P.13.10
Unexpected findings in array analysis with possible implications for dystrophinopathy

L. Ginjaar 1, D. Van Heusden 1, C.G.C. Dutch University Hospitals 2, M.J.V. Holfer 1
1 Leiden University Medical Center, Clinical Genetics, Leiden, Netherlands; 2 Medical Faculties, Clinical Genetics, Leiden, Netherlands

Since the introduction of array analysis in molecular diagnostics for patients suffering from mental retardation and developmental disorders as well as prenatal diagnostics many unexpected findings were made. So far, more than 20 different cases with possible implications for dystrophinopathy have been reported to our centre. About an equal amount of gross deletions and duplications within the DMD gene were observed. In nearly all cases array results could be confirmed and gross deletions/duplications were accurately mapped by means of Multiplex Ligation Probe Amplification of the DMD gene. In about half of the families the mutations found may have implications for the patients and/or their families. In the majority of cases with duplications, no effect was found by means of segregation studies in families. Also some FISH experiments showed that duplications were located in other parts of the genome. A number of duplications extended towards both the 5′ or 3′ end of the DMD gene and even further. Most deletions were internal gene deletions except for one and in the majority of cases novel DMD carriers were identified. In a few cases the mutations found yielded new information on their pathogenicity. For instance, deletions which could not be found in DMD databases possibly indicating that these mutations might be harmless deletions occurring in the general population. In one case prenatal DNA analysis identified a male fetus with an out-of-frame deletion indicating a possible novel DMD patient.

http://dx.doi:10.1016/j.nmd.2013.06.604

P.13.11
Proteomic analysis of cardiomyopathic tissue from the aged mdx model of Duchenne muscular dystrophy

A. Holland, K. Ohlendieck
National University of Ireland Maynooth, Biology Department, Kildare, Ireland

Senescent heart tissue from the mdx mouse model of Duchenne muscular dystrophy exhibits distinct pathological aspects of X-linked cardiomyopathy. In order to establish proteome-wide alterations in dystrophin-deficient hearts during aging, cardiomyopathic tissue from young versus aged mdx mice was examined by label-free LC-MS/MS analysis. Significant age-dependent alterations were established for 67 proteins, of which 28 proteins were found to be decreased and 39 proteins were shown to be increased in their expression levels. Drastic changes were demonstrated for proteins involved in ion transportation, the immune response, the cellular stress response, muscle contraction, metabolic regulation and the extracellular matrix. An immunoblotting survey of young and old wild type versus mdx hearts confirmed these proteomic findings and illustrated the effects of natural aging versus dystrophin deficiency. These proteome-wide alterations suggest a disintegration of the basal lamin structure and cytoskeletal network in dystrophin-deficient cardiac fibres, increased levels of antibodies in a potential autoimmune reaction of the degenerating heart, compensatory binding of excess iron and a general perturbation of metabolic pathways in dystrophy-associated cardiomyopathy. The proteomic technologies and analytical tools used in this study to identify significant proteins of interest have the potential to be brought forward and provide new therapeutic and diagnostic biomarkers for dystrophinopathy-related cardiomyopathy.

http://dx.doi:10.1016/j.nmd.2013.06.605

P.13.12
An objective method for immunofluorescence analysis of dystrophin levels in muscle from DMD patients in clinical studies

Prosensa Therapeutics BV, Leiden, Netherlands

Duchenne muscular dystrophy (DMD) is characterized by absence or very low (trace) expression of dystrophin at the sarcolemmal membrane of the muscle fibers. In clinical studies aiming to restore dystrophin expression, dystrophin levels are measured in muscle biopsies by immunofluorescence analysis of cross-sections or western blot analysis of total protein extracts. However, appropriate quantification poses a technical challenge as dystrophin levels may be low and/or variable between fibers in the same biopsy.

We have developed an immunofluorescence method and automated image analysis that measures the dystrophin intensity per individual fiber in a biopsy. It reproducibly detects even small differences in dystrophin levels. Muscle cross-sections co-stained for dystrophin and spectrin are imaged by confocal microscopy and image analysis is performed using Definiens software. Using a customized algorithm, and the sarcolemmal spectrin signal as a mask, the software automatically segments each image into individual muscle fibers, measures the varying dystrophin intensity per individual fiber, and objectively produces a histogram of the distribution in the fiber population, including revertant fibers. Duplicated analysis of the biopsies on the same or multiple days and by different operators was shown to be reproducible, objective and able to distinguish between different low dystrophin levels in Becker muscular dystrophy and DMD samples. Moreover, in DMD patients treated with antisense oligonucleotides to restore dystrophin expression, comparisons of the dystrophin intensity distribution histograms of pre- and post-treatment muscle biopsies showed increases in dystrophin intensities of entire muscle fiber populations post-treatment.

http://dx.doi:10.1016/j.nmd.2013.06.606

P.13.13
Electrical impedance myography in DMD: A multi-center study of reliability and relationships to strength and function

C. Zaidman 1, J. Bohorquez 2, L. Wang 3, J. Florence 1, A.M. Connolly 1, D.M. Farzouf 1, G. Williams 4
1 Washington University, Neurology, St. Louis, MO, United States; 2 Convergence Medical Devices, Woburn, MA, United States; 3 Kennedy Krieger Institute, DART Therapeutics, Inc., Center for Genetic Muscle Disorders, Baltimore, MD, United States; 4 DART Therapeutics, Inc., Woburn, MA, United States

Determine reliability of electrical impedance myography (EIM) in boys with Duchenne muscular dystrophy (DMD) and healthy controls and compare results to measures of strength and function.

EIM is a non-invasive, rapid to apply, painless, and quantifiable technique that is sensitive to muscle pathology. It could potentially serve as a biomarker of disease in DMD.

Sixty-one boys with DMD and 31 healthy boys, aged 3–12 years, were recruited and evaluated at five centers. Assessments included EIM of unilateral arm and leg muscles (EIM system1103, Convergence Medical Devices, Inc.), six minute walk test (6MWT), North Star Ambulatory Assessment (NSAA), timed functional tests (TFT), and strength (hand held dynamometry). EIM measurements were repeated by a second examiner. EIM, TFT, and dynamometry data were normalized based on the healthy subject data and percent predicted of normal values were calculated.

All boys tolerated testing well. Although results of EIM from single muscles showed good reliability and correlation with function and strength, an average measure of EIM from each subjects biceps brachii,