

CYTOGENETIC FINDINGS IN MENTALLY RETARDED IRANIAN PATIENTS

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ABSTRACT

We conducted a cytogenetic study on 865 individuals with idiopathic mental retardation (MR) who were admitted to the Cytogenetics Department of the Iran Blood Transfusion Organisation (IBTO) Research Centre, Tehran, Iran; these were performed on blood samples using conventional staining methods. Chromosome anomalies were identified in 205 of the patients (23.6%). The majority were Down's syndrome cases ($n = 138$). In 33 males, a positive fragile X anomaly was found. The remainder ($n = 34$) had other chromosomal abnormalities including structural chromosome aberrations ($n = 23$), marker chromosomes with an unknown origin ($n = 3$), sex chromosome aneuploidy ($n = 6$) and trisomy 18 ($n = 2$). The contribution of chromosome aberrations to the cause of MR in this group of patients is discussed.

Keywords: Chromosome abnormality, Idiopathic mental retardation (MR), Iranian patients.

INTRODUCTION

Mental retardation (MR) is characterized by destruction in intellectual abilities, and by an inability to adapt to the environment and the social situation. Mental retardation is found in individuals either as an

isolated finding, or as part of an underlying disorder [1]. The worldwide prevalence of MR is about 2.3% [2]. Despite large studies being conducted to discover the etiology of MR, in less than 50% of MR cases is the cause identified and the genetic defect known to be responsible for 17-47% of MR cases [3].

It is known that numerical and structural chromosomal anomalies are one of the most common causes of MR seen in these patients [4-12]. Identification of the causes of MR in a patient is of great importance because of the consequences it has for the prognosis, risk of occurrence in other family members, and prevention. Mental retardation is the reason for a substantial portion of referrals of patients and families to the genetic counseling unit. Here we summarize the result of a cytogenetic study performed on 865 mentally retarded Iranian patients consecutively referred to the Cytogenetics Department of the Iran Blood Transfusion Organisation (IBTO) Research Centre, Tehran, Iran.

MATERIALS AND METHODS

Blood samples were collected from 865 idiopathic MR patients who were referred to IBTO for cytogenetic study. There were 287 females and 578 males. The median age of the patients was 9.5 years. The patients enrolled in this study had unexplained MR. In addition, some of them showed stigmata of dysmorphism, malformations, growth retardation, family history of MR, developmental delay, miscarriages, infertility/subinfertility suggestive of a familial chromosomal translocation/inversion.

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Chromosomal analysis was performed on phytohemagglutinin (PHA)-stimulated peripheral lymphocyte cultures of the patients using standard cytogenetic methods [13,14]. A cytogenetic test for fragile X was performed upon request.

Briefly, peripheral blood lymphocytes were cultured in 5 mL RPMI 1640 (Gibco®; Invitrogen, Paisley, Scotland, UK), supplemented with 20% (v/v) fetal bovine serum (GIBCO®; Invitrogen) and 10 µL/mL phytohemagglutinin (PHA) (GIBCO®; Invitrogen) at 37°C. After 72 hours of incubation, 40 µL colcemid (10 µg/mL) (GIBCO®; Invitrogen) was added to the cells. The cells were incubated at 37°C for about 10 mins. The suspension was centrifuged, and the pellet was resuspended in 5-10 mL KCL (0.075 M) for about 20 mins. at 37°C. After centrifugation, the cells resuspended in fixative (3v methanol:1v acetic acid) (Merk, Frankfurt, Germany). The fixative was changed at least three times. Using a Pasteur pipette, a drop was dropped onto the slide. The chromosomes were viewed under phase contrast to assess quality of the metaphases and nuclei. The chromosomes were treated with trypsin, then stained with Giemsa (GTG-banded) after aging.

Twenty to 30 metaphases were analyzed per individual and in cases of suspected mosaicism, the

numbers of metaphases were increased to a total of 100 for analysis. A resolution of 450-band stage was considered as a minimum; for a more detailed structural analysis, 550-700-band stage was preferred. The routine analysis was based on GTG-banded staining. For patients with structural chromosome abnormalities or marker chromosomes, a chromosome study of the parents was recommended and performed if the parents were alive and available (some of the patients lived in orphanages) or cooperated.

