Çukurova Üniversitesi Tıp Fakültesi Dergisi
Journal of Cukurova University Faculty of Medicine

Araştırma Makalesi / Research Article

Cytogenetic and Molecular Investigation in Children with Possible Fragile X Syndrome

Frajil X Sendromu Olduklarından Şüphelenilen Çocuklarda Sitogenetik ve Moleküler Araştırmalar

Onur Özer[1], Osman Demirhan[1], Erdal Tunc[1], Hüseyin Bağcı[2], Dilara Karahan[1], Nilgün Tanrıerdi[1], Bertan Yılmaz[1], Ali Irfan Güzel[3], Ibrahim Keser[2]

[1]Çukurova Üniversitesi Tıp Fakültesi, Tıbbi Biyoloji ve Genetik Anabilim Dalı. ADANA

[2]Akdeniz Üniversitesi Tıp Fakültesi. Tıbbi Biyoloji ve Genetik Anabilim Dalı, ANTALYA

[3]Recep Tayyip Erdoğan Üniversitesi Tıp Fakültesi, Tıbbi Biyoloji ve Genetik Anabilim Dalı. RİZE

Çukurova Üniversitesi Tıp Fakültesi Dergisi (Journal of Cukurova University Faculty of Medicine) 2012; 37(2): 76-83

ABSTRACT

Objective: Fragile X syndrome (FXS) is the most common cause of inherited mental retardation and is due to a mutation in the X-linked FMR1 gene. Molecular genetic testing and chromosome analysis are indicated for this disorder. In this context, we tried to determine the frequency of the FXS, and other chromosomal abnormalities of Turkish pediatric neurology outpatients.

Materials and Methods: Cytogenetic and molecular screenings were performed to estimate the prevalence of the fragile X in 107 patients with mental retardation, language disorders, hyperactivity, developmental delay or fragile X syndrome phenotype. Only 26 out of 107 patients were screened, molecularly.

Results: Cytogenetically fragile X-positive cells was found in 8 cases (7.5%) of 107 patients; in 4.7% of males and in 2.8% of females. The autosomal fragile sites (FS) was found in 14 (13.1%) cases. One (0.9%) patient had pericentric inversion of chromosome 9. Molecular analysis were performed for 26 patients and all patients showed normal CGG expansion.

Conclusion: In diagnosis of fragile X syndrome, chromosome analysis must be run in conjunction with the molecular studies. It is recommended that all members of the fragile X family under risk should be screened both by cytogenetic and molecular methods. Genetic counseling can be useful to patients and families considering genetic testing.

Key Words: Fragile X syndrome, FMR1 gene, cytogenetic and molecular screenings

ÖZET

Amaç: Kalıtımla geçen mental retardasyonun en yaygın nedeni Frajil X sendromudur (FXS). X kromozomu üzerinde bulunan FMR1 geninde meydana gelen bir mutasyon, bu sendroma yol açmaktadır. Tanı için kromozom analizi ve moleküler genetik testlerden birine veya her ikisine birden başvurulabilmektedir. Bu çalışmada, hasta grubundaki (türk pediyatrik nöroloji hastalarında) FXS frekansı belirlenmeye çalışılmıştır.

Yöntem: Bu çalışmada mental retardasyon, konuşma güçlüğü, hiperaktivite ve gelişme geriliği gibi şikayetleri bulunan veya frajil X fenotipi gösteren 107 hastada fragile X yaygınlığını belirlemek için sitogenetik ve moleküler taramalar yapıldı. 107 hastanın sadece 26'sı moleküler olarak değerlendirildi.

Bulgular: Sitogenetik olarak incelenen 107 olgunun 8'inde frajil X pozitif hücrelere rastlandı. Buna göre erkek olguların % 4.7'sinde, kadın olguların ise % 2.8'inde frajil X pozitif hücrelere rastlandı. 14 olguda (%13.1) ise otozomal frajil bölgelere rastlandı. Bir olgunun 9. kromozomunda perisentrik inversiyona rastlandı. Moleküler analizi yapılan 26 olgunun tamamının CGG tekrar sayısı artışı bakımından normal oldukları tespit edildi.

