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MEETING ABSTRACTS
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ORAL PRESENTATIONS

A1
Malignant hyperthermia mutations and correlation with the severity of the anesthetic complication and the level of the in vitro contracture tests
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BMC Anesthesiology 2014, 14(Suppl 1);A1

Background: Malignant hyperthermia (MH) is a complication of anaesthesia appearing as an acute potentially lethal hypermetabolic state in people carrying a genetic anomaly expressed in the skeletal muscle. MH susceptibility can be diagnosed by in vitro contracture test (IVCT) with halothane and caffeine requiring muscular biopsy, or by looking for the MH mutations directly in DNA extracted from the blood. Studies showed an influence of the type of mutation (genotype) on the various expressions (phenotypes) of the MH susceptibility. The aims of this study are to look for any correlation between the presence of a MH mutation and 1) the severity of the anesthetic complication and 2) the force of the contractures observed in the IVCT.

Materials and methods: Observational analytical retrospective anonymized study based on the informations contained in the Lille MH Database. The criteria of inclusion were: 1) anesthetic probands classified as MHS according to the IVCT and/or the presence of a MH mutation in the RYR1 gene in at least one family member and 2) existence of sufficient clinical information concerning the crisis. The severity of the MH crisis was classified as: death, survival after stay in intensive care unit ICU, survival without stay in ICU. The results of the IVCT: maximal force of contracture at halothane 2%, caffeine 2mmol/L, halothane 3%.

Results: 92 MHS probands were included in the study: 63 men (68%) and 29 women (32%) over a period going from 1964 to 2013. The age at the moment of the crisis is 18.9 years (DS 13.3). Mutations were found in 59 families (64%) including 53 causal mutations (58%) and 6 probable (6%). No significant difference was observed in the degree of severity between the 2 populations. The force of contracture of the IVCT was significantly higher in the group with MH (Table 1).

Conclusions: In this study, the severity of the MH crisis is not related to the presence of MH mutations. It is well known that the severity is more dependent on environmental factors, age of the crisis, exposure time to the volatile anesthetic agents, administration of dantrolene. On the other hand, the presence of a MH mutation in gene RYR1 modifies the muscular phenotype since the force of contracture in the IVCT is significantly more important in this group. These results support the genetic heterogeneity of the disease.

A2
Malignant Hyperthermia testing in probands with NO adverse anesthetic reaction
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BMC Anesthesiology 2014, 14(Suppl 1);A2

Background: Malignant hyperthermia (MH) is characterized by an adverse reaction to volatile anesthetic, and/or succinylcholine. Typically, following an adverse anesthetic reaction or positive family history, patients will undergo caffeine-halothane contracture (CHCT) and/or genetic testing. However, sometimes patients with no individual or family history of anesthetic reaction are referred for MH testing due to a variety of reasons. The objective of our study was to investigate reasons for referrals in non-anesthetic cases, and assess their phenotype.

Materials and methods: Following institutional research ethics board approval, all the CHCT-tested probands at our MH center were identified. Patients with anesthetic reactions were excluded. Reasons for referrals, baseline CK, genetics results, histopathology were analyzed and compared between patients with positive and negative CHCT results. Response to dantrolene among patients with positive CHCT was also assessed. Wilcoxon rank sum tests, and fisher’s exact test were used for numerical, and categorical parameters, respectively.

Results: Between 1992-2012, 152 probands with non-anesthetic reaction were identified. Of these, 104 (68.4%) had positive CHCT. Reasons for referrals included unexplained high creatine kinase-CK (50.6%), post-viral chronic fatigue (41.4%), post-exercise rhabdomyolysis (7.9%), and heat stroke (0.6%). Fifty-nine patients with high CK (76.6%), and 36 patients with post-viral chronic fatigue (57.1%) had positive CHCT based on the standardized North American CHCT test protocol. The viral illness included influenza, Epstein-Barr, and cytomegalovirus. The fatigue was defined as muscle pain, weakness, and cramps, interfering with functional ability, lasted more than three months after the onset of viral illness.

Thirty-eight (36.5%) patients with positive CHCT had abnormal histomorphology, which included central cores, and multi-minicores. Three patients carried causative mutations in Ryanodine receptor-I (RYR-I); of these, 2 were referred for unexplained high CK, and 1 was referred for exercise-induced rhabdomyolysis. Forty patients with positive CHCT (38.4%) were
### Table 1 (abstract A1) Severity of malignant hyperthermia reaction and force of contracture of the in-vitro-contracture test

<table>
<thead>
<tr>
<th>Severity</th>
<th>N=92</th>
<th>No mutation</th>
<th>Mutation</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td>29</td>
<td>10 (35%)</td>
<td>19 (65%)</td>
<td>0.85* (NS)</td>
</tr>
<tr>
<td>Alive stay ICU</td>
<td>28</td>
<td>5 (18%)</td>
<td>23 (82%)</td>
<td>0.15* (NS)</td>
</tr>
<tr>
<td>Alive no stay ICU</td>
<td>35</td>
<td>18 (52%)</td>
<td>17 (48%)</td>
<td>0.17* (NS)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Force of contracture</th>
<th>Mean+/SD</th>
<th>Mean+/SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2% g</td>
<td>0.99+/-1.02</td>
<td>2.46+/-1.86</td>
</tr>
<tr>
<td>Caf 2mmol g</td>
<td>0.58+/-0.58</td>
<td>1.29+/-0.97</td>
</tr>
<tr>
<td>H3% g</td>
<td>1.43+/-1.33</td>
<td>4.24+/-2.43</td>
</tr>
</tbody>
</table>

Although negative patients may be happy to result negative, they remain worried regarding anesthesia own and of their relatives. The worry for General Anesthesia (GA) does not disappear with a negative test and it may be very difficult explaining that other possible causes may be involved in MH like reactions. In some MHN probands it may be easier to find an explanation, as it is possible an alternative diagnosis or because… the not clear or wrong anesthetic report but what about the others. In some of similar cases, we decide to perform a genetic analysis and in one patient MHN patient we found a "no causative mutation". At this point the question is 1) can be assure the MHN patients regarding GA with trigger agents? 2) Are there other possible investigations to give more assurance to such patients? 3) Genetic testing should be prescribed also in the MHN probands?

We present the data of our MH laboratories highlighting the IVCT and genetic results obtained on our probands and the suggestions and information that we give to the MHN probands as well as some questions to the auditorium.

### A5

Update of the quality assurance (QA) results of halothane and caffeine analysis of all current participating malignant hyperthermia units

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**BMC Anesthesiology 2014, 14(Suppl 1):A5**

**Background:** The provision of a Quality Assurance Scheme for members of the European Malignant Hyperthermia Group (EMHG) is to ensure that the concentrations of halothane and caffeine in the tissue bath are as stipulated in the in vitro contracture test (IVCT) protocol. Confirmation of the correct concentrations in the tissue bath is an essential part of the IVCT protocol. The focus of this presentation will be to provide an update of the QA results from the analysis of both halothane and caffeine from all of the participating units over a period of three years. The aims are to show

1. How many malignant hyperthermia (MH) units have achieved the target concentrations of halothane for nominal vaporiser settings of 0.5% (0.11mM) and 2.0% (0.44mM) and caffeine concentrations of 0.5mm and 2.0mm?

2. How these halothane concentrations can vary within each lab?

**Results:** Each lab is aware of its own results. Anonymised results from January 2012 to January 2014 will be presented from each participating MH unit. These will include halothane results for vapouriser settings of 0.5% and 2.0%. The muscle bath concentration is usually lower than the required concentration, but halothane concentrations can also be found to be higher. Halothane concentrations can also vary within each lab.

