# 43RD EMGH MEETING 2025
# ABSTRACTS

The European Malignant Hyperthermia Group (EMHG) was formed in 1983 and comprises all MH investigation centres who follow the EMHG testing protocol. This includes centres from Europe, North America, Brazil, Australia, New Zealand and from South Africa. The EMHG annual meeting is usually hosted by one of these centres and the 43rd annual EMHG meeting took place in Cape Town, South Africa, on 24–25 April 2025, marking the first time this prestigious event was hosted in this country. Delegates from around the globe attended, contributing to a robust and diverse academic programme. The event featured both clinical and scientific presentations, which significantly contributed to its success. Three distinguished guest speakers—a paediatric neurologist, a veterinary surgeon, and an anaesthetist—delivered insightful talks, offering unique interdisciplinary perspectives. Abstracts from the meeting are published in this edition of the South African Journal of Anaesthesia and Analgesia (SAJAA).

**Prof Thierry Girard**
Chairman of EMHG, Basel, Switzerland

**Dr Hannah Brand**
Meeting organiser, MHCSA South Africa

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SASA MAIN CONFERENCE

# EMHG ABSTRACTS 2025

## Myopathies with especial relevance to *RYR1* variants

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Congenital myopathy with central nuclei is the most common form of congenital myopathy in the Western Cape province of South Africa, despite a lower prevalence in other populations. Internationally, the incidence of all congenital myopathies is estimated at 6 per 100 000 live births. Centronuclear myopathy (CNM) is an umbrella term for a group of rare genetic muscle disorders presenting with muscle weakness that can range from mild to profound. Pathologically characterised by the central location of the muscle fibre nucleus on muscle biopsy. There are three subtypes of CNM classified by inheritance – X-linked, autosomal dominant and autosomal recessive forms caused by different genetic mutations.

Dominant and recessive *RYR1* gene mutations are associated with a wide range of inherited myopathies, including CNM. The *RYR1* gene is located on chromosome 19q13.1, contains 106 exons and encodes *RYR1*, which is the main sarcoplasmic reticulum calcium release channel. It plays a crucial role in excitation–contraction coupling (ECC). It is the commonest variant linked to congenital myopathies (50%), especially CNM for the South African group. Dominant *RYR1* mutations may also cause malignant hyperthermia (MH), an altered pharmacogenetic response to halogenated anaesthetics and muscle relaxants in susceptible but otherwise healthy individuals.

This talk aims to give an overview of CNM, including its epidemiology, clinical presentation and natural history. We will share the local experience of our multidisciplinary neuromuscular service at the Red Cross War Memorial Children's Hospital in the Western Cape of South Africa. Our research describing a phenotypic and genotypic analysis of our patients with centronuclear myopathy will be presented. We will highlight the challenges we face in the African continent and the realities of resource limitation. Current international collaborative efforts looking at future therapeutic strategies will be shared.

## Capture myopathy – an unintended hazard of working with wildlife

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Capture myopathy is a condition that is described mainly in wild animals and is usually associated with stress and physical exertion following prolonged capture and handling episodes. This can be the result of chasing, handling, restraint or transport. Four forms of this metabolic condition have been described – hyper-acute, acute, sub-acute and chronic, with significant morbidity and mortality described. The prognosis is generally poor, and attempts at treatment with non-specific supportive treatment have not shown a good success rate. The condition is mostly seen in ungulates, with the highest incidence in antelope and bovid species.

Southern Africa has significant wildlife conservation and wildlife ranching programmes that rely on the capture and translocation of both common and endangered animals, so the impact of this condition can be of major significance. Many cases have been described, and the incidence and severity of the condition vary from species to species. Understanding the management and behaviour of the various wildlife species can be key to reducing the incidence.

Although a significant number of cases have been seen, relatively little research has been done on the condition, primarily due to the managemental intricacies of performing research on wild animals. Some significant studies have, however been done, which shed light on the underlying pathophysiological processes and clinical presentation.

These processes, potential treatment and similarities to human conditions will be discussed. A historical adage that has been used in the wildlife translocation industry still holds true – prevention is better than cure.

## Using exome sequencing to investigate phenotype-genotype discordance in the UK MH population

**Catherine Daly,[1] James Poulter,[2] Marie-Anne Shaw,[2] Sarah Hobson,[1] Philip Hopkins[1,2]**

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The in vitro contracture test (IVCT) has remained the gold standard for malignant hyperthermia (MH) diagnosis partly because of the continuing prevalence of phenotype-genotype discordance. Within the 52% (480/927) of susceptible UK families who have a diagnostic variant, 18% (91/480) have some degree of discordance, that is, at least one individual negative for the familial *RYR1* variant but with a positive IVCT. As part of the unit's exome programme, 23 of these families were sequenced in an effort to identify potential genetic causes of discordance.

One MHS genotype +ve, one MHS genotype -ve, and one MHN individual, with sufficient good quality DNA, were selected from each discordant family for exome sequencing. MHS static halothane contractures were ≥ 0.2 g. The sequencing data were processed and an annotated variant list for each family was generated. Initially, variants with either a gnomAD minor allele frequency > 0.001 (heterozygous variants) or > 0.03 (homozygous or potential compound hets.) were removed, followed by those with CADD score < 15 and REVEL score < 0.5. Subsequently, variants found in the MHN samples were also removed. Variants were retained if they were in genes that featured in any of the following four NHS National Genomics Panels – malignant hyperthermia, acute rhabdomyolysis, congenital myopathy, or skeletal muscle channelopathy. In addition, variants flagged as pathogenic or likely pathogenic by ClinVar with genotype tissue expression (GTex) > 10 were also included.

Fifteen discordant individuals had at least one variant with a REVEL score > 0.5, three had variants in *RYR1*, including one likely pathogenic variant, and one had two variants in *CACNA1S*, both with REVEL scores > 0.9. The resulting gene list was uploaded to the Enrichr[1] web tool to produce a ranked list of diseases associated with these genes. These disorders may allude to several pathways that could be investigated further to expand the genetic testing offered to MH patients.

| Genes (no. of families with variants in this gene) | ClinVar 2019 disease terms |
|---|---|
| *RYR1*(3)*; *COL6A2*(2); *COL6A1*(1) | congenital muscular dystrophy |
| *DES* (1); *CAV3*(1); *SDHA* (1)* | cardiomyopathy |
| *DGUOK* (1); *SCN4A* (3); *SDHA* (1)* | mitochondrial diseases |
| *SCN4A* (3); *SDHA* (1)* | Leigh syndrome |
| *GAA* (3); *PHKA1*(1) | glycogen storage disease |
| *DES* (1); *CAV3*(1) | limb-girdle muscular dystrophy |
| *DES* (1); *SDHA* (1) | primary dilated cardiomyopathy |
| *NEB* (3) | nemaline myopathy |

* ?compound heterozygous variant

### Reference

1. Chen EY, Schmidt B, Maskell DL. A hybrid short read mapping accelerator. BMC Bioinformatics. 2013;128(14). https://doi.org/10.1186/1471-2105-14-67

## Referrals to the Melbourne Hospital MH diagnostic Royal unit

**Gillies RL, Bjorksten A, Hockey B, Surah A**

*Malignant Hyperthermia Diagnostic Unit, Royal Melbourne Hospital, Australia*

The work of our MH investigation unit has progressed over the last 24 years. Changes are reflected in the characteristics of the clinical scenarios investigated, the format of referrals, referrals from neuromuscular specialist colleagues and the increasing use of genetic screening for other disorders. The authors present a summary of referral characteristics for patients including emerging patterns and reflect on the importance of collective understanding and joint decision making with expert genetic colleagues.

