

PW 19:
Mitochondrial disorders

PW19-228	<p><u>ADULT ONSET ATAXIC NEUROPATHY AS ISOLATED MANIFESTATION OF MITOCHONDRIOPATHY</u> POUGET J¹, SALORT-CAMPANA E¹, AZULAY JP¹, UZENOT D¹, FIGARELLA-BRANGER D², PAQUIS V³ (1) Neuromuscular Reference Center, Marseille, FRANCE. (2) Neuropathology Department, Marseille, FRANCE. (3) Mitochondriopathy Reference Center, Nice, FRANCE.</p>
	<p>Objective : to report 2 cases of isolated ataxic neuropathy with onset in adult age and associated with mitochondriopathy.</p> <p>Background : Patients with mitochondriopathy exhibit a wide range of neurologic symptoms and peripheral neuropathy can be considered to be an unusual clinical manifestation for a patient with a mitochondrial disease. In addition to a chronic axonal sensorimotor neuropathy, sensory ataxic neuropathy is more evocative and usually included in complex syndromes : NARP, SANDO, MNGIE. Mitochondriopathy is usually not considered as etiology for adult onset sensory ataxic neuropathy.</p> <p>Design/methods : We had the opportunity to observe 2 cases of sporadic adult-onset sensory ataxic neuropathy which can be attributed to multiple mitochondrial DNA deletions.</p> <p>Results : The first case concerns a 67 yrs man with ataxic symptoms onset at 47 yrs. ENMG revealed normal motor potentials and absent sensory potentials in the four limbs. Nerve biopsy showed a severe loss of myelinated fibres without any regenerative process. Muscle biopsy demonstrated a very few ragged red and Cox negative fibres. In skeletal muscle, we found multiple mt-DNA deletions by long-range PCR and southern blot analysis. No mutation were identified in the POLG1, TWINKLE and ANT1 genes. Ocular muscles involvement appeared only late in the clinical course at 65 yrs. The second case concerns a 50 yrs old woman who complained of ataxia since 20 yrs. A slow progression was reported, compatible with an unaided walking at 50 yrs. Ocular muscles were completely spared, even at 50 yrs and the peripheral neuropathy was isolated. Electrophysiological study showed no sensory potential in four limbs and reduced motor amplitude in lower limbs. In skeletal muscle, we found few mt-DNA deletions by long-range PCR and southern blot analysis. We analysed the coding region and exon/intron boundaries of the POLG1 gene by sequencing. The patient was heterozygous for both c.1391 T>C (M464T) and c.2302 A>G (K768E) misense mutations.</p> <p>Conclusion : Mitochondriopathy as a cause of isolated ataxic neuropathy has to be considered in adults, even after 40 yrs. This form is probably underdiagnosed and prevalence underestimated. Muscle mt-DNA analysis can be proposed as a main diagnostic marker.</p>

PW19-229	<p><u>HETEROGENEITY PHENOTYPIC AND HETEROPLASMY IN MTDNA T8993G MUTATION IN NARP AND MILS (PATIENTS FROM ARGENTINA)</u> MARTINEZ PEREA MDC¹, LISTE H², POSADAS MARTINEZ MM³ (1) HOSPITAL AERONAUTICO CENTRAL.JEFE SERVICIO NEUROLOGIA INFANTIL, BUENOS AIRES, ARGENTINA. (2) HOSPITAL AERONAUTICO CENTRAL.NEUROLOGIA, BUENOS AIRES, ARGENTINA. (3) HOSPITAL AERONAUTICO CENTRAL.EX ROTANTE UDH.SERV.NEUROLOGIA INFANTIL, BUENOS AIRES, ARGENTINA.</p>
To contact the author:: mdeposadas@intramed.net.	<p>The mitochondrial DNA point mutation T8993G has been associated with NARP and maternally inherited Leigh syndrome (MILS). Usually there is a correlation between the percentage of mutated mtDNA and clinical severity. Objectives: The aim is to show different clinic phenotypes, multisystemic disorders and different percentage to mtDNA mutation and heteroplasmy in the same family with diagnosis of NARP and others members maternally inherited with Leigh's syndrome (MILS). Material and Methods: We present four members of an Argentinean family with mtDNA T8993G point mutation who had diverse clinical manifestations and different percentage of mtDNA mutation. Diagnosis was established according to modified Walker Criteria based in clinical, functional, molecular, metabolic parameters and was followed for 11 years. Case 1: IF. Female 14 years, early onset, seizures at 8 months, motor milestones delayed, nasal speech, swallowing difficulties, ataxia, and neuropathy. CT scan showed lesions in basal ganglia and atrophy of the brain. Case 2: NF. Female 13 years old, presented sudden severe weakness at 5years old, fever and lethargy. CT scan showed hypointense signal in basal ganglia. Case 3: BA. Female 40 years old, presented rheumatoid arthritis, neuropathy. Case 4: LF. Female 18 years old, presented convergent strabismus and abnormal movements at 15 years old. Molecular test showed mtDNA T8993G point mutation. They did not show cardiological disturbances. Results: The percentage of mtDNA T8993G point mutation in blood was: Case 1:74%, in muscle biopsy 92%. Sural nerve biopsy showed axonal degeneration. Case 2: 86%. Case 3: 36%. Case 4: show mtDNA T8993G point mutation. CONCLUSION: Phenotypic heterogeneity in this family show both Leigh's and NARP phenotypes. High mitochondrial DNA T8993G mutation is not always associated with typical features and severity of Leigh's and NARP syndromes. There was no correlation in our patients between proportion of mutant mitochondrial DNA in blood and clinical features. Acknowledgments: to Dr. Bindhoff, Middlesbrough General Hospital. United Kingdom. Dra. Lei Li, Moelcular Laboratory , Childrens Hospital Los Angeles. USA. Buenos Aires. Argentina: Dra. A. Taratutto, FLENI, Dra.Marcela Garcia Alvarez.</p>

