Chromosomal abnormalities in 457 Turkish patients with MCA/MR

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SUMMARY: Celep F, Sönmez FM, Karagüzel A. Chromosomal abnormalities in 457 Turkish patients with MCA/MR. Turk J Pediatr 2006; 48: 130-134.

The evaluation of multiple congenital abnormalities and/or mental retardation (MCA/MR) is always a challenge to clinicians. The recognition of specific physical or behavioral characteristics can vastly improve diagnostic yield. Chromosomal abnormalities account for a high percentage in the etiology of MCA/MR. In this study, frequency of chromosomal abnormalities was 4.81% of 457 patients. Chromosomal abnormalities and polymorphisms were detected in 65 (14.21%) (structural and numerical chromosomal abnormalities in 22 patients and polymorphisms in 43) of 457 MR and/or MCA patients. Our results show that chromosomal abnormalities contribute much to the causation of multiple malformations and/or MR. It is essential that fluorescence in situ hybridization (FISH) be used in conjunction with standard methods in order to maximize obtainable information for better management of patients with MR and/or MCA.

Key words: multiple congenital abnormalities, mental retardation, chromosomal abnormalities, FISH.

Frequency of mental retardation (MR) and developmental disorders is variable in childhood (2-3%). The etiology of MR is complex, with an unknown cause in more than 50% of mentally retarded patients[2,10]. Mendelian disorders, chromosomal abnormalities, or environmental factors can act as a single cause or in combination. Important causes are chromosomal abnormalities, which are detectable in 4-34.1% of patients, depending on the patient selection and techniques. It is difficult to give an accurate estimate of the contribution of cytogenetic abnormalities to MR because of variability in parameters[2,10,31]. Identification of these chromosomal abnormalities may explain the cause of MR/multiple congenital abnormalities (MCA) syndromes and has important implications for genetic counseling. Visible duplication or deficiency of any of the autosomes is almost invariably associated with mental handicap, post-natal growth deficiency and dysmorphic features. Routine cytogenetic analysis indicates that chromosomal abnormalities constitute 40% of severe (IQ<55) and 10-20% of mild MR (IQ 55-70); however, rearrangements involving less than 1 or 2 megabases are undetectable even at the highest resolution and could account for a substantial number of idiopathic cases[12].

Until recently, chromosomal studies were performed according to standard procedures and included analysis of 15-20 GTG banded metaphases on peripheral blood lymphocyte cultures. Since the end of the 1980s, several patients have been described with translocations not detected by conventional light microscopy, but elucidated by fluorescence in situ hybridization (FISH) with whole chromosome paints or subtelomeric probes[19]. For the past decade, molecular cytogenetics has played an increasingly important role in the research and diagnosis of MR[4,12,31].

The aim of the present study was to determine the incidence of chromosomal abnormalities in 457 patients with MR and/or MCA in the Black Sea coastal region who were referred by clinicians from Pediatrics for genetic evaluation. No similar study has been carried out in this region to date.

Material and Methods

Clinical evaluation of the patients

Patients from the Pediatrics Clinic, with unrecognized patterns of minor and major anomalies and MR, were selected for this investigation. Patients with Down, Turner, Klinefelter and fragile-X syndrome were excluded. There were 457 selected patients (245 males and 212 females).

Metaphase chromosome spreads were obtained from phytohemagglutinin-stimulated cultures of peripheral blood lymphocytes on the level of 400-550 bands. GTG-banded chromosome preparations were examined in a minimum of 20 metaphases for each patient and karyotyped according to International System for Human Cytogenetic Nomenclature (ISCN) criteria[13]. FISH was undertaken using whole chromosome painting (WCP) libraries and α-satellite DNA probes and examined in a minimum of 100 metaphases for each patient.

Results

A chromosomal abnormality and polymorphisms were diagnosed in 65 (14.21%) of the 457 patients. We determined structural and numerical chromosomal abnormalities in 22 (33.84%) patients (Table I) and polymorphisms in 43 (66.15%) patients (Table II) of 65 patients. In the group with structural and numerical abnormalities (22 patients), 11 patients (16.92%) had translocations (Table IA), six patients (9.23%) had duplications and deletions (Table IB), and five patients (7.7%) had other abnormalities (ring, marker chromosome, etc.) (Table IC).

