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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.

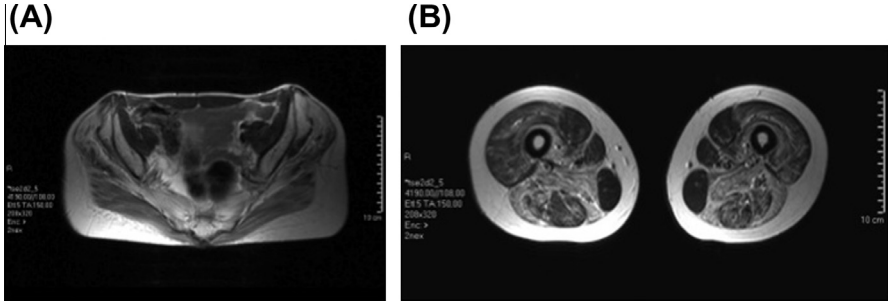

Fig. 1. Muscle RMN imaging of pelvis (A) and thighs (B) showing fatty replacement of the gluteus and atrophy in the rectus femoris, in the three vasti muscles and in the long biceps femoris; the sartorius, gracilis and the adductor longus are spared.

In addition Sanger sequencing does not pick up large rearrangements, as deletions or duplications which remain undetected.

CGH analysis is able to unravel rearrangements due to copy number variations mutations (CNVs) representing about 5–6% of genetic mutations causing inherited diseases [9].

We approached the molecular analysis in our patient by using a customized CGH array focused on 50 genes known to cause neuromuscular disorders. The identification of the rearrangement of the TRIM32 gene addressed the following analysis by PCR and Sanger sequencing of the gene and permitted to reach a molecular diagnosis.

The TRIM32 gene is composed of two exons but the entire open reading frame is contained in the second exon encoding for a protein of 653 amino acids; the protein sequence is made of several conserved domains in particular the six NHL repeats are believed to be

Table 1
List of the 50 genes included in the CGH array.

Gene symbol	Chr	5′ end	3′ end	Nb exons	Size	Spacing	Nb of probes
DMD	X (−)	33267847	31049257	86	2218590	185	12017
EMD	X (+)	153260781	153262951	6	2170	15	141
LMNA	1 (+)	154350885	154376721	12	25836	62	420
SYNE1	6 (−)	153000427	152484064	149	516363	92	5594
SYNE2	14 (+)	63389236	63762997	116	373761	92	4049
MYOT	5 (+)	137231273	137251473	10	20200	62	328
CAV3	3 (+)	8750296	8763753	2	13457	46	292
CAPN3	15 (+)	40427393	40491863	30	64470	62	1048
DYSF	2 (+)	71534068	71767430	58	233362	92	2528
SGCG	13 (+)	22652891	22797880	8	144989	92	1571
SGCA	17 (+)	45598190	45608997	10	10807	31	351
SGCB	4 (−)	52599403	52581680	6	17723	46	384
SGCD	5 (+)	155686145	156127379	9	441234	92	4780
TCAP	17 (+)	35074925	35076388	2	1463	15	95
TRIM32	9 (+)	118489202	118503404	2	14202	31	462
FKRP	19 (+)	51940943	51953682	4	12739	31	414
TTN	2 (−)	179380595	179098785	316	281810	62	4579
POMT1	9 (+)	133367910	133389014	20	21104	31	686
FCMD - FKTN	9 (+)	107360032	107443566	11	83534	31	2715
POMT2	14 (−)	76857170	76810952	21	46218	31	1502
DES	2 (+)	219991343	219999705	9	8362	15	544
CRYAB	11 (−)	111287683	111284560	3	3123	15	203
FLNC	7 (+)	128257719	128286564	48	28845	31	937
ZASP	10 (+)	88418301	88485804	16	67503	62	1097
BAG3	10 (+)	121400872	121427319	4	26447	31	860
VCP	9 (−)	35062739	35046065	17	16674	15	1084
FHL1	X (+)	135056527	135121176	8	64649	62	1051
COL6A1	21 (+)	46225091	46249491	35	24400	31	793
COL6A2	21 (+)	46342361	46377191	29	34830	46	755
DOK7	4 (+)	3434831	3466007	7	31176	62	507
LAMA2	6 (+)	129245879	129879503	65	633624	92	6864
LARGE	22 (−)	32646510	31998963	16	647547	92	7015
POMGNT1	1 (−)	46458564	46426840	23	31724	15	2062
SEPN1	1 (+)	25999154	26017400	13	18246	31	593
COL6A3	2 (−)	237987659	237897301	44	90358	62	1468
ITGA7	12 (−)	54387994	54364521	25	23473	31	763
TNXB	6 (−)	32185129	32116911	44	68218	31	2217
ITGA9	3 (+)	37468817	37836285	28	367468	92	3981
TPM3	1 (−)	152431333	152400814	13	30519	31	992
NEB	2 (−)	152299335	152049999	150	249336	31	8103
ACTA1	1 (−)	227636566	227633516	7	3050	15	198
TPM2	9 (−)	35680153	35671990	11	8163	15	531
TNNT1	19 (−)	60352486	60335909	14	16577	31	539
CFL2	14 (−)	34252796	34249243	5	3553	15	231
MTM1	X (+)	149487605	149592374	15	104769	31	3405
DNM2	19 (+)	10689655	10803679	22	114024	31	3706
BIN1	2 (−)	127581434	127521978	20	59456	31	1932
RYR1	19 (+)	43616080	43770144	106	154064	31	5007
MYH7	14 (−)	22974810	22951687	40	23123	31	751
TMEM16E	11 (+)	22171298	22261489	23	90191	62	1455
Total				1738	7587524		103598

