

Malignant Hyperthermia: Investigation for the Uninitiated

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Introduction

Malignant Hyperthermia (MH) is a rare pharmacogenetic disorder. Ever since it was first described, a foolproof diagnostic test for MH has been sought. However, this has been no easy task.

An MH reaction is a clinical chameleon, with fulminant clinical presentations marked by variable non-specific signs that have historically resulted in up to 70% mortality. Susceptibility cannot be diagnosed by clinical examination, and it demonstrates considerable genetic heterogeneity with variable penetrance. The diagnosis and follow up investigation of MH represents a complex challenge that is as interesting as it is frustrating. Nevertheless, important steps have been made over the last four decades. This paper aims to summarise the progress so far, particularly for those anaesthetists who are not directly involved in the testing of MH patients.

Why should we test for MH?

The question may be asked, given that the In Vitro Contracture Test (IVCT) is an invasive and costly procedure that is neither 100% sensitive nor specific, why bother with the IVCT at all? Why not use a non-invasive test instead? A Devil's advocate may argue that, with the ability to avoid triggering agents, the easiest solution would be to label all at risk patients as MH susceptible (MHS), and treat them accordingly.

Despite its shortcomings, an IVCT result may be of great benefit to the family of a tested proband or close relative. A negative result allows all of the progeny of a proband to avoid further testing. Furthermore, abandoning MH testing would result in an ever-increasing population for whom volatile-free anaesthesia would be mandatory. This is a costly proposition, would burden many adults unnecessarily with the MHS label, and would make anaesthesia for at risk children considerably less flexible.

As this paper outlines, despite improvements in both the understanding of MH and the tools to investigate it, there is not yet a validated alternative to replace IVCT as our standard test. The future development of non-invasive (in particular genetic) tests relies on a diagnosis based on the IVCT. Linkage analysis (see below) requires an independent confirmatory test before a mutation can be considered causative and used as a screening test for MH.

Diagnostic Testing

An ideal diagnostic test will have high sensitivity (the ability to pick up all true cases, i.e. low false negative rate) and high specificity (the ability to pick up only true cases, i.e. low false positive rate). Diagnostic tests in a clinical setting are, however, rarely perfect.

Given the invasive nature of IVCT, efforts have been directed at a non-invasive diagnostic technique. Prior to the availability of the IVCT, these included tests of red cell and platelet function, anthropometrical analysis and electromyography. None have proved adequately sensitive or specific.¹ Other widely available tests that have been considered include histopathology and elevated creatine phospho-kinase levels.

Histopathology

Mezin et al² described significant differences in the histological properties of MHS, MHE and MHN muscle specimens. The presence of a tetrad of non-specific lesions (muscle fibre atrophy or hypertrophy, numbers of internal nuclei and myofibrillar necrosis) was noted only in their MHS group (in 35%) and not in the MHE or MHN group. No specific histopathological finding has yet been associated with MH. Following these findings, von Breunig et al³ investigated a large sample of patients suspected of MH. In contrast to Mezin et al's results, no significant differences were found in each diagnostic group's histology and, interestingly, no subject in any group exhibited the tetrad of non-specific changes.

Whether or not a specific MH myopathy exists remains controversial. To date, results demonstrate poor sensitivity and specificity, even where there are statistically significant differences between groups. Therefore, the role of histological examination in MH diagnosis appears limited. The principal benefit may be in diagnosing previously unrecognized specific myopathies.

Elevated Resting Creatine Phosphokinase Levels

Creatine phosphokinase (CK) is an enzyme found in striated muscle. Its primary function is to maintain the energy balance between resting and exercising muscle. Serum CK levels may be elevated in many pathophysiological conditions, including statin drug use, post-exercise, myopathies (e.g. Duchenne Muscular Dystrophy (DMD) and Central Core Disease (CCD)) and trauma.

Given that some myopathies are associated with elevated resting CK levels and the theory (now disproved) that myopathy of any kind increases the risk of MH susceptibility, the association of MH susceptibility with high resting CK levels has been considered. Results, and conclusions drawn, vary significantly in trials comparing CK levels with the IVCT.