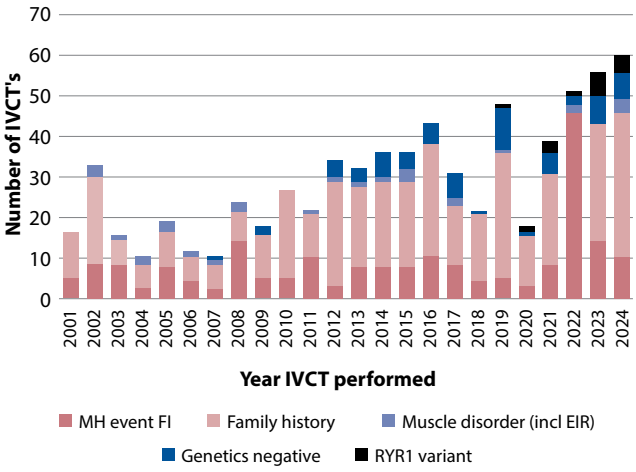



**Figure 1:** Reasons for performing IVCT

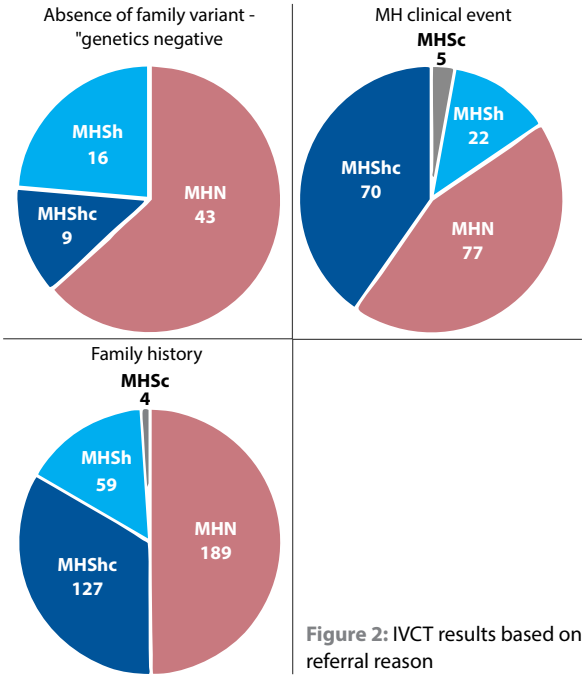
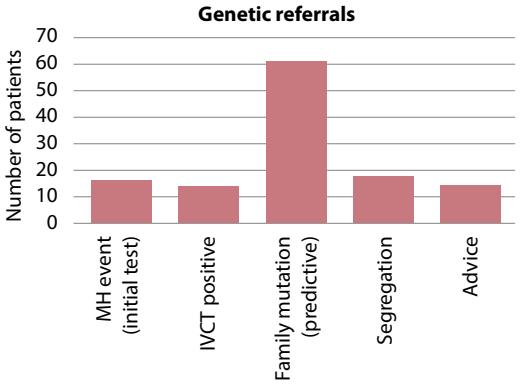



**Figure 2:** IVCT results based on referral reason



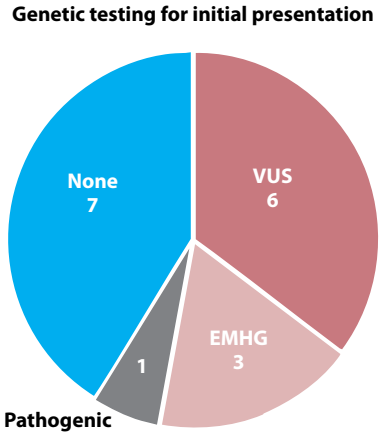




**Figure 3:** Genetic counselling

Formal genetic clinic with counselling has been offered in our unit since 2020. In that time, we have processed 152 referrals (with another 70 sent on to other genetic units' interstate). Seventeen patients underwent genetic testing to investigate an initial presentation.

| Variant | IVCT | Classification |
|---|---|---|
| RYR1 c.640A>G p.Thr214Ala | MHShc | VUS |
| RYR1 c.4178A>G p.Lys1393Arg | MHN | VUS |
| CACNA1S c.2979C>A p.Ser993Arg | MHN | VUS |
| CACNA1S c.4060A>T p.Thr1354Ile | MHSh | VUS |
| RYR1 c.742>A p.Gly248Arg | | EMHG |
| RYR1 c.14497C>T p.His4833Tyr | | EMHG |
| RYR1 c.529C>T p.Arg177Cys | | Pathogenic |
| RYR1 c.1840C>T p.Arg614Cys | | EMHG |
| RYR1 c.7204C>T p.Arg2402Trp plus TRPV1 c.2165G>A p.Arg722His | | VUS |
| RYR1 c.8773G>A p.Glu2925Lys, c.9970G>T p.Val3324Phe | | VUS |

**Figure 4:** Genetic testing for initial presentation

IVCT's were performed on 16 patients where an *RYR* variant was reported as an incidental finding often as part of a workup for non-specific muscle symptoms.

| | |
|---|---|
| c.5989G>A (p.Glu1997Lys) | MHN |
| c.6575A>G (p.Tyr2192Cys) | MHN |
| c.4217C>T (p.Thr1406Met) | MHN |
| c.4038C>A (p.Asn1346Lys) | MHN |
| c.11132C>T (p.Thr3711Met) | MHN |
| c.2461C>T (p.HIS821Asn), c.8071T>A (p.Tyr2691Asn) | MHN |
| c.2654G>A (p.Glu879Lys) | MHN |
| c.2654G>A (p.Arg885His) | MHN |
| c.11132C>T (p.Thr3711Met) | MHSc |
| c.9571G>C (p.Gly3191Arg) | MHSh |
| c.6961A>G (p.Ile2321Val) | MHSh |
| c.7025A>G (Asn 2342Ser) | MHSh |
| c.2654G>A (p.Arg885His) | MHSh |
| c,11543G>A (p.Arg3848Lys) | MHSh |
| c.4038C>A (p.Asn1346Lys) | MHSh |
| c.6650G>A (p.Gly2217Asp) | MHSh |

**Figure 5:** IVCT's on *RYR1* variants reported as incidental findings

## Influence of genetic testing on IVCT results – a retrospective analysis

**Lother B,[1] Pichler A,[1] Helf D,[1] Skirde J,[1] Jelting Y,[2] Johannsen S[1]**

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*[2] Institute of Human Genetics, Würzburg University, Germany*

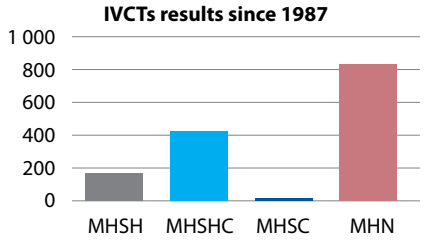
**Introduction:** Based on the subjective impression that the number of $MHS_H$ diagnoses at the Wuerzburg centre for malignant hyperthermia has been markedly increasing in recent years despite an unchanged IVCT protocol,[1] representing an early and successful example of stratified medicine. In 2001, our group also published a guideline for the use of DNA-based screening of malignant hyperthermia susceptibility. We now present an updated and complete guideline for the diagnostic pathway for patients potentially at increased risk of developing malignant hyperthermia. We introduce the new guideline with a narrative commentary that describes its development, the changes to previously published protocols and guidelines, and new sections, including recommendations for patient referral criteria and clinical interpretation of laboratory findings.","container-title":"Br J Anaesth","DOI":"10.1093/bja/aev225","ISSN":"1471-6771 (Electronic a systematic re-evaluation of the test results was the aim of this study. The focus was on potential changes in patient characteristics and testing indications over time, as well as the evolving role of human genetic testing in this context.

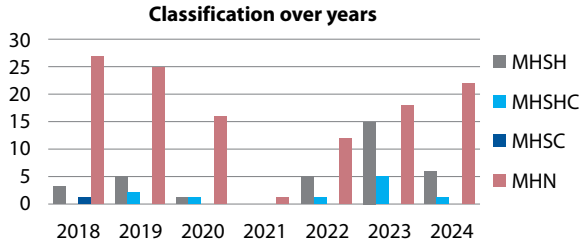
**Methods:** Results of all IVCTs of our unit since its foundation in 1987 were anonymously exported from our local data storage system and analysed retrospectively. Sets with incomplete or inconsistent data were excluded. Results from 2018 to 2024 were split into subgroups according to IVCT results ($MHS_{HC}$, $MHS_H$, $MHS_C$ and MHN). In the groups, data of genetic analysis if available were related to IVCT results.

**Results:** Of the 166 data sets 10 (6%) were classified $MHS_{HC}$, 35 (21%) $MHS_H$, 1 (1%) $MHS_C$ and 120 (72%) MHN. 52% of the $MHS_{HC}$ group and 7% of the $MHS_H$ group were associated with

a pathogenic or likely pathogenic variant. Contractures of less than 1 mN above threshold were observed in 25% of the $MHS_H$ muscle bundles compared to only 3% in the $MHS_{HC}$ group.

**Discussion:** The number of IVCTs performed between 2018 to 2020 and 2022 to 2024 was similar. However, in the first period, the average percentage of $MHS_H$ results was 11% while it was 30% in the later period. One contributing factor was the number of performed genetic investigations. In the recent years from 2022 to 2024 each eligible patient was offered genetic testing before performing IVCT. With a much higher percentage of pathogenic variants in the $MHS_{HC}$ population a selection bias towards more $MHS_H$ diagnoses on IVCTs is generated. Additionally, the higher rate of borderline results compared to the $MHS_{HC}$ may reflect a certain degree of false positives.

