CYTOGENETICS OF AUTISM

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ABSTRACT:

Autism is a complex neurodevelopmental disorder that impairs communication and social skills. With the exception of chromosomes 14 and 20 abnormalities, rest of the chromosomes have been associated with autistic behavior. Abnormalities of chromosome 15 and structural and numerical abnormalities of the sex chromosomes have been the most frequently documented.

AIM :

In the present study, cytogenetic analysis of the peripheral blood samples of patients who have been diagnosed positive for autism was performed to identify the structural or numerical chromosomal abnormalities by karyotyping.

RESULTS :

The study showed no complex rearrangements or other chromosomal abnormalities. The negative results obtained in the present study may be due to the influence of some environmental factors during their embryogenesis, since autism is a complex multifactorial disorder or may be due relatively small sample size.

CONCLUSION:

Integration of results from advanced Molecular techniques would support the understanding of genetic background of autism in the future.

Keywords: *Cytogenetics, autism, PDD, GTG banding*

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INTRODUCTION:

Autism is a chronic, nonprogressive developmental disorder and one of the most heritable complex genetic disorders in psychiatry. Despite this high heritability, autism has a heterogeneous etiology, with multiple genes and chromosomal regions likely to be involved. It affects four in every 10,000 children, and the ratio of affected males to females is 3:1(APA, 1994)[1]. Autism cannot be traced to a Mendelian (single-gene) mutation and thought to be a complex multifactorial disorder. It is a strongly genetic, heritable complex disorder with an estimated heritability of greater than 90%, thought to be caused by genetic and environmental factors. Studies have examined the occurrence of chromosome abnormalities in a large sample of patients with autism and related pervasive developmental disorders (PDDs)[2]. Review of literature reveals abnoramlity in chromosome 15 to be the most frequent occurrence on karyotyping of patient with this disorder [3]. Children with autism generally have problems in three crucial areas of development — social interaction, language and behaviour and they do not follow the typical patterns of development and autistics have been described as being in their "own world".[4] Prevalence of autism is estimated to be 1–2 per 1, 000.[5] Research into biological factors implicated in autism focused on four areas: neurological, biochemical and genetic abnormalities, and problems during pregnancy and/or birth[6].

The specific causes of autism are unknown; although many genetic and environmental causes of autism have been proposed, its theory of causation is still incomplete. Mutations in different sets of genes may be involved in different autistic individuals. Autism cannot be traced to a Mendelian (single-gene) mutation or to a single abnormality. Teratogens are also related to the risk of autism in rare cases.[7] Families that have one child with idiopathic autism, there is an increased risk of having another child with autism. This recurrence risk is estimated to be about four percent which is greater than that found in families that do not have a child with autism.[8] The environment also plays a major role in the development of autism.

Each child with autism is likely to have a unique pattern of behaviour.[9] Children with some symptoms of AUTISM SPECTRUM DISORDER (ASD) but not enough to be diagnosed with classical autism are often diagnosed with PDD-NOS (Pervasive developmental disorder not otherwise specified). Children with autistic behaviours but well-developed language skills are often diagnosed with Asperger's syndrome. Girls with autistic symptoms may have Rett syndrome, a sex-linked genetic disorder. Although it varies significantly in character and severity, it occurs in all ethnic and socioeconomic groups and affects every age group. Males are four times more likely to have ASD than females.[10]

Studies of people with ASD have found irregularities in several regions of the brain and abnormal levels of serotonin or other neurotransmitters in the brain. This suggest that ASD could result from the disruption of normal brain development early in foetal development caused by defects in genes that control brain growth and that regulate how brain cells communicate with each other, possibly due to the influence of environmental factors on gene function.[11] Some of the common syndromes associated with autism are Asperger's syndrome, Rett syndrome, Prader-Willi Syndrome, Angelman Syndrome and Fragile X syndrome.[12]

To date, there have been a number of reports in the literature of chromosome aberrations in autism covering a broad spectrum of anomalies, including terminal and interstitial deletions, balanced and unbalanced

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translocations and inversions. Furthermore, instances of marker chromosomes and autosomal or sex chromosome aneuploidies have been reported in autism.

Ashley-Koch et al. identified an autistic disorder family in which the three siblings had inherited from their mother an identical paracentric inversion on the long arm of chromosome 7, with the breakpoints appearing to coincide with two chromosome 7 common fragile sites. This family is of particular interest in relation to recent genetic linkage studies. The majority of these chromosomal abnormalities arise de novo, but the functional significance of these aberrations in autism remains to be established.[3] Six major reviews of sibling, twin and family studies have all concluded that a genetic etiology exists for many cases of autism/PDD.[13] Fragile X (FRAXA) syndrome has been shown to be associated with autistic disorder in several studies.[14]

The above said reviews provide preliminary evidence of linkage and association between chromosomal abnormalities and autistic disorder. While these findings are intriguing, they are preliminary and require further study. Hence a study was undertaken to perform a cytogenetic analysis on the blood samples of children's with autism by culturing the lymphocytes for 72 hrs and staining them using standard GTG banding technique to explore the role of chromosomal abnormalities in autism.