Ellis⁴ stated that elevated CK levels "may be useful for screening in those few families in which people susceptible to MH have high CK levels". However, Paasuke et al⁵ concluded on the basis of their investigation that, "diagnosing MH susceptibility by ... serum CK level must be abandoned". More recently, Weglinski et al⁶ advised that, "Individuals with high CK levels must be treated as MHS until proven otherwise", despite acknowledging serious methodological flaws in their study. Overall, the balance of opinion leads to the conclusion that serum CK levels are not sensitive or specific enough to be useful for diagnosis in individual patients.

In vitro contracture testing: The Standard

IVCT History

In 1970, Kalow et al⁷ described the first in vitro test for MH based on the knowledge that isolated muscle contracts in the presence of caffeine and that MH reactions were usually accompanied by severe muscular rigidity. Muscle sampled from patients who had survived fulminant MH reactions was exposed to caffeine and halothane. Significant increases in tension with exposure to caffeine (and potentiation with the addition of halothane) in test cases were noted in comparison to controls.

In 1971, Ellis et al⁸ expanded on Kalow's work with exposure to halothane, methoxyflurane, suxamethonium and lignocaine at body temperature. Positive results corresponded well with positive family history of MH and elevated CK levels. Ellis's work is the basis of the present IVCT used throughout Europe and Australasia. In 1984, the European MH Group (EMHG) published a protocol for IVCT⁹ to standardize investigation throughout different testing facilities. All of the Australasian testing centers (Melbourne, Palmerston North, Perth and Sydney) use the EMHG IVCT protocol. A separate North American MH Group (NAMHG) protocol exists.

EMHG IVCT protocol

In summary, biopsy of vastus medialis or lateralis occurs under non-triggering anaesthesia. Muscle tension is measured while caffeine or halothane is added to the tissue bath in incremental concentrations. Tissue viability is maintained by carboxygenation and confirmed by repetitive electrical stimulation. Three tests are performed: static caffeine (SC), static halothane (SH) and dynamic halothane (DH).

The "threshold" is the lowest drug concentration at which a sustained increase of at least 0.2g baseline tension occurs. A test is positive if the threshold occurs at or before 2% v/v halothane or 2 mmol/l caffeine. Three diagnostic test results are possible. MH Susceptible (MHS) individuals have positive halothane and caffeine thresholds. MH Normal (MHN) individuals have negative halothane and caffeine thresholds. MH Equivocal (MHE) individuals have either a positive caffeine or halothane threshold, but are clinically regarded as MHS. Only abnormal muscle contracts with halothane exposure, but even normal muscle will usually contract in response to high caffeine concentrations.

Given that IVCT is not only invasive, but is also a costly and scarce resource, careful consideration is given to which family members are tested first. Children under 10 yrs are not considered suitable for IVCT.

Accuracy of the IVCT under the EMHG protocol

It is inherently difficult to accurately assess the sensitivity and specificity of a diagnostic test such as the IVCT, where no proven alternative exists. Led by Ording, the EMHG performed a comparison of IVCT results from patients with previous fulminant MH reactions (MHS group) and a group of low risk subjects (controls).¹⁰ MHS diagnosis was confirmed on the basis of a high score on the MH Clinical Grading Scale (MHC GS)¹¹ that predicts the degree of likelihood that clinically fulminant reactions truly represent MH.

Results estimate that the (EMHG) IVCT has at least 99% sensitivity and 94% specificity. It should be emphasized that the EMHG IVCT thresholds have been deliberately set to reduce the clinically dangerous result of a false negative. It is accepted that to maintain high sensitivity that there must be some trade-off in

specificity. Several reports of false negative IVCT results have been published.¹² On careful examination, the EMHG protocol had not been rigidly adhered to, and this finding appears to exonerate the IVCT.

While the validity of the MHCGRS has not been formally tested, the lack of any reports where patients diagnosed MHN have subsequently had MH reactions supports the excellent reported sensitivity. Some consider that the estimated specificity of 94% is unduly optimistic. This may in part relate to IVCT results derived from patients with known or occult neuromuscular diseases other than MH. IVCT for this subgroup of patients is considered to be non-specific.¹³

The problem with MHE

The single biggest “thorn in the side” of the IVCT is the MHE result. It remains uncertain what this result means in terms of real clinical risk. While experts recommend the conservative approach of considering MHE as equivalent to MHS, there is little to support this in the literature. The MHE group probably contributes a significant number of false positive IVCT results. This contention is based on the relative paucity of causative mutations (5%, local unpublished results) found in MHE patients and few case reports of clinically fulminant MH reactions in this group.