PW19-230	<p><u>SENSORY ATAXIC NEUROPATHY WITH OPHTALMOPLEGIA AND DYSARTHRIA IN MOROCCAN PATIENT WITH HOMOZYGOTE MUTATION IN POLG GENE</u> RAFAI MA¹, BOULAAJAJ F², JARDEL C. ³, LOMBES A. ⁴, BOUREZGUI M⁵, EL MOUTAWAKIL B⁶, SLASSI I⁷</p> <p>(1) (1) Service de Neurologie- explorations Fonctionnelles, Chu Ibn Rochd, casablanca, MOROCCO. (2) (1) Service de Neurologie- explorations Fonctionnelles, Chu Ibn Rochd, casablanca, MOROCCO. (3) (2) Unité INSERM, Bâtiment Babinski, Hôpital Pitié Salpêtrière, paris, FRANCE. (4) (2) Unité INSERM, Bâtiment Babinski, Hôpital Pitié Salpêtrière, paris, FRANCE. (5) (1) Service de Neurologie- explorations Fonctionnelles, Chu Ibn Rochd, casablanca, MOROCCO. (6) (1) Service de Neurologie- explorations Fonctionnelles, Chu Ibn Rochd, casablanca, MOROCCO. (7) (1) Service de Neurologie- explorations Fonctionnelles, Chu Ibn Rochd, casablanca, MOROCCO.</p>
To contact the author:: neuroblanca@gmail.com	<p>Introduction Mitochondrial cytopathies represent very heterogonous a group of affections related to a dysfunction of the respiratory chain of mitochondrion. Their expression is polymorphic and pluristemic. The progressive external ophtalmoplegia is one of the most characteristic symptoms and may be the onset clinical presentation. Its association with a ataxiant neuropathy and a dysarthria defines a new entity: the SANDO syndrome (sensory ataxic neuropathy with dysarthria and ophtalmoplegia).</p> <p>Case report We report a new observation, a 21 year old young patient with first degree consanguinity, presented ataxia with axonal sensory-motor neuropathy and sensitive prevalence at the ENMG, associated ophtalmoplegia, a ptosis and a dysarthria evolving since the 7 years age.</p> <p>Résultats Serum index Lactate - Pyruvate is increased and the neuromuscular biopsy revealed presence of many Red Ragged Fibers (RFF). Brain MRI was normal. The diagnostic of a mitochondriopathy type SANDO was the most probable. Molecular studies in genomic DNA (POLG gene) found homozygote mutation type: 1789 C>T.</p> <p>Discussion The SANDO syndrome is a rare mitochondriopathy currently descripted (only 6 cases are brought back to our knowledge). It's characterized clinically by the association of progressive external ophtalmoplegia with dysarthria and ataxiant neuropathy.</p> <p>Conclusion The role of nuclear DNA mutation to determine mitochondrial dysfunction is not completely known.</p>

