The American Journal of Pathology, Vol. 185, No. 10, October 2015

The American Journal of PATHOLOGY
ajp.amjpathol.org

MOLECULAR PATHOGENESIS OF GENETIC AND INHERITED DISEASES

Differential Isoform Expression and Selective Muscle Involvement in Muscular Dystrophies

Sanna Huovinen,*† Sini Penttilä,† Panu Somervuo,‡ Joni Keto,‡ Petri Auvinen,‡ Anna Vihola,§ Sami Huovinen,¶ Katarina Pelin,‖ Olayinka Raheem,† Juha Salenius,** Tiina Suominen,† Peter Hackman,§ and Bjarne Udd†§††

From the Department of Pathology, Fimlab Laboratories, Tampere University Hospital, Tampere; the Neuromuscular Research Center† and the Division of Vascular Surgery,** Department of Surgery, Tampere University Hospital and University of Tampere, Tampere; The Institute of Biotechnology‡ and the Department of Biosciences,‖ Division of Genetics, University of Helsinki, Helsinki; the Department of Medical Genetics,§ Folkhälsan Institute of Genetics, Haartman Institute, University of Helsinki, Helsinki; the Department of Electrical Engineering,¶ University of Oulu, Oulu; and the Department of Neurology,†† Vaasa Central Hospital, Vaasa, Finland*

Accepted for publication June 22, 2015.

Address correspondence to Sanna Huovinen, M.D., Department of Pathology, Fimlab Laboratories, PO Box 2000, FIN-33521 Tampere, Finland. E-mail: sanna.huovinen@fimlab.fi.

Despite the expression of the mutated gene in all muscles, selective muscles are involved in genetic muscular dystrophies. Different muscular dystrophies show characteristic patterns of fatty degenerative changes by muscle imaging, even to the extent that the patterns have been used for diagnostic purposes. However, the underlying molecular mechanisms explaining the selective involvement of muscles are not known. To test the hypothesis that different muscles may express variable amounts of different isoforms of muscle genes, we applied a custom-designed exon microarray containing probes for 57 muscle-specific genes to assay the transcriptional profiles in sets of human adult lower limb skeletal muscles. Quantitative real-time PCR and whole transcriptome sequencing were used to further analyze the results. Our results demonstrate significant variations in isoform and gene expression levels in anatomically different muscles. Comparison of the known patterns of selective involvement of certain muscles in two autosomal dominant titinopathies and one autosomal dominant myosinopathy, with the isoform and gene expression results, shows a correlation between the specific muscles involved and significant differences in the level of expression of the affected gene and exons in these same muscles compared with some other selected muscles. Our results suggest that differential expression levels of muscle genes and isoforms are one determinant in the selectivity of muscle involvement in muscular dystrophies. *(Am J Pathol 2015, 185: 2833–2842; http://dx.doi.org/10.1016/j.ajpath.2015.06.018)*

The human body contains >300 anatomically different skeletal muscles, all structurally adapted to perform unique functional roles.[1] One established factor separating phenotypically different muscle groups is their fiber type composition.[2] Paravertebral muscles and the soleus muscle of the calf are postural muscles containing predominantly slow type I fibers, which are resistant to fatigue. In contrast, in muscles with mainly phasic functions, such as the triceps brachii muscle of the upper limb and the orbicularis oculi muscle, the fast type IIA oxidative-glycolytic and fast type IIB/X glycolytic fibers needed for midterm aerobic and short-term anaerobic activity are more numerous.[3] Muscular dystrophies are characterized by the differential and sometimes extremely selective involvement of muscles.[4–7] However, only a few muscles contain remarkable fiber type predominance, and the heterogeneity of how the different muscles react on muscle disease causing mutations cannot be explained only by this basic variation in fiber type proportions. From a therapeutic point of view, understanding the reasons for some muscles to escape the dystrophic effects of disease-causing mutations would be highly relevant.

Examples of the selective muscle involvement pattern used for this study are late-onset tibial muscular dystrophy (TMD; Udd myopathy), caused by mutations in the last exon 363 of the titin (*TTN*) gene,[8–10] early-onset Laing distal myopathy (LDM), caused by mutations in the rod

Supported by Tampere University Hospital Research grants 9L107, 9P056, and 9R053 and Finnish Academy, Health Sciences, grant 115735.
P.H. and B.U. contributed equally to this work as senior authors.
Disclosures: None declared.

