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ORIGINAL ARTICLE

Treatment with ataluren in four symptomatic Duchenne carriers. A pilot study

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Duchenne muscular dystrophy (DMD) is a devastating X-linked neuromuscular disorder caused by dystrophin gene deletions (75%), duplications (15-20%) and point mutations (5-10%), a small portion of which are nonsense mutations. Women carrying dystrophin gene mutations are commonly *unaffected* because the wild X allele may produce a sufficient amount of the dystrophin protein. However, approximately 8-10% of them may experience muscle symptoms and 50% of those over 40 years develop cardiomyopathy. The presence of symptoms defines the individual as an affected "*symptomatic* or *manifesting* carrier". Though there is no effective cure for DMD, therapies are available to slow the decline of muscle strength and delay the onset and progression of cardiac and respiratory impairment. These include ataluren for patients with nonsense mutations, and antisense oligonucleotides therapies, for patients with specific deletions. Symptomatic DMD female carriers are not included in these indications and little data documenting their management, often entrusted to the discretion of individual doctors, is present in the literature.

In this article, we report the clinical and instrumental outcomes of four symptomatic DMD carriers, aged between 26 and 45 years, who were treated with ataluren for 21 to 73 months (average 47.3), and annually evaluated for muscle strength, respiratory and cardiological function. Two patients retain independent ambulation at ages 33 and 45, respectively. None of them developed respiratory involvement or cardiomyopathy. No clinical adverse effects or relevant abnormalities in routine laboratory values, were observed.

Key words: Duchenne muscular dystrophy, nonsense mutations, DMD symptomatic/affected carriers, manifesting carriers, ataluren

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Introduction

Duchenne muscular dystrophy (DMD) is an X-linked devastating disorder affecting muscles and heart in young boys [1-3], caused by mutations in the dystrophin gene. These are most commonly due to deletions (75%), followed by duplications (15-20%) and point mutations (5-10%) which include nonsense mutations (nmDMD) [4].

Women carrying a dystrophin gene mutation on one of two X-chromosomes have long been considered as *unaffected* because the wild X allele may produce a sufficient amount of dystrophin protein. However, approximately 8-10% of DMD carriers experience muscle symptoms [5,6], while 50% of those over 40 years, develop a cardiomyopathy [7,8]. The presence of symptoms defines the affected individual as a "*symptomatic* or *manifesting* carrier" [9-16]. Both terms have been widely used since 1974, when Moser and Emery [9] first defined "manifesting

carriers" as "*females with a history of Duchenne muscular dystrophy in their pedigree who have symptomatic weakness*".
DMD carriers can develop muscle weakness and/or myalgia, cramps, fatigue, or show enlarged calf muscles (pseudo-hypertrophy) [5, 6,17]. Increased levels of serum creatine kinase (CK) up to ten times the upper normal limit were observed in approximately 40-50% of carriers, especially in childhood [18,19]. Additionally, a considerable percentage of carriers may develop cardiomyopathy in adult age [7,8,20]. Therefore, the symptoms may range from a severe Duchenne-like to a very mild Becker-like phenotype.
Several mechanisms leading to reduced dystrophin production were hypothesized to explain the variable onset of clinical manifestations and, in particular, the role played by skewed X- chromosome inactivation (XCI). XCI is a mechanism that equalizes the X-linked gene dosage between males and females by inactivation of one of the two X chromosomes in females, during early embryonic development [21]. Accordingly, in females, a mosaic of two cell types is formed expressing either the maternal or the paternal X chromosome. *Random XCI* results in an equal (50:50) distribution of the maternal and paternal X chromosome, while a *skewed XCI* produces an unequal (> 50 %) distribution due to a preferential inactivation of the maternal or the paternal X chromosome. However, the normal range of skewed XCI is broad, ranging from 65:35 [22, 23] to 70:30 [24, 25] or even 80:20 [26]. Usually, the term *extremely skewed XCI* indicates the preferential inactivation of one X chromosome in 90-95% of cells [26]. Skewing might be caused by limited survival of one X-chromosome due to its dysfunction or lethality [27], and indeed, a translocation affecting the DMD gene locus was identified in some symptomatic female carriers [28-31]. Accordingly, symptomatic DMD carriers may have a preferential inactivation of the X-chromosome carrying the wild allele, with onset and severity of DMD symptoms related to the amount of dystrophin produced, which depends on the degree of inactivation [31-33].
While there is no current cure for DMD, available therapies may slow the decline of muscle weakness and delay the onset of heart and respiratory involvement. Among others, steroids, ACE-inhibitors and beta-blockers, are the gold standard of treatment [34,35]. In the last decade, different therapeutic approaches have been proposed in patients with dystrophin gene deletions or duplications with encouraging results. Among these we remember gene therapy, which consists in introducing into muscles a transgene that codes for a truncated version of the DNA complementary to dystrophin (cDNA) [36], and exon skipping techniques with antisense oligonucleotides which convert an out-of-frame into an in-frame [37] mutation, both developed and tested in clinical trials. For DMD patients with stop codon mutations [4], potential drugs such as gentamicin [38-42] and ataluren (PTC124)[41] were explored as an alternative approach. These drugs allow ribosomal readthrough of premature stop codons, enabling the production of a functional dystrophin, which may ameliorate disease progression [42-45]. About 10-15% of DMD patients could potentially benefit from treatment with ataluren [42-45]. This drug has been available in Europe since 2014 [41] under the commercial name Translarna®.
In 2017, McDonald and al. [42] presented the results of a phase 3, multicentre, randomized, double-blind, placebo-controlled trial (ACT DMD) that assessed the ability of ataluren to stabilize ambulation, with a focus on a pre-specified subgroup of patients with ambulatory decline. The primary endpoint of change in 6-min walk distance (6MWD) from baseline to week 48, expecting a difference of at least 30m between ataluren-treated and placebo-treated patients, was not reached (difference 13.0m [SE 10.4], 95% CI-7.4 to 33.4; p = 0.213). However, a benefit with ataluren was observed in the subgroup of patients with a better baseline, of 6MWD between 300 and 400m (difference vs placebo 42.9m [SE 15.9], 95% CI 11.8-74.0; p = 0.007), which was further determined in subsequent reports [43-45]. Articles recently published on the long-term ataluren treatment indicated a delay in loss of ambulation, as well as effects on cardiac and respiratory parameters and upper limb motor function, even after loss of ambulation [45-47]. An early treatment plan with ataluren has also been suggested [48,49].
Symptomatic DMD carriers are not included in these treatment plans [50] as limited data is available regarding their management [6,51,53], rendering physicians to make decisions based on their own discretion. We previously reported [54] the results of nine months of treatment with ataluren in a 26-year-old symptomatic nmDMD carrier, who showed improved motor skills. In this article, we report the outcomes of four symptomatic nmDMD carriers, one from Israel and three from Italy, treated with ataluren for an average period of 47.3 months.

