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Abstract

More than a billion humans worldwide are predicted to be completely deficient in the fast skeletal muscle fibre protein α -actinin-3 due to homozygosity for a premature stop codon polymorphism, R577X, in the *ACTN3* gene. The R577X polymorphism is associated with elite athlete status and human muscle performance, suggesting that α -actinin-3 deficiency influences the function of fast muscle fibers. Here we show that loss of α -actinin-3 expression in a knockout mouse model results in a shift in muscle metabolism towards the more efficient aerobic pathway, and an increase in intrinsic endurance performance. In addition, we demonstrate that the genomic region surrounding the 577X null allele displays low levels of genetic variation and recombination in individuals of European and East Asian descent, consistent with strong, recent positive selection. We propose that the 577X allele has been positively selected in some human populations due to its effect on skeletal muscle metabolism.

Background

The Institute for Neuromuscular Research (which is part of the Children's Hospital at Westmead and affiliated with the University) has a major focus on the diagnosis and treatment of children with muscle diseases, such as muscular dystrophy. However, we also have an interest in individuals at the other end of the spectrum - elite athletes. If we can understand what factors improve muscle performance then that may, in turn, have applications for the treatment of people with muscle disease.

We are particularly interested in a gene, called the *ACTN3* gene, which contains the recipe for making a specific protein called alpha-actinin-3. This protein is found in fast-twitch skeletal muscle fibres (the cells that are required for rapid, forceful movement, e.g. in sprinters and weight-lifters). Several years ago members of our team showed that there is a common variation of the *ACTN3* gene that does not actually make any of this protein. We call this version of the gene 577X. Amazingly, it turns out that almost 20% of the general population (and over one billion people worldwide) carry two copies of this 577X version, and are thus completely deficient in alpha-actinin-3.

To determine whether the absence of alpha-actinin-3 had an impact on muscle function, we collaborated with the Australian Institute of Sport to look at how common the 577X version was in elite athletes from a variety of sports. We showed that alpha-actinin-3 deficiency was extremely rare in sprint athletes, suggesting that this protein plays a crucial role in the function of fast-twitch muscle fibres. Interestingly, the frequency of alpha-actinin-3 deficiency was actually higher in endurance athletes than in the normal population, suggesting that the loss of this protein may actually provide a benefit for endurance performance.

This research was published in 2003 and received some media attention at the time; good lay summaries were published in *New Scientist* (<http://www.newscientist.com/article.ns?id=dn4092>) and *The Bulletin* (<http://bulletin.ninemsn.com.au/bulletin/EdDesk.nsf/All/171A35355EDDABB7CA256D8C0002E39A>).

Since these results were published, similar studies have been performed in groups of athletes in Finland and Greece, and both studies found the same results: a very low frequency of alpha-actinin-3 deficiency in sprinters, and a higher frequency in endurance athletes. The effect on sprint athletes is particularly strong: of the 74 Olympic-level sprint athletes that have so far been tested, not a single one has been alpha-actinin-3 deficient. In addition, a number of groups have shown that the 577X variant also affects muscle function in the general population, with individuals with alpha-actinin-3 deficiency being slightly weaker and having reduced sprint performance compared to individuals with one or two functional copies of the ACTN3 gene.

We are now focussed on *how* the loss of alpha-actinin-3 influences the function of muscle. To answer this question, we have spent the last few years developing a strain of mice engineered to be completely deficient in alpha-actinin-3. Overall, these "knockout" mice are quite healthy, as you would expect given that the 20% of humans who are also deficient in alpha-actinin-3 do not suffer from any serious disease. However, we have now shown that the muscle of knockout mice displays an increase in a particularly efficient form of metabolism, known as oxidative metabolism. This shift towards a more efficient metabolic pathway could explain why alpha-actinin-3 deficiency is so common amongst endurance athletes.

Finally, we have used DNA samples from 96 individuals from around the world to examine the evolutionary past of the 577X variant. Our analysis suggests the alpha-actinin-3 deficiency actually provided some benefit to the ancestors of modern Europeans and Asians following their migration out of Africa, resulting in it rapidly increasing in frequency in these populations due to natural selection. We hypothesise that the benefit provided by alpha-actinin-3 deficiency was due to more efficient muscle metabolism, which may have allowed them to adapt to the more hostile environments of Eurasia.

This work is the subject of our paper to be published in *Nature Genetics* in early September.