PW19-231	<p>SEVERE SENSORY GANGLIONOPATHY AS THE PREDOMINANT PRESENTATION OF DNA POLYMERASE GAMMA (POLG) MUTATION MAISONOBE T¹, JARDEL C², TCHIKVILADZÉ M³, LAFORÉT P³, HAUW JJ⁴, BOUCHE P⁵, LOMBÈS A⁶</p> <p>(1) Département de Neurophysiologie Clinique; Laboratoire de Neuropathologie R. Escourolle, AP/HP GHU Pitié- Salpêtrière; INSERM U582; UPMC-Paris6, Paris, FRANCE. (2) Biochimie métabolique; AP/HP GHU Pitié- Salpêtrière; INSERM U582; UPMC-Paris6, Paris, FRANCE. (3) Centre de référence des maladies Neuromusculaires, Institut de Myologie, AP/HP GHU Pitié-Salpêtrière; UPMC-Paris6, Paris, FRANCE. (4) Laboratoire de Neuropathologie R. Escourolle, AP/HP GHU Pitié-Salpêtrière; UPMC-Paris6, Paris, FRANCE. (5) Département de Neurophysiologie Clinique; AP/HP GHU Pitié- Salpêtrière; UPMC-Paris6, Paris, FRANCE. (6) INSERM U582; Biochimie métabolique; AP/HP GHU Pitié- Salpêtrière; UPMC-Paris6, Paris, FRANCE.</p>
To contact the author:: a.lombes@institut-myologie.org.	<p>Genetic alterations of the catalytic subunit of the polymerase gamma gene (<i>POLG</i>) have been recently involved in very diverse clinical diseases ranging from early severe Alpers-Huttenlocher syndrome to late adult diseases presenting with a very diverse combination of ophthalmoplegia, ataxia, epilepsy, peripheral neuropathy, gastro-intestinal symptoms, psychiatric disturbances, Parkinson syndrome... Polymerase gamma is the sole DNA polymerase that is responsible for the replication of mitochondrial DNA. Its reported mutations have been very diverse, affecting any of the three functional domains of the protein (polymerase catalytic, exonuclease or linker domains). They have been associated with recessive as well as dominant inheritance. Furthermore several <i>POLG</i> mutations have significant frequency in the general population despite <i>in vitro</i> demonstration of their impact on the protein function and/or stability. In conclusion mutations of the <i>POLG</i> gene appear to be a major cause of a number of human neuromuscular disorders, the range of which remains to be determined.</p> <p>We here report the clinical, electrophysiological, and muscle histological characteristics of a series of 10 patients with severe sensory ganglionopathy as prominent clinical presentation. These investigations allowed to diagnose the mitochondrial origin of the disease. Genetic analyses demonstrated the presence of <i>POLG</i> mutations with recessive inheritance in most of these patients. Severe sensory ganglionopathy should thus be considered as a clinical presentation highly suggestive of <i>POLG</i> mutations.</p>

PW19-232	<p><u>CO-EXISTENCE OF DNA POLYMERASE GAMMA (POLG) AND MITOCHONDRIAL DNA (MTDNA) MUTATIONS MAY HAVE IMPACT ON DIAGNOSIS AND GENETIC COUNSEL</u></p> <p>MEDJA F¹, JARDEL C², NELSON I¹, TCHIKVILADZÉ M³, LAFORÊT P³, EYMARD B³, LOMBÈS A⁴</p> <p>(1) INSERM 582; UPMC-Paris6, Paris, FRANCE. (2) Biochimie métabolique, AP/HP GHU Pitié- Salpêtrière; INSERM U582; UPMC-Paris6, Paris, FRANCE. (3) Centre de référence des maladies Neuromusculaires, Institut de Myologie, AP/HP GHU Pitié-Salpêtrière; UPMC-Paris6, Paris, FRANCE. (4) INSERM U582; Biochimie Métabolique, AP/HP GHU Pitié Salpêtrière; UPMC-Paris6, Paris, FRANCE.</p>
To contact the author:: a.lombes@institut-myologie.org.	<p>Genetic alterations of the catalytic subunit of the polymerase gamma gene (<i>POLG</i>) have been recently involved in very diverse clinical diseases ranging from early severe Alpers-Huttenlocher syndrome to late adult diseases presenting with a very diverse combination of ophthalmoplegia, ataxia, epilepsy, peripheral neuropathy, gastro-intestinal symptoms, psychiatric disturbances, Parkinson syndrome... Polymerase gamma is the sole DNA polymerase that is responsible for the replication of mitochondrial DNA. The functional consequences of its alterations are defects of the mtDNA maintenance with either quantitative (depletion) or qualitative anomalies (mutations and deletions) of the mtDNA. These alterations are responsible for the defect in oxidative phosphorylation that underlies the onset of symptoms.</p> <p>The presence of mtDNA alterations is therefore an intrinsic part of the diagnostic criteria that lead to the search for <i>POLG</i> mutations. However, once created by the mutated <i>POLG</i>, qualitative mtDNA alterations have an evolution that is independent from the <i>POLG</i> mutation. They may be amplified as reported in normal ageing process. They also may be transmitted according to the maternal transmission of mtDNA and therefore independently from the <i>POLG</i> allele.</p> <p>We present two pedigrees that illustrate the questions raised by the co-existence of <i>POLG</i> and mtDNA mutations with respect to the relative responsibility of the mutations in the clinical presentation and the consequence on the mode of transmission of the symptoms.</p>

