Int J Pharm Bio Sci 2012 July; 3(3): (B) 531 - 544

Research Article **Cytology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

CLINICAL AND CYTOGENETIC ANALYSIS OF PERVASIVE DEVELOPMENTAL DISORDER (PDD), MENTAL RETARDATION (MR) AND BROAD AUTISM PHENOTYPE (BAP) FROM SOUTH INDIAN POPULATION

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ABSTRACT

The aim of study is to analyze the clinical and cytogenetic investigation on patients with PDD, MR and BAP from South Indian population. Cytogenetic analysis of 11 patients with PDD, 3 patients with BAP and 12 patients with MR were carried out by using human leukocyte culturing method and clinical analysis were carried out for all the cases with the help of physicians. A significantly higher number of chromosomal aberrations were observed in all groups when compared to the controls ($p < 0.001$). This study shows the significantly higher number of chromosomal aberrations in all the cases through karyotyping and chromosomal aberration analysis, which has to be further investigated deeply to detect the possible genetic causes of autism to provide genetic counseling, awareness and management of autistic children.

KEYWORDS: Pervasive Developmental Disorder (PDD), Broad Autism phenotype (BAP), Mental Retardation (MR), Autism, Chromosomal Aberration.

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INTRODUCTION

Pervasive developmental disorders (PDDs) are characterized by the impairments in reciprocal social interaction, communication, and language and by the presence of stereotypic/repetitive patterns of behavior and interests[1]. PDDs are also sometimes referred to as autism spectrum disorders, and represent a class of disorders sharing similar features and including distinct diagnoses like autistic disorder, Asperger syndrome (AS), PDD not otherwise specified (PDD-NOS) disorder, and childhood disintegrative disorder (CDD). The term PDD refers to the broad class of disorders comprising all three specific diagnoses[2,3]. The prevalence of autistic disorder has increased in recent surveys and current estimates of prevalence are around 20/10,000, whereas the prevalence for PDD not otherwise specified is around 30/10,000 in recent surveys. Combined all together, recent studies that have been examined the whole spectrum of PDDs have consistently provided and estimates in the 60–70/10,000 range, making PDD one of the most frequent childhood neurodevelopmental disorders[4].

Mental Retardation (MR) is a variable, heterogeneous manifestation of central nervous system (CNS) dysfunction. It is characterized by significantly sub average intellectual functioning, existing concurrently with the related limitations in two or more of the following adaptive skill areas such as community use, self-direction, health and safety, leisure, work and functional academics[5]. But in the BAP is generally considered to be a subclinical set of characteristics or traits that index familiarity or genetic liability to autism. This conception holds that BAP is milder but qualitatively similar to diagnose the autism phenotype. BAP characteristics were first observed by Kanner[6] and the behavioral features of this BAP in relation to performance on a measure of social-cognition in an attempt to tease out this complex clinical picture and to identify the markers of underlying neuropsychological systems of genetic significance to autism[7].

Studies focusing on finding the location of chromosomal abnormalities and the breakpoints may be greatly useful in the identification and mapping of genes associated with the disease. Although some reports on the chromosomal abnormalities in autism are available in literature, a majority of them arise de novo, and the functional significance of these abnormalities in autism remains to be investigated. Further, a multitude of research studies is being undertaken in all the major countries; but in our country, very few studies on the genetics of autism have been reported. Hence, this study was undertaken to throw more light on the genetic nature of the disease using parameters such as frequency and pattern of chromosomal changes. This study also involves a correlative analysis of cytogenetics of PDD, BAP and mental retardation because of the fact that a sizeable proportion of the autistic people are mentally retarded too.

METHODS AND MATERIALS

PARTICIPANTS

A total number of 27 cases of autism spectrum disorder were referred to us for karyotyping analysis through the Dr. Rama Rau Polyclinic, Kilpauk, Chennai, Tamilnadu, India. Participants included twenty seven children (21 boys and 6 girls) with Pervasive Developmental Disorders (PDD) aged between 4 to 15 years. Clinical and cytogenetic analysis of 11 patients with PDD, 3 patients with BAP, 13 patients with MR were carried out with equal number of age and sex matched control sample. A written informed consent was obtained from their parents. The study was approved by the University Human Ethical Committee of the VIT University.

