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TWO SIBLINGS WITH FRAGILE X SYNDROME

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SUMMARY

The fragile X syndrome is the most common specific cause of familial mental retardation. It is associated with fragility at Xq27.3. Because of variations in route of inheritance and clinical features, it can only be diagnosed through genetic studies. However, cytogenetic expression levels of fragility in lymphocytes show variation, and it is not always possible to identify affected males with a lower degree of fragility expression using cytogenetic techniques.

In this study, two siblings with Martin-Bell phenotype are reported. Although they had typical clinical findings and positive family history, fragility at Xq27.3 could not be demonstrated in the first cytogenetic studies. Two years later, in repeated cultures of the siblings, 2%- and 5%-fragility was found. The conflicting results of repeated cultures confirmed that the expression variability of fragility might be the reason for false negative cytogenetic results in affected patients with low fragility ratios.

Key words: *Fra (X)(q27.3), Mental Retardation, Martin Bell Phenotype*

ÖZET

FRAJİL X SENDROMU

Ailesel mental retardasyonu nedenleri arasında ilk sırada yer alan Frajil X sendromu, Xq27.3 bölgesindeki frajilite ile birliktelik gösterir. Kalıtım ve klinik bulgularındaki değişkenlik nedeni ile kesin tanısı ancak genetik çalışma ile koyulabilir. Ancak, sitogenetik ekspresyon düzeyleri de değişkenlik göstermektedir ve düşük ekspresyon düzeyi olan hastalara sitogenetik yöntemlerle her zaman tanı koyulamayabilir.

Bu çalışmada, Martin Bell fenotipi gösteren iki olgu sunulmaktadır. Tipik klinik bulgularına ve pozitif aile öyküsüne rağmen ilk sitogenetik çalışmada Xq27.3'te frajilite gözlenememiş, ancak iki yıl sonra tekrarlanan lenfosit kültürlerinde 2% ve 5% oranlarında frajil X saptanmıştır. Bu olguların tekrarlanan kültürlerindeki çelişkili sonuçlar, düşük frajilite oranlarına sahip hastalarda frajilite değişkenliğinin yanlış negatif sonuçlara neden olabileceğini desteklemektedir.

Anahtar Kelimeler: *Fra (X)(q27.3), Mental Retardasyon, Martin Bell Fenotipi*

The fragile X syndrome is the most common cause of familial mental retardation (1). This X-linked mental retardation is associated most typically with conspicuous facial features of high forehead, prominent lower jaw and large ears. Patients with fragile X syndrome may have mild connective tissue abnormalities that lead to fine skin, hyperextensible metacarpophalangeal joints and mitral valve prolapse. Another characteristic finding is macroorchidism, which becomes prominent after puberty (2). Many patients have behavioural difficulties such as hyperactivity,

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poor eye contact, short attention span, hand flapping and/or biting, cluttered speech and tactile defensiveness (3).

The syndrome is associated with fragility at Xq27.3a, a rare folate-sensitive fragile site (FSFS). Abnormality is demonstrated under specific conditions. FSFSs are induced by thymidylate stress, which may occur as a result of thymidine/folate deficiency or addition of methotrexate into the medium (4). Only a fraction of cells express this fragility, thus necessitating the screening of a large number of cells. The degree of expression in affected males varies from less than 4% to more than 50%. At least two fragile X-positive cells are necessary for diagnosis (5). The number of cultures and cells required for analysis depends on phenotype and family history.

CASE REPORTS

Case 1

A five-year-old boy was admitted to the hospital because of tonic seizures during sleep. Physical examination revealed macrocephaly, with a head circumference of 53 cm (mean value for this age in Turkey: 49.7±1.2 cm) (6), large ears and large testicles (1.5x2 cm.). Distal phalanxes of index and middle fingers were hypoplastic. The patient was hyperactive and uncooperative, giving an impression of mental retardation. He had mild autistic behaviour characterised by difficult eye contact and poor verbal communication.

