

A Cytogenetic Study of Children with Developmental Delay Mental Retardation

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ABSTRACT The presented study was carried out to identify the chromosomal abnormalities in children with mental retardation and associated anomalies, registered at Kasturba Hospital, Manipal and in and around clinics during 2005-2009. Four hundred and twenty children were subjected to clinical and G-banded cytogenetic evaluation. Of these, 245 children reported as autosomal trisomy and variants who were initially diagnosed for Down syndrome, 14 had structural abnormalities, who were mentally retarded with associated congenital anomalies. The rest were found to have normal chromosomal karyotypes although they were diagnosed with developmental delay and associated malformations. In conclusion, the study suggests that G-banded karyotyping is a routine clinical test for Mental retardation (MR) patients with or without congenital anomalies, albeit molecular karyotyping needs to be applied for detection of submicroscopic chromosome alterations.

INTRODUCTION

Mental retardation (MR) is a variable, heterogeneous manifestation of central nervous system dysfunctions, and is a lifelong disability which presents in infancy or the early childhood years. Since MR cannot be diagnosed until the child reaches the age of 5 years when standard measures of intelligence become reliable and valid, the term Global Developmental Delay is used to address the abnormality of the child. Global developmental delay is defined as the delay in attainment of developmental milestones at the expected age and is implied by deficits in learning and adaptation (Majnemer and Shevell 1995). Mental retardation/developmental delay can occur in any family and cuts across all racial, ethnic, educational, social and economic backgrounds. WHO (2005) reported that about 450 million people suffer from mental or behavioral disorders in India. Developmental Delay and Mental Retardation (DD/MR) occurs between 1-3% of general population (Kabra and Gulati 2003). Mental retardation is characterized by significant limitations both in intellectual functioning and adaptive behavior, expressed as abnormal conceptual, social, and adaptive skills. DD/MR can be diagnosed by tests for intelligence quotient (IQ) and adaptive behavior. The degree of impairment can be divided into mild (IQ 50-

70), moderate (IQ 35-50), and severe (IQ 20-35). The causes may be heterogeneous such as genetic, metabolic, environmental or infections. Accomplishment of the genetic causes of mental retardation is one of the greatest challenges for clinicians and scientists. In the present study we have examined 420 children with DD/MR with or without congenital anomalies for cytogenetic defects.

MATERIALS AND METHODS

Clinical evaluation and cytogenetic analysis were carried out on 420 children (237 males and 183 females) diagnosed with developmental delay, multiple congenital anomalies and associated disorders of mental retardation. The patients were referred for cytogenetic analysis from KMC, Manipal and other healthcare centers in and around Udupi district. Chromosomal preparations were obtained from cultured PHA stimulated peripheral blood lymphocytes employing laboratory standardized technique (Moorhead et al. 1960) and were Giemsa banded according to Seabright (1971). A minimum of 50 metaphases were analyzed by using automated Ikaros software (Version 5.0) and further 50 metaphases were assessed in certain cases to exclude mosaicism. The karyotypes were constructed according to the guidelines of International System for Human Cytogenetic Nomenclature (2009).

RESULTS AND DISCUSSION

Cytogenetic investigation of DD/MR children with or without congenital anomalies is an important factor in the etiology of the disorder. A

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major challenge for human geneticists is to detect new causes for mental retardation, which, although present in about 3% of the population, is unexplained in more than half of all the cases. Our results show that some of these children possess numerical as well as structural rearrangements. Usually the children with multiple congenital anomalies may not exhibit chromosomal abnormalities due to developmental defects during embryonic and fetal development. However, these anomalies associated with mental retardation may be due to chromosomal abnormalities at macro- or micro- level which may not be detected by conventional cytogenetic analysis. Trisomy 21 is the most frequent genetic cause of mental retardation and contributes to about 30% of all moderate to severe cases of mental retardation. Kaur et al. (2003) reported 110 cases (76.79%) of Down syndrome among 140 cases of mental retardation. In the present study, 246 (58.5%) children were clinically diagnosed as Down syndrome. Of these, 208 (84.55%) cases were with trisomy 21, 11(4.47%) cases with Robertsonian translocation and 5(2.03%) cases were mosaic down syndrome with instances of duplication, inversion, and reciprocal translocation 5(2.43%) were also observed (Table1). Rest of the children 17(6.91%) were found to have normal chromosomal karyotypes. The dysmorphic features in children with normal chromosomal complement, might be the presence of extra chromosomal material at micro level on chromosome 21 or non- homologues, which cannot be detected by the conventional cytogenetic technique due to limited resolution (5-10 Mb). Recent studies (Prandini et al. 2007) on Down syndrome indicate that mental retardation phenotype may be caused by genomic imbalance. The impact of phenotypic variability depends on HSA21 gene mutation, as proposed by Rachidi and Lopes (2007), and variations in gene expression due to a gene duplication which induces functional alterations at cellular levels were found to cause brain morphological defects, behavioral alterations and mental retardation in Down syndrome.

