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The differential gene expression profiles of proximal and distal muscle groups are altered in pre-pathological dysferlin-deficient mice

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Abstract

The selective pattern of muscle involvement is a key feature of muscular dystrophies. Dysferlinopathy is a good model for studying this process since it shows variable muscle involvement that can be highly selective even in individual patients. The transcriptomes of proximal and distal muscles from wildtype C57BL/10 and dysferlin deficient C57BL/10.SJL-*Dysf* mice at a prepathological stage were assessed using the Affymetrix oligonucleotide-microarray system. We detected significant variation in gene expression between proximal and distal muscle in wildtype mice. Dysferlin defiency, even in the absence of pathological changes, altered this proximal distal difference but with little specific overlap with previous microarray analyses of dysferlinopathy. In conclusion, proximal and distal muscle groups show distinct patterns of gene expression and respond differently to dysferlin deficiency. This has implications for the selection of muscles for future microarray analyses, and also offers new routes for investigating the selectivity of muscle involvement in muscular dystrophies.

Keywords: Dysferlin; Muscular dystrophy; Expression profile

1. Introduction

Microarray analysis as a tool to study muscular dystrophy has been used in an increasing number of studies in both patients and animal models with mutations in a variety of different muscular dystrophy associated genes. These studies have identified some secondary changes, which appear to be common to muscular dystrophy in general. The compilation of particular expression profiles from patients and animal models of specific types of muscular dystrophy may eventually delineate reproducible 'molecular signatures' of disease.

While it is well recognised that a characteristic and subtly different pattern of muscle involvement can be observed in various forms of muscular dystrophy, the mechanism by which some muscles are apparently more or less susceptible to the effects of the underlying mutation remains entirely unknown. One possibility is that a broad range of normal expression profiles in different skeletal muscle groups might condition their response to disease. If the patterns of gene expression in these muscle groups responded differently to the presence of a muscular dystrophy associated mutation, this would provide support for this hypothesis.

Dysferlinopathy (MIM*603009) is a good model of variability in muscular dystrophy. Mutations in dysferlin give rise to a number of clinically distinct presentations

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including a proximal, limb-girdle form of muscular dystrophy and two forms of distal myopathy, Miyoshi myopathy (MM) and distal myopathy with anterior tibial involvement (DMAT) [1,2]. A very highly selective pattern of muscle involvement is seen in individual affected patients and identical dysferlin mutations have been shown to cause a variable phenotype within the same family or population group [2,3]. However no genetic variants, which modify this phenotype are currently known. This variable but selective muscle involvement in dysferlinopathy suggests that it can act as a paradigm for understanding selectivity of muscle involvement in muscular dystrophies in general. Due to the obvious barriers to studying different human muscle groups we chose to study gene expression patterns in two muscle groups in a new mouse model of dysferlinopathy and its matched wildtype strain.

The SJL mouse strain, a well characterised model of dysferlinopathy, has a number of phenotypes unrelated to the dysferlin mutation. Since SJL descends from a wild-derived strain of Swiss mice which are genetically distinct from the common laboratory mouse [4–6] there is not an appropriate control strain available. In order to overcome these disadvantages we transferred the dysferlin mutation from SJL onto C57BL/10 by repeated backcrossing. Affected mice develop a muscular dystrophy which appears comparable to human dysferlinopathy and shows more severe pathological changes in the affected limb muscles than the SJL strain.

We have used oligonucleotide microarray analysis to demonstrate that gene expression indeed varies in the proximal and distal musculature of wildtype mice and that dysferlin deficiency even at a prepathological stage alters this pattern. Changes in gene expression in the mutant animals show some overlap with previously reported studies of muscular dystrophy, indicating that changes at the transcript level are an early marker of the dystrophic process.

2. Material and methods

2.1. Animals

The dysferlin mutation from SJL was detected by PCR using standard techniques as previously described [5]. C57BL/10.SJL-*Dysf* mice were generated by repeatedly backcrossing the dysferlin mutation from the SJL strain onto C57BL10ScSnHim. Offspring of N2- and all further generations were genotyped for the dysferlin mutation and only heterozygous animals were used for breeding. To obtain homozygous animals to be used for experiments, offspring from generations N8 and N9 were mated with littermates. For muscle harvesting the animals were anesthetized with trichloracetaldehydemonohydrate (400 mg/kg, ip). Muscle was flash frozen in isopentane prior to sectioning for immunohistochemistry or routine histological staining. Small sections of muscle were macerated in treatment buffer (50 mM Tris pH6.8, 100 mM DTT, 2% SDS, 0.02% bromophenol Blue, 5% glycerol) and heated to 95 °C before loading on standard SDS-PAGE gels. Dysferlin was detected using antibody NCL-Hamlet by standard methods. For Evan's blue dye (EBD) visualisation of muscle membrane integrity the EBD (5 mg/ml solution in PBS) was injected intraperitoneally, the mice sacrificed after 24 h and muscle harvested for sectioning. Force generation from isolated gastrocnemius muscle induced to contract by electrical stimulation (1ms spikes, 2.5–3.5 mA) of the ischiadic nerve was measured by a force transducer that was attached to the muscle tendon. The muscle preload was 0.14 N for wild types ($n=4$) as well as for C57BL/10.SJL-*Dysf* mutants ($n=3$). Pyruvate kinase levels from peripheral blood were measured using standard techniques. Mice were obtained locally and all animal procedures were approved by the local animal care and use committee.

2.2. RNA processing

Quadriceps muscles and tibialis anterior muscles were dissected from four wildtype (C57BL/10, female, 3-week-old) mice and four C57BL/10.SJL-*Dysf* (female 3-week-old) mice. RNA samples were mixed into four different sex-, age-, diagnosis and muscle group matched pools to eliminate possible individual variations in gene expression. Total RNA isolated from homogenized samples using acid phenol extraction (TRIzol reagent, Invitrogen) was reverse transcribed using an oligo-dT primer coupled to a T7 RNA polymerase binding site. Double-stranded cDNA was made biotinylated-cRNA synthesized using T7 polymerase. The cRNA was hybridized for 16 h to an array, followed by binding with a streptavidin-conjugated fluorescent marker, and then incubated with a polyclonal anti-streptavidin antibody coupled to phycoerythrin as an amplification step. Samples were analysed on Affymetrix murine genomes U74 set chip A, B, C (A-Chip: 12,000 genes; B-Chip: 12,444 clones; C-chip: 11,934 clones) (Affymetrix, Santa Clara, CA) which represents 36,378 named genes and EST sequences. Each analysis was performed in triplicate. Therefore, each RNA pool derived from multiple animals was hybridised to three independent microarrays to control for intra-experimental sources of noise.

2.3. GeneChip data analysis

Microarray Analysis Suite 5.0 was used to calculate signal intensities, detection calls (present or absent) and signal log ratios. Each dysferlin deficient replicate was compared with each control replicate (a total of 9 comparisons for each of the 4 branches of the analysis). Transcripts defined as differentially regulated met the criteria of: (1) consistent increase/decrease call across all replicates based upon Wilcoxon's signed rank test (2)