Sonuç: Frajil X sendromunun tanısında kromozom analizi ve moleküler yöntem birlikte kullanılmalıdır. Frajil X pozitif bireylerin bulunduğu ailelerin tüm bireylerinin hem sitogenetik ve hem de moleküler olarak taranmalarında fayda görülmektedir. Hastalara ve hasta ailelerine genetik danışmanlık verilmesinde yarar görülmektedir.

Anahtar sözcükler: Frajil X sendromu, FMR1 geni, sitogenetik ve moleküler taramalar

INTRODUCTION

FXS is the most common cause of inherited mental retardation. The causative gene for FXS is FMR-1 and this gene was identified by Fu et al[1]. In the literature, there are some mental retardation cases linked to sex chromosomes. For example Martin and Bell described a family of sex linked mental retardation without dysmorphic features[2]. Lubs observed a marker X chromosome in a family with mentally retarded males[3]. But for FXS, the clinical features other than mental retardation include subtle dysmorphism, behavioral abnormalities and macroorchidism in postpubertal males. Because of the phenotype being subtle, clinical diagnosis may be difficult especially in young children. Hence, all cases of mental retardation without obvious cause should be tested for FXS, especially in young children. The reported prevalances of FXS, based on cytogenetic screening, is 0.4-0.8/1000 in males and 0.2-0.6/1000 in females[4]. Carpenter et al. studied 36 patients with a family history of MR and found 13.9% to be fragile X-positive, whereas Froster-Iskenius et al. .studied more than 200 patients with a family history of MR and found only 3.6% to be fragile X positive[5,6]. In another study of us, we found that the frequency of FXS in 120 Turkish children with intellectual disability is to be 11.7%[7]. However, the sensitivity of the cytogenetic test is not 100% for detecting full mutation carrying males, it is also low for detecting full mutation and pre-mutation female carriers, and it is virtually zero for detecting male pre-mutation carriers. Recently, a comparison of the results of direct DNA analysis for the CGG repeat were shown to be in complete agreement with the cytogenetic diagnosis of FXS in a study of 434 mentally retarded Japanese patients[8]. Molecular screening studies of Turkish male patients with MR of unknown etiology gave a prevalence of 3%[9-11]. In the present study, cytogenetic and molecular screenings were performed to estimate the prevalence of the fragile X in 107 patients with mental retardation, language disorders, hyperactivity, develop-mental delay or fragile X syndrome phenotype.

MATERIALS AND METHODS

107 children (95 boys and 12 girls) diagnosed at the Department of Pediatric Neurology of Çukurova University, Faculty of Medicine were included in this study. These children were selected on the basis of clinical criteria compatible with mental retardation, language disorders, hyperactivity, epilepsy, developmental delay or FXS phenotype. All 107 subjects with MR were referred to our Medical Biology and Genetics Department at the Faculty of Medicine of Çukurova University for cytogenetic investigation of fragile X and molecular analysis. The molecular analysis of the CGG repeat expansions of the FMR1 gene were performed by the Molecular Genetic Laboratory, Department of Medical Biology and Genetics, Faculty of Medicine, Akdeniz University, Antalya. For cytogenetic analysis, each child was examined for fragile X chromosome and other chromosome aberrations. In all cases, the metaphase chromosomes were obtained either by standard methods or by basal medium without folic acid. For each case at least 100 metaphases were evaluated. All numerical or structural anomalies were recorded according to the International System for Human Cytogenetic Nomenclature 2009[12]. Molecular detection of Fragile X was performed by employing salting out DNA extraction method using whole blood followed by quantitative fluorescence Polymerase Chain Reaction (QF-PCR) amplification of the FMR1 gene (5'UTR CGG Repeat) to detect normal, pre-mutated and full mutated alleles using Fragile X detection kit (Abbott Laboratories, Abbott Park, Illinois, USA).. Briefly, DNA was extracted from peripheral blood samples of the subjects (25 male and 2 female), who had been clinically diagnosed as Fragile X. DNA samples were then amplified with FMR1- and gender-specific primers in a thermal cycler (Applied Biosystems, Foster City, CA). The PCR

