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FRAGILE X SYNDROME

CLINICAL AND CYTOGENETIC STUDIES

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ABSTRACT - Three families with the fragile X syndrome were studied with the aim to establish the most frequent clinical signs in the affected individuals and heterozygous women. The clinical evaluation, IQ level measurements and cytogenetic studies were performed in 40 subjects, 20 males and 20 females. The fragile X diagnosis was confirmed in all the male individuals with mental retardation. In the postpubertal subjects the most frequent clinical signs were inner canthal distance < 3.5 cm, macro-orchidism, long and narrow face and high arched palate while in the prepubertal subjects the behavioral characteristics as hyperactivity and poor eye contact were the most frequent and were observed in all patients. Twenty six percent of the heterozygous women presented with mental retardation and showed clinical signs rather than behavioral ones. All male individuals with mental retardation were observed as having fragile X [fra(X)] in lymphocytes culture. Sixty three percent of women showed fra(X). There was a positive correlation between the frequency of fra(X) and the clinical characteristics. We emphasize the importance of the clinical evaluation in the study of familial mental retardation and in the screening of isolated cases with suspect of having the fragile X syndrome.

KEY-WORDS: fragile X syndrome, mental retardation, X-linked mental retardation.

Síndrome de X frágil: estudo clínico e citogenético

RESUMO - Três famílias com a síndrome do X frágil foram estudadas com o objetivo de estabelecer as características clínicas mais frequentes nos indivíduos afetados e nas mulheres heterozigotas. Avaliação clínica, de nível intelectual e citogenética visando a expressão do sítio frágil do cromosssomo X na região Xq27.3 foram empregadas em 40 indivíduos, 20 do sexo masculino e 20 do sexo feminino. Foi confirmado o diagnóstico da síndrome do X frágil em todos os indivíduos do sexo masculino com deficiência mental. Nos indivíduos pós-puberais as características clínicas mais frequentes foram: distância intercantal interna inferior a 3,5 cm, macroorquidia, face estreita e alongada, e palato alto. Nos indivíduos pré-puberais as características de comportamento como hiperatividade e pobre contato ocular foram observadas em todos os pacientes. Vinte e seis por cento das mulheres heterozigotas apresentaram deficiência mental e nestas as características clínicas foram mais importantes. Todos os indivíduos do sexo masculino com deficiência mental apresentaram o cromossomo X frágil [fra(X)] em cultura de linfócitos. Sessenta e três por cento das mulheres demonstraram o fra(X). Houve correlação positiva entre a frequência de fra(X) e as características clínicas. Enfatizamos a importância da avaliação clínica no estudo de deficiência mental familial e na triagem de casos isolados com suspeita da síndrome do X frágil.

PALAVRAS-CHAVE: síndrome do X frágil, deficiência mental, deficiência mental ligada ao X.

The fragile X syndrome is second only to Down syndrome as genetic cause of mental retardation, accounting for approximately 50% of all cases of X-linked mental retardation. Its prevalence is 1 in 4000 males[20]. In Brazil, it had been estimated as 8% of mentally retarded males and 4% of mentally retarded females[13]. This syndrome affects males and is characterized by mental retardation, large or prominent ears, a long and narrow face and macro-orchidism[8]. Its is associated with a fragile site at Xq27.3 which is expressed in folate deficient media[18]. A gene called FMR-1 (Fragile Mental Retardation-1) was identified at that region and it was shown that in the overwhelming

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majority of cases the mutation is caused by the amplification of a CGG trinucleotide repeat[16,21,24]. The number of CGG repeats in the FMR1 gene of the normal population varies from 6 to 50. Unaffected carriers of the fra(X) mutation have an increased number of repeats, usually between 60 and 200, and are said to have a premutation. Subjects with mental retardation have over 200 repeats and are said to have a full mutation.

We present here the analysis of three large fra(X) families. Our aim was to evaluate the most frequent clinical findings in the affected males and heterozygous females and to correlate them with the expression of the fragile site at Xq27.3.

MATERIAL AND METHODS

Patients

Forty individuals (Fig 1) from three unrelated families with X-linked mental retardation were ascertained through the out-patient medical genetics clinic of the Hospital of the Faculty of Medicine of Ribeirão Preto (University of São Paulo, São Paulo, Brazil). There was a total of 20 males and 20 females with age ranging from 4 to 54 years and from 4 to 75 years, respectively.