Attempts have been made to further separate MHE test results using additions to the standard IVCT. The plant alkaloid ryanodine has been investigated. Wappler et al¹⁴ demonstrated that its use can differentiate the majority of standard MHE results as either MHS or MHN. The use of ryanodine is now recommended as an optional extra IVCT testing agent by the EMHG, but is not yet a formal requirement. Similar trials with 4-chloro-m-cresol (4CmC) have failed to clearly differentiate MHE results.¹⁵

The Genetics of MH: DNA Analysis

With its introduction into MH investigation, it was hoped that DNA analysis would be the panacea for the problems associated with the imperfect and invasive IVCT. However, the genetics of MH have proven much more complex than had been hoped and its practical applicability is, as yet, limited.

General Genetic Theory & Techniques

Television crime programs invariably portray “DNA fingerprinting” as easy, akin to ordering coffee and doughnuts, available at the drop of a hat, and miraculously never failing to “collar” the bad guy. This portrayal belies the time-consuming, expensive and complicated process that is faced in reality.

Like most medical specialties Genetics embraces large amounts of jargon. To this end a glossary is provided below.

Allele:	Alternative forms of the same gene
Autosomal:	Pertaining to chromosomes other than the sex chromosomes
Discordance:	Disagreement between the phenotypic test result and the genotype. May be due to a false positive or a false negative test result.
Exon:	The region of a gene that actually contains the coding sequence. Exons are separated by introns, which are regions that do not contribute to the final gene product.
Gene:	Unit of inheritance composed of DNA, with a specific function. Most genes contain information for making specific proteins.

Genetic Marker:	A polymorphic segment of DNA with a known physical location on a chromosome whose inheritance can be followed. Can be part of a gene.
Locus:	Unique chromosomal location that defines the specific gene or DNA sequence.
LOD score:	A statistical estimate of whether two loci are likely to lie near each other on a chromosome and are therefore likely to be inherited together. LOD score of 3=logarithm of the odds (to base 10) means the odds are 1000:1 in favour of genetic linkage.
Linkage:	The tendency for genes and other genetic markers to be inherited together because of their location near one another on the same chromosome.
Polymorphism:	Genetic characteristic with more than one common form in a population. Commonly used to describe a mutation without discernable clinical effect.
Proband:	The family member through whom a family's medical history comes to light. (Also known as: index case.)

The Process of DNA Analysis

The ultimate goal of a genetic test is to determine the DNA sequence of a disease causing mutation.

In general, investigation starts with only a disease, identified in the case of MH by IVCT results. To identify the disease causing gene, linkage analysis is employed. This uses DNA markers to identify a region of DNA that is inherited with the disease. Because recombination occurs freely between homologous chromosomes during meiosis, the closer the marker is to the gene under investigation the more likely it is to be inherited with it. The marker and disease inheritance are then compared. If certain statistical criteria are satisfied (see LOD score above), the disease gene can be identified and tracked through a proband's pedigree.

Once the disease gene is known, specific mutations are sought. Given that in the case of the main MH-related gene, a single base substitution in more than 15,000 nucleotides may be the target, this is an enormous task. Although it is possible to sequence a whole gene and to systematically compare with it with the "normal" sequence, it is prohibitively time-consuming and expensive to do this on a regular basis.

To narrow down the general location of a mutation, the process of single-stranded conformation polymorphism (SSCP) analysis is used. This method analyses DNA in segments of 200-300 nucleotides at a time. The secondary structure of ssDNA is reliant on its base composition. Any mutation will change the secondary structure of the DNA. Alterations in conformation tend to affect migration through acrylamide gel, with visible changes from normal in the characteristic banding pattern on electrophoresis. Once the presence of a mutation is identified by SSCP analysis on a specific exon, it can be sequenced and precisely identified using a semi-automated DNA sequencer.

Once a mutation is sequenced, investigators must then confirm that it is causative for the disease. This firstly requires linkage analysis within the proband's pedigree, to determine if the mutation and the disease state are inherited consistently. Secondly, the normal sequence of the gene must be shown to be highly conserved throughout other species, so that a mutation could plausibly cause a disease state. Thirdly, the

mutation should be absent in 100 unrelated controls. The ultimate, but also the most difficult, confirming step is functional analysis of the mutant protein.