PW19-233	<p>CEREBELLAR ATAXIA ASSOCIATED TO COQ10 DEFICIENCY GONZÁLEZ-PÉREZ P¹, RIVAS-INFANTE E², SÁNCHEZ-ALCÁZAR JA³, NAVAS LLORET P⁴, BAUTISTA-LORITE J⁵ (1) University Hospital Virgen del Rocío, Seville, SPAIN. (2) University Hospital Virgen del Rocío, Seville, SPAIN. (3) Pablo de Olavide University, Seville, SPAIN. (4) Pablo de Olavide University, Seville, SPAIN. (5) University Hospital Virgen del Rocío, Seville, SPAIN.</p>
To contact the author:: palgonp@yahoo.es.	<p>OBJECTIVES: We present a new case of cerebellar ataxia associated to a coenzyme Q10 (CoQ10) deficiency and apparently responsive to CoQ10 supplementation after five months receiving this treatment. Skin biopsy, as well as muscle biopsy, confirmed this deficiency.</p> <p>METHODS: A 24-year old woman is followed up since she was 14 because of a progressive childhood-onset cerebellar ataxia. Neurological examination disclosed nystagmus and a mild left VI cranial nerve paresis, disartria, limbs and truncal ataxia with prominent dysmetria in finger-nose-finger and heel-knee tests, universal arreflexia, bilateral Babinski sign, aquilea retraction and progressive pes cavus. Bilateral distal weakness in the anterior compartment muscular group in lower limbs was present. Walking was not possible without aid. Mental retardation, developmental motor delay and seizures were not observed. There were not neurological family history nor consanguinity.</p> <p>RESULTS: Genetic and acquired cerebellar ataxia causes were excluded. MRI revealed a prominent cerebellar atrophy. CK was mildly elevated. Muscle biopsy sample was morphologically normal. Skin biopsy was also performed. The measurement of the activities of the mitochondrial chain respiratory enzymes in muscle extracts, as well as in fibroblasts, revealed that the activity of II and II-III complexes were markedly reduced suggesting a block at CoQ10 level.</p> <p>According to this, the patient is receiving 1500 mg of CoQ10 daily from five months ago. Cerebellar function is evaluated every two months using the Scale for the Assessment and Rating of Ataxia (SARA). After informed consent was obtained, we filmed the patient one time before and twice after introducing the CoQ10 supplementation (VIDEO). In addition to the subjective improvement the patient feel, the scores in the SARA are also improving progressively.</p> <p>CONCLUSION: A CoQ10 deficiency should be ruled out in patients with an undefined cerebellar ataxia. Skin biopsy could be an alternative to muscle biopsy in these cases.</p>