The patients were divided into five different groups according to age, sex and the presence of mental retardation: group 1, post-pubertal mentally retardated males; group 2, prepubertal mentally retardated males; group 3, mentally retardated females; group 4, obligate heterozygous non-mentally retarded and group 5, siblings that do not fit in the other groups.

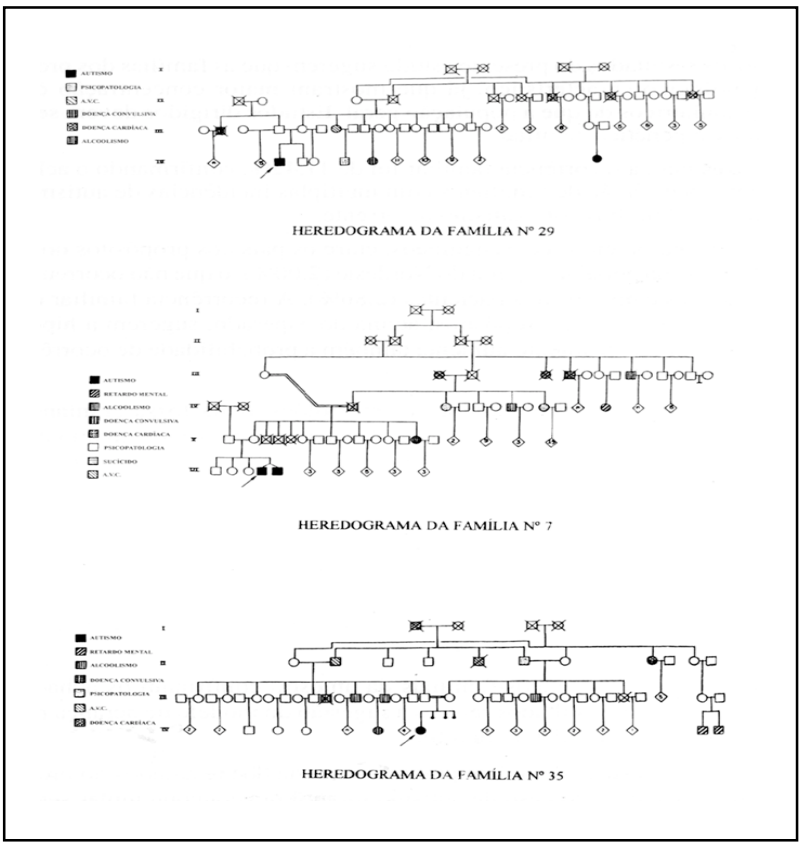

Fig 1. A) Family 1. B) Family 2. C) Family 3

normal male *mentally retarded male* *fra(X) positive male*

◯ *normal female* ◐ *mentally retarded female* ◑ *fra(X) positive female*

Clinical studies

All patients were evaluated clinically. Anthropometric measurements were performed according to Hall et al.[9] and included: weight, height, head circumference, inner canthal distance, outer canthal distance, ear length, total hand length, middle finger length, penile length and testis volume. The testicular volume was measured with the Prader orchidometer. A testicular volume of more than 25 ml was measured using the formula $\pi/6(\text{length})(\text{width}^2)$. Macro-orquidism was defined as testicular volume greater than 30 ml for post-pubertal males and greater than 4 ml for prepubertal males[8].

Cognitive profile

Cognitive function was quantified in all subjects by the tests: INV-Forma C, G-36, Goodenough and Vineland. The subjects were diagnosed as having a normal intelectual performance or being mentally retarded (mild, moderate, severe or profound).

Cytogenetic studies

The cytogenetic investigation were done according to preparation and analysis proposed by the Comittee convened at the 4º International Workshop on Fragile X Syndrome[10]. Chromosome analysis was performed on peripheral lymphocytes cultured for 72 hours in three culture systems for eliciting the Xq27.3 fragile site:

A. TC199 (Adolfo Lutz) with 5% Fetal Bovine Serum (FBS)(Gibco) and 26 µg/ml of Trimetoprim (Sigma)[2].

B. RPMI 1640 (Gibco) with 20% FBS (Gibco) and $2x10^{-7}$M FUdR (Sigma)[7].

C. RPMI 1640 (Gibco) with 20% FBS(Gibco) and 300 mg/L Thymidine (Sigma)[19].

One hundred GTG banded metaphases for each system culture were analyzed. The protocols A and B were used for males and A, B and C for females. A fragile X frequency of 4% or more were considered positive.