Fragile X syndrome (FXS), a form of commonly inherited disorder is recognized as the second most common cytogenetic cause of mental retardation next to Down syndrome, and it is mainly caused by massive expansion of CGG triplet repeats encoded by mutated fragile X MR (FMR1) gene located at Xq27.3 (Feng et al.

Table 1: Chromosomal complements reported in suspected children with Down syndrome

<i>Male children</i>	<i>No.</i>	<i>Female children</i>	<i>No.</i>
46,XY	03	46,XX	14
47,XY+21	119	47,XX+21	89
46,XY47,XY+21	2	46,XX/47,XX+21	3
46,XY,rob (13;21)(q10q10)	2		
46,XY,rob (14;21)(q10q10)	2	46,XX,rob (14;21)(q10q10)	3
46,XY,rob (15;21)(q10q10)	1	46,XY,rob (21;21)(q10q10)	1
46,XY,rob (21;21)(q10q10)	1	46,XY,rob (21;22)(q10q10)	1
46,XY,dup (21)(q11-q12)	1	46,XX,dup (21)(q11-q12)	2
47,XY+inv (21)(q11-q12)	1	47,XX,t(2;12) (P13;q24.2)	1

1995) but cytogenetic visualization of the fragile X chromosome is one of the main tools which help in its diagnosis (Sutherland 1977). We observed three patients with clinical features of FX syndrome and carried out cytogenetic analysis on them and the results were confirmed by PCR analysis using specific primer sets. Cytogenetic observation was positive in one and negative in two children.

Deletions and/ duplications may cause diverse phenotypes, which may depend both on its size and location, and almost all invariably causing mental retardation. The minimum size of 15 million bases (roughly amounts to single chromosome band) can be detected under the microscope on metaphase spreads. An example of such a cytogenetically visible deletion involves short arm of chromosome 5, characterized by mental retardation and cat-like crying in childhood (Mainardi et al. 2001). Upon karyotype examination of patients we observed a deletion of varying sizes on short arm of chromosome 5 [(46,XY,del(5)(pter...p13:) in a male and 46,XX,del(5)(p13.3;p15.3) in a female child]. We observed deletion (18q-) and duplication (18q+) in two male children respectively, who were diagnosed for developmental delay/mild MR children with dysmorphic facial features (Table 2). An earlier report on 18q deletion and 18q duplication syndromes (Arguedas and Batchelor 2009; Ceccarini et al. 2007) evidenced to have similar features of reduced intellectual functioning and developmental delay. We had one familial case with 46,XY, ins(6p)(p24;p13p16) mat and three reciprocal translocation of 46,