Caffeine analysis results collected over the past 24 months will also be presented from each participating lab.

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### A6

A case of prolonged exertional rhabdomyolysis in a MHS individual

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**BMC Anesthesiology 2014, 14(Suppl 1):A6**

**Background:** We report a case of prolonged exercise-induced rhabdomyolysis (ER) in an otherwise healthy male malignant hyperthermia susceptible (MHS) individual.

**Case report:** A 42-year-old healthy Caucasian male contacted our malignant hyperthermia (MH) hotline due to cramps and muscle pain 1 week after moderate exercise. He was already diagnosed MHS in 1988 by in vitro contracture test (IVCT), after a suspected MH episode during general anesthesia.

At present, he reported severe muscle pain and cramps in both upper legs. The discomfort has started after moderate endurance training with...
mild muscle soreness for 2 days. On the 3rd day, pain increased, accompanied by severe muscle cramps in both upper legs. Symptoms were worsened by cold temperature. Due to pain, muscle weakness and swelling in both upper legs, he had sought medical attention on day 5 in a surgical outpatient clinic, where an ultrasound examination showed a "homogeneous increase in muscle density of both quadriceps muscles". No laboratory tests except d-dimer were performed and after exclusion of an acute thromboembolic event, the patient was dismissed. Due to ongoing pain, he contacted our MH-Hotline 7 days after the exercise. He still felt sick and reported darker urine during the past 3 days. Body temperature was not yet measured. His general practitioner had already taken a blood examination one hour before the telephone call. One day later, laboratory analysis was available and showed a CPK of 39,628 U/l and myoglobin of 2,863 ng/ml, consistent with rhabdomyolysis. Also liver enzymes were elevated, but kidney function and potassium were normal. The patient reported a body temperature of 37.5°C in the morning. Due to the high CPK levels 7 days after exercise (normally, peak levels are expected within 48 to 96 h), we recommended an immediate admission to our unit and decided to start with dantrolene 2.5 mg/kg IV. After the initial dose of dantrolene he was admitted to an intermediate care unit for the first 24h. Dantrolene was not continued since he was clinically stable. No clinical signs of a compartment syndrome could be detected. Pain continuously decreased and laboratory parameters slowly returned to normal within the next 2 weeks except of a CPK of 330 U/l. Body temperature and kidney function remained normal during the whole period. Any other reason for rhabdomyolysis than MHS diagnosis, i.e. viral or bacterial infection, hypothyresis, medication, drugs or dietary supplements could be ruled out. A MRI examination showed an inhomogeneous enhancement of contrast medium and edema of both quadriceps muscles. The patient was dismissed from hospital 3 days later, additional genetic testing has been performed. Unfortunately, previous CPK levels of the patient were unknown and in 1988, when IVCT was performed, histology was not regularly done.

Conclusions: With respect to this case, many questions concerning ER remain open. First, there are no guidelines for the acute management and the specific treatment of ER. There is some evidence from the literature supporting initial fluid therapy, but insufficient data exist, whether or to which patients dantrolene should be administered and how much and how long it should be given. There are no parameters defined that indicate how long the patient should be followed.

Secondly, there are only sparse data that provide some guidance on what we should recommend to the patient to avoid further relapses of ER. Finally, no sufficient data exist about the therapeutic value of preventive measures such as oral dantrolene or carnitine phosphate.

A7 Dantrolene in the treatment of malignant hyperthermia: a case report
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BMC Anesthesiology 2014, 14(Suppl 1):A7

Background: Malignant hyperthermia (MH) is a rare disorder, occurring in 1 per 5,000 to 1 per 50,000 anaesthetic procedures and is usually fatal if untreated. Relapse occurs in 33% of patients in spite of adequate management. Since emergency treatment is required, few reports document the time course of events relative to treatment.

Case report: The patient, a 19 year old healthy male, who suffered childhood asthma, was anaesthetised for a planned mandible sagittal split and bimaxillary osteotomia due to prognathia. Anaesthesia was induced with propofol 140mg and succinylcholinum 100mg was administered for muscle relaxation. Relaxation was not optimal but intubation was performed without problems. Anaesthesia was maintained with remifentanil (0.6-2.3µg/ kg/min) and desflurane (4.9-7vol%). After 15 minutes the airway pressure was 30cmH2O, which was initially interpreted as broncho-obstructive due to his childhood asthma, however, it was probably a sign of muscle rigidity. The patient was given minute ventilation at 8.2 litres, with pCO2 subsequently remaining stable for 2 hours. However, this increased to 100kPa and the patient began to sweat. The patient's temperature rose from 35.5°C 15 minutes after the start of the procedure to 37.9°C after 2.5 hours, but 15 minutes later the patient's temperature was 38.9°C. MH was diagnosed and dantrolene 380mg administered. Minute ventilation was increased to 12 litres FIO2 100%. Cooling was performed with cold intravenous Ringer Acetate and surface cooling with ice. After 25 minutes the temperature was 37.6°C. The patient remained on the ventilator for 11 hours after anaesthesia. There was mild recrudescence at approximately 6 hours, with blood pH gradually falling from 7.43 to 7.31, and pCO2 increasing from 4.9 to 6.8. A 200mg dantrolene infusion was therefore administered over 2 hours, prior to extubation of the patient, such that the total dose of dantrolene administered was 580mg (29 vials). When the patient came round he reported severe muscle pain. With regard to surgery, the mandible sagittal split was performed, but the maxillary surgery was cancelled, leaving the patient unable to close his mouth or bite. Over the following 2 weeks, prior to the second surgery, the patient was only able to drink and lost 6kg. Second surgery was performed in trigger free anaesthesia without problems.

In vitro contracture test revealed MH susceptible results (halothane contracture 2.8g and caffeine 1.3g). The patient has p. 552 Arg>Tyr mutation in RYR1.

Conclusions: This report describes the time course for the onset of clinical features of MH, and associated changes in laboratory parameters, and during relapse. It clearly demonstrates the need for, and efficacy of, dantrolene, when used immediately. Since the introduction of intravenous dantrolene, mortality from MH has fallen from 80% to less than 5%. The European Guidelines for the management of a MH crisis recommend the immediate use of dantrolene sodium in combination with other symptomatic treatments as soon as a MH crisis is suspected, and recognise that an adult patient requires up to 50 vials for effective treatment.

A8 Intraoperative tachycardia, hypercapnia, hyperthermia and muscle stiffness in a dantrolene unavailability case. Can calcium channel blockers be valuable?
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BMC Anesthesiology 2014, 14(Suppl 1):A8

Background: The case reported involved the constellation of the above signs and exceptional circumstances meant that the right drug was not available at the same time. Calcium channel blockers were given for life-saving reasons. However their value for that purpose is questionable and may be dangerous.

Case report: Caucasian healthy man, 19 years old, was anaesthetised with propofol bolus, remifentanil perfusion and sevoflurane maintenance. No muscle relaxants were used.

Tachycardia was treated at 30 minutes with propranolol EV. Hypercapnia was evident at 50 minutes. Hyperthermia was confirmed at 60 minutes and specific attitudes were taken: sotalol 180mg IV was given. At 65 minutes, muscle stiffness with ventilatory difficulties (Paw>40 cmH2O, CO2>65 mmHg, SaO2<82%), axillar temperature=48.5 ºC (0.2ºC/min) - verapamil 5mg EV was given.

