scoliosis, ankle contractures and ataxia. There was no cardiac or respiratory involvement. EMG was normal. Brain MRI at 6 years showed cerebellar atrophy and mild under-opercularisation of the left Sylvian fissure; when repeated at 9 years, there was mild progression of cerebellar atrophy and additional supratentorial sulcal prominence suggestive of volume loss. Muscle MRI showed generalized increase in T1 signal in the lower limb with normal STIR sequences. CK was raised (800-1614 IU/L). Muscle biopsy showed fibre size variability, increased internal nuclei, fatty endomysial infiltration, few regenerating fibres, type 1 predominance and some minicores. Minor changes of uncertain significance on laminin-α and a-dystroglycan staining were observed. Respiratory chain enzyme studies were normal. Whole-exome sequencing revealed 2 missense MSTO1 variants. The first variant (c.766C>T p.(Arg256Trp)), affecting a conserved residue in the tubulin domain of the protein, is reported in the gnomAD dataset with an allelic frequency of 0.00003, while the second (c.1435C>T p. (Pro479Ser)) is novel. In silico tools predict both variants as damaging. Phasing of the variants is in progress. This case confirms a consistent phenotype associated with recessive *MSTO1* gene mutations and suggests that progressive cerebellar atrophy can be a feature of the condition.

http://dx.doi.org/10.1016/j.nmd.2018.06.217

P.190

Novel variant of *GOSR2* gene in a patient presenting with mitochondrial myopathy and epilepsy

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GOSR2 gene is a Golgi vesicle transport gene which encodes for the Golgi SNAP receptor complex member 2 protein. This protein mediates transport between the medial and trans Golgi compartments. Homozygous mutations in *GOSR2* gene (variant c.403G>T, p.G144W) have been associated with progressive myoclonic epilepsy. Patients reported had mildly elevated creatine kinase but normal muscle biopsy and no symptoms of myopathy. One reported case with compound heterozygous *GOSR2* mutations (previously described mutation c.430G>T and a novel splice site mutation c.336+1G>A) presented with congenital muscular dystrophy. Here we report a case of congenital hypotonia and epilepsy with muscle biopsy findings consistent with mitochondrial myopathy. Patient was found to have compound heterozygous *GOSR2* mutations (paternally inherited previously described pathogenic mutation c.430G>T and a maternally inherited novel mutation c.22dup that was classified as likely pathogenic. We hypothesize that the novel variant of *GOSR2* gene found in this patient could be contributing to both pathologies (mitochondrial myopathy and epilepsy) together. Additionally, we hypothesize that *GOSR2* gene may be involved in mitochondrial structure formation and may play a role in the development of mitochondrial myopathy.

http://dx.doi.org/10.1016/j.nmd.2018.06.218

P.191

Single muscle fiber analysis of extraocular and skeletal muscles in a CPEO patient harboring a pathogenic point mutation in the *MT-TN* gene

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Chronic progressive external ophthalmoplegia (CPEO) is a frequent feature of mitochondrial disorders and usually associated with mitochondrial DNA (mtDNA) mutations. Skeletal muscle (SKM) tissue is most frequently used for biochemical analyses and mitochondrial genome testing. However, extraocular muscle (EOM) is the clinically most affected tissue but usually not available for routine work-up. Consequently, systematic data on EOM is limited and the reason for preferential clinical affection remains unclear. We addressed this unsolved question by histochemical and genetic analyses of EOM and SKM single muscle fibers in a patient with isolated CPEO caused by a heteroplasmic point mutation in the *MT-TN* gene. The histochemical analysis showed higher absolute numbers of cytochrome c oxidase (COX)-deficient EOM fibers compared to COX-deficient SKM fibers. However, genetic analyses by restriction fragment length polymorphism revealed no significant difference in the mutation loads between COX-negative single muscle fibers in EOM compared to SKM. Quantitative single fiber real-time PCR revealed higher mtDNA copy numbers in single muscle fibers of EOM compared to SKM. COX-negative single muscle fibers of EOM and SKM showed significantly higher mtDNA copy numbers compared to COX-positive fibers suggestive of a compensatory mtDNA proliferation. We show that high loads of the *MT-TN* mutation correlate with a biochemical loss of COX activity in single muscle fibers of EOM and SKM at a similar threshold. The higher absolute numbers of COX-negative fibers in EOM compared to SKM might be caused by facilitated segregation of the mutation into the EOM providing thereby a possible explanation of the preferential ocular manifestation in CPEO.

