

growing knowledge resulting from the advances in human genome sequencing.

doi:10.1016/j.nmd.2011.06.820

P1.61

Physicians ability to recognize Duchenne muscular dystrophy on clinical basis: Need for educational programs

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Despite of the fact that Duchenne muscular dystrophy (DMD) is the most common form of muscular dystrophy in childhood, this condition is not easily recognized by pediatricians and orthopedists. We carried out a study to assess the knowledge those physicians have about DMD. Forty medical doctors from public and private hospitals and 76 medical students were surveyed (from the second, fourth and sixth grades). They answered the questions: Q1. A three year-old boy with frequent falls has an orthopedic (OD), neurologic (ND), cardiac, rheumatic or other disease? Q2. The creatine phosphokinase levels were checked. Would you change your answer of Q1? Q3. What is the diagnosis? Q4. DMD is an orthopedic, neurologic, cardiac, rheumatic or other disease? Q5. What do you know about the treatment of DMD? Seventy-five percent of the orthopedists answered OD and none of them chose ND for Q1. Considering the pediatricians, 54% chose ND and 32% OD. Eighty-four percent of the second grade medical students, 69% of those in the fourth grade and 83% of the sixth grade answered ND for Q1. Fourty-two percent of the orthopedists chose muscular dystrophy as the diagnosis (16.7% DMD) and the pediatricians, 54%. Fifty percent of the orthopedists and 43% of the pediatricians think steroids are useful for DMD patients. The parents of patients with muscle diseases usually first seek attention of pediatricians and orthopedists. Our data clearly showed that a high percentage of those physicians had difficulties thinking of DMD only on clinical basis. As the early diagnosis is of great importance to start treatment quickly and delay the complications, our work stress the need of educational programs for physicians to call their attention for diseases with high mortality such as DMD. Stimulating the participation of undergraduate medical students in an outpatient clinic of muscular dystrophy patients allowed a high percentage of them to correctly consider the diagnosis.

doi:10.1016/j.nmd.2011.06.821

P1.62

What is the best age at which to perform population screening for Duchenne muscular dystrophy?

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A number of centres, notably Wales, have performed population screening for Duchenne muscular dystrophy (DMD) over many years, while others have begun recently or are considering screening. There has been considerable debate in the literature and at public fora as to the best age at which to perform population screening for Duchenne muscular dystrophy. If population screening is performed as part of newborn screening programs, with results relayed to parents shortly after birth, there is debate as to whether such early testing 'robs' parents of the "normal" time before a diagnosis is made and might interfere with the bonding process. On the other hand later testing increases the likelihood of having further affected boys born into the same family. So what is the best age to perform

screening? It is becoming increasingly common for "Facebook" to be the forum for the Duchenne community to search for services being provided locally, to seek advice and support and gauge what is happening internationally. We therefore canvassed the Duchenne community through Facebook as to the reaction of parents whose son had been diagnosed with DMD through newborn screening or at a later age. The general consensus appears that, parents were "happy" with, or rationalised, whatever diagnostic process they had experienced. For example, when diagnosed later: "I loved our three years before Duchenne." When diagnosed through newborn screening: "We were able to plan our next pregnancy." Perhaps the most crucial comment is from one mother: "There is no good time to find out that your son has Duchenne." Therefore, from this survey, it is perhaps best to consider the most cost effective and logistically feasible age to screen the population in different countries, to ensure that through informed choice, as few families as possible find their son or sons have DMD.