cycles applied were as follows; 15 cycles of 98.5 °C for 10 seconds, 58 °C for 1 minute and 75 °C for 6 minutes, followed by 15 cycles of 98.5 °C for 10 seconds (with 0,1°C increments/cycle), 56 °C for 1 minute and 75 °C for 6 minutes. At the end of PCR, the products were purified and then the capillary electrophoresis was carried out in an ABI 3130 Genetic Analyzer (ABI, Foster City, CA) using POP7 polymer. GeneMapper software was used to analyze the fragile X data to identify gender and CGG repeat size of samples. Analysis of the results and calculation of the peak areas were performed using GeneMapper 4.0 software (Applied Biosystems).

RESULTS

One hundred seven children were included in this study, 12 girls (11.2%) and 95 boys (88.8%). The mean age of children was determined as 11.63 (1.5-18). Eight children (7.5%) were cytogenetically diagnosed as fragile X syndrome (Table 1)(Figure 1). The frequency of fragile X-positive cells was found 4.7% in males and 2.8% in females. Other autosomal FS were observed in 14 (13.1%) children. One of 107 patients (0.9%) had pericentric inversion of chromosome 9(p11;q12) (Table 2)(Figure 1).

PCR and GeneMapper software analysis of the CGG repeat sequences characterizing fragile X showed no evidence of abnormal CGG repeat expansion in the 26 children analyzed. The PCR products were also assessed by electrophoresis. The number of repeats ranged from 21 to 39 copies. All had similar size CGG triplet repeat expansions. The 29 repeat alleles (50%) were the most common followed by the 30 and the 31 repeat alleles. In two child we found fragility at Xq27.3 region cytogenetically, but according to molecular analysis we didn't any evidence of abnormal CGG repeat expansion in FMR1 gene (Table 1).

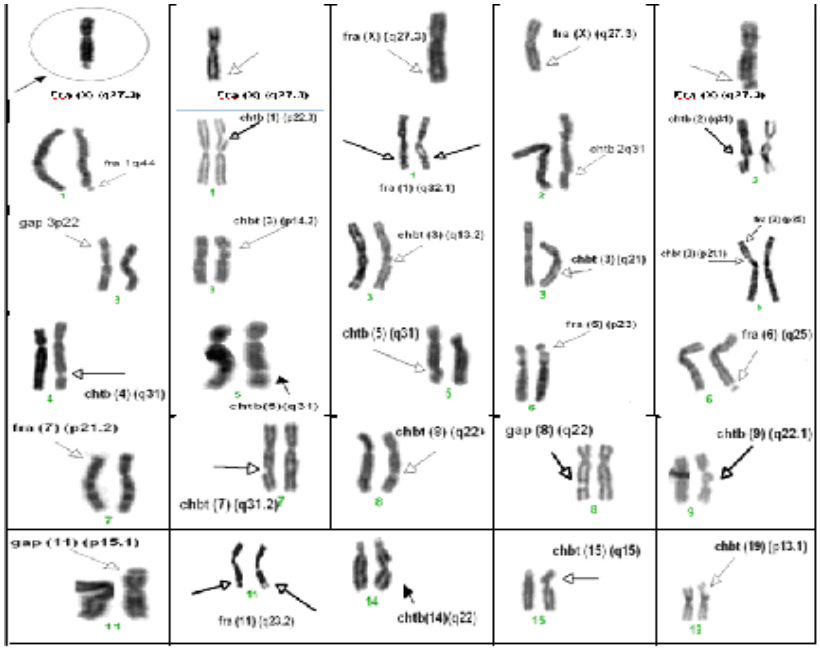

Figure 1. Cytogenetic expression of the fra(X)(q27.3) and some autosomal FS seen in our patients.

