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Case report

'Amish Nemaline Myopathy' in 2 Italian siblings harbouring a novel homozygous mutation in Troponin-I gene

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Abstract

Amish Nemaline Myopathy is a severe form of nemaline myopathy associated to mutation in *TNNT1* gene, firstly reported among the Old Order Amish. Here we report two Italian siblings who manifested, by the age of 7 months, progressive and severe muscle weakness and wasting, respiratory insufficiency, *pectus carinatum* deformity and failure to thrive. Muscle biopsy was consistent with nemaline myopathy and novel homozygous missense mutation in *TNNT1* was found. Our cases expand the mutational spectrum of *TNNT1*, confirm the invariable peculiar clinical phenotype also outside the Amish population, and suggest that *TNNT1* should be considered for molecular analysis in NM patients with chest deformities and progressive contractures.

Key words: Pectus carinatum; Nemaline myopathy; Rod bodies; Tropomyosin I; *TNNT1*.

1. Introduction

Nemaline myopathies (NEMs) are a heterogeneous group of hereditary myopathies characterized by skeletal muscle weakness and the presence of rod-like structures in skeletal muscle fibres. So far, 13 autosomal genes have been identified as causative for NEM. These comprise *ACTA1, NEB, TPM2, TPM3, CFL2, KHLH40, KLHL41, KBTBD13, LMOD3, MYPN, MYO18B, TNNT1* and *TNNT3* [1–13]. NEM related to *TNNT1* mutations are very rare.

A recessive form caused by a homozygous c.505G>*T* nonsense mutation in *TNNT1* was first reported in children of common Amish descent [13]. This mutation is associated to a peculiar and severe phenotype, named 'Amish Myopathy'(ANM), characterized by early onset tremors of the jaw and lower limbs, proximal contractures, generalized hypotonia and progressive weakness associated with severe pectus carinatum and muscle atrophy, leading to respiratory insufficiency and death in the second year of life.

Additional recessive loss-of-function mutations in *TNNT1* have recently been reported in non-Amish ethnic groups with similar recessive NM [14–17] including a homozygous deletion encompassing *TNNT1* and *TNNI3* that was recently described in a severe form of ANM with dilated cardiomyopathy [18]. Moreover, an autosomal dominant *TNNT1* missense mutation has been recently reported in several members of an extended family with Ashkenazi Jewish ancestry affected by NM and characterized by a mild phenotype with considerable clinical heterogeneity [19].

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Here we report on 2 additional familial cases of NM associated to a novel homozygous missense mutation in *TNNT1*.

2. Case reports

The proband (patient #1) is a young girl, the second child of non-consanguineous parents originated from a small town of the Southern Italy (about 5000 p). She was born following an uncomplicated pregnancy. Birth weight was below the 50th centile. By the age of 4 months she started to present progressive muscle weakness, chest deformity, difficulty in swallowing and breathing. At 7 months her parents began to notice severe failure to thrive. Our first neurological examination was performed at the age of 1.5 years. Weight and length were below the 3rd centile (weight 5 kg and length 63 cm, respectively). She had severe and generalized muscular weakness and atrophy, chest deformity (*pectus carinatum*), rigid spine and multiple joint contractures (Fig. 1). The baby had normal interaction and the ocular movement was spared. Her maximum motor ability was to rotate the head in lying position retaining only poor distal movements of the hands. She was hospitalized because of poor respiratory and nutritional conditions, and soon had to be treated with tracheostomy and gastrostomy.

Her eldest sister (patient #2) presented the same clinical course. She was born uncomplicated pregnancy. Club feet were observed since birth. At 4 months she started to present generalized muscle weakness and at age 8 months she was hospitalized for failure to thrive. She underwent tracheostomy and gastrostomy soon after. A muscle biopsy performed at the age of 10 months showed features consistent with Nemaline Myopathy (Fig. 2). She is now 11 years old, she is a very skilled girl, and is able to speak and write using a tablet, having residual muscle strength in the upper limbs. Clinical evaluation shows severe growth failure (weight 10 kg and length 80 cm, <<3rd centile), generalized muscular atrophy, contractures and *pectus carinatum*.

