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Large family cohorts of lymphoblastoid cells provide a new cellular model for investigating facioscapulohumeral muscular dystrophy

Takako I. Jones [a,1], Charis L. Himeda [a,1], Daniel P. Perez [b,*], Peter L. Jones [a,1,**]

[a] The Department of Cell and Developmental Biology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655, USA
[b] FSH Society, 450 Bedford Street, Lexington, MA 02420, USA

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Abstract

Facioscapulohumeral muscular dystrophy (FSHD) is associated with aberrant epigenetic regulation of the chromosome 4q35 D4Z4 macrosatellite repeat. The resulting DNA hypomethylation and relaxation of epigenetic repression leads to increased expression of the deleterious *DUX4-fl* mRNA encoded within the distal D4Z4 repeat. With the typical late onset of muscle weakness, prevalence of asymptomatic individuals, and an autosomal dominant mode of inheritance, FSHD is often passed on from one generation to the next and affects multiple individuals within a family. Here we have characterized unique collections of 114 lymphoblastoid cell lines (LCLs) generated from 12 multigenerational FSHD families, including 56 LCLs from large, genetically homogeneous families in Utah. We found robust expression of *DUX4-fl* in most FSHD LCLs and a good correlation between DNA hypomethylation and repeat length. In addition, *DUX4-fl* levels can be manipulated using epigenetic drugs as in myocytes, suggesting that some epigenetic pathways regulating *DUX4-fl* in myocytes are maintained in LCLs. Overall, these FSHD LCLs provide an alternative cellular model in which to study many aspects of D4Z4, DUX4, and FSHD gene regulation in a background of low genetic variation. Significantly, these non-adherent immortal LCLs are amenable for high-throughput screening of potential therapeutics targeting *DUX4-fl* mRNA or protein expression.
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1. Introduction

Facioscapulohumeral muscular dystrophy (FSHD), affecting ~1:7500–15,000 individuals, is the most prevalent muscular dystrophy that indiscriminately afflicts children and adults of all ages and both genders [1–5]. All forms of FSHD are genetically and epigenetically linked to the chromosome 4q35 D4Z4 macrosatellite array, with the interplay between these factors accounting for much of the high variability in disease penetrance and severity characteristic of the disease [1,6–15]. The predominant form of the disease, FSHD1 (OMIM 158900), represents >95% of reported cases and results from large DNA deletions within the 4q35 D4Z4 repeat array [16,17]. Healthy, genetically unaffected individuals are typically defined as having more than 10 D4Z4 repeat units (RUs) on both 4q chromosome arms (generally 25–35 RUs and as high as 120 RUs per array [18,19]), whereas individuals with genetic FSHD1 have a contracted D4Z4 array in the range of 1–10 D4Z4 RUs on one 4q chromosome arm, consistent with an autosomal dominant mode of inheritance (Fig. 1) [20]. These polymorphic FSHD1-sized D4Z4 contractions are not sufficient for pathogenesis; development of FSHD also requires a disease permissive allele of the chromosome 4q subtelomere (4A) in *cis* with the contracted array [19,21–23]. FSHD2 (OMIM 158901), the far less common form of FSHD, presents with similar clinical features as FSHD1, but is caused by unlinked mutations in genes encoding chromatin regulatory proteins [7,8,24,25]. However, FSHD2 is also genetically linked to the 4q35 array by the requirement of at least one permissive 4A-type subtelomere and a specific range of D4Z4 RUs (~11–28 RUs) in order to develop disease [7,23]. Thus, with these genetic requirements, FSHD2 is considered a digenic disease [8,25].

* Corresponding author. FSH Society, 450 Bedford Street, Lexington, MA 02420, USA. Fax: +1 781 862 1116.
E-mail address: Daniel.Perez@fshsociety.org (D.P. Perez).
** Corresponding author. Department of Pharmacology, University of Nevada, Reno School of Medicine, 1664 N Virginia Ave., Reno, NV 89557, USA. Fax: +1 775 784 6956.
E-mail address: peterjones@med.unr.edu (P.L. Jones).
[1] Current address: Department of Pharmacology, University of Nevada, Reno School of Medicine, 1664 N Virginia Ave., Reno, NV 89557, USA.

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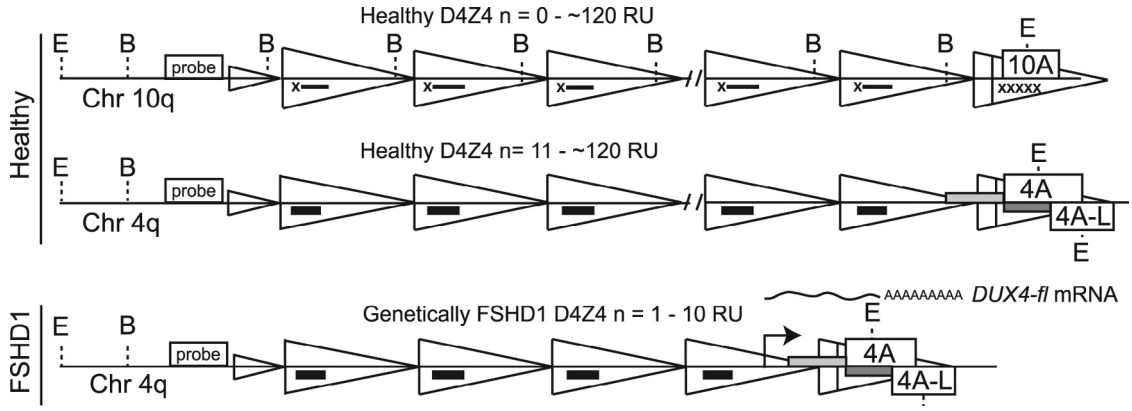