The arterial blood gases: pH=7.23, K+ =6.2mEq/L. Insulin and dextrose was used.

The dantrolene was delivered at 80min but the clinical improvement delayed their administration to 120min. 40 mg guided by K+ blood analysis. At 48h the patient had severe legs oedema and at 72h blood CK = 12,700 U/L. He was discharged at one week and had fully recovered from renal and pereoneal nerve dysfunctions in the following months.

This patient was treated in the maxillofacial department, which is away from the main hospital unit. The dantrolene unavailability is an issue of the anaesthesiologist’s responsibilities. We did not carry out that specific checklist and were not aware of that situation. Portugal does not have laboratories for definitive diagnosis of malignant hyperthermia (MH) but the Larach clinical grading scale to predict patients susceptibility give 70 points; in the range of 50 points or more classified as almost certain. However, discussion of clinical signs and its sequential identification might question the therapeutic timing. If the therapy was implemented by the hypercapnia diagnosis, might the clinical evolution be different? And would the drug arrive in time for a life-saving situation? Is there any clinical indication for rechecking the local pharmacy? We do not exclude the possibility that clinical improvement might have been a consequence of previous therapeutic measures; however, there was a temporal drug-effect.
A9
Carnitine palmitoyltransferase 2 deficiency, malignant hyperthermia and anesthesia
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BMC Anesthesiology 2014, 14(Suppl 1):A9

Background: Carnitine palmitoyltransferase (CPT) deficiencies are a common autosomal recessive disorder resulting in a defect in mitochondrial fatty acid oxidation. The CPT system is made up of two separated proteins located in the outer CPT1 and inner CPT2 mitochondrial membranes. CPT1 catalyses the formation of acylcarnitine from carnitine and long chain fatty acid-CoA. Acylcarnitine then crosses the inner mitochondrial membrane where it combines with CoA to form acyl-CoA, a process catalyzed by CPT2. Acyl-CoA is then available to undergo beta-oxidation. Deficiencies of both CPT1 and 2 have been described and leave patients unable to derive energy from fatty acid oxidation. Once immediate glucose and glycogen stores are exhausted, hypoglycemia may occur. This process conducts to rhabdomyolysis with muscle pains, hyperkalemia, metabolic acidosis and myoglobinuria. In severe cases acute renal failure, cardiac arrest and death ensue.

Case report: A 35 years old woman was referred for a consultation to our malignant hyperthermia center before anesthesia for in vitro fertilization (IVF). She never had an anaesthesia. Her parents had 2 other healthy children. All family's members had had general anesethesia without problems. At 18 year old, after massive rhabdomyolysis during an interactive viral infection, the patient had a DNA analysis that revealed 1237Arg and Ser113Leu, mutations in CPT 2 gene. The patient was diagnosed as CPT2 deficiency. From these data she over went 4 crises of massive rhabdomyolysis, during which the CK levels exceed 90.000 IU/l. The patient is in good physical status, renal functions are normal, liver enzymes are mildly elevated.

Conclusions: 1. Is there indication for muscle biopsy and in vitro contractile test (IVCT)? What is the risk and what is the benefit from IVCT?
2. Does this patient need a special genetic counseling before pregnancy?
3. IVF needs a great number of sedations - what is the sedation plan?
4. The pregnancy may aggravates her general status and the delivery may be a challenge for patient as well as for us - anesthesia plans.

A10
Malignant hyperthermia as a rare cause of SIRS after cardiac surgery
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BMC Anesthesiology 2014, 14(Suppl 1):A10

Background: Use of extracorporal circulation (cardiopulmonary bypass) during cardiac surgery can cause a systemic inflammatory response. This so called “post-perfusion-syndrome” (PPS) occurs in about a quarter of patients and results in clinical signs and symptoms of “systemic inflammatory response syndrome” (SIRS) in 2-10% of patients. This condition is clinically associated with mild hyperthermia, acidosis, tachycardia and vasoplegia. It is generally treated with cristalloid infusions and vasopressors, and is mostly subsided by the next morning, at the latest after 48h.

Malignant hyperthermia is associated with a severe combined (respiratory and metabolic) acidosis, hyperlactatemia, hypercapnica, hyperthermia, grossly elevated serum levels of creatine kinase (CK) and acute renal failure.

Case report: We present a 23-year old patient, who gradually developed multi-organ failure following mitral valve repair for severe secondary mitral regurgitation due to dilated cardiomyopathy. His past medical history included partial hepatectomy for hepatoblastoma in childhood (1982) and partial lung resection due to recurrent pneumothoraces in 2002. Induction of general anesthesia was carried out with thiopentone, sufentanil and cis-atracurium and was maintained with isoflurane as the hypnotic agent. Following surgery, the patient showed increased infusion requirements and was highly vasopressor-dependent. Due to oliguric renal failure and refractory metabolic acidosis, continuous veno-venous hemodialfiltration was instituted early. Increased volume and vasopressor needs gradually decreased within the first 30 hours. In conjunction with two episodes of body temperature peaks of > 39°C in „non-CRRT-intervals“, the deferred but disproportionate elevation of serum-creatinkinase activity led to the clinical suspicion of malignant hyperthermia (Table 1). Much later, long after hospital discharge, MH was confirmed by in vitro testing.

Table 1 (abstract A10) Metabolic parameters and adrenergic drug application during the hospital stay of the patient.

<table>
<thead>
<tr>
<th></th>
<th>D0</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
<th>D7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate (mmol/l)</td>
<td>10.2</td>
<td>3.8</td>
<td>2.5</td>
<td>1.5</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.22</td>
<td>7.38</td>
<td>7.49</td>
<td>7.47</td>
<td>7.49</td>
<td>7.45</td>
</tr>
<tr>
<td>WBC (Г/Л)</td>
<td>28.91</td>
<td>20.12</td>
<td>18.33</td>
<td>19.71</td>
<td>27.93</td>
<td>19.53</td>
</tr>
<tr>
<td>CK (Л/Л)</td>
<td>1159</td>
<td>1645</td>
<td>17926</td>
<td>25119</td>
<td>19828</td>
<td>12908</td>
</tr>
<tr>
<td>CK-MB (µg/L)</td>
<td>67.4</td>
<td>65.9</td>
<td>168.7</td>
<td>62.2</td>
<td>26.1</td>
<td>4.4</td>
</tr>
<tr>
<td>TNI (mg/ml)</td>
<td>17.50</td>
<td>13.50</td>
<td>9.03</td>
<td>6.59</td>
<td>3.34</td>
<td>1.20</td>
</tr>
<tr>
<td>NA (µg/min)</td>
<td>130</td>
<td>40</td>
<td>50</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VP (л/л)</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DBX (µg/min)</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
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<td>1000</td>
</tr>
<tr>
<td>ADR (µg/min)</td>
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<td>30</td>
<td>16</td>
<td>20</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>MIL (µg/h)</td>
<td>4000</td>
<td>4000</td>
<td>4000</td>
<td>4000</td>
<td>4000</td>
<td>4000</td>
</tr>
</tbody>
</table>

Conclusions: High volume requirements and vasopressor-dependency occur commonly following surgical mitral valve repair, especially in patients with severely impaired left ventricular function. Differential diagnosis of hyperthermia on the ICU includes microbial triggers of the systemic inflammatory process, which could have been the case in this patient, where an infection might have been suspected. PPS and SIRS do not prompt any suspicion of malignant hyperthermia. Nevertheless, rare causes of both, hyperthermia and volume-depletion should be taken into account, even if the clinical course is rather mild in a way so that MH appears almost unlikely.

