

Inheritance of Two Benign Genetic Variants Causes a Muscular Dystrophy

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One of the most common forms of muscular dystrophy, fascioscapulohumeral dystrophy (FSHD), is clinically characterized by the initial onset of weakness in the facial muscles, which progresses to muscles of the shoulder blade (scapula), upper arm (humerus) and ultimately the trunk and lower limbs. Two years ago, an international team of researchers, including the laboratory of Dr. Stephen Tapscott (Human Biology Division), discovered that FSHD is caused by the expression of a specific transcription factor, *DUX4*, in adult skeletal muscle (Lemmers *et al.*, 2010; Snider *et al.*, 2010). Two genetically distinct types of this disease (FSHD1 and FSHD2) result in clinically indistinguishable symptoms, yet previously, the researchers only understood how *DUX4* was activated in FSHD1 patients. In a recent paper by the same research team, Lemmers *et al.* (2012) now report how they successfully deciphered the corresponding mechanism of *DUX4* activation for FSHD2.

The enduring collaboration that led to this important advancement in understanding mechanisms of muscular dystrophy has involved, among others, Drs. Silvère van der Maarel and Richard Lemmers (Leiden University), Dr. Daniel Miller (University of Washington), Dr. Rabi Tawil (University of Rochester), and past and present members of the Tapscott Lab (including Dr. Lauren Snider, Dr. Galina Filippova and former graduate student Dr. Linda Geng). A major source of funding for this work has come from a Program Project Grant (P01) awarded by NIH's National Institute of Neurological Disorders and Stroke. This grant has been headed by Dr. Tapscott and administered through the Fred Hutchinson Cancer Research Center.

In earlier studies, the researchers found that *DUX4* is normally expressed in the germline, where it activates gametogenic and early developmental genes (Snider *et al.*, 2010; Geng *et al.*, 2012). In contrast, *DUX4* is epigenetically repressed in healthy somatic cells, including differentiated muscle tissue. Multiple copies of the *DUX4* retrogene lie within a D4Z4 macrosatellite repeat array (composed of a tandemly repeated 3.3 kilobase DNA unit) that is located near one end of chromosome 4 (Chr 4). FSHD1 is caused by D4Z4 array contraction, which results in DNA hypomethylation and decreased repressive heterochromatin (*i.e.*, chromatin relaxation) in this region of Chr 4. Healthy individuals have 11 to 100 D4Z4 repeat units, whereas individuals with FSHD1 have fewer than 11 repeats. In all types of FSHD, chromatin relaxation makes the epigenetic

repression of *DUX4* less efficient, resulting in variegated *DUX4* expression in a subset of skeletal muscle nuclei. However, contraction of the D4Z4 array, by itself, does not cause FSHD. A tightly linked Chr 4 haplotype permissive of *DUX4* expression is also required. This haplotype contains a DNA sequence variant distal to the last D4Z4 repeat that signals polyadenylation of *DUX4* mRNA and stabilizes the mRNA. In individuals affected by either FSHD1 or FSHD2, the *DUX4* transcription factor damages muscle by activating stem cell and germline genes, which are toxic to muscle cells, as well as β -defensin 3, which inhibits muscle cell differentiation (Geng *et al.*, 2012).

Though the release of *DUX4* from epigenetic repression is most commonly caused by D4Z4 contraction, a substantial number of people with contraction-independent FSDH are genetically diagnosed each year. Because people suffering from this second type of FSHD are also known to exhibit DNA hypomethylation and relaxed chromatin at the D4Z4 array, Lemmers *et al.* performed a pedigree analysis on several families with a history of FSHD2 (see figure). They did so to determine whether an epigenetic modifier of the D4Z4 array, located somewhere else in the genome, causes FSHD2 when combined with the FSHD-permissive *DUX4* allele. The researchers first measured D4Z4 hypomethylation in study participants and their unaffected relatives. They found that not only do FSHD2-affected individuals have much lower levels of D4Z4 methylation compared to the general population, but the hypomethylation-trait was present in some other family members as a genetic trait that was inherited independently from the FSHD-permissive Chr 4 haplotype. Next, the authors sequenced the exomes (*i.e.*, the coding portions of all genes known to be expressed) of twelve individuals in seven families with the hypomethylation trait. This revealed rare mutations in one of the two copies of *SMCHD1* on Chr 18 as the cause of the hypomethylation-trait in all but one family. All mutations in this gene were found to lower the amount of *SMCHD1* protein produced in somatic cells substantially, suggesting that a single functional copy of *SMCHD1* is 'haploinsufficient' and does not produce enough *SMCHD1* protein to prevent *DUX4* de-repression. Finally, using experimental gene knockdown with RNA interference, the researchers demonstrated that reduced *SMCHD1* expression in muscle cells cultured from healthy individuals triggered *DUX4* expression at levels matching those seen in individuals with FSHD2. Therefore, FSHD2 is caused by the independent inheritance of an FSHD-permissive Chr 4 haplotype (signaling polyadenylation of *DUX4* mRNA) and rare variants in *SMCHD1* that cause D4Z4 hypomethylation, neither of which by itself causes disease.