AIM AND OBJECTIVE

The main aim of the study was to perform a cytogenetic analysis on the peripheral blood samples of patients who have been diagnosed positive for autism according to Diagnostic and statistical manual of mental disorders (DSM-IV), in order to rule out the possibility of chromosomal abnormalities by karyotyping.

Karyotype of the patient with chromosomal abnormality is to be further confirmed by molecular cytogenetic technique.

MATERIALS AND METHODS:

The study group involved 12 individuals with ASD and 3 age matched controls. After approval from the Ethics Committee, written informed consent was obtained from 12 individuals with ASDs and 3 age matched controls in the range of 2-4yrs, 4-6yrs and 6-8yrs. These subjects attended the Department of Speech Hearing and language Sciences and Vidhya Sudha-school for special children. Selection criteria for entering the study were: (1) a diagnosis of autism(DSM-IV) (2) chronological age between 2 and 8 years; (3) IQ < 30; Pregnancy, medical, and developmental histories together with a three to four generation pedigree were obtained from each patient. Physical examinations were performed and particular attention was paid to growth parameters, to any dysmorphic trait or minor anomaly especially involving the face, limbs and skin; and to abnormal muscle tone or reflexes, to the presence of involuntary movements, or coordination abnormalities. 3ml of blood was collected from all subjects in order to look for chromosomal anomalies by karyotyping.

CHROMOSOME PREPARATION AND GTG BANDING:

Approximately 1ml of freshly drawn, heparinised whole blood was added into a Culture flask containing 8ml of RPMI 1640 medium, supplemented with 2ml of fetal bovine serum and the lymphocytes were stimulated to proliferate with the addition of 400 μl of Phytohemagglutinin and the cells were cultured for 72hours at 37°C with 5% CO_2. At 66½ hr 100μl of Ethidium Bromide was added and the metaphases were arrested with the addition of 100μl of Colchicine - a spindle fibre inhibitor at 67th hr. After incubating for 1h, the contents were transferred to a centrifuge tube and centrifuged at 1000rpm for 10 min. The supernatant was discarded and the pellet was disturbed and 8ml of hypotonic solution was added and incubated for 20min at 37°C. The contents were again centrifuged at 1000rpm for 10min. The supernatant was discarded and Carnoy's fixative was added drop wise while disturbing the pellet using a vortex to avoid clumping and incubated at room temperature for 20 min. 2 – 3 washes was given with carnoy's fixative in order to obtain a white pellet. The pellet was casted onto a clean glass slide and aged at 60°C overnight. The slides were banded with trypsin and giemsa and analyzed under the microscope. 25 metaphases were analyzed and appropriate results were documented.

RESULTS:

Peripheral blood samples from 12 autistic children's and 3 age matched controls were cultured and processed after which 25 metaphases from each sample were analysed in 100X magnification and karyotyped using cytovision software to rule out the chromosomal abnormalities. The present findings do not support the existence of structural or numerical chromosomal abnormalities in this series. None of the individuals presented with chromosomal disorders.

Figure 1&2: Demonstrates the Karyotype of normal male and female obtained after processing the sample and being