A11
Malignant hyperthermia on ICU – sudden attack of the “snake”
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BMC Anesthesiology 2014, 14(Suppl 1):A11

Background: Fulminant malignant hyperthermia (MH) is a rare emergency that should be known to every medical professional although practical exposure is a rarity even for experienced anesthesiologists. The vast majority of documented MH cases occurred during general anesthesia in the operating room following application of volatile anesthetics and/or depolarizing muscle relaxants. With increasing...
utilization of the anesthetic conserving device AnaConDa® for sedation of intensive care patients, who could benefit from reduced duration of mechanical ventilation and earlier hospital discharge, volatile anesthetics made their way into the intensive care unit (ICU).

**Case report:** With oral and written consent of the patient, we report the case of a 59-years old male, who was hospitalized due to persistent lumbalgia. On the second day of his hospital stay, increasing lumbalgia led to ICU admission and required tracheal intubation and mechanical ventilation. Sedation was maintained by propofol and sufentanil. A chest radiography revealed bilateral pulmonary infiltration, suggestive of influenza pneumonia. Mosfloxacin, piperacillin/tazobactam and oseltamivir were started immediately. When after four days of mechanical ventilation an additional sedative became necessary, the anesthetic conserving device for sevoflurane sedation was installed. Suddenly, following five hours of sevoflurane administration, hemodynamic instability characterized by rapidly dropping arterial blood pressure occurred. Arterial blood gas analysis revealed severe acidosis (\(pH\) 7.17; \(pCO_2\) 70.4 mmHg; \(pO_2\) 104 mmHg (fiO2 50%), base excess 9.8; lactate 0.6 mmol/L). A rapid increase of body temperature from 39.6 °C to 40.7 °C within 30 min was noticed. Creatine kinase and myoglobin levels were significantly elevated to maximum levels of 3455 U/L and 4197 μg/L due to acute rhabdomyolysis. As soon as MH was suspected, sevoflurane application was discontinued and Dantrile® was administered intravenously. After treatment the hemodynamic and metabolic situation gradually improved. The subsequent ICU stay remained uneventful and the patient was extubated on day 17 without neurological deficits. Nine month after the suspected MH event, the patient underwent muscle biopsy and in-vitro-contracture-testing at the Wuerzburg MH unit. His MH susceptibility was confirmed by significant contractures at the defined thresholds after halothane and caffeine exposure.

**Conclusions:** The presented case underlines the significance of MH as differential diagnosis in intensive care patients with hemodynamic and metabolic breakdown, if volatile anesthetics are used for sedation. Hence, awareness of the symptoms of MH and immediate initiation of adequate treatment can be crucial not only in the operating room.

**Note:** This abstract was awarded by the EMHG as best clinical presentation.

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**A12 Update of the EMHG database on genetic variants in type 1 ryanodine receptor and their possible impact on phenotype**

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**BMC Anesthesiology 2014, 14(Suppl 1):A12**

**Background:** The type 1 ryanodine receptor (RYR1) is expressed in human skeletal muscle and plays a key role in calcium homeostasis. Most patients with malignant hyperthermia (MH) have mutations in the RYR1 gene. Today, the gold standard for MH diagnosis is an invasive open muscle biopsy followed by the in-vitro-contracture-test (IVCCT). Less invasive diagnosis using molecular genetic methods is of increasing importance, but knowledge about genetic variants in RYR1 is limited. The European MH Group (EMHG) RYR1 mutation database is not up-to-date and lacks information on a significant number of more recently identified variants or further knowledge on variants already in the database. Therefore, the need for an update in order to expand the use of molecular genetic testing is required.

**The aim of this study was to collect information about variants in the RYR1 gene and their functional effect. Finally this information will be imported into the EMHG RYR1 mutation database.**

**Materials and methods:** PubMed was used for data collection. For the search, the three keywords “Malignant Hyperthermia”, “Mutation” and “Ryanodine Receptor Calcium Release Channel” were used; the operator was “AND”. Publication dates from the beginning of 2006 to the end of 2012 were selected. However, some older and newer studies were also included if they seemed important for the gain of information. Additionally, the considered papers had to be relevant for the human species. The software EndNote X7 (Thomson Reuters, Carlsbad, CA, USA) was used for administration of references and data was collected and processed in Microsoft Excel for Mac 2011 (Microsoft Corporation, Redmont, WA, USA).

**Results:** Altogether 62 publications were found according to the search algorithm. In the newly created database, an overall of 316 variants were gathered. 143 variants were not yet in the EMHG database, 129 of them not functionally characterized or non-pathogenic, 13 functionally characterized and putatively causative and one causative (p.H4833Y), respectively.

Among another 11 variants which were already in the EMHG database two (p.R330H, p.R2336H) can be classified as causative (Figure 1).

**Conclusions:** This study shows that important additional information on variants in RYR1 was published since 2006. However, only three mutations (p.R330H, p.R2336H and p.H4833Y) can be newly classified as causative mutations according to the EMHG guidelines. The main reason for this disappointing conclusion is the fact that the criterion “co-segregation with the disease in at least two pedigrees” according to the EMHG guidelines was missing in otherwise MH causative mutations. Nevertheless, this update of the database gives easier access to genetic information and emphasizes the increasing significance of genetic testing.

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**A13 Advantage from massive parallel sequencing of RYR1 and CACNA1S in diagnostics of malignant hyperthermia susceptibility**

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**BMC Anesthesiology 2014, 14(Suppl 1):A13**

**Background:** Mutations in RYR1 (ryanodine receptor type 1) are linked to the majority of malignant hyperthermia (MH) families (75%) and some cases of Exertional Heat Stroke. Two CACNA1S variants associated with MH have been functionally characterized. Historically, because of the large size of the RYR1 gene, MH families were screened for 31 diagnostic RYR1 variants only. Methods based on PCR and Sanger sequencing were used. The new opportunity arose since next generation sequencing techniques became available. With this technique sequencing is considerably quicker therefore even long gene sequences such as RYR1 can be easily screened for variations.
Material and methods: We used Next-Generation Sequencing to look for coding sequence non-synonymous variants of RYR1 and CACNA1S. We applied two different target enrichment methods, Long-Range PCR and HaloPlex (Agilent), to be able to focus on particular regions of the genome.

Results: In our study we included a group of 80 unrelated MHS individuals who were previously screened for diagnostic mutations in RYR1 and were found negative. Another group consisted of discordant individuals, whose in vitro contracture test (IVCT) phenotype was at odds with their genotype for a familial diagnostic RYR1 mutation. The third studied cohort (n=38) were Exertional Heat Stroke patients, IVCT tested susceptible or normal.

Conclusions: Our results illustrate that Next Generation Sequencing provides broad genetic information quicker and at lower cost than conventional approaches. To improve understanding of malignant hyperthermia genetics, screening of susceptible individuals only for a limited number of diagnostic RYR1 variants is not sufficient. Studying the full length of RYR1 and CACNA1S coding sequence may also resolve some phenotype/genotype discordant cases. Finally, Next Generation Sequencing techniques provide opportunities for studying larger gDNA fragments or the whole genome, which may result in discovering some other genes linked to MH.