RESULTS

Table 1 shows the clinical findings observed in mentally retarded post-pubertal males (group1). The most frequent findings were inner canthal distance < 3.5 cm (100%), macro-orchidism (81%), long and narrow face (77%), high arched palate (70%), avoidance of eye contact (61%), prominent ears (54%), large ears (46%), hand calluses (38%) and hyperactivity (38%). Some of these findings are shown on Figures 2 and 3.

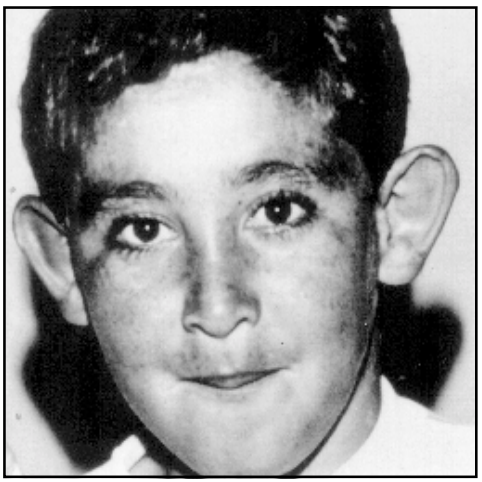

Fig 2. Patient III20 (family 1) with large and prominent ears.

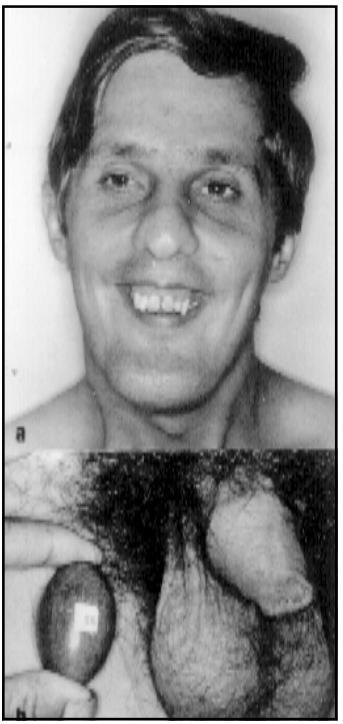

Fig 3. (a) Patient III3 (family 1) with long and narrow face and (b) macro-orchidism (testicular volume= 56 ml).

Table 1. Clinical findings in mentally retarded postpubertal males (group 1).

Findings	Family 1			Family 2						Family 3				Total
	III 3	III14	III20	II4	II8	II10	III3	III4	III8	V23	V24	V27	V37	
Age (years)	27	22	13	43	35	31	19	18	14	24	21	16	19	
Ears > 7 cm	-	-	+	+	+	+	-	+	+	-	-	-	-	6/13
Prominent ears	-	-	+	-	-	-	-	+	+	+	+	+	+	7/13
Inner canthal distance <3.5 cm	+	+	+	+	+	+	+	+	+	+	+	+	+	13/13
Long and narrow face	-	+	+	+	+	+	-	-	+	+	+	+	+	10/13
High arched palate	+	+	+	-	+	-	-	+	+	+	+	-	+	9/13
Hyperactivity	-	-	+	-	-	-	+	+	+	+	-	-	-	5/13
Avoidance eye contact	+	-	+	+	+	+	+	+	-	-	+	-	-	8/13
Hand calluses	-	-	+	+	+	-	-	+	-	-	+	-	-	5/13
Macro-orchidism/	+	+	ne	-	+	ne	+	+	+	-	+	+	+	9/11
testicular volume Right;Left (ml)	56;56	58;58		20;20	54;54		36;36	50;50	31;30	25;25	61;44	44;21	38;38	
Brachydactyly	+	-	-	-	-	-	-	-	-	+	-	-	-	2/13
Myopia	-	+	-	-	-	-	-	-	-	-	-	-	-	1/13
Hyperextensible joints	-	-	+	-	-	-	-	-	-	+	+	-	+	4/13
Strabismus	-	-	-	-	+	-	-	-	-	-	-	-	-	1/13
Hernia	-	-	-	-	-	-	-	-	-	-	-	+	+	2/13

+, present; -, absent; ne, not evaluated.