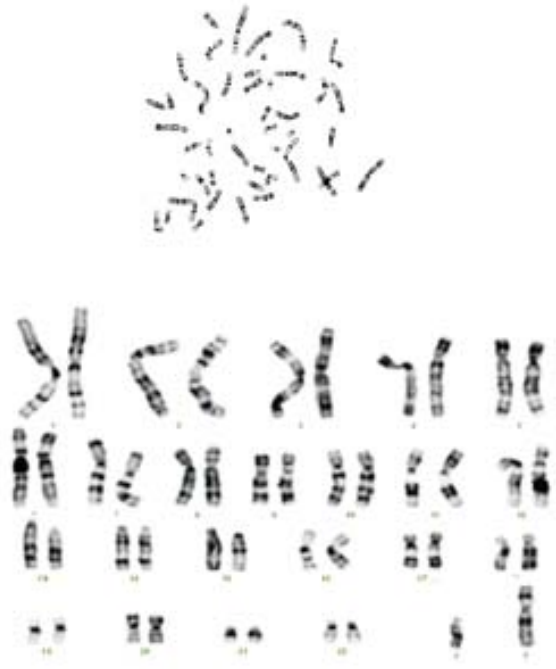

Fig. 1. KARYOTYPE – NORMAL MALE X1000

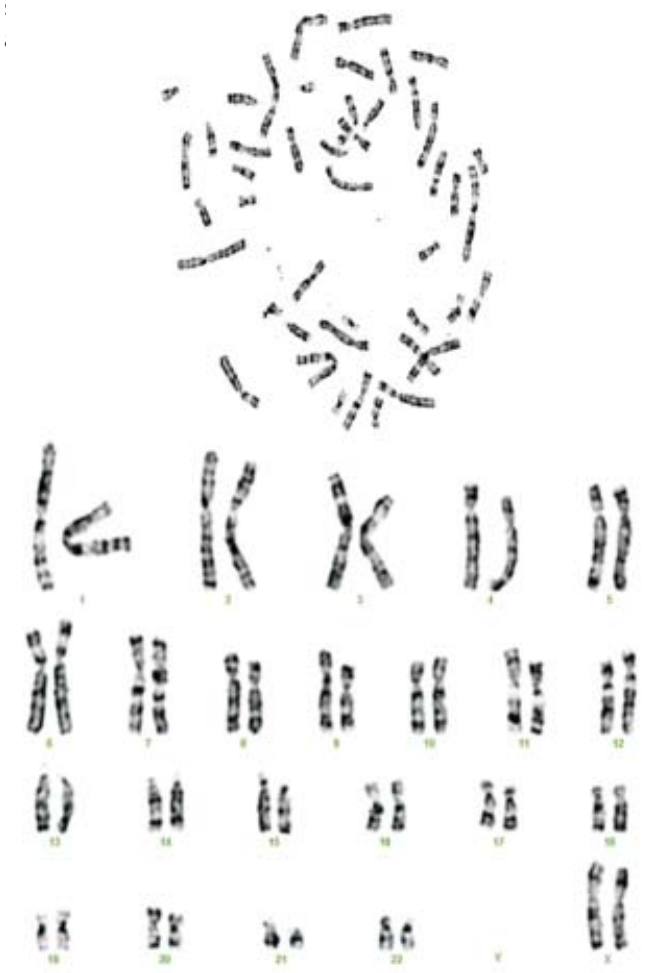

FIG 2.KARYOTYPE –NORMAL FEMALE X1000

DISCUSSION:

The findings of the present study are not of much significance and none of the cases out of 12 samples showed any chromosomal abnormalities. Chromosomal abnormalities detected by cytogenetics or molecular cytogenetics are of major aid to locate several genes. About 3-5% of autistic patients have a chromosomal abnormality visible with cytogenetic methods. Almost all chromosomes have been involved including translocations and inversions resulting in disruption of genes at the breakpoints and balanced chromosomal rearrangements are observed at a significantly increased rate. The findings reported in some earlier surveys reported a higher rate of chromosomal abnormalities. The higher rates found in these samples may be attributed to geographic and/or sample characteristics.[15]

Identification of chromosomal disorders has two current purposes. The first is identification of specific aetiology. The second is establishing accurate recurrence risk for the parents and other family members that may lead to prevention through identification of disorders which increase the risk of autistic disorder. Current cytogenetic banding methods are limited in resolution for the detection of genetic imbalances, and generally only detect deletions or duplications of 2-3 Mb in size. FISH analysis greatly increases the resolution, routinely allowing detection of deletions or duplications of cosmid sized genomic clones (30-40 kb). However, most FISH applications require a targeted testing approach to rule out imbalance of a specific genomic region.

The negative results obtained in the present study could be attributed to the fact that autism is a complex anomaly with a multifactorial inheritance. The manifestation of the disease in individuals of the present study could be due to some environmental factors during their embryogenesis or due to geographic and/or sample characteristics and also could be due to relatively small sample size. Though there are many genes involved, environmental factors also have an influence on the developing child, which should also be considered. Identification of susceptible genes and underlying clinical or genetic heterogeneity in patients with autism will help to understand the aetiology of autism.

CONCLUSION:

Although there is clear evidence that genetic factors play a role in autism and many genes are likely to be involved, in the present study, no chromosomal abnormalities were observed involving 12 patient samples and 3 age matched controls. Even though karyotyping has limited efficiency in detecting subtle chromosomal abnormality; it still serves as the basis for identifying gross chromosomal aberrations**.** This suggests high resolution banding and Identification of susceptible autistic genes by molecular techniques and increased sample size would help in further diagnosis.

Our findings in autism are preliminary and require replication. If replicated, it is possible that autistic disorder may share a common risk. To conclude, the present findings do not support the existence of chromosomal abnormalities and chromosomal anomalies may be more common in co-occurring conditions of ASD rather than traditional autism. Integration of results from large sample size and advanced molecular techniques would support the understanding of genetic background of autism in the future.

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