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Cytogenetic Investigations in Mentally Challenged Individuals

Surbhi Mahajan, Anupam Kaur and Jai Rup Singh

Centre for Genetic Disorders, Guru Nanak Dev University, Amritsar 143 005, Punjab, India

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ABSTRACT Mental retardation means persistently slow learning of basic motor and language skills during childhood and a below-normal global intellectual capacity as an adult. Its incidence is reported to be 2-3% worldwide. Chromosomal aberrations account for 15% of mentally retarded individuals. In the present report, 183 mentally challenged individuals were cytogenetically analyzed. Peripheral lymphocytes were cultured using standard methods with modifications and metaphases were analyzed using automated karyotyping programme. The chromosomal aberrations were seen in 32 cases (17.4%) of which 22 (12.0%) were males and 10 (5.4%) females, most of these were in microcephalic individuals. Some of the chromosomal aberrations were: Yqh+, deletion Xq28, ring chromosomes, marker chromosomes, translocations, fragile sites etc. Thus cytogenetic investigations are important to screen cases with mental disability before proceeding to molecular/Fluorescence in situ hybridization analyses in these cases.

INTRODUCTION

Mental retardation (MR) is the manifestation of brain dysfunction that originates during developmental period, resulting in the limitation of two or more adaptive skills, that is, communication, self care, home living, social skills, self direction, health, safety, leisure and work (Medicine net.com 2011). Mental retardation results as a defect in the structure and function of neuronal synapse (Chechlacz et al. 2003). The incidence of mental retardation (IQ<70) is found to be 2-3% worldwide (Lewis 2007). Its incidence in developing countries is about 2-3 times more as compared to developed countries. Males are found to be more affected than females. The risk of mental retardation is found to be higher in children with congenital structural defects (Decoufle et al. 2001).

The cause of mental retardation may be genetic (30%) or environmental, congenital (for example, fetal exposure to teratogenic agents, chromosome disorders), or acquired (for example, central nervous system infection, head trauma). Chromosomal aberrations account for 15% of mentally retarded individuals (Mulley et al. 1992). Monosomies and trisomies are reported to be more frequent than tetrasomies, pentasomies, double aneuploids, polyploidy, etc., whereas subtelomeric rearrangements account for 5% of mental retardation /malformation syndrome (Archer et al. 2005). Several types of structural aberrations are also known to cause mental retardation, the common ones being deletion, duplication, inversion, translocation and isochromosome formation (Flint et al. 1995; Walter et al. 2004; Holinski-Feder et al. 2007).

Microcephaly is a common feature found in cases with mental retardation. It is a neurodevelopment condition in which there is reduction in cerebral cortex volume. The brain weight and the skull volume are markedly reduced. Skull is core shaped with receding chin and forehead. Language development is poor and mental retardation is severe to profound (McCreary et al. 1996). It has been reported to be 0.34 per 1000 births in rural Maharashtra (Datta and Chaturvedi 2000). The frequency of microcephaly is greater in populations having higher rate of consanguinity. These individuals have characteristic facial features like cerebral palsy, hypotonia, spasticity, growth retardation, epilepsy, prenatal asphyxia, respiratory distress syndrome and strabismus.

SUBJECTS AND METHODS

In the present study, cytogenetic investigations were carried out in 183 mentally challenged individuals during the period from 2002-2007. These included familial as well as isolated cases with neurological or behavioral disorder associated with mental retardation. Most of these were referred from various parts of Punjab to the Centre for Genetic Disorders and some of the indi-

Corresponding Author:
Dr. Anupam Kaur
Reader
Centre for Genetic Disorders
Guru Nanak Dev University
Amritsar, Punjab (143005)
Phone: +91-0183-2258802, Ext.:3447; 9872239393
Fax: +91-0183-2258820
E-mail: anupamkaur@yahoo.com

viduals were from Pingalwara and Dr. Vidya Sagar Institute of Mental Health, Amritsar. Detailed pedigree analysis, clinical examination of each case was done and recorded. The recogniseable cases of Down Syndrome were excluded. Blood samples were collected after the informed consent.