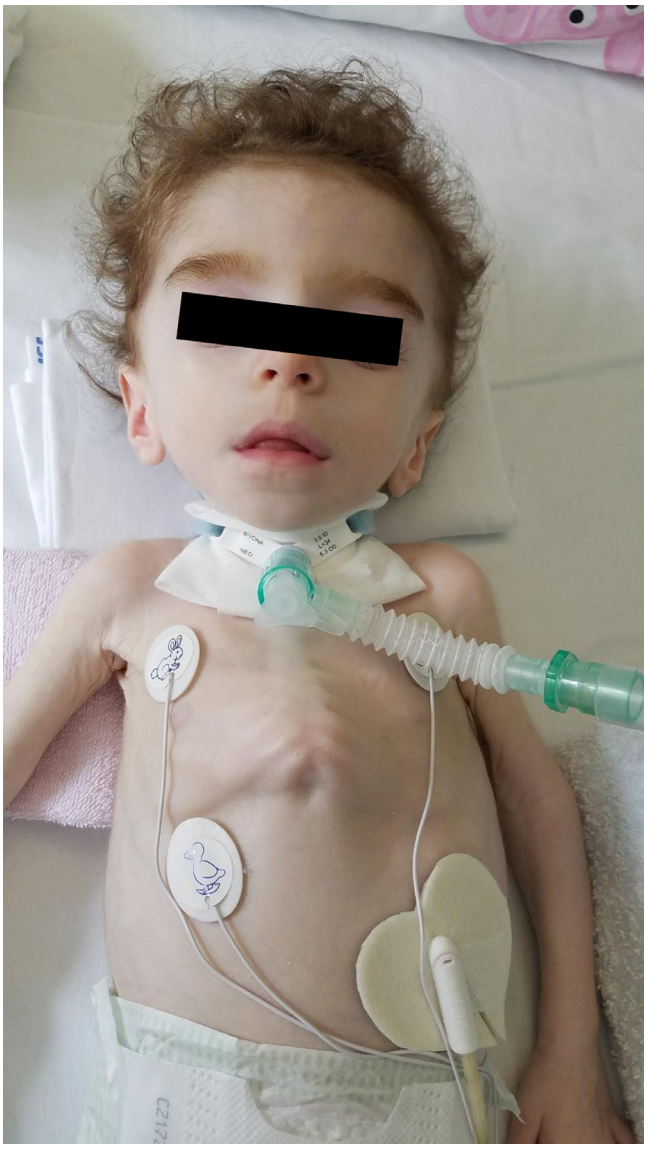

Fig. 1. Picture of the proband: in the picture is shown the severe pectus carinatum that characterised the clinical phenotype of Amish Myopathy.

2.1. Molecular genetic testing methods

Genomic DNA was extracted from peripheral blood with standard methods after obtaining signed informed consent. DNA was analysed for a targeted neuromuscular disease (NMD) panel that included 95 myopathy-related disease genes as already described [20]. The Illumina Variant Studio data analysis software was used to annotate the variants. Validation and segregation analysis of the identified variants in *TNNT1* (NM_003283) was performed by Sanger sequencing.

2.2. Muscle biopsy

Muscle biopsy, performed in pt#2 at 10 months of age, showed histopathological findings consistent with severe nemaline rod myopathy. The specimen (*m. vastus lateralis*) showed marked variation in fibre size with numerous hypotrophic or atrophic fibres mostly belonging to Type 1, slow twitching fibres (Fig. 2). Diameter of fibres varied from less than 1 um in severe atrophic fibres, up to 13.5 μm in larger size fibres. Average diameter of type 1 fibre was 6.47 μm, whereas average diameter of type 2 fibres was calculated at 8.61 μm. Gomori trichrome staining highlighted several inclusions of granular reddish-purple material, consistent with nemaline-rod bodies in isolated fibres. Nemaline bodies were particularly abundant in smaller Type 1 fibres.

2.3. Genetic results

NGS analysis revealed a novel homozygous c.661G>*T*; p.(E221X) mutation in exon 12 of *TNNT1*. Sanger sequencing confirmed the NGS data of the proband and detected the same homozygous mutation in her elder sister as well as heterozygosity for their parents.