RESULTS

In our study of 865 screened subjects (287 females, 578 males), anomalies were identified in 205 of the patients (23.6%). The majority were Down’s syndrome cases ($n = 138$, 15.9% of all the screened MR cases, and 67.3% of the cases with chromosome abnormalities). In 33 males, a positive fragile X anomaly was found (3.8% of all the screened MR cases, and 16% of the cases with chromosome abnormalities).

The remainder ($n = 34$, 3.9% of all the screened MR cases, and 16.5% of the cases with chromosome abnormalities) had other chromosomal abnormalities (Table 1), mainly structural chromosome aberrations

Table 1. Structural and numerical chromosome anomalies in patients with mental retardation.

#	Sex-Age	Clinical Data (in addition to MR)	Karyotype (structural chromosomal anomalies)	Familial/ De Novo
1	F-9	developmental delay; history of three sepsis cases in family (probability of autosomal recessive immunodeficiency); epicanthic fold	47,XX,del(2)(q22q32.2)+r(2)(q22;q32.2)[81]/46,XX,del(2)(q22;q32.2)[9]	<i>de novo</i>
2	F-?	further clinical data not available	46,XX,del(6)(p25)[7]/46,XX[46]	<i>de novo</i>
3	M-13 mths	developmental delay	46,XY,del(18)(q23)	<i>de novo</i>
4	F-2	developmental delay; external ear duct stenosis; depressed nasal bridge; stoned peripheral fitness	46,XX,del(18)(q21.3)[18]/46,XX[33]	<i>de novo</i>
5	F-9	respiratory problems at birth; cardiovascular problems; protrusion of back of head and forehead bossing; unable to hold neck erect; autism	46,XY,del(4)(p15.31)	<i>de novo</i>
6	F-?	further clinical data not available	46,XX,del(11)(q23.2)	<i>de novo</i>
7	M-7	open mouth; micrognathia; high arched palate; recurrent respiratory infections	46,XY,del(22)(q11.2)	<i>de novo</i>
8	F-3.5	developmental delay; epicanthic fold; moon face	46,XX,del(5)(p15.3)[8]/46,XX	<i>de novo</i>
9	F-?	developmental delay; asphyxy; restlessness	46,XX,del(5)(p15.2)	not known