CLINICAL ANALYSIS

Clinical analysis were carried out for all 27 cases with the help of the physicians, all the clinical features that are needed to classify the

autism spectrum disorder (ASD) were recorded from all the 27 cases. Information which includes age of patient, sex, language problems, lack of eye contact, laughing, hyperactive, sleep disorders, defective speech, mental retardation, IQ, attention and concentration problems, movement, playing difficulties were recorded.

CYTOGENETIC ANALYSIS

2 ml of intravenous blood was collected from every patient and equal number of age and sex matched control sample by using sodium heparin coated vaccutainer. The cytogenetic studies were carried out in all the cases and equal number of age and sex matched controls sample to find out the karyotype and chromosomal aberrations, which includes chromosome break, chromatid break, ring chromosome and dicentric chromosome. Chromosome preparations were obtained from PHA-stimulated peripheral blood lymphocytes by using modified method of Hungerford, 1965[8]. At least fifty well spread metaphase plates were scored by direct microscopic analysis. Well spread metaphases were photographed under oil immersion objective lens (100X) of Leica DM2000 microscope with Metasystems camera and the photomicrographs of banded spreads were karyotyped using automatic IKaros software (Metasystems). The karyotype was described according to the International System for Human Cytogenetic Nomenclature[9].

RESULTS

CLINICAL ANALYSIS

Clinical analysis all the cases of Pervasive Developmental Disorder, Broad Autism Phenotype and Mental Retardation observed few clinical variations in Language Problems, Lack of eye contact, Laughing, Hyperactive, Sleep disorders, Defective speech, Mental retardation, IQ, Attention and Concentration Problem, Movement, Playing Difficulties which were presented in Table 1A,2A and 3A.

Table 1A
The Clinical Features of Pervasive Developmental Disorders (PDD)

S.NO	CLINICAL FEATURES	PDD 1-1	PDD 2-1	PDD 3-1	PDD 4-1	PDD 5-1	PDD 6-1	PDD 7-1	PDD 8-1	PDD 9-1	PDD 10-1	PDD 11-1
1	Age at Reporting	11yrs	7yrs	4yrs	9yrs	15yrs	13yrs	10yrs	12yrs	15yrs	6yrs	13yrs
2	Sex	Male	Male	Male	Male	Male	Male	Male	Female	Female	Female	Male
2	Language Problems	No	Yes	Yes	No	No	No	Yes	Yes	No	No	Yes
3	Lack of eye contact	Yes	No	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes
4	Laughing	Normal	Abnormal	Normal	Normal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal
5	Hyperactive	Yes	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes	Yes
6	Sleep disorders	No	No	Yes	No	Yes	No	No	Yes	Yes	No	Yes
7	Defective speech	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	Yes	Yes
8	Mental retardation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	IQ	54	57	60	55	63	50	65	70	58	55	55
10	Attention and Concentration Problems	Yes	Yes	No	Yes	No	Yes	No	No	No	Yes	Yes
11	Movement	Normal	Normal	Abnormal	Abnormal	Abnormal	Normal	Normal	Normal	Abnormal	Normal	Normal
12	Playing Difficulties	No	Yes	No	No	No	No	Yes	No	Yes	No	No
13	Karyotype	46,XY	47,XY+21	46,XY	47,XY+21	46,XY	46,XY	46,XY	46,XX	46,XX	46,XX	46,XY

IQ Description: 130+: Very superior; 120-129: Superior; 110-119: High average; 90-109: Average; 80-89: Low average; 70-79: Borderline; Below 70: Extremely low

Table 2A
The Clinical Features of Mental Retardation (MR)

