

Extending the clinical and mutational spectrum of *TRIM32*-related myopathies in a non-Hutterite population

INTRODUCTION

TRIM32-related myopathies represent a phenotypic spectrum of a rare autosomal recessive muscle disorder. The disease is described as a mild and progressive myopathy without characteristic clinical features. Originally classified as limb-girdle muscular dystrophy (LGMD) 2H (OMIM #254110), the disorder was first

identified in the Hutterite population and the homozygous *TRIM32* founder mutation, p.Asp487Asn, was identified as the cause of this disease.¹ Only seven patients with definite non-Hutterite *TRIM32*-related myopathy have been reported in the literature. Apart from two missense mutations residing in the NHL repeats of *TRIM32*, only deletions, frameshift and nonsense mutations have been reported.² Having applied next generation sequencing technologies to over 1000 patients with suspected genetic muscle disorders, we present nine patients with *TRIM32*-related myopathies and three patients with a homozygous *TRIM32* variant of unknown significance (VUS).

SUBJECTS AND METHODS

DNA samples from 1000 patients with unexplained limb-girdle muscle weakness and/or elevated serum creatine kinase (CK) levels were gathered through the MYO-SEQ project. Samples were processed and whole exome sequencing (WES) performed as described previously.³ Additional patients with *TRIM32*-related myopathy were diagnosed by the Northern Molecular Genetics Service (NMGS) diagnostic laboratory through panel sequencing of 32 LGMD genes. Variants were classified according to ACMG guidelines.⁴ MRI imaging was performed for eight patients on a 1.5T MRI platform. Muscle biopsies for all patients were analysed following standard histological techniques.

RESULTS

Genetic findings

Of the 1000 MYO-SEQ patients, we identified 36 with rare coding variants in *TRIM32* (minor allele frequency <1%; numbered 1–36 in online supplementary table 1). Twenty-six patients had single heterozygous variants; these were discarded from our analysis as the variants were unlikely to be pathogenic in this autosomal recessive disease. Two further patients were excluded from our analysis: patient 18 was heterozygous for a pathogenic *DES* mutation and patient 10 was homozygous for a pathogenic *CAPN3* mutation. This resulted in seven patients with suspected pathogenic *TRIM32* variants and one patient with a homozygous VUS. We included four additional patients harbouring homozygous rare *TRIM32* variants: three from NMGS and one for whom WES was performed in Tehran. Two patients harboured a pathogenic variant and two had a VUS. Pathogenic variants outside the NHL repeats were frameshift

mutations (online supplementary figure 1); missense mutations in the coiled-coil region and intervening region were classified as a VUS.⁴

Clinical phenotypes

The clinical findings of patients with pathogenic *TRIM32* variants are described in the upper panel of online supplementary table 2. The main presenting symptoms were related to proximal lower limb weakness. Axial, facial and periscapular muscles were variably involved. Serum CK levels were moderately increased (≤ 2000 U/L). Forced vital capacity was normal in the eight patients for whom we had reliable spirometry. There were no patients

with a cardiomyopathy. A few striking clinical features were noted for the patients carrying a homozygous *TRIM32* VUS (lower panel of online supplementary table 2), including marked distal upper limb weakness for patient 16 and childhood onset and high CK for patient 38.

Muscle imaging

MRI scans of the patients with pathogenic *TRIM32* variants revealed a preferential affection of the posterior thigh compartment, evolving to a diffuse involvement of the anterior thigh in later stages (figure 1). A consistent involvement of the posterior lower leg compartment and the tibialis anterior muscle was observed as well as