Continue

Table 1. Continued

10 11	M-15 M-9	low-set ears; epicanthal folds; speech problems low-set ears; epicanthal folds; micrognathia	46,XY,del(5)(p15.2) brother: 46,XY,del(5)(p15.2) parental chromosome study not done	paternal/ maternal
12	M-17 mths	developmental delay; minor dysmorphic features	46,XY,t(16;17)(q22;p13) father: 46,XY,t(16;17)(q22;p13)	paternal
13	F-14 mths F-20	severe developmental delay; minor dysmorphic features	45,XX,der(7)t(7;22)(q36.2;q11.1~11.21), -22 mother: 46,XX,t(7;22)(q36.2;q11.1~11.21)	maternal
14	F-47	depression; history of self injury and suicide	46,XX,t(2;3)(q23;p25)	not known
15	F-8	apparent dysmorphic features; developmental delay; abnormal EEG	46,XX,t(1;4)(q21;p16),add(22)(p13)	<i>de novo</i>
16	F-10 mths F-21	developmental delay; no apparent dysmorphic features	47,XX,t(11;22)(q23;q11.2),+der(22)t(11;22)(q23;q11.2) mother: 46,XX,t(11;22)(q23;q11.2)	maternal
17	F-8 F-?	learning disability	46,XX,inv(6)(p23p21) mother: 46,XX,inv(6)(p23q21)	maternal
18	M-11 M-35	epilepsy; right hemiplegia due to head trauma at 4 years old; strabismus	46,XY,inv(6)(q22.1q25.1) father: 46,XY,inv(6)(q22.1q25.1)	paternal
19	M-3 F-27	bilateral talipes equinovaiuse	46,XY,inv dup(10)(p11.2q26.3) mother: 46,XY,inv(10)(p11.2q26.3)	maternal
20	M-8	developmental dela; prominent nose; speech and behavior problems	46,XY,add(15)(pter)	<i>de novo</i>
21	F-?	further clinical data not available	46,XX,add(15)(p13)	not known
22	M-14 mths	frontal bossing; small jaw; low-set ears; deepset eyes; strabismus; drooping upper eyelid (left side); widely-spaced eyes; short nose; long philtrum; downcurved upper lip; camptodactyly; hypotonia	46,XY,dup(7)(q21.2q32)	<i>de novo</i>
23	F-?	developmental delay; hypotonia in hand and leg (power: 2/5); lack of eye contact; dysmorphic features; gastrointestinal reflex; cardiovascular defects (oval fromen); small head circumference; retarded growth; edema in one foot; third toe of right foot longer than the others; low breast line; fever of unknown origin; spcial nose feature	46,XX,r(18)(q21.2qter)	<i>de novo</i>
Karyotype (numerical chromosomal anomalies)				
1	M-19	developmental delay; minor dysmorphic features	47,XY+mar[8]/46,XY[12]	<i>de novo</i>
2	F-15	primary amenorrhea	47,XXX[22]/46,X+mar[8]/48,XXX,+mar[2]/45,X[2]	<i>de novo</i>
3	F-8	further clinical data not available	47,XX,+mar	<i>de novo</i>
4	F-13 F-?	further clinical data not available	45,X(n=2)	<i>de novo</i>
5	M-7 M-21	further clinical data not available	48,XXYY(n=2)	<i>de novo</i>
6	M-19	further clinical data not available	47,XXY	<i>de novo</i>
7	M-3	further clinical data not available	48,XXXX/49,XXXXY	<i>de novo</i>
8	M-3 mths M-20 days	some of the typical clinical symptoms of trisomy 18	47,XY,+18(n=2)	<i>de novo</i>

($n = 23$). The chromosomal anomalies in these patients were mostly of *de novo* origin except in six cases (patients #12, #13, #16, #17, #18 and #19). In five cases parental chromosome study could not be performed (patients #9, #10, #11, #14 and #21). Marker chromosomes with an unknown origin found in three *de novo* cases. Sex chromosome aneuploidy was detected in six patients. Twelve cases had inversion 9q which is believed to be a normal variant.

DISCUSSION

There is great variation in the frequency of the reported chromosomal abnormalities found in MR patients. A cytogenetic study of 419 MR school children in southern Taiwan, by Shiue *et al* [2], found chromosomal abnormalities in 22.43% of the cases, with trisomy 21 occurring in 77 cases (18.38%). Sex chromosome aneuploidies were found in three cases (0.72%). Structural abnormalities of autosomes were found in 13 cases (3.10%) (2). Another study of 341 MR children in Taiwan found chromosomal abnormalities in 89 cases (20.3%) including 63 of trisomy 21 (10.7%) and 13 of fragile X (3.8%) [4].

Coco and Penchaszadeh [5] reported on a cytogenetic study in 200 MR children in Argentina. They found chromosomal abnormalities in 42 (21%) with 26 cases having structural chromosome defects [5].

Two studies were performed in The Netherlands. One study done in Amsterdam (in the south of The Netherlands) indicated that a chromosomal base in 22.1% of the patients was responsible for their MR. Of these, 14.3% were Down's syndrome patients, and 6.1% had other chromosomal abnormalities [6]. Another study done in Amsterdam indicated that 20 patients had chromosomal anomalies (7.5%) in 266 karyotyped MR children. Interestingly, these were mainly structural chromosome aberrations [7].

A study performed in Poland showed that the incidence of abnormal karyotypes in MR patients was 10.1% [8]. However, the percentage of chromosome aberrations found in patients with non specific mental retardation was 2.2% [8]. A study done by Butler and Singh [9] in America showed that 39 out of 201 (6.6%) institutionalized MR patients had abnormal chromosome with Down's syndrome noted in 31 of the patients.

