RESEARCH REPORT

1. Proposal number: 2008B1972

2. Title of experiment: Tropomyosin mutations associated with muscle weakness and nemaline myopathy: Structure and Function of the thin filament studied by X-ray diffraction

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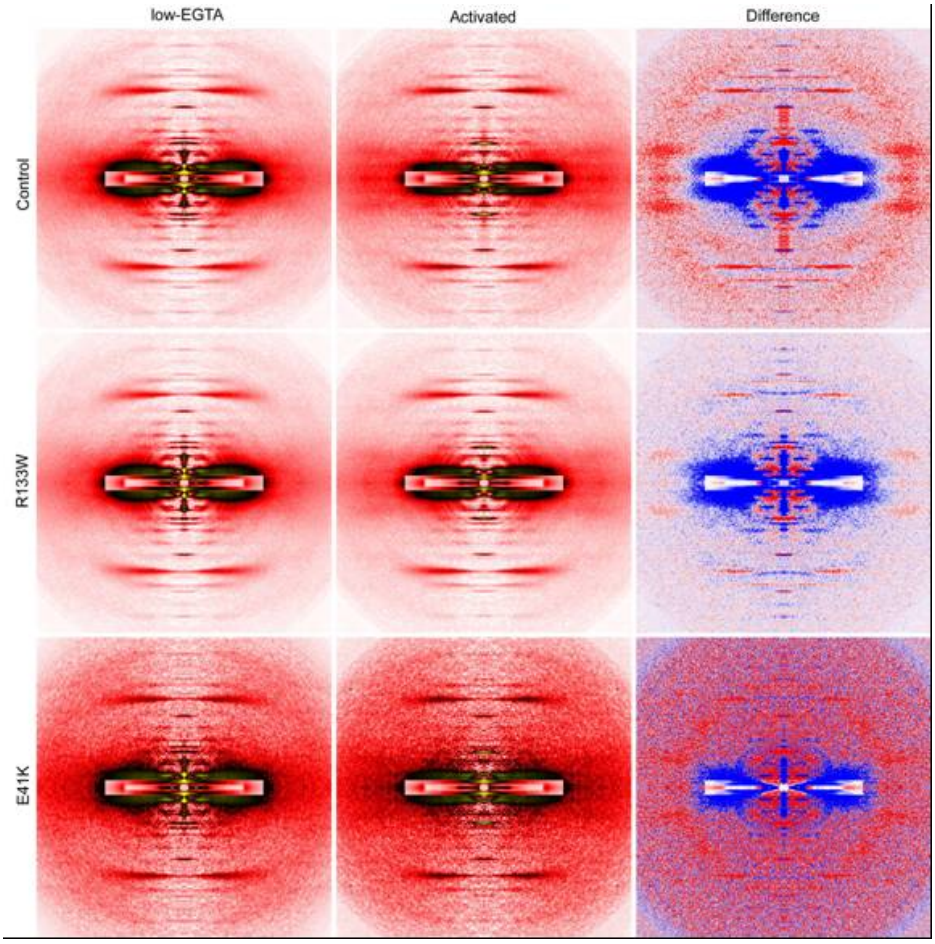
4. Beamline used: BL45XU (small-angle scattering station)

5. Research background and purpose: Nemaline myopathy is an under-appreciated congenital neuromuscular disorder (Sanoudou & Beggs, 2001), classically defined by weakness with limb, facial, masticatory and respiratory muscles involvements. The overall frailty is handicapping for most of the daily motor activities, and has a dramatic negative impact on the quality of life. To date, no cure exists. Understanding the mechanisms underlying muscle weakness in nemaline myopathy appears to be primordial and urgent in order to develop potential therapeutic strategies.