http://dx.doi.org/10.1016/j.ajpath.2015.06.018

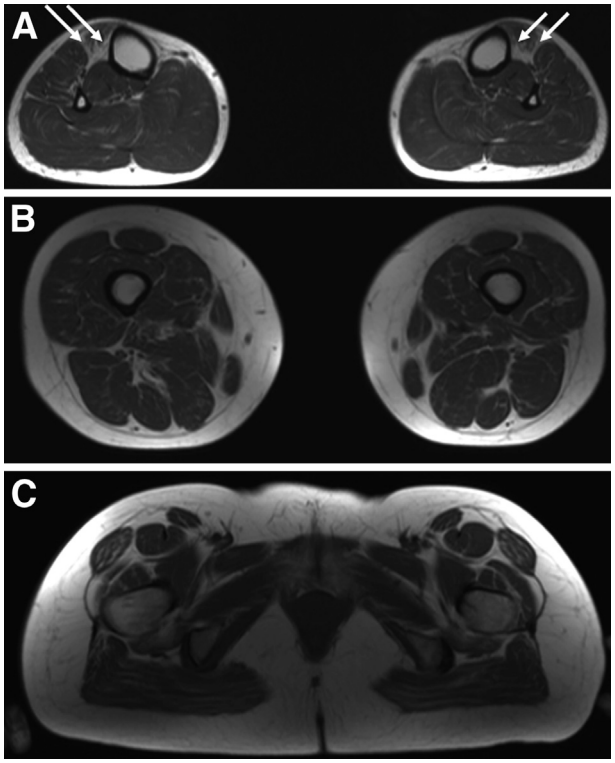

Figure 1 Selective dystrophic involvement of muscles in a 39-year-old female Laing distal myopathy patient caused by dominant mutation K1729del in exon 36 of the *MYH7* gene. **A:** Magnetic resonance images show replacement with fatty connective tissue in the tibialis anterior and extensor hallucis longus muscles in the lower legs (**arrows**). **B** and **C:** No major changes are discerned in the thigh (**B**) and pelvic muscles (**C**).

domain of the slow myosin heavy chain gene (*MYH7*),[11,12] and adult-onset hereditary myopathy with early respiratory failure (HMERF), caused by mutations in exon 343 of A-band *TTN*.[13–15]

The *MYH7* gene has 40 exons and encodes slow/β-cardiac myosin heavy chain protein (MyHC I), which is the major myosin heavy chain in human ventricular cardiac myocytes and is expressed in all type I slow skeletal muscle fibers.[2] Modulations of the expression, including alternative splicing, of mRNA of *MYH7* and some other key sarcomeric genes have been observed in cardiac muscle in some pathological conditions, such as cardiomyopathies and heart failure.[16,17] Mutations in *MYH7* rod domain exons 32 to 38 cause LDM,[11,12] with highly selective involvement of the anterior compartment muscles of the lower legs, typically tibialis anterior and extensor hallucis longus muscles (Figure 1).

TTN is the largest gene in nature, with a coding sequence of 100 kb in 363 exons and with alternative splicing generating many different isoforms.[18–21] Mutations associated with HMERF have been reported in the kinase domain of *TTN* encoded by exon 358 and, more frequently, in a Fn3 119 domain of A-band titin encoded by exon 343 of the gene.[13–15] The disease is distinguished by the combination of adult-onset myopathy with respiratory failure early in the clinical course and myotilin immunoreactive inclusion bodies in the myofibers.[14,22,23] The unexplained characteristic muscle magnetic resonance imaging findings in HMERF disease are selective early involvement of the semitendinosus muscle on the thigh and the obturatorius muscles on the pelvic level (Figure 2). In the lower leg muscles, the first changes frequently appear in the tibialis posterior and flexor digitorum longus muscles.[13–15,24]

Mutations in the two last exons, 362 and 363, encoding the extreme C-terminus of the molecule, residing in the periphery of the M-band, lead to TMD, a distal myopathy characterized by the late onset of the disease and rimmed vacuolar myopathology in the affected muscles.[6,8,9] The hallmark of TMD is the severe and selective fatty replacement in the tibialis anterior muscle, followed later by the involvement of the long toe extensor muscles (Figure 3).[25]

We hypothesized that unknown molecular diversities (ie, fingerprints) in different muscles account for the propensities of the muscles to escape (or not) the harmful effects of the mutations. Alternative splicing allows for a multitude of diversification of gene functions.[26] Many disease-causing mutations directly involve errors in alternative splicing events, both in cis and in trans,[27,28] and the

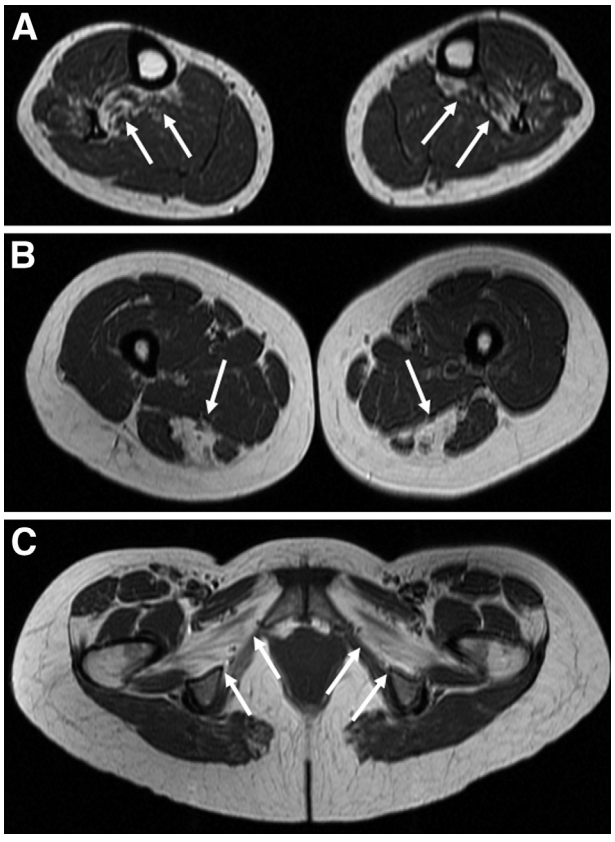

Figure 2 **A:** Muscle magnetic resonance imaging in a 48-year-old female hereditary myopathy with early respiratory failure patient with a dominant heterozygous p.C30071R mutation in A-band *TTN* gene shows selective dystrophic changes in the lower legs and early fatty degenerative replacement in the tibialis posterior and flexor digitorum longus muscles (**arrows**). **B** and **C:** In the thigh (**B**), highly selective replacement is observed in the semitendinosus muscles, and in the pelvis (**C**), it is seen in the obturatorius muscles (**arrows**).