While the overall frequency of chromosomal abnormalities in these reports was similar, there are reports of either low or high percentages of chromosomal aberrations in other studies. For example,

Celep *et al.* [10] reported the percentages of chromosomal abnormalities in 457 Turkish MR Patients to be only 4.81%. 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 multiple congenital anomaly (MCA) patients. On the other hand, a study done in Slovakia revealed a very high percentage of chromosome abnormalities in MR patients. Of 324 MR patients, 104 (53.0%) had chromosomal aberrations [11].

The differences between the incidences of chromosomal abnormalities in the literature could be caused by the criteria for patient selection, and the techniques applied [cytogenetics only or in combination with molecular cytogenetics such as fluorescent *in situ* hybridization (FISH) and comparative genomic hybridization (CGH)]. In our study of 865 screened subjects, chromosomal anomalies were identified in 205 of the patients (23.6%). The majority were Down's syndrome cases ($n = 138$, 15.9%). Interestingly, we found three cases with marker chromosomes (0.34%). Liehr and Weise [15] found that the incidence of marker chromosomes is about 0.288% in MR patients.

In general, van Karnebeek *et al.* [3] showed that the mean yield of chromosome aberrations in classical cytogenetics is about 9.5% (variation: 5.4% in school populations to 13.3% in institute populations; 4.1% in borderline-mild MR to 13.3% in moderate-profound MR; more frequent structural anomalies in females). They also indicated that for fragile X anomalies, yields were 5.4% (cytogenetic studies) and 2.0% (molecular studies) [3].

The incidence of fragile X positive cases in our study is slightly higher than some other reports although we only employed cytogenetic tests for fragile X. For example, Butler and Singh [9] reported 2.0% fragile X positive in his cases, while in our study it was 3.8%. Nevertheless, our results indicate that the diagnostic contribution of the fragile X screening could be considered equally important as conventional chromosome banding techniques for the detection of structural chromosome abnormalities.

Some of the chromosome aberrations were detected in more than one case. For example: in two cases, chromosome 2 was involved with a very close breakpoint of q22 and q23 (Table 1; patients #1 and #14); in two cases, chromosome 4 with breakpoints p16 and p15.3 (Table 1; patients #5 and #15); and in

another two cases, chromosome 6 with breakpoints p25 and p23 (Table 1; patients #2 and #17). Even more interesting, chromosome 11 in two cases and chromosome 18 in another two cases had the same breakpoints (Table 1; patients #6 and #16, and #4 and #23, respectively). This could be very interesting because some of the genes responsible for MR may be located in these breakpoints. For example, the chromosomal breakpoints for the two MR patients (patients #1 and #14) were at 2q22 and 2q23, respectively. Heterozygous mutations or deletions of the ZEB2 gene, which is located near to 2q22, is known to be responsible for Mowat-Wilson syndrome, with MR being one of the main features of this syndrome (16). Furthermore, heterozygous mutations/deletions of the DPAGT1 gene located at 11q 23 (the breakpoint for patients #6 and #16) may reduce up to 88.0% of the mature mRNA and cause clinical features including MR [17].

CONCLUSIONS

In conclusion, the results of this study illustrate the contribution of chromosomal abnormalities to the pathogenesis of MR in this group of mentally retarded Iranian patients. Therefore, we recommend cytogenetic analysis for every individual with idiopathic MR.

This can help the management of the MR patient much better. In addition, by discovering the cause of MR, *e.g.*, deletion or duplication/trisomy of a chromosomal segment resulting from a paternal/maternal balanced translocation, prenatal diagnosis could be applied for future pregnancies, thus preventing the birth of another MR infant(s) through therapeutic abortion, which is allowed in Iran. Furthermore, since a routine cytogenetic analysis gives a minimum resolution of only 4-10 Mb, other advanced molecular cytogenetic techniques would be helpful for the diagnosis of the MR patients with normal karyotype, as mentioned by some researchers [12,18].

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