S.NO	CLINICAL FEATURES	MR 1-1	MR 2-1	MR 3-1	MR 4-1	MR 5-1	MR 6-1	MR 7-1	MR 8-1	MR 9-1	MR 10-1	MR 11-1	MR 12-1	MR 13-1
1	Age at Reporting	6yrs	9yrs	8yrs	12yrs	14yrs	11yrs	9yrs	13yrs	8yrs	12yrs	9yrs	15yrs	8yrs
2	Sex	Male	Male	Male	Male	Male	Male	Male	Female	Female	Female	Male	Male	Male
2	Language Problems	No	Yes	Yes	No	No	No	Yes	Yes	No	No	Yes	No	No
3	Lack of eye contact	Yes	No	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes
4	Laughing	Normal	Abnormal	Normal	Normal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Normal	Normal
5	Hyperactive	Yes	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes	Yes	Yes	No
6	Sleep disorders	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes	No	Yes	No	Yes
7	Defective speech	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes	No
8	Mental retardation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	IQ	65	54	57	61	59	62	61	71	59	51	63	65	53
10	Attention and Concentration Problems	No	Yes	Yes	Yes	No	Yes	No	No	No	Yes	Yes	Yes	No
11	Movement	Normal	Normal	Abnormal	Abnormal	Abnormal	Normal	Normal	Normal	Abnormal	Normal	Normal	Normal	Abnormal
12	Playing Difficulties	No	Yes	No	No	No	No	Yes	No	Yes	No	No	No	No
13	Karyotype	47,XY+21	46,XX	47,XY+21	47,XY+21	46,XX	47,XY+21	46,XX	46,XY	46,XY	46,XY	46,XY	46,XY	

IQ Description: 130+: Very superior; 120-129: Superior; 110-119: High average; 90-109: Average; 80-89: Low average; 70-79: Borderline; Below 70: Extremely low

Table 3A
The Clinical Features of Broad Autism Phenotype (BAP)

S.NO	CLINICAL FEATURES	BAP 1-1	BAP 2-1	BAP 3-1
1	Age at Reporting	15yrs	12yrs	10yrs
2	Sex	Male	Male	Male
2	Language Problems	No	Yes	Yes
3	Lack of eye contact	Yes	No	No
4	Laughing	Normal	Abnormal	Normal
5	Hyperactive	Yes	Yes	Yes
6	Sleep disorders	No	No	Yes
7	Defective speech	Yes	Yes	Yes
8	Mental retardation	Yes	Yes	Yes
9	IQ	62	52	58
10	Attention and Concentration Problems	Yes	Yes	Yes
11	Movement	Normal	Abnormal	Abnormal
12	Playing Difficulties	No	Yes	Yes
13	Karyotype	46,XY	47,XY+21	46,XY

IQ Description: 130+: Very superior; 120-129: Superior; 110-119: High average; 90-109: Average; 80-89: Low average; 70-79: Borderline; Below 70: Extremely low

CYTOGENETIC ANALYSIS

The entire workload of the cytogenetic study in patients with pervasive developmental disorders, those with broad autism phenotype, mental retardation and were discussed sequentially below.

PERVASIVE DEVELOPMENTAL DISORDERS (PDD):

In Pervasive Developmental Disorders, the giemsa banded metaphase analyses revealed 47, XY+21 trisomy for 2 cases of PDD presented in Figure 1, 6 cases of PDD revealed normal 46, XY male karyotype and 3 cases of PDD revealed normal 46, XX female karyotype. The frequency of chromosome aberrations per cell in the PDD patient was (0.28) much higher than that of the male (0.014) and female (0.012) control at the level of $p < 0.001$, presented in Table 1B and Figure 2.