Patients and methods

Patients

Four symptomatic female carriers were included. Demographic and clinical data, including country of origin, age at first symptoms, age at muscle biopsy, time between first symptoms and muscle biopsy, age at genetic confirmation, time between first symptoms and laboratory abnormality or genetic confirmation were retrospectively collected. Age at first and last visit, age at informed consent, prior and concomitant medications, age at start- and end-date of ataluren treatment, and age at loss of ambulation were also collected.

Methods

Data on motor function were assessed at the baseline and at the last visit, using the 6MWT, North Star Ambulatory Assessment (NSAA) total score and, when available, dynamic tests (Gowers time, time to climb 4 steps) and upper limb power test (PUL) [55]. When available forced vital capacity (FVC) and left ventricular ejection fraction (LVEF) were evaluated. Ataluren was taken orally with the dosage adjusted according to the patients' weight.

Statistical analysis

The small sample size prevented statistical analysis therefore only qualitative data (i.e. mean, standard deviation, range) will be shown when applicable.
The study was approved by the Ethical Committee of the University of Campania Luigi Vanvitelli (approval n. 763 of 23/11/2018).

Results

Case descriptions

IL001

The patient was diagnosed at the age of 40 years as a carrier of a stop codon mutation (c.8038C > T; p.R2680*) in the *DMD* gene and extremely skewed XCI (89:11) following the diagnosis of her son with DMD. The patient began to have frequent falls at the age of 30, follo wed by gradually increasing difficulty in climbing stairs. At age 43, upper extremity weakness became evident, along with low back pain, progressive gait difficulty, and falls. Clinical examination was notable for hyperlordosis, proximal and distal muscle weakness (with approximately 50% reduction in muscle strength on MRC scale), absence of deep tendon reflexes, and Trendelenburg gait. She had no respiratory or cardiac symptoms or signs; echocardiography was normal and CK levels were 8.4 times the upper normal limit. At age 44, treatment with ataluren was started at a dose of 2,000 mg per day orally. During the first year of treatment, she reported progressive improvement in balance, gait and endurance, absence of falls and resolution of low back pain. Repeated quantitative muscle strength testing showed a gradual increase in proximal upper extremity muscle strength, while that of the proximal lower extremities was unchanged. The 6MWT increased from 280 meters at baseline to 300 meters after 9 weeks, 322 meters after 56 weeks, and 300 meters after 92 weeks of treatment, respectively. After two years of treatment, she gained weight (7% of her original weight) and walking again became difficult. Her 6MWT declined 6 months later to 263 meters, and after 3 years of treatment to 220 meters, accompanied by an additional gain of weight to 16% of her original weight. Muscle strength showed a slight decline. The dose of ataluren was adjusted accordingly to the weight gain, but gait became increasingly difficult and recurrent falls occurred. At age 48, gait became dependent on support and at the age of 49 she lost ambulation. Treatment was stopped at the age of 49. CK levels were activity-dependent and ranged from 3.1 to 4.1 times the upper normal limit. No adverse events were observed.