Peripheral lymphocytes of the proband were cultured in RPMI 1640 with 10% fetal calf serum and stimulated with phytohemagglutinin. For the expression of fragile sites, cultures were also set up in folate deficient medium, that is, M199 for all the cases. Slides were made using standard techniques and G-banding was done using trypsin (10%) (Benn and Perle 1992). A total of 50 metaphases were scanned for each case of which 10 metaphases were karyotyped (Cytovision, Applied Imaging).

RESULTS

In the present study of 183 subjects chromosomal abnormalities were seen in 32 cases of which 22 were males and 10 females (Table 1). Their age group ranged from 2.5-40 years. Thirty subjects out of 183 had microcephaly of which thirteen showed abnormal karyotype (Table 2). Fragile sites were observed in eleven cases (6.0%). Autosomal fragile sites were observed in 7 cases, fragile site at 9q12 was seen in 4 cases (Fig. 1), at chromosome 2 in two cases, at 6p21 in one case, while fragile site at Xq27.3 was seen in another case.

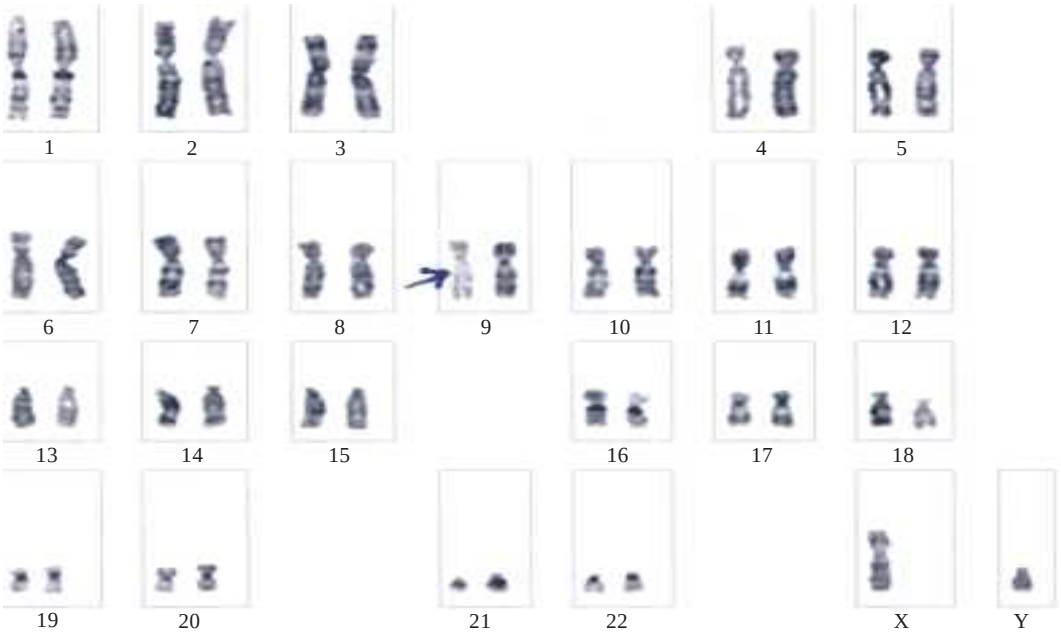
Table 1: Total cases 183

Sex	*Normal karyotype*	*Abnormal karyotype*	*Total number*
Male	97	22	119
Female	54	10	64

There was a case MM6 with ring chromosome 7 and one with ring chromosome 22 (Fig. 2). Large heterochromatin was observed in Y chromosome in three cases (R-404, R-436, R-436F), whereas one case had small Y chromosome (1087). In case R-334, R-463, deletion of chro-