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# Metabolomic and lipidomic fingerprints of malignant hyperthermia susceptibility in human skeletal muscle

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*[6] Department of Chemistry, Middle East Technical University, Turkey*
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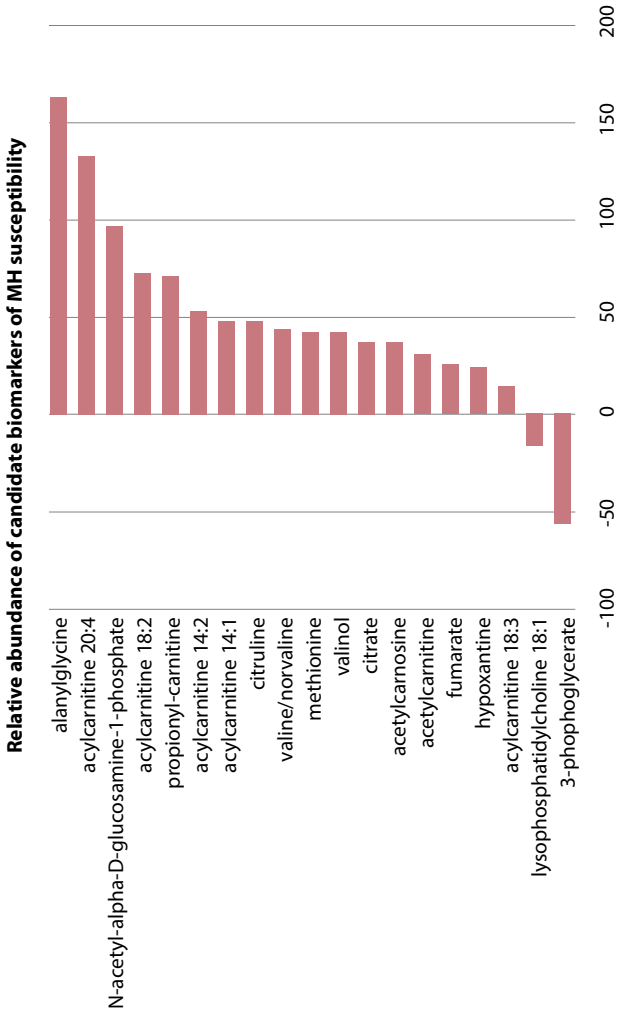
*[9] Hospital Universitari i Politécnic la Fe, Biomedical Research Institute la Fe, Spain*

**Introduction:** We aim to develop a minimally invasive test for diagnosing malignant hyperthermia susceptibility (MHS) by identifying its biochemical fingerprints in human skeletal muscle using metabolomics.

**Methods:** Frozen muscle samples from MHS and MHN patients were analysed at CEMBIO (Madrid) after exposure to caffeine or halothane. Lipidomics were conducted at Koellensperger's Lab (Vienna) using freshly harvested muscle from patients undergoing contracture testing. Metabolites with significant fold-changes between MHS and MHN were ranked by effect size. Adsorption kinetics of various extractive phases were assessed for optimal extraction time of candidate biomarkers by solid phase microextraction (SPME) in vivo.

**Results:** Metabolomics of biobank muscle (36 MHS, 48 MHN) revealed significant fold-changes in amino acids, oligopeptides, citrate, fumarate, hypoxanthine, and long-chain acylcarnitines, all upregulated in MHS. Lipidomics of fresh muscle (7 MHS, 7 MHSh, 11 MHN) showed elevated long-chain unsaturated



acylcarnitines, triglycerides, cholesterol ester, phospholipids, and plasmalogens in MHS, while cardiolipin and shorter-chain lipids were downregulated. Differences between MHS and MHN were evident before pharmacological challenges. SPME probes dip-coated with hydrophilic lipophilic balanced particles enabled optimal biomarker adsorption within 15 minutes.

**Conclusion:** Metabolomics and lipidomics profiles distinctive of MHS and MHN skeletal muscle will enable shortlisting candidate biomarkers as actionable targets to diagnose MH susceptibility using a metabolite risk score.[1]

Funding – Research Grant from the Canadian Institute of Health Research (CIHR) through the European Joint Programme on Rare Diseases (EJP RD) Joint Transnational Call 2022 for Rare Diseases.

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## Cost-efficiency of genetic screening for malignant hyperthermia susceptibility

**Cezar Souza,[1] Carlos A Ibarra Moreno,[1] Leslie Biesecker,[2] Lusine Abrahamyan,[3] Sheila Riazi[1]**

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**Introduction:** Malignant hyperthermia susceptibility (MHS) is a pharmacogenetic condition requiring an invasive and costly muscle biopsy for diagnostic confirmation by contracture testing. Genetic screening to diagnose MHS is a non-invasive alternative that offers wider testing accessibility at reduced costs. We evaluated the cost-consequence of cascade genetic screening as fist-line test for MHS diagnosis compared to the reference standard muscle biopsy contracture testing.

**Methods:** We conducted a retrospective cost-consequence analysis from January 2020 to December 2024 at the University Health Network, Toronto. Patients referred due to suspicion of MHS were offered genetic screening by DNA analysis of *RYR1* and *CACNA1S* genes at the initial clinical encounter. Cascade genetic testing was offered to the first- and second-degree relatives of those who harboured a genetic variant diagnostic for MHS. We compared the costs of initial genetic screening and cascade testing against those that would have accrued if the index case and their relatives had undergone contracture testing in first place, accounting also for ancillary expenses such as travel costs and lost wages.

**Results:** Genetic variants diagnostic for MHS were detected in 50 (8.1%) out of 613 patients screened. The total healthcare costs of cascade genetic screening for MHS in index cases and their first- and second-degree relatives were $6 572 000 CAD, compared to $9 420 000 CAD that would have resulted from performing muscle biopsy as first-line testing strategy. When considering indirect costs, the cascade genetic screening strategy resulted in substantial financial societal savings exceeding $5 million CAD.

**Conclusion:** Cascade genetic testing as a first-line screening tool significantly reduces healthcare and societal costs compared to muscle biopsy-based diagnostics for MHS. Furthermore, non-invasive genetic screening evokes minimal patient aversion, bolstering test accessibility and diagnostic uptake of patients with suspected MHS and their relatives.

## Use of calcium to treat hyperkalemia during a malignant hyperthermia crisis: review of international recommendations

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*[2] SUNY at Buffalo, USA*

**Introduction:** Hyperkalaemia may be experienced during an MH crisis and recommended treatment varies among international experts. Though the mechanism of action is poorly understood, calcium successfully mitigates this electrolyte abnormality. MH crisis results from uncontrolled sarcoplasmic Ca2+ release in susceptible patients.[1] An Influx of extracellular calcium contributes to the calcium overload of the myoplasm, contributing to the crisis.[2] A recent study demonstrates that calcium treatment for hyperkalaemia works through calcium dependent myocyte conduction propagation.[3] Does use of calcium contribute to severity of the MH crisis or recrudescence?

**Methods:** We reviewed and documented variation in the most recent international recommendations for management of hyperkalaemia during the MH crisis.

**Results:**

Hyperkalaemia Management During MH Crisis (all accessed 2/15/25)

| www.MHAUS.org<br>United States | www.EMHG.org<br>Europe | www.malignanthyperthermeia.org.au<br>Australia/New Zealand | www.anaesthetists.org<br>www.ukmhregistry.org<br>United Kingdom |
|---|---|---|---|
| • **CaCl2 or Ca gluconate**<br>• NaHCO3<br>• glucose/insulin<br>• B2 agonist<br>• dialysis/ECMO | • glucose/insulin<br>• **CaCl2 or Ca gluconate**<br>• B2 agonist<br>• dialysis | • hyperventilation<br>• glucose/insulin<br>• **CaCl2 or Ca gluconate** | • NaHCO3<br>• glucose/insulin<br>(spec. ped infusion notes)<br>• **Calcium- in extremis** |

**Conclusion:** Studies suggest use of calcium for treatment of hyperkalemia during MH crisis should be considered after other

strategies are inadequate or failing. International expert review will unify current recommendations, improve patient care.

### *References*


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## Scoliosis and malignant hyperthermia


**Andréa O Gomes,[1] Leonardo Tsuji,[2] Joilson M Santos,[1] Pamela V Andrade,[1] Adham A Castro,[2] Andre Y Aihara,[2] Helga CA Silva[1]**

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*[2] Department of Diagnostic Imaging, Federal University of São Paulo, Brazil*


**Introduction:** Malignant hyperthermia (MH) is a pharmacogenetic disorder characterised by uncontrolled hypermetabolism following exposure to halogenated anaesthetics and/or succinylcholine in genetically susceptible individuals. Variants in the **RYR1** gene are also associated with various congenital myopathies, characterised by dysmorphisms and muscle weakness. Scoliosis is frequently observed in patients with several myopathies affecting axial musculature, such as those linked to **RYR1** gene variants.[1,2] The progressive weakness can result in spinal misalignment, favouring the development of progressive curvatures.