A14
The introduction of a targeted next generation sequencing diagnostic service for MH

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BMC Anesthesiology 2014, 14(Suppl 1):A14

Background: In this paper we describe how we sought approval and are implementing a diagnostic service for malignant hyperthermia (MH) using clonal targeted next generation sequencing. Approval required submission of a gene dossier to the UK Genetic Testing Network. This document included:

1. An overview of MH and the evidence for involvement of RYR1 and CACNA1S;
2. Details of the genes;
3. Current diagnostic approaches;
4. Proposed sequencing strategy;
5. Gene coverage with proposed strategy;
6. Validation strategy;
7. Genetic epidemiology of MH;
8. Test characteristics (sensitivity, specificity, PPV, NPV);
9. Cost benefit of new test;
10. Referral criteria

Following adoption of the dossier by the UGTN and validation of the sequencing strategy in a diagnostic facility, we are now in a position to offer testing. Testing will be offered to families where MH has been confirmed by IVCT and to new index cases. The cost of the sequencing is £530, compared to £3,500 for the IVCT. For index cases, the referring physician will be advised of the pre-test probability for their patient having MH as they may consider IVCT to be more cost-effective when the pre-test probability is low.

Diagnostic reports will be issued in accordance with the joint guideline of the UK Association of Clinical Genetic Science (ACGSS) and the Dutch Society of Clinical Genetic Laboratory Specialists (VKGL). Variants will be classified using a 5 class system: Reports for classes 1 – 3 will advise IVCT. Variants will be assigned to a class depending on their reported frequency in databases (dbSNP, 1000 Genomes, EVS), segregation analysis and functional analysis.

A15
Targeted exon capture and NGS to investigate an undefined myopathy reveal RYR1 variants

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BMC Anesthesiology 2014, 14(Suppl 1):A15

Background: The family under investigation consists of parents and two daughters, one being the proband. The mother and the proband have elongated facial features. The father and second daughter appear normal. The older daughter presented for elective tonsillectomy aged 8 years. She had severe masseter spasm after suxamethonium. The rest of the procedure was carried out under total intravenous anaesthesia. No blood gas analysis could be done, but a creatine kinase next day was significantly elevated (2934). This led to study of both parents. There was no family history of malignant hyperthermia but an undefined myopathy was suspected in mother and daughter. Both mother and father were diagnosed malignant hyperthermia (MH) susceptible by in vitro contracture test (IVCT). This prompted a DNA analysis for variants associated with MH.

Materials and methods: Standard histochemistry, biochemistry and electron microscopy were carried out on muscle tissue from the mother. DNA from all four family members was analysed by targeted exon capture and next generation sequencing using the Ion Torrent platform. B-lymphoblastoid cells were generated from all family members and assayed for abnormal calcium release.

Results: The mother and both daughters carry a premature stop codon in ryanodine receptor subtype 1 (RYR1) as well an uncharacterized RYR1 variant inherited from the father. The mother also carries a second uncharacterized RYR1 variant, not inherited by either daughter. Muscle histology showed two cox-negative fibres suggestive of a mitochondrial disorder but not definitive. Calcium release assays using B-lymphoblastoid cells suggest a hypersensitive RY1 channel in all four family members.

Conclusions: The RYR1 variants identified cannot be definitively associated with susceptibility to MH, although the functional assays in B-lymphoblastoid cells suggest a hypersensitive channel. It is possible that the undefined myopathy is associated with another gene and the MH susceptible result by IVCT is unrelated to this condition. Further analysis of the family is required for a definitive diagnosis.

A16
Preferential allele amplification leading to RyR1 misgenotyping in a malignant hyperthermia susceptible individual

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BMC Anesthesiology 2014, 14(Suppl 1):A16

Background: Many current methods for the detection of gene variants relevant for inherited disorders like malignant hyperthermia (MH) rely on the polymerase chain reaction (PCR). A positive PCR result for the genetic region of interest is required for downstream analysis like sequencing, restriction digestion or probe hybridization. However, it is sometimes overlooked that in order to obtain a true genotype, both paternal and maternal alleles must be represented in the PCR product. We present the

Table 1(abstract A14) 5 Class System

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
<th>Interpretation</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Clearly not pathogenic</td>
<td>MH not confirmed or excluded</td>
</tr>
<tr>
<td>2</td>
<td>Unlikely to be pathogenic</td>
<td>MH not confirmed or excluded</td>
</tr>
<tr>
<td>3</td>
<td>Variant of unknown significance (VUS)</td>
<td>MH not confirmed or excluded</td>
</tr>
<tr>
<td>4</td>
<td>Likely to be pathogenic</td>
<td>Consistent with diagnosis</td>
</tr>
<tr>
<td>5</td>
<td>Clearly pathogenic</td>
<td>Confirms diagnosis</td>
</tr>
</tbody>
</table>
case of a preferential PCR amplification of one ryanodine receptor subtype 1 (RYR1) mutant allele that led to apparent homozygosity of a proband.

**Material and methods:** A woman, 23 y.o. with family history of MH susceptibility was initially genotyped as homozygous carrier of the MH causative RYR1 mutation 2434Gly>Arg. This unusual genotype was investigated by further PCR and sequencing.

**Results:** Sequencing of the genomic region from the biological parents of the proband revealed that only the father was a mutation carrier. This result was confirmed by using an additional, extended PCR that covered the target exon plus adjacent introns, casting doubt on the initial genotype call of the daughter. Using the same extended PCR in the daughter, indicated that she indeed carried a wild-type along with the mutated RYR1 allele. We endorsed this genotype over the initial one because the presence of additional intronic polymorphisms (SNPs) provided evidence that both parental alleles were represented in the extended amplicon. Moreover, taking advantage of the Inherited Disease Panel and a next generation sequencing platform (Ion Torrent, Personal Genome Machine, Life Technologies), we could confirm the heterozygosity for the RYR1 mutation by a completely different method.

**Conclusions:** These results highlight that PCR amplification bias of one parental allele may completely mask the presence of the second parental allele, giving rise to apparent, but false, homozygosity. An appropriate quality control to establish the diploid nature of the material to be sequenced is thus important for accurate PCR-based genotyping, particularly for the diagnosis of autosomal dominant disorders like MH. For this purpose, we suggest the use of extended regions reaching neighboring SNPs.

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A17

**RYR1 mutation screening 1992 – 2014: a genetic report on 22 years from the Würzburg MH unit**

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**BMC Anesthesiology 2014, 14(Suppl 1):A17**

**Background:** The ryanodine receptor type 1 (RYR1) is one of the biggest known muscle proteins (4 x 565 kDa) acting as the major calcium release channel of skeletal muscle. A large number of mutations have been identified in the corresponding gene (RYR1) giving rise to a variety of clinical phenotypes: Malignant Hyperthermia susceptibility (MHS) and the group of congenital (core) myopathies.

**Materials and methods:** In 1992, we began to screen MHS individual by PCR and restriction enzyme digestion for the few then known RYR1 mutations. At around 1995, we switched to Sanger sequencing of selected exons (mutation hot-spots) and for a number of years, we analysed five exons in MHS (exons 17, 39, 40, 45, 46) and four in myopathic subjects (exons 95, 100, 101, 102). From 2002 on, we also included whole-genome sequencing of all 106 exons by Sanger or NGS technologies (starting from 2011). In total, we have screened 1,029 unrelated index cases. The great majority of malignant hyperthermia (MH) individuals had a positive in vitro contracture test (IVCT) result (MHS or MH-equivalent MHE) or a likely clinical MH episode. Patients with congenital myopathies were referred on the basis of their clinical presentation.