http://dx.doi.org/10.1016/j.nmd.2018.06.219

P.192

Severe isolated mitochondrial myopathy in childhood

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Mitochondrial disorders often present as multisystemic diseases, but some patients only show symptoms in a single organ or tissue. Isolated myopathy as solitary manifestation of mitochondrial disease is relatively rare. They can present with myalgia, exercise intolerance, proximal muscle weakness or external ophthalmoplegia. The clinical course is variable; from rapidly progressive to static. Here we describe three pediatric patients with severe mitochondrial myopathy. The three male patients were from non-related parents. They presented at the mean age of 2.5 years with rapidly progressive myopathy characterized by proximal muscle weakness, shoulder girdle atrophy, and profound weakness of neck muscles with dropped head. They developed severe muscular hypotonia and two lost the ability to sit and walk. Non-neurologic manifestations were: respiratory insufficiency (2/3), apneas (1/3) and poor weight gain (3/3). Cardiac evaluation was normal (3/3). CK and lactic acid levels were increased in all. Brain MRI was unremarkable (3/3); MRS showed lactate peak (1/3). Treatment with coenzyme Q10, carnitine, riboflavin and thiamine did not show any effect. Muscle biopsies revealed increased mitochondrial proliferation with ragged red (2/3) and COX deficient fibers (3/3). Respiratory chain activity in muscle (2/3), showed severe complex I reduction (case 1) and multiplex complex deficiency (case 2). Mitochondrial DNA content was severe reduced (case 2). Molecular studies identified: case1, a homoplasmic variant on *MT-TL1* gene m.A>G3302, case2, biallelic mutations on *TK2* gene c.547C>T, (p.Arg183Trp) and c.416C>T (p.Ala139Val), case 3, a heteroplasmic variant on *MT-TL1* m.A>G3243. Mitochondrial disorders should be considered in the differential diagnosis of early onset severe isolated myopathy. Since they can be related to mitochondrial or nuclear DNA mutations, molecular diagnosis is essential for prognosis and genetic counseling.

http://dx.doi.org/10.1016/j.nmd.2018.06.220

P.193

Unexpected genetic diagnosis of mitochondrial disease in three consanguineous Turkish families

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Mitochondrial diseases are a clinically heterogeneous group of disorders caused by dysfunction of the mitochondrial respiratory chain. Some mitochondrial disorders affect a single organ, while many involve multiple systems such as skeletal muscle, brain, heart and liver, leading to diagnostic difficulties. Here we present three patients who were originally suspected to have a primary disease of skeletal muscle, leukodystrophy and brain malformation. Patients were recruited from three paediatric neurology clinics in Turkey: Izmir, Malatya and Diyarbakir. Whole exome sequencing (WES) was performed using Illumina exome capture (38 Mb target). Data analysis was carried out on the RD-Connect Genome-Phenome Analysis Platform (https://platform.rd-connect.eu/). Standard filtering criteria with MAF<1% and high/moderate VEP were used, as well as a list consisting of >5,000 medically interpretable genes. We identified a homozygous frameshift variant (p.Glu41GlyfsTer10) in *NDUFA12* and a homozygous missense variant (p.Gln85His) in *NDUFS3*, both associated with Leigh syndrome due to mitochondrial complex I deficiency (OMIM# 256000). We also identifid a homozygous nonsense variant (p.His158ProfsTer8) in *TACO1* associated with mitochondrial complex IV deficiency (OMIM# 220110), the second patient to be described worldwide so far. All the variants were highly pathogenic and were absent in the control population, suggesting they were disease-causing. Critical clinical review and metabolic analysis confirmed the mitochondrial deficiency. Next generation sequencing has the advantage of allowing an unbiased genetic diagnosis. We described three cases that had been initially diagnosed as myopathy, brain malformation and leukodystrophy, and WES resulted in the diagnoses of mitochondrial disorders. Importantly, this will allow for appropriate clinical management of these patients.

http://dx.doi.org/10.1016/j.nmd.2018.06.221

P.194

Deficiency of the iron-sulphur cluster assembly protein ISCU causes impaired biogenesis or stability of respiratory chain complex I, II and IV in muscle