Abnormal behavior features were observed most frequently in prepubertal males (group 2) (Table 2). All the prepubertal patients had hyperactivity and avoidance of eye contact, 50% had hand biting and hand flapping. The clinical manifestations most frequently observed in this group were macro-orchidism (66%), prominent ears (50%), hyperextensible joints (50%) and calluses (50%). Table 3 shows the clinical features on mentally retarded females (group 3). The findings observed most frequently in this group were hyperactivity (75%), poor eye contact (75%), inner canthal distance <3.5 cm (50%), high arched palate (50%) and long face (50%). Two heterozygous (group 4) did not show any abnormal clinical signs. The historical information most frequently observed were premature menopause, learning disabilities and depression. The presence of a long and narrow face was observed in 63% of the patients (Table 4). The clinical findings were observed most frequently in mentally retarded females (group 3) than in females with normal cognitive profile (group 4) (P= 0.0410). Three individuals from group 5 had some clinical manifestation of fragile X syndrome (Table 5).

Two (F2:III4 and F3:V27) of 13 ascertained postpubertal males were moderately mentally retarded. All others had severe mental retardation. All prepubertal males were severe mentally retarded. On group 3, two (F2:III1 and F3:V47) had severe mental retardation, one (F1:III8) moderate

Table 2. Clinical findings in prepubertal mentally retarded males (group 2).

Clinical findings	Family 1	Family 3			
	IV6	V28	V45	V46	Total
Age (years)	4	10	9	7	
Long face	-	-	+	-	1/4
Large ears	+	-	-	-	1/4
Prominent ears	+	-	-	+	2/4
High arched palate	-	-	-	+	1/4
Hyperextensible joints	+	-	+	-	2/4
Macro-orchidism/testicular volume (ml)	ne	+/4	+/4	-	2/3
Hand calluses	-	+	+	-	2/4
Hyperactivity	+	+	+	+	4/4
Hand flapping	-	+	-	+	2/4
Hand biting	-	+	+	-	2/4
Avoidance of eye contact	+	+	+	+	4/4

+, present; -, absent; ne, not evaluated.

Table 3. Clinical findings in mentally retarded females (group 3).

Clinical findings	Family 1	Family 2		Family 3	Total
	III8	III1	III9	V47	
Age (years)	16	25	12	4	
Historical information					
Hand flapping	-	-	-	-	0/4
Hand biting	-	+	-	-	1/4
Hyperactivity	+	+	-	+	3/4
Poor eye contact	+	+	-	+	3/4
Physical manifestation					
Inner canthal distance < 3percentile	-	+	+	-	2/4
Large ears	-	-	-	-	0/4
Prominent ears	-	-	-	-	0/4
Long face	-	+	+	-	2/4
High arched palate	-	+	+	-	2/4
Hyperextensibles metacarpal-phalangeal joint	-	-	-	-	0/4
Hand calluses	-	-	-	-	0/4

+, present; -, absent.

Table 4. Clinical findings in heterozygous females (group 4).

Findings	Family 1						Family 2		Family 3			Total
	I2	II2	II6	II7	II8	III5	I4	II3	IV3	IV6	IV9	
Age (years)	68	50	45	37	33	24	75	47	51	39	28	
Historical information												
Premature menopause (years)	na	+/38	+/40	m	m	m	+/40	-/46	na	m	m	3/4
Cardiac murmur	-	-	+	-	-	-	-	-	-	-	-	1/11
Learning disabilities	-	-	-	+	+	-	-	-	-	+	-	3/11
Special education	-	-	-	-	-	-	-	-	-	-	-	0/11
Depression	-	-	-	-	+	-	-	-	-	+	-	2/11
Hand biting	-	-	-	-	-	-	-	-	-	-	-	0/11
Poor eye contact	-	-	-	-	+	-	-	-	-	-	-	1/11
Physical manifestation												
Large ears	+	-	-	-	-	-	+	-	-	-	-	2/11
Prominent ears	-	-	+	-	+	-	+	-	-	-	-	3/11
High arched palate	-	-	-	-	+	-	-	-	-	-	-	1/11
Long face	+	-	+	+	+	-	-	+	-	+	+	7/11
Cardiac murmur	-	-	+	-	-	-	-	-	-	-	-	1/11
Hyperextensible joints	-	-	-	-	-	-	-	-	-	-	-	0/11

+, present; -, absent; na, not available; m, menstruating.