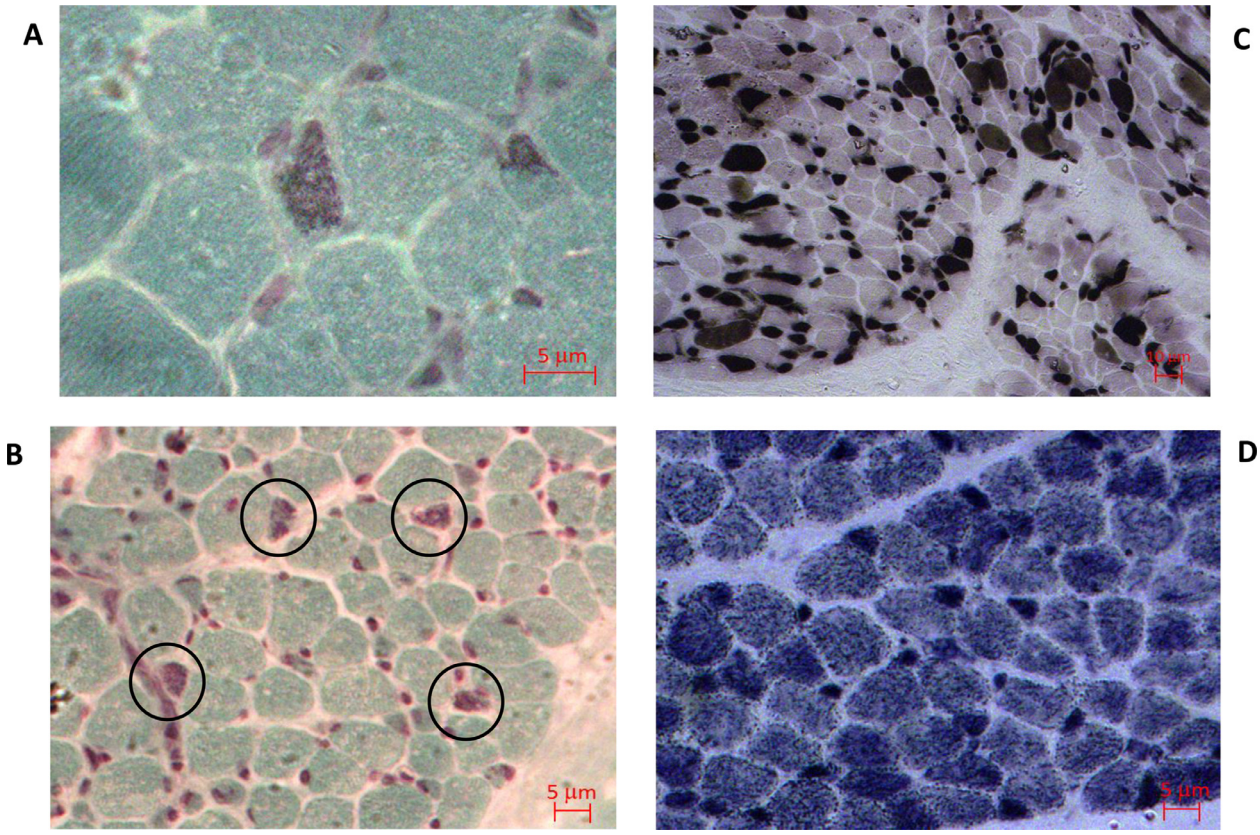

Fig. 2. Histopathological features of muscle biopsy from patient 2. (A) Modified Trichrome Gomori staining (magnification 60× oil) demonstrates numerous fucsinophil rod bodies, almost filling the sarcoplasm, suggestive of a nemaline myopathy. (B) Modified Trichrome Gomori staining (magnification 40×) also shows variation in fibres size and prevalence of nemaline bodies in the smaller fibres (circles). (C) and (D) ATPase 4,3 and NADH stainings (magnification 40×) reveal that most of the atrophic and hypotrophic fibres are dark type 1 fibres, confirming type 1 atrophy as expected in a tropomyosin defect.

The p.E221X mutation converts the Glu^{221} to a premature stop codon and the resultant protein is predicted to lack 58 aminoacids of its highly conserved C-terminus causing a loss of the binding sites for both the Troponin inhibitory subunit (TnI) and the Troponin Ca^{2+}-binding subunit (TnC) in the T2 region of TnT (Fig. 3).

3. Discussion

Here we report on a novel truncating mutation in *TNNT1* that results in a severe congenital nemaline myopathy indistinguishable from the Amish Myopathy. The remarkable features in our sibs are severe muscle hypotrophy, stiffness and *pectus carinatum*, together with severe failure to thrive by the age of eight months and early need of nutritional and respiratory support.

As in previous reported cases, prominent features at muscle biopsy were hypotrophy of type 1 fibres and prevalence of rod bodies in smallest type 1 fibres.

The rods bodies are primarily restricted to type 1 fibres in most individuals with *TNNT1* mutations [13,14]. These findings confirm the idea that since troponin T1 localizes to the type 1 muscle fibres, these are more profoundly affected.