Table 2: Cases with abnormal karyotype

S. No.	*Case No.*	*Age/Sex*	*Karyotype*
1	R-272	2.5yrs/Male	47,XY,+21
2	R-334	8yrs/Female	47,XX,-20,+21/46,XX (30%:70%)
3	R-405	10yrs/Male	46,XYqh+
4	R-415	8yrs/Male	46,XY,fra(9)(q12) (100% cells)
5	R-427F	-/Male	46,XY,fra(2)(q)/46,XY (10% cells)
6	R-427M	-/Female	46,XX,fra(7)(q35)/46,XX,fra(9)(q12)/46,XX (10% cells)
7	R-430B	14yrs/Male	46,XY,+3,-4,-4,+mar/46,XY,-3,-18,+mar,+mar/46,XY (10%;10%;80%)
8	R-434F	-/Male	47,XY,+21
9	R-435	5yrs/Male	46,XY,r(22)
10	R-436	1.5yrs/Male	46,XYqh+
11	R-436F	-/Male	46,XYqh+
12	R-442	9yrs/Male	46,XY,fra(9)(q12) (100% cells)
13	R-446	14yrs/Female	46,XX,fra(2)(q35)/46,XX (20%:80%)
14	R-447	11yrs/Male	46,Y,fra(X)(q27.3)/46,XY (10%:90%)
15	R-463	11yrs/Male	45,XY,-21/46,XY (10%:90%)
16	R-471	35yrs/Female	46,XX,+mar
17	R-478	3.5yrs/Male	46,XY,fra(2)(p11.2) (100% cells)
18	R-527	35yrs/Male	47,XY,+mar/46,XY (40%:60%)
19	962	15yrs/Male	46,Y,del(X)(q28)
20	962B	12yrs/Male	46,Y,del(X)(q28)
21	962M	-/Female	46,X,del(X)(q28)
22	1020	6yrs/Female	47,X,-9,+mar,+mar/46,XX (20%:80%)
23	1055	6.6yrs/Male	46,XY(fragile sites in 10% cells)
24	1087	13yrs/Male	46,XYqh-
25	1090	12yrs/Male	46,XY,fra(9)(q12) (100% cells)
26	1090B	1.5yrs/Male	46,XY,fra(9)(q12) (100% cells)
27	1132	6yrs/Male	46,XY,fra(6)(p21) (100% cells)
28	MM4	15yrs/Female	46,XX,del(2)(p25-p22)/46,XX (10%:90%)
29	MM5	11yrs/Female	45,XX,-C/46,XX (10%:90%)
30	MM6	18yrs/Female	46,XX,r(7)(p21q35)/46,XX (10%:90%)
31	MM7	8yrs/Female	46,XX with satellite association in 19.4% cells
32	MM8	40yrs/Female	46,XX with satellite association in 25% cells



(c)

Fig. 1. Fragile site at 9q12 (46,XY,fra(9)(q12))

mosomes 20 and 21 was seen respectively and deletion(2)(p25-p22) was seen in MM4. Mosaic variegated aneuploidy was observed in MM5 with random loss of a C group chromosome. In this case 32% cells were found to have 45 chromosomes. Satellite associations, were observed in MM7 and MM8.