IT001

This patient's medical history has been previously published [55]. Briefly, a 33-year-old female came to our observation at the age of 12. First medical evaluation at 18 months for delayed motor milestones and very high CK levels (56.4 times the upper normal limit). Muscle biopsy revealed a dystrophin mosaic pattern with marked reduction/absence of dystrophin in most fibres and others with normal protein expression. MLPA testing identified no deletions/duplications. The patient started treatment at age 12 with deflazacort and antioxidant drugs (Vitamin C, Vitamin E, Ubiquinone) with a slight worsening of muscle strength. The ability to get up from the floor was lost at age 10. Later, NGS analysis identified a stop codon causative mutation c.7792C > T (p.Gln2598*) in exon 53 of the *DMD* gene, and treatment with ataluren was started at the age of 26. The 6MWT at that time was 100 meters and Power of the Upper Limb (PUL) [55] was 29/74. Both cardiac and respiratory parameters were normal. CK values were 2.5 times the upper normal limit. Soon after the beginning of treatment, the patient experienced subjective well-being and improvement in strength. Unfortunately, due to a traumatic fracture of the femur requiring surgical intervention, the treatment was suspended for two months. Upon resumption of ataluren treatment, the patient was unable to walk, and PUL declined to 27/74. However, 12 weeks later she had recovered the ability to walk with support, with a 6MWT of 76m. After 24 and 36 weeks, the patient was able to walk independently without support, and the 6MWT value was 52m and 55m, respectively. PUL values increased to 29/74 after 12 weeks of treatment and remained stable in subsequent evaluations. She lost independent ambulation at the age of 30. CK values ranged between 1.2 and 1.9 the upper reference limit. Both cardiac and respiratory parameters remained stable during the 73 months of follow-up.

IT002

This is a 33-year-old patient, which had her first muscle weakness symptoms appear at age 17. A muscle biopsy, performed at the age of 21, showed a dystrophic pattern associated with abnormal dystrophin immune-histochemical staining. Genetic analysis identified a heterozygous nonsense mutation c.8038C > T, (p.Arg2680*) in exon 55 of the *DMD* gene. She began treatment with ataluren at the age of 29. At that time her 6MWT was 255.2 meters and she was unable to get up from the floor, but still able to climb four standard steps in 15.4 seconds. Both FVC and LVEF parameters were within normal limits. Eighteen months after treatment, the 6MWT declined to 181.4 meters, and the time to rise from the floor increased to 30 seconds. FVC and LVEF were unchanged. CK values decreased from 2.3 to 1.9 times the upper reference limit. However, after 48 months of treatment, at her last follow-up visit, the patient retained ability to walk independently, the 6MWT improved to 200 meters while time to rise from the floor declined to 28sec. The FVC declined to 90% and the LVEF decreased by 10%, though remaining within the normal limits. The patient never stopped treatment which was well tolerated.

IT003

This is a 45-year-old patient, who presented muscle weakness at the age of 35. A muscle biopsy, performed at the age of 41, showed dystrophic changes and a binding reduction of dystrophin in all muscle fibers. Genetic analysis identified the point mutation c.1373C > T in the *DMD* gene. At the age of 43, the patient began treatment with ataluren. The 6MWT test at that time was 370 meters; she was unable to independently get up from the floor, but was still able to climb four standard steps within 7.3 seconds. CK values were 5 times the upper reference limit. Both FVC and LVEF were within the normal limits. The patient chose to suspend the treatment after 21 months. Nevertheless, on her last visit, findings showed stabilization, with a 6MWT of 360 meters and time of rising from the floor of 7 seconds. FVC and LVEF parameters were unchanged.

Aggregated results and analysis

A summary of the main characteristics of the four symptomatic carriers treated with ataluren, is shown in Table I. Details of muscle function are reported in Figure 1, while FVC and LVEF, at baseline and at the last visit, are reported in Table II.

Three patients are from Italy and one from Israel. The age of onset of first symptoms ranged from 18 months to 35 years (mean age 20.9 ± 14.9 years). In three carriers, muscle biopsy was performed

Table I. Demographic characteristics of the nmDMD carriers treated with ataluren.

Patient ID	Current age (years)	Age at (OoS) (years)	Age at MB (years)	MB Findings	Time between OoS and MB (years)	Age at GC of nmDMD (years)	Time between MB and GC (years)	Time between OoS and GC (years)	Prior and concomitant medications
IL001	51	30	n. p.			38		8	None
IT001	33	1.6	1.6	Mosaic pattern of dystrophin positive/ negative fibres	0	24	22	25	Deflazacort, antioxidants, calcium and vitamin D3
IT002	33	17	21	Mosaic pattern of dystrophin positive/ negative fibres	4	21	0.6	0.6	None
IT003	45	35	41	Mosaic patter of dystrophin positive/ negative fibres	6	42	1	7	None
Mn	**40.5**	**20.9**	**21.2**		**3.3**	**21.2**	**7.9**	**10.1**	
SD	**9.0**	**14.9**	**19.7**		**2.5**	**8.9**	**12.2**	**9.0**	
Range	33 - 51	1.6 - 35	1.6 - 41		0 - 6	21 - 42	0.6 - 22	0.6 - 25	

Abbreviations. OoS: Onset of Symptoms; MB: Muscle Biopsy; GC: Genetic Confirmation.

Table II. Clinical data in the treated patients, at baseline and at the last visit.