Table 5. Clinical findings in group 5.

Findings	Family 1		Family 2		Family 3				Total
	II3	III4	III5	III6	V21	V25	V29	V39	
Sex	m	f	f	m	f	f	m	f	
Age (years)	54	23	17	15	29	19	9	9	
Long and narrow face	+	-	-	-	-	-	-	-	1/8
Large ears	-	-	-	-	-	-	-	-	0/8
Prominent ears	+	-	-	-	-	-	-	-	1/8
Macro-orchidism	-			-			-		0/3
Learning disabilities	-	-	-	-	-	-	-	+	1/8
Depression	-	+	-	-	-	-	-	-	1/8

+, present; -, absent; f, female; m, male.

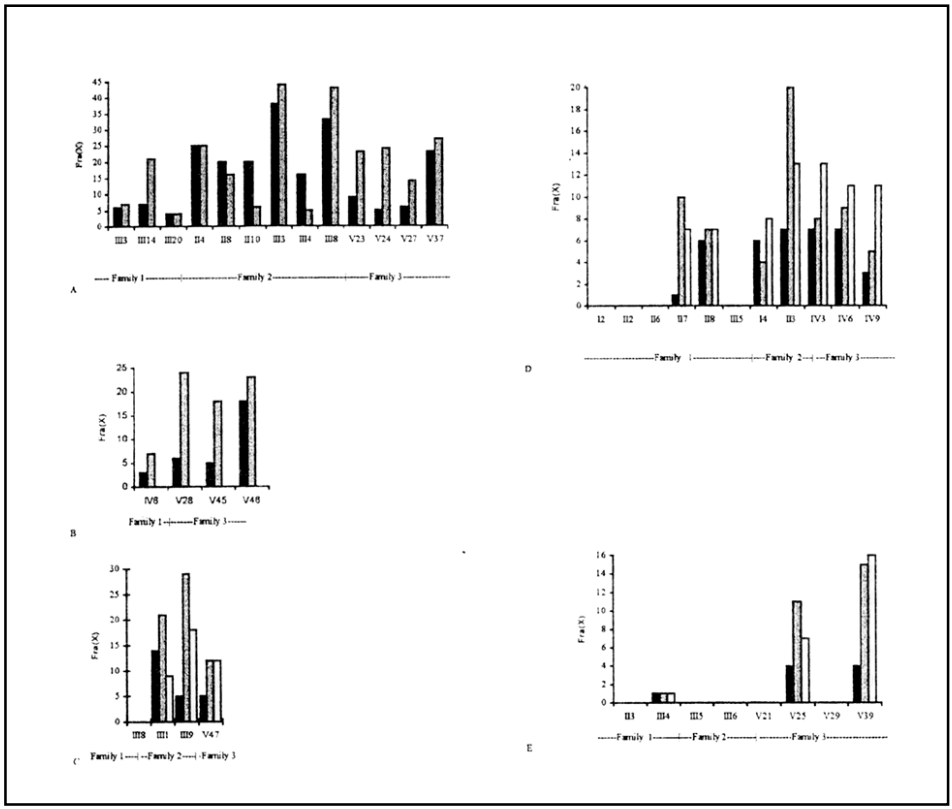

Fig 4. Cytogenetics results. A) Group 1. B) Group 2. C) Group 3. D) Group 4. E) Group 5.
protocol A; protocol B; protocol C.

and one (F2:III9) mild. All the obligate heterozygous had normal intellectual performance, but three (F1:II7; II8 and F3:IV6) had learning disabilities. All the patients in group 5 had normal intellectual performance, except one (F3:V39) which had learning disabilities.

The cytogenetic studies are shown on Figure 4. All the mentally retarded males were fra(X) positive. One mentally retarded female was fra(X) negative. 63% (7/11) obligate heterozygous were fra(X) positive. The four fra(X) negative heterozygous were from family 1. Two individuals from group 5 were fra (X) positive and one had 1% of fragility of the Xq27.3 region. The median expression of fra(X) varied between the families. In family 1 it was 7.5%; in family 2, 24.25%: and in family 3, 26.25%.

