THE RESULTS OF MOLECULAR AND CYTOGENETIC ANALYSIS IN 6 FAMILIES WITH FRAGILE - X SYNDROME IN TURKEY

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ABSTRACT

Objective: The aim of this study was to optimize the diagnosis of the fragile X syndrome in six large families with fragile X syndrome in Turkey.

Methods: Southern blot analysis was performed to identify the mutations of the FMR 1 gene localized on FRAXA locus using StB12.3 probe among 36 members (19 males, 17 females) of fragile X families and controls (8 males, 8 females) following cytogenetic analysis by fragile X induction methods.

Results: Eleven males and 9 females had full mutations, while 7 males and 3 females had normal range of CGG repeats. One female who was found positive by cytogenetic analysis had mosaic mutation (Y2[II-3] with 6.0, 5.2, 2.8 kb fragment sizes). Five females had premutations and 1 male had atypical fragment pattern.

Conclusion: We suggest that, diagnosis of fragile X syndrome is not possible only by cytogenetic analysis. For appropriate counseling it is recommended that all members of the fragile X family under risk should be screened both by cytogenetic and molecular methods.

Key Words: Fragile X syndrome, DNA analysis, Mosaicism, StB12,3 probe.

INTRODUCTION

Fragile X syndrome is one of the most frequent genetic causes of mental retardation (MR); with an incidence of 1/4.000 in males and 1/5.000 in females (1,2). The mutations associated with the syndrome have been characterized (3), and the fragile X mental retardation 1 (FMR 1) gene has been cloned (3,4). Fragile X mutations consist of an increase in the size of a target fragment containing a trinucleotide (CGG)n repeats located in the 5'-UTR region of the FMR-1 gene (5-7). The triplet repeats in FMR-1 gene normally vary in size. Between 6 to 54 repeats are considered normal, between 54 and 200, and more than 200 repeats are considered premutation and full mutation, respectively (8-10). A normal man carrying premutated allele is called "normal transmitting male" (NTM) and has a high risk of having affected grandsons (11). Some affected individuals could be mosaics with coexistence of premutations and full mutations (11-13). These expansions would be expressed as a fragile site at Xq27.3 locus (FRAXA) by cytogenetic analysis using international fragile X induction systems (14,15).

MATERIALS AND METHODS

The subjects investigated in our laboratory were families of six probands with mental retardation, developmental delay and behavioral problems. Together with 6 affected individuals 36 relatives were investigated both by cytogenetic and molecular analysis. All families were from Antalya or its surrounding regions. Sixteen normal persons (8 males, 8 females) were used as controls. For fragile X induction, TC 199 with 5% FCS and TC 199 with MTX (10^{-7} M final concentration) were used. We have scored at least 100 and 200 metaphases for males and females, respectively.

Genomic DNA from 10 ml peripheral blood with EDTA was extracted by the salting-out method (16). Five micrograms of genomic DNA were double digested by EcoRI/EagI, then blotted and hybridized with probe StB12.3 according to Rousseau et al (5).

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RESULTS

The results of the DNA and cytogenetic analysis of our families are given in Table I. Among the 17 females at risk, 5 premutation, 9 full mutation, 1 mosaic mutation were identified; whereas in the 19 males at risk, 11 full mutations, and 1 atypical hybridization pattern were identified by Southern blot. Seven males and 3 females were found normal after DNA analysis.

Fig. 1a shows the hybridization pattern of the Family Y. Y5 (II-4) was a cytogenetically positive female with 2 percent of fragile X expression like her sister Y2(II-3) and her mother Y8(I-2). Y5(II-4) had three fragments which were 6.2, 5.2, and 2,8 kb instead of the expected 5.2 and 2.8 kb fragments in normal females by EcoRI/EagI double digestion. Y3 (III-1) and Y4 (III-2) had full mutation (approximately a 7.0 kb-fragment) (Fig.1a).

Table I. Summary of molecular and cytogenetic results of families with fragile X syndrome.

FAMILIES	SEX	FRAGILE X (%)	DNA MUTATION *				
		TC199 + TC199 (MTX 10^{-7})	N	P	F	M	A
FAMILY A							
A1	M	-	+				
A2	F	8			+		
A3	M	8			+		
A4	M	13			+		
A5	F	-	+				
A6	F	13			+		
A7	M	3			+		
A8	M	8			+		
FAMILY Y							
Y1	M	-	+				
Y2	F	2				+	
Y3	M	7			+		
Y4	M	32			+		
Y5	F	2			+		
Y6	M	16			+		
Y7	F	3			+		
Y8	F	2		+			
FAMILY B							
B1	M	20			+		
B2	F	3		+			
B3	M	-					+
B4	M	-	+				
B5	F	-		+			
B6	F	2		+			
FAMILY S							
S1	M	13			+		
S2	F		+				
S3	F	2		+			
S4	M	-	+				
FAMILY TR							
TR1	M	-	+				
TR2	F	5			+		
TR3	M	18			+		
TR4	F	16			+		
TR5	F	2			+		
FAMILY TL							
TL1	M	24			+		
TL2	F	-	+				
TL3	M	-	+				
TL4	F	5			+		
TL5	M	-	+				

* N= Normal, P= Premutation, F= Full mutation, M= Mosaic, A= Atypical pattern.