Table 1. Summary of clinical features, cytogenetic and molecular findings in 32 suspected children screened for FXS

Pat. no	M/F	Age	Cytogenetic abnormalities	Molecular genetic	Clinical findings
1	M	6	46,XY	Normal	MR
2	M	4	46,XY	Normal	Developmental delay
3	M	4	46,XY	Normal	Hyperactivity
4	M	7	46,XY	Normal	Epilepsia
5	M	10	46,XY	Normal	MR
6	M	5	46,XY	Normal	MR
7	M	11	46,XY	Normal	Cerebral gigantism
8	M	3	46,XY	Normal	MR
9	M	8	46,XY,fraX(+), 5%	Normal	Developmental delay
10	M	8	46,XY	Normal	MR
11	M	9	46,XY	Normal	Epilepsia, MMR
12	M	10	46,XY	Normal	MR
13	M	5	46,XY	Normal	MR
14	M	5	46,XY,fraX(+), 5%	Normal	Epilepsia
15	M	4	46,XY	Normal	MMR
16	F	3	46,XX	Normal	MR
17	M	9	46,XY	Normal	MR
18	M	3	46,XY	Normal	MMR
19	M	3	46,XY	Normal	Autism
20	M	5	46,XY	Normal	MR
21	M	7	46,XY	Normal	MR
22	M	6	46,XY	Normal	MR
23	M	10	46,XY	Normal	MR
24	M	4	46,XY	Normal	MR
25	M	11	46,XY,aut.fra. 5%	Normal	MR
26	M	9	46,XY,aut.fra. 8%	Normal	MR
27	F	7	46,XX,fraX(+), 10%	-	MR
28	F	7	46,XX,fraX(+), 12%	-	MR
29	F	1	46,XX,fraX(+), 10%	-	MR
30	M	11	46,XY,fraX(+), 8%	-	MR
31	M	11	46,XY,fraX(+), 12%	-	MR
32	M	8	46,XY,fraX(+), 15%	-	MR

Table 2. Summary of autosomal fragility findings in 14 suspected children screened for FXS.

Pat No	M/F	Age	Cytogenetic Abnormalities	Molecular genetic	Clinical findings
1	M	11	46,XY,aut.fra.5%	Normal	MR
2	M	9	46,XY,aut.fra.8%	Normal	MR
3	M	7	46,XY,aut.fra.6%	-	MR
4	M	14	46,XY,aut.fra.6%	-	MR
5	M	5	46,XY,aut.fra.8%	-	MR
6	M	9	46,XY,auto.fra.8%	-	MR
7	F	5	46,XX,aut.fra.8%	-	MR
8	M	7	46,XX,aut.fra.8%	-	MR
9	M	13	46,XX,aut.fra.10%	-	MR
10	M	14	46,XY,aut.fra.10%	-	MR
11	M	5	46,XY,aut.fra.6%	-	MR
12	M	9	46,XY,aut.fra.8%	-	MR
13	M	3	46,XY,aut.fra.15%	-	MR
14	M	4	46,XY,aut.fra.15%	-	MR