In a family deletion of Xq28 was seen in two

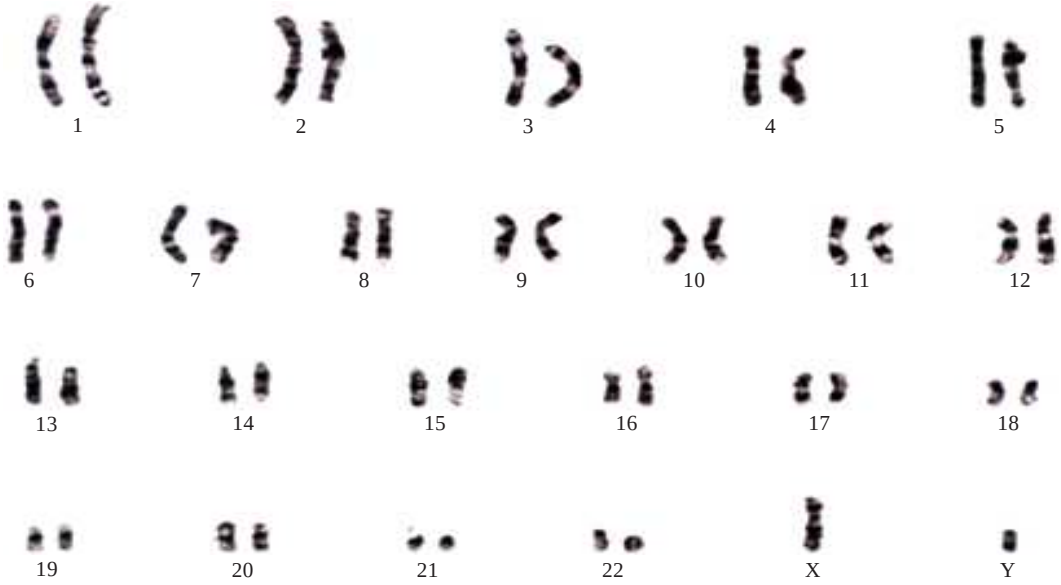


Fig. 2. Karyogram showing ring 22 [46,XY,r(22)]

brothers and their mother (962, 962B, 962M). The brothers were having Hunter syndrome. The mother was phenotypically normal.

Marker chromosomes were observed in 4 cases. In a family with two mentally retarded siblings (R-430, R-430b), one brother had normal chromosomal constitution while other showed 46,XY,+3,-4,-4,+mar/47,XY,-3,-18,+mar,+mar/46,XY (10%:10%:80%) chromosomal constitution.

DISCUSSION

In the present study, 183 mentally retarded individuals were cytogenetically analyzed. Chromosomal anomalies were seen in 17.4% cases and most of these were microcephalic (Tables 1, 2). In a study of 1760 mentally challenged subjects, Dave and Shetty (2010) observed chromosomal anomalies in 31.5% cases (555) where 14.2% (250) were Down syndrome. In a similar study of 150 female mental retardation subject by Dutta et al. (2009), chromosomal abnormalities were seen in 3 (2%) and 30 cases (20%) were diagnosed as Down syndrome.

The most common cause of mental retardation is fragile X syndrome (Xq27.3) with a prevalence of 1 in 1250 males and 1 in 2500 females (Sherman et al. 1985; Hagerman and Silverman 1991). In the present study, fragile sites were observed in 4.9% cases. Only one case showed fragile X and others showed autosomal fragile sites at chromosome 2, 6 and 9. Dave and Shetty (2010) reported fragile sites in 1% cases (19/1760) which included fragile X, fragile sites at 2q, 6q, Xp and 8q. In a study of 179 children with mental retardation indicated that FRAXA were less frequent in the studied population (Dutta et al. 2009). In a study of 100 subjects, fragile X expression and other fragile sites 3p14, 6q26, 7q36, 8q24, 9q32, 10q26 were seen (Chetan et al. 2001). In another study (Chetan et al. 2002), fragile X was seen in 26 subjects out of 300. Association of fragile site at 2q11 and 9q12 with schizophrenia was observed by Chen et al. (1998). Fragile site at 2q31 in four mental retardation subjects has also been reported (Manjunatha et al. 2002), and is a common fragile site first reported by Sutherland and Hetch (1985) and another common fragile site at 2q33 was reported by Hecht and Hecht (1984). There is an association of mental retardation with a rare fragile site at 2q11 having a frequency of 3%, reported by Tukun et al. (2000). In a study of 85 cerebral palsy cases, Kadotani et al. (2001) reported 6p21 fragile sites in 10 cases.

In one of our case (MM5), mosaic variegated aneuploidy was seen. But no such correlation, of clinical phenotype and mosaic variegated aneuploidy has been cited in literature. Mosaic variegated aneuploidy is considered as a disease predisposing to cancer (Jacquemont et al. 2002).