Patient ID	Age at first visit (years) for Ataluren	Ataluren Start Date	Duration of treatment (months)	L.o.A. (years)	6MWD at Ataluren Start Date (meters)	6MWD at Ataluren End Date (or last visit)	FVC (%) at Ataluren Start Date	FVC (%) at Ataluren End Date (or last visit)	LVEF (%) at Ataluren Start Date	LVEF (%) at Ataluren End Date (or last visit)
IL001	43	05/2015	58	49	280	u.t.p.	n.p.	n.p.	n.p.	n.p.
IT001	26	10/2017	73	30	100	u.t.p.	68	64	65	64
IT002	29	11/2019	48		255,2	200	85	90	66	60
IT003	43	10/2021	21		370	360	97	95	64	63
Mn	**35.2**		**47.3**	**39.5**	**251.3**	**280**	**83.3**	**83.0**	**65.0**	**62.3**
SD	**9.0**		**21.2**	**13.4**	**112.3**	**113.1**	**14.6**	**16.6**	**0.82**	**1.7**
Range	26-45		21-73		100-370	200-360	68-97	64-95	64-66	60-64

Abbreviations. L.o.A.: Loss of ambulation; FVC: Forced Vital Capacity; LVEF: Left Ventricle Ejection Fraction; u.t.p.: unable to perform; n.p.: not performed.

with an average time between the onset of symptoms and muscle biopsy of 1.3 years (range 0-6). The average age at molecular confirmation of the diagnosis was 21 years, ranging from 3 to 38. Patient IT001 was additionally treated with deflazacort, antioxidants, calcium and vitamin D3. The other three had no additional drug therapy.

Treatment with ataluren was started at the mean age of 35.8 (range 26-45 years); the start date of treatment with ataluren is between May 2015 and October 2021. The average follow-up period was 47.3 months, ranging from 21 to 73. Two patients, IL001 and IT003, discontinued treatment during follow-up: at age 49 due to the loss of ambulation and after 21 months on a voluntary basis, respectively.

At the first visit, 6MWT was available for all patients, showing a mean value of 251.3 ± 112.3m, and at last visit in two patients (IT002 and IT003), with a mean value of 280 m ± 113.1m. NSAA total score was available for only one patient (IT002) who had a baseline score of 13/34 that improved to 15/34, at last visit. Time of rising from the floor was available for only one patient (IT003) and passed from 7.3 seconds at baseline to 7.0 at last visit. PUL test was available for only one patient (IT002) who had a baseline score of 29/74 that was stable at last visit.

None of the patients developed respiratory involvement or cardiomyopathy. Percentage FVC values were available for three out of four patients, and passed from 83.3 ± 14.6 to 83.0 ± 16.6 percent of those expected. LVEF values were available in 3 out of 4 carriers and

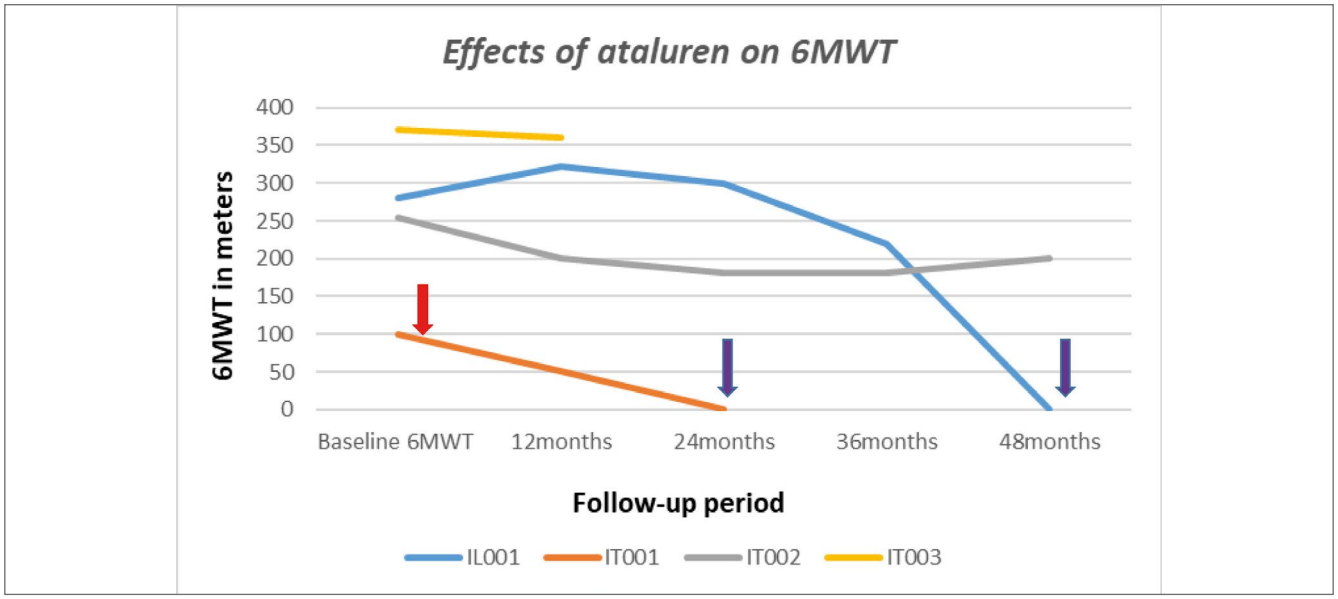

Figure 1. 1 Effects of ataluren on 6 MWT during the follow up The red arrow indicates the time of the femur fracture The purple arrows indicate after how many months of treatment LoA occurred.

varied on average from 65.0 to 62.3%. During the follow-up, two patients retained the ability to walk independently. However, patient IT001 lost her ambulation at age 30, and patient IL001 at 49 years. None of the patients reported side effects.