History of MH Genetics

On a molecular level it has long been presumed that the abnormality of muscle seen in MH lies with calcium regulation. More specifically, the calcium release channel or Ryanodine receptor (named for its activation by the plant alkaloid ryanodine) of the sarcoplasmic reticulum was implicated as the "prime suspect". With the aid of the extensive work on Porcine Stress Syndrome (the pig equivalent of MH), investigators began closing in on what was thought to be "the MH gene" in humans.

In 1989, the Ryanodine receptor type 1 (RYR1) gene was sequenced, cloned and mapped to the long arm (q) of chromosome 19. In 1990, McCarthy et al¹⁶ demonstrated that known genetic markers on a discrete region of chromosome 19q were tightly linked (i.e. reliably inherited) with the MHS trait in three Irish families. During the same year, MacLennan et al¹⁷ showed that markers for the same region of chromosome 19 were tightly linked (LOD score=4.2) with the MHS trait in Canadian pedigrees.

The first actual mutation was sequenced from the porcine model in 1991. The same mutation was then identified in a small number of MH families. Over the intervening years, an ever-increasing number of RYR1 mutations have been identified (see below). The molecular pathology of the RYR1 receptor abnormality appears to allow it to open to non-physiological stimuli (triggers) and to remain open for abnormal periods.¹⁷

Failure to fulfil potential

After promising beginnings it was to become clear that the genetics of MH were anything but simple. Despite significant increases in the understanding of the molecular pathology of MH, there has been only modest progress in its clinical applicability. Genetic investigation of MH has not yet fulfilled the potential that many had hoped for. Although large numbers of RYR1 mutations are known, linkage studies have determined that MH susceptibility is linked to the RYR1 locus in only about 50% of affected families. Mutations have been identified in less than 50% of these, although this could approach 70% with more thorough screening methodology.¹⁸ It is disappointing to note that less than a quarter of MH susceptible patients in many studies have thus far had mutations identified.

Additionally, genetic testing has failed to shed any further light on the true nature of MHE patients, who remain a clinical headache for MH investigators using the EMHG protocol. Finally, there have been multiple reports of discordance, causing confusion as to how confident we can be that genetic diagnosis is a reliable alternative to IVCT.

Why has DNA analysis failed to deliver?

Structure of the Ryanodine Gene

The sheer size and complexity of the RYR1 gene is an inherent problem. It is among the largest genes described in humans, consisting of more than 15,000 coding nucleotides. It is divided into 106 exons, two of which are alternatively spliced. To date at least 175 RYR1 mutations have been identified in humans. Of these, 78 may be considered causative for MH. Seventeen are also causal for Central Core Disease (CCD) and a further 50 are causal for CCD and related disorders alone. Central core

disease is a myopathy characterised by muscle hypotonia (floppy infant syndrome), delayed motor development, symmetrical proximal weakness, and raised CK levels. CCD is often associated with clinical MH susceptibility. Forty-seven are (silent) polymorphisms with no discernible clinical effect. Mutations are predominantly clustered in three parts of the RYR1 gene, in so-called “hot-spots”.¹⁹

Discordance

The biggest single impediment to progress in MH genetics is the lack of reliable phenotyping for the condition. There are many reports of discordance between DNA analysis and IVCT results.²⁰ Both false positives and false negatives have been reported (see above).

Given that linkage analysis already incorporates small degrees of uncertainty with the use of LOD scores, protocols used in proving a mutation causative are conservative. If discordance is present (even in a single member of a pedigree), this casts doubt on the causative nature of a mutation. MacKenzie et al²¹ demonstrated that by lowering IVCT thresholds in the NAMHG protocol they could achieve RYR1 linkage in a large pedigree. Using the original thresholds yielded no consistent linkage. This work is not directly applicable to IVCT under the EMHG protocol where the MHE group is the most likely to cause false positive results. Given the degree of uncertainty of what an MHE result actually represents, local practice is to omit MHE results in linkage analysis where possible.

Jurkat-Rott et al²⁰ surmise from their results that if it were not for the likely inaccuracies of IVCT, MH susceptibility could be linked to RYR1 in more than 85% of families. Multiple mutations may also be contributing to discordance (see unexpected findings below).