PW19-234	<p><u>EXERCISE-INDUCED RHABDOMYOLYSIS AS ISOLATED CLINICAL EXPRESSION OF COQ10 DEFICIENCY. A NEW PHENOTYPE.</u> GONZÁLEZ-PÉREZ P¹, RIVAS-INFANTE E², SÁNCHEZ-ALCÁZAR JA³, NAVAS LLORET P⁴, BAUTISTA-LORITE J⁵ (1) University Hospital Virgen del Rocío, Seville, SPAIN. (2) University Hospital Virgen del Rocío, Seville, SPAIN. (3) Pablo de Olavide University, Seville, SPAIN. (4) Pablo de Olavide University, Seville, SPAIN. (5) University Hospital Virgen del Rocío, Seville, SPAIN.</p>
To contact the author:: palgonp@yahoo.es.	<p>OBJECTIVES: We present a new phenotype associated to CoQ10 deficiency characterised by a late-onset recurrent myoglobinuria and exercise intolerance without muscle weakness nor CNS involvement. CoQ10 supplementation was introduced and by now the patient remains asymptomatic.</p> <p>METHODS: A 24-year old man was followed up because of episodes characterised by myalgias and myoglobinuria associated to long-duration physical exercises in the last two years. These symptoms disappeared after hours remaining asymptomatic. There was not neurological family history nor consanguinity.</p> <p>RESULTS: Neurological examination was normal. A high CK level was detected in one of the episodes (18.000 IU/L) being normal in asymptomatic periods. The aerobic forearm exercise test was normal. An open muscle biopsy taken from <i>biceps brachii</i> was performed but routine and immunohistochemical stainings did not show morphological abnormalities. Carnitine palmitoyl transferase deficiency was ruled out. The measurement of the activities of the mitochondrial chain respiratory enzymes in muscle extracts, as well as in fibroblasts, revealed that the activity of II, I-III and II-III complexes and CoQ10 were markedly reduced. This patient is receiving 1500 mg daily of CoQ10. He has not referred exercise intolerance any more and routine analyses have not demonstrate high CK level since then.</p> <p>CONCLUSIONS: - Late-onset and isolated recurrent myoglobinuria constitutes a new phenotype associated to a CoQ10 deficiency. Unlike other reports, this patient has not developed muscle weakness nor CNS abnormalities. We do not know if the early-onset CoQ10 treatment has played a key role avoiding the presence of other symptoms. - Mitochondrial chain respiratory enzymes study should be performed in patients with an undefined exercise intolerance because if a CoQ10 deficiency is confirmed, CoQ10 supplementation is recommended. Skin biopsy could be an alternative to muscle biopsy to confirm a CoQ10 deficiency.</p>

PW19-235	<p>SEARCH FOR PARTNERS OF MITOFUSIN 2, A MITOCHONDRIAL FUSION PROTEIN MUTATED IN CHARCOT-MARIE-TOOTH DISEASE TYPE 2A (CMT2A) GUILLERY O¹, PUCELLE M¹, FLORENCE M¹, LOMBÈS A¹, ROJO M² (1) INSERM U582 - Institut de Myologie - Groupe Hospitalier Pitié-Salpêtrière, Paris, FRANCE. (2) Institut de Biochimie et Génétique Cellulaires - UMR5095 CNRS- Université Victor Segalen, Bordeaux, FRANCE.</p>
To contact the author:: manuel.rojo@ibgc.u-bordeaux2.fr.	<p>Charcot-Marie-Tooth disease (CMT) is a frequent, genetically heterogeneous group of peripheral neuropathies. Mutation of the <i>MFN2</i> gene provoke CMT2A, and probably represent the commonest cause of CMT2. The deficiencies provoked by <i>MFN2</i>-mutations and the physiopathological mechanisms of CMT2A remain unknown. In order to advance in the understanding of mitofusin function and CMT2A physiopathology, we setup to identify partners of Mfn2 and Mfn1, two ubiquitous GTPases of the mitochondrial outer membrane that are required for mitochondrial fusion and display 60% sequence identity.</p> <p>We have demonstrated by immunoprecipitation that Mfn2 and Mfn1 interact with each other and with OPA1, another mitochondrial GTPase involved in fusion (1). In addition, others have identified putative mitofusin-partners (Mfn2-binding Stoml2 and Mfn1-binding MIB) using proteomic approaches (2,3). We engineered soluble forms of Mfn2 and Mfn1 and used them as baits in yeast-two-hybrid (Y2H) screens. Surprisingly, none of these known partners was “fished” in our Y2H-screens.</p> <p>The Mfn1-NT being toxic to yeast cells, this Y2H-screen was performed with an inducible promoter. It yielded a high number of clones (>80). The high proportion of clones that were out of frame or in antisense orientation point to unspecific interactions with the toxic (misfolded?) bait. A first Mfn2-screen yielded 3 clones encoding a predicted protein of unknown function (hypothetical Mitofusin2 binding protein - hM2BP). We have cloned hM2BP and are currently investigating its localization and properties. A second Mfn2-screen yielded 3 independent clones of a second putative partner (2PP) reported to be involved in the maintenance of mitochondrial morphology and in mitochondrial apoptosis. We pursue the study of 2PP-Mfn interactions and their putative role in mitochondrial dynamics.</p> <ol style="list-style-type: none"> 1. Guillery et al. (2008) <i>Biol Cell</i> in press. 2. Hajek et al. (2007) <i>J Biol Chem</i> 282(8), 5670-5681 3. Eura et al. (2006) <i>J Cell Sci</i> 119(Pt 23), 4913-4925