Discussion

In this study, we report our experience with the stop codon read-through therapy ataluren in four symptomatic nmDMD female carriers. We identified that this treatment showed a measurable improvement and/or stabilization in muscle performance. This effect was limited, and in two cases was temporary, but in all cases the treatment was started late in the course of their disease. Earlier treatment initiation may have provided better results.

Ataluren (Translarna® by PTC Therapeutics) received conditional approval in 2014 by the EMA [41] in ambulant nmDMD patients aged ≥ 5 years. The benefits of early treatment have also been highlighted [48,49]. Michael et al. [46] reported in 2021 the Swedish experience on long-term cumulative ataluren treatment in 11 patients with nmDMD, over a median period of 6.3 years. The results indicated a delay in loss of ambulation, as well as a slower decline in FVC and upper limb motor function even after loss of ambulation, suggesting treatment with ataluren *should be started as soon as the diagnosis is confirmed, monitored closely and, in case of sustainable benefit, continued even after loss of ambulation*. Limited data is available on non-ambulatory nmDMD patients treated with ataluren. Ebrahimi-Fakhari et al. [47] reported their experience in four non-ambulatory nmDMD patients. Routine investigations included cardiac function, pulmonary function tests and muscle strength. They compared changes in left ventricular fractional shorting, forced volume vital capacity and BMI from two defined time-periods (18-26-month period prior to and after ataluren start). Mean age at loss of ambulation was 10.1 ± 0.5 years, mean age when initiating ataluren treatment 14.1 ± 1.4 years. Serial echocardiography, pulmonary lung function tests, and assessment of muscle strength indicated mild attenuation of disease progression after initiation of ataluren treatment. No adverse clinical effects, or relevant abnormalities in routine laboratory values were reported. They concluded ataluren appeared to modestly ameliorate the clinical course in their patients with a good safety profile.

Following these encouraging results, the competent institutional authorities were asked to extend the treatment beyond the loss of ambulation, underlining that the disease progressively affects respiratory muscles and the heart, similarly to skeletal muscles.

In this scenario, symptomatic DMD carriers still remain a neglected population [50] with limited high-quality evidence to guide their treatment, and with available evidence mainly based on expert opinions and clinical experience. The prevalence of skeletal muscle dystrophy among Duchenne female carriers including asymptomatic carriers, is estimated to be between 2.5-19% [5,6], while the occurrence of dilated cardiomyopathy is between 7.3-16.7% [7,8].

Viggiano et al. [31] observed that DMD carriers with moderate/severe muscle involvement (in particular those with onset of symptoms before 15 years) exhibit a skewed or extremely skewed XCI, while carriers with mild or late onset muscle involvement present a random XCI. Moreover, when comparing carriers with muscle to those with heart-manifestations, the former group showed a higher degree of skewing [31-33]. The frequency of cardiomyopathy in carriers increases with age [13,16,52-54] and studies begin to appear on how and when to best treat these patients [53,54].

In this report, the first to the best of our knowledge on long-term treatment with ataluren in nmDMD symptomatic carriers, we evaluate the results in four patients treated for 17 to 73 months. The response to treatment was promising in all patients, with a tendency towards improvement or stabilization in muscle performance, at least in the first

periods. A separate comment deserves the response of patient IT001, who, despite having experienced a femur fracture forcing her to immobility for two months, with a consequent loss of ambulation, was able to resume it after only 12 weeks from resuming treatment. During follow-up, two patients retained the ability to walk independently at the age of 33 (IT002) and 45 (IT003) respectively, while the other two (IL001 and IT001) lost it, at the age of 49 and 30 respectively. The loss of ambulation and the age at which this occurs, seem to be related to the age of onset and severity of the symptoms, and to duration of the disease. In fact, patient IL001 presented severe muscle symptoms at around 30 years and patient IT001 had a very early onset of symptoms (18 months), and lost the ability to get up from the floor at 10 years. Responsiveness to treatment with ataluren may depend on a variety of factors. The most likely factor is the delay in initiation of treatment, which is indicated by the magnitude of weakness or gait dysfunction. In all our patients, treatment was started late around 21 years after the onset of symptoms, at a time when some motor functions such as the ability to get up from the floor or climb stairs were already lost. We know that, in progressive and degenerative diseases, starting treatment early gives better results. Even for patients suffering from DMD, the tendency of current therapeutic approaches is to start treatments as soon as possible [48,49]. In this regard, larger clinical trials, possibly on younger females are required to assess the role of ataluren and its long-term impact on disease progression in symptomatic nmDMD carriers. The degree of the skewed XCI pattern may also have an effect on severity of symptoms and responsiveness as well. However, XCI analysis was studied in only two of our cases (IL001 and IT001), and gave different results: an extremely skewed XCI pattern in IL001 (89:11) and a random pattern in IT001 (60:40). Interestingly the latter showed symptoms at a very early age, arguing against this assumption at least in this case. It is intriguing to correlate the weakness and the response to treatment to the level of dystrophin expressed in muscles. However, this was not possible because a western blot analysis of dystrophin was not available in the three patients, in whom muscle biopsy was carried out.

