

**PW 15:
Congenital myopathies –
Nemalin myopathies,
tropomyosin mutations
and others**

PW15-182	<p>MUTATIONS IN THIN FILAMENT RELATED PROTEINS ASSOCIATED WITH NEMALINE MYOPATHIES AND CONGENITAL FIBRE TYPE DISPROPORTION (CFTD)</p> <p>MONNIER N¹, DROUHIN S¹, MARTY I², JOUK PS³, LABARRE-VILA A³, MEZIN P³, LUNARDI J¹</p> <p>(1) Biochimie et Génétique Moléculaire, CHU Grenoble, Grenoble, FRANCE. (2) Inserm U836, Grenoble Institut des Neurosciences, Grenoble, FRANCE. (3) Centre de Référence des Maladies Neuromusculaires, CHU Grenoble, Grenoble, FRANCE.</p>
To contact the author:: jlunardi@chu-grenoble.fr.	<p>Background: Mutations in genes coding for thin filament components have been identified in structural congenital myopathies including nemaline myopathy, congenital fibre type disproportion, Cap disease and actin myopathy. Nemaline myopathies (NM) are characterized by the presence of rod structures in muscle and classified in seven sub-groups according to onset age and clinical features. Dominant, recessive and <i>de novo</i> mutations have been identified mostly in <i>ACTA1</i> and <i>Neb</i> genes and, to a lower extend, in <i>TPM2</i>, <i>TPM3</i>, <i>TNNT1</i> and <i>CFL2</i> genes. Congenital fibre type disproportion (CFTD) is characterised by consistent hypotrophy of type 1 compared to type 2 fibres and associated with mutations in <i>ACTA1</i>, <i>TPM3</i> and <i>SEPN1</i> genes.</p> <p>We have studied a panel of 47 patients presenting with NM (37) or CFTD (8) and analysed <i>ACTA1</i>, <i>TPM2</i>, <i>TPM3</i>, <i>TNNT1</i> and <i>CFL2</i> genes by exon sequencing. When possible, <i>NEB</i> linkage studies have also been performed.</p> <p>Mutations have been identified in <i>ACTA1</i> gene in 8 NM cases and one CFTD case , in <i>TPM3</i> gene in 1 NM case and one CFTD case, in <i>TPM2</i> gene in 2 NM cases and linkage to <i>NEB</i> gene in two further cases of NM. No mutation has been identified in <i>TNNT1</i> and <i>CFL2</i> genes in the remaining 24 NM cases (2/3).</p> <p><i>De novo ACTA1</i> missense mutations were mostly associated with severe or intermediate forms of NM or CFTD whereas missense mutations in <i>TPM2</i>, <i>TPM3</i> but also <i>ACTA1</i> genes were identified in dominant milder forms of NM. Furthermore, a recessive non-sense <i>TPM2</i> mutation was identified in a new form of nemaline myopathy associated with a non-lethal multiple pterygium syndrome (Escobar variant).</p> <p>These results are in good agreement with the previously reported studies and confirm that mutations in additional genes or <i>NEB</i> gene must account for the majority of NM cases.</p>

PW15-183	<p><u>NO LINKAGE TO KNOWN LOCI IN A LARGE CONSANGUINEOUS FAMILY WITH NEMALINE MYOPATHY AND RIGID SPINE</u> MAKRI S¹, VILMA-LOTTA L², TERKI N³, LAING NG⁴, WALLGREN-PETTERSSON C² (1) Service de neurologie. Etablissement Hospitalier Spécialisé Ali Ait Idir, Alger, ALGERIA. (2) Department of Medical Genetics, University of Helsinki, and the Folkhälsan Institute of Genetics, Helsinki, FINLAND. (3) Service d'anatomopathologie. CPMC, Alger, ALGERIA. (4) Neuromuscular Research Institute, Perth, AUSTRALIA.</p>
To contact the author:: makrisamira@yahoo.fr.	<p>Nemaline myopathy (NM) is a rare congenital myopathy characterized by muscle weakness and the presence of nemaline (rod) bodies in the muscle fibers. NM is a genetically heterogeneous condition of which rigid spine syndrome is a rare feature. Here we describe a sib pair from a consanguineous family. Their motor development was normal, and presentation was at ages 12 years and 6 years with axial myopathy, limitation in flexion of the cervical and dorsolumbar spine and restrictive respiratory insufficiency. The patients did not show limb or facial weakness, smallness of muscle bulk or joint contractures. Scoliosis and severe retrocollis were noted in the elder sib who died at 14 years during acute respiratory infection. CK levels were normal. Electromyography showed a myogenic pattern in the deltoid muscle. Cardiac investigations revealed no abnormality. Biopsy of the deltoid muscle with Gomori trichrome method showed nemaline bodies in subsarcolemmal region of the muscle fibers in both children. There is uniformity of type 1 fibres and absence of type 2 fibres in the first one, predominance of type 1 fibres, fibre type disproportion and incomplete differentiation of fibre types in the younger. Linkage analyses and/or sequencing excluded the six known NM genes <i>ACTA1</i>, <i>NEB</i>, <i>TPM2</i>, <i>TPM3</i>, <i>TNNT1</i> and <i>CFL2</i> and also the following candidate genes: <i>SEPN</i>, <i>TTN</i>, <i>DES</i>, <i>MYOT</i> and <i>RYR</i>. In conclusion, further linkage and sequencing studies are warranted to identify the causative genetic defect in this familial case of nemaline myopathy with rigid spine.</p>