**Objective:** To investigate the association between scoliosis and MH susceptibility.

**Methods:** The Institutional Research Ethics Committee approved this research and all participants provided their written informed consent. Participants included all patients referred for investigation due to personal/family history of MH crisis. The investigation included clinical/neurological evaluation, vastus lateralis muscle biopsy with histochemistry, in vitro contracture test (IVCT), molecular analysis (whole exome sequencing), and radiological studies of the spine. Spinal radiographs were analysed to measure the Cobb angle, a standard radiographic method used to quantify spinal curvature and determine the presence of scoliosis. The measurements followed a standardised protocol to ensure accuracy and consistency. Cobb angle values were classified as negative (< 10°) or positive (≥ 10°), indicating the absence or presence of significant scoliosis, respectively.

**Results:** We have already enrolled 39 patients (23 MH-susceptible-MHS and 16 MH-negative-MHN). A significant variation in Cobb angle values was observed among 14 patients (7 MHN/7 MHS) analysed so far, with a predominance of lower angles. One MHN (14%) and three MHS (42,8%) patients had Cobb angles greater than 10°, classified as positive, while the majority had values below this threshold.

**Conclusion:** Although there was not a significant difference in the frequency of scoliosis between the MHS/MHN groups, the frequency in both groups was higher than in normal Brazilian samples.[3] A direct relationship between scoliosis and MH susceptibility has not yet been fully established, but the presence of scoliosis in individuals with RYR1-associated myopathies may suggest an increased risk in MHS individuals.

*Funding: CAPES, FAPESP

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## Genetic characteristics of Brazilian MH patients


**Mariz Vainzof,[1] Brandow Willy Souza,[1] Lucas S Souza,[1] Antonio FR Junior,[1] Felipe TGR Vasconcelos,[1] Joilson M Santos,[2] Pamela V Andrade,[2] Helga CA Silva[2]**

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*[2] Brazilian Malignant Hyperthermia Unit, Department of Anesthesiology, Pain and Intensive Care, Federal University of São Paulo, Brazil*


**Introduction:** Malignant hyperthermia (MH) is a pharmacogenetic hypermetabolic syndrome expressed as an acute crisis after exposure of susceptible individuals (MHS) to halogenated agents/succinylcholine. MH crisis prevalence is variable, from 1:10 000 in children to 1:50 000 in adults. MH susceptibility has been associated with genes codifying proteins of the excitation-contraction complex of the skeletal muscle, with a frequency of 1:217–1:2 750 in the general population.[1] Most families present variants in the *RYR1* gene (50–60%). Rare families present variants in the *CACNA1S* (1%), *STAC3* (< 1%), or *ASPH* genes.[1,2]

**Objective:** To present genetic characteristics of an MH Brazilian sample.

**Methods:** The Institutional Research Ethics Committee approved this research and all participants provided their written informed consent. The investigation included vastus lateralis muscle biopsy with histochemistry, in vitro contracture test (IVCT), and molecular analysis (whole exome sequencing).

**Results:** From 1997 to 2024, 349 families were referred to evaluation in our MH unit and 166 of them were already investigated for MH susceptibility with the IVCT and/or molecular analysis (107 positive families, 59 MHN families). Among the positive families, 83 had a personal/family history of MH crisis. A whole exome molecular analysis was performed in 61 of these

positive MH families (30 probands and 31 relatives). No variants were found in 21 families (34.4%), while variants in genes related to MH were found in 40 (65.5%) families. Variants in the *RYR1* gene were found in 37 families (60.6%). *RYR1* gene variants were predominantly pathogenic (P: at least 14 families), followed by variants of uncertain significance (VUS: 10), probably pathogenic (PP: 9), benign (B: 3), and probably benign (PB: 1). More than one variant in the *RYR1* were found in five individuals. Two patients with variants in the *RYR1* gene had concomitant variants in the *CLCN1* and *DMD* genes. Variants in the *CACNA1S* gene were found in three families (4.8%), all of them with concomitant variants in the *RYR1* gene (*RYR1/CACNA1S*: VUS/PB, PP/PP, P/PP respectively). Three families presented variants in the *STAC3* gene (4.8%). The *STAC3* variant was the same in all the three unrelated non-consanguineous Afro-Brazilian families (c.851G>C: p. Trp284Ser), present in homozygous state in the probands.[3] No variants in the *ASPH* gene were found.

**Discussion:** In this preliminary Brazilian sample, the frequency of *RYR1* gene variants was similar to previous reports in other countries, but there was a higher frequency of *STAC3/CACNA1S* variants.

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## *STAC3* disorder: a common cause of congenital myopathy in Southern African patients

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The spread of this variant worldwide and the allele frequency higher in the African/African-America ancestry than the Admixed Americans, strongly indicates that the *STAC3* c. 851 G>C (p.Trp284Ser) variant has an African origin which may be due to an ancient mutation with migration and population bottlenecks.

*STAC3* disorder, previously known as Native American myopathy, is characterised by congenital myopathy, hypotonia, musculoskeletal and palatal anomalies and susceptibility to malignant hyperthermia. Essop et al. report on the frequency of *STAC3* c.851 G>C in a cohort of 127 patients presenting with congenital hypotonia that tested negative for spinal muscular atrophy and/or Prader-Willie syndrome. They present a clinical retrospective, descriptive review of 31 Southern African patients homozygous for *STAC3* c.851 G>C. A carrier rate of 1/56 and a predicted birth rate of 1/12 500 were estimated from a healthy cohort. A common haplotype spanning *STAC3* was identified in four patients. Of the clinical group, 93% had a palatal abnormality, 52% a spinal anomaly, 59% had talipes equinovarus multiplex congenita and 22% had a history of malignant hyperthermia.

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## Evaluation of genetic variants in the *RYR1, CACNA1S* and *STAC3* genes to estimate the genetic prevalence of malignant hyperthermia with data from the Leipzig institute of human genetics, Update 2025

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**Background:** MH is thought to be a rare disorder and its exact genetic disease prevalence is unknown. The estimated prevalence of MH-associated genetic variants ranges from 1:3 000 to 1: 839. Clinical MH episodes are even less frequent presumably due to incomplete penetrance. The objective of the presented study was to analyse the frequency of genetic variants in three MH genes within a non-MH related cohort. In Brno 2024, we already presented the first results. Since then, we have refined our analysis, updated the variants and enhanced the database.

**Methods:** After approval from the local ethics committee, NGS exome datasets of 13 692 individuals were screened for variants in the *RYR1*, *CACNA1S* and *STAC3* genes. The cohort consisted of patients and individuals who were genetically analysed at the institute of human genetics, University Hospital Leipzig, due to a broad spectrum of questions and consultation reasons, but not regarding MH. Only independent individuals were investigated. Variants with an allele frequency > 0.1% were excluded. Synonymous variants and artifacts were also excluded, with a focus on missense variants, nonsense variants, deletions and insertions. All variants were annotated and classified according to the ACMG and EMHG criteria.

**Results:** After analysing 13 692 datasets, we detected 691 *RYR1*, 315 *CACNA1S* and 51 *STAC3* variants.

According to ACMG: *RYR1*: 3 pathogen (P) variants in 14 individuals, 11 likely pathogen (LP) variants in 20 individuals. *CACNA1S*: 2 pathogen variants in two individuals, two likely pathogen variants in two individuals. *STAC3*: 1 pathogen variant in three individuals, associated to Native American Myopathy, not MH. In total, 18 P or LP variants in 38 unrelated individuals out of 13 692. Genetic prevalence is 1:360. When only considering the variants from the EMHG database, the prevalence decreases to 1:805. This rate drops to 1:1.141 when only considering the variants curated and annotated by VCEP for *RYR1*.

**Conclusion/Discussion:** This study demonstrates that the prevalence of pathogenic or likely pathogenic MH-associated

genetic variants is 3 to 10 times higher in a population seeking genetic counseling for reasons besides MH, compared to the general population. Furthermore, when focusing solely on the pathogenetic variants from the EMHG database, the prevalence aligns closely with that reported in other studies.

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## Rare insertion in the *RYR1* gene causing malignant hyperthermia in a familial case with clinical variability


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Variants in the *RYR1* gene, which encodes the ryanodine receptor type 1 (RyR1), are associated with several neuromuscular disorders, including malignant hyperthermia (MH), central core disease (CCD), multiminicore disease, King-Denborough syndrome, and exercise-induced rhabdomyolysis. The *RYR1* gene plays a crucial role in calcium release from the sarcoplasmic reticulum in skeletal muscle cells, essential for muscle contraction. Heterozygous variants in *RYR1 gene* are responsible for ~75 % of all MH susceptible (MHS) cases. About 700 *RYR1* variants have been identified to date,[1] but only 66 of these have been recognised as diagnostic by the European Malignant Hyperthermia Group (EMHG). Missense variants are largely the most common ones, while insertions and duplications are rarer, accounting for less than 10% of all *RYR1* mutations.