Respiratory involvement or cardiomyopathy was not detected in any of our patients during follow-up. This may be due to treatment with ataluren, but may be due to a late onset phenotype, which cannot be excluded. Adverse clinical events were not reported by the patients, nor relevant abnormalities were observed in routine laboratory values.

We are aware that the study has several limitations, which include the small number of carriers treated, use of retrospective data and lack of comparable untreated symptomatic DMD carriers. However, the estimated number of nmDMD patients is approximately 10-15%, and symptomatic nmDMD female carriers represent an even smaller percentage, indicating that our collection of patients is rare. Despite these limitations, we believe our study aims to draw the attention of clinicians and institutions to the condition of symptomatic DMD carriers and the need to offer them the therapeutic opportunities provided for affected males.

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Conflict of interest statement

The Authors have no conflicts of interest to declare that are relevant to the content of this article.

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Author's contributions

Conceptualization, methodology, writing original draft preparation, writing review, editing and supervision LP; investigation and data collection AD, MS, LPa, DZ, LR, AT; writing review AD, AT.
All authors have read and agreed to the published version of the manuscript.

Editor's Note

Following acceptance of the manuscript for publication, we learned that the EMA's Committee for Medicinal Products for Human Use (CHMP), on 26 January 2024, confirmed, following review, its initial recommendation not to renew the Conditional marketing authorization for Translarna (ataluren).

References

1 Carter JC, Sheehan DW, Prochoroff A, et al. Muscular Dystrophies. Clin Chest Med. 2018;39(2):377-89. https://doi.org/10.1016/j.ccm.2018.01.004.

2 Monaco AP. Dystrophin, the protein product of the Duchenne/Becker muscular dystrophy gene. Trends Biochem Sci. 1989;14(10):412-5. https://doi.org/10.1016/0968-0004(89)90290-9.

3 Kamdar F, Garry DJ. Dystrophin-Deficient Cardiomyopathy. J Am Coll Cardiol. 2016;67 (21):2533-46. https://doi.org/10.1016/j.jacc.2016.02.081.

4 Aartsma-Rus A, Ginjaar IB, Bushby K. The importance of genetic diagnosis for Duchenne muscular dystrophy. J Med Genet. 2016;53:145-151. https://doi.org/10.1136/jmedgenet-2015-103387

5 Mercier S, Toutain A, Toussaint A, Genetic and clinical specificity of 26 symptomatic carriers for dystrophinopathies at pediatric age. Eur J Hum Genet. 2013;21(8):855-63. https://doi.org/10.1038/ejhg.2012.269.

6 Ishizaki M, Kobayashi M, Adachi K, et al. Female dystrophinopathy: Review of current literature. Neuromuscul Disord. 2018;28(7):572-81. https://doi.org/10.1016/j.nmd.2018.04.005.

7 Politano L, Nigro V, Nigro G, et al. Development of cardiomyopathy in female carriers of Duchenne and Becker muscular dystrophies. JAMA. 1996;275(17):1335-8. PMID: 8614119.

8 Adachi K, Hashiguchi S, Saito M, et al. Detection and management of cardiomyopathy in female dystrophinopathy carriers. J Neurol Sci. 2018;386:74-80. https://doi.org/10.1016/j.jns.2017.12.024.

9 Moser H, Emery AE. The manifesting carrier in Duchenne muscular dystrophy. Clin Genet. 1974;5(4):271-84. https://doi.org/10.1111/j.1399-0004.1974.tb01694.x.

10 Norman A, Harper P. A survey of manifesting carriers of Duchenne and Becker muscular dystrophy in Wales. Clin Genet. 1989;36(1):31-7. https://doi.org/10.1111/j.1399-0004.1989.tb03363.x.

11 Barkhaus PE, Gilchrist JM. Duchenne muscular dystrophy manifesting carriers. Arch Neurol. 1989;46(6):673-5. https://doi.org/10.1001/archneur.1989.00520420093029.

12 Giliberto F, Radic CP, Luce L, et al. Symptomatic female carriers of Duchenne muscular dystrophy (DMD): genetic and clinical characterization. J Neurol Sci. 2014;336(1-2):36-41. https://doi.org/10.1016/j.jns.2013.09.036.

13 Imbornoni L, Price ET, Andrews J, et al. Diagnostic and clinical characteristics of early-manifesting females with Duchenne or Becker muscular dystrophy. Am J Med Genet A. 2014;164A(11):2769-74. https://doi.org/10.1002/ajmg.a.36728.

14 Lee SH, Lee JH, Lee KA, et al. Clinical and genetic characterization of female dystrophinopathy. J Clin Neurol. 2015;11(3):248-51. https://doi.org/10.3988/jcn.2015.11.3.248

15 Zhong J, Xie Y, Bhandari V, et al. Clinical and genetic characteristics of female dystrophinopathy carriers. Mol Med Rep. 2019;19(4):3035-44. https://doi.org/10.3892/mmr.2019.9982

16 Cruzeiro MM, Vale TC, Marrone CD. Symptomatic female carriers of mutations in the Duchenne muscular dystrophy gene. Arq Neuropsiquiatr. 2020;78(9):598-9. https://doi.org/10.1590/0004-282X20200061.