Pericentric inversion 9 was observed in 12 (18.5%) of 65 patients (Table IIA). Polymorphisms, 22p+,15p+,13p+, were detected in four (6.15%) and chromosomal heteromorphisms including 1qh+ (n=10), 9qh+ (n=14) and 16qh+ (n=3) were in 27 (41.53%) of 65 patients (Table IIB).

The majority of patients (85.79%) had normal karyotypes. Patients with structural aberrations and their parents were evaluated cytogenetically and by FISH analysis. In a t(4;15) patient (Patient 2 in Table IA) with MCA, motor MR was cytogenetically detected as 4q+. After cytogenetic examination, FISH analysis of this patient showed paternal origin of t(4;15)[5]. Translocations, t(12;15) and t(3;12), in two patients (Patients 8, 4 in Table IA) were of maternal origin and

Table I. Structural and Numerical Chromosome Anomalies in the MCA/MR Patients

No.	Sex	Karyotype
	A-Translocations	
1	M	45,XY,t(13q;15q)
2	M	46,XY,ish der(4)t(4;15)(q35;?)pat(wcp4+,D15Z1+),inv(9)
3	M	45,XY,t(14q;21q)
4	F	46,XX,t(12;15)(p12;p11)mat(wcp12+,wcp15+)
5	F	46,XX,t(21;21)(q10;q10)[36]/46,XX,13p+[5]
6	M	46,XY,t(16;18)(p13.2;p11.3)
7	M	46,XY,der(7)t(7;13)(p12;p11)
8	F	46,XX,t(3;12)(q25.2;p12)mat(wcp3+,wcp12+)
9	M	45,XY,t(13q;14q)
10	M	46,XY,t(5;11)(q34.1;q22.1)
11	M	46,XY,t(13q;15q)
	B-Deletion and duplications	
12	M	46,XY,del(22)(p10)
13	F	46,XX,del(9)(p13→pter)
14	F	46,XX,del(21)(q22)
15	M	46,XY,dup(19)(p13→pter)
16	M	46,XY,dup (18)(p11.1;p11.22)
17	F	46,XX,dup(9)(p21;p24)
	C-Other structural and numerical chromosomal abnormalities	
18	M	46,XY,r(21)(p11.2;q22.2)
19	M	46,XY,18q+
20	M	46,XY,4q+
21	F	47,XX,+mar
22	M	45,XY,-21

MR: Mental retardation. MCA: Multiple congenital abnormalities.

Table II. Specification of MCA/MR Patients With Polymorphisms

No.	Sex	Karyotype
	A-Pericentric inversion	
1	F	46,XX,inv(9)(p13;q21)
2	M	46,XY,inv(9)(p13;q21)
3	F	46,XX,inv(9)(p13;q21)
4	M	46,XY,inv(9)(p13;q21)
5	M	46,XY,inv(9)(p13;q21)
6	F	46,XX,inv(9)(p13;q21)
7	M	46,XY,inv(9)(p13;q21)
8	F	46,XX,inv(9)(p13;q21)
9	M	46,XY,inv(9)(p13;q21)
10	M	46,XY,inv(9)(p13;q21)
11	M	46,XY,inv(9)(p13;q21)
12	M	46,XY,inv(9)(p13;q21)
	B-Heteromorphisms	
13	F	46,XX,22p+
14	F	46,XX,15p+
15	F	46,XX,15p+
16	F	46,XX,13p+
17-19	F	46,XX,16qh+
20-24	M	46,XY,1qh+
25-29	F	46,XX,1qh+
30-35	F	46,XX,9qh+
36-43	M	46,XY,9qh+

also in balanced form by FISH technique. Also, a patient (Patient 18 in Table IC) with the ring chromosome 21, r(21), had a brother with trisomy 21. Parent and other siblings of this patient were normally karyotyped.

Discussion

In general, a routine chromosome analysis should be used as a starting point for any cytogenetic investigation of developmental delay and/or MR. Based on family history and clinical phenotype, subsequent workups can follow various pathways for a diagnosis. Depending on clinical indications, additional chromosome counts may be needed to rule out mosaicism, and appropriate band levels should be reached to detect small aberrations in targeted regions. The list of new molecular cytogenetic methods with potential applications in diagnosis and prognosis of MR is growing, thanks to rapid advances in the Human Genome Project and its related areas. The FISH technique is important as a reliable and sensitive method for elucidating the nature of structural chromosomal abnormalities that cannot be resolved by conventional banding techniques alone[12,15,26,31]. Chromosome 'painting' was suggested as the method of choice to detect subtle translocations that were often observed in patients with unexplained MR, with or without dysmorphic features[3]. The yield from FISH studies for MCA/MR can be vastly improved by utilizing clinical information on recognizable genotype/neurobehavioral phenotype correlation[9].