In a molecular study of 36 families with at least one patient with malignant hyperthermia crisis and/or MHS classification in the in vitro contracture test (IVCT), we identified one family with two affected siblings carrying an insertion of 18 pb in exon 91 of the *RYR1* gene, resulting in an in frame insertion of 6 amino acids (c.12828_12829insGAGGGCGCGGCGGGGCTC: p.G4284_T4285insAAGLEG). This insertion was found at a frequency of 0,0007% in gnomAD and was not present in normal 1200 Brazilian controls. This variant has been previously reported in ClinVar as VUS (rs1159068582). In our family, we were able to increase the odds of pathogenicity, even though it remains classified as VUS (PM2_sup, PM4, PS4_sup, PS3_sup).

The sister had an atypical reaction to anaesthesia. Muscle biopsy at age 18 showed positive IVCT (MHShc). Histopathological analysis identified type I fibre atrophy. During clinical evaluation, she presented club-foot, exercise intolerance, high-arched palate, proximal paresis of the four limbs, hyporeflexia, but normal creatine kinase (CK) level. Her brother underwent a muscle biopsy at age 25 due to the family history of MH. His histology was normal, but he had a positive IVCT (MHShc). Furthermore, he had club-foot, ptosis, kyphosis, high-arched palate, and CK levels increased by 20-fold. He reported that two of his three children also had ptosis and strabismus.

We studied the relative expression of *RYR1* in mRNA extracted from their muscle biopsies and identified a ~50% reduction in its expression, suggesting a hypomorphic allele. A study evaluating the role of this insertion in protein structure is ongoing.

The pathomechanisms of *RYR1* gene variants are diverse and can be categorised as gain-of-function, loss-of-function, and decreased expression of the *RYR1* gene.[2] Small insertions can often lead to loss of function or improper protein folding, especially considering that a recurrent duplication in a very close region in exon 91 (p.Thr4288_Ala4290dup) has been described in patients with HM and exertional rhabdomyolisis.[3] It will be very important to clarify the mechanism involved with this insertion in the MH phenotype. This study adds evidences to the association of this variant with the *RYR1*-related phenotype and the study of additional family members possibly will be able to reach its classification as probably pathogenic.

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## Malignant hyperthermia in Slavonic cohort – the "grey" zone of *RYR1* VUS


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**Introduction:** The genetic background of malignant hyperthermia (MH) was identified only in approximately half of MH-susceptible (MHS) patients, differing between

geographical areas. Variants recognised as being sufficiently functionally characterised to be used in diagnostics for MH are on the European Malignant Hyperthermia Group (EMHG) list of diagnostic variants. However, the list may be a bit biased in favour of better-described and published MHS cohorts.

**Methods:** We performed a retrospective analysis of clinical data and molecular genetic characteristics conducted on MH-risk patients referred during 20+ years of diagnostics at the Academic Centre for Malignant Hyperthermia of Masaryk University, Brno, Czechia.

**Results:** MH susceptibility was confirmed in 94 families. 79% of our MHS probands were diagnosed based on MH-related adverse anaesthesia complications in personal/family history. Diagnostic MH variants in the *RYR1* gene were detected in 48% of MHS patients. In 18% of MHS patients, 14 different VUS were detected in *RYR1* (Table I). In total, 11 of our VUS can be described as PPb PPc according to the EMHG Scoring Matrix, and most of them are unique to families. Three VUS were detected in more than one unrelated family: *c.1589G>A*, *c.1598G>A* and *c.6742C>T*. As the EMHG plans to introduce a list of "Variants unlikely to cause MH", which would be extremely helpful for clinical counselling, we also listed the VUS in *RYR1* with negative IVCT.

**Table I:** Variants of uncertain significance in the *RYR1* gene connected with clinical information and IVCT results

| ***RYR1* variant (NM_000540.2)** | **EMHG Scoring Matrix** | **Number of probands** | **Reason for referral** | **Adverse anaesthesia complications** | **IVCT** |
|---|---|---|---|---|---|
| c.49G>T p.(Asp17Tyr) | PPb | 1 | Myopathy | no | $MHS_{hc}$ |
| c.178G>A p.(Asp60Asn) | PPb PPc | 1 | Postoperative hyperthermia | yes | $MHS_{hc}$ |
| c.946C>T p.(Arg316Cys) | PPb PPc | 1 | MH crisis in family | yes | $MHS_{hc}$ |
| c.1589G>A p.(Arg530His) | PPb PPc | 2 | MH crisis – trismus | yes | $MHS_{h}$ |
| | | | MH crisis – typical | yes | $MHS_{hc}$ |
| c.1598G>A p.(Arg533His) | PPb PPc | 2 | Incidental finding in genetic testing | no | not performed |
| | | | MH crisis | yes | $MHS_{hc}$ |
| c.1762C>T p.(Leu588Phe) | PPb PPc | 1 | MH crisis, CCD | yes | $MHS_{h}$ |
| c.2198G>A p.(Gly733Glu) | BSa BPa | 1 | Myopathy | no | MHN |
| c.3257G>A (p.Arg1086His) | BSa | 1 | Incidental finding in genetic testing | no | MHN |
| c.6385G>A p.(Asp2129Asn) | PPb PPc | 1 | MH crisis | yes | $MHS_{h}$ |
| c.6742C>T p.(Arg2248Cys) | PPb PPc | 2 | MH crisis | yes | $MHS_{h}$ |
| | | | MH crisis in family | yes | $MHS_{h}$ |
| c.6863T>C p.(Leu2288Ser) | PPb PPc | 1 | MH crisis | yes | $MHS_{hc}$ |
| c.7035C>A p.(Ser2345Arg) | PPb PPc | 1 | MH death in family | yes | $MHS_{hc}$ |
| c.7087T>C p.(Cys2363Arg) | PPb PPc | 1 | MH death in family | yes | $MHS_{hc}$ |
| c.7210G>A p.(Glu2404Lys) | PPb PPc | 1 | Postoperative rhabdomyolysis | yes | $MHS_{hc}$ |
| c.7268T>A p.(Met2423Lys) | PPb | 2 | Myopathy | no | $MHS_{h}$ |
| | | | Incidental finding in genetic testing | no | MHN |
| c.7904A>T p.(Glu2635Val) | PPb BSa | 1 | Incidental finding in genetic testing | no | MHN |
| c.10648C>T p.(Arg3550Trp) | BPa | 1 | Incidental finding in genetic testing | no | $MHS_{h}$ |

**Discussion and conclusion:** Unfortunately, with our relatively small MHS cohort, none of our VUS met the strict criteria needed for a variant to be added to the EMHG list of diagnostic variants. This may change with the sharing of data among MH centres. Also, unsurprisingly, the interpretation of some variants is very problematic (e.g. *c.7268T>A*) and sharing knowledge of MH experts can be very beneficial for counselling.

**Declarations:** Some of the text and data have been previously submitted to BJA as part of a manuscript (currently under review). This research was supported by Specific University Research provided by MŠMT (MUNI/A/1733/2024, MUNI/A/1771/2024), and supported by MH CZ – DRO (FNBr, 65269705) and mainly supported by funds from AZV—Czech Health Research Council (NU21-06-00363).

## The natural course of a MHS patient with pathogenic *RYR1* variant evaluated with a cardiopulmonary exercise test

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**Introduction:** Malignant hyperthermia (MH) is an autosomal dominant trait. MH-susceptible (MHS) individuals develop hypermetabolic syndrome when exposed to volatile anaesthetics and/or depolarising neuromuscular blockers.[1,2] Additionally, some patients can develop hypermetabolic states during exercise. It has been postulated that these patients can develop myopathic features during adult life.

**Objective:** To analyse the natural evolution of an MHS individual by a cardiopulmonary exercise test (CPET).[3,4]

**Methods:** The Institutional Ethics Committee approved this research and all participants provided their written informed consent. The incremental CPET was performed in the Ultima CPX™ system (MedGraphics Corporation, St Paul, MN, USA) which controls the ergometer (Lode Corival, Netherlands). The 12-minute protocol was in ramp format, a load of 10W/min followed by a five-minute recovery period. Blood gas analyses were also conducted during the CPET.