**Results:** Among the 305 individuals screened for the MHS hot-spot, we identified 16 different sequence variants in 46 individuals (15.8 %). In 41 patients, the mutation met the criteria for causality of the European MH group (EMHG) leaving only 5 individuals with unclassified variants. In the myopathy group of 474 index cases, 37 variants were identified in 72 individuals by screening the "CCD-hot-spot" (15.2 %). In this group, only three mutations in 16 patients fulfilled the causality criteria of EMHG. Thus, for 56 individuals, the screening gave no clear-cut results.

**A18 Knowledge on malignant hyperthermia: as rare as the disease? A nation wide survey**

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**BMC Anesthesiology 2014, 14(Suppl 1):A18**

**Background:** Knowledge on malignant hyperthermia (MH) has expanded vastly during the past decades, but not everyone is up to date on all this newfound knowledge. To assess the current level of knowledge on MH, a survey was performed among Dutch anaesthesia personnel. Research questions were: What is the current general knowledge of anaesthesia personnel about MH; furthermore, do anaesthesiologists (in training) know more than (trainee) nurse anaesthetists; and, does experience with a MH crisis and/or triggerfree anaesthesia result in a better overall knowledge score?

**Materials and methods:** The survey consisted of an online questionnaire, responders were recruited via Dutch social media groups for anaesthesia personnel. The survey entailed 12 questions, 3 of which assessed the existing knowledge on MH. The other 9 questions assessed the assessed the existing knowledge on MH. The questions covered various important aspects of MH: knowledge on the incidence, triggers, symptoms, prevention, (importance of early) recognition, and treatment. The maximum possible score was 12 points. Crosstabs and one way anova analysis was performed with SPSS Statistical software package version 21 for statistical analysis, P <0.05 was taken to represent significance. Correctness of the answers was assessed in relation to the available literature and EMHG guidelines on the subject.

**Results:** A total of 104 (n=104) responders entered the survey, of which 22 were anaesthesiologists; 20 residents; 42 nurse anaesthetists; 17 trainee nurse anaesthetists; 3 did not specify their profession. Among responders 52 subjects had no previous experience with MH in practice, as opposed to 51 who did have experience (1 subject did not specify), the latter group had a significantly higher knowledge score. Results of the knowledge questions and total knowledge scores are shown in table 1.

**Conclusions:** Knowledge on MH is not quite as rare as the disease but certainly needs improvement as evidenced by this survey. Anaesthesiologists and residents have significantly better knowledge than (trainee) nurses anaesthetists. Yet the highest average knowledge score of 4.68 out of a maximum of 12 points is disappointing reflecting insufficient basic knowledge on MH. These observations call for improved knowledge dissemination of this rare but very dangerous complication of anaesthesia. Means by which this might be achieved is simulation education as
Role of sarcoplasmic reticulum junctional proteins in skeletal muscle strength

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BMC Anesthesiology 2014, 14(Suppl 1):A19

Background: Skeletal muscle constitutes approximately 40% of body mass, and age-induced decrease of muscle strength impinging on daily activities and on normal social life in the elderly. Loss of muscle strength has been recognised as a debilitating and life threatening condition also in cachexia in cancer patients and in clinical conditions associated with prolonged bed rest. Skeletal muscle dihydropyridine receptors (Cav1.1) act as Ca2+ channels and voltage sensor to initiate muscle contraction by activating ryanodine receptors, the Ca2+ release channels of the sarcoplasmic reticulum. Cav1.1 activity is enhanced by a retrograde stimulatory signal delivered by the ryanodine receptor. JP45 is a membrane protein interacting with Cav1.1 and the sarcoplasmic reticulum Ca2+ storage protein casquestrin (CASQ1).

We hypothesized that JP45 and CASQ1 form a signalling pathway which modulates Cav1.1 channel activity.

Materials and methods: We isolated flexor digitorum brevis (FDB) muscle fibres from JP45 and CASQ1 double knock-out mice (DKO) and tested whether there were differences in Ca2+ homeostasis between the different mouse lines.

Results: Our results show that Ca2+ transients evoked by tetanic stimulation in DKO fibres, result from massive Ca2+ influx due to enhanced Cav1.1 channel activity. This enhanced activity causes an increase of muscle strength both in vitro and in vivo.

Conclusions: We conclude that skeletal muscle contraction is strengthened through the modulation of Cav1.1 channel activity by JP45 and CASQ1.

Acknowledgements: This work was supported by funds from Swiss Muscle foundation, A.F.M., S.N.F and Department of Biomedicine University Hospital Basel. This study was also supported by Research Grant no. GGP08153 from the Italian Telethon ONLUS Foundation to F.P. and grants from the NIH/NIA (AG13934 and AG15820) to O.D.

References

A20

Gene expression in the context of malignant hyperthermia status and ageing

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BMC Anesthesiology 2014, 14(Suppl 1):A20

Background: This study aims to investigate gene expression in skeletal muscle in relation to age and MH status, focussing on particular genes of interest. Expression patterns have been analysed to address the question of whether major causative malignant hyperthermia (MH) mutations influence skeletal muscle ageing. In this context we have examined gene expression in MH susceptible, both with (MHS+) and without mutation (MHS-), and MH normal (MHN) material from in vitro contracture test (IVCT) muscle biopsies.

Material and methods and results: Affymetrix data, derived from Affymetrix HG_U133Plus2.0 arrays on a cohort of 59 patient muscle cDNA samples, were analysed for potential genes of interest. These 59 samples comprised an age range of 10-71 years and included 27 MHS+, 13 MHS- and 19 MHN. Linear models (LM) were applied using R studio 3.0.10. LM1 incorporated three independent variables: age, sex and malignant hyperthermia (MH) status and one dependent variable, expression. LM2 included just the sex and MH status variables, LM3 included only the age and MH status, and LM 4 included all 3 variables but left out the age-MH status interaction term. Model comparison was completed using Akaike Information Criterion to establish the model that best fitted the observed data. The results were interpreted to ascertain those genes that may be of interest in the context of age, MH status and interaction between these two variables.

The genes selected through this process, along with additional genes of interest based on criteria, such as involvement in store-operated calcium entry (SOCE) in skeletal muscle, were then subjected to TaqMan Gene Expression Analysis on a cohort of 108 cDNA samples, derived from the NIH/NIA (AG13934 and AG15820) to O.D.
## A21

**Caenorhabditis elegans as a model organism for RYR1 variants and muscle ageing**

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BMC Anesthesiology 2014, 14(Suppl 1):A21

**Background:** Malignant Hyperthermia (MH), central core disease (CCD), exertional heat stroke (EHS) and late-onset axial myopathy have been attributed to mutations in ryanodine receptor type 1 (RYR1). The RyR1 protein is over 5000 amino acid residues long, making manipulation of the mammalian gene difficult. The ryanodine receptor in *Caenorhabditis elegans* is unc-68, which has 40% amino acid identity to the human protein.

**Material and methods: and results:** Due to the compact nature of the *C. elegans* genome, the unc-68 gene is only 27 kb and is entirely contained in the fosmid clone WRM069cA02. Using recombineering, single base pairs were changed in this fosmid to establish nine different variants:

- Four implicated in MH:
  - p.G341R c.1021G>A
  - p.R2163C c.6489G>A
  - p.R2545H c.7631G>A
  - p.R2458H c.7373G>A
- One implicated in EHS:
  - p.R163C c.487C>T
- Two implicated in CCD:
  - p.R4661H c.14083G>A
  - p.A4940T c.14820G>A
- Two implicated in Late-onset axial myopathy:
  - p.K3452Q c.10354A>C
  - p.R163C c.487C>T

Using these altered fosmids, transgenic strains were developed by microinjection.

