Facioscapulohumeral (FSHD1) and other forms of muscular dystrophy in the same family: is there more in muscular dystrophy than meets the eye?


Abstract

We report on two unrelated Brazilian families with members affected by two different forms of muscular dystrophy. In the first one, the 35-year-old male proband has limb-girdle muscular dystrophy with proximal weakness, elevated creatine kinase and a myopathic muscle biopsy. All the proteins known to be associated with limb-girdle muscular dystrophy were normal. Two of his sisters also complained of muscle weakness. The oldest sister showed clinical signs consistent with facioscapulohumeral muscular dystrophy, confirmed through molecular analysis. She presented a 30 kb EcoRI/BlnI fragment which was found in another six relatives, but surprisingly not in the affected proband or the other sister. In the second family, a 57-year-old male with a typical facioscapulohumeral muscular dystrophy phenotype has a 17 kb EcoRI/BlnI fragment, which was also present in other affected relatives. However in a 14-year-old severely affected male cousin, confined to a wheelchair since age 12, but without facial weakness, the small fragment was absent. These families illustrate the importance of testing all affected individuals in a family.

Keywords: Facioscapulohumeral; Muscular dystrophy; Brazilian families

1. Introduction

Facioscapulohumeral muscular dystrophy (FSHD1) is an autosomal dominant muscle disorder, mapped to 4q35 [1]. In most patients, probe p13E-11 (D4F104S1) detects a polymorphic EcoRI fragment smaller than 35 kb, which has 35–300 kb in normal individuals, and consists of multiple copies of a tandemly repeated 3.3 kb KpnI unit. It has been suggested that deletions of integral number of these units might affect nearby genes by altering the chromosomal structure, inducing position effect variegation [2]. Since the subtelomeric region of chromosome 10 (10q26) is highly homologous to 4q35, molecular diagnosis is confirmed through the use of the restriction enzyme BlnI, which cleaves only the 10q26 units into small, non-detectable fragments [3]. Clinically, FSHD1 is characterized by progressive weakness of facial, shoulder girdle and upper arm musculature, with occasional lower limb involvement and a remarkable inter and intrafamilial variable expression ranging from asymptomatic carriers to severely affected children.

Limb-girdle muscular dystrophy (LGMD) is also a clinically and genetically heterogeneous disorder, characterized by proximal weakness, ranging from severe childhood to milder adult forms. Fifteen genes, six autosomal dominant (AD), LGMD1A (5q22), LGMD1B (1q11), LGMD1C (3p25), LGMD1D (6q23), LGMD1E (7q) and LGMD1F (5q31) and 9 autosomal recessive (AR), LGMD2A (15q15), LGMD2B (2p13), LGMD2C (13q12), LGMD2D (17q12), LGMD2E (4q12), LGMD2F (5q33), LGMD2G (17q11), LGMD2H (9q31) and LGMD2I (19q13) responsible for LGMD have already been mapped and/or identified [4,5]. Linkage analysis in Brazilian affected families has shown that there is still further genetic heterogeneity for both AD as well as AR forms [6].

2. Case reports

2.1. Family 1

The proband (III-12), a 35-year-old Brazilian male was referred to our Center in 1997, with a clinical course suggestive of LGMD. He had progressive weakness of the upper and lower limbs with onset in childhood, diminished
reflexes but no facial involvement. He could walk on his toes and heels but had some difficulty in climbing stairs. His family (Fig. 1) comprises seven sisters and five brothers, and his healthy parents (mother aged 69 and father aged 72), are non-consanguineous. His electromyography was myopathic. Laboratory studies following informed consent, showed a serum creatine-kinase (CK) activity elevated 16-fold above normal. DNA analysis performed before the muscle biopsy excluded mutations in the caveolin gene. Muscle biopsy showed minimal histological changes, with conserved myofibrillar network, discrete connective tissue infiltration and 90% type II fiber predominance. Muscle protein immunohistochemical and western blot analysis revealed normal pattern for the following proteins: dystrophin, the four sarcoglycans, calpain, dysferlin and telethonin.

Based on these results he was diagnosed as having probably a still unclassified form of LGMD.

Six of his sisters (aged 51, 49, 45, 41, 38 and 36, respectively) and two brothers (aged 50 and 46, respectively) were personally examined in our center. They were all asymptomatic with exception of two females. Individual III-10, aged 38, complained of muscular weakness, had calf hypertrophy, and a serum CK 5-fold above normal. She refused further investigations. Individual III-1, aged 51, although referred to us as having probable LGMD, showed a typical mild FSHD phenotype. She had scoliosis since age 13, difficulty in lifting her arms above the horizontal line and in climbing stairs, winged asymmetrical scapulae, mild facial, upper and lower limb muscle weakness and absent lower limbs reflexes. Her serum CK was increased 4-fold.