MM6 had ring chromosome 7 in 10% cells. Ring chromosomes are formed as a result of breakage and reunion in the distal p and q arms accompanying loss of genetic materials in these regions. Ring chromosome 7 has been reported in only 16 cases (Kaur et al. 2008). A male child, R-435 had ring chromosome 22. In ring chromosome 22, loss of p arm and satellite materials have been reported to have minimal clinical consequences the size of distal deleted q arm segment affects the overall phenotype (Luciani et al. 2003). Most of the cases reported in literature with ring 22 are males.

In our study, deletion of the p arm of chromosome 2 was observed in MM4. She had microcephaly, dysmorphic face, irregular dentition, micrognathia, speech retardation, delayed milestones and epilepsy. Dysmorphic features, hand abnormalities associated with growth and developmental delay have been reported by Ferguson-Smith (1973).

We observed large heterochromatic region in Y chromosome in three cases whereas, there was one case having smaller Y chromosome. These may be normal polymorphic variables and are also found in normal population. Similar observation was reported by Dave and Shetty (2010), where abnormalities of Y chromosome included long Y, small Y and inversion Y in 9.3% cases (164/1760).

In our study, deletion of chromosome 21 was present in R-463. Similarly, Ahlbom et al. (1998) reported a large interstitial deletion of the q arm of chromosome 21, congenital hypothyroidism, and hyperopia in a girl with mental retardation. Similarly, mosaic karyotype with deleted chromosome 21, was observed in a boy with cleft palate/lip, severe mental retardation and seizures (Sliuzas et al. 2006). In a similar study of cases with developmental disabilities, deletion in chromosomes 2q, 9q, 15q and Yq, was seen by Ravnan et al. (2006). Mosiac karyotype with deletion 21 was present in a boy with cleft palate/lip, severe mental retardation and seizures.

Marker chromosomes were observed in two cases (R-527, 1027). In a study of 98 individuals, marker chromosome was found in one case (Santos et al. 2000).

In the present study, a family of Hunter syndrome (962), Xq28 deletion was seen in two brothers and their mother. The test for mucopolysaccharidosis was positive in brothers confirming Xq28 deletion. It is an X linked recessive disorder affecting males. Important features include coarse face, enlarged abdomen, ear infections, sleep apnea and hernias. It occurs due to absence of enzyme iduronate-2-sulphatase that inhibits the breakdown of a mucopolysaccharide called glycosaminioglycans. The accumulation of glycosaminioglycans in brain leads to delayed development and mental retardation (Wraith et al. 2008).

R-430 and his brother had features of Schwartz-Jampel syndrome, characterized by abnormalities of the skeletal muscles, muscle weakness, myotonic myopathy; bone dysplasia; joint contractures; growth delays and dwarfism. R-430 had normal karyotype, while R-430b had 46,XY,+3,-4,-4,+mar/47,XY,-3,-18,+mar,+mar/46,XY (10%:10%:80%).

Satellite association was seen in MM7 and MM8 in19.4% and 25% of the cells respectively (Table II). The phenomenon of satellite association involving a specific position of satellite chromosomes with their satellite directed toward each other was first observed in mitotic human chromosomes (Ferguson-Smith and Handmaker 1961) and also reported by Ohno et al. (1981). In satellite association, fusion of two nuclei tends to stretch mechanically leading to the risk of breakage and thus predisposing to translocations. Gorla et al. (1998) reported satellite association in a patient with severe mental retardation. Anuradha et al. (2002) reported these associations in recurrent abortions.

Mental retardation is a heterogeneous disorder. Subtelomeric or subtle aneusomies at the chromosome ends are significant cause of idiopathic mental retardation. Chromosomal anomalies were observed in 17.4% of cases using classical cytogenetics in this region of Punjab. Thus cytogenetics is the reliable method for diagnosis of chromosomal aberrations. Every case with mental retardation should be studied cytogenetically and standard lymphocyte culturing should be accompanied with treated lymphocytes in a medium with low folic acid for the expression of fragile sites. However, these cases should be followed with molecular cytogenetics (fluorescence in situ hybridization) and other molecular investigations for the detection of micro deletions and insertions.

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