Recent advances demonstrate that nemaline myopathy is primarily due to mutations in genes coding skeletal muscle sarcomere proteins and, in particular, proteins of the thin filament such as tropomyosin (Michele *et al.*, 1999; Donner *et al.*, 2002; Tajsharghi *et al.*, 2007). Tropomyosin mutations are relatively subtle mutations, typically missense mutations changing just one DNA nucleotide and resulting in the substitution of just one amino acid in the protein. This raises the question of the mechanisms by which such minor changes result in muscle weakness. The present proposal specifically aimed at investigating how different tropomyosin mutations alter thin filament structure and function leading to muscle weakness in nemaline myopathy.

6. Experimental methodology: We performed X-ray diffraction experiments on membrane-permeabilized muscle fibres coming from biopsy sample specimens from patients carrying different tropomyosin mutations (E41K and R133W) associated with nemaline myopathy, and from healthy controls. The experiments were carried out using the SPring-8 synchrotron radiation facility (Harima, Hyogo, Japan). On the day of experiment, muscle fibres were dissected and mounted in arrays of 30 fibres. X-ray diffraction patterns were recorded for each array of 30 membrane permeabilized fibres in relaxing (low [Ca^{2+}]) and activating (high [Ca^{2+}]) solutions by using a cooled CCD (charge-coupled device) camera (C4880, Hamamatsu Photonics, 1000 × 1018 pixels) in combination with an X-ray image intensifier (V5445P, Hamamatsu Photonics). The wavelength was 0.09 nm, and the specimen-to-detector distance was ~2 m. To compensate for the relatively small dynamic range of the detector, absorber masks made of aluminum and copper were placed at the center of the image intensifier. The exposure time was ~2 s, and usually several to tens of patterns were summed to obtain a final image to be analyzed. The four quadrants of the image were folded after correction for the fiber inclination, and the background was subtracted.

7. Research results: Briefly, in the figure, the left column corresponds to recordings in the relaxing solution, the middle in activating solution, and the right is the difference. In the "difference" patterns, the red color represents the area in which intensity was increased after activation, and the blue color, decreased. In fibres from controls, the area for the 2nd actin layer line (ALL) (tropomyosin LL) was clearly enhanced. Other reflections, including the 6th (59A) and 7th (51A) LLs, were also clearly enhanced. In fibres from patients carrying a tropomyosin mutation (R133W) and diagnosed with myopathy, these enhancements were much weaker, suggesting that the regulatory system was not fully turned on (explaining muscle weakness). On the other hand, in fibres from patients carrying another tropomyosin mutation (E41K) and with myopathy, the patterns were less visible because of insufficient counting statistics, but the enhancement of the 2nd and 6th ALL was still evident.



8. Current/future issues: This was the first time that X-ray diffraction patterns were successfully recorded in humans in health and disease. All the pilot results taken together are promising and show that distinct tropomyosin mutations differently alter thin filament structure and function leading to muscle weakness in nemaline myopathy. Nevertheless, more experiments using X-ray diffraction are required to clearly define the function impairments caused by the various tropomyosin mutations in nemaline myopathy.

9. References:

Donner, K., Ollikainen, M., Ridanpaa, M., Christen, H. J., Goebel, H. H., De Visser, M., Pelin, K. & Wallgren-Pettersson, C. (2002). Mutations in the beta-tropomyosin (TPM2) gene--a rare cause of nemaline myopathy. Neuromuscul Disord 12, 151-158.

Michele, D. E., Albayya, F. P. & Metzger, J. M. (1999). A nemaline myopathy mutation in alpha-tropomyosin causes defective regulation of striated muscle force production. J Clin Invest 104, 1575-1581.

Sanoudou, D. & Beggs, A. H. (2001). Clinical and genetic heterogeneity in nemaline myopathy--a disease of skeletal muscle thin filaments. Trends Mol Med 7, 362-368.

Tajsharghi, H., Ohlsson, M., Lindberg, C. & Oldfors, A. (2007). Congenital Myopathy With Nemaline Rods and Cap Structures Caused by a Mutation in the beta-Tropomyosin Gene (TPM2). Arch Neurol 64, 1334-1338.

10. Status of publication: More experiments using X-ray diffraction are needed before submitting the data to a scientific journal.

11. Key words: Muscle disease, gene mutation, tropomyosin, structure and function.