Fig. 1. Positions of restriction enzyme sites, probe, and PCR primers used to distinguish D4Z4 sequence. Scheme depicting the chromosome 4q35 D4Z4 macrosatellite array, which is contracted in FSHD1, leading to epigenetic changes specific to the contracted chromosome. De-repression of the pathogenic allele leads to aberrant expression of the *DUX4-fl* mRNA from the distal-most D4Z4 repeat unit (RU). The 4q35 and 10q26 D4Z4 arrays are distinguished from each other by combined *Eco*RI (E) + *Avr*II (B) digestion followed by Southern blotting and probing with p13E-11 (probe). DNA methylation changes were assayed by BSS specific for the distal D4Z4 RU of 4qA and its allelic variant 4qA-L [12] (gray bars) or all D4Z4 RUs (black bars).

Despite the clear genetic distinctions between these two forms of FSHD, the effects on D4Z4 chromatin are quite similar. In FSHD1, the 4q35 deletions result in the loss of regulatory heterochromatin that significantly alters the local epigenetic landscape of the contracted allele and is characterized by allele-specific DNA hypomethylation [12,26–30]. FSHD2 mutations reside in the genes encoding the epigenetic machinery responsible for establishing and maintaining repression of the D4Z4 arrays; therefore, FSHD2 is characterized by DNA hypomethylation of both 4q35 D4Z4 arrays and often both 10q26 arrays as well [7,8,25]. Thus, although epigenetic dysregulation is characteristic of FSHD in general, FSHD1 and FSHD2 subjects can be distinguished from each other and even individually diagnosed based on the DNA methylation profiles of the 4q35 D4Z4 arrays and disease permissive alleles [11,31,32]. In addition, some FSHD1 subjects may also have FSHD2-type mutations, with the combined effect presenting as a very severe form of FSHD, thus characterizing these genes as modifiers of disease severity [9]. To date, there have been two FSHD1 modifier genes identified, SMCHD1 and DNMT3B [8,25], both of which encode epigenetic regulator proteins, further highlighting the importance of the 4q35 epigenetic status with respect to disease presentation.

In both forms of FSHD, chromatin de-repression at the 4q35 D4Z4 macrosatellite has similar downstream molecular consequences, resulting in the aberrant expression of a pathogenic isoform of the *DUX4* (double homeobox 4) gene, *DUX4-fl*, in skeletal muscle [15,23,26,33–38]. Each D4Z4 RU encodes a copy of *DUX4* [33]; however, only *DUX4-fl* produced from the distal-most 4q35 D4Z4 RU is stably expressed in FSHD. This is due to an array-distal polyadenylation signal (PAS) in the noncoding exon 3 of *DUX4*, which is only present in permissive 4q alleles (termed 4qA) [23,35]. This PAS is required to stably express *DUX4* mRNAs in somatic cells and is essential for developing both forms of FSHD. However, the *DUX4* PAS is absent from about half of the 4q chromosomes (4qB alleles) in the human population. These non-permissive 4qB chromosomes lack exon 3 of the *DUX4* gene and are therefore unable to generate polyadenylated *DUX4* mRNA in somatic cells, supporting the requirement for stable *DUX4* expression in the development of FSHD [21,23,35,39,40]. Interestingly, the highly homologous chromosome 10q26 D4Z4 arrays have the A-type exon 3 sequence distal to the array; however, the putative *DUX4* PAS is a sequence variant that does not signal polyadenylation, thus explaining why D4Z4 contractions on chromosome 10 are not associated with FSHD [21,23,39].

With the identification of *DUX4* as the key pathogenic gene in FSHD, the field is now engaged in designing DUX4-targeted therapies [23,33,35,41,42]. Therapies can also be targeted upstream of *DUX4* expression, as the epigenetic disruption at the FSHD locus is a major determinant in disease presentation and progression. In addition, there are multiple genetic and epigenetic modifiers of FSHD severity, which provide additional therapeutic targets [7,9,12,25,36,43]. However, the fundamental nature of FSHD presents several obstacles to traditional drug development and screening (e.g., *DUX4* is a primate-specific gene expressed at very low levels primarily in differentiated FSHD skeletal myocytes, which are not amenable to high-throughput screening [35–37]). In addition, the high variability of genetic and epigenetic features found among FSHD patients can confound studies that are under-powered. Thus, there is a great need in the field for large numbers of well-characterized and freely available cohorts of cells, both FSHD and related healthy controls, as well as the development of readily screenable cellular models of FSHD. We and others have previously shown that primary blood cells recapitulate the epigenetic signature of the FSHD locus seen in skeletal myocytes [26,31]. Here we perform an initial characterization of 114 lymphoblastoid cell lines (LCLs) derived from healthy and FSHD1 affected individuals from 12 multigenerational families to provide valuable new tools for the FSHD field. Previous studies using the genetically homogeneous families in the Utah region have been informative in early clinical descriptions and linkage studies for FSHD [20,44–48]. The

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