DISCUSSION

The most prominent feature and significant problem of FXS is mental retardation. The clinical features other than mental retardation include subtle dysmorphism, behavioral abnormalities and macroorchidism in postpubertal males. Clinical diagnosis may be difficult especially in young children. Therefore, all cases of mental retardation without obvious cause should be tested for FXS. A number of genetic epidemiological studies from various geographical areas have previously been done, based on cytogenetic screening or DNA testing[13-14]. In general population, the prevalence of FXS is found to be around in 1 in 4000 males[15]. The prevalence of FXS in Asian population was reported to range from 0% to 11%. In selected populations of mentally retarded patients, an overall prevalence rate of 4.8% of fragile X was reported by Proops et al. About 40% of X-linked mental retardation and 4% of all MR has been attributed to FXS[16]. Cytogenetic screening studies of mentally retarded Saudi patients gave an FXS frequency of 8.5% (7.86% of males and 0.65% of females)[17]. Carpenter et al. studied 36 patients with a family history of MR and found 13.9% to be fragile X-positive cases, whereas Froster-Iskenius et al. studied more than 200 patients with a family history of MR and found only 3.6% to be fragile X positive[5,6]. Iqbal et al. studied 81 patients with a family history of MR; among these, 12 patients (14.8%) were found to be fragile X-positive, which is similar to the report by Carpenter et al[5,17]. In the present study, 7.5% of 107 children with MR was found to be positive for fragile X detected by cytogenetic analysis. Also, in a previous study by our group, cytogenetic screening in 120 children with MR, language disorders, attention deficit hyperactivity, or developmental delay has shown that the frequency of FXS in Turkish population to be 11.7%[7]. Our cytogenetics incidence of fragile X was consistent with the results of the fragile X screening studies performed in the other populations with MR. Inconsistency in the results of these studies may stem from the technical aspects of cytogenetic analysis for FXS, usually a longer exposure to colchicine causes more chromosomal condensation, which turns fragile X more difficult to be detected by microscopic analysis. Furthermore, the wide range of frequencies may be due to different population studied, different sizes of the studied populations, different selection criteria, or may be due to the methods used to diagnose FXS.

The sensitivity of the cytogenetic test is not 100% for detecting full mutation carrying males, which is also low for detecting full mutation and premutation female carriers and virtually zero for detecting male pre-mutation carriers. It is now apparent that the false-positive rate for cytogenetic testing was significant in both affected and carrier individuals[18]. Thus, all potential carriers in fragile X families who were tested negative by cytogenetic tests should also be confirmed with molecular DNA techniques for fragile X, which are thought to be more sensitive. For these reasons, cytogenetic testing is not recommended to be replaced by molecular genetic testing. Nevertheless, cytogenetic analysis should be done initially for all cases referred for fragile X testing to detect cases with other chromosome abnormalities besides FRAXA or FRAXE.

In the present study, molecular screening of the fragile X showed no evidence of abnormal CGG repeat expansion in 26 children. Moreover, the expression of cytogenetic fragility at Xq27.3 which has been previously reported in two children also showed no evidence of abnormal CGG repeat expansion (Table 1), and there was no correlation between the frax A or E and (CGG)n repeat length. Inconsistency in the results may stem from the difficulty of distinguishing the FRAXA locus from the two other FS loci, FRAXE and FRAXF. Both FRAXE and FRAXF are located in the similar region, which is at Xq27.3-28. From our data, the (CCG)n repeat were found to have increased to 21 to 39 repeats with a peak at 29 repeats in the

children. Molecular screening in MR children has shown that the frequency of FXS to be 2% in Central Java, Indonesia[19]. Among Japanese MR cases FXS varied between 0.8% and 2.7%, and FXS frequency has been demonstrated as 2.8% in a population of Chinese MR individuals[8]. Also, molecular screening studies of Turkish male patients with MR of unknown etiology had a fragile X prevalence of 3%[9]. In some studies conducted on Turkish families variable results were obtained. According to one study, the 55.6% of 36 members in 6 families with fragile X had full mutations in their FMR gene[20], but in another study, 12.9% of 132 cases were found to have a full mutation in index cases with FXS[10]. Also, the 15.6% of 32 members in eight fragile-X affected families that had been clinically and cytogenetically assessed had full mutations[11].

In addition to fragile-X screenings, we screened our patients in terms of autosomal FS. The 5-20% of cells in 14 children (13.1%) had autosomal FS, higher expression of autosomal FS in our study population was however unexpected. In another study by our group, cytogenetic screening in children with MR, language disorders, attention deficit hyperactivity, or developmental delay has shown that a statistically significant difference in the autosomal FS between the study and control groups ($p<0.05$) [7]. Routine cytogenetic analysis has shown that chromosomal anomalies are responsible from 40% of severe (IQ<55) and 10-20% of mild MR (IQ 55-70)[9]. Curry et al. also claimed that 4-28% of individuals with MR had chromosomal abnormalities, the frequency of which increased with the severity of MR and the presence of congenital anomalies[21]. Expressed FS can lead to interchromosomal recombination. Demonstration of the autosomal FS can be a problem in some families, and presumably in some individuals, due to the risk of new mutations.

