PERGAMON

Neuromuscular Disorders 15 (2005) 471–475

www.elsevier.com/locate/nmd

Recurrent somatic mosaicism for D4Z4 contractions in a family with facioscapulohumeral muscular dystrophy

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Received 7 December 2004; received in revised form 10 March 2005; accepted 23 March 2005

Abstract

Autosomal dominant facioscapulohumeral muscular dystrophy (FSHD) is caused by contraction of the D4Z4 repeat on 4q35. We describe a FSHD family of unusual genetic complexity presenting with two independent mitotic contractions of D4Z4 in two successive generations. In addition, a non-pathogenic FSHD-sized allele of approximately the same size is interfering with the DNA diagnosis in this family. Interestingly, this allele is not recognized by the probes 4qA and 4qB representing two distal variants of 4qter, suggesting the presence of yet another, infrequent variant of 4qter.

Keywords: FSHD; Facioscapulohumeral muscular dystrophy; D4Z4; Mosaicism; Non-penetrance

1. Introduction

Autosomal dominant facioscapulohumeral muscular dystrophy (FSHD; MIM 158900) is the third most common inherited muscular disorder with an incidence of 1:20,000. FSHD is foremost characterized by progressive weakness and atrophy of facial, shoulder girdle, upper arm, foot extensor and pelvic girdle muscles. Also, non-muscular features can be part of the disease as >50% of patients suffer from subclinical high-tone hearing loss and with an almost equal frequency retinovasculopathy is observed in patients with FSHD [14].

FSHD is caused by contraction of the polymorphic D4Z4 repeat in the subtelomere of chromosome 4q (4qter). While in the control population, D4Z4 alleles of 11–100 units (residing on *Eco*RI fragments of 41–350 kb) can be encountered, most FSHD patients carry one array of 1–10 units (*Eco*RI fragments of 10–38 kb) [18,21].

In the subtelomere of the long arm of chromosome 10q (10qter) an almost identical and equally polymorphic repeat array resides [1,3]. In contrast to chromosome 4, repeats on this chromosome may vary between 1 and 100 units without pathological consequences. The chromosomal origin of individual repeat units can be identified by the use of two restriction enzymes: *Bln*I is recognizing a single restriction site within the chromosome 10-derived unit but not the chromosome four-derived unit while the opposite holds true for *Xap*I [9].

Distal to D4Z4, two alleles of the 4q subtelomere have been identified, designated 4qA and 4qB, that are almost equally common in the Caucasian population [19]. Analysis of 4qA and 4qB in the Dutch FSHD population showed that contraction of D4Z4 is not sufficient to cause disease as FSHD is invariably associated with D4Z4 contractions with the 4qA variant of the chromosome 4q subtelomere [6]. Indeed, we recently could confirm the presence of FSHD-sized D4Z4 alleles residing on chromosome 4qB in healthy

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doi:10.1016/j.nmd.2005.03.005

individuals providing strong evidence that these alleles do not cause FSHD or have a strongly reduced penetrance [8].

Mitotic contractions of D4Z4 leading to somatic mosaicism for the disease allele are observed in almost 50% of de novo FSHD families. Depending on gender, residual repeat size and proportion of cells carrying the disease allele, mosaic individuals are affected or asymptomatic carriers [17]. A predominance of asymptomatic female carriers of a mitotic rearrangement has been reported repeatedly [5,17,25]. Detailed analysis of somatic mosaicism in a panel of 11 mosaic FSHD families showed that these mitotic rearrangements likely occur during early zygotic cell division by a gene conversion mechanism between sister chromatids [7].

We describe here a FSHD family of unusual complexity for geneticists and counselors as it combines recurrent somatic mosaicism for D4Z4 contractions in two successive generations, a low-penetrant FSHD allele and an interfering non-pathological FSHD-sized allele.