Fig. 1b shows the hybridization pattern of the Family B. B2 (II-4) and her mother B6(I-2) were cytogeneticially positive, and had premutations with increasedr repeat size (0.2 kb). B3(II-3) was cytogenetically negative and clinically normal male who had atypical hybridization pattern probably due to incomplete digestion, instead of the expected 2.8 kb fragment for normal males (Fig.1b). The instability and abnormal methylation of the CGG repeats were in agreement with clinical phenotype in all members of tested families. Also, there was anticipation of CGG repeats in families with three generations, as expected.

DISCUSSION

The family members of fragile X syndrome patients with or without clinical features should be screened by molecular methods. Coexistence of the full and premutation carriers has been described in fragile X families. Mosaicism has been detected with a higher prevalence in males (5,9,10,13,17). In the present study one female (Y2, in Table 1.) who was cytogenetically positive was found to be mosaic by DNA analysis (Fig.1). The ratio and the distribution of mosaicism in tissues is not known. Y5's (II-4) mother Y8(I-2) has the premutation (5.2 and 3.0 kb), her sister Y2 (II-3) has the mosaic and her brother Y6 (II-2) has the full mutation patterns ranging from 6.0 to 6.6 kb. B2(II-4) has the premutation and her mentally retarded son (III-1) has a full mutation (Fig.1b). Our data suggests that the families of the fragile X syndrome should be definetly investigated by molecular tests. The proportion of methylation plays an important role in the expression of FMR 1 gene. Our data shows an agreement with previously reported studies (18-20). For appropriate genetic counseling cytogenetic analysis should be performed on clinically suspected fragile X cases to rule out other chromosome abnormalities. In addition, all relatives under risk should be screened by molecular tests.

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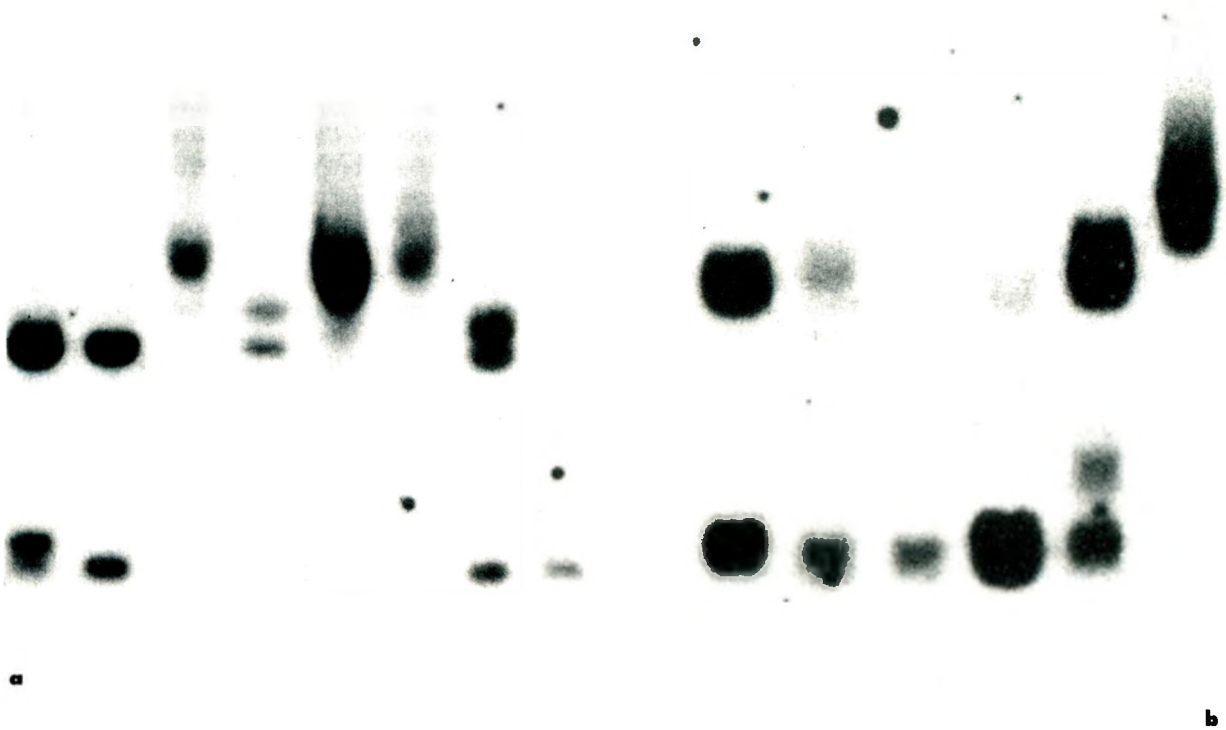

Fig. 1.: Pedigrees and StB12.3 hybridization patterns of EcoRI/EagI double-digested DNA samples; a) from Family Y; Y2(II-3): mosaic female with normal allele, b) from Family B; B2(II-4): a female with premutation, B3(II-3): a male with atypical hybridization pattern, and B4(II-2): normal male.

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