PW19-236	<p><u>EVIDENCE FOR INCREASED PROTEIN NITRATION IN VESSEL WALL OF MITOCHONDRIAL DISEASE PATIENTS</u></p> <p>VATTEMI G¹, MARINI M¹, MECHREF Y², MENEGUZZI A³, TONIN P¹, GRIGOLI L¹, DI CHIO M⁴, TEDESCO V⁴, LOVATO L¹, FILOSTO M¹, SCARPELLI M¹, CHIAMULERA C⁴, MINUZ P³, NOVOTNY M², TOMELLERI G¹</p> <p>(1) Department of Neurological Sciences and Vision, Section of Clinical Neurology, University of Verona, Verona, ITALY. (2) Department of Chemistry, Indiana University, Bloomington, USA. (3) Department of Biomedical and Surgical Sciences, Section of Internal Medicine, University of Verona, Verona, ITALY. (4) Department of Medicine and Public Health, Section of Pharmacology, University of Verona, Verona, ITALY.</p>
To contact the author:: giuliano.tomelleri@univr.it.	<p>Mitochondrial diseases (MD) are multisystemic disorders, mostly affecting central nervous system, skeletal and cardiac muscle. To evaluate the hypothesis that in MD the vessel wall, in particular the vascular endothelium, may be affected by increased oxidative stress causing a reduction in nitric oxide (NO) availability, muscle biopsy specimens from sixteen patients with MD and different phenotypes have been studied. We tested the pathobiology of vasculature in MD by assaying the presence of 3-nitrotyrosine in muscle biopsies followed by the proteomic identification of proteins that undergo tyrosine nitration. We then studied the expression and the activity of nitric oxide synthase (NOS) enzymes that are responsible for the production of NO.</p> <p>3-nitrotyrosine was specifically located in the small vessels of muscle tissue and the staining was stronger and evident in a much higher percentage of vessels from MD patients compared to age-matched controls. We then identified 11 specific proteins that are nitrated under pathological conditions and that are mainly involved in energy metabolism and located in mitochondria. In MD muscle biopsies we observed at protein level an up-regulation of endothelial NOS (eNOS) which is specifically located in the vascular endothelium, while no significant difference was present for neuronal NOS and inducible NOS. Total NOS activity, iducible NOS activity as well as activity of constitutive isoforms of NOS (eNOS and neuronal NOS) was increased in patients' muscle biopsies compared to control muscles although the difference was not statistically significant.</p> <p>The present results provide evidence that vessel walls are a target of oxidative/nitrative stress in patients affected with mitochondrial respiratory chain dysfunction.</p>

PW19-237	<p><u>ATYPICAL METABOLIC MYOPATHY WITH STRUCTURAL AND FUNCTIONAL ALTERATION IN MITOCHONDRIA</u> ROMERO-DIAZ VJ¹, OCHOA-MORALES H², DE LA FUENTE-CORTEZ B², GARESSE R³ (1) Dept. Histology, School of Medicine, U.A.N.L., Monterrey, MEXICO. (2) Dept. Genetic, School of Medicine, U.A.N.L., Monterrey, MEXICO. (3) Dept. Biochemistry, Insto. Invest.Biomedicas, Fac. Medicina,U.A.M., Madrid, SPAIN.</p>
To contact the author:: vikromero@email.com.	<p>The metabolic myopathies are characterized for alterations in the biochemical anabolic or catabolic reactions and they can be found in the different groups of illnesses of the metabolism, are presented for any of the 11 enzymes specific involved in the corporal production of energy. Generally they are detected in the childhood or adult life, not are fatal and the biopsy confirms the diagnosis. The mitochondrial myopathies frequently are congenital and affect other systems besides the muscle, present abnormalities in the mitochondrial metabolism and are characteristics the crystalloid inclusions detected by microscopy. In base to this we present the finds of the structural aspects and biochemical observed in 5 cases of myopathy in four children, 3 males and 1 female with ages from 20 months to 9 years and one male adult of 29 years. The muscle biopsy taking from the quadriceps was processed for stain techniques of routine, enzymatic and not enzymatic histochemistry for studies by light and electronic microscopy, and biochemical test for detect mutations in mtDNA. The muscular tissue presented mainly morphologic changes due to an accentuated atrophy and/or hypertrophy of both fibers type with a lower mean or over the normal diameter average for the age, with irregularities in the oxidative reactions by hyperactivity or hypo activity in the sarcolemma and in the sarcoplasm, besides with deficit in the phosphorylase and excess of glycogen and lipids. Joined to the variable quantity of mitochondria (much diminished or very numerous) besides the structural changes in the form, in size, and aspect or density of cristae and mitochondrial matrix, but in none of the cases crystalloid inclusions were seen. Molecular studies of the mtDNA showed 80% of the tRNA Leu with mutation in A3248G, in 3 of 5 analyzed cases. The results indicated mitochondrial myopathy in some cases.</p>