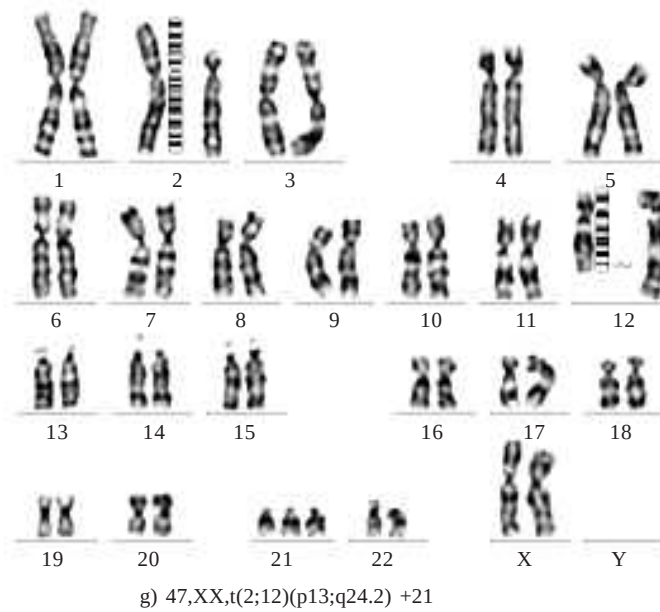
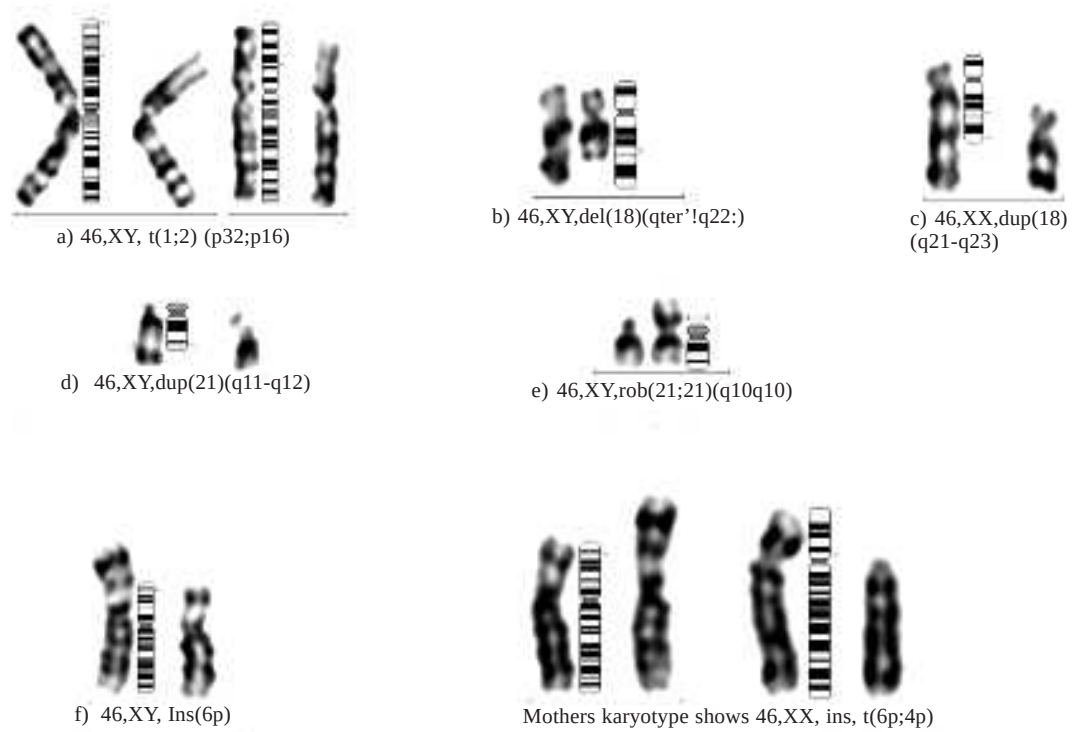


Fig.1 (a to g). Chromosomal karyotypes in developmental delay and mental retardation

Table 2: Diagnostic clinical manifestations and chromosomal karyotypes in developmental delay and mental retardation

Referral diagnosis and clinical manifestations	Age/ sex	Chromosomal karyotype
Moderate to mild mental retardation (Fragile X syndrome?)	2years/ male	46,XY,Fra(X)(q27.3)
Hydrocephaly, develop mental delay with and congenital anomalies	1years/ male	47,XY+mar(?)
Development delay with multiple congenital anomalies	6 months/male	46,XY del(18)(q22-q23)
Developmental delay with dysmorphic features.	1year / male	46,XY,dup(18)(q21-q23)
Developmental delay with /Multiple congenital anomalies	2 years/ male	46,XY, ins(6p)(p24;p13p16)mat
Developmental delay/ mild MR	2years/ male	46,XY, t(1;2)(p32;p16)
Features of Cri Du Chat syndrome	2months/male	46,XY, del(5)(pter....13:)
Dysmorphic features	1month/ male	47,XY+18
Developmental delay with multiple congenital anomalies	2years / female	47,XX+13
Dysmorphic features	1month/female	46,XX/47,XX+18
Multiple congenital anomalies	1m/F	46,XX,del(13)(q32-q34)
Dysmorphic features	1m/F	46,XX,del(5)(p13.3-p15.3)
Failure to thrive	3m/F	46,XX,dup(14)(q32.3)

XY, t(1;2) (p32;p16), 46,XX,t(11;16), and 47,XX,t(2;12) – two of them had mild mental retardation whereas later two children had moderate MR. The children who exhibited abnormal phenotypic features with mild to moderate mental retardation may have genetic imbalances which may not be detected by conventional cytogenetic methodology. Since the identification of submicroscopic subtelomeric rearrangements as a cause of idiopathic MR, testing for submicroscopic observations has become an important clinical evaluation system for the etiological diagnosis of unexplained DD/MR. Novel molecular karyotyping methods such as, Fluorescence *in situ* hybridization (FISH), Multiplex ligation-dependent probe amplifications (MLPA), and Microarray analysis can detect submicroscopic

chromosome alterations at higher resolution. Mental retardation may not be cured but can be prevented. Hence, it is important to ascertain the cause of mental retardation, to enable reduction of recurrence risk and where MR is transmissible, proper genetic counseling could be provided.

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