This important study identifies *SMCHD1* as an epigenetic modifier of the D4Z4 array and the causal genetic determinant of FSHD2. *SMCHD1* is a member of the 'Structural Maintenance of

Chromosomes' (SMC) gene family. SMC genes code for proteins that regulate chromatin repression in a wide variety of organisms. *SMCHD1* itself was first identified in mice, where mutations of this gene epigenetically influence the probability that the coat color gene *agouti* is expressed in different patches of skin, resulting in mice with mottled fur. Given the profound epigenetic effects of *SMCHD1* in humans, mice and many other organisms, the authors suggest that we may find *SMCHD1* to be a genetic modifier of other human diseases as well.

[Lemmers RJLF, Tawil R, Petek LM, Balog J, Block GJ, Santen GWE, Amell AM, van der Vliet PJ, Almomani R, Straasheijm KR, Krom YD, Klooster R, Sun Y, den Dunnen JT, Helmer Q, Donlin-Smith CM, Padberg GW, van Engelen BGM, de Greef JC, Aartsma-Rus AM, Frants RR, de Visser M, Desnuelle C, Sacconi S, Filippova GN, Bakker B, Bamshad MJ, Tapscott SJ, Miller DG, van der Maarel SM](#). 2012. Digenic inheritance of an *SMCHD1* mutation and an FSHD-permissive D4Z4 allele causes facioscapulohumeral muscular dystrophy type 2. *Nature Genetics*, advanced online publication, released on 11 November 2012, doi:10.1038/ng.2454.

Also see: [Geng LN, Yao Z, Snider L, Fong AP, Cech JN, Young JM, van der Maarel SM, Ruzzo WL, Gentleman RC, Tawil R, Tapscott SJ](#). 2012. DUX4 activates germline genes, retroelements, and immune mediators: implications for facioscapulohumeral dystrophy. *Developmental Cell* 22:38-51.

[Lemmers RJ, van der Vliet PJ, Klooster R, Sacconi S, Camaño P, Dauwerse JG, Snider L, Straasheijm KR, van Ommen GJ, Padberg GW, Miller DG, Tapscott SJ, Tawil R, Frants RR, van der Maarel SM](#). 2010. A unifying genetic model for facioscapulohumeral muscular dystrophy. *Science* 329:1650-1653.

[Snider L, Geng LN, Lemmers RJ, Kyba M, Ware CB, Nelson AM, Tawil R, Filippova GN, van der Maarel SM, Tapscott SJ, Miller DG](#). 2010. Facioscapulohumeral dystrophy: incomplete suppression of a retrotransposed gene. *PLoS Genetics* 6:e1001181.

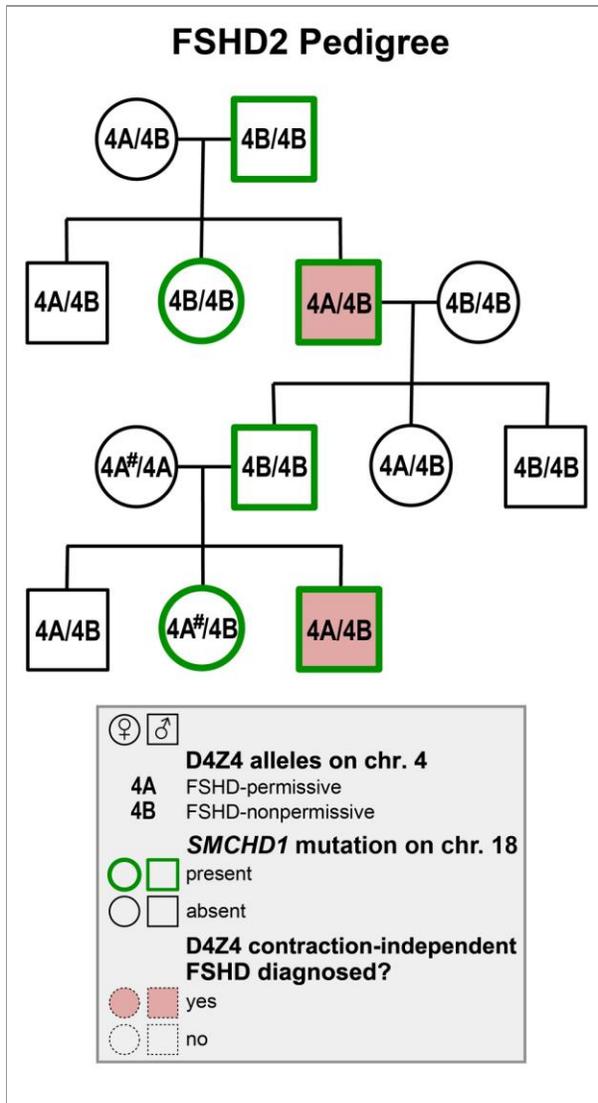


Image courtesy of Stephen Tapscott

Idealized composite FSHD2 pedigree, showing independent segregation of a muscular dystrophy-permissive D4Z4 allele (4A) and any of several mutated forms of the SMCHD1 gene (green outline), which segregate with D4Z4 CpG hypomethylation. Only individuals with digenic inheritance of both the FSHD-permissive D4Z4 allele and a mutated SMCHD1 allele develop D4Z4 contraction-independent, type 2 FSHD (i.e., FSHD2). The 4B allele of the D4Z4 array is not permissive of FSHD. Rare subtypes of the 4A allele (marked with #) are also considered to be FSHD-nonpermissive.