**Results:** A 37-year-old female MHS patient with a pathogenic variant in the *RYR1* gene (*c.487C>T* or *p.Arg163Cys*) was submitted to a CPET in 2019 and 2025. The first CPET showed an aerobic capacity within normal limits; absence of cardiocirculatory, ventilatory, and gas exchange limitations during the CPET. A slightly elevated lactate at rest 25 mg/dl (reference value < 15 mg/dl) with a high lactate/workload ratio (Lac/W). The second CPET detected an aerobic capacity slightly reduced and signs of cardiocirculatory limitation (early lactate threshold-AT, tachycardic response pattern to metabolic demand, reduced oxygen pulse, plateau in the curve after the AT, and increased Lac/W ratio at the peak of exercise). There was an increased ventilatory response ($VE/VCO_2$) due to heightened ventilatory drive from rest (hypocapnia) and ventilatory inefficiency during exercise. There were no signs suggestive of ventilatory or gas exchange limitations for oxygen.

**Conclusion:** Over a relatively short period (six years), there was a decrease in physical performance. The CPET is an objective and reproducible test that can be used to detect subtle physiologic changes in MHS patients.

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## Unravelling diagnostic metabolic signatures of malignant hyperthermia: insights from porcine and human skeletal muscle

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Malignant hyperthermia (MH) is a life-threatening pharmacogenetic disorder triggered by volatile anaesthetics and muscle relaxants. Current diagnostic methods rely on highly invasive skeletal muscle contracture testing, hindering at-risk individuals from early detection.[1] Our study aims to develop a minimally invasive diagnostic approach employing solid phase microextraction (SPME) to isolate metabolite biomarkers from skeletal muscle.

We used a porcine model expressing the *RYR1 p.R615C* variant to replicate human MH physiology[2] and first constructed a comprehensive skeletal muscle atlas under basal conditions via untargeted metabolomics and lipidomics by CE-MS, HILIC-UHPLC-MS, and RP-UHPLC-MS in both positive and negative ion modes. This atlas establishes critical baseline profiles of metabolites and lipids within the porcine muscle, providing a valuable reference for recognising disease-specific perturbations. Then, biceps femoris muscle samples were collected in two phases: (P1) baseline, and (P2) after general systemic MH induction with inhaled sevoflurane (> 50% increase in baseline end-tidal $CO_2$) at defined intervals (0–40 min every five min) to capture acute biochemical changes. Notably, untargeted analysis revealed marked increases in amino acids and energy metabolites, indicating heightened energy turnover and oxidative stress. Small peptides and vasoregulatory molecules also exhibited significant shifts, highlighting potential disruptions in muscle signalling pathways. In addition, elevated carnosine also indicated adaptive pH regulation under extreme metabolic stress. These findings were corroborated in human muscle samples obtained from MH-negative ($n$ = 49) and MH-positive ($n$ = 41) patients following contracture testing. In line with the porcine model, MH-positive patients exhibited increased alanylglycine and carnosine levels. Additionally, acylcarnitines

emerged as significant markers in humans, highlighting the translational relevance of these observations.

By integrating results from both porcine and human studies, we identified promising biomarkers to guide SPME fibre coating design for a minimally invasive MH test. The development of the skeletal muscle atlas proved pivotal in distinguishing basal metabolic profiles from MH-induced perturbations, enabling a clear definition of clinically relevant biomarkers. This innovative approach could reduce the use of invasive biopsy, facilitate MH screening, and enhance patient safety and care.

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## Lightning struck twice in the same place: Mitochondrial myopathy and malignant hyperthermia – case report

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**Introduction:** Mitochondrial diseases comprise a specific group of syndromes characterised by defects in energy metabolism, leading to impaired energy production and aerobic metabolism, particularly affecting high-energy-demand tissues. Malignant hyperthermia (MH) is a pharmacogenetic disorder expressed as a hypermetabolic reaction, triggered in MH-susceptible (MHS) patients by halogenated agents and succinylcholine, due to excessive intramuscular calcium release. Current literature demonstrates that patients with mitochondrial myopathies are not considered to have a higher risk of developing MH than the general population. Both groups belong to the larger category of rare diseases, which often present multiple clinical challenges in anaesthetic management. Our objective was to report a fatal atypical reaction to anaesthesia in a family where we identified a mitochondrial dysfunction in the mother of the proband, a *RYR1* gene variant (VUS) in his father, and a history of exercise-induced rhabdomyolysis in his paternal first cousin.

**Case details:** A 2-year-old boy, ASA 1, underwent an adenoidectomy and bilateral tympanostomy. He presented previous diagnosis of ptosis, strabismus, cleft palate, pectus carinatum, and cryptorchidism. Anaesthesia was induced with sevoflurane and nitrous oxide. After 80 minutes, he developed rigidity, hyperthermia of 43 °C (peaking at 45 °C), and a non-specified arrhythmia. MH was suspected, sevoflurane was discontinued and total intravenous anaesthesia was performed. Management included hyperventilating with 100% oxygen, active cooling, dantrolene (16 mg/kg), and supportive medications, but the patient developed four successive cardiac arrests and died. Blood gas analysis showed severe acidosis, hyperkalaemia, hyponatraemia, and hyperlactataemia. The post-mortem exam found no specific pathology, and DNA samples were not collected. His mother had ptosis, micrognathia, exercise intolerance, myalgia, high CK levels (218IU/L – normal range up to 170 IU/L) and muscle biopsy indicating a mitochondrial myopathy. Her IVCT was negative for MH. The father carried a variant in the *RYR1* gene (NM_000540/ NM_001042723: exon 51: c.G8197T:p.G2733C), which was absent in all databases and was classified as *likely pathogenic* according to the ACMG criteria and as a *variant of uncertain significance (VUS)* by the ClinGen MHS Variant Curation Expert Panel. A male paternal cousin presented exercise intolerance, myalgia, myoglobinuria, high CK levels, and the same genetic variant.

**Conclusion:** This report demonstrates that different diseases can coexist within the same family, leading to atypical adverse reactions, and increasing the complexity of both the anaesthetic management and clinical investigation. The index patient exhibited multiple dysmorphic features and his atypical reaction to anaesthesia may have been related to an underlying subclinical myopathy, possibly resulting from mixed inheritance from both parents. In atypical anaesthetic reactions, a negative MH investigation raises the possibility of other conditions that may cause complications during anaesthesia. This report highlights the need for comprehensive genetic and clinical evaluations to manage complex phenotypes in families with rare diseases and atypical anaesthetic responses, emphasising multidisciplinary approach in overlapping conditions, from pre-anaesthetic planning to postoperative care, ensuring the best perioperative outcome.

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## Risk factors for adverse events in anaesthesia for patients with mitochondrial diseases: a scoping review

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**Introduction:** To map the available scientific data on types of anaesthesia performed in patients with established mitochondrial disease and to investigate the relationship between mitochondrial disease subtype, demographic data, anaesthesia type, surgical intervention, and the occurrence of adverse events (AEs).

**Methods:** We conducted a scoping review of articles in all languages using Cochrane Library (1995–2022), Embase (1947–2022), LILACS (1982–2022), SciELO/Web of Science (2002–2022), PUBMED/MEDLINE e SCOPUS (1960–2022) databases. The search terms included "mitochondrial myopathy", or "mitochondrial disease", or "mitochondrial defect" or "Leigh Syndrome", or "MERRF syndrome", or "MELAS syndrome", or "Kearns Sayre syndrome" (KSS), or "mitochondrial", and "anaesthesia". Additional articles were identified through reference list searches. We selected articles describing patients with a confirmed diagnosis of mitochondrial disease who underwent any type of anaesthesia and documented postoperative care. Data extraction from the articles was performed independently by three authors using predefined data fields.

**Results:** AEs occurred in 3.2% of anaesthetic procedures in large case series (30 patients or more) and in 26% of individual case reports reviewed. Reported AEs included acute metabolic decompensation with lactic acidosis and/or electrolytic dirturbances, acute dysfunction of cardiac or cerebral tissues, ventilatory insufficiency, hyperthermia, muscle weakness/rigidity, and atypical response to non-depolarising neuromuscular blockade. There was no correlation between AEs and age, sex, ASA physical status classification, type of surgery, or specific mitochondrial disease classification. However, AEs were associated with emergency/urgency surgical procedures, and the use of some drugs (opioids and non-depolarising neuromuscular blockers). The data analysed did not support a higher risk of malignant hyperthermia in patients with mitochondrial diseases.

**Conclusions:** The available data from case reports and series demonstrate that all types of anaesthesia have been used in patients with mitochondrial disease. The occurrence of AEs was associated with the use of opioids and non-depolarising neuromuscular blockers, and emergency surgeries, which may be considered risk factors for decompensation in this population. Specific precautions are necessary throughout the perioperative period, from pre-anaesthetic evaluation to postoperative care.