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Iron–sulphur cluster containing proteins are essential for iron homeostasis and respiratory chain function, with ISCU being among the most conserved proteins in evolution. Deficiency of the mitochondrial isoform due to deleterious mutations in the ISCU gene is associated with mitochondrial myopathy and exercise intolerance known as hereditary myopathy with lactic acidosis (OMIM #255125). Biochemical investigations demonstrate a characteristic profound complex II (succinate dehydrogenase, SDH) deficiency in addition to complex I and complex IV (cytochrome c oxidase, COX) deficiency. Muscle histopathology is typically associated with SDH deficiency as well as regional COX deficiency. We analyzed the expression of complex I to V of the respiratory chain by immunohistochemical and western blot analyses using antibodies to subunits of the five different enzyme complexes in muscle tissue of patients with homozygous or compound heterozygous deleterious *ISCU* mutations. The monoclonal antibodies we used were from Abcam and directed to subunit NDUFB8 (complex I), SDHB (Complex II), UQCRC2 (complex III), MTCO1 (complex IV), ATPB (complex V) and VDAC1 (mitochondrial marker). There was a reduced expression of complex I, II and IV subunits as demonstrated by western blot analysis. Immunohistochemistry showed that the deficiency was restricted to the same regions as the enzyme histochemical deficiency. The results demonstrate that the peculiar regional respiratory chain enzyme deficiency seen in hereditary myopathy with lactic acidosis is associated with deficiency of protein subunits of the corresponding respiratory chain complexes. Lack of ISCU protein thus affects the biogenesis, assembly or stability of several of the respiratory chain complexes, which appears to be an important mechanism that explains the enzyme deficiency and clinical symptoms.

http://dx.doi.org/10.1016/j.nmd.2018.06.222

P.195

A novel multiplex chromogenic immunoassay for evaluating mitochondrial respiratory chain complex I and complex IV defects in diagnostic muscle biopsies

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The investigation of clinically suspected mitochondrial disease (mtD) includes performing a skeletal muscle biopsy for biochemical/histochemical assessment of mitochondrial respiratory chain (RC) defects. COX-SDH histochemistry detects RC-complex IV (CIV) defects, but RC-complex I (CI) defects cannot be detected histochemically. CI/CIV defects are common in mtD. Immunohistochemical evaluation of RC-complex defects relies on reduced amount of the assembled complex associated with catalytic deficiency, detectable with RC subunit-specific monoclonal antibodies. Our aim was to design a dual chromogenic immunoassay (DCI) for evaluating CI/CIV defects in diagnostic muscle biopsies. In the DCI optimised protocol, primary antibodies (Abcam), TOMM20 (mitochondrial mass), NDUFB8 (CI) and MTCO1 (CIV) were coincubated (TOMM20+CI and TOMM20+CIV), and then TOMM20 developed to yellow and the other marker to teal (Discovery/Ventana Systems) with colocalising antibodies visualising as green. Control sections stained as a mosaic dark green (type I fibres) and light green (type II fibres) pattern. Completely CI/CIV-deficient fibres stained yellow, and partly CI/CIV-deficient fibres stained yellow-green, and were easily detectable due to good visual colour contrast. The DCI and COX-SDH assays were performed in serial frozen sections. 23 biopsies were assessed: 15 with genetically confirmed mtD (mtDNA rearrangements/point mutations/depletion), 4 with high clinical/histological suspicion of mtD, and 4 unaffected controls. % COX and CI/CIV-deficient fibres were counted in two random fascicles, with high concordance amongst % COX-negative and CI/CIV-deficient fibres. The DCI detected more CI-deficient fibres in 7/19 cases and more CIV-deficient fibres in 5/19 cases compared to COX-negative fibres (average 6%). Most COX-negative fibres had dual CI+CIV defects with DCI. Segmental and partial CI/CIV defects were detectable. Equivocal COX-SDH stained fibres were often strongly CI/CIV-immunodeficient. In conclusion, our multiplex DCI reliably detects CI/CIV defects comparable in sensitivity to the COX-SDH histochemical assay, is easy to evaluate due to a good visual contrast between CI/CIV positive and negative fibres and can be easily co-opted to routine diagnostic work. Studies are underway to develop a quadruple chromogenic immunoassay for digital evaluation of CI/CIV defects.

http://dx.doi.org/10.1016/j.nmd.2018.06.223

MITOCHONDRIAL DISEASES I (Oral)

I.4

Skeletal muscle manifestations in mitochondrial disease

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Besides their critical role in bioenergetics, mitochondria additionally display complex dynamic behaviors within cells, including fusion, fission, directed transport and targeted destruction (mitophagy). The relevance of these processes to human disease has been intensively documented over the last several years. While oxidative phosphorylation defects are classically associated with muscular dysfunction, more recent work indicates that genetic defects in mitochondrial dynamics are also associated with myopathy in mice and humans. Indeed, the long, cylindrical geometry of skeletal myofibers places unique demands on the mitochondrial population and limits functional homogeneity along the length of the fiber. Our efforts to measure mitochondrial dynamics in intact skeletal muscle has provided insight into the role of organelle behavior in modulating disease. We find that heterogeneity in mitochondrial fusion rates is not only a defining characteristic between mus-