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Case Report

Dystrophin Exon 29 Nonsense Mutations Cause a Variably Mild Phenotype

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ABSTRACT

Background: Nonsense mutations in the dystrophin gene usually result in a severe Duchenne muscular dystrophy phenotype.

Findings: We describe a 7-year-old boy with a rare pathogenic mutation in exon 29 c.3940C>T p.(Arg1314Ter) resulting in exon skipping, in turn rescuing the phenotype from a severe Duchenne type to a milder Becker muscular dystrophy type. No adults have been described with this mutation to date.

Conclusions: Exon skipping of exon 29 results in a higher level of functional dystrophin. Some cases of muscular dystrophy may still require muscle biopsy to determine optimal management and pharmaceutical treatment options.

Key Words: Dystrophin, Exon 29, Nonsense mutation, Becker muscular dystrophy, Exon skipping

INTRODUCTION

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are allelic X-linked disorders of the dystrophin gene at the Xp21 locus. The dystrophin gene is the largest gene known and contains 79 exons[1]. The prevalence of DMD in Ireland is around 3 per 100,000 cases[2].

DMD is usually evident from infancy/early childhood and is characterised by delayed motor milestones, a waddling gait, Gower's sign and later calf hypertrophy. Muscle disease is rapidly progressive and many boys with DMD are wheelchair bound as young teenagers. Approximately 30% of patients will have learning difficulties and as many as 80% will develop cardiomyopathy; although only 10% of affected boys will die from heart failure[3]. At a cellular level the dystrophin gene is responsible for producing the protein dystrophin which acts to couple the sarcolemmal cytoskeleton with the extracellular matrix via the dystrophin glycoprotein complex. Loss of this stability makes the muscle more susceptible to damage when exposed to mechanical stress. As muscle damage progresses there is secondary hypertrophy due to infiltration with adipose tissue[4]. Dystrophin is virtually absent from all muscle cells (>98%) in DMD[5].

In contrast, the muscle seen in BMD patients has a variable amount of dystrophin present and constitutes a milder skeletal muscle phenotype. The mean age of onset is 11 years. Loss of the ability to walk may not occur until 40 or 50 years. Often cramping on exercise is the presenting complaint, followed by difficulties in running and climbing stairs.

Antisense-mediated exon skipping therapy is a promising therapeutic approach to skip over the mutated exon, restoring the open reading frame and producing a partially functional dystrophin. Essentially exon skipping converts what would be a severe DMD phenotype into a milder BMD phenotype. Exon 51 skipping has been the target of clinical trials but this will only be directly applicable to about 13% of all DMD individuals[6]. Knowledge of other potential skipping targets is critical to the development of antisense oligonucleotides (AONs) for many other DMD patients. Multiple AON 'cocktail' therapies have recently been used in the mouse model, as a way of expanding the coverage for deletion mutations in exons 45-55 that can be restored[7]. The drug Etiplirsen has recently been approved by the FDA to allow exon 51 skipping[8]. With the advent of treatments, databases of mutations are now being compiled to collect information about the type and frequency of DMD mutations – such as TREAT NMD DMD[9].

