

RESEARCH - BMHA LOTTERY GRANT- SUMMARY

Lottery Funding – After two application attempts £150,000 was awarded to the BMHA to fund research into the further development of a DNA blood test for MH families, the ultimate aim of such research being to replace the present MH test method which requires an invasive muscle biopsy. The funding began April 2005. The grant employs a full time research technician and a part time administrative assistant.

Advantages – DNA testing if widely used would i) reduce costs to the NHS for MH screening avoiding operating theatre and staff time required for muscle biopsy testing ii) minimise the inconvenience and unease to patients iii) increase numbers of people tested and allow testing of young