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Genetics of Fragile X Syndrome: A Systematic Data from the Indian Population

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KEY WORDS Fragile X syndrome; Indian population; frequency; diagnosis; genetic coun-selling.

ABSTRACT Fragile X syndrome is the commonest form of X-linked disorder. Its frequency among MR ranges from 6-9%. There are a few reports available on Fragile X syndrome from Indian population and we have screened for 300 MR subjects with 26 subjects (8.6%) showing Fragile X chromosome expression in 3-40% of lymphocyte cultures. Herein, we have discussed frequency of Fragile X in the Indian population. Among the subject groups, there were 7 families with multiple sibs being affected and 3 mothers of the affected subjects showed carrier status. The combined data from the Indian population is presented in this study for better understanding of the population dynamics of this syndrome.

INTRODUCTION

Fragile X syndrome is the most common X-linked genetic disorder associated with MR with a prevalence of around 1 in 1250 males and 1 in 2500 females. (Sherman et al. 1985; Hagerman 1992). Fragile X chromosome derives its name from the characteristic appearance of hypochromatic constriction at the tip of X chromosome at Xq 27.3 locus and it is visualized in the cells cultured in folic acid deficient medium (Sutherland 1979c). Most of the Fragile X patients show triad of clinical features, viz., MR, triangular face and macro-orchidism Fragile X syndrome has been reported by several groups from many countries and from different ethnic population.

Though there are several reports accrued in the literature on the diagnosis, frequency and treatment for Fragile X syndrome all round the globe, a systematic data on diagnostic criteria, association, frequency and cytogenetic studies of fragile X syndrome from Indian population is lacking. The present study was carried out to assess the frequency of Fragile X syndrome in the Indian population, to study the genetic segregation patterns in the families to establish genotype-phenotype correlations and for offering genetic counselling services among high risk families in detail, keeping in view of the vast information available in the literature.

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SUBJECT AND METHODOLOGY

300 subjects attending MR clinic at National Institute of Mental Health and Neurosciences, Bangalore, were selected for the study following exclusion and inclusion criteria, where in subjects with MR, core clinical symptoms suggestive of Fragile X were included and patients with metabolic disorders, common trisomies, multiple congenital anomalies were excluded from the study group (Table 1). As Fragile X syndrome shows male predominance, as may as 238 males were selected. 62 females were analysed to study the phenotype and genotypic nature of the syndrome. Most of the subjects showed mild to severe degree of MR.

The fragile Xq 27.3 was made to express in TC 199 medium which is deficient in folic acid content with low serum and a high pH (Table 2). Culture were also set up in RPMI 1640 medium with inducers FudR and MTX. 100 cells were screened for each cultures and repeat cultures were set up for confirmation in positive subjects. A cut off point of 4% of Fragile X expression was taken as positive for the syndrome in males and 2% in females which are confirmed through repeat blood cultures, since some of the autosomal fragile sites at the tip of the long arm can mislead the fragile X diagnosis through cytogenetic assesment (Chetan et al. 2001) and only good G banded metaphase (>100) preparations were analysed for scoring the presence of Fragile X chromosome. The fragile X site was confirmed by two different observers to minimize the biased ascertainment. Some of the cytogenetically positive patients were subjected for mo-

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

Total Subject	*Families*	*Male*	*Age/yrs*	*Female*	*Age/yrs*
300	272	238	1 yr- 39 yrs	62	1 yr - 26 yrs

Table 2: Culture protocol used for fragile X expression

Medium	*FBS(ml)*	*pH*	*Duration*	*Inducer*	*Final conc.*	*Duration*
RPMI1640	10-15	7.0-7.2	72 HRS	-	-	-
TC 199	5-8	7.4-7.6	72 HRS	-	-	-
RPMI 1640	10-15	7.2-7.4	96 HRS	MTX	0.01 mg/ml	Final 24 hrs
RPMI 1640	10-15	7.2-7.4	96 HRS	FudR	10^{-7} M	Final 24 hrs

lecular assessment to know the mutational status at FMR-1 gene, a preliminary data regarding molecular studies have been repre-sented elsewhere (Sujatha et al. 1998).

RESULTS

Twenty six subjects from 16 families showed fragile X expression in 3 to 40% of cells observed in different culture conditions (Table 3). 23 subjects were males aged between 4 to 3 yrs and 3 females aged between 3 to 8 yrs. One family had a monozygotic twin (P IV 12 and 13) with almost similar clinical symptoms. Three mothers among affected subjects showed carrier status with low percent of fragile X expression. Seven families in the present study showed more than one sibs being affected by fragile X chromosome.

DISCUSSION

Fragile X syndrome is a genetic disorder often expressing fragility at 27.3 region and is inherited in co-dominance fashion with 30% penetrance in female and 80% of penetrance in males(Sherman 1985). Fragile X syndrome was first reported by Lubs (1969) and a detailed account on the population genetics of Fragile X syndrome was reported by Jacobs et al. (1983), Sherman et al (1985) and Arinami et al. (1986). There are a few