doi:10.1016/j.nmd.2011.06.822

P1.63

How to deal with unexpected mutations in healthy persons

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A 43-years old normal male was referred to our center for clinical evaluation. Family history revealed that he has two daughters, 15 and 9 years, respectively. The youngest one has a history of bilateral coloboma, hearing loss and attention deficit disorder of unknown cause which prompted the family to look for a genetic service. Whole genome oligonucleotide array CGH analysis performed in another laboratory Genome DxReport, Gaithersburg-MD 20877 revealed that she carries a 179 kb deletion in the dystrophin gene which was inherited from her father. Clinical and neurological examination showed that the father is completely asymptomatic. He is able to run, jumps and plays soccer regularly without any difficulty. His serum CK was borderline (223 μ L normal up to 189 μ L). DNA analysis, through MLPA, confirmed an in-frame deletion encompassing exons 38–44. This deletion has apparently not been described before. Muscle biopsy showed no myopathic alterations. Immunofluorescence analysis for dystrophin, using antibodies against the N-terminal, rod domain and C-terminal regions of the proteins showed a normal and continuous sarcolemmal pattern of distribution. Through western blot analysis, using the same antibodies, a strong dystrophin band of ~390 kDa was observed, compatible with the size expected for the transcript of his deleted gene. Improvement in DNA technology is increasingly identifying unexpected mutations in healthy persons. This raises an important problem in interpreting the results, defining prognosis and genetic counseling of at-risk family members. Supported by FAPESP-CEPID, INCT, CNPq and ABDIM.

doi:10.1016/j.nmd.2011.06.823

CONGENITAL MUSCULAR DYSTROPHIES: POSTER PRESENTATIONS

P2.1

Candidate biomarkers in merosin-deficient congenital muscular dystrophy

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Merosin-deficient congenital muscular dystrophy (MDC1A) is a rare disorder presenting at birth or in early childhood with hypotonia, weakness, and a dystrophic appearing myopathy. There are targeted pharmacological agents in development approaching the point of moving into CMD clinical trials. Rational clinical trial design dictates the need for sensitive and specific end points and outcome measures. To compare and initially validate blood serum protein expression profiles in MDC1A. Surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) technology was used to analyze the serum of 14 patients with MDC1A compared to age-, gender- matched controls. Our studies have identified a number of identifiable protein peaks using CM10, IMAC30, H50 and NP20 chip arrays (SELDI-MS) that were significantly different between MDC1A and age, gender matched controls. Further we identified 3 candidate protein peaks significantly elevated compared to the controls. *m/z* respectively, (A) 4647, (B) 7772, and (C) 9300. Further characterization of the proteins is currently being pursued. These results are encouraging that specific proteins associated with the CMD disease process can be identified in patient's serum using relatively non-invasive techniques.

doi:10.1016/j.nmd.2011.06.824

P2.2

A benign form of MDC1A in Korean siblings with a novel LAMA2 mutation S.Y. Huh^a, Y.E. Park^b, D.S. Kim^c

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Merosin-deficient congenital muscular dystrophy (MDC1A, MIM #607855) is characterized by early onset of profound muscle weakness in infancy, high CK level, and normal intelligence despite the diffuse white matter change in the brain. Although it is typically a disease of infancy or early childhood, there are some cases with much benign phenotypes. A 20-year-old man was evaluated for chronic non-progressive gait disturbance and diffuse white matter change on brain MRI. The birth histories were unremarkable. As he grew older, his motor milestones were slightly delayed. He could sit unassisted at 6 months, independent walk at 2 years. He had been unstable on walk and had difficulty in running since childhood. He was operated for the Achilles tendon contractures at age 15, and became to walk better. The brain MRI, taken because of posttraumatic headache, showed a diffuse white matter change on T2WI. Family history was remarkable for his elder sister who had similar problems. Examination showed mild scoliosis, joint contractures, generalized muscle atrophy. Neurological finding were 4+ MRC strength in limbs with generalized areflexia. The blood creatine kinase activity was elevated to 963 IU/L. The nerve conduction velocity was slowed in all tested nerves, while the needle EMG was compatible with active myopathy. Visual and somatosensory evoked potential study showed delayed latencies. Immunohistochemistry showed lack of merosin. DNA analysis revealed a novel heterozygous nonsense mutation in exon 34 of the LAMA2 (c.6160 C>T (p.Gln2054X)) in both proband and his sister. We were not able to identify the second mutation from the other allele. Our patient shows that MDC1A occasionally can present as exceptionally mild benign phenotype mimicking limb-girdle muscular dystrophy. The electrophysiologic studies and imaging study also showed evidence of subclinical central and peripheral demyelination which is accompanied by merosin deficiency.