PW19-238	<p><u>TRANSMISSION OF MITOCHONDRIA AND MITOCHONDRIAL DNA NUCLEOIDS DURING MITOSIS</u> GUILLERY O¹, MISEREY-LENKEI S², LOMBÈS A¹, GOUD B², ROJO M³ (1) INSERM U582 - Institut de Myologie - Groupe Hospitalier Pitié-Salpêtrière, Paris, FRANCE. (2) UMR 144 CNRS/Institut Curie, Paris, FRANCE. (3) Institut de Biochimie et Génétique Cellulaires - UMR5095 CNRS-Université Victor Segalen, Bordeaux, FRANCE.</p>
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<p>To contact the author:: manuel.rojo@ibgc.u-bordeaux2.fr.</p>	<p>Mitochondria are dynamic organelles that represent a single cellular compartment interconnected by continuous fusion and fission. The balance of these antagonizing reactions determines overall mitochondrial morphology, which ranges from elongated branched filaments to small punctuate structures. During mitosis organelles like nucleus, Golgi apparatus and endoplasmic reticulum are dramatically rearranged and rearrangements are required for progression of cell division. In contrast, little is known about the mitochondrial inheritance, which not only ensures the transmission of organelles' membranes and proteins, but also that of the mitochondrial DNA (mtDNA), which encodes essential components of the respiratory chain as well as RNA molecules required for their intramitochondrial translation. This genome is organised in hundreds of nucleoids, nucleoprotein complexes containing 2-8 copies of mtDNA that distribute throughout mitochondria. Transmission of this mtDNA is essential to understand the appearance and transmission of severe, maternally transmitted mitochondrial diseases provoked by mtDNA mutations.</p> <p>In this work, we focus on evolution of mitochondrial morphology and associated mtDNA nucleoids transmission during mitosis, which we investigate in mitotic HeLa cells. We show that, in contrast to the fragmenting Golgi apparatus, mitochondria retain their filamentous morphology throughout mitosis. Mitochondria are excluded from the area occupied by chromosomes and the mitotic spindle, but distribute homogenously throughout dividing cells. We show that the majority of filamentous mitochondria contain numerous mtDNA-nucleoids and that nucleoids distribute, like mitochondria, throughout the dividing cell. Finally we show that Drp1-mediated mitochondrial fission is not essential for mitochondrial transmission, progression though mitosis and cytokinesis.</p>
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<p>PW19-239</p>	<p>THE USE OF AFFYMETRIX RE-SEQUENCING CHIP FOR MITOCHONDRIAL DNA (MITOCHIP) IN CLINICAL DIAGNOSIS NELSON I¹, FILAUT S², JARDEL C², LOMBÈS A³ (1) INSERM U582; UPMC-Paris6, Paris, FRANCE. (2) Biochimie Métabolique, AP/HP GHU Pitié-Salpêtrière; INSERM U582; UPMC-Paris6, Paris, FRANCE. (3) INSERM U582; Biochimie Métabolique, AP/HP GHU Pitié-Salpêtrière; UPMC-Paris6, Paris, FRANCE.</p>
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The incidence of patients with neuromuscular diseases and a suspected mitochondrial diseases is very high. Most patients present with characteristics that are suggestive of a mitochondrial DNA (mtDNA) alteration including mosaic pattern of muscle histological alterations (cytochrome c oxidase deficient muscle fibres or ragged-red fibres), combined defect of the oxidative phosphorylation pathway, or a pattern of inheritance compatible with maternal transmission. In addition mtDNA alterations have to be excluded in patients without suggestive anomalies, for example in very young patients who are known to lack typical muscle histological alterations, or in the presence of an isolated complex I defect, which does not induce these histological alterations even when the underlying genetic alteration resides in one of the 7 mtDNA complex I structural subunit genes. As a consequence, analysis of the mtDNA sequence is a recurrent demand in centres specialized in neuromuscular disorders. Several techniques have been progressively developed to answer that demand including screening methods such as denaturing gradient gel electrophoresis, denaturing high performance liquid chromatography, the Surveyor technology based on the clivage of mispaired bases.