PW15-184	<p>MUTATION ANALYSIS OF LARGE GENES: THE EXAMPLE OF NEBULIN LEHTOKARI VL¹, LUNKKA-HYTÖNEN M¹, KELLINSALMI M¹, PELIN K², WALLGREN-PETTERSSON C¹ (1) Folkhälsan Institute of Genetics and University of Helsinki, Faculty of Medicine, Helsinki, FINLAND. (2) University of Helsinki, Faculty of Biosciences, Helsinki, FINLAND.</p>
<p>To contact the author:: vilma.lehtokari@helsinki. fi.</p>	<p>Mutation detection in nemaline myopathy (NM) is challenging due to the size of the main causative gene, nebulin (<i>NEB</i>), with 183 exons. Using denaturing high performance liquid chromatography (dHPLC) we have identified 91 different exonic and intronic point mutations as well as small deletions and insertions in <i>NEB</i> in 85 families. Carefully optimised dHPLC offers efficient identification of heterozygous mutations. The identification of the first big deletion in <i>NEB</i> erasing the entire exon 55 (Anderson, 2004) revealed the importance of a convenient method for tracking larger alterations (large deletions, insertions or amplifications) affecting whole exons of <i>NEB</i>. Multiplex Ligation-Dependent Probe Amplification (MLPA) is a promising method for the detection of DNA copy number changes. In MLPA, a copy is made of each target exon by hybridization of two probes to each exon. The probes are ligated and then amplified in a multiplex-PCR reaction using universal fluorescent-labelled primer pair. The PCR fragments produced are analysed by fragment analysis methods, and the relative copy numbers of the target exons are calculated. Our ongoing MLPA studies currently include synthetic self-designed probes for 30% of <i>NEB</i> exons. The initial series consists of DNA samples from nine NM patients with one known <i>NEB</i> mutation each. Altogether 167 different probe pairs are required for analysis of all <i>NEB</i> exons, as the same eight probe pairs will hybridise to the highly homologous exons 82-105 in the central region of <i>NEB</i>. In addition to these ongoing mutation analyses we screen the families for the deletion of exon 55 using Anderson's PCR-based method. Where myoblast RNA is available we perform RT-PCR in order to sequence the constitutively spliced regions of <i>NEB</i> mRNA. RT-PCR has revealed three novel mutations in <i>NEB</i>. Efficient mutation identification in <i>NEB</i> requires combination of multiple methods.</p>