The patient was the first child of unrelated healthy parents born after an uneventful pregnancy and delivery. The parents reported that his developmental milestones were late: walking without help, speaking, control of urinary and anal sphincters were gained at two years of age.

On laboratory examination, the patient's blood and urine paper chromatography for aminoacid, blood T3, T4, TSH levels, computerized axial tomography of brain and X-rays of hands and feet were found to be normal. EEG showed irregularity on the background activity and bilateral synchronous paroxysmal discharge of 1-2 Hz sharp and slow waves. Neuropsychological examination with Stanford-Binet test showed an IQ of 62.

Ten months later, the parents reported two more seizures. The patient was put on 30 mg/kg/day valproate treatment, and had no seizures for the following two years. However, his parents stopped giving him valproic acid because he refused to take the medicine. Two months later, his seizures recurred, and he began taking medicine again.

During that two-year period, the patient's appearance became more characteristic of fragile X. In addition, he had a brother with a similar appearance. Because of the patient's typical findings and positive family history, he was re-evaluated. On laboratory examination at that time, EEG showed paroxysmal activity, magnetic resonance of the brain revealed hypomyelinization, and Stanford-Binet test showed an IQ of 70.

Case 2

The second child of this family, an 18-month-old boy, came to our attention because of his brother. After an eventful pregnancy, this child was born by Caesarean section, due to delayed effective uterine contractions. Like his older brother, his development was also delayed, with the following milestones: his first word came at 12, he walked with help at 16 months, he was still not able to walk without help at admission.

On physical examination, he was found to have large ears, despite his normal head circumference of 48 cm (N: 47±1.3 cm) (6). He appeared to be hyperactive, and a Denver Developmental Screening test showed his development as abnormal.

On laboratory investigation, blood and urine aminoacid levels were normal. Magnetic resonance imaging (MRI) findings were compatible with hypomyelinization.

Because of his brother's typical findings, the decision was made to analyze the chromosomes of both children for fragile X.

Cytogenetic Analysis and Results

Cytogenetic analyses were done on peripher-

al blood lymphocytes. Whole blood was cultured 72 hours at 37C in 5 ml folate-depressed medium prepared according to the following protocol: 100ml M199 w/o folic acid, 5 ml fetal calf serum, 5 ml phytohemagglutinin, 100 IU/ml penicillin, 100 g/ml streptomycin. 0.04 g/ml colchicine was added at the 71st hour (7). Two hundred giemsa (5%) stained metaphases were analysed for each patient. Slides were decolored after screening, and GTG banding was performed on metaphase chromosomes to define the fragile chromosomes.

Fragility at Xq27.3 was not found at first time. However, when cultures were repeated two years later, 2% and 5% fragility was observed in Case 1 and Case 2, respectively (Fig. 1). The mother's chromosomes were normal in 200 metaphases obtained from peripheral lymphocytes under folate-depressed culture conditions.

DISCUSSION

After the discovery of the association of Martin-Bell phenotype with fragility at Xq27.3 (8), early diagnosis of this most frequent heritable cause of mental retardation has become more important. A better knowledge of the clinical characteristics and variations are necessary to recognize this condition. This may lead to early diagnosis, giving families the advantages of better help, more information and genetic counselling, including prenatal diagnosis.

Fragile X syndrome can only be diagnosed through genetic studies. However, significant variation has been reported for cytogenetic expression of fragility (9, 10, 11, 12, 13). Fragile-site expression is strongly correlated with the repeat number of unstable trinuclotide (CCG) sequences at Xq27.3, which vary in length (14). Variability is a result of amplification, which may be due to unequal cross-over during female meiosis (15, 16). However, recombination can also occur during mitosis of somatic cells (16, 17). Slippage of Okazagi fragments at DNA synthesis may be responsible for the variation of repeat numbers among the different cells of an individual (18). Repeat number variation may cause somatic mosaicism, which may result in heterogenous expression of fragility (18). It has also been noted that sampling from different tissues may be responsible for variations of expression in an individual (19). Steinbach et al reported that expression is generally higher in peripher-

