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Cytogenetic Investigations in Autistic Children: A Preliminary Study on the Detection of Fragile X Chromosome

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A review of literature reveals that there are several reports accrued on the association of autism and fragile X syndrome [1], [2], [3], [4], [5], [6], [7], [8]. However, there have been no published reports on the prevalence of the fragile (X) syndrome in populations of autistic males, for those by Watson et al [9], and Benzech and Noel [10], [11] who reported that 4 cases out of 76 autistic males showed fragile site at Xq (27) region and 24 cases of 43 fragile (X) (q27) males screened respectively.

In view of the association of fra (X) syndrome and autism, a pilot study was initiated to establish the association and prevalence of fragile X chromosome in austic children from India for the first time.

Material and Methods

Four males and two females, age range from 1 to 9 years, who were clinically diagnosed as having infantile autism or had austic features constituted the material for the present study. Table I, shows the family birth history and some important features pertaining to these cases. Lymphocytes cultures were put up according to the modified microculture method of Arakaki and Sparkes [12]. The chromosomal analyses were done using GTG [13], CBG [14] and QFQ [15] banding techniques. Replicate cultures were put up for fragile (X) expression following the method of Brookwell et al [16] and at least 50 cells were screened for fragile (X) and the constitutive fragile sites expression.

Results

The results of the cytogenetic studies of all these cases are given in tables III and IV respectively.

The chromosome complement in all these cases revealed normal karyotypes. However, minor chromosomal variations were detected in these patients, which include large secondary constrictions on chromosome 9, enlarged short arm on 22, large satellites on 21 & 22 and a large Y chromosome (see table III).

The fragile (X) chromosome was not found in any of these cases. However, there were fragile sites noticed in autosomes, which include 1, 2, 3, 5, 6 chromosomes, of which the most commonly observed fragile sites in most of the cases were at 3p14 & 6p23 band regions. The former is a common fragile site or even called as FUdR- sensitive or constitutive fragile site and the latter folate sensitive fragile site (see table IV).

Table I - Details of family birth history and important features characteristic of autistic children

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M - Male
F - Female
There was no parental consanguinity in any of the cases.
There was no H/O Inf. death in the family.

Table IIa - Summary of the dermatoglyphic featurees observed in 6 autistic children

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Table IIb - Summary of the dermatoglyphic featurees observed in 6 autistic children

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UL: Ulnar loop
W: Whorl
AR: Arch
TFRC: Total finger ridge count

Table III - Chromosome complement and minor chromosomal variations observed in six autistic children

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p + : Enlarged short arm, s+: Giant satellite, qh/ph: Secondary constriction on the long/short arm, + : Observed, -: Not observed.

Table IV - Fragile sites and breakpoints in chromosomes from peripheral lymphocyte cultures

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Class 1 : Folate sensitive fragile site, Class 2 : Distamycin inducible fragile site, Class 3 : BrDU

requiring fragile site, Class 4 : Common fragile sites (Folate sensitive & constitutive fragile sites), Class 5 : Possible rate fragile sites, +ve : Positive band region, -ve : Negative band region

Dermatoglyphic features of all these cases are presented in table II. Bilateral simian creases as well as sydney 1ines in the palm were noticed in two cases.

Discussion

Recently it has been reported that 2-4 children in every 10,000 are diagnosed as autistic with a 4:1 male to female ratio [5].

The developmental disability manifests before 2 years of age [17], [18]. Autism was described as a clinical entity by Kanner [19] as there are several clinical features present.

Regarding the aetiology of autism, no specific causes are known, although, several possible causes eg. environmental factors' genetic factors; chemical imbalance, brain dysfunction and emotional disturbances have been postulated [17], [18], Of these, recently, it has been shown that genetic factors in particular gained much importance in establishing the aetiology of autism. Several reports have been published on the association of fragile (X) syndrome and autism [9], [10], [11].

Although several reports have been published regarding association of fragile (X) syndrome and autism, there have been no published reports on the prevalence of the fragile (X) syndrome in populations of autistic males, except for the findings of Watson et al [9] who observed that the association of fragile (X) chromosome expression in 4 out of 76 autistic males studied had a 5.3% frequency in the total study population of autistic males which is comparable to the frequency of classical fragile (X) syndrome, with a frequency of 0 to 9% in patients who have severe mental retardation without other chromosomal disorders and thus forms an unspecified group of mental retardation. Also, they speculate that the cause of the profound developmental disorder is the fragile (X) syndrome in a small proportion of persons diagnosed for infantile autism. Also the frequency of fragile (X) expression among autistic males is not high as compared to that present among severely retarded, non-autistic males. However, reports of Benzech and Noel [10], [11] showed a high frequency of fragile (X) males (24 cases out of 43 fragile (X) males) in mentally retarded subjects with moderate to severe degree of mental retardation.

Of late the association between the presence of a fragile site at (X) (p22) and autistic/psychotic behaviour in children [4] has been seriously reviewed [6], [8], in that the fragile (X) (p22) is not associated with infantile autism but is attributed to psychiatric disorders: Jenkins et al [8] opines that, fragile (X) (p22) is truly a heritable or constitutive folate-sensitive/FUdR-inducible fragile site, as they have, observed fra (X) (p22) expressed in non autistic, mentally retarded, unrelated individuals screened for fragile (X) (q27) expression.

In all the 6 cases studied, the karyotypes revealed normal features. However, minor chromosomal variations were observed, which includes large secondary constrictions on 9, 16, 19; large satellites on 21, 22; enlarged short arm on 22 and the presence of a large Y chromosome (table III). The minor chromosomal variations and polymorphism observed are probably of no direct significance in influencing the phenotypes of their hosts, as they are also observed in general population and in men tally retarded subjects [21].

None of these six autistic children were found to have the marker (X) chromosome. However, there were fragile sites and breakpoints observed on autosomes. (table IV). Of these only the 6p23 is a heritable fragile site. The 3p14 is a constitutive site and can be used as control', for the conditions conducive to fragile (X) (q27) expression [22].

Further study on the expression of these sites in parents and sibs of the affected child and scoring more cells for fragile (X) chromosome expression on a larger same pie of autistic cases has to be made to know whether, they are truly heritable or constitutive folate-sensitive/FUdR inducible fragile sites.

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