PRESENTATION

We present a 7-year-old boy with a mild BMD phenotype

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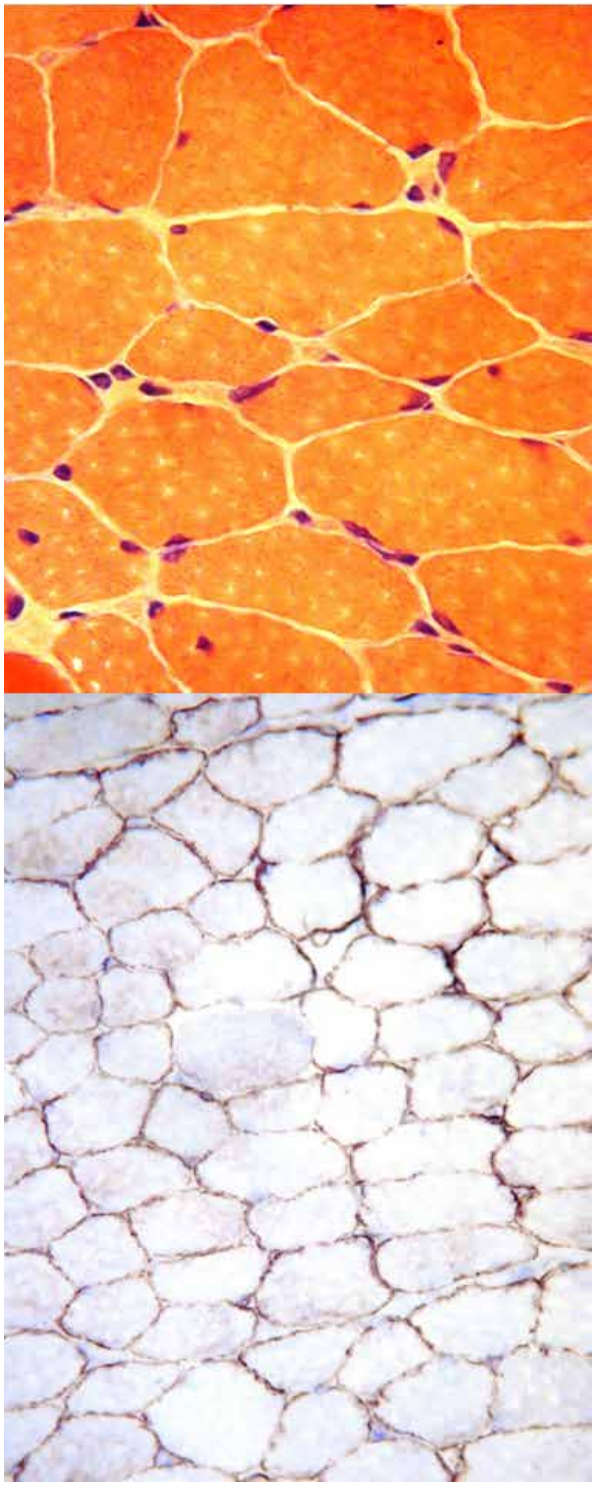

Fig 1. H&E image (top)

H&E shows mild variation in the fibre shape and size. Occasional internal nuclei are present but the typical features of a dystrophy are not seen. There are no split fibres; there is no fibrosis or fibre necrosis.

Dystrophin 1 immunohistochemistry (bottom)

Dystrophin 1 immunohistochemistry shows generally good circumferential staining with areas of partial loss including one completely negative fibre.

