was seen in 2 unrelated cases from our study which was first reported by Buhler et al. (1982) in a severally retarded male. This fragile site was heritable as the mother of the index case was a carrier for the same fragile site.

As per the suggestions of Williams and Howell (1976) the individual with a fragile site may be at a slightly increased risk of producing abnormal children, this statement was also confirmed by the studies of Sutherland (1985) which seems to be true for the mother of case No.4, she has three affected children and severe retardation associated with two fragile sites 4q 31 and 2q 31 and a normal sib who is the carrier of only one fragile site 2q31. It is thought that the deleterious effect is more likely to occur when the chromosome with the fragile site is carried by the egg than the sperm. Samadder et al. (1993) suggested that, there was a deficiency of offsprings expressing the fragile site, when transmission was through fathers, suggesting gametic selection or the phenomenon of genomic imprinting.

Though, molecular or biochemical nature of a fragile sites is still not clear, events related to its molecular structure is known, in that, both high levels and low levels of thymidine causes perturbation in the levels of deoxyribonucloside triphosphate (dNTP) pools. Such a pool imbalance leads to a decrease in the fidelity of DNA replication (Kunkel and Loeb 1979; Kunkel et al. 1982). High levels of thymidline leads to elevated levels of thymidine triphosphate (dTTP), which in turn inhibits the ribonucleotide reductase catalyzed reduction of cytidine diphosphate to deoxycytidine triphosphate (dCTP) available for DNA synthesis. An elevated level of dCTP is seen with low levels of thymidline. This implies that folate sensitive fragile sites are expressed, when either dTTP or dCTP is depleted. Thus, the DNA at these specific loci is susceptible to disruption by low levels of either dCTP or dTTP during DNA synthesis, probably due to the formation of many single stranded gaps. It has been suggested (Taylor and Hagerman 1983) that a DNA region containing many such gaps would be a poor substrate for packaging and a fragile site is a region of DNA controlling expression of a fragile site is located at or very close to the fragile site (Sutherland and Hecth 1985). Sutherland et al. (1985) proposed that the DNA which is expressed as a folate sensitive fragile site is a repetitive alternating sequence of polypurine/polypyrimidine rich DNA which would lead to the production of single strand gaps when either dCTP or dTTP is limiting. Brinboim and Sederoff (1975), have reported polypurine/polyrimidline rich DNA sequence in Drosophilia which was found to be mainly composed of a simple repeating subunits (AAGAG/TTCTC). It is also known that Z-DNA is composed of alternating pyrimidlines and purines. In Z-DNA the repeating sequence is d(CA), d(GT), which occur in short

stretches through out Human genome (Hamada and Kakunaga 1982), which also would result in a single strand lesions, when replicating under conditions of limiting dCTP and dTTP pools. It is also thought that, some of the longer stretches of Z-DNA are responsible for the common fragile sites which are weakly induced by thymidylate stress and strongly induced by the DNA polymerase alpha-inhibitor aphidicolin (Glover et al. 1984). Fragile sites occur from the amplification of a naturally occurring polypurine/ polypyrimidine sequence. The degree of amplification could account for the differences obser-ved in the case of inducible fragile sites and also for the other rare sites (Sutherland 1985).

More recently, Jones et al. (1995) have shown p(CCG)n repeat to with in a 100 kb region of 11q 23.3, (Fra 11q 23.3 is a rare folate sensitive fragile site), expansion of the p(CCG) tri nucleotide is responsible for the expression of FRA 11B. Thus, it is clear that autosomal fragile sites also resemble the fragile X in its molecular nature, in being a trinucleotide repeat genetic disorder. Like fragile X and other fragile sites so far characterized, a CpG island adjacent to FRA11B was found to be methylated when p(CCG)n repeat exceeds certain limits. Also the characterization of the autosomal fragile site FRA 16A provided evidence that methylation is a consequence of trinucleotide expansion, rather than the manifestation of an intrinsic imprinting system. In fragile X syndrome, hypermethylation following p(CCG)n repeat expansion results in the loss of transcription of FMR1 gene. Similarly loss of transcription of such a hypothetical gene as a result of fragile site expression would be responsible for the clinical-phenotype observed in the cases with various autosomal fragile site manifestation.

However, whether the presence of the autosomal fragile sites alter the various transcriptional activity of these genes in question, remain unknown. But in the present context whenever there is a positive autosomal fragile site manifested, the phenotype-genotype correlations become a major difficulty for the assessment of the genetic disorder, we presume, that in such situation the genes in vicinity of the autosomal fragile site would bear a contiguous effect as far as the functional aspect of the closely set genes on either side of the fragile site. Thus, leading to a altered structural and functional transcriptional activity and thereby manifestation of abnormal protein synthesis, which has a direct bearing on the early CNS development and differentiation.

SUMMARY

Genetic and environmental etiological factors accounts for a majority of mentally retarded subjects. Many reports advocate, chromosomal fragile sites as potential markers in various pathological conditions. We looked in to the role of fragile sites for association with mentally retarded subjects. We studied 300 MR subjects, of which 28 patients (9.3%) from 23 families showed 19 different fragile sites expressing in 4-60% of cells in various culture conditions. 4 families had multiple sibs being affected for a particular fragile site. In this context, we could clearly say that, in the absence of any other etiological factors, these fragile sites play a vital role as the causative factors. Though the exact nature and functions of these sites are not clear, genes in the vicinity of these fragile sites would bear contiguous effect, leading to altered structural and functional proteins, which has a direct bearing on the early CNS development leading to mental retardation.

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