PW15-185	<p>MECHANISMS OF ROD FORMATION IN INHERITED MYOPATHIES VANDEBROUCK A¹, DOMAZETOVSKA A¹, COOPER ST¹, ILKOVSKI B¹, NORTH KN¹ (1) The Institute for Neuromuscular Research, The Children's Hospital at Westmead, Westmead, AUSTRALIA.</p>
<p>To contact the author:: avandebrouck@hotmail.f r.</p>	<p>Protein aggregates or rods are the primary pathological feature in nemaline myopathy. Mutations in the gene encoding skeletal muscle α-actin (<i>ACTA1</i>) are responsible for about 20% of nemaline myopathy cases associated with cytoplasmic rods, as well as cases of intranuclear rod myopathy. "Nemaline" rods also occur as a secondary feature in some mitochondrial disorders, in particular in association with complex I deficiency. The mechanisms of rod formation are not well understood – particularly when they can occur in diverse disorders with very different structural and metabolic defects. Therefore we sought to determine their composition and structure.</p> <p>We have developed a tissue culture model in which to study mutations in <i>ACTA1</i> identified in patients. The expression of mutant actin-EGFP leads to formation of cytoplasmic or intranuclear rod-like structures in muscle cells, similar to those observed in patient muscle. We are also able to induce rod formation in cells transfected with wild-type actin-EGFP by depleting ATP, as a model of oxidative stress (and mitochondrial myopathy). We have determined that these different rods have altered biochemical properties, varying in their protein composition and actin conformation. Using Fluorescence Recovery After Photobleaching (FRAP) we have also seen a difference in actin turnover in different rods suggesting that they have very different actin dynamics.</p> <p>In summary, we have demonstrated that rods form secondary to different pathogenic processes (mutations in <i>ACTA1</i> and ischemia/ATP depletion) have different structural properties and biochemical dynamics. Characterisation of these different rods will ultimately help in understanding the mechanism of their formation and also the impact they have on cellular function in disease.</p>

PW15-186	<p>FUNCTIONAL STUDIES OF BETA-TROPOMYOSIN (TPM2) NUUTINEN E¹, MARTTILA M¹, OLLILA S², DONNER K³, PELIN K², WALLGREN-PETTERSSON C¹</p> <p>(1) The Folkhälsan Institute of Genetics, Helsinki, FINLAND. (2) Department of Biological and Environmental Sciences, University of Helsinki, Helsinki, FINLAND. (3) Hospital District of Helsinki and Uusimaa, Helsinki, FINLAND.</p>
<p>To contact the author:: elina.t.nuutinen@helsinki.fi.</p>	<p>Tropomyosins together with the troponin complex regulate the binding of actin to myosin during muscle contraction. In humans tropomyosins are encoded by at least four different genes, <i>TPM1-4</i>. Mutations in the beta-tropomyosin (<i>TPM2</i>) gene have been reported in patients with nemaline myopathy (NM), cap myopathy and distal arthrogyrosis. The aim of the study is to shed light on the pathogenetic pathways leading from six mutations identified in <i>TPM2</i> to the structural abnormalities seen in the patients' muscle fibres and to clinical muscle weakness. Here, we study the aberrant protein products and their effects on the binding of beta-tropomyosin to actin and on the ability of the tropomyosin molecules to form dimers. To confirm our preliminary results, based on <i>E.coli</i>-produced beta-tropomyosin, suggesting altered affinity, we are now expressing WT and aberrant variants of beta-tropomyosin proteins in a baculovirus expression system. This ensures acetylation of the NH2 terminus, which is important for the affinity of beta-tropomyosin to actin, and for the dimerisation of tropomyosin molecules. The mutations are introduced into mouse <i>TPM2</i>-cDNA clones with Stratagenes Quick Change® Site Directed Mutagenesis Kit. Actin binding is monitored by an <i>in vitro</i> cosedimentation assay, where G-actin is polymerised to F-actin and beta-tropomyosin binding to actin is analysed with SDS-PAGE and Coomassie blue staining. The dimerisation assay utilises baculovirus system-expressed alpha-tropomyosin also. The formation of homo- or heterodimers is evaluated using Western blot analysis. The study is ongoing.</p>