Table 1B

The Cytogenetic Analysis of Pervasive Developmental Disorders (PDD)

S.NO	CASE NO.	KARYOTYPE	TOTAL NO.OF CELLS SCORED	NUMBER OF CELLS WITH AB	CH. ABERRATIONS				TOTAL NUMBER OF CH. ABS	% CH. ABS	NO.OF ABS PER CELL
					CH. BKS.	CHD. BKS.	DIC. CH.	RG. CH.			
	MALE CONTROL (n=10)	46,XY	500	7	7	0	0	0	7	1.4	0.014
	FEMALE CONTROL (n=10)	46,XX	500	6	6	0	0	0	6	1.2	0.012
1	PDD1-1	46,XY	50	10	11	4	2	0	17	34	0.34
2	PDD 2-1	47,XY+21	50	9	12	1	0	2	15	30	0.30
3	PDD 3-1	46,XY	50	8	11	1	0	0	12	24	0.24
4	PDD 4-1	47,XY+21	50	11	9	8	0	1	18	36	0.36
5	PDD 5-1	46,XY	50	16	14	4	0	1	19	38	0.38
6	PDD 6-1	46,XY	50	10	7	6	0	0	13	26	0.26
7	PDD 7-1	46,XY	50	9	12	0	0	0	12	24	0.24
8	PDD 8-1	46,XX	50	7	8	2	0	0	10	20	0.20
9	PDD 9-1	46,XX	50	11	8	5	0	0	13	26	0.26
10	PDD 10-1	46,XX	50	12	8	3	1	0	12	24	0.24
11	PDD 11-1	46,XY	50	10	9	4	0	0	13	26	0.26
	TOTAL		**550**	**113**	**109**	**38**	**3**	**4**	**154**	**28**	**0.28**
	CH=CHROMOSOME; CHD: CHROMATID; BRS: BREAKS; DIC;DICENTRIC; RG: RING; ABS: ABERRATIONS; AB:ABERRATION										
	The chromosome aberrations per cell in the patient = 0.28										
	The chromosome aberrations per cell in the male control = 0.014 and female control is = 0.012										
	In the patients the frequency of chromosomal aberrations is significantly higher than that of the control at the level of $p < 0.001$.										

Mental Retardation (MR)

In Mental Retardation disorders the giemsa banded metaphase analyses revealed 47, XY+21 trisomy for 4 cases of MR, 6 cases of MR revealed normal 46, XY male karyotype and 3 cases of MR revealed normal 46, XX female karyotype. The frequency of chromosome aberrations per cell in the MR patient was (0.272) much higher than that of the male (0.014) and female (0.012) control at the level of $p < 0.001$, presented in Table 2B and Figure 2.

Table 2B

The Cytogenetic Analysis of Mental Retardation (MR)

S.NO	CASE NO.	KARYOTYPE	TOTAL NO.OF CELLS SCORED	NUMBER OF CELLS WITH AB	CH. ABERRATIONS				TOTAL NUMBER OF CH. ABS	% CH. ABS	NO.OF ABS PER CELL
					CH. BKS.	CHD. BKS.	DIC.CH.	RG.CH.			
	MALE CONTROL (n=10)	46,XY	500	7	7	0	0	0	7	1.4	0.014
	FEMALE CONTROL (n=10)	46,XX	500	6	6	0	0	0	6	1.2	0.012
1	MR 1-1	47,XY+21	50	10	11	4	2	0	17	34	0.34
2	MR 2-1	46,XY	50	9	12	1	0	2	15	30	0.30
3	MR 3-1	47,XY+21	50	8	11	1	0	0	12	24	0.24
4	MR 4-1	47,XY+21	50	11	9	8	0	1	18	36	0.36
5	MR 5-1	46,XY	50	16	14	4	0	1	19	38	0.38
6	MR 6-1	47,XY+21	50	10	7	6	0	0	13	26	0.26
7	MR 7-1	46,XY	50	9	12	0	0	0	12	24	0.24
8	MR 8-1	46,XX	50	7	8	2	0	0	10	20	0.20
9	MR 9-1	46,XX	50	11	8	5	0	0	13	26	0.26
10	MR 10-1	46,XX	50	12	8	3	1	0	12	24	0.24
11	MR 11-1	46,XY	50	10	9	4	0	0	13	26	0.26
12	MR 12-1	46,XY	50	7	5	5	1	0	11	22	0.22
13	MR 13-1	46,XY	50	6	6	6	0	0	12	24	0.24
	TOTAL		**650**	**126**	**120**	**49**	**4**	**4**	**177**	**354**	**0.272**
	CH=CHROMOSOME; CHD: CHROMATID; BRS: BREAKS; DIC;DICENTRIC; RG: RING; ABS: ABERRATIONS; AB:ABERRATION										
	The chromosome aberrations per cell in the patient = 0.272										
	The chromosome aberrations per cell in the male control = 0.014 and female control is = 0.012										
	In the patients the frequency of chromosomal aberrations is significantly higher than that of the control at the level of $p < 0.001$.										