In order to establish these strains as a suitable model for studying RYR1 variants phenotyping assays were completed to assess the effects of halothane and caffeine on each of the strains developed. The rationale for this approach is based on the intravertebrate test (IVCT). Both halothane and caffeine assays were carried out in 5-median, 1mM, 1.5mM, 2.0mM, 2.5mM concentrations of halothane were used, with halothane dissolved in DMSO prior to dilution in a liquid medium. The lowest concentration used is the lowest dose of halothane that will fully anaesthetise the worms and the highest dose is the maximum dosage from which worms can recover. Worms were immerses for 60 seconds and then body bends counted. The curves for each test were analysed according to the protocol of EMHG. The curves for each test were analysed to determine if there were contractures.

**Results:** Two animals in the control group (0.3 ±0.14 g), two animals in the 5mg statin group (0.2 ±0 g) and three animals in the 20 mg statin group (0.22 ±0.02) had greater than or equal to 0.2 g contracture in the presence of 2% halothane. One animal in the 5mg statin group (0.28 g) and two group of the 20 mg statin group (0.26 ±0.02 g) had greater than or equal to 0.2 g contracture in the presence of 2mM caffeine. But these contractures were minimal and there was no statistical difference in the number of animals per group with this change (chi-square, p not significant).

**Conclusions:** A preliminary analysis of these findings suggests that doses of 5-20 mg/kg of simvastatin were not sufficient to cause contractures and do not increase the risk of MH crisis in healthy individuals in monotherapy.

## A22

**Chronic use of low dose simvastatin does not alter the IVCT in normal rats**

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BMC Anesthesiology 2014, 14(Suppl 1):A22

**Background:** Statins are currently the preferred agent for the treatment of hypercholesterolemia. However, myalgia, increased serum levels of creatin kinase (CK) and even rhabdomyolysis may occur, characterizing the cholesterol - lowering agents myopathy (CLAM), with a multifactorial aetiology. In statin monotherapy, the incidence of CLAM is 0.1 to 0.5 % and it is dose related. RYR1 (ryanodine 1) gene alteration was observed in the patients with CLAM, an animal model of MH (malignant hyperthermia) showed hyper metabolism after simvastatin, simvastatin induced in vitro contractures in muscle of animal model and susceptibility to MH by IVCT (in vitro contracture test) was diagnosed in patients with CLAM.

On the other way, postoperative rhabdomyolysis occurred in patients treated with cholesterol-lowering drugs, leading to the suggestion of suspend these medications prior to surgery or do not use succinylcholine in patients taking these drugs. But this stance is challenged by other groups. Thus, this study examined, in rats chronically exposed to simvastatin dose previously associated with muscle alteration, the outcome of the IVCT.

**Materials and methods:** With approval of the local ethics committee, 30 male Wistar rats of 8 weeks of age were divided into 3 groups: A - control, B - Statin 5 mg/kg/day; C - Statin 20 mg/kg/day. For two months, via intragastric intubations using stainless curved feeding needle, the animals in groups B and C received simvastatin daily, diluted with carboxymethyl cellulose (CMC) 0.5 %, group A received only CMC. Next, the animals were anesthetized for removal of the vastus lateralis muscles, used in the IVCT according to the protocol of EMHG. The curves for each test were analysed to establish whether there were contractures.

**Results:** Two animals in the control group (0.3 ±0.14 g), two animals in the 5mg statin group (0.2 ±0 g) and three animals in the 20 mg statin group (0.22 ±0.02) had greater than or equal to 0.2 g contracture in the presence of 2% halothane. One animal in the 5mg statin group (0.28 g) and two group of the 20 mg statin group (0.26 ±0.02 g) had greater than or equal to 0.2 g contracture in the presence of 2mM caffeine. But these contractures were minimal and there was no statistical difference in the number of animals per group with this change (chi-square, p not significant).

**Conclusions:** A preliminary analysis of these findings suggests that doses of 5-20 mg/kg of simvastatin were not sufficient to cause contractures and do not increase the risk of MH crisis in healthy individuals in monotherapy.
Conclusions: Medical record coding for MH typically includes both incident cases as well as a history of MH. The positive predictive value of about 70% for MH in this study are consistent with other studies of ICD-9 accuracy in the US. However, epidemiologic studies based on coded diagnosis of MH should carefully distinguish between incident cases related to anesthesia, cases unrelated to anesthesia and diagnosis based on history only.

A24
MH in Russia: obstacle races
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BMC Anesthesiology 2014, 14(Suppl 1):A24

Background: The problem of malignant hyperthermia (MH) was awakened in Russia in 2012, when 3 y.o. girl died in private hospital in Saint Petersburg. MH is well known, but well forgotten by most of anaesthesiologists in Russia since we have a lack of diagnostic and treatment tools. Even if MH crisis takes place we have no specific treatment at the moment and a few opportunities for diagnosing MH susceptibility (MHS): dantrolene is still not registered, capnography isn’t available in probably 2/3 of operating theatres all over the country, there is only one genetic lab performing search for causative MH mutations and in vitro contracture test lab is not equipped with proper devices.

MH is not a part of any national governmental or healthcare ministry program, so our work is not sponsored and is totally volunteered. Our small team is full of enthusiasm, but this can’t cover expenses for lab equipment. We had received financial support from two commercial sponsors, but it was not too big and we were not able to buy all the necessary things. We had received a great support from our French colleagues who shared their experience, knowledge, soft- and hardware and even some important lab pieces.

This report is intended to be a kind of “Call for help”. If your lab has some equipment to share (old, not in use and even out of service) we would appreciate any contribution and support.

A25
Development and evolution of the MHUA5 cognitive aid for malignant hyperthermia
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BMC Anesthesiology 2014, 14(Suppl 1):A25

Background: Cognitive aids help the stressed practitioner in an emergency setting carry out complex tasks. Ideally, they ensure the completion of key steps and prevent unnecessary ones. Malignant hyperthermia is an emergency event that lends itself to the use of a cognitive aid. Experts associated with the Malignant Hyperthermia Association (MHUA) first developed a cognitive aid in the form of a “poster” in the mid-1980s.

Methods and results: MH experts based the “poster” on review of the current literature, personal experience, and experience gained from the MH Hotline. The first four versions (1991, 1993, 1995, and 1998) were formatted as a list of steps. In the first ten years, changes were mainly to content rather than design. For example, dantrolene administration was prioritized - Step 1 “GET HELP GET DANTROLENE,” changing the anesthesia circuit tubing and CO2 absorbent were no longer recommended; and specific dosages were given for insulin and glucose for the treatment of hyperkalemia. Released in 2003, the fifth version of the MH poster had a completely different format with three separate sections for diagnosis (including masseter spasm and sudden cardiac arrest in a child), acute treatment, and post acute care. The sentences were converted to bullet points and formatted in columns to make reading easier. Bolded text was also used to highlight common errors, such as failing to use sterile water to dilute the dantrolene, and the avoidance of calcium channel blockers. The next two versions (2005 and 2008) retained this format with added information on myoglobinuria and the maintenance of adequate urine output. The latest version (2011), has a dramatically different look. The most notable changes are the use of color, pictures, and an algorithm style rather than bullet-point steps.

Over 20,000 posters have been distributed over past several decades. The use of the posters/algorithms has been incorporated into simulation-based scenarios involving MH; the posters have been translated into different languages, and modified for inclusion in the Stanford Emergency Manual.