Other possible sites for mutations

So what about the remaining 15%? At least six other genes/loci have been identified or considered as alternatives that segregate with MHS (when not linked to RYR1). These include three genes (on chromosomes 1, 7 and 17) coding for subunits of the di-hydropyridine (DHP) receptor, which activates the RYR1. Only one of these genes (on chromosome 1) has had causative mutations identified in a small number of MH families. Two other loci on chromosomes 3 and 5 (where genes have not yet been identified), and the possibility of a seventh as yet unidentified locus, have been published.¹⁹

Unexpected Findings

From the time it was first recognised in 1960 as a familial condition, MH has appeared to follow autosomal dominant inheritance on the evidence of many pedigrees. This typically has meant that once one parent of the proband is identified as susceptible, the other is presumed normal. A group of studies has challenged both presumptions. Mauritz et al²² noted that in 44 families where both parents underwent IVCT, three families had positive test results in both parents. These authors believed that these results could be explained by inbreeding in the families concerned. However, Islander et al¹² showed similar results. In 101 Scandinavian MHS probands (where both parents had IVCT), six sets were both MHS, twenty sets were MHS/MHE and a further six sets were MHE/MHE. Monnier et al²³ also found a similar proportion (six

of 104 French families where both parents underwent IVCT) of MHS/MHS parents. Furthermore, they identified RYR1 mutations in both parents for four sets of apparently unrelated parents.

Explanations that were considered for the Scandinavian results included inaccuracies in IVCT (especially false positive phenotyping in the MHE group). If the reported specificities of IVCT are accurate, this cannot by itself explain both parents being positive in almost one third of families. Furthermore, there is no evidence of inbreeding using other genetic markers in their study group. Using the French data, it is postulated that the incidence of MHS genotype is much more common than the previously estimated 1:10,000 (i.e. it is likely to be closer to 1:2000) and that the degree of incomplete penetrance has been significantly underestimated. This would explain both the higher than expected dual family mutation and the low numbers of MH reactions observed. Finally, Islander et al¹² describe two families where the proband was susceptible (on the basis of fulminant reactions under anaesthesia) and had MHN/MHN parents. While some uncertainty surrounds these cases, the authors feel this is "most likely explained by recessive inheritance or the occurrence of new mutations".¹²

These findings have significant bearing on the practical utility of genetic screening. On this basis, Monnier et al²³ recommend that, where possible, both parents of a proband should be tested and both IVCT and DNA analysis should be employed whenever possible in each MH family.

Practical Bottom Line of Genetic Testing

The proposed benefits of DNA analysis for MH testing are, firstly, to reduce the number of patients that must undergo the invasive and imperfect IVCT and, secondly, to allow testing of those patients not suitable for IVCT (principally the very old and the very young). However, because of the technical and theoretical constraints detailed above, DNA analysis has many practical limitations. It cannot be rationally used as the primary diagnostic test for MH susceptibility.

While it is technically possible to sequence the full RYR1 gene in any proband (e.g. a patient who has a clinically suspicious reaction under anaesthetic), it would take at least three months of fulltime work for a skilled laboratory scientist. If an RYR1 mutation did exist, the chances of finding a mutation should approach 85%. However, the reality is somewhat less optimistic. The likelihood of finding an RYR1 mutation for "all-comers" is less than 5%. In this cost-conscious age, a more pragmatic approach is taken. As a general rule, only probands who are MHS on IVCT are considered for new DNA analysis. Locally, the investigation is restricted largely to the RYR1 "hotspots", and the screening of 15 exons takes up to three weeks for a batch of six different patients. This carries an estimated 50% chance of finding an RYR1 mutation in the proband (Personal communication M. Davis, MH Geneticist).

Once a family mutation is discovered and proven causal, DNA analysis for MH comes into its own. SSCP analysis can be used and a specific change in banding pattern on electrophoresis can diagnose MH susceptibility in a close family member. This takes less than forty-eight hours and allows the family member to avoid the invasive IVCT. However, if the mutation is absent on SSCP analysis, since there is an estimated 6% probability (see above) of multiple MH mutations, the relative under investigation must be shown to be MHN by IVCT before they can be safely considered to be not at risk.

What does the future hold for DNA analysis in MH?

At present, the use of DNA analysis aids MH diagnosis in a large number of families. Additionally, it enables diagnosis in the elderly and the very young, who are unsuitable for IVCT. However, the proportion of families where causative mutations have been identified remains disappointingly low. While it is reasonable to assume that more mutations will be discovered, it seems that, clinically, DNA analysis for MH diagnosis has reached a plateau. It is unlikely that significant improvements will be made until a test is developed that can produce accurate phenotypes.