We here report the results of a trial of a re-sequencing chip for mtDNA in 35 patients from La Pitié-Salpêtrière. This project has been conducted within the frame of a national trial of the French Mitochondrial Diseases network, using Affymetrix Gene Chip mitochondrial Resequencing 2.0 Array. Financial support from the GIS-MR provided the service of PartnerChip company for DNA handling (amplification, purification, digestion, hybridization) and data generation. Advantages and pitfalls of that approach are discussed.

PW19-240

APOPTOSIS AND OXIDATIVE STRESS IN MITOCHONDRIAL MYOPATHIES, THE SEQUENCE OF EVENTS

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<p>To contact the author:: g.fayet@institut-myologie.org.</p>	<p>In addition to their key role in the cell energy production, mitochondria are critical actors in apoptosis and oxidative stress. Increased susceptibility to apoptosis and oxidative stress has been shown in cellular models of defects in the oxidative phosphorylation pathway similar to those encountered in mitochondrial diseases. However extent of both apoptosis and oxidative stress as well as their responsibility in the clinical outcome of human mitochondrial diseases is till controversial and remains to be elucidated in the prospect of potential therapeutic approaches of mitochondrial diseases.</p> <p>Using an <i>in situ</i> approach to address in muscle from patients with mitochondrial myopathies, we address the relationship between respiratory defect, mitochondrial proliferation and mutation load on the one hand and apoptosis and oxidative stress on the other hand. Eleven biopsies from patients with typical mitochondrial myopathy and large size mitochondrial DNA (mtDNA) deletion were analyzed with respect to the presence of apoptosis (immunoreaction of activated caspase 3), and of oxidative lesions (immunological demonstration of the presence of 8-hydroxy-deoxyguanosine or nitrotyrosine, and overexpression of superoxide dismutase 1 and 2). Serial muscle sections allowed correlative analysis of these anomalies with respiratory defect (cytochrome c oxidase activity) and mitochondrial proliferation (succinate dehydrogenase activity) in 3000 individual muscle fibres. They demonstrate that oxidative stress occurs early in the sequence of events that link respiratory defect to oxidative stress to mitochondrial proliferation to apoptosis. Amount of normal and mutant mtDNA in individual muscle fibres will now be addressed by a quantitative PCR approach.</p>
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<p>PW19-241</p>	<p><u>L-CARNITINE SUPPLEMENTATION RESTORES SOME MITOCHONDRIAL FUNCTIONS IN AGED RATS.</u> LE BORGNE F¹, RIGAULT C¹, PROST J², BERNARD A¹, MAZUE F¹, DEMARQUOY J¹ (1) INSERM U866 - Université de Bourgogne, Dijon, FRANCE. (2) Université de Bourgogne - UPRES 4183, Dijon, FRANCE.</p>
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In mammals, age causes structural and functional changes in the skeletal muscle. In human, muscle changes start during the fourth decade of life and cause progressive disabilities. Besides these changes, associated alterations in body composition favor the onset of many metabolic disorders, such as type 2 diabetes and hypertension. Decrease in the synthesis rate of mitochondrial proteins occurs with age, and is likely to be caused by a reduction in mitochondrial biogenesis and ATP production. Aging is also characterized by an alteration in the hormonal and the nutritional status.

During this study, we determined oxidative and metabolic changes occurring in muscle during aging and evaluated the effect of a nutritional supplementation of L-carnitine on these parameters.

We observed a decrease in muscle L-carnitine level during aging; a decrease in oxidative capacities in lipolytic muscle was also observed. On the other hand, an increase in markers of the oxidative stress was found. All together these changes lead to a decrease in muscle activity and an increase in fat deposit.

We evaluated the effect of a L-carnitine supplementation on several parameters related to muscle physiology and oxidative stress. We described that supplementing old rats with 30 mg/kg b.w. L-carnitine during 12 weeks (i) allowed to restore L-carnitine level in muscle cell, (ii) restored muscle oxidative activity in the soleus, a lipolytic muscle, (iii) induced positive changes in body composition: a decrease in abdominal fat mass and an increase in muscle capabilities without any change in food intake (iv) reduced several parameters linked to the oxidative stress and (v) increased the global antioxidant activity of the plasma.

These data suggested that L-carnitine supplementation in old animals may limit the oxidative stress, increase muscle capacities, be beneficial for body composition and could attenuate the progressive decline in some mitochondrial functions that occurs with age.