PW15-187	<p><u>BENEFICIAL EFFECTS OF THE CA²⁺ SENSITIZER EMD 57033 ON THE REGULATION OF MUSCLE CONTRACTION IN FIBRES CARRYING A NOVEL E41K BETA-TROPOMYOSIN MUTATION</u></p> <p>OCHALA J¹, LARSSON L¹ (1) Uppsala University, Uppsala, SWEDEN.</p>
<p>To contact the author:: julien.ochala@neurofys.uu.se.</p>	<p>A novel E41K beta-tropomyosin mutation, associated with muscle weakness and congenital myopathy, was recently identified. This mutation directly affects contractile characteristics of single skinned muscle fibres, i.e. (i) decreased shortening speeds at saturated Ca²⁺ concentration (apparent rate constant of force redevelopment k_{tr} and unloaded shortening speed V₀); and (ii) depressed sensitivity of force to Ca²⁺ concentration, contributing to the muscle weakness. To investigate if these mutation-related alterations are reversible, we exposed fibres to the Ca²⁺ sensitizer EMD 57033 and studied a broad range of contractile parameters. Results showed that 30 µM of EMD 57033 (i) had no effects on shortening speeds at saturated Ca²⁺ concentration (k_{tr} and V₀); but (ii) increased Ca²⁺ sensitivity of force, in fibres carrying the E41K beta-Tm mutation, emphasizing a potential therapeutic role of this drug in patients carrying the mutation. To improve the understanding of the mechanisms underlying the improvement in Ca²⁺ sensitivity of force in fibres exposed to EMD 57033, stiffness was evaluated in various Ca²⁺ concentrations. Ca²⁺ sensitivity of stiffness was also enhanced after exposure to the Ca²⁺ sensitizer, suggesting that the changes in the Ca²⁺ activation of force were predominantly due to an increase in the relative number of attached cross-bridges at different Ca²⁺ concentrations.</p>

PW15-188	<p>A NEW CASE OF CONGENITAL MYOPATHY WITH CRISTALLOID INCLUSIONS STOLTENBURG-DIDINGER G¹, FARDEAU M¹, EYMARD B², LAFORET P², VOIT T¹, CLAEYS K¹ (1) Unité de Morphologie Neuromusculaire, Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, FRANCE. (2) Centre de Référence Neuromusculaire Paris-Est, Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, FRANCE.</p>
To contact the author:: g.stoltenburg@institut-myologie.org.	<p>Congenital myopathies are inherited muscle diseases which most often manifest in early life and are distinguished by structural muscle biopsy abnormalities. We describe the morphology of a patient with a slowly progressive proximal myopathy with unique crystalloid inclusions in type 2 muscle fibres. The male patient presented at the age of 24 years with a mild muscle weakness and exercise intolerance. As first symptoms at the age of 20y, he had noticed fatigability and difficulty to keep up with peers in sports. He underwent two muscle biopsies in the deltoid muscle (24,35y), in which pathomorphology didn't change. Some muscle fibres showed a large number of small inclusions, single or multiple, in their periphery or centre. The inclusions were strongly eosinophilic, and fuchsinophilic by the Gomori trichroom method. With oxidative reactions in enzymohistochemistry, the inclusions were almost invisible. They showed no activity in myofibrillar ATPase. The inclusions were selectively present in type 2 fibres. The inclusions were negative for the immunohistochemical reactions for vimentin, desmin, ubiquitin, alpha-actinin and the subtypes of myosin heavy chain. At the ultrastructural level, the inclusions were composed of vesicular profiles, approximately 20 nm in cross-diameter, connected by radially arranged bars. The architecture was highly regular, with a hexagonal pattern of distribution and spacing of circular profiles. The crystalloid inclusions showed an oval or trapezoid shape, and were arranged along the myofibrils in their longitudinal axis, where they displayed fine parallel dense lines. Within several of the hexagonal crystalloid structures, small areas containing glycogen granules could be observed. At these sites, the highly structured hexagonal pattern was interrupted. The inclusions were morphologically different from tubular aggregates and nemaline bodies. The inclusions were not related to any normal cellular organelle. We suggest that this is the fourth case of a new congenital myopathy with characteristic intracytoplasmic inclusions.</p>

