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Cytogenetic Studies of Idiopathic Mental Retardation: A Report

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ABSTRACT A majority of subjects with mental retardation and congenital anomalies cannot be classified under any known malformation syndromes. Major problems in understanding the mechanisms involved in the production of this abnormal phenotype and consequently in providing accurate and informed genetic counseling are therefore challenging. The application of recently developed chromosome banding techniques including the high resolution banding and FISH studies to population surveys has enabled accurate identification of individual chromosomes , specific existence and understanding of various chromosomal aberrations and their association with mental retardation. The present study was carried out to delineate any such chromosomal aberrations and their association in subjects with idiopathic mental retardation, multiple congenital malformations and various syndromes.

INTRODUCTION

The mental retardation (MR) of unknown cause is grouped under "idiopathic mental retardation" category. Idiopathic MR subjects show learning disabilities and borderline intelligence whose IQ range is 71-85.

Cytogenetic abnormalities among the subjects with Idiopathic Mental retardation with chromosomal abnormalities have been recorded; like 22q-13.3 deletion in language disability and speech in Autism (Manning et al. 2004), deletions in the 2qter and 6qter regions is seen in an idiopathic mental retarded subject by Li Rong and Zheng (2004).

In a study by Luciani et al. (2003), telomeric 22q13 deletion occurred as a result of a ring chromosome, simple deletion and translocations. The deletion was shown to be highly variable, ranging from 160 kb to 9 Mb. It was also shown that the parental origin of these deletions was more paternal (74%) than maternal (26%). A study by Sismani et al. (2001) detected an 8q subtelomeric deletion in an idiopathic mentally retarded subject by using FISH and MAPH Telomere Assay. A mild developmental delay and dysmorphism and very blue iris in a patient who showed 15q24, q26 1.1 interstitial deletion was reported by Spruijt et al. (2003).

Idiopathic subjects also show chromosomal anomalies in the form of translocations. A *de novo* balanced translocation between 17p 13.3 and 20q 13.33 was identified by Walter et al. (2004). A study by Anderlid et al. (2002) identified one de novo unbalanced translocation and three unbalanced translocation inherited in a patient suffering with idiopathic mental retardation. Granzow et al. (2000), identified an unbalanced cryptic translocation der (5) t (3; 5) (q 27- p15.3) in a family with three cases of unexplained mental retardation and dysmorphic features using the Multiplex FISH Assay. Another unbalanced cryptic translocation between chromosomes 8 and 13: der (13) t (8; 13) (q 24.3- q34) in two sisters was identified by Kleefsrta et al. in 2000.

FISH studies in 84 families with idiopathic mental retardation (Ewa Bocian et al. 2004) had revealed a large number of aberrations such as: 46 XY t(7,10) (q36 q26), 46 XY der(13) t(4,13) (p16 q34), 46 XY der(2) t(2,7) (q37 q36), 46 XX der(4) t (4,21) (p16 q 22), 46 XY der(6) t(4,6) (q35 q27), 46 XX der(13) t(X, 13) (q28 q34), 46 XY der(10) t(10,19) (q26, p13.3), 46 XY del (4) (p16.1 p16.3).

Cryptic unbalanced chromosomal rearrangements in telomeric bands of human chromosome constitute a significant cause of idiopathic mental retardation. This was supported by numerous investigations, namely Ghaffari et al. (1998), Coco and Penchaszadeh (1982), Flint et al. (1995) Arunkumar (1998), Baker et al. (2002).

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Keeping in view the literature on genetic studies in idiopathic mental retardation, the present investigation comprises the study of 100 mental retardation subjects. The report shows that 12 of the subjects had subtle chromosomal defects and details of the genetic implications are discussed in brief (Venkatesh 2006).

MATERIALS AND METHODS

100 patients consisting of 76 males and 24 females in the age group between 1-30 years belonging to different parts of India were selected and included in the study on the basis of mental retardation, multiple congenital malformations, various syndromes and anomalies of the central nervous system (CNS). All patients with syndromes of known gross chromosomal abnormalities like, Down syndrome, Klinefelter syndrome, Turner syndrome and patients with biochemical disorders were excluded from the present study.