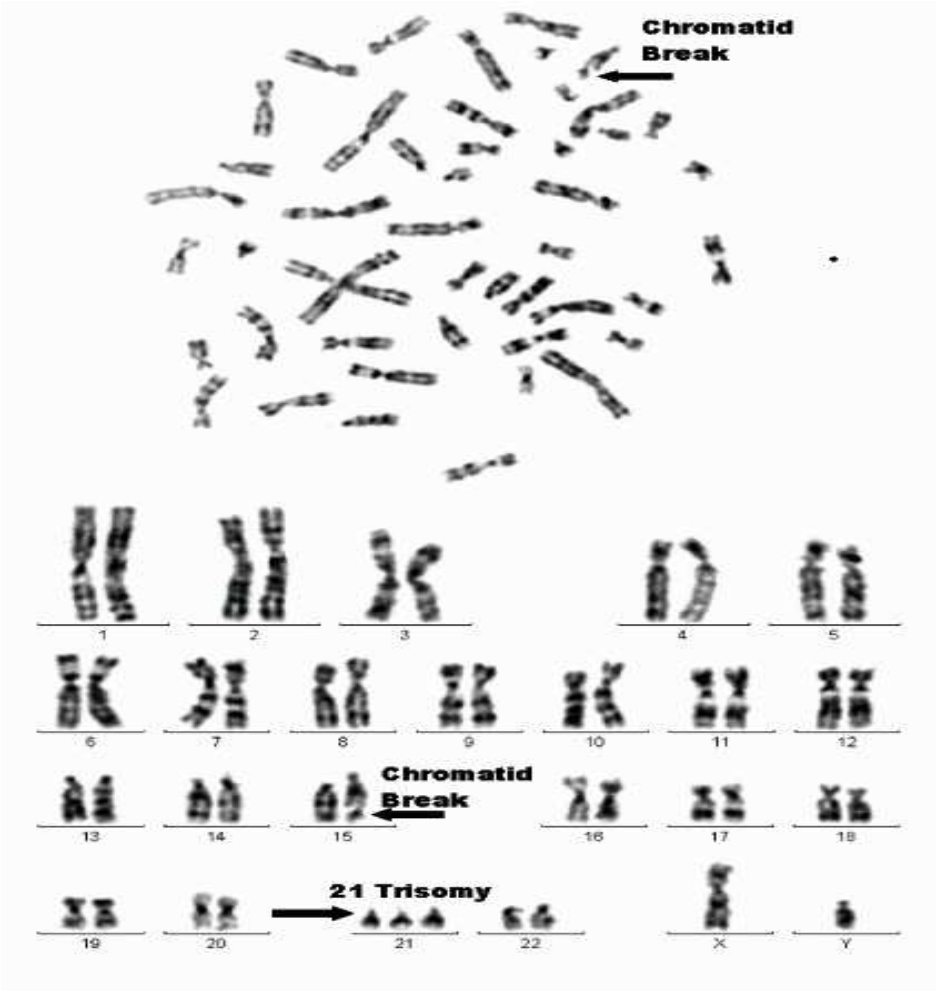
Broad Autism Phenotype (BAP)

In Broad Autism Phenotype disorders the giemsa banded metaphase analyses revealed 47, XY+21 trisomy for 1 case of BAP, and rest of 2 cases of BAP revealed normal 46, XY male karyotype. The frequency of chromosome aberrations per cell in the BAP patient was (0.32) much higher than that of the male (0.014) control at the level of $p < 0.001$, presented in Table 3B and Figure 2.