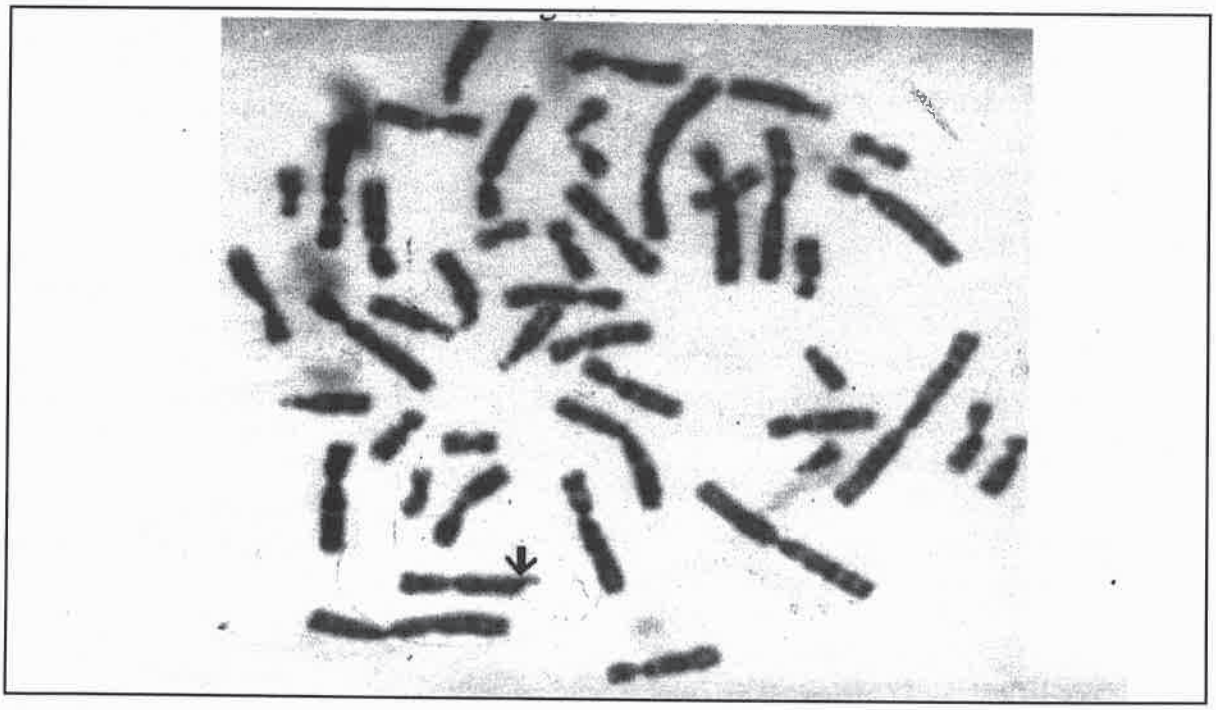

Figure 1: Fragile X chromosome in one metaphase from Case 2.

al blood lymphocytes (20). Nevertheless, fragile X has been found in different cells, indicating that it is not a cell-specific phenomenon (21). Cantu and Jacobs suggested that the fragile site could not be expressed in all cell lineages of an individual. It has also been noticed that the occurrence of fragile X in a cell did not indicate its presence in previous divisions (10). As a matter of fact, a very small difference in expression from time to time in the same individual's lymphocytes has been reported (22). Although Silverman et al (23) reported that expression of fragile X in more than four percent of cells was precisely associated with Martin Bell phenotype, it is not possible to define the lowest expression limit for Martin Bell phenotype. Tommerup et al reported that some affected males with a lower degree of fragility could not be identified using cytogenetic techniques (24).

Our conflicting results from repeated cultures confirmed that the expression variability of fragility might be the reason for false negative cytogenetic results in affected patients with low fragility ratios.

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