A positive correlation was observed between clinical findings and the frequency of chromosome fragile site Xq27.3 (Spearman´s coefficient = 0.3921 p= 0.032). The individual clinical fra(X) characteristics were most frequently observed in males than in females (p=0.0005). The fragile site frequencies were observed most frequently in males (p=0.0016).

DISCUSSION

The three main clinical features associated with the fragile X syndrome are macro-orchidism, large and prominent ears and a long and narrow face. Approximately 80% of affected postpubertal males have one or more of these features[8]. In the group 1 the clinical features seen were similar to

those observed by Nolin et al.[14]. Macro-orchidism was reported in all patients described by Nolin et al[14] and was documented in 81% of our cases. This study found a higher incidence of narrow inner canthal distance (100% *v* 88%). The narrow inner canthal distance associated with normal outer canthal distance is related to the long palpebral fissures observed in anthropometric studies[1,12]. Fifty percent of adult males have long ears and 30% prominent ears[8]. In our study and that of Nolin et al.[14] prominent ears were observed most frequently than large ears. Avoidance of eye contact was observed most frequently in our study (61% *v* 41%) while hyperactivity was less frequent (38% *v* 59%).

The classical clinical features were not found in prepubertal males. The behavioral features are more important diagnostically[8]. In our study, all patients had attention problems and hyperactivity, 50% had hand biting and 50% had hand flapping.

Fifty five percent of mentally retarded females have some clinical manifestation of fragile X syndrome[6]. Cronister et al.[3] studied 26 mentally retarded females and observed long face, hyperextensible metacarpophalangeal joints and flat feet more frequently than other features. Our data, including only four females, show that a long narrow face, highly arched palate and inner canthal distance <3.5 cm where present in 50% of the cases. Historical findings were more important for the diagnosis in our study as in Cronister´s study.

Only 14 % of normal functioning females have some clinical features of fragile X syndrome[6]. Learning disabilities, language problems, emotional problems, chronic affective disorders and schizotypal features are present in more than 40% of the heterozygous[23]. The clinical features most frequently observed in our study were long narrow face (63%), prominent ears (27%) and large ears (18%). Our historical data were not particularly informative, except that 3 in 4 heterozygous had experienced premature menopause. This datum must be an over estimation, as in 2 females the age of menopause was not recorded and we have analyzed only 11 females. Cronister et al.[3] found 13% and Schwartz et al.[17] observed 26% of premature menopause. Learning disabilities and depression were the other historical manifestation recorded.

Overall, the difference between the presence of the fragile X phenotype in mentally retarded and in normal functioning females was statistically significant (p=0.04010). The clinical manifestation were more frequently observed in males than in females (p=0.0005).

Wisniewiski et al.[22] observed 15,1% of mild; 49,15% of moderate; 26,4% of severe and 9,4% of profound mental retardation in males. One female was mild mentally retarded and another had moderate mental retardation among seven females evaluated. In our study 88.2% of males were diagnosed as having severe mental retardation. Twenty three percent of females were ascertained as having mental retardation. This difference can be partially explained because of the psychological tests used to quantify the cognitive function. We used non verbal tests and the fra(X) individuals have been reported as having deficits in non-verbal reasoning, spatial reasoning and short-term memory[4].

In our sample all the mentally retarded males have positive cytogenetic expression of fra(X). The median expression was recorded as lower than the ones reported in the literature (16.9% v 25%)[15]. This can be explained because of the low median observed in family 1, contrasting to the others two families.

This study shows only one mentally retarded female that did not express fra(X). Sixty three percent of the obligate heterozygous expressed fra(X). The 4 individuals that had a negative expression were from family 1. This is the family that had the lowest median value for fra(X) supporting the hypothesis of an intrafamilial lower expression. This could be explained due to a shorter expansion of the triple repeat mutation. Posterior molecular analysis of these families would clear up these observations.

A positive correlation between clinical features and fra(X) expression (Spearman´s coefficient of 0.3921) suggested that these findings are very important for the clinical screening for fragile X syndrome in familial cases of mental retardation or isolated cases[5,11].

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