Among the one hundred patients with unknown cause of mental retardation, multiple congenital malformation and syndromes were selected for the present study, 60 (50 males and 10 females) patients had mild degree of mental retardation, 25(16 males and 9 females) patients had moderate degree of mental retardation, 15(10 males and 5 females) have multiple congenital malformations (Table 1). These subjects were selected from in-patient and out-patient services of Mental Retardation clinic and from the Child Guidance Clinic (CGC) unit at NIMHANS, Bangalore, India. Since the evaluation of mental retardation in young infants was difficult, patients less than one year of age were not included in the present study. Among those included between one year and thirty years of age, a definite abnormality of the CNS was readily identified apart from some degree of mental retardation, multiple congenital malformations and syndromes.

Fifty intellectually and phenotypically normal, healthy individuals from the same age group were selected to serve as controls.

The chromosome preparations were made from peripheral blood lymphocytes using the standard method of Arakaki and Sparkes (1963) modified by Manjunatha (1988). The slides were GTG banded using Seabright's (1971) method of chromosome banding. Karyotypes were made according to the classification adopted by the Paris conference (1971).

For the Fluorescent In Situ Hybridization (FISH) analysis the cultures were setup similar to those for GTG banding. Both centromeric and Locus Specific Indicator (LSI) probes (Vysis) were used. The denatured probe was applied to the marked region on the slides and a coverslip was placed over the slide .The slides were placed in the Hybridization chamber (HYBRITE, Vysis) overnight at 37^0 C. The slides were counter-stained with DAPI or PI and observed under Leica DM LB2 Microscope, using the appropriate filters. For the Interphase nuclei a minimum of 100 cells for good signals and at least 5 images were captured. For metaphase spread, a minimum of 15 spreads were analyzed and 3 images were captured.

RESULTS

The cytogenetic investigations were done in 100 subjects with idiopathic mental retardation, multiple congenital malformations and 50 controls. Among the subject group, males and females between the age group 1-30 yrs were selected and studied. A systematic survey on the presence of any subtle chromosomal aberrations revealed that 12 subjects have chromosomal structural anomalies (Table 2), such as, translocations, deletions, inversions, and ring chromosome formations. These findings were compared with the data on the control population.

Among the 12 subjects positive for various chromosomal anomalies, 8 were males aged between 2 1/2 to 17 years and 4 were females aged 3 to 23 years.

Out of the 12 positive families studied, 1 family showed first degree consanguinity, the rest showing no consanguineous union. In these 12 families, one family had two sibs affected with the same chromosome anomaly in their karyotype.

DISCUSSION

The findings varied from inversions of the chromosomal regions, deletions, ring formations,

Table 1: Degree of M.R. / MCA syndromes among the patient Groups (n = 100)

Abnormality	*Age group*	*Males*	*Females*	*Tota1*
Mild M.R	1-30 yrs	50	I 0	60
Moderate M.R	1-30 yrs	16	9	25
MCA	1-30 yrs	10	5	15
Total	1-30 yrs	76	24	100

Table 2: Different chromosomal anomalies found among the positive subjects in the present study (n=12) (see Fig 1 and 2)

Case No	*Chromosomal anomaly*	*Sex*	*Age(in years)*	*Clin. Status / Diagnosis*
1	del(15)(q11q13)	Male	8	Moderate MR/PWS
2	del(15)(q11q13)	Male	10	Mild MR/PWS
3	del(15)(q11q13)	Male	13	Severe MR
4	inv(9)(p13q21)	Male	4	Mild MR
5	inv(9)(p11q13)	Male	2 1/2	MCA
6	inv(9)(p11q13)	Male	9	Severe MR
7	inv(X)(q13q27)	Female	17	Mild MR
8	del(X)(pter→q21)	Female	16	Moderate MR
9	r(15)	Male	6	Mild MR / MCA
10	r(22)	Male	12	Mild MR
11	t(1;7)(p21;q11.2)	Female	3 1/2	Severe MR
12	t(3;10)(q28.2;q21.1)	Female	16	Moderate MR / MCA

Table 3: The percentage of chromosomal abnormalities reported previously by various authors in subjects with mental retardation.