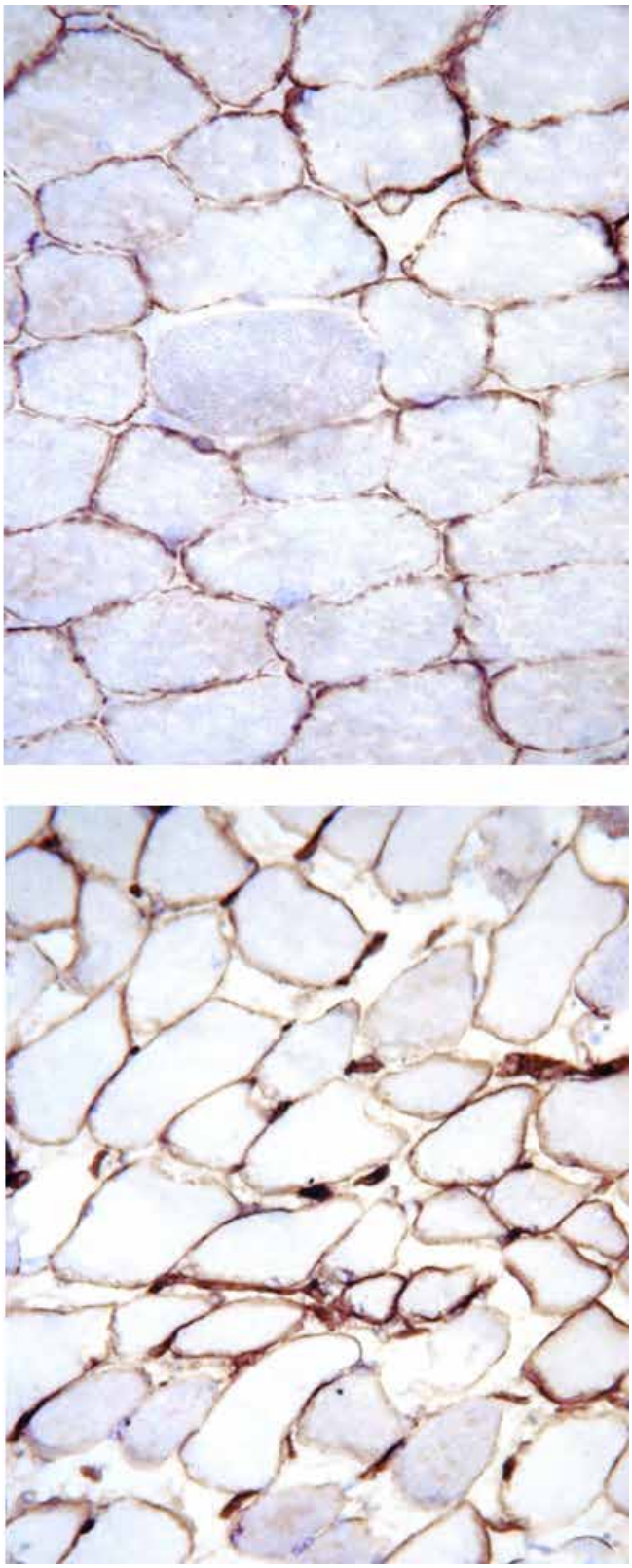

Fig 2. Dystrophin 2 (top)

Dystrophin 2 staining is similar to dystrophin 1.

Utrophin (bottom)

Utrophin immunohistochemistry shows diffuse circumferential upregulation.

and a pathogenic mutation in exon 29 c.3940C>T p.(Arg1314Ter).

He presented to paediatric services at the age of 5 years with developmental delay. He was born at 34 weeks gestation with a birth weight of 4lbs. His neonatal course was unremarkable. His motor milestones were delayed and he had severe speech delay with only a few words at 5 years old. Upon presentation, he could walk upstairs and run. He had some difficulties coming down stairs and could not jump or pedal a bicycle, but could climb and perform Gower's manoeuvre without difficulty.

RESULTS

Initial investigations included a creatine kinase level of 10,000 U/L. His initial genetic screening was negative for DMD and hence a muscle biopsy was performed. This was suggestive of a mild dystrophy but the typical features of Duchenne or Becker dystrophy were not present and only showed mild changes in dystrophin (Figures 1 and 2). His muscle strength in all groups of muscles at age seven was at least 4/5. He had a MRI of his brain which was entirely normal. His echocardiogram and array cytogenetic testing analysis were completely normal. Testing of muscle DNA confirmed an exon 29 mutation c.3940C>T p.(Arg1314Ter) which was then identified in blood DNA, allowing carrier testing in family members.

DISCUSSION

There have been only a few case reports of exon 29 nonsense mutations in the literature and each of them describe an encouraging milder phenotype (Table 1). The variant in our patient is extremely rare and is predicted to produce a truncated dystrophin leading to a severe phenotype. We were unable to take this further with RT-PCR analysis due to hospital funding restrictions, however, evidence in the literature from a 5-year-old male with the same mutation as our patient showed that exon 29 was aberrantly spliced out of dystrophin mRNA transcripts resulting in an in-frame deletion at the RNA level[10]. As a result, there is a higher level of functional dystrophin and a milder phenotype. [A nonsense mutation in exon 27 has previously been reported as promoting exon skipping[11] which may support a similar mechanism for the exon 29 skipping].

Another paper describes 3 patients within the same family as having a mild phenotype (Exon 29 4148C>T). The first, a 58-year-old man, who is wheelchair bound for longer distances and has kyphoscoliosis and cardiomyopathy. His 23-year-old nephew has mild symptoms only and his 26-year-old nephew has a raised CK only[12].

