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Case report

A patient with limb girdle muscular dystrophy carries a TRIM32 deletion, detected by a novel CGH array, in compound heterozygosis with a nonsense mutation

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Abstract

Limb girdle muscular dystrophy 2H is a rare autosomal recessive muscular dystrophy, clinically highly variable, caused by mutations in the TRIM32 gene. Here we describe a 35-years-old who experienced progressive muscle weakness. The muscle biopsy revealed an unspecific pattern of atrophic and hypertrophic fibers; the immunohistochemistry for several proteins was normal. Comparative genomic hybridization (CGH) analysis showed a heterozygous deletion of the entire TRIM32 gene. On the other allele we identified the R316X nonsense mutation. The genetic diagnosis of LGMD2H in this case was reached by using a novel high throughput diagnostic tool.

Keywords: Limb girdle muscular dystrophy; Array CGH; TRIM32

1. Introduction

Limb girdle muscular dystrophies (LGMD) are a group of diseases in which the molecular characterization is challenging due to an high genetic heterogeneity but similar clinical manifestations. Limb girdle muscular dystrophy 2H (LGMD2H, MIM 254110) is due to mutations in the TRIM32 gene and is inherited with an autosomal recessive pattern. Clinically it is described a wide variability without a specific hallmark of the disease; onset is usually within the 2nd or 3rd decade of life and the progression is slow; most patients remain ambulatory into the 6th decade of life [1].

The first mutation in the TRIM32 gene (D487N) was identified in a genetically isolated population, the Hutterite [2,3], and was associated to a slowly progressive proximal muscular weakness and wasting. The same mutation has subsequently been shown to be responsible also for a different clinical phenotype, the sarcotubular myopathy syndrome (SMT) [4]. Recent studies [5–7] have identified other mutations in the TRIM32 gene in non-Hutterite patients leading to a muscular phenotype. The variations described are small mutations (missense and frameshift) and are all located in the C-terminal domain of the protein precisely in the NHL domain (named after NCL-1, HT2A and LIN-41 similarity) known to be involved in protein–protein interactions.

Borg et al. [7] reported a compound heterozygosis for a TRIM32 30 kb intragenic deletion and a frameshift mutation in a Swedish family with a complex phenotype of LGMD2H and SMT. The majority of the mutations present in literature and databases (www.lovd.nl/TRIM32) are small mutations reported in homozygosis in

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the patients but the segregation in the family is not always studied; therefore some cases considered as homozygous could instead hide the presence of gross rearrangement on the other allele.

Among the new high throughput molecular diagnostics tools, CGH analysis is focused into the identification of Copy Number Variations (CNVs) the prevalence of which in neuromuscular disorders (NMDs) may be underestimated because they are missed by standard genomic DNA analyses (as PCR). We used a CGH approach, focused on a group of genes known to cause NMDs, to study a sporadic Italian patient with LGMD phenotype; here we describe the clinical, histopathological, muscle magnetic resonance (MRI) finding and the results of the molecular analysis in the patient.

2. Case report

A 35 years old women came to our attention because of muscle weakness and muscle pain mostly in the lower limbs. Family history was negative for neuromuscular disorders and the two older brothers and both the parents were healthy; there was no consanguinity in the family. She achieved normal motor milestones but she referred not to be able to run as fast as the other kids at school. She did not complain of any disability until the age of 25 when she experienced a progressive muscle weakness and difficulty into climbing stairs; nowadays she refers pain and stiffness of the legs soon after a walk.

The CK was slightly raised (2× normal) and the electromyography (EMG) of both biceps brachialis and tibialis muscles showed a myopathic pattern with decrease in duration of the action potential. On physical examination she has marked hypotrophy and weakness of the pelvic girdle muscles in particular of the glutei; there was no evidence of calf hypertrophy. Cardiac examination (including ECG) was normal.

The muscle biopsy (vastus lateralis) revealed an aspecific pattern of atrophic and hypertrophic fibers and there were no signs of sarcotubular aggregates; the immunohistochemistry for dystrophin, sarcoglycans, dysferlin and caveolin was normal. The muscle RMN (Fig. 1) showed morphological changes in several muscles in particular is evident a fatty replacement of the gluteus and atrophy in the rectus femoris, in the three vasti muscles and in the long biceps femoris; the sartorius, gracilis and the adductor longus are spared.

We performed a CGH analysis by using a customized array, the NMD Chip array, developed as part of the NMD Chip EU project. Briefly, RefSeqGene, position, size and exon number were retrieved for the 50 genes involved in progressive NMD, congenital myopathies and congenital muscular dystrophies, from the Gene Table 2009 [8] (Table 1); probe design was manufactured by Roche–Nimblegen in a 12-plex arrays (135,000 probes/sub-array).

The TRIM32 gene (Hg18 Ch9:118.489.202-118.503.404) is covered by 462 probes and the result of the CGH in our patient is consistent with an heterozygous deletion (score −0.5); the deletion was confirmed also by real time PCR. In the NMD Chip array the genomic regions flanking the TRIM32 gene are not covered by probes, therefore is not possible to define precisely the break points of the deletion.

We screened by PCR and sequencing the other TRIM32 allele and we identified a nonsense c.1837 C > T (R613X) mutation located in the C terminal NHL domain, known to be an hot spot; the mutation has not been reported to date and is expected to result in a truncated not functional protein (Fig. 2).

3. Discussion

Molecular diagnosis of monogenic diseases with high genetic heterogeneity is a challenging task and LGMD represent such a case because clinical presentation infrequently suggests a specific protein defect; also laboratory analysis often produce unspecific results and multiplex Western blot analysis, an useful initial approach, not always is able to identify the primarily affected protein.

To date the molecular genetic diagnosis of LGMD is based on a gene by gene screening through standard PCR techniques and sequencing, which might be time consuming and expensive depending of course on the number of exons represented in the genes.

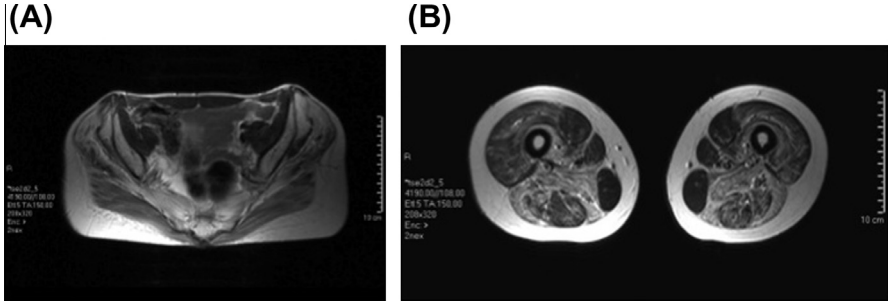

Fig. 1. Muscle RMN imaging of pelvis (A) and thighs (B) showing fatty replacement of the gluteus and atrophy in the rectus femoris, in the three vasti muscles and in the long biceps femoris; the sartorius, gracilis and the adductor longus are spared.