Number	*Investigation*	*Year*	*Subjects studied (n)*	*Subjects with chromosomal anomalies (n)*	*%*
1.	Lubs and Lubs	1973	54	4	7.4
2.	Jacobs PA	1978	475	57	12.0
3.	Faed et al	1979	756	103	13.6
4.	Gripenberg et al	1980	1062	349	32.8
5.	Kondo et al	1980	449	37	8.1
6.	Narahara K	1981	74	11	14.9
7.	Nelson and Smart	1982	720	148	20.5
8.	Rasmussen et al	1982	1905	354	18.5
9.	Ambani et al	1984	709	248	35.0
10.	Fryns et al	1984	1991	424	21.3
11.	Reddy and Thomas	1985	200	39	19.5
12.	Kanata S	1986	121	14	11.6
13.	Kanata S	1986	56	9	16.1
14.	Hedge et al.	1989	1314	312	23.7
15.	Latha P	1996	100	18	18.0
16.	Bhaskar Rao GV	1999	200	40	20.0
17.	Hong et al	1999	604	69	11.3
18.	Riegel et al	2001	254	15	5.9
19.	Anderlid et al	2002	111	10	9.0
20.	Koolen et al	2004	210	14	6.7
21.	Soggard et al	2005	132	9	7.0
22.	Present Study	2007	100	12	12.0

translocations to various chromosomal regions (Table 2, Fig 1a-e and 2). Previous studies by various authors (Table 3) have also found out the association between the chromosomal anomalies and mental retardation. Multiple congenital anomalies, as a separate group do not specifically associate with any chromosomal anomaly. However, when mental retardation is also present there is an association with chromosomal anomalies. Although various syndromes have so far been associated with a "specific chromosomal anomaly" as in the case of Prader-Willi syndrome, del 15 (p11-13), there are still a lot more syndromes for which a definite chromosomal anomaly is not attributed. This study has tried to find out any such association and was successful to some extent.

Hence, it can be thought that, the association of these various chromosomal abnormalities with idiopathic mental retardation, congenital malformations and syndromes might have a definite etiology, but their implications in phenotype-genotype correlations are not well understood. However, they are useful for management, prevention, treatment amongst the high risk family members.

The application of the technique of high resolution banding, coupled with FISH analysis

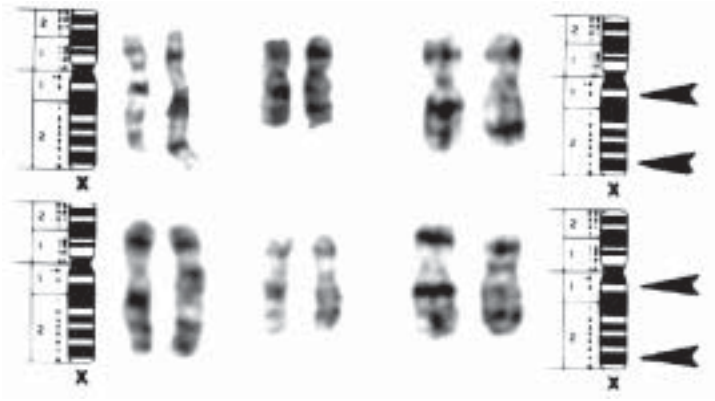

Fig. 1a. Cut-out showing invX (Case No. 7)

Fig. 1b. Cut-out showing r15 (Case No. 9)

Fig. 1c.Cut-out showing r22 (Case No. 10)