Conclusions: The MHUA5 MH treatment poster was one of the, if not the first cognitive aid to be used in anesthesia practice. It has evolved from a simple list of steps to a visually appealing and easy to follow algorithm that incorporates all key treatment elements and common diagnostic questions. Although it is impossible to measure how the “poster” has influenced the recognition and treatment of MH, the fact that it is widely distributed and continues to be in demand attests to its probable effectiveness.

A26
French guidelines for the management of malignant hyperthermia
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Background: Malignant hyperthermia (MH) is a complication of anaesthesia appearing as an acute potentially lethal hypermetabolic state in people carrying a genetic anomaly expressed in the skeletal muscle. Clinical guidelines aim to help health professionals and patients make the best decisions about treatment or care for a particular condition or situation. The guidelines are typically written in statement form by a reputable organization. The authors of guidelines review the research literature and take advice from experts to gather the current evidence on which to base the recommendations in a guideline. Doctors, nurses and other health care professionals are encouraged to follow clinical guidelines where appropriate [http://www.patient.co.uk/guidelines.asp].

The French Society of Anesthesiology and Intensive Care (SFAR) decided in 2012 to ask the French MH Group (FMHG) to write Guidelines for the management of Malignant Hyperthermia. The FMHG gathers the professionals in charge of the Five MH Centers involved in the diagnosis of MH by performing in vitro contracture test (IVCT), genetic analysis and/or an expert advice. Pr Renée Krivosic-Horber, Dr. Anne-Frédérique Dalmas Dalmases Centre HM Lille, Pr Yves Nivoche Centre HM Robert Debré Paris, Pr Jean-François Payen Chu Grenoble, Pr Joël Lunardi, Madame Nicole Monnier, Pr Julien Faure Centre de Biologie Moléculaire CHU de Grenoble, Dr Alexandre Moerman Génélique Clinique CHRU de Lille, Dr Thierry Girard MH Center Basel Suisse was asked to give his opinion as a french speaking international expert.

Three meetings and many exchanges by mail between the experts and with the colleagues in charge of the Guidelines Board of the SFAR were necessary to obtain a consensus from all the participants. Eventually the text was published on the site of the SFAR in October 2013. http://www.sfar.org/article/1080/recommandations-d-rsquo-experts-pour-le-risque-d-rsquo-hyperthermie-maligne-en-anesthesie-reanimation-sfar-crc-12-septembre-2013. Dr. Krivosic-Horber has been invited to present the Guidelines with a written paper in the annals, at the annual meeting of the SFAR in Paris in September 2014.

Index of the Guidelines:
1) Screening of the risk of MH susceptibility during the “consultation d’anesthesie” (which is legally mandatory in France).
2) How to do the diagnosis of MH susceptibility (IVCT or Genetic?)
3) How to perform an anaesthesia free of MH risk.
2) Management of the MH crisis: presented as a poster with diagnosis and treatment on one side and stock and preparation of dantrolene on the other side.
3) What to do after the crisis.
4) The addresses of the MH centers and a map showing the type of diagnosis they can provide.
POSTER PRESENTATIONS

P1
Malignant hyperthermia and beyond - a virtual BioBank
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Background: In order to address the complexities of Malignant Hyperthermia (MH) and genetically related disorders it is necessary to access and interrogate easily all information collected over the last 40 years, in addition to incorporating the continuous supply of new information.

Material and methods: With all types of data held in a single database we are interested in a number of projects including:

• A more detailed analysis of in vitro contracture test (IVCT) data to better define phenotype for research purposes, and to assess the effects of specific mutations on the range of phenotypes. We have previously conducted an analysis of the effects of a number of ryanodine receptor type 1 (RYR1) mutations on IVCT and MH reaction phenotypes.
• We have previously published preliminary work considering the possibility of genetic modifiers, and this is a topical area with the role of common versus rare variants as contributors to complex disorders being hotly debated. It will be possible to initiate small projects on the effects of previously identified single nucleotide variants and SNV-SNV interactions on phenotype.
• To analyse observations such as discordancy in greater depth.
• To assess the statistical power for large-scale studies such as a Genome Wide Association Scan.

Results: In Leeds, we have initiated a relational database, developed using FileMaker Pro®. This software was specifically chosen due to its versatility to set permissions and access rights ensuring that all data stored is kept secure. A private, restricted access file, currently holds nearly 10,000 records, with one individual being represented as one record. It contains names, family codes, date of birth and diagnostic identification numbers. This file preserves anonymity by assigning individuals within families a unique identification number, subsequently acting to link all other data. This private file is held on a different server from the second wider access ‘public’ file, whose data is linked to the private file only by the unique identifier. This ‘public’ file holds a range of information in the form of tables:

• The family table describes familial relationships, which will be suitable for exporting to .ped files, enabling this data to be imported directly into genetic analysis programs.
• The process table which holds sample collection and storage information, together with the procedures undertaken e.g. histology, exome sequencing, preparation of myoblasts.
• The phenotype and IVCT tables contain information such as clinical details and raw IVCT data, allowing for a more detailed analysis in addition to the clinical definition of MH5 versus MHN.
• The genotype table contains all genotypic information to date such as mutation testing results.

Conclusions: We are interested in expanding this database by bringing together Leeds data with other European data. We have the ability to create accounts only for specified users and management through privilege sets, ranging from full access, data entry and read only. This would enable users to assess the feasibility and initiate large-scale collaborative programmes of research. Thus through use of this database, supported by the Leeds centre, we hope to build collectively a pan European resource and international virtual biobank.

P2
100 Telephone conversations about malignant hyperthermia
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Background: We sought to analyse the telephone calls concerning MH to the Department of Anaesthesiology and Intensive Care Medicine, University Hospital, Leipzig, Germany.

Material and methods: A total of 100 documented telephone inquiries from January 2011 until March 2014 were summarized, analysed for caller and cause of contact.

Results: 100 inquiries were analysed: 76 patients and 24 medical doctors called. Out of the 24 medical doctors, 16 were anaesthesiologists. Reason for malignant hyperthermia (MH) inquiry: Positive family anamneses (37%), own anaesthetic accident (29%) or general information about MH (34%). In the group of positive family anamneses, 34 patients with MH or their relatives and three medical doctors sought information. The described anaesthetic accident ranged from reaction to death during an operative procedure. Inquiries based on an anaesthetic accident were addressed by a patient 20 times and by a medical doctor 9 times.

Some curious questions were asked:
Anaesthetist: “Patient had an anaesthetic incident forty years ago. What should I do?” (anaesthetic management)
Patient: “My anaesthetist refused the anaesthesia as long as I’m not tested for MH!” (suspicious family history)
Intensive care physician: “Our patient on the ICU must immediately be tested for MH because he has a rhabdomyolysis and we don’t know why!” (no triggering substances)
Patient: “My uncle had problems during anaesthesia. Now our family should be tested. How do I proceed?”
General practitioner: “MH was suspected in a muscular biopsy.”

Conclusions: MH is a known but with uncertainties connected disorder especially in association with muscular diseases. Approximately three-quarter of inquiries were made from patients. Patients profit from the care of specialized MH centre. However, further educational work about MH by specialized centres is still necessary.

Cite abstracts in this supplement using the relevant abstract number, e.g.: Petersen et al. 100 Telephone conversations about malignant hyperthermia. BMC Anesthesiology 2014, 14(Suppl 1)P2