Original Research Article

The cytogenetic and molecular study of Fragile X syndrome: A leading cause of Autism

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Abstract

Background: Fragile X syndrome is common cause of intellectual disability in the patient of autism. Thorough genetic work up, cytogenetic and molecular, is important to establish the diagnosis to have therapeutic and prognostic approach. **Aim:** to conduct the karyotyping to find out the fragile site in the X chromosome and to find the mutation by polymerase chain reaction in the FMR1 gene **Material and methods:** 21 patients (19 males and 2 females) were included in our study with autistic features and clinical features suggestive of fragile X syndrome such intellectual disability, phenotypic features like protruding and large ears, thin and triangular face, strabismus, club foot etc. Genetic familial history, Karyotyping was in the special medium (Thymine and folate deficient medium) to identify the fragile site in the X chromosome. Later on PCR was carried out to find the mutation in the FMR1 gene. **Results:** In 3 patients fragile X site was observed in karyotyping and of these two cases of mutation in FMR1 gene was observed. The patient with the autism should be tested for the Fragile X Syndrome and to categorise premutations or full mutation for genetic counselling to family members. **Conclusion:** Thorough Genetic work up which includes karyotyping and molecular studies for FXS in autistic children has to be carried out to establish the diagnosis.
Key Word: Fragile X syndrome.

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INTRODUCTION

Autism is a neuro developmental disorder, the prototypic developmental disorder (PDD). It is characterized by the triad of limited or absent verbal communication, lack of reciprocal social interaction or responsiveness and restricted, stereotypic and ritualistic pattern of interests and behavior.[1] Autism and autistic spectrum disorder are common cause of intellectual disability (mental retardation). Autism is diagnosed according to the DSM IV. Autism shows genetic heterogeneity and AUTS1 to AUTS18 have been mapped on different loci on different chromosomes[2,3].Cohen *et al*[4] reported that many genetic conditions such Fragile X Syndrome, Down syndrome, Rett syndrome, Cohen syndrome, Angelman syndrome, Smith-Lemli-Opitz syndrome are associated with autism.

Fragile X Syndrome (FXS): In 1943, Fragile X Syndrome (FXS) was first described by Martin and Bell.[5] FXS, genetic disorder is commonly inherited cause of intellectual disability after Down syndrome. Members are always affected or carrier in the family tree of the patient with FXS. The genetic defect is dynamic. On karyotype prepared with lymphocytes cultured in folate and thymidine depleted medium, the long arm of X chromosome shows constriction followed by thin strand of genetic material at X_q 27.3. Because of the constriction and thin strand, Fragile X term. The band X_q 27.3 is also known as fragile X mental retardation gene (***FMR1*** gene). The function is to produce fragile X mental retardation protein (**FMRP**). This protein binds messenger RNA in neurons and dendrites.[6] There are two causes in which FMRP protein is not formed and is responsible for the FXS. First, the gene ***FMR1*** containsa

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repeating base pair triplet (CGG) expansion. And second, the point mutation in the ***FMR1*** gene or deletion of gene. Phenotypic presentation of FXS is dependent on the number of CGG repeats in the first exon at 5' end of the band X_q 27.3. Normal individuals have 5-54 repeats. Individuals with 45-54 repeats are unaffected but may pass a permutation as a risk on to future generations. Premutation individuals have 55-199 repeats and more than 200 repeats is a full mutation. In permutation, FMRP is not affected but enhanced production FMR1 mRNA. Primary Ovarian insufficiency and fragile x syndrome associated tremor/ataxia (FXTAS) may be the presentation due toxicity of FMR1 mRNA. In full mutation, hypermethylation of cysteine bases that restrict protein binding occurs and thus inactivation of gene FMR1 and absent protein. Repeats are unstable from generations to generation posing difficulty in predicting the risk. Degree of methylation has direct correlation to the signs and symptoms Fragile X Syndrome. The microstructure of white matter is abnormal in the inferior longitudinal and uncinate fasciculus in brain.[7]