PW15-189	<p><u>RIGID SPINE SYNDROME, SEVERE MICROCEPHALY, NORMAL INTELLIGENCE AND SHORT STATURE: A NEW ENTITY?</u> SPEHRS-CIAFFI V¹, RICHARD P², LOBRINUS JA³, JEANNET PY¹ (1) Pediatric Neuromuscular Center, CHUV, Lausanne, SWITZERLAND. (2) Service de Biochimie, Hôpital de la Salpêtrière, Paris, FRANCE. (3) Division of Pathology, CHUV, Lausanne, SWITZERLAND.</p>
To contact the author:: virginia.spehrs-ciaffi@chuv.ch.	<p>Introduction: Rigid spine syndrome is a non specific term used to describe patients presenting early rigidity of the spine due to axial muscle contractures, muscle weakness, limb-joint contractures and often respiratory failure. It is seen in rigid spine muscular dystrophy type 1 (RSMD1) with mutations in the SEPN1 gene, and occasionally in Emery-Dreifuss muscular dystrophy, some congenital myopathies and in collagen VI related disorders. In the present study, we report the clinical, morphological and genetic analysis of a patient with rigid spine syndrome, short stature and microcephaly of unknown cause despite thorough investigations.</p> <p>Case report: This 14 year-old boy was initially followed in the neuropaediatric clinic for severe microcephaly with normal cognitive development. He was born at term with in-utero growth retardation and walked at 16 months. When first seen at 35 months he had a Gowers' sign with no other signs of weakness. His growth parameters always remained below the 3rd percentile. Rigid spine and muscular weakness were first noted at 8 years old. Thereafter he presented fairly rapidly progressive axial and proximal limb weakness, elbow and knees retractions, nasal speech and frequent low respiratory tract infections. Serum CK and brain MRI were normal. Muscle biopsy showed dystrophic changes and normal expression of all tested proteins on immunohistochemistry including emerin, alpha-dystroglycan and collagen VI. His echocardiogram showed a left ventricular function in the low normal limit. Respiratory function showed progressive restrictive disease and non-invasive ventilation is currently being started. No SEPN1 and LMNA gene mutations were found.</p> <p>Conclusion: To our knowledge the association of rigid spine syndrome, severe microcephaly (with normal intelligence and no brain abnormality) and short stature in a child with congenital muscular dystrophy has not been previously reported. Given the severity of the microcephaly, genetic testing for the alpha-dystroglycanopathy genes are planned in this patient.</p>

PW15-190	<p><u>INDUCIBLE MOUSE MODEL OF VISCERAL MYOPATHY BY SMOOTH MUSCLE-SPECIFIC INACTIVATION OF THE SERUM RESPONSE FACTOR GENE</u> MERICSKAY M¹, BLANC J¹, TRITSCH E¹, MORIEZ R², AUBERT P², NEUNLIST M², FEIL R³, LI Z¹</p> <p>(1) Pierre & Marie Curie University Paris 6 - CNRS UMR7079 Physiology and Physiopathology, Paris, FRANCE. (2) INSERM U539 Institut des Maladies de l'Appareil Digestif, Université de Nantes, Nantes, FRANCE. (3) Interfakultäres Institut für Biochemie, Universität Tübingen, Tübingen, GERMANY.</p>
To contact the author:: merics@ccr.jussieu.fr.	<p>SRF regulates the expression of muscle genes and immediate early genes and plays a crucial role in the heart and the skeletal muscles. Here, we investigated the consequences of inactivating SRF in adult gastrointestinal smooth muscle cells (SMCs). SRF-floxed mice were crossed with SM-CreER^{T2}(ki) mice expressing a tamoxifen-inducible recombinase in SMCs. The mutant mice developed severe dilation of the intestinal tract associated with food stasis in the lumen 13 days after tamoxifen treatment. Mutant mice displayed cachexia and died between days 13 and 22. The dilation was associated with a thinning of the muscularis propria and was also observed in the urinary bladder. <i>Ex vivo</i> colonic contraction was impaired in the mutant before the occurrence of the dilation phenotype. The expression of several genes, including those encoding smooth muscle actin, the heavy chain of smooth muscle myosin and smoothelin, was 60 to 70% lower in mutants than in controls, and mutants also had a lower F/G actin ratio. <i>Conclusions:</i> SRF plays a central role in maintaining visceral smooth muscle contractile function in adults. Mice with a SMC-specific SRF mutation develop a severe motility disorder resembling chronic intestinal pseudoobstruction induced by visceral myopathy in humans, and may be used as an inducible model of this disorder. The dilatation of the visceral organs is similar to the dilatation of the heart chambers when SRF is inactivated in the adult heart and suggests the existence of common pathological pathways in the cardiac and intestinal pumps.</p>