Table 3B

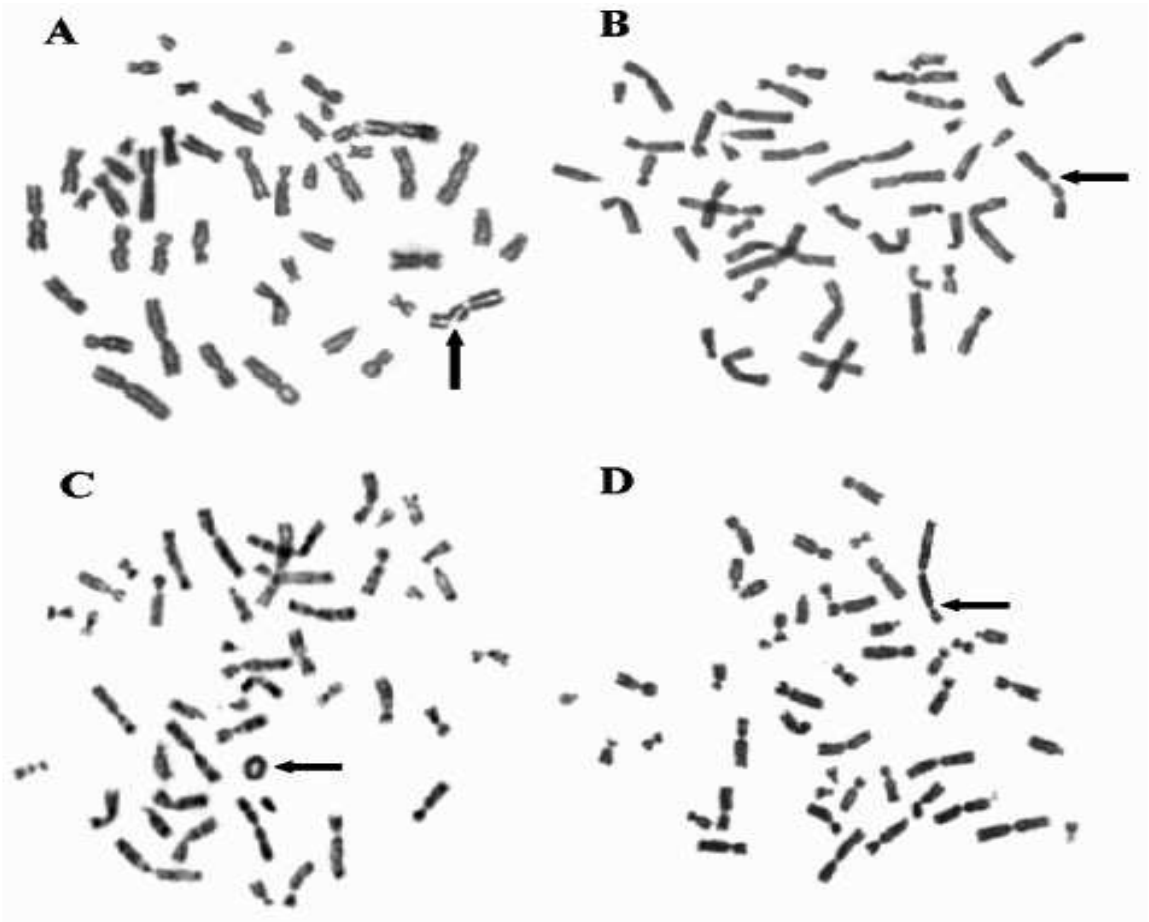
The Cytogenetic Analysis of Broad Autism Phenotype (BAP)