Although type 2 fibres hypertrophy has been reported in *TNNT1* patient, presumably as compensatory adaptation to hypotrophic type 1 fibres [15], we did not detect this in our patients possibly because muscle sample was taken at a very early stage before any adaptatory mechanism could occur. *TNNT1* encodes troponin T type 1, a 35- to 37-kDa protein with variable N-terminal and conserved middle and C-terminal regions, which is exclusively found in type 1 fibre and serves to anchor the troponin complex (along with troponin C and I) onto the tropomyosin–actin thin filaments. Multiple transcript variants encoding different isoforms have been found for this gene and four major *TNNT1* isoforms are expressed in muscle with inclusion or exclusion of exon 5 and exon 12′ (a longer version of exon 12) which were reported to be alternatively spliced exons [21–24]. Troponin T regulates the conformational changes in the thin filaments during excitation–contraction–coupling of slow skeletal muscle and truncating *TNNT1* variants have repeatedly been demonstrated to prevent the incorporation of TnT into the myofilament leading to progressive muscle degeneration and weakness [13,23,25]. Our patients harbour a new c.661G>*T* mutation in exon 12 of *TNNT1* converting the Glu^{221} to a premature stop codon. The mutation abolishes the binding sites for the Troponin inhibitory subunit (TnI) and the Troponin Ca^{2+}-binding subunit (TnC) which are crucial for correct assembly of the troponin complex [25]. Excluding the Dutch cases who have an exon 14 deletion in compound heterozygosity

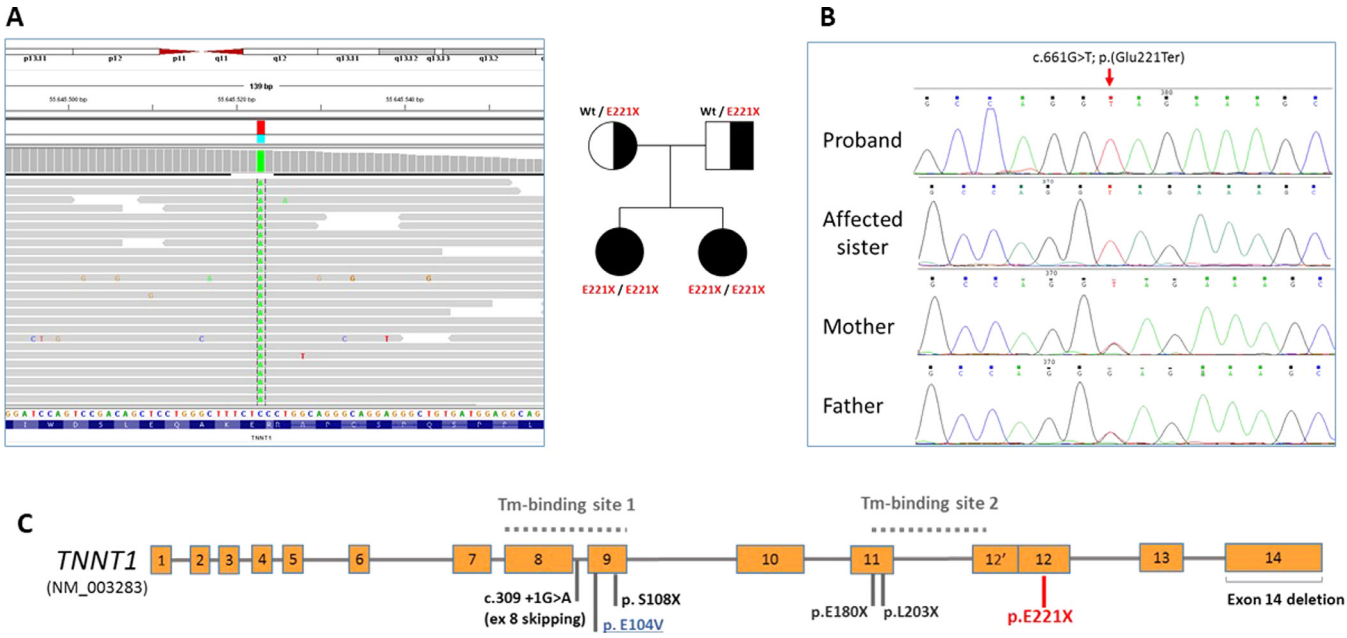

Fig. 3. Genetic results and pedigree. (A) IGV alignment of Bam and VCF files in proband, generated using the targeted NGS panel, showing the novel homozygous *TNNT1* variant. (B) Electropherograms showing homozygosity for the c.661G>*T*; p.(E221X) substitution in sibs and heterozygosity for their parents. (C) Schematic diagram of *TNNT1* gene with previously reported recessive (black) and dominant (blue) *TNNT1* mutations and the novel mutation identified in our family (red).

with the exon 8 skipping mutation [14], all the AR *TNNT1* mutations described so far, are homozygous nonsense or frameshift mutations involving the functionally important and evolutionarily highly conserved middle and/or the C-terminal regions of the troponin T1 protein. These domains contain binding sites for tropomyosin, TnI and TnC and presumably do not tolerate structural variations [13–15].

4. Conclusion

Our cases expand the mutational spectrum of *TNNT1* and confirm the invariable peculiar clinical phenotype also outside the Amish population, corroborating the hypothesis that this gene should be considered for molecular analysis in NM patients with chest deformities and progressive contractures.

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