doi:10.1016/j.nmd.2011.06.825

P2.3

Monoamine oxidase inhibitors reduce mitochondrial ROS accumulation and dysfunction in patients with collagen VI myopathies

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Muscular dystrophies (MDs) are a family of genetic disorders characterized by progressive muscle weakness and premature death. Several studies documented the key role of increased formation of reactive oxygen species (ROS) in the pathophysiology of MDs. The source of ROS, however, is still controversial as well as their major molecular targets. Based on the results that we obtained in experimental murine models of MDs, namely (i) *Col6al*^{-/-} mice, a model of Bethlem myopathy and Ullrich congenital MD and (ii) *mdx* mice, a model of Duchenne MD, we investigated whether the mitochondrial enzymes monoamine oxidases (MAOs) cause oxidative stress and mitochondrial dysfunction in myoblasts from patients affected by Bethlem myopathy and Ullrich congenital MD. To address this issue myoblast cultures from patients were treated with well-characterized inducers of oxidative stress, such as hydrogen peroxide, and mitochondrial ROS accumulation was measured with Mitotracker Red. Interestingly, myoblasts from dystrophic patients generated larger amounts of ROS than cells from healthy donors, which was matched by a rise in MAO-B protein level. As already shown in cells from the animal models (Menazza et al. Hum Mol Genet 2010;19:4207–15) increased ROS formation was significantly reduced by MAO inhibition with pargyline. In keeping with MAO causing increased oxidative stress in CoVI diseases, Ullrich congenital MD cultures generated larger amounts of ROS in response to a MAO substrate, such as tyramine, a finding that matches the increased MAO activity of dystrophic mice. Importantly, reduced accumulation of ROS was paralleled by improved mitochondrial function, as assessed by accumulation of tetramethyl rhodamine methyl ester (TMRM). Taken together, these findings confirm the relevance of MAO-dependent ROS formation in MDs, thus providing the rationale for future clinical trials by using compounds, such as MAO inhibitors, that are already widely used for neurological diso.

doi:10.1016/j.nmd.2011.06.826

P2.4

Searching for pulmonary outcome measures in 'Early Onset' COL6-related myopathy

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COL6 gene mutations result in a myopathy with variable severity, often with progressive respiratory and orthopedic complications. A simple functional classification has shown genotype-phenotype correlations (Briñas et al. 2010). The definition of disease course and appropriate outcome measures is crucial for the design of clinical therapeutic trials. We studied 23 patients with onset of symptoms in the first two years and abnormal

The aim of this study was to characterize the clinical and genetic features of a 4-year-old female with merosin-deficient congenital muscular dystrophy type 1A (MDC1A). MDC1A is the most common form of congenital muscular dystrophy. MDC1A is caused by mutation of the laminin α -2 gene (LAMA2), localized to chromosome 6q22-q23. Clinical presentation, as well as the results of neuro-imaging, electrophysiology and molecular genetic tests were used to evaluate a patient with MDC1A. The patient exhibited severe hypotonia and marked proximal weakness at 6 months of age, as well as delayed development. Presentation on theme: "Congenital Muscular Dystrophy Biomarker Discovery" Presentation transcript: 1 Congenital Muscular Dystrophy Biomarker Discovery James Collins MD, PhD Assistant Professor Division of Neurology Cincinnati Children's Hospital Medical Center. 2 Disclosures Research Foundation Grant: Cure Congenital Muscular Dystrophies partnered with S.A.M. (A 17 Gene expression profiling CMD Fukuyama-type CMD and Merosin - deficient patients expression profile in muscle up-regulation extracellular matrix and basement membrane component genes unique expression pattern dystrophin-deficient muscle Unique profile of FCMD compared to MDC1A Taniguchi, M., et al. *Biochem Biophys Res Commun*, (2): p.