PW15-191

**AMYOPLASIA DUE TO AN ABNORMAL DIFFERENTIATION OF THE
APPENDICULAR MYOBLASTS**

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Amyoplasia (OMIM 108110) is the most common form of arthrogryposis multiplex congenita (AMC). It is a rare, sporadic condition characterized by decreased skeletal muscle mass, typical contractures and limb positioning at birth and normal intellectual development. Common associated findings are a round face with slightly small jaw and frontal midline capillary haemangioma. Typically, no other malformations are present. Pathogenesis is not yet very clear; it could be the consequence of myopathic, neuropathic or vascular abnormalities. We report a case of amyoplasia in a young girl. She is the 5th and last child of a non consanguineous couple. The pregnancy was considered as normal although the mother noted that foetal movements were poor. At birth the baby presented the characteristic contractures of four limbs with hardly any muscles mass in the four limbs. She also had on her round face a capillary haemangioma. She acquired head control and sitting position at normal age and her cognitive function at 6 years was completely normal. The MRI performed at 2 years confirmed that the muscles of the four limbs were replaced by a fibrous and fatty tissue whereas the paravertebral muscles had a normal signal. Muscle biopsies performed during club foot surgery showed very few muscle cells without organization in the right hallux abductor. This case of amyoplasia suggested a specific impairment of the appendicular muscles maturation contrasting with a normal maturation of the axial muscles. Work is in progress to test candidate genes involved in myoblast proliferation and migration of limb's buds.

Key Words: Amyoplasia, arthrogryposis multiplex congenital, appendicular muscles, myogenic factors, myogenesis

PW15-192	<p>HISTOLOGICAL, METABOLIC AND GENETIC INVESTIGATIONS OF EXERTIONAL HEAT STROKE ABRIAT A¹, KOZAK G², BROSSET C¹, FIGARELLA-BRANGER D³, MONNIER N⁴, COZZONE PJ², PELLISSIER JF³, LUNARDI J⁴, BENDAHAN D² (1) Département d'Anesthésie Hôpital Laveran, Marseille, FRANCE. (2) CRMBM UMR CNRS 6612, Marseille, FRANCE. (3) Département d'Anatomo-Pathologie, CHU Timone, Marseille, FRANCE. (4) Département de Génétique, CHU la Tronche, Grenoble, FRANCE.</p>
To contact the author:: david.bendahan@univmed.fr.	<p>Exertional heat stroke (EHS) occurs in young, healthy individuals engaged in a strenuous physical activity and is accompanied by hyperthermic and lost of consciousness. Given that EHS and Malignant Hyperthermia (MH), a subclinical myopathy due to abnormal calcium handling, share a few clinical features, one could wonder whether calcium handling and muscle energetics are altered in EHS patients as it is the case in MH susceptible subjects (1).</p> <p>In the present study, we have analyzed the results of 182 subjects included in a survey of military personnel who have had EHS between 2004 and 2006. Each subject was included in the study after informed written consent was obtained. A muscle biopsy was excised from the biceps muscle and divided into three samples. A histological analysis was performed on the first sample while the second sample was used for in vitro contracture tests (IVCT) (4). Genetic analysis of the third sample was performed in order to check the existence of mutations commonly found in MHS subjects (2). Muscle energetics was analyzed non invasively during a standardized rest-exercise-recovery protocol using ³¹P magnetic resonance spectroscopy (MRS) as previously described (3).</p> <p>According to the IVCT, 14.4 % of the subjects were classified as MHS (i.e. both halothane and caffeine tests were abnormal) and 15.6% were equivocal for Halothane (i.e. the halothane but not the caffeine test was abnormal). IVCT with caffeine were abnormal in 20% of the subjects so that overall half of the subjects displayed IVCT abnormalities. Histological analyses did not reveal significant alterations whereas the non invasive investigation of muscle energetics, assessed with MRS, was abnormal for 25% of the subjects. Among the subjects with abnormal IVCT, 24% displayed metabolic abnormalities during the standardized exercise test. The preliminary genetic investigations performed in 8 subjects did not show mutations in the RYR1 gene.</p> <p>Overall, 60% of the subjects investigated displayed infraclinical abnormalities after an EHS episode. Although we did not find typical histological or metabolic abnormalities but rather heterogeneous alterations, subjects with EHS must be investigated through a multidisciplinary approach in order to better understand the metabolic and genetic bases, if any, of EHS.</p> <ol style="list-style-type: none"> 1. Bendahan D. et al. <i>Anesthesiology</i> 88: 96-107, 1998. 2. Monnier N and Lunardi J. <i>Ann Biol Clin (Paris)</i> 58: 147-156, 2000. 3. Bendahan D et al. <i>Anesth Analg</i> 93: 683-689, 2001. 4. Bendahan D. <i>Acta Anaesthesiol Scand</i> 48: 1019-1027, 2004.