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ScienceDirect

Neuromuscular Disorders 25 (2015) 371–374

www.elsevier.com/locate/nmd

Clinical Casebook

A normal life without muscle dystrophin

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Received 27 November 2014; received in revised form 9 February 2015; accepted 10 February 2015

Abstract

Here we summarize the clinical history of Ringo, a golden retriever muscular dystrophy (GRMD) dog, who had a mild phenotype despite the absence of muscle dystrophin. Ringo died of cardiac arrest at age 11 and therefore displayed a normal lifespan. One of his descendants, Suflair, born April 2006, also displays a mild course. Dystrophin analysis confirmed total absence of muscle dystrophin in both dogs. Muscle utrophin expression did not differ from severely affected GRMD dogs. Finding what protects these special dogs from the dystrophic degeneration process is now a great challenge that may open new avenues for treatment. But most importantly, the demonstration that it is possible to have a functional muscle, in a medium-large animal even in the absence of dystrophin, brings new hope for Duchenne patients.

Keywords: GRMD mild course; Functional muscle no dystrophin; Muscular dystrophy; Mild course no utrophin up-regulation

Introduction

This is the story of Ringo, the famous golden retriever muscular dystrophy (GRMD) dog, born July 2003 and deceased August 2014. Grossly elevated serum creatine-kinase and DNA testing confirmed he had inherited the GRMD mutation from his GRMD mother [1]. As a newborn, he was so different from his affected littermates that it was difficult to believe he carried the dystrophin mutation. In order to be convinced, we repeated his genotyping 5 times, from different blood samplings. While his littermates and other GRMD dogs from our colony were dying during their first year of life or were severely affected, Ringo, who was unaware of his genetic condition, behaved as if he were a normal dog. He could run, stand on his pelvic limbs, opened doors and flirted with all the female dogs from the kennel. He escaped several times and, without asking anyone's permission, fertilized 4 different GRMD carrier females, one of them twice. As a result, he had 49 descendants, 29 males and 20 females. Among the affected offspring, Suflair, born April 2006 (currently 8 yrs and 10 months old), also shows a mild phenotype. As previously reported [2], histopathological analysis in repeated muscle biopsies from Ringo and Suflair showed typical features of a dystrophic process such as fiber size variation, splitting, necrosis, central nuclei, rounded fibers, in a pattern comparable to severely affected dogs. Dystrophin was also totally absent in their muscle. Utrophin analysis revealed its presence in muscle fibers sarcolemma from both Ringo and Suflair, as also observed in the severely GRMD affected dogs, while it was present only in the neuromuscular junction in normal control. Utrophin expression analysis through Western blot was also investigated in Ringo and Suflair as compared to one normal and three severely affected dogs. The expression was upregulated in all affected dogs independently of the severity of their clinical course [2] and therefore could not provide an explanation for their milder phenotype. Serum CK was always elevated (around 20,000 u/l, normal up to 220 u/l).

On December 2011, Ringo (aged 8 years, 8 months) had a unilateral testicular tumor (seminoma) which had to be

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http://dx.doi.org/10.1016/j.nmd.2015.02.007
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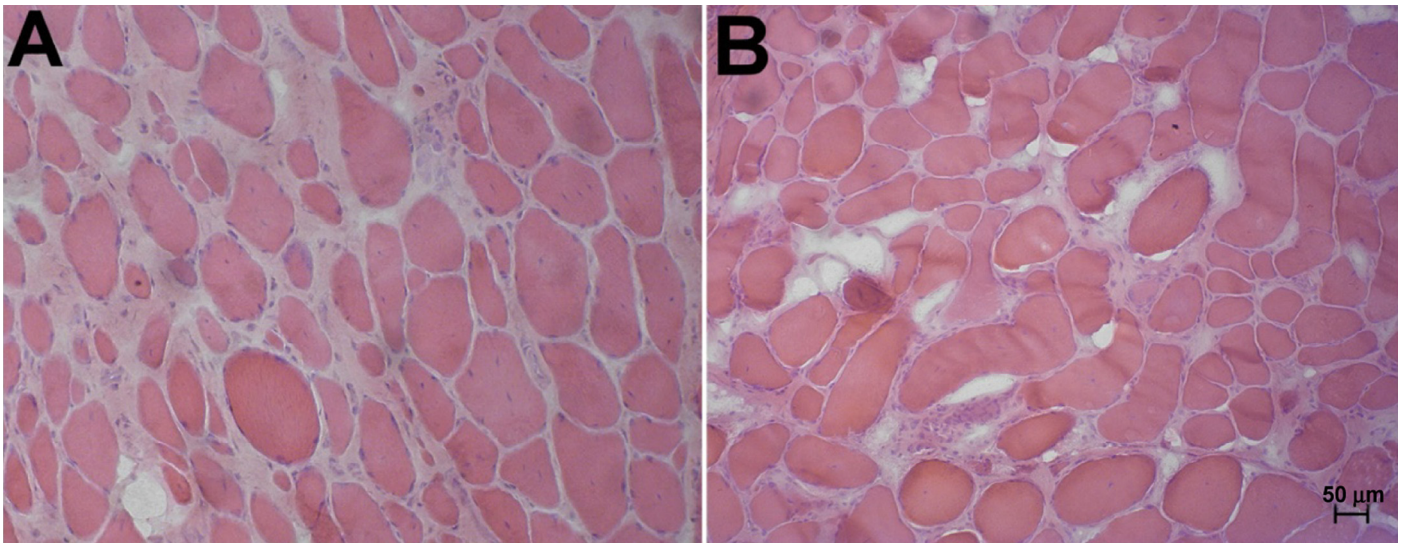

