ICNC-0743  Long-term follow-up in patients with congenital myasthenic syndrome due to RAPSN mutations

Introduction: Congenital myasthenic syndromes (CMS) are a heterogeneous group of genetic disorders, all of which impair neuromuscular transmission. Rapsyn (RAPSN) mutations account for approximately 14-27% of all CMS patients. We describe the clinical and molecular genetic findings as well as long-term follow-up data of 10 patients with CMS due to RAPSN mutations.

Methods: Ten patients from 8 unrelated families with genetically confirmed CMS due to mutations in the RAPSN gene were included. They were followed up serially in our clinics over a mean period of 14.5 years.

Results: We present a comprehensive description of the clinical and molecular findings. Two patients were homozygous and eight heterozygous for the common p.Asn88Lys mutation. In three of the heterozygous patients we identified three novel mutations (c.869T>C; p.Leu290Pro, c.1185delG; p.Thr396Profs*12, and c.358delC; p.Gln120Serfs*8). Conclusion: In our cohort the RAPSN mutations lead to a relatively homogeneous phenotype, characterized by fluctuating ptosis, occasional bulbar symptoms, neck muscle weakness, and mild proximal muscle weakness with exacerbations precipitated by minor infections. Interestingly, episodic exacerbations continue to occur during adulthood. Crises after age 6 were characterized by proximal limb girdle weakness, bulbar symptoms, and ptosis, and not so much by respiratory insufficiency. All patients presented during neonatal period and responded to cholinergic agonists. In most affected patients, additional use of 3,4-diaminopyridine resulted in significant clinical benefit. The disease course is stable except for intermittent worsening.

Daniel Natera-de Benito*(1);
(1)Hospital Universitario de Fuenlabrada, Madrid, Spain;
ICNC-0736  Early-onset congenital myopathies and congenital myasthenic syndromes: Genetic testing replacing invasive testing! An illustrative case series

Background: Early-onset neuromuscular disorders have overlapping manifestations. Early diagnosis is critical for treatment and prognostication. Often, conventional investigations (Electrophysiology and muscle biopsy) fail to reveal confirmed diagnosis. Study methods: Series of 9 children with early-onset neuromuscular disorders (June 2014-September 2015). Results: There were 5 children with congenital myopathies (NEB, ACTA1A, RYR1, MYH7-, DES+), and 4 children with CMS (2 with COLQ, one each with CHNRE-, CHRNA1-). Male:Female was 3:6. All had symptom onset before 12 months age: intrauterine-2, at birth-4 and later-3. Seven of 9 had consanguineous parentage, with positive family history in 4. Ophthalmoplegia and fatigable weakness were distinguishing features between two groups: all children with CMS and none with congenital myopathies. Children with myopathies were more likely to have severe symptoms in neonatal period: feeding, breathing difficulties (5/5) and contractures (4/5), more likely to have failure-to-thrive, and less likely to attain independent walking. Investigations like CPK, nerve conduction studies were not contributory in any child. Repetitive nerve stimulation test was positive in all children with CMS and child with Myosin storage myopathy. None of our children underwent EMG, while one underwent muscle biopsy. All children underwent targeted gene sequencing panel. Seven of 9 mutations were previously unreported/novel. Two infants with neonatal-onset myopathy died of respiratory failure. Two children with COLQ mutations worsened with Pyridostigmine, but responded to Salbutamol. Conclusion: The definitive diagnosis of congenital myopathy or CMS using targeted gene panels is quicker, non-invasive and should supersede invasive tests like EMG and muscle biopsy. This also aids in antenatal diagnoses.

Ramesh Konanki(1); Lokesh Lingappa*(1); Shah, N.M.(2)*;
(1)Department of Neurology, Rainbow Hospital for Women and Children, Hyderabad, Telangana;(2)Department of Neurology, Rainbow Hospital for Women and Children, Hyderabad, India;
ABSTRACT BOOK PLATFORM

Wednesday 4 May
Neurmuscular disorders - diagnosis

ICNC-0732   Fat infiltration is non-uniform along the proximodistal muscle axis in Duchenne Muscular Dystrophy

Introduction
The progressive replacement of muscle tissue by fat in Duchenne muscular dystrophy (DMD) has been studied using quantitative MRI between, but not within individual muscles. The latter is important to better understand the pathophysiology of muscle degeneration in this disease.