17 Papa R, Madia F, Bartolomeo D, et al. Genetic and early clinical manifestations of females heterozygous for Duchenne/Becker muscular dystrophy. Pediatr Neurol. 2016;55: 58-63. https://doi.org/10.1016/j.pediatrneurol.2015.11.004.

18 Percy ME, Andrews DF, Thompson MW. Serum creatine kinase in the detection of Duchenne muscular dystrophy carriers: effects of season and multiple testing. Muscle Nerve. 1982;5(1):58-64. https://doi.org/10.1002/mus.880050111.

19 Zhang J, Meng Q, Zhong J, et al. Serum MyomiRs as Biomarkers for Female Carriers of Duchenne/Becker Muscular Dystrophy. Front Neurol. 2020;11:563609. https://doi.org/10.3389/fneur.2020.563609.

20 Florian A, Rösch S, Bietenbeck M, et al. Cardiac involvement in female Duchenne and Becker muscular dystrophy carriers in comparison to their first-degree male relatives: a comparative cardiovascular magnetic resonance study. Eur Heart J Cardiovasc Imaging. 2016;17(3):326-33. https://doi.org/10.1093/ehjci/jev161.

21 Monk M, Boubelik M, Lehnert S. Temporal and regional changes in DNA methylation in the embryonic, extraembryonic and germ cell lineages during mouse embryo development. Development. 1987;99(3):371-382. https://doi.org/10.1242/dev.99.3.371.

22 Orstavik KH, Orstavik RE, Halse J, et al. X chromosome inactivation pattern in female carriers of X linked hypophosphataemic rickets. J Med Genet 1996;33:700-703. https://doi.org/ 10.1136/jmg.33.8.700

23 Satoh M, Ogikubo S, Yoshizawa-Ogasawara A. Correlation between clinical phenotypes and X-inactivation patterns in six female carriers with heterozygote vasopressin type 2 receptor gene mutations. Endocr J 2008; 55:277-284. https://doi.org/ 10.1507/endocrj.k07-083

24 Matthews PM, Benjamin D, Van Bakel I et al. Muscle X-inactivation patterns and dystrophin expression in Duchenne muscular dystrophy carriers. Neuromuscul Disord 1995;5:209-220. https://doi.org/10.1016/0960-8966(94)00057-G

25 Amos-Landgraf JM, Cottle A, Plenge RM et al. X chromosome-inactivation patterns of 1,005 phenotypically unaffected females. Am J Hum Genet 2006;79:493-499. https://doi.org/ 10.1086/507565

26 Orstavik KH X chromosome inactivation in clinical practice. Hum Genet 2009;126:363-373. https://doi.org/10.1007/s00439-009-0670-5

27 Van den Veyver IB. Skewed X inactivation in X-linked disorders. Semin Reprod Med. 2001;19:183-191. https://doi.org/10.1055/s-2001-15398.

28 Emanuel BS, Zackai EH, Tucker SH. Further evidence for Xp21 location of Duchenne muscular dystrophy (DMD) locus: X;9 translocation in a female with DMD. J Med Genet. 1983;20(6):461-463. https://doi.org/10.1136/jmg.20.6.461.

29 Nevin NC, Hughes AE, Calwell M, et al. Duchenne muscular dystrophy in a female with a translocation involving Xp21. J Med Genet. 1986;23(2):171-3. https://doi.org/10.1136/jmg.23.2.171.

30 Zatz M, Vianna-Morgante AM, Campos P, Diament AJ. Translocation (X;6) in a female with Duchenne muscular dystrophy: implications for the localisation of the DMD locus. J Med Genet. 1981;18(6):442-7. https://doi.org/10.1136/jmg.18.6.442.

31 Viggiano E, Ergoli M, Picillo E, et al. Determining the role of skewed X-chromosome inactivation in developing muscle symptoms in carriers of Duchenne muscular dystrophy. Hum Genet. 2016;135(7):685-98. https://doi.org/10.1007/s00439-016-1666-6.

32 Juan-Mateu J, Rodríguez MJ, Nascimento A, et al. Prognostic value of X-chromosome inactivation in symptomatic female carriers of dystrophinopathy. Orphanet J Rare Dis. 2012;7:82. https://doi.org/10.1186/1750-1172-7-82.

33 Viggiano E, Picillo E, Cirillo A, et al. Comparison of X-chromosome inactivation in Duchenne muscle/myocardium-manifesting carriers, non-manifesting carriers and related daughters. Clin Genet. 2013;84(3):265-70. https://doi.org/10.1111/cge.12048.

34 Bushby K, Muntoni F, Urtizberea A, et al. Report on the 124th ENMC International Workshop. Treatment of Duchenne muscular dystrophy; defining the gold standards of management in the use of corticosteroids. 2-4 April 2004, Naarden, The Netherlands. Neuromuscul Disord. 2004;14(8-9):526-34. https://doi.org/10.1016/j.nmd.2004.05.006.

35 D'Amario D, Gowran A, Canonico F, et al. Dystrophin Cardiomyopathies: Clinical Management, Molecular Pathogenesis and Evolution towards Precision Medicine. J Clin Med. 2018;7(9):291. https://doi.org/10.3390/jcm7090291.