Fig. 1. H&E staining illustrating the histopathological features of (A) biceps muscle from Ringo at the age of 10 years and 9 months, as compared to (B) muscle histopathology observed in a late stage GRMD dog, two weeks before his death.

removed surgically. After that, he started to gain weight and have more difficulty running. In the last year of his life, he had difficulties standing up, but could walk and eat normally.

His last muscle biopsy, taken at the age of 10 years, 9 months, showed a histopathological pattern similar to the one observed in a late stage GRMD dog with a significant replacement of muscle fibers by connective tissue and fat (Fig. 1).

Last August, Ringo died suddenly of cardiac arrest, one month after turning 11 years old.

On necropsy, the endocardium revealed thickening of the right and left atrioventricular valves, myocardial hypertrophy with eccentric dilated ventricular chambers. Microscopic analysis revealed diffuse myxoid degeneration (endocardiosis) in the region of the atrioventricular valve endocardium. In the epicardium and myocardium regions, there was intense fatty infiltration. This replacement of the myocardium by fat was more pronounced in the right ventricle region where there were almost no cardiomyocytes. Other regions show replacement of cardiac striated muscle by fibrous tissue (multifocal fibrosis). *C*omputed tomography confirmed that the *causa mortis* was heart failure caused by dilated cardiomyopathy. A moderate atrioventricular valve endocardiosis as well as fat and fibrotic tissue replacement mostly in the left ventricle were also observed.

Dilated cardiomyopathy is the most prevalent form of cardiomyopathy in non-dystrophic dogs, with increased risk in large dogs and with advancing age [3,4].

Post-mortem muscle MRI acquired in a 3.0 T MRI system (Intera Achieva, Philips Healthcare, Best, The Netherlands) showed atrophic muscles with a variable degree of fat infiltration. In the pelvic limb, evidences of fat infiltration were observed in the extensor digitorium longus (EDL) muscle. The estimated fat percentage from three-point DIXON images was of 21% for the EDL, while the other lower leg muscles presented normal appearance with up to 6% of fat. Interestingly, the thigh presented a large amount of intermuscular fat combined to fat infiltration (up to 28%) in several muscles (Fig. 2), similar to what is observed in DMD boys [5]. Increased intermuscular fat and alterations in T2 weighted muscle images have also been described in GRMD dogs [6–8]. However, in all these studies, the dogs were up to 12 months old, and the lesions observed in MRI were related to necrosis and inflammation, but not to fat infiltration. It is possible that the more pronounced muscle fat infiltration, which until now was not observed in younger GRMD dogs, is related to Ringo's advanced age.

According to Dubowitz [9], we should value our exceptions. Indeed, Ringo and Suflair have been the subject of many investigations. Our ultimate goal is to find what is different in these two dogs since it could represent a novel therapeutic target for Duchenne dystrophy. The good news is that Ringo and Suflair are not an exception anymore. A colony of Labrador muscular dystrophy dogs (LRMD) has been recently identified with the same characteristics as Ringo and Suflair [10]. These asymptomatic LRMD dogs, unrelated to our GRMD dogs, have no muscle dystrophin, nor do they have utrophin expression different from severely affected dogs.

Finding what protects these special dogs from the dystrophic degeneration process is now a great challenge that may open new avenues for treatment. But most importantly, these findings demonstrate that it is possible to have a functional muscle even in the absence of dystrophin, and this brings new hope for Duchenne patients.

Acknowledgements

We are very grateful to many people who gave us invaluable help during all these years, in particular the