What about “gene therapy” for MH? Gene therapy is in its infancy at present. While in the distant future it may be possible to replace abnormal RYR1 genes in MHS individuals, it is unlikely that MH will be prioritised for this very complicated and expensive process, given that non-triggering anaesthesia is a wholly acceptable preventative strategy.

Novel Non-invasive tests: The Holy Grail of MH

With the limitations of DNA analysis becoming apparent, investigators have worked tirelessly to find an alternative (and more accurate) diagnostic test for MH. The elusive “holy grail” is a highly sensitive, highly specific, non-invasive test.

B Lymphocytes

Sei et al²⁴ first discovered that B lymphocytes express the RYR1 in 1999. It was also determined that 4-chloro-m-cresol (4CmC), a known RYR stimulant, induced calcium release from these lymphocytes. It was hypothesised that MHS individuals would exhibit altered calcium release in this setting. B lymphocytes from MHS, MHN and normal controls (NAMHG protocol) were stimulated using either 4CmC or caffeine. Statistically significant differences were found between MHS and MHN group results. However, the individual ranges overlap significantly, so investigators acknowledge their inability to clinically discriminate between groups. It is important also to note that this *in vitro* model may not be truly representative of calcium regulation in skeletal muscle. A plethora of genes that may enhance or mitigate an MH response are not represented in B lymphocytes. This fact, and the small number of MH mutations from loci other than RYR1, suggest that this test is at present unable to replace the standard IVCT.

Myotubes

Advances in biotechnology have now enabled the culture of myotubes for testing from samples of adult muscle stem cells. Myotubes are effectively fully functional, multinucleated muscle units. Moderately large numbers of myotubes can be cultured from a relatively non-invasive needle biopsy. Klinger et al (2002)²⁵ used measurement of extracellular (EC) pH as an indicator of altered intracellular calcium metabolism to compare myotubes cultured from MHS and MHN individuals. Briefly, the process involves enzymatic dissociation of cells from muscle tissue, 4-6 days in a growth medium, 4-6 days in a differentiation medium, and then testing by exposure to 4-CmC with pH measurement. Results demonstrate considerable discrimination between MHS and MHN myotubes on the basis of EC acidification for a range of different 4-CmC concentrations. The potential benefits for this mode of MH testing include non-invasiveness, the potential to repeat tests on stored specimens and the ease with which samples may be sent to distant testing sites. However, numbers in this trial were

small (n=16), MHE patients were not included and the process is extremely technically demanding.

To address some of the concerns above, Girard et al²⁶ measured halothane-induced increases in intracellular calcium ($\text{HII}[\text{Ca}^{++}_i]$) in myotubes taken from MHS, MHE and MHN patients. Overlaps in the ranges between groups occurred, so a result above the 95th percentile for the normal group was accepted as MHS. Results regrettably demonstrate that this test, in its present form, cannot replace the IVCT. A maximum of 75% of the MHS patients were identified. It remains unknown whether the remaining undiagnosed 25% were due to false positive IVCT results, or to an over-estimation of true "normal" $\text{HII}[\text{Ca}^{++}_i]$. Nevertheless, Girard's group remains optimistic about the utility of this test used in conjunction with genetic screening. They estimate a significant proportion of individuals could be diagnosed MHS without requiring an invasive IVCT.

Nuclear Magnetic Resonance Spectroscopy

This modality is able to measure adenosine triphosphate (ATP) and other phospho-monoesters along with pH in muscle non-invasively. Several studies have shown delayed reconstitution of pH and ATP with increases in phosphocreatine in MHS patients. Imperfect discrimination, high technological requirements, and low specificity limit the applicability of this test so far for diagnostic purposes.¹

Summary

More than four decades of perseverance by the international MH community has achieved major advances in our understanding of the molecular pathology of MH. Improvements in our ability to diagnose MH have been significant, but non-invasive diagnosis remains limited to a disappointingly small proportion of MH families. For the moment, the ultimate prize of a "highly sensitive, highly specific non-invasive test" remains elusive. However, it is hoped that in the not too distant future an accurate non-invasive diagnostic test will be found and that MH DNA analysis will be able to realise its full potential.

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