The paper describing the 5-year-old patient with the same exon 29 mutation as our patient (c.3940C>T) notes he had a raised CK level only and doesn't report the developmental delay or speech impairment present in our case[10]. This patient formed part of a database of 229 DMD/BMD patients in East China. He was one of the only two patients with BMD to have a point mutation. All other point mutations resulted in DMD. Our patient had a normal array test and no other causes were identified for the developmental delay and we cannot be certain these features are definitely due to the DMD but such features are recognised in other boys with the condition with other mutations.

Canine models have also been used to explore a mild muscular dystrophy phenotype. Ringo was the most notable dog; a Brazilian Golden Retriever born with complete absence of dystrophin and a clinically mild phenotype and normal lifespan[13]. Vieira et al recently described a 3-generation family of Labrador retriever dogs with no signs of muscle weakness in the setting of markedly increased creatine kinase activity and absent dystrophin[14]. There are parallels with this case and canine models may be helpful in elucidating further therapeutic intervention in boys with DMD as it is useful to know that exon 29 skipping results in a higher level of functional dystrophin.

This case report consolidates a small cohort of patients demonstrating skipping of exon 29. We would hope to continue to observe a mild phenotype in this patient. Expanding knowledge of exon skipping is critical to the on-going research of this mechanism as a possible therapy. It

TABLE 1.

Published patients with exon 29 mutations

Patient	Phenotype	Exon	Mutation	Ref.
58 yr old male	Wheelchair long distances, kyphoscoliosis, cardiomyopathy	29	c.4148C>T	12
26 yr old male	Raised CK only	29	c.4148C>T	12
23 yr old male	Mild symptoms	29	c.4148C>T	12
5 yr old male	Raised CK only	29	c.3940C>T	10
7 yr old male	Mild skeletal muscle weakness, raised CK and learning difficulties	29	c.3940C>T	Our patient
Family of male patients	Early onset dilated cardiomyopathy	29	c.4148C>T	16

is also invaluable in reassuring patients and parents that the clinical course of this genotype is a seemingly milder one.

Given that our patient's clinical phenotype and muscle dystrophin confer a diagnosis of Becker Muscular dystrophy, although muscle biopsy is not routinely recommended in general diagnostic work-up of cases, this case illustrates that in a small number of cases, muscle biopsy may be helpful in determining the exact phenotype classification. In this case, treatment with approved small molecule compounds such as Ataluren, an oral medication that suppresses nonsense mutations[15] (ameliorating the effect of nonsense mutations within the Dystrophin gene), may not be necessary as our patient would therefore not fit the criteria for treatment on closer examination, thus saving costs and unnecessary side effects. Of note, the ACT DMD trial (48-week randomised placebo controlled phase III trial) demonstrated no statistically significant differences in a 6 minute walk test against placebo. Even etiplirsen the exon 51 skipping drug whilst showing an elevation of 11-21% in dystrophin, did not provide substantial evidence of clinical effectiveness[8]. Further studies using western blotting may help in quantifying the dystrophin expression, and RNA analysis may explain a clearer mechanism for the exon skipping. Identification of adults with this particular mutation will shed light on the evolving phenotype of this mutation, and follow-up of these childhood cases will shed further light on the natural history and progression of the disorder and the effectiveness of treatments.

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COMPETING INTERESTS

None

AUTHOR CONTRIBUTIONS

Conception: Rebecca Moore, Sandya Tirupathi, Patrick J. Morrison

Manuscript writing: Rebecca Moore, Patrick J. Morrison

Provision of Histopathology and descriptions: Brian Herron

Echocardiography: Andrew Sands

Final approval of manuscript: Rebecca Moore, Sandya Tirupathi, Brian Herron, Andrew Sands, Patrick J. Morrison

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All authors have seen and approved the final manuscript and none have any competing interests. All authors have agreed to the submission to the journal and the manuscript is not currently under submission in any other journal.

This research was carried out according to our institution's guidelines. Permission was granted to access all relevant patient data.

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