S.NO	CASE NO.	KARYOTYPE	TOTAL NO.OF CELLS SCORED	NUMBER OF CELLS WITH AB	CH. ABERRATIONS				TOTAL NUMBER OF CH. ABS	% CH. ABS	NO.OF ABS PER CELL
					CH. BKS.	CHD.BKS.	DIC.CH.	RG.CH.			
	MALE CONTROL (n=10)	46,XY	500	7	7	0	0	0	7	1.4	0.014
1	BAP 1-1	46,XY	50	10	11	4	2	0	17	34	0.34
2	BAP 2-1	47,XY+21	50	9	12	1	0	4	17	32	0.32
3	BAP 3-1	46,XY	50	7	10	5	0	0	15	30	0.30
	TOTAL		**150**	**26**	**33**	**9**	**2**	**4**	**47**	**32**	**0.32**
	CH=CHROMOSOME; CHD: CHROMATID; BRS: BREAKS; DIC;DICENTRIC; RG: RING; ABS: ABERRATIONS; AB:ABERRATION										
	The chromosome aberrations per cell in the patient = 0.32										
	The chromosome aberrations per cell in the male control = 0.014										
	In the patients the frequency of chromosomal aberrations is significantly higher than that of the control at the level of $p < 0.001$.										



➡ *- Left arrow indicate the chromatid break in chromosome number 15*
➡ *- Right arrow indicates the 21 trisomy*

Figure 1
Karyotyping analysis showing 47, XY+21 Trisomy (Down Syndrome) in Pervasive Developmental Disorder (PDD).



A. Arrow indicate the chromosome break in 46, XY chromosome complement
B. Arrow indicate the chromatid break in 46, XX chromosome complement
C. Arrow indicates the ring chromosome.
D. Arrow indicate the dicentric chromosome

Figure: 2
Various types of chromosome aberrations observed in PDD, MR and BAP cases of autism spectrum disorders

DISCUSSION

The current objective of this study is to provide a better patients picture of the relationship among patients with PDD exhibiting symptoms of MR and BAP via clinical and chromosomal aberration analysis in south Indian population. They are mostly preoccupied with self-stimulatory behavior that affects their learning ability and concentration skills. The lack of attention to commands also affects learning, speech and communication and their overall functions[10,11]. In this study most of the children were affected for Communication skills and communication was by gestures instead of words. Most of them had self talking and unnecessary laughing behaviors

There is much variation in the capacity to use vocal communication in young children with ASD, which likely contributes to the wide range of speech and language skills. Some children with ASD have been found to use a limited consonant inventory and less complex syllabic structure, while others show adequate complexity of vocalizations[12,13,14] found that compared to children with developmental delays (DD), preschool children with ASD used a comparable proportion of syllables containing consonants but a significantly greater proportion of syllables with atypical phonation,

such as squeals, growls, and yells. Deficits in the capacity to use conventional and symbolic gestures have been documented in numerous studies[15]. Although speech delay is a common feature of children with ASD, there are many stages of communication development that precede spoken words and provide a foundation for the emergence of verbal language[16].

In this study the most of the participants can express their needs through gestures though not the exact information. When they play with peers, very minimal or no interaction is seen. They show irritability when they were asked to do an activity which is not of their choice. People with autism show significant social and communication deficits when compared to their mental age peers. Even high functioning verbal patients with autism behave and communicate in appropriately, remain deficient in social interactional skills later in life[17,18]. The children with ASD showed comparable or higher use of communication to request and protest, but significantly less use of gaze shifts, shared positive affect conventional gestures, coordinated gestures with vocalizations and eye gaze, and communication for joint attention[19].

One of the most significant stresses for family caregivers and support staff is the extent of behavior problems exhibited by children and adults with developmental disabilities[20,21]. In this study, the participants' activity levels are poor in performance and difficult in modulating, sensory systems mostly in tactile and vestibular system. They show difficulty in continuing an activity and mostly exhibit randomness and lacks purposefulness unless they are constantly persuaded. Most of the participants are hyperactive in choice of their activities. They mostly display imitation rather than creating own activity.