In addition several reports have documented a variety of neurodevelopmental abnormalities and mental retardation in individuals with FSs. Although, the nature of the fundamental genetic defects is unknown, it is likely to be situated at the locus of the microscopically observable FSs. The higher expression of autosomal FSs in our patients can lead to a local blockage of several genes in that region around the FSs, leading to a variety of neurodevelopmental abnormalities and mental retardation in individuals.

We reported one patient (0.9%) with pericentric inversion 9 (p11;q12) (Table 2). Pericentric inversion of the heterochromatic region of chromosome 9 [inv(9)(p11q13)] is a common heteromorphism found in 1–3% of the population, and is considered to be a normal variation. But, Liu et al. (1997) has suggested that pericentric inversion 9 is associated with various diseases and also appear to be unfavorable for human reproduction[22]. Kunugi et al. (1999) found that 4% schizophrenics are carriers an inv[9], and the incidence of inv[9] among Japanese schizophrenics was significantly higher than the general population[23]. Demirhan et al.(2003) also reported an unusually increased prevalence (5.2%) of inv[9] in Turkish patients with schizophrenia[24]. This may indicate that the effect of qh region on the development of schizophrenia would not be major one, but it may be a risk-increasing factor. Further studies are necessary to elucidate the role of pericentric inv[9] in FRX individuals and individuals with psychiatric problems.

In conclusion, in our cytogenetic analysis, 7.5% of fragile X-positive cases among 107 mentally retarded Turkish children is similar to reports in other parts of the world. From these results, it has been understood that the higher expression of autosomal FSs could also lead to MR. Cytogenetic studies are critical, since constitutional chromosome abnormalities have been identified as frequently or more frequently than fragile X mutations in mentally retarded individuals referred for fragile X testing. Our molecular data did not show any evidence of fragile X mutation (in the 26 children analyzed). In spite of this, DNA analysis for fragile X syndrome should be performed as part of a comprehensive genetic evaluation that includes routine

cytogenetic analysis. Further studies in large sample numbers are also needed to elucidate this association. Inter-population studies for X-linked disorders will also be helpful.

Acknowledgments

This project was supported by the Cukurova University Research Grant TF.2009YL11.