Based on her phenotype, she was tested for the FSHD1 gene. DNA was extracted from whole blood, digested with EcoRI and EcoRI/BlnI and hybridized with probe p13E-11, as previously reported [7]. A 4q fragment of 30 kb was detected, strongly supporting the diagnosis of FSHD1. The diagnosis was put beyond doubt by further analysis using pulse field gel electrophoresis (PFGE) which, through identifying four EcoRI fragments and finding two Bln-resistant fragments (one of them of 30 kb), establishes the 30 kb fragment to be on chromosome 4.

We then tested the other relatives. The small FSHD1 fragment was present in III-5 and III-11, both asymptomatic, but surprisingly not in the 35-year-old proband (III-12). The FSHD1 fragment was also found in the father II-1, aged 72 and in IV-1, aged 22 (not personally examined by us but both said to be asymptomatic) as well as in individuals IV-13 and IV-14 (aged 19 and 16), who had a very mild facial weakness.
2.2. Family 2

In this family (Fig. 2), five affected individuals were personally examined by us. They all had a typical mild FSHD1 phenotype with facial and upper limb weakness. The only exception was individual IV-17, a 14-year old severely affected boy who had no facial weakness, and was confined to a wheelchair since age 12. His CK was increased 4.5 fold and his clinical course resembled Duchenne dystrophy, with proximal weakness. However, no deletion was found in the dystrophin gene.

After informed consent, 12 individuals were tested for the FSHD1 gene. All clinically affected patients had an EcoRI/BlnI fragment of 17 kb, except individual IV-17. Unfortunately individual IV-17, refused to have a muscle biopsy for dystrophin or other muscle protein analysis. Further analysis of III-3 by PFGE, as in family 1, confirmed the chromosome 4 origin of the (17 kb) EcoRI/BlnI fragment.

3. Discussion

The present families illustrate complicated situations that may occur in the diagnosis and genetic counseling of neuromuscular disorders.

In family 1, individual III-1 although mildly affected, has a clinical course characteristic of FSHD1. If she would be the proband, the diagnosis of FSHD1 would be established and her brother III-12 would be probably classified as having the same disorder. Although he has no facial weakness, FSHD1 4q35 has been reported recently in patients, mostly males, presenting with facial-sparing scapular myopathy [8]. The finding of seven normal LGMD associated proteins in III-12 makes it unlikely that he has one of the known forms of AR-LGMD and therefore his diagnosis remains open.

In family 2, individual IV-17, although more severely affected, was considered to also have FSHD, with atypical phenotype, and therefore his mother an asymptomatic FSHD1 carrier. Although there were two asymptomatic individuals (II-3 and II-5) in this family, we could not detect any FSHD1 fragment among the seven clinically normal individuals who analyzed molecularly.

On the other hand, in family 1, the FSHD1 fragment was found in four asymptomatic members, aged over 20 (respectively 72, 45, 36 and 22). A correlation between the size of
the FSHD1 fragment and the severity of the phenotype as well as age at onset has been suggested [9–11]. However, small fragments (ranging from 17 to 30 kb) have been observed by us in several asymptomatic carriers (unpublished observations), which makes it very difficult to provide a prognosis based on the size of the EcoRI fragment.

Clinical anticipation in FSHD1 has been first suggested by us [12] and supported by others [10,11]. In family 1, it is not possible to verify if a ‘de novo’ mutation occurred in individual II-1 or if the FSHD1 fragment had been inherited. However, it shows that asymptomatic carriers may occur in at least two generations (individual II-1 and his daughters III-5 and III-11).

The molecular mechanism underlying FSHD1 is still unknown. It has been suggested that deletions of the 3.3 kb KpnI repeats can alter the structure of the heterochromatin, due to its proximity to the 4q telomere, and therefore activate or inactivate nearby genes, inducing position effect variegation [2]. The existence of asymptomatic carriers, more frequently among females than males [13] is intriguing. It has recently been suggested that retrotransposons could be mediators of phenotype variation in mammals [14]. According to the authors, the nature of retrotransposons activity and the very large number of genes that can be affected may produce phenotypic variation even between genetically identical individuals.

From our clinic population of around 70 families with FSHD, there are about 350 known relatives of index cases. If we consider the prevalence of hereditary neuromuscular disorders in the population to be very approximately 1/1000, the finding of two families with an additional neuromuscular disorder would be about three times higher than expected.

Therefore, although the presence of different neuromuscular disorders in the same genealogy could be only a coincidence, it is tempting to speculate that some epigenetic mechanism might turn individuals more prone to pathological mutations, as long as non-paternity is excluded. This mechanism might provide an explanation for the two families here reported or the association of Charcot-Marie-Tooth (CMT1A) neuropathy and FSHD1 already reported in three families [15]. It is noteworthy that a ‘mariner’ transposon-like element located near a recombination hotspot responsible for inherited peripheral neuropathies has been reported [16]. Understanding such mechanisms as well as why some individuals are protected from the clinical manifestation of pathological mutations will be of utmost importance for future therapeutic approaches.

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