Several cytogenetic studies with MR and/or MCA have been reported in the literature[7,8,14,18,21,29,32]. Approximately 1 of 500 phenotypically normal individuals has a visible balanced chromosomal rearrangement when analyzed at the resolution level of 400 bands[11]. Chromosomal anomalies are found in 4-34.1% of individuals with MR[9,31]. The incidence of chromosomal abnormalities in children with developmental disorder may be as high as 15%, with the genetic condition thought to be the direct causal factor for the development delay in 9% of patients[6]. Microcephaly, short stature, hypertelorism, nasal and auricular anomalies and hand anomalies were the most common features among cytogenetically abnormal patients[10].

The aim of the present investigation was to determine the incidence of chromosomal abnormalities in patients with unexplained MR who in addition showed stigmata of dysmorphology, malformations and growth retardation, or a family history suggestive of a familial translocation. The patients selected from Pediatric Clinics were sent to routine cytogenetics and molecular cytogenetics laboratory for evaluation. In the present study, we excluded patients with Down, Turner, Klinefelter and fragile X syndromes. In our series, we detected chromosomal abnormalities and heteromorphisms in 65 patients. Numerical and structural chromosomal abnormalities were detected in 22 (4.81%) patients. These results correspond to the literature. Reciprocal translocations are, in many cases, inherited from one of the parents but they can also occur de novo and are one of the most common structural aberrations of human chromosomes, found in 0.093-0.095% of all live-borns. We suggest that FISH technique is valuable in resolving several structurally abnormal cases such as the patient with unbalanced 4;15 translocation[5], 3;12 translocation, 12;15 translocation, ring chromosome 21 and duplication chromosome 18. While the translocation 4;15 was of paternal origin, others were maternal.

It has been suggested that the size of the deletion may have a direct bearing on the severity of the phenotype, based on observations in individual reports[23]. In this study, deletions were only seen in three patients.

Pericentric inversions of qh regions are regarded as chromosomal variations that do not manifest severe consequences and have thus been termed heteromorphisms. These rearrangements are not evolutionarily selected against since they only involve encoding heterochromatic material, which apparently has no effect on the phenotype of the individuals. Mental and growth retardation were reported in some cases of inv(9)[16,24,28]. We detected these variations in 12 (18.5%) of 65 patients.

In duplication chromosome 18p, we investigated whether or not this is a translocation from other chromosomes, but found no signals on other chromosomes by FISH technique. Ring chromosome 21 is a rare chromosome aberration often associated with MR and dysmorphic features[20,22,25].

The majority of patients (85.79%) had normal karyotypes; however, some of these patients might have submicroscopic rearrangements. But we did not detect these abnormalities by conventional cytogenetic analysis. This study further reinforces the necessity of FISH investigations as an adjunct to conventional cytogenetic analysis in the characterization of chromosomal abnormalities. As this technique becomes more widely used, more accurate assessment of complex rearrangements can be anticipated[27]. More effective methods for diagnosis of MCA/MR will continue to emerge in the future. It is always a challenge to transfer new technology from a research setting to clinical applications. Three large studies[1,17,30] have shown that in 3-7% of patients, subtelomeric aberrations can be the cause of unexplained moderate-to-severe MR or developmental delay with dysmorphism. Indeed, if the phenotype is classical for a chromosome aberration and the results with subtelomeric probes are normal, one should consider extending the study to markers covering the entire genome within useful distances. Therefore, we consider FISH with multiple subtelomeric probes to be a valuable diagnostic tool that should be implemented in all clinical cytogenetics laboratories. We will primarily plan to detect submicroscopic aberrations in patients with unexplained MR by FISH using multiple subtelomeric probes in the follow-up of this study.

The re-evaluation of MCA/MR syndromes will affect the impact of genetic counseling in families where balanced carriers exist. Purposes of this study were to prevent recurrence with genetic counseling and to determine the incidence of structural and numerical aberrations in patients with unexplained MR/MCA.

Acknowledgement

We gratefully thank Biologist T. Köseahmet and Chemist F. Yenisey for their help in the cytogenetic studies.

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