A recent FISH subtelomere study found one out of ten unselected patients with ASD had a subtelomeric 2q ter deletion[22]. In our experience all the cases were negative for subtelomeric rearrangements. Children with Down syndrome have autism more commonly than expected. The incidence was at least 7% in one study[23]. This finding suggests that chromosome abnormalities may lower the threshold for the expression of autism. In this study the giemsa banded metaphase analyses pervasive Developmental disorders revealed 47, XY+21 trisomy for 2 cases of PDD presented in Figure 1, 6 cases of PDD revealed normal 46, XY male karyotype and 3 cases of PDD revealed normal 46, XX female karyotype. In Mental Retardation disorders the karyotyping analysis revealed 47, XY+21 trisomy for 4 cases of MR, 6 cases of MR revealed normal 46, XY male karyotype and 3 cases of MR revealed normal 46, XX female karyotype. In Broad Autism Phenotype disorders the giemsa banded metaphase analyses revealed 47, XY+21 trisomy for 1 case of BAP, and rest of 2 cases of BAP revealed normal 46, XY male karyotype. The frequency of chromosome aberrations per cell in the PDD patient was (0.28), for MR patient was (0.272) and for BAP patient was (0.32) presented in Table 1B, 2B and 3B. The frequency of chromosome aberrations in all the three classes of autism cases showed significantly higher number of aberrations when compared with male (0.014) and female (0.012) control at the level of $p < 0.001$. Recent studies estimated a rate of 3-5% of cytogenetic anomalies in autistic disorders[24,25]. A study showed chromosomal anomalies to be found rarely in children with classical autism. The same study showed chromosomal anomalies to be common in children diagnosed to have PDD and autistic traits[26]. Our results from this study also confirm these findings.

Genetic factors play a very important role in the causation of autistic traits in children with intellectual disability. Among these, children falling into the category of classical autism do not have associated chromosomal abnormalities, whereas those with autistic traits and PDD have chances of associated chromosomal abnormalities present. It is mandatory that a detailed cytogenetic evaluation has to be recommended in all subjects with ASD, more so if the subject

additionally shows intellectual disability, abnormal EEG patterns or seizures, muscular hypotonia, severe motor and gait problems or dysmorphic features.

CONCLUSION

A multitude of research studies are being undertaken in all major countries; but in India, very few studies on the genetics of Autism have been published. Until recently, the diagnosis of autism had been achieved purely on a clinical basis, given the lack of diagnostic genetic markers. Causes and contributing factors for autism are poorly understood. Evidence suggests that prevalence is rising, but the extent to which diagnostic changes and improvements in ascertainment contribute to this increase is unclear. Both genetic and environmental factors are likely to contribute etiologically. The present study shows chromosomal aberrations were significantly higher in autism cases when compared with control samples. In conclusion, it should be noted that detailed cytogenetic studies are recommended for all children with autistic disorders, particularly if they have mental retardation, EEG anomalies, or convulsions, muscle hypotonic, severe motor disorders, and signs of minor developmental anomalies[27] the mothers should also be studied. As autistic conditions are largely genetically determined, the detection of chromosomal anomalies as a probable cause of autistic disorders is very significant in genetic consultations genetic counseling, awareness and management of autistic children.

Our biggest wish today is that everyone here will become an advocate for these vulnerable children and families. We need to get parents out of shame and denial. We need to campaign for availability of appropriate services. We need to educate the government and the public about ASD; we need to get schools to open their gates for our children.

ACKNOWLEDGEMENT

The authors are indebted to patients and family members for providing us with blood samples. The authors would also like to thank the management of VIT University for providing the facilities to carry out this work. The author S.Rajiv is grateful to VIT University for providing the financial assistance during this tenure.

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