Methods
We studied fat infiltration along the proximodistal muscle axis within all individual lower leg muscles using the Dixon technique on a 3T MR scanner in 22 DMD patients (mean age 9.3±3.1 years, range 5-16 years). Fat fractions were reported as a mean value per muscle per slice. Generalized estimating equations were used to evaluate the effect of location on the proximodistal axis on fat percentage (%fat), assuming a parabolic relation.

Results
Higher %fat was observed in distal and proximal muscle segments compared to the muscle belly. This difference in %fat along the axis ranged from 5-40%. Location along the proximodistal muscle axis had a significant effect on %fat for all muscles but the gastrocnemius lateralis and peroneus muscles.

Discussion
Mechanical strain is not distributed uniformly along the proximodistal muscle axis in healthy skeletal muscle (1), and dystrophin is concentrated near the musculotendinous junction in mice. (2,3) We propose that stress-induced muscle degeneration starts at the level of the myotendinous junction and becomes more homogeneous in later stages of the disease. This needs to be taken into account when using muscle biopsies and quantitative MRI as biomarkers for DMD. It also points to mechanical disruption of the membrane as one of the key factors in the pathophysiology of DMD. [1] Shinn D.D. et al. J Apply Physiol 2009; [2] Tidball JG et al. Am J Pathol 1991 [3] Samitt CE et al. Muscle Nerve 1990;

M. T. Hooijmans(1); N. Doorenweerd(1); J. Burakiewicz(1); Anastasopoulos*, C.(2)*; A.G. Webb(1); J.J.G.M Verschuuren(3); E.H. Niks(3); H.E. Kan(1);
(1) C. J. Gorter Center, Radiology, Leiden University Medical Center, Leiden; (2) C. J. Gorter Center, Radiology, Leiden University Medical Center, The Netherlands; (3) Neurology, Leiden University Medical Center, Leiden, Zuid-holland;
Cerebral diffusion weighted magnetic resonance spectroscopy suggests membrane damage or structural deficits in neuronal and glial cells in Duchenne muscular dystrophy patients

DMD is associated with learning disabilities and neurodevelopmental disorders in addition to progressive muscle weakness[1]. Dystrophin is expressed in neuronal, glial and endothelial cells in which its function is unclear[2]. Cerebral diffusion tensor imaging previously indicated microstructural alterations with increased water apparent diffusion coefficient (ADC) and reduced fractional anisotropy throughout the white matter [3]. However, diffusion of water in biological tissues is non-specific, occurring inside, outside, and through cellular structures. Diffusion weighted spectroscopy (DWS) can provide ADCs of cell-type specific metabolites[4,5]. DWS and T1-weighted MR scans were obtained in ten patients with DMD (mean age 16.2, range 10-22 years) and six age-matched controls. The DWS spectra were zero-order phased, eddy current and frequency shift corrected, and quantified using LCModel with a simulated basis set to compute the ADCs of N-acetylaspartate (exclusively neuronal), creatine (ubiquitous) and choline (predominantly glial)[5]. Variances were assessed using an F-test and an unpaired t-test was used to assess differences in ADCs (p<0.05). ADCs of tNAA and Cho were significantly higher (p=0.036 and p=0.026 respectively) and that of tCr higher (p=0.054) in DMD patients. Variance was significantly higher in tNAA ADC in DMD patients (p=0.029). Our results suggest that metabolite ADC increases are non-cell-type specific. Combined with earlier results of increased water ADC this may indicate leaky membranes, allowing exchange with extracellular space similar to what occurs in muscle cells in DMD patients[3,6]. Alternatively, there may be structural deficits, such as changes to mitochondria or the cytoskeleton, within the cells that are non-specific.