2. Materials and methods

2.1. Patient

The proband (II-1) is a 27-year-old man. His first complaints were at the age of 14, when he mentioned difficulties in lifting his right arm above shoulder level. Pictures at earlier age showed that already at age 12 his facial muscles were affected. On physical examination he had striking asymmetry in strength and tropism in the upper part of his body, including abdominal muscles. Right arm was severely affected (Medical Research Council (MRC) grades 2–4) except for most distal muscles (MRC grades 4–5$^-$). For the left arm most grades were close to normal (MRC grades 4–5) except for muscles involved in external rotation (MRC grade 3$^+$) and extension in shoulder joint (MRC grade 3$^-$). Scapular fixators were severely affected bilaterally with scapula presented in trapezius muscle at right. In lower limbs calf muscles (predominantly m. tibialis anterior: at left grade 3, at right grade 3$^+$) and thigh muscles (predominantly m. quadriceps femoris: at left 3$^-$, at right 3$^+$) and adductors in hip joint were affected almost symmetrically. CT scan of the shoulder girdle showed reduced absorption of X-ray beam with corresponding muscle atrophy, most severely presented for right m. deltoideus, m. pectoralis minor and m. triceps brachii. Semiquantitative evaluation correlated with MRC grades. No muscle biopsy of the patient was available.

Both parents (I-1 and I-2) and the proband's sister (II-2) had neither symptoms nor signs on physical examination. EMG of the proband showed myopathic changes and CK levels were slightly increased. Father's EMG showed only suspective data for muscle involvement and normal CK levels. Mother's and daughter's EMG were normal.

2.2. DNA isolation and analysis

High molecular weight DNA was isolated from peripheral blood lymphocytes (PBL) via a standard phenol extraction method. For pulsed-field gel electrophoresis (PFGE), 5 μg genomic PBL-DNA embedded in agarose plugs was used. For D4Z4 repeat sizing, DNA was digested with *Eco*RI, *Eco*RI/*Bln*I or *Xap*I, separated by linear gel electrophoresis (LGE) or PFGE (for PFGE genomic DNA was digested with *Eco*RI/*Hin*dIII instead of *Eco*RI), blotted and hybridized with probes p13E-11 and 9B6A as described [11]. For determining the allelic variant of 4qter with probes 4qA and 4qB, *Hin*dIII digested DNA was used as described [6]. After exposure to phosphorimager screens, hybridization data were analyzed using the Image Quant software (Molecular Dynamics).

3. Results

D4Z4 analysis by PFGE and hybridization with probe p13E-11 showed three four-type repeats in the proband (II-1) based on their resistance to *Bln*I and sensitivity for *Xap*I. Two of them, 18 and 96 kb in size, showed reduced signal intensity indicative for a mitotic contraction of the 96 kb maternally derived array to 18 kb. Quantification of the signal intensity revealed that 60% of the patient's cells had the contracted allele while the remainder carried the unchanged maternal allele. Apart from the 18 kb repeat, a second FSHD-sized *Bln*I-resistant allele of 32 kb was identified as well as two chromosome 10-derived alleles of 120 and 48 kb based on the *Bln*I or *Xap*I sensitivity (Fig. 1a).

Surprisingly, this FSHD-sized repeat of 32 kb was also present in his asymptomatic sister (II-2) and was inherited from the father (I-1) who presented with a mosaic genotype for this allele. In more detail, the father carried chromosome 10-derived repeats of 200 and 48 kb, respectively, and four-derived repeats of 170, 110 and 32 kb, the latter two representing two mosaic alleles based on their reduced signal intensity. Quantitation of allele intensities in the father showed that the 32 kb allele was present in 80% of the cells while the remainder carried the 110 kb allele from which the 32 kb allele originated. In the daughter, chromosome 10-derived repeats were identified at 200 and 48 kb as well as a second FSHD-sized chromosome four-derived allele of approximately 32 kb based on the doubled signal intensity.

Indeed, in the mother (I-2) also a FSHD-sized *Bln*I-resistant allele of approximately 32 kb was visualized. It was inherited by the daughter (II-2) who therefore also presented, like her brother (II-1) with two, almost equally sized FSHD alleles (Fig. 1a). In addition, the mother carried a chromosome four-derived allele of 96 kb as well as chromosome 10-derived alleles of 120 and 48 kb.