MATERIAL AND METHODS

Our study included 21 patients (19 males and 3 females) of varying age from above 3 years to 13 years. The study was conducted in the one of the reputed genetic centre in Mumbai. The patient were autistic and with symptoms of fragile X syndrome were referred to our centre for genetic evaluation in Mumbai. Genetic evaluation includes family, developmental cognitive and neuropsychiatric history. Dysmorphic features were noted such as musculoskeletal, craniofacial, ears, eyes and extremities. The clinical and dysmorphic study was carried out by the clinical geneticist. Patient with autistic features and the signs and symptoms of Fragile X Syndrome were included in the study. Genetic test such as karyotyping and karyotyping on the lymphocytes cultured in the special medium (folate and thymidine depleted medium) were carried out to find the fragile X site on the q arm of the X chromosome. Polymerase chain reaction had done to find out the mutation in the *FMR1* gene. Sequencing of the first exon at the 5' end of band of X_q 27.3 is carried out to find the number of CGG repeats to categorise the patients with premutations and full mutations for better understanding of disease and counselling. But we have not included sequencing in our study. Exclusion criteria: Patient with autism which falls in the category of Rett syndrome were excluded from our study.

RESULTS

Table 1

Total no. of Cases	Males (boys)	Females (girls)
21	19	2

Table 2:

Age group	Number of patients
3 - 6	10
7-9	6
9-12	5

Table 3:

TOTAL POSITIVE CASES for karyotyping and FMR1 gene	Positive Karyotype	Positive FMR1 Mutation
3	3	2

Table 4:

Clinical features	Present in No. of patients	Total no. of cases	Percentage %
Autistic features (poor eye contact, social avoidance, and hand flapping or biting)	6	21	28.57%
Seizure	7	21	33.33%
Hallucal crease	4	21	19.04%
Thin face with Prominent ears	19	21	90.47%
Strabismus	2	21	9.52%
Club foot	2	21	9.52%
History of Asphyxia	7	21	33.33%

Autistic features were found in 6 out of 21 patient (28.57%). 7 patients (33.33%) were history of seizures. Thin face and prominent ears were present in 90.47%. Strabismus and club foot were in present in 9.52% each. (Table4). Intelligence quotient (IQ) is in range 20 to 70.

DISCUSSION

In our study of Fragile X Syndrome of 21 patients includes 19 male and 2 females. Males are more commonly affected than the females.[2] Males with full mutations presents with the Fragile X syndrome.[8] It shows X linked inheritance. Premutations or fragile X syndrome is present in the nearly all the mothers of affected males. Premutations are passed by the affected males to their daughters. Sons remains unaffected as they receives only Y chromosome from the father and not the X chromosome. Only half of the females with full mutations presents with the fragile X syndrome, half remains unaffected because of the inactivation of other X chromosome. Females with fragile X syndrome exhibit less severe intellectual disability than males with this disorder. Affected females can pass full mutation to their children. Those males with premutations are usually range in between unaffected to mildly affected and transmits stable premutations to their daughters none to sons. Females with premutations either are unaffected or mildly affected with fragile X syndrome. But they might have a 20% chance of having fragile X associated primary ovarian insufficiency, a condition in which female reached menopause before the age of 40. CGG repeats are unstable and and increase during oogenesis. If the oocyte contains more than 200 repeats and fertilized, male child will have fragile X syndrome and female child will have 50% chance of fragile x syndrome.[9] Very few patients have the mutations in the FMR1 gene.[10] In our study 2 out of 21 patients showed mutation in FMR1 gene. It is advisable to conduct the sequencing in the mother of patients so that risk estimation can be assess for future pregnancy. But in our study we have not done sequencing. De novo mutations have not been reported.[11] 28.57 % of patients meets the diagnostic criteria of autism in our study which is 30% reported by the Claudia Bagni. The minor difference can be attributed to less number patients in the study. Cognitive impairment is important clinical sign of fragile X syndrome. Cognitive impairment is present in all the patient of fragile X syndrome though intelligence quotient varying from 20-70.[8] Female and less affected males may have IQ higher IQ nearly 80. Patient with premutations can be normal or slightly less IQ. Approximately 30% of boys with FXS meet the diagnostic criteria for autism, and these children have the lowest developmental and adaptive behavior scores of those with FXS. Clinical phenotype long face with protruding large ears was a most constant feature in almost all the patients (90.47%). Hallucal crease is also important finding was present in 19.04%. One third of the patients, 33.33% was associated with the seizures. Strabismus and club foot were in present in 9.52% each. Intelligence quotient (IQ) is in range 20 to 70.These clinical phenotype are helpful in diagnosing the fragile X Syndrome[12]

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