References

Doorenweerd *, N.(1)*;Ece Ercan(2);Melissa Hooijmans(2);Jedrak Burakiewicz(2);Kieren Hollingsworth(3);Volker Straub(4);Andrew Webb(2);Jos Hendriksen(5);Jan Verschuuren(2);Erik Niks(2);Hermien Kan(2);Itamar Ronen(2);
(1)Leiden University Medical Centre, C.J.Gorter Centre for High Field MRI, Leiden, The Netherlands;(2)Leiden University Medical Centre, Leiden, The Netherlands;(3)Newcastle Magnetic Resonance Centre, Campus for Ageing and Vitality, Newcastle University, Newcastle upon Tyne;(4)John Walton Muscular Dystrophy Research Centre, International Centre for Life, Newcastle University, Newcastle upon Tyne;(5)Kempenhaeghe Epilepsy Center, Heeze, The Netherlands;
ICNC-0707  Alpha-dystroglycanopathies (αDGpathy): Diagnostic yield, from new sequencing techniques depends on deep clinical phenotyping

Introduction: αDGpathies are heterogeneous group of disorders ranging from severe congenital muscular dystrophy (CMD) forms with and without structural brain anomalies to limb-girdle muscular dystrophy (LGMD). Methods: Thirty patients from 27 families with the clinical and pathological diagnosis of αDGpathy were included in the study. Molecular work-up included whole exome sequencing including CNV analysis. Results: The mean age of the patients (17 boys, 13 girls) was 7 years (6 months-19 years), of 22 (73%) being symptomatic before 6 months of life. Presenting symptoms were; developmental delay (n=14), hypotonia (n=4), difficulty in climbing stairs (n=4), respiratory and feeding problems (n=2), seizure (n=1). The phenotypic categorization of patients in order of clinical severity is; Walker-Warburg Syndrome (n=1), muscle-eye-brain syndrome (n=2), CMD with mental retardation (n=9), CMD with cerebellar involvement (n=5), LGMD-MR (n=6), LGMD-No MR (n=4). Consanguinity was present in 20 families, and 41% (n=11) had affected sibling. MRI was available in 22 patients, and 16 (72%) had abnormal findings. Homozygous and compound heterozygous mutations in αDGpathy genes were detected in 18 of 22 (82%) probands. Five patient’s genetic work-up is pending. Mutations in POMT1 were the most prevalent (n=5) in our cohort, followed by FKRP (n=3), POMT2 (n=3), POMGNT1 (n=3), TMEM5 (n=1), FKTN (n=1), GMPPB (n=1), and SGK196 (n=1). Of those 5 are novel mutations. Conclusion: Our etiological yield with next generation sequencing techniques has been 82% so far. This is probably above the expected level, and highlights the importance of a multidisciplinary expert group for clinical, pathological and genetic work-up.

Didem Ardicli*, D.(1)*; Mert Karakaya, M.(2); Gökknur Haliloğlu, G.(1); Beril Talim, B.(3); Haluk Topaloglu, H.(1); Sebahattin Cirak, S.(2);
(1) Hacettepe University- Children’s Hospital, Department of Pediatric Neurology, Turkey; (2) Cologne University Hospital, Department of Pediatrics and Institute of Human Genetics, University Hospital Cologne, Germany; (3) Hacettepe University- Children’s Hospital, Department of Pediatric Pathology, Department of Pediatric Pathology, Turkey;
ICNC-0716  Novel genotypes and phenotypes revealed by whole exome sequencing studies in familial Charcot-Marie-Tooth disease