36 Chamberlain JR, Chamberlain JS. Progress toward Gene Therapy for Duchenne Muscular Dystrophy. Mol Ther. 2017;25(5):1125-31. https://doi.org/10.1016/j.ymthe.2017.02.019.

37 Wood MJ. To skip or not to skip: that is the question for Duchenne muscular dystrophy. Mol Ther. 2013;21:2131-2 https://doi.org/10.1038/mt.2013.252.

38 Politano L, Nigro G, Nigro V, et al. Gentamicin administration in Duchenne patients with premature stop codon. Preliminary results. Acta Myol. 2003;22:15-21. PMID: 12966700.

39 Finkel RS. Read-through strategies for suppression of nonsense mutations in Duchenne/ Becker muscular dystrophy: aminoglycosides and ataluren (PTC124). J Child Neurol. 2010;25(9):1158-64. https://doi.org/10.1177/0883073810371129.

40 Politano L. Read-through approach for stop mutations in Duchenne muscular dystrophy. An update. Acta Myol. 2021;40(1):43-50. https://doi.org/10.36185/2532-1900-041

41 PTC Therapeutics. PTC Therapeutics receives conditional approval in the European Union for Translarna for the treatment of nonsense mutation Duchenne Muscular Dystrophy. 2014, available online: http://ir.ptcbio.com/releasedetail.cfm?releaseid=863914 (accessed on 26 November 2023).

42 McDonald CM, Campbell C, Torricelli RE, et al. Ataluren in patients with nonsense mutation Duchenne muscular dystrophy (ACT DMD): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2017;390(10101):1489-1498. https://doi.org/10.1016/S0140-6736(17)31611-2.

43 Campbell C, Barohn RJ, Bertini E, et al. Meta-analyses of ataluren randomized controlled trials in nonsense mutation Duchenne muscular dystrophy. J Comp Eff Res. 2020;9(14):973-984. https://doi.org/10.2217/cer-2020-0095.

44 Michorowska S. Ataluren-promising therapeutic premature termination codon readthrough frontrunner. Pharmaceuticals (Basel). 2021;14(8):785. https://doi.org/10.3390/ph14080785.

45 Mercuri E, Muntoni F, Osorio AN et al. Safety and effectiveness of Ataluren: Comparison of results from the STRIDE Registry and CINRG DMD Natural History Study. J. Comp. Eff. Res. 2020;9:341-60. https://doi.org/10.2217/cer-2019-0171.

46 46. Michael E, Sofou K, Wahlgren L, et al. Long-term treatment with Ataluren. The Swedish experience. BMC Musculoskelet Disord. 2021;22(1):837. https://doi.org/10.1186/s12891-021-04700-z.

47 Ebrahimi-Fakhari D, Dillmann U, Flotats-Bastardas M, et al. Off-Label Use of Ataluren in four non-ambulatory patients with nonsense mutation Duchenne Muscular Dystrophy: Effects on cardiac and pulmonary function and muscle strength. Front Pediatr. 2018;6:316. https://doi.org/10.3389/fped.2018.00316.

48 Ruggiero L, Iodice R, Esposito M, et al. One-year follow up of three Italian patients with Duchenne muscular dystrophy treated with Ataluren: is earlier better? Ther Adv Neurol Disord. 2018;11:1756286418809588. https://doi.org/10.1177/1756286418809588.

49 Bitetti I, Mautone C, Bertella M, Manna MR, Varone A. Early treatment with Ataluren of a 2-year-old boy with nonsense mutation Duchenne dystrophy. Acta Myol. 2021;40(4):184-186. https://doi.org/10.36185/2532-1900-062.

50 Politano L. Females with dystrophinopathy: A neglected patient population. Dev Med Child Neurol. 2023;65(8):1001-1002. https://doi.org/10.1111/dmcn.15525

51 Hogrel JY, Zagnoli F, Canal A, et al. Assessment of a symptomatic Duchenne muscular dystrophy carrier 20 years after myoblast transplantation from her asymptomatic identical twin sister. Neuromuscul Disord. 2013;23(7):575-9. https://doi.org/10.1016/j.nmd.2013.04.007.

52 Lim KRQ, Sheri N, Nguyen Q, et al. Cardiac Involvement in Dystrophin-Deficient Females: Current Understanding and Implications for the Treatment of Dystrophinopathies. Genes (Basel). 2020;11(7):765. https://doi.org/10.3390/genes11070765.

53 Sarkozy A, Quinlivan R, Bourke JP, et al. 263rd ENMC International Workshop: Focus on female carriers of dystrophinopathy: refining recommendations for prevention, diagnosis, surveillance, and treatment. Hoofddorp, The Netherlands, 13-15 May 2022. Neuromuscul Disord. 2023;33(3):274-284. https://doi.org/10.1016/j.nmd.2023.01.003.

54 D'Ambrosio P, Orsini C, Nigro V, et al. Therapeutic approach with Ataluren in Duchenne symptomatic carriers with nonsense mutations in dystrophin gene. Results of a 9-month follow-up in a case report. Acta Myol. 2018;37(4):272-4. PMID: 30944907

55 Pane M, Mazzone ES, Fanelli L, et al. Reliability of the Performance of Upper Limb assessment in Duchenne muscular dystrophy. Neuromuscul Disord. 2014;24(3):201-6. https://doi.org/10.1016/j.nmd.2013.11.014.