

CHROMOSOME STUDIES IN MALES AFFECTED BY DUCHENNE OR BECKER MUSCULAR DYSTROPHY

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ABSTRACT

We studied cytogenetically 48 male patients with Duchenne or Becker muscular dystrophy. All of them showed normal X chromosomes. Fragility of Xp21 was investigated in 1400 G-banded chromosomes of 28 patients and only one break was observed at this band (0.07%). This low frequency of breakage excludes Xp21 as a fragile site in these patients.

INTRODUCTION

Duchenne (DMD) and Becker (BMD) muscular dystrophy are X-linked allelic diseases characterized by progressive degeneration and weakness of skeletal muscles, due to the absence of or defect in the protein dystrophin (Hoffman *et al.*, 1987, 1988). The DMD/BMD locus is mapped at band Xp21. Association of the chromosomal segment Xp21 and Duchenne dystrophy was first established from a study of DMD phenotype females, carriers of X; autosome translocations (Jacobs *et al.*, 1981; Zatz *et al.*, 1981; Verellen-Dumoulin *et al.*, 1984; Boyd *et al.*, 1986). The abnormal phenotype resulted from expression of the DMD gene on the translocated X chromosome, due to preferential inactivation of the structurally normal X. The normal gene at Xp21 could have been altered by the break, giving origin to the abnormal phenotype. In this case, the break would be the mutational event and both males and females with breaks at this site would be affected, resulting in sporadic

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cases of the dystrophy. Alternatively, a defective gene already present at Xp21 could turn this band fragile, favouring breaks and rearrangements. Inherited cases of the disease would then be expected in families of patients with a structurally abnormal X.

Molecular deletions at the DMD/BMD locus have been described in 40 to 70% of affected males (Forrest *et al.*, 1987, 1988; Koenig *et al.*, 1987; Lindlof *et al.*, 1989; Passos-Bueno *et al.*, 1990), and duplications occur at a frequency of about 6% (Hu *et al.*, 1990). These mutations could be associated with a higher frequency of chromosomal breaks at Xp21.

The purpose of this paper was the cytogenetic study of DMD and BMD male patients in order to determine the frequency of breaks and rearrangements at Xp21.

MATERIAL AND METHODS

We studied 48 unrelated male patients with DMD or BMD: 42 DMD, 3 BMD and 3 DMD or BMD. Diagnosis was established based on rate of progression of the disease, physical examination, electromyography, muscle biopsy and serum creatine kinase (CK) and pyruvate kinase (PK) activities. Criteria for differential diagnosis were: age of onset of the signs (3 to 5 years for DMD and 5 to 15 years for BMD), rate of progression (faster in DMD and slower in BMD) and age of inability to walk (before 12 years for DMD and over 16 for BMD).

Heterozygosity risks for the proband's mother were estimated for each case through Bayesian calculation as reported previously (Zatz and Otto, 1980, 1986).

Chromosomes were analyzed in cultured lymphocytes with G-banding in at least three cells from each patient. In 28 patients (23 DMD, 3 BMD and 2 DMD or BMD) chromosomal breaks and gaps at Xp21 were scored for 50 cells from each patient. In 32 patients (29 DMD, 1 BMD and 2 DMD or BMD) only metaphase chromosomes were analyzed, and in 16 (13 DMD, 2 BMD and 1 DMD or BMD) prometaphase chromosomes were also studied.

RESULTS

All 48 patients showed normal X chromosomes. One DMD patient was a carrier of a familial balanced translocation - t(1;9)(p22;q13)mat. The translocation was present in 16 other relatives of the proband. Another DMD patient showed a rare fragile site - fra(16)(q22) - inherited from his mother.

Among the 28 patients who were investigated for the occurrence of breaks at Xp21, among the 1400 cells analyzed, a break at this site was observed in one cell from a DMD patient (0.07%). If we consider only the 23 DMD patients, the break

frequency at Xp21 was 0.09%. The probability that the mother of this patient is a DMD carrier was estimated as 4.4%.

The mother of the patient with a fra(16) showed a break at Xp21 in one of 50 metaphases analyzed (2%). However, the probability of her being a heterozygote was estimated as 1.3%.

DISCUSSION

None of our patients showed cytogenetically detectable deletions at Xp21, in accordance with the data of Spowart *et al.* (1982) who did not detect any abnormality in high-resolution banded chromosomes of nine DMD cases. Previously reported patients, who had such deletions, showed a combination of phenotypes which included muscular dystrophy, chronic granulomatous disease, glycerol kinase deficiency, adrenal hypoplasia, retinitis pigmentosa and ornithine transcarbamylase deficiency (Clarke *et al.*, 1986; Chelly *et al.*, 1988). Our patients did not show clinical signs other than those of the dystrophy, except for mental retardation, which was present in about 50% of the cases. Therefore, cytogenetically detectable deletions are restricted to those cases in which the dystrophy is associated with other clinical signs.

The extremely low frequency of breaks at Xp21 that we observed indicates that this band is not a fragile site in the affected individuals and that the DMD/BMD gene does not cause chromosome breaks.

The balanced translocation t(1;9) present in a DMD affected boy was casually associated with the dystrophy and was segregating in the family for at least four generations. The fragile 16 also was not related to the DMD phenotype.

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RESUMO

Estudamos citogeneticamente 48 pacientes de sexo masculino, casos isolados de distrofia muscular de Duchenne ou Becker. Todos apresentaram cromossomos X normais. Fragilidade da banda Xp21 foi investigada em 28 pacientes e somente uma célula apresentou quebra cromossômica nessa região.

no total de 1400 células analisadas (0,07%). A baixa frequência de quebra exclui Xp21 como um sítio frágil nestes pacientes.

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