Charcot-Marie-Tooth (CMT) disease is a clinically and molecularly heterogeneous familial peripheral neuropathy disorder. Direct Sanger sequencing and more recently next generation (gene panel as well as whole exome) based sequencing have been used to confirm the molecular etiology of CMT. We used SNP-CGH arrays followed by whole exome sequencing (WES) to identify the genetic basis of idiopathic CMT in two autosomal recessive and one autosomal dominant family. In a consanguineous (Gypsy Romanov) family with axonal sensory motor polyneuropathy (CMT-II), WES identified homozygosity for a novel splicing mutation (c.376-9T>G) in MPV17 which was confirmed by RT-PCR. Autosomal recessive mutations in MPV17 are known to cause Mitochondrial (hepatocerebral) DNA depletion syndrome 6. In family 2, two sisters with Mondini anomaly, deafness, facial dysmorphism, and peripheral neuropathy were found to have compound heterozygous mutations (c.1715C>T; c.94C>T) in the novel gene ASB2 (ankyrin repeat and suppressor of cytokine signaling/SOCS box-containing), which plays a role in protein degradation with the elongin BC complex. In family 3, a man and his daughter with apparent autosomal dominant simplex CMT, WES revealed an extremely rare heterozygous missense mutation (c.161C>T) in PPP2R4 which encodes PP2A that regulates expression of HSP27 encoded by HSPB1. Interestingly, heterozygous mutations in PPP2R4 cause CMT type 2F and hereditary distal motor neuropathy type IIB. In conclusion, our clinical and WES studies suggest mutation of MPV17 gene as a new cause for uncomplicated CMT. Additionally, we suggest ASB2 and PPP2R4 as novel genes involved in autosomal recessive and dominant peripheral neuropathy, respectively.

Majed Dasouki(1,2)*; Yiran Guo(3); Hakon Hakoranson(4); Everett G. Hall(5); Diana S. Acevedo(5); Irfan Saadi(5)

(1)Department of Genetics, King Faisal Hospital & Research Center, Riyadh, Saudi Arabia; (2)Department of Neurology, University of Kansas Medical Center, Kansas City, KS;
(3)Center for Applied Genomics, The Children’s Hospital of Philadelphia, Philadelphia, PA;
(4)Center for Applied Genomics, The Children’s Hospital of Philadelphia, Philadelphia, PA; Department of Pediatrics, The Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA
(5)Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS
ICNC-0754  Spinal muscular atrophy syndromes: involvement beyond the anterior horn cell

Introduction: Spinal muscular atrophies are a group of inherited disorders characterized by motor neuron loss in the spinal cord and lower brainstem, muscle weakness and atrophy. While clinical manifestations are typically confined to anterior horn cells, less common phenotypes may include more extensive or multisystem involvement (SMA plus). Advances in Next Generation Sequencing (NGS) have accelerated the identification of causative genes and shown to be more comprehensive and able to provide diagnostic results in a timely fashion.

Methods: Utilising clinical, neurophysiological, radiological and pathological assessments combined with NGS technologies, we performed 13 exomes in 8 families with SMA plus. The aim of the study was to define phenotypes and causative genes, provide pathophysiological insights and guide diagnostic approaches.

Results: Various SMA plus syndromes were identified revealing symptoms beyond the anterior horn cell, including bulbar and/or cortico-motor neurone dysfunction, visual, hearing or cognitive impairment/ degeneration, epilepsy, achalasia and endocrinopathy. Five families had mutations in known SMA plus genes (VRK1, ASAH1, FBXO38, PLAG26 and AAAS) with expanded phenotypes related to the gene observed. One potentially novel candidate gene was discovered with functional studies underway and no mutation was found in two families. Neurophysiological, radiological and pathological investigations were important in characterising phenotypes. Taken together, these assessments were critical in attaining molecular genetic diagnosis, in both single gene testing and NGS approaches.

Conclusion: Understanding the diverse clinical and genetic phenotypes of SMA plus syndromes is essential in developing a diagnostic strategy and emphasises the importance of a multidisciplinary approach to navigate the complexities of molecular genetic diagnosis.

Teoh*, H.L.(1,2)*; Sampaio, H.(1,2); Mowat, D.(2);; Buckley, M.(4); Roscioli, T.(5); Farrar, M.(1,2); (1) Sydney Children’s Hospital, Department of Neurology, Sydney, Australia; (2) School of Women’s and Children’s Health - University of New South Wales, Discipline of Paediatrics, UNSW Medicine, Australia; (3) Department of Medical Genetics, Sydney Children’s Hospital, NSW, Australia; (4) Prince of Wales Hospital, SEALS laboratory, Randwick, Australia; (5) Sydney Children’s Hospital, Department of Medical Genetics, Sydney, Australia;