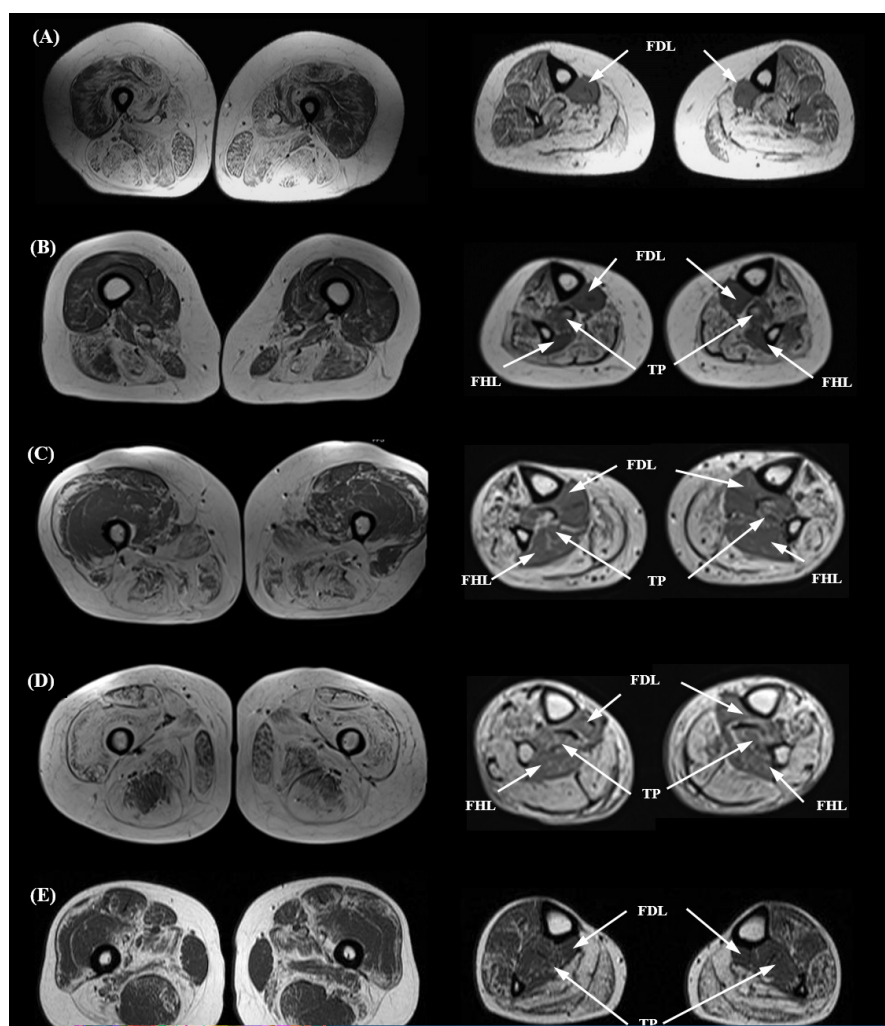


Figure 1 T1-weighted axial MRI of the lower limbs of five patients identified with pathogenic *TRIM32* variants. MRI images at mid-thigh level on the left and mid-calf level on the right for: (A) patient 14 at approximately 14 years of disease duration; (B) patient 15 at 18 years of disease duration; (C) patient 27 at 8 years of disease duration; (D) patient 28 at 28 years of disease duration; (E) patient 37 at 5 years disease duration. (A–C and E) MRI images at thigh level, with slight differences in the exact level of acquisition of the image, revealed a preferential involvement of posterior compartment muscles. (D) End-stage involvement of all thigh muscles was observed for patient 28. (A–E) MRI images at calf level revealed a similar pattern for all patients, with predominant involvement of the posterior compartment. White arrows indicate relatively spared muscles. FDL, flexor digitorum longus; FHL, flexor hallucis longus; TP, tibialis posterior.

the peronei muscles in later stages. There was a relative sparing of the flexor hallucis longus, flexor digitorum longus and tibialis posterior muscles. MRI images for the patients carrying a homozygous VUS did not reveal any consistent pattern (online supplementary figure 2).

Histological features

Muscle biopsies showed non-specific myopathic or dystrophic changes. Vacuoles containing basophilic material were noted in scattered muscle fibres of patients 14, 15 and 39 (online supplementary figure 3). No specific histopathological features were noted on the biopsies of patients carrying a homozygous VUS in *TRIM32*.

DISCUSSION

To our knowledge, other studies have never yielded such a large cohort of patients with non-Hutterite *TRIM32*-related myopathy and thus we present an extended understanding of this rare neuromuscular disorder. The highly conserved NHL domain was the focal region of the pathogenic missense variants in this study, with pathogenic variants outside the NHL repeats being frameshift variants. A caveat to WES is that it is commonly considered to be intractable to copy number variation (CNV) and repeat expansion detection. However, specialised analytical software now permits such analysis. Indeed, a heterozygous 63.5 kb deletion overlapping with p.Arg613Ter was identified in patient 36's exome using a modified version of PennCNV on a custom Illumina Infinium Array.⁵ Repeat expansion disorders—including facioscapulohumeral dystrophy, myotonic dystrophy type 1 and 2 and oculopharyngeal muscular dystrophy—were excluded in case of clinical suspicion.

The phenotypes of the patients harbouring novel variants were not strikingly different from those of the patients with known pathogenic variants. Consistent with the current literature, distal upper limb weakness seems to be exceptional while axial, facial and periscapular muscles were variably involved. Despite *TRIM32*-related myopathy being described as a 'mild' myopathy, four patients in our cohort were wheelchair-dependent. Our data do not provide evidence in favour of cardiac or respiratory involvement. MRI imaging revealed a consistent pattern of muscle involvement. The histological features in our patients encompassed the LGMD2H and 'sarco-tubular myopathy' spectrum that has been described in the literature: non-specific myopathic changes

or dystrophic features sometimes accompanied by vacuoles.²

Additionally, we describe the phenotypes of three patients harbouring a homozygous missense *TRIM32* VUS. For these patients, no other candidate variants in known myopathy genes were identified. Despite some phenotypic characteristics contrasting with those of the patients with definite pathogenic variants, we cannot exclude that these variants are causal in the disease; future functional data will provide insight into their pathogenicity.

Overall, we report nine patients with *TRIM32*-related myopathy harbouring 10 pathogenic *TRIM32* variants and introduce three patients with a homozygous *TRIM32* VUS. Complemented with deep phenotyping, our application of WES enabled patients with *TRIM32*-related myopathy to be identified. We propose that similar approaches of targeted sequencing and thorough curation of phenotypic information will expedite future *TRIM32*-related myopathy diagnoses.

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Competing interests VS is or has been a principal investigator for trials sponsored by Sanofi Genzyme, GSK, Prosenza/Biomarin, Ionis Pharmaceuticals and Sarepta. He has received speaker honoraria from Sanofi Genzyme. He is or has been on advisory boards for Acceleron Pharma, Audentes Therapeutics, Biomarin, Bristol-Myer Squibb, Italfarmaco S.p.A., Nicox, Pfizer, Sanofi Genzyme, Santhera, Sarepta Therapeutics, Summit Therapeutics, Tivrosan and TrophyNOD.

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Ethics approval Ethical approval was granted by the Newcastle and North Tyneside research ethics committee (REC #09/H0906/28) and by the local ethics committees of the participating centres.

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