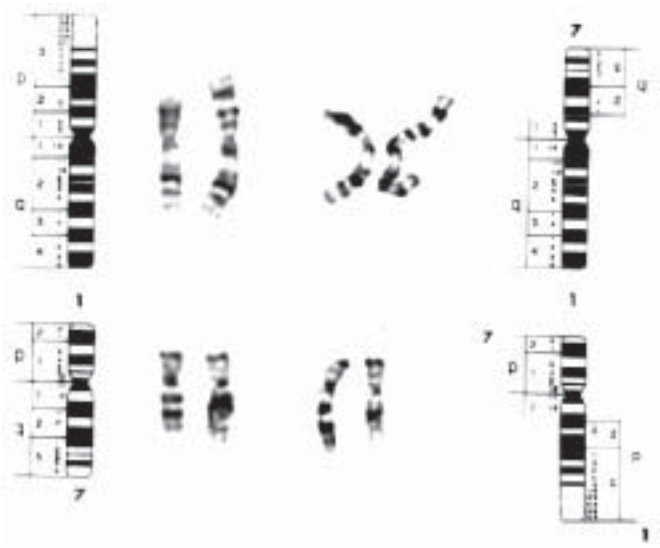

Fig. 1d. Cut-out of 1 and 7 chromosome showing breakpoints of translocation t(1;7)(p21:q11.2)(Case No. 11)

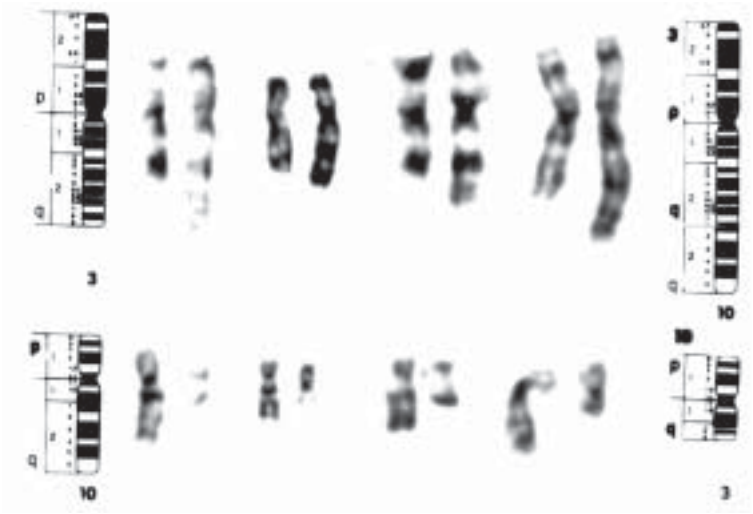

Fig. 1e. Cut-out of 3 and 10 chromosome with translocation breakpoints t(3;10) (q28.2; q21/1) Case No. 12

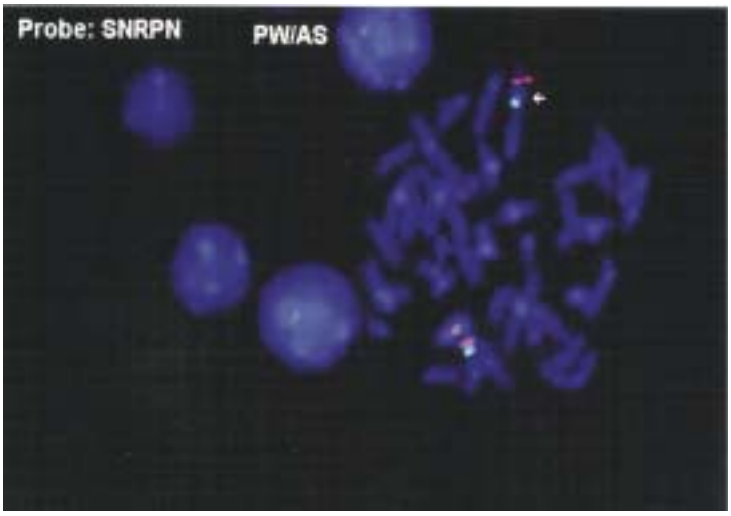

Fig. 2. FISH image showing 15(q11-13) microdeletion in PWS (Case No. 1)

if applied to the routine cytogenetic screening programmes, would definitely help in identifying the subtle chromosomal aberrations, which otherwise could be missed in the normal conventional banding methods. Specialized techniques like PCR probe analysis and chromosome painting, CGH, CGH array and Multicolor FISH analysis would definitely provide us with the full molecular details of the disease under consideration and would be of great help in establishing the proper phenotype-genotype correlations. This helps us in providing accurate genetic counseling to the affected and also to the members of the families at high-risk, also for a better management and therapeutic regimen.

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