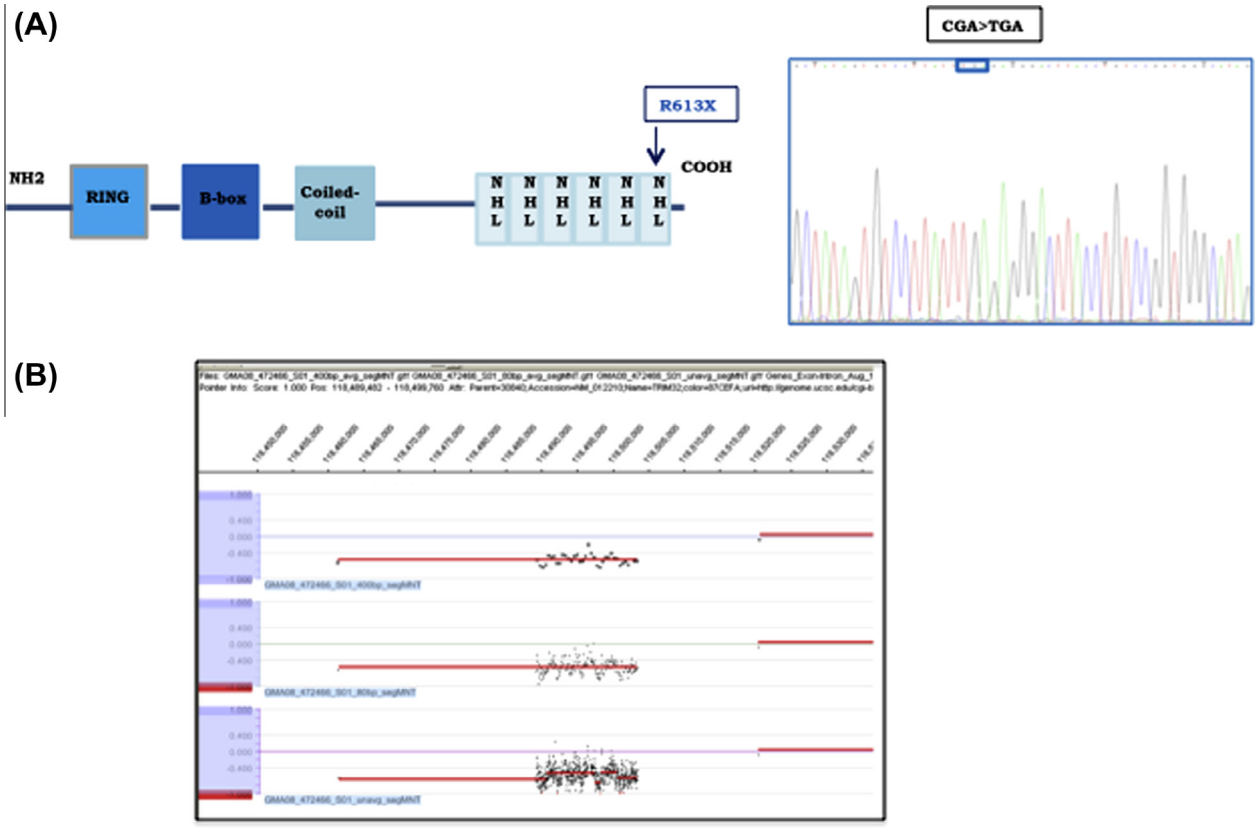

Fig. 2. (A) Schematic representation of TRIM32 domain structure and localization of the nonsense mutation identified in our patient (B) Array CGH analysis results: deletion of the entire TRIM32 gene (different averaging windows, from the top: 10×, 2×, 1×). The score detected (−0.5) is consistent with heterozygosity for the deletion.

responsible for homodimerization of the protein, fact which is necessary for the ubiquitin ligase activity. In fact, the TRIM32 protein is believed to act as an E3 ubiquitin ligase through its N-terminal ring for proteosome degradation of proteins and to activate miRNAs through the C-terminal NHL repeat [10].

Although TRIM32 appears to be expressed ubiquitously, it is still not clear why certain mutations of TRIM32 would result in a phenotype relatively confined to skeletal muscle; only one mutation (P130S), located in the B-box domain of the protein, has been associated to a non-muscular phenotype, the Bardet Biedel syndrome type 11 (#MIM 209900)[11].

Interestingly, Saccone et al. [5] reported that mutations associated with muscular phenotypes impair the self-dimerization of TRIM32; moreover they found that the effect of the homozygous mutations was comparable to the deletion of the entire CC and NHL protein domains. To date there is only one case in literature [7] of two brothers with compound heterozygous deletion and point mutation in TRIM32 gene; the phenotype in these patients is of an early-onset STM associated to LGMD2H.

The point mutation (Cys521Valfs*13) described by Borg et al. [7] is expected to result in a truncated protein lacking three out of six NHL domains, instead they report that the truncated protein is not detectable in patients muscle.