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## Activated charcoal filters for fast elimination of inspiratory sevoflurane during paediatric anaesthesia – preliminary data of a prospective interventional study

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**Introduction:** In acute malignant hyperthermia (MH) crises both EMHG and MHAUS recommend using activated charcoal filters (ACF) for fast elimination of the triggering volatile anaesthetic (e.g. sevoflurane). However, these filters were originally developed for decontaminating anaesthesia machines in preparation for trigger free anaesthesia. But in an acute MH crisis the patient will exhale far more volatile anaesthetic compared to a contaminated anaesthetic machine. There is only one laboratory study testing ACF in the acute MH setting using an olive oil flask to simulate the patient.[1] Therein, the adsorption capacity of the ACF was exceeded within 67 min after isoflurane and 83 min after sevoflurane exposure. Therefore, MHAUS recommend changing ACF after 60 min when used during an acute MH. However, there are studies needed to evaluate the use case of ACF in acute MH.

**Methods:** We conducted a prospective single centre interventional nonrandomised pilot study in which MH negative paediatric patients received mask induction with sevoflurane followed by a transition to total intravenous anaesthesia during elective surgery. In the test group ($n$ = 9) ACF were attached to both the inspiratory and exspiratory limp of the breathing hose as soon as the sevoflurane vapour was turned off. Inspiratory sevoflurane concentration was measured for a total of 60 minutes using ion mobility spectrometry with gas chromatographic preseparation. In the control group ($n$ = 10) no ACF were attached. In both groups a fresh gas flow of 10 $L \cdot min^{-1}$ was maintained. Primary outcomes were time to < 5 ppm of inspiratory sevoflurane and any occurrence of rebounds > 5 ppm of inspiratory sevoflurane.

**Results:** In our preliminary data the patients showed no significant differences in weight (13.6 ± 2.5 vs. 13.8 ± 4.6 kg) and age (2 ± 1 vs. 2 ± 1 years) between the groups. Reduction of inspiratory sevoflurane to < 5 ppm was faster in the ACF group (2.7 ± 3.5 min) compared to the control group with only two cases reached inspiratory sevoflurane concentrations of < 5 ppm after 60 min. No rebound to > 5 ppm sevoflurane was observed in this study.

**Conclusions:** ACF show an effective elimination of inspiratory and expiratory sevoflurane with no signs of saturated charcoal capacity. Therefore, ACF should be considered safe during the first 60 minutes of a paediatric MH crisis. However, this study

cannot draw conclusions regarding the effectiveness or safety of ACF in an adult MH crisis.

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## Optimising TIVA/TCI delivery through processed EEG monitoring: clinical principles and pitfalls

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Processed electroencephalographic (EEG) monitoring has become an increasingly valuable tool for guiding total intravenous anaesthesia (TIVA) and target-controlled infusion (TCI), particularly with propofol-based regimens. While the bispectral index (BIS) remains the most widely used commercial device, a growing body of evidence supports the importance of understanding the underlying EEG patterns that inform these indices. This talk reviewed practical EEG features relevant to anaesthetic depth and described strategies for real-time EEG-guided titration of hypnotic agents.

Key EEG signatures, including alpha-delta coupling, frontal alpha oscillations, and burst suppression, were explored in relation to pharmacokinetic-pharmacodynamic interactions during TIVA. Special attention was given to the dynamic nature of EEG under different anaesthetic states and the influence of patient-specific factors such as age, comorbidity, and surgical stimulation. Examples were presented where EEG patterns diverged from BIS numerical trends, emphasising the limitations of relying on index values alone. Additionally, the presentation highlighted common pitfalls, including artefact misinterpretation, electromyography contamination, and age-related changes in EEG expression, which can confound depth assessment.

Through clinical vignettes and real-time EEG trace reviews, the talk aimed to familiarise anaesthetists with practical interpretation of raw and density spectral array data to enhance TIVA/TCI delivery. The conclusion reinforced that processed EEG should be used as an adjunctive tool—interpreted within the clinical context—to refine individualised anaesthesia, improve patient safety, and potentially mitigate the risk of intraoperative awareness.

## EMQN best practice guidelines for genetic testing and reporting in *RYR1*-related disorders

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The '*EMQN Best Practice Guidelines for Genetic Testing and Reporting in RYR1-related disorders'* are designed with the purpose to aid clinical genetic laboratories in testing, and unequivocal and comprehensive reporting of *RYR1* variants for the benefit of patients and their family members. These guidelines are supported by experts in the field of anaesthesia, (paediatric) neurology, clinical genetics and clinical laboratory genetics.

The ryanodine receptor type 1 is a large receptor and calcium channel that regulates calcium release from the sarcoplasmic reticulum resulting in muscle contraction. This receptor is encoded by the *RYR1* gene and expressed predominantly in skeletal muscle. Pathogenic *RYR1* variants can cause several allelic disorders: malignant hyperthermia, a hypermetabolic reaction to certain anaesthetics, in otherwise healthy individuals, as well as both autosomal dominant and recessive congenital myopathies. In general, *RYR1* gain-of-function variants are associated with malignant hyperthermia susceptibility, whereas dominant-negative and loss-of-function variants are associated with dominant and recessive myopathies, respectively. However, a small subset of *RYR1* variants is associated with a combination of dominant malignant hyperthermia susceptibility with either a dominant or a recessive myopathy. The apparent discrepancy between molecular mechanisms and different phenotypes is currently poorly understood.

As a consequence, the context-dependent interpretation of *RYR1* variants is challenging in diagnostic genetic testing. In particular, it is not trivial to assign a possible associated risk for an allelic disorder for an individual or family members (i.e. the risk of malignant hyperthermia susceptibility in families with a myopathy and *vice versa*).

## Patients' perceptions of muscle biopsy and malignant hyperthermia investigation, from referral to diagnosis – a quantitative survey study

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**Introduction:** Although malignant hyperthermia investigations, IVCTs, have been conducted since the 1980s, database research reveals that there are no published systematic evaluations of how patients experience the entire investigation process. By examining patients' experiences with the MH investigation – before, during, and after the muscle biopsy – a significant knowledge gap could be addressed, thereby enhancing the patient-centred nature of the entire investigative process from both a medical and care perspective. Person-centred care is a holistic approach based on three core principles: the relationship between patient and caregiver, the context of care, and the needs of the patient. Understanding past patients' experiences is crucial to ensure that future MH patients receive care that aligns with this holistic approach.

**Aim:** To investigate adult patients' perceptions of muscle biopsy and the malignant hyperthymia investigation, from referral to diagnosis, during the years from 2018 to 2024 at the Swedish Malignant Hyperthermia unit.

**Method:** A quantitative survey study with an inductive approach is planned at the Swedish malignant hyperthermia unit. Research participants will be identified via the MH unit's database of patients who have undergone MH investigation. After obtaining ethical approval from the Swedish Ethical Review Authority, a questionnaire will be sent to all patients 18 years or older at the time of their muscle biopsy investigation at the MH unit in Lund between 2018 and 2024.

The surveys will be compiled, and the data will be analysed using descriptive and comparative statistics. If possible, a linear regression analysis will be conducted to identify relationships between explanatory variables and response variables. Responses will be compiled with the help of Data Analysis and Register Centre (DARC) at the Department of Health and Medical Governance, CPUA Representative for Region Skåne.

**Result and Conclusion:** None yet. According to the time plan data collection will take place between May and August 2025, and a result is expected to be presented in a master's thesis in December. The purpose of the study is, by the investigation of adult patients' perceptions of muscle biopsy and the malignant hyperthymia investigation, to identify potential areas for improvement in the process. Highlighting these areas is expected to enhance the quality of the investigation process. By systematically investigating patients' experiences the unit aims to improve and personalise the care provided.

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## *MYH1* variants in MH susceptible individuals


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**Background:** *MYH1*, the gene encoding myosin heavy chain 1, has recently been implicated in recurrent rhabdomyolysis.[1] *MYH1* is highly, and almost exclusively, expressed in skeletal muscle. We describe variants in this gene identified through exome sequencing and their possible involvement in oligogenic combinations associated with MH susceptibility.

**Methods:** With Ethics Committee approval, genomic DNA from 98 unrelated MH- susceptible individuals underwent exome sequencing. Libraries were prepared using the IDT xGen Research Panel. Following alignment to the GRCh38 human genome, variants from each sample were combined into one gvcf file and all variants jointly genotyped. Variants with minor allele frequency > 0.01 in any cohort of the Genome Aggregation Database, gnomAD v4.1, or CADD score < 15 were excluded. Filtered variants from each sample where we found a variant in *MYH1* were used as input for oligogenic variant analysis (ORVAL v3.0.0 https://orval.ibsquare.be/input). Within ORVAL we explored the involvement of *MYH1* variants in gene pairs and gene modules associated with the two highest ranked categories of pathogenicity score.