REFERENCES

1. Fu YH, Kuhl DP, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, et al. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. Cell. 1991; 67: 1047-1058.
2. Martin JB, Bell J. A pedigree of mental defect showing sex-linkage. J Neurol Psychitr. 1943; 61: 54-157.
3. Lubs HA. A marker X chromosome. Am J Hum Genet. 1969; 21: 231-244.
4. Gustavson KH, Blomquist HK, Holmgren G. Prevalence of the fragile X syndrome in mentally retarded boys in a Swedish county. Am J Med Genet. 1986; 23: 581-7.
5. Carpenter NJ, Leichtman LG, Say B. Fragile Xlinked mental retardation. A survey of 65 patients with mental retardation of unknown origin. Am J Dis Child. 1982; 136: 392–8.
6. İskenius UF, Felsch G, Schirren C, Schwinger E. Screening for fra (X) (q) in a population of mentally retarded males. Hum Genet. 1983; 63: 3-157.
7. Demirhan O, Taştemir D, Somer DR, Firat S, Avcı A. A Cytogenetic Study in 120 Turkish Children with Intellectual Disability and Characteristics of Fragile X Syndrome. Yonsei Med J. 2003; 44: 583 -592.
8. Hofstee YO, Arinomi T, Hamaguchi H. Comparison between the cytogenetic test for fragile X and the molecular analysis of the FMR1 gene in Japanese mentally retarded individuals. Am J Med Genet. 1994; 51: 466-470.
9. Tunçbilek E, Alikaşifoğlu M, Boduroğlu K, Aktaş D, Anar B. Frequency of fragile X syndrome among Turkish patients with mental retardation of unknown etiology. Am J Med Genet. 1999; 84: 202-3.
10. Bilgen T, Keser I, Mihci E., Haspolat S, Tacoy S., Luleci G. Molecular analysis of fragile X syndrome in Antalya Province. Indian J Med Sci. 2005; 59(4): 150-155.
11. Uyguner ZO, Wollnik B, Kayserili H, Tükel T, Basaran S, Apak MY. Establishment Of A Nonradioactive Molecular Diagnosis Of Fragile-X Syndrome. Turk J Med Sci. 2000; 30: 253–260.
12. 1Shaffer LG, Slovak ML, Campbell LJ. An International System for Human Cytogenetic Nomenclature. Published in collaboration with Cytogenetic and Genome Research, 2009.
13. Webb T. The epidemiology of the fragile X syndrome. In: Davies KE, (ed). The fragile X syndrome. Oxford: Oxford University Pres, 1989.
14. Sherman SL. Epidemiology. In: Hagerman RJ, Cronister AC, (eds). Fragile X syndrome: Diagnosis, treatment, and research. Baltimore: The Johns Hopkins University Pres, 1996.
15. Turner G, Webb T, Wake S, Robinson H. Prevalence of Fragile X syndrome. Am J Med Genet. 1996; 64: 196-197.
16. Proops R, Mae M, Jacobs PA. A study of mental retardation in children in the island of Hawaii. Clin Genet. 1983; 23: 81-96.
17. Iqbal MA, Sakati N, Nester M, Ozand P. Cytogenetic Diagnosis of Fragile X Syndrome: Study of 305 Suspected Cases In Saudi Arabia. Ann Saudi Med. 2000; 20: 3-4.
18. Mannens M, Slater RM, Heyting C, Bliek J, De Kraker J, Coad N, et al. Molecular nature of genetic changes resulting in loss of heterozygosity of chromosome 11 in Wilms' tumours. Hum Genet. 1988; 81: 41-48.
19. Faradz SMH, Buckley M, Tang L, Leigh D, Holden JJA. Molecular screening for fragile X syndrome among Indonesian children with developmental disability. Am J Med Genet. 1999; 83: 350-351.
20. Keser I, Lüleci G, Aklan M. The results of molecular and cytogenetıc analysıs in 6 famılıes wıth Fragıle - X syndrome in Turkey. Marmara Medical Journal. 2000; 13(1): 7-10.
21. Curry CJ, Stevenson RE, Aughton D, Byrne J, Carey JC, Cassidy S, et al. Evaluation of mental retardation: Recommendations of a consensus conference. American College of Medical Genetics. Am J Med Genet. 1997; 72: 468-477.
22. Liu YC, Lee ML, Chen CP, Lee CC, Lin SJ, Chao MC, et al. Inversion and enlargement of the heterochromatin region of chromosome no. 9 among Taiwanese. Tzu Chin Med J. 1997; 9(3): 159–167.
23. Kunughi H, Lee KB, Nanko S. Cytogenetic findings in 250 schizophrenics: evidence confirming an excess of the X chromosome aneuploidies and pericentric inversion of chromosome 9. Schizophr Res. 1999; 40: 43– 47.
24. Demirhan O, Taştemir D. Chromosome aberrations in a schizophrenia population. Schizophr Res. 2003; 65: 1–7.

Yazışma Adresi / Address for Correspondence:
Prof. Dr. Osman DEMİRHAN
Cukurova University, Faculty of Medicine,
Department of Medical Biology and Genetics,
01330 ADANA
Tel: 90-322-3387140, Fax: 90-322-3386572,
Email:osdemir@cu.edu.tr

geliş tarihi/received :18.04.2012
kabul tarihi/accepted:23.05.2012