They hypothesize that the effect of the deletion associated to the frameshift mutation is a disruption of self-dimerization and de-stabilization of the truncated protein; the loss of E3 ubiquitin ligase activity located in the NHL domain could account for the activation of other proteins degradation pathways and for the SMT aspect at the biopsy. In our patient the nonsense mutation is expected to result into the loss of the last NHL repeat only and considering that the TRIM32 coding sequence is encoded by a single exon is unlikely that this mutation lead to nonsense mediated decay [12].

In the patient we describe the clinical phenotype and muscle biopsy had no hallmark distinctive for a specific form of LGMD therefore the molecular analysis would have been a multistep process of screening of several genes.

We adopted a novel high throughput tool, a customized CGH array for neuromuscular genes (created by the NMD Chip consortium), which lead us to the identification of a gross rearrangement involving the whole TRIM32 gene; this, together with a nonsense mutation on the other allele identified with PCR and direct sequencing, turned out to be the molecular defect in the patient.

The precise percentage of CNVs in neuromuscular genes is unknown and only recently the use of CGH array has increased the number of data regarding deleterious copy number imbalances in these group of diseases [13].

Having the exact genetic diagnosis is today considered mandatory for being enrolled in clinical trials, as well as for genetic counselling, risk figures and reproductive choices. In conclusion, the use of new high throughput approach in the diagnostic routine of neuromuscular disorders will improve and speed up the diagnosis of rare diseases as we have proved for this rare case of LGMD2.

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