Table 3: Fragile X expression (%) in 26 subjects in various culture conditions

Family	*Subject Code*	*Sex*	*Age*	*TC199*	*RPMI/MTX*	*RPMI/FudR*	*RPTC199*
1	A III2	M	5 yrs	4%	2%	4%	6%
2	B IV1	M	5 yrs	12%	7%	8%	-
3	C III1	M	6 yrs	4%	6%	3%	4%
4	D III1	M	14 yrs	5%	3%	0%	8%
5	EIII1	M	22 yrs	36%	13%	8%	-
6	F V1	F	3 yrs	5%	3%	0%	3%
7	G III6	M	4 yrs	20%	16%	12%	-
8	H V1	M	7 yrs	8%	4%	6%	6%
	H V2	F	4 yrs	4%	3%	0%	4%
9	I III5	M	26 yrs	18%	8%	10%	24%
	I III6	M	20 yrs	16%	10%	6%	20%
10	J V2	M	18 yrs	40%	32%	19%	-
	J V6	M	11 yrs	30%	30%	7%	-
	J V7	M	8 yrs	32%	8%	16%	-
11	K IV1	M	5 yrs	15%	7%	6%	20%
12	L III1	M	4 yrs	23%	8%	5%	17%
13	M III3	M	33 yrs	11%	5%	4%	10%
	M III6	M	26 yrs	15%	6%	8%	11%
14	N IV2	M	9 yrs	8%	6%	5%	7%
	N IV3	F	8 yrs	4%	8%	6%	5%
15	O IV1	M	9 yrs	8%	4%	4%	9%
	O IV2	M	7 yrs	5%	4%	4%	7%
	O IV3	M	4 yrs	10%	6%	4%	12%
16	P IV11	M	17 yrs	25%	8%	5%	22%
	P IV12	M	12 yrs	30%	7%	5%	27%
	P IV13	M	12 yrs	10%	4%	4%	10%

Table 4: Reports on the frequency of Fragile X Syndrome cases from Indian population.

S.No.	*Region*	*Author*	*No. Screened*	*Fx positive*	*(%)*
1	Western India	Parikh et al. 1999	849	165	19.43
2.	Northern India	Jain & Verma 1997	370	31	8.38
3.	Southern India	Present Study	300	26	8.67
4.	Southern India	Mallikarjuna Rao 2001	132	7	5.30
5.	Northern India	Deepti et al. 2001	120	9	7.51
6.	Western India	Murthy et al. 1991	113	5	4.42
7.	Southern India	Sujatha Bhaskaran et al. 1998	98	7	7.14
8.	Eastern India	Sharmila Saha & Uma Dasgupta 1999	90	5	5.55
9.	Eastern India	Babu Rao et al. 2001	60	3	5.0

reports available on the frequency, association, molecular studies on Fragile X syndrome from the Indian population (Table 4).

In our study, 300 patients were subjected for cytogenetic analysis of which twenty six subjects showed Fragile X chromosome in 3 to 40% of cells in different culture conditions. Culture conditions with TC 199 yielded better results than induced culture with RPMI 1640, suggesting depletion of folic acid is sufficient for fragile X expression. Subjects with higher percentage of expression always showed consistency even in repeat cultures. 3 female obligate carriers (mothers) of subjects (Family 9,10 and 16) showed fragile X expression in 2-6% of cells, with normal clinical symptoms except in mother of family 9 where, mild psychosis and depression was noticed. In the present sudy 7 families showed multiple sibs with fragile X syndrome (families H,I,J,M,N,O and P), thus, conforming to the nature of inheritance of the disease. Some of the autosomal fragile sites like 3p14 and $9qh^{+}$ was noticed in high percentages (10-60%) in fragile X subjects and these features could be used as potential markers for the proper cytogenetic analysis of fragile X chromosomes (Chetan et al. 2001).

Most of the fragile X syndrome subjects showed core clinical features like, MR, triangular face, lop ears, macro-orchidism, connective tissue disorder, autism, hyperactivity, gaze aversion, learning disability, cognitive behavioural prob-lems and seizures (Girimaji et al. 2001). A few subjects also showed some of the rare clinical symptoms like true microcephaly (FVI) and cerebral palsy (Family 8).

Since the first report on Fragile X family from our centre (Manjunatha et al. 1988), later there have been few reports on the Fragile X syndrome frequencies from the Indian sub population (Table 4) with a varied frequency of manifestation (4-19%), which are in accordance with the world-wide reports (Blomquist et al. 1983; Bundey et al. 1985, Arinami et al. 1986), most of the study suggesting 4-9% of fragile X frequency among mentally retarded population, except for a report from Western India (Parikh et al. 1999) showed a higher frequency of 19.43%. However, It is evident from our study that, cases with typical symptoms suggestive of Fragile X syndrome, history of MR in the families following exclusion/inclusion criteria for selection of subjects, gives a frequency of 8.66% (26 among 300) for Fragile X syndrome which is the most frequent cause of MR next to Down's syndrome (Russel 1985).

Recent advances in the molecular diagnostics of fragile X syndrome has helped in the accurate identification of number of CGG repeats at the FMR-1 locus (Warren and Nelson 1994). Preliminary data on the cytogenetically positive fragile X subjects from our study were tested with PCR and Southern hybridization techniques for molecular confirmation (Sujatha et al. 1998). In view of the accrued sparse information on the fragile X syndrome from India, data regarding cytogenetics, molecular studies such as allelic frequencies, mutation status and haplotyping is necessary for better understanding of the population dynamics of fragile X syndrome and for offering proper and accurate genetic counselling services to the high risk families.

SUMMARY

Fragile X syndrome is the most common genetic cause of MR next to Down Syndrome. We have screened 300 MR patients and showed 8.6% of Fragile X etiology. We employed cytogenetic methods and confirmed few cases with molecular techniques. Our frequency on Fragile X syndrome among MR subjects is in accordance with the world literature and also confirms to the other data available from the Indian population, concerning genetics of fragile X syndrome.

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