**Results:** Four missense variants and one nonsense variant (all heterozygous) in *MYH1* were found in five individuals (Table I). There is insufficient evidence that these *MYH1* variants or variants found in other genes in the five individuals contribute a single gene effect in MH susceptibility. The *MYH1* variants featured in predicted pathogenic combinations and derived gene modules. Genes with high skeletal muscle tissue expression found in combination with *MYH1* in more than one patient were: *EIF4G1*, *KIF1C*, *HTT*, *OBSCN*, *ORAI1*, *SPEG*, *TTN*. The combinations with the highest likelihood of being pathogenic were mostly predicted to operate through a digenic mechanism (rather than monogenic/modifier or dual molecular diagnosis). But no genes can be formally implicated from this analysis.

**Discussion and Conclusion:** This preliminary analysis suggests that *MYH1* should be evaluated in larger sample sets in a potential oligogenic model.

**Table I:** *MYH1* variants found in MH-susceptible individuals. ENF, European non-Finnish

| ***MYH1* variant** | | **Minor allele frequency** | | | ***In silico* pathogenicity** | |
|---|---|---|---|---|---|---|
| **Nucleotide** | **Amino Acid** | **gnomAD total** | **gnomAD ENF** | **UK Biobank** | **CADD** | **REVEL** |
| c.3847C>T | p.Arg1283Cys | 0.0008354 | 0.001061 | 0.00117 | 24.3 | 0.507 |

| | | | | | | |
|---|---|---|---|---|---|---|
| c.3039C>T | p.Leu982Phe | – | – | – | 24.1 | 0.704 |
| c.2110G>A | p.Glu704Lys | 0.001769 | 0.00226 | 0.00222 | 23.8 | 0.753 |
| c.1960C>G | p.Leu654Val | 0.00000124 | 0.000001697 | 0.00000204 | 19.5 | 0.48 |
| c.580C>T | p.Gln194Ter | 0.0001995 | 0.0002602 | 0.00031 | 38 | – |

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## Some observations on *RYR1* variant prevalence and clinical incidence of MH in the UK

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**Background:** It has become increasingly apparent that there is considerable disparity between the population prevalence of *RYR1* variants associated with MH and the incidence of clinical MH reactions. Here, I revisit the clinical incidence of MH reactions, the number of uneventful anaesthetics that index cases have before they present with a clinical reaction and the relationship between variant prevalence and clinical incidence.

**Methods:** An estimate of the crude incidence of clinical reactions in the UK was based on a five year period (1988–92) when the number of new cases was at its peak. Official population statistics were used, while the number of general anaesthetics administered was estimated from publicly available data. There were detailed records of the anaesthetic history of 375 index cases and 1 037 MH susceptible relatives. The prevalence of 28 variants in *RYR1* that are recurrent in the UK population was estimated using UK Biobank data (https://afb.ukbiobank.ac.uk/gene/RYR1) . The relative risk for a carrier of each variant to develop an MH reaction was calculated by dividing the number of carriers of each variant (estimated from the population prevalence) by the number of UK families harbouring the variant.

**Results:** From 1988–92 there were 155 index cases referred to the UK MH Unit and shown to be MH susceptible by the IVCT. The crude incidence of MH was 0.534 cases per million people per year. The crude incidence of MH in those exposed to general anaesthesia was 9.06 per million per year, or an incidence of 1:110 000 anaesthetics. For each anaesthesia exposure, approximately 50% of index cases developed a clinical reaction (range 0–11 prior exposures): this was not dependent on the *RYR1* variant found in the family. Combining these two analyses suggests that ~ 1:55 000 of the population is at risk of developing a clinical reaction. Examination of relative risk of a clinical reaction in carriers of different *RYR1* variants suggested an almost 150-fold difference across the 28 variants studied. The number of families harbouring each variant was not related to the population prevalence of the variant. Interestingly however, there was a strong inverse correlation between variant population prevalence and the relative clinical risk associated with each variant.

**Discussion and Conclusion:** These data suggest that individuals who have developed an MH reaction have a risk of developing MH of ~ 50% with each anaesthetic exposure irrespective of the *RYR1* variant they carry. However, the contribution of the different variants to the biological threshold required to impart that risk appears to vary greatly. The relationship between prevalence and clinical risk also suggests that gain of function *RYR1* variants impart a negative selection pressure proportional to their biological effect. These data can also inform about the risk of *RYR1* variants found incidentally: if the variant has a MAF > 0.000021 and has not been associated with an MH reaction in the relevant population, we can be 95% confident that the carrier has a risk of developing MH no greater than the population risk.

## Human phenotype ontology terms relevant to malignant hyperthermia

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**Background:** The search for new genetic variants associated with the risk of MH may be constrained by the over-simplification or lack of precision in describing the clinical event. In genetic studies we have tended to group together all patients with a clinical presentation associated with MH susceptibility and a positive IVCT. However, many patients have been investigated, for example, on the basis of a reaction involving muscle rigidity in response to succinylcholine without the metabolic features required by the definition of malignant hyperthermia agreed by the EMHG. A number of bioinformatic tools developed for the interrogation of genomic data include functionality to incorporate phenotype terms in their algorithms. This might be useful for MH and, if so, the EMHG should take a lead.

**Methods:** A mapping exercise of terms relevant to MH was carried out against terms already found in the Human Phenotype Ontology (HPO, https://hpo.jax.org/) project. I categorised terms relating to MH under "hypermetabolism", "muscle clinical", "muscle histology" and "complications": imprecise or incorrect terms in HPO were identified as were missing terms.

**Results:** HPO terms were mapped and existing terms within HPO are presented in italic font with my comments, where appropriate in parentheses. Interestingly, there is an HPO term *malignant hyperthermia* (probably better listed just as a "disease"), under the parent term *abnormal temperature regulation* (imprecise). *Malignant hyperthermia* is subdivided into *anaesthetic-induced malignant hyperthermia* (superfluous) and *exercise-induced malignant hyperthermia* (incorrect). The number of existing and proposed HPO terms under different categories are summarised in Table I.

**Table I:** Existing and proposed HPO terms relevant to MH

| Category | Existing HPO terms | Proposed new terms | Comments |
|---|---|---|---|
| Hypermetabolism | 13 | 13 | – |
| Muscle clinical | 21 | 17 | 2 imprecise terms |
| Muscle histology | 21 | 0 | – |
| Complications | 12 | 0 | – |

**Discussion and Conclusion**: Clinical terms relevant to an MH reaction need to be expanded, including to specify clinical features when they occur during anaesthesia. There was one HPO term relating to the IVCT – *reduced in vitro contracture test threshold*. I suggest that inclusion of more granular phenotyping terms related to the IVCT responses may be helpful.

## Variant-specific association between risk of clinical reaction and IVCT responses

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**Background:** We have previously demonstrated that IVCT responses vary according to which *RYR1* variant is carried by an MH susceptible individual.[1] There are limited data, however, that link specific variants, IVCT responses and predisposition to a clinical MH reaction.

**Methods:** We selected 22 variants in *RYR1* that are recurrent in the UK MH cohort. All but one of these (*p.Asp3986Glu*) is classified as pathogenic or likely pathogenic. We determined the relative risk for a carrier of each variant to develop an MH reaction by dividing the number of carriers of each variant (estimated from the prevalence of the variant in the UK population, https://afb.ukbiobank.ac.uk/gene/RYR1) by the number of UK families harbouring the variant. We then determined the correlation between relative risk of a clinical reaction and strength of halothane and caffeine contractures in respective variant carriers.[1] In secondary analyses we determined the association between the REVEL score for each variant and the clinical risk and the IVCT responses.

**Results:** We found significant correlations between the relative clinical risk for each variant and the IVCT contracture responses (Table I). There was a weaker association between the relative clinical risk and REVEL score, which became non-significant in a sensitivity analysis (omission of data for *p.Ser1728Phe*).

**Table I:** Results of correlation analyses

| *Variable 1* | Variable 2 | $r_s$ | P (2-tailed) |
|---|---|---|---|
| Relative clinical risk | Static halothane response | 0.60 | 0.003 |
| Relative clinical risk | Static caffeine response | 0.77 | 0.00003 |
| Relative clinical risk | REVEL score | 0.47 | 0.03 |
| REVEL score | Static halothane response | 0.20 | 0.37 |
| REVEL score | Static caffeine response | 0.05 | 0.82 |

**Discussion and Conclusion:** These data suggest an association between IVCT responses and the risk of carriers of different *RYR1* variants developing a clinical MH reaction. These analyses assume that carriers of different variants are equally likely to be exposed to triggering anaesthesia.

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