

## Epigenetic variability is a modifier of facioscapulohumeral muscular dystrophy

Takako I. Jones<sup>1,2,3</sup>, Chia-Yun Sun<sup>2,3</sup>, Céline Debarnot<sup>3,4</sup>, Charis Himeda<sup>1,2,3</sup>, Charles P. Emerson, Jr<sup>1,2,3</sup>, and Peter L. Jones<sup>1,2,3</sup>

<sup>1</sup>Department of Cell and Developmental Biology, University of Massachusetts Medical School, Worcester, MA, USA

<sup>2</sup>The Eunice Kennedy Shriver National Institute of Child Health and Human Development Sen. Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD

<sup>3</sup>Boston Biomedical Research Institute, Watertown, MA, USA

<sup>4</sup>European School of Biotechnology of Strasbourg, France

Contact: [peter.jones@umassmed.edu](mailto:peter.jones@umassmed.edu)  
Ph: 774-455-1581

### Abstract

Facioscapulohumeral muscular dystrophy (FSHD), the most prevalent myopathy afflicting both children and adults, is strongly associated with epigenetic changes of the 4q35-localized macrosatellite D4Z4 repeat. Recent studies propose that FSHD pathology is caused by the misexpression and missplicing of the *DUX4* (double homeobox 4) gene, encoded within the repeat array, resulting in production of a pathogenic protein, DUX4-FL. We have analyzed *DUX4* mRNA and protein expression in a large collection of myogenic cells and muscle biopsies derived from muscles of FSHD1 affected subjects and their unaffected first-degree relatives. We confirmed that stable DUX4-fl mRNA and protein were expressed in myogenic cells and muscle tissues derived from FSHD affected subjects, including several genetically diagnosed adults yet to show clinical manifestations of the disease; however, there was great individual and familial variation in the levels of DUX4-FL. In addition, we found DUX4-fl mRNA and protein expression in muscle biopsies and myogenic cells from genetically unaffected relatives of the FSHD subjects, although at a significantly lower frequency. These results establish that DUX4-fl expression *per se* is not sufficient for FSHD muscle pathology. To investigate if subtle differences in the epigenetic status of the 4q35 region could account for the wide variation in DUX4-fl expression among FSHD1 subjects and potentially the spurious expression in certain unaffected controls, family cohorts of myogenic cells from FSHD1 subjects were tested for their sensitivity to small molecules that can alter the chromatin state. We find that myogenic cells from FSHD1 subjects are overall epigenetically poised to express DUX4 compared with unaffected subjects; however, FSHD1 subjects show individual differences in their capacity to express DUX4-fl in response to DNA demethylation and blocking histone deacetylation. Therefore, individual differences in the epigenetic status likely impacts progression of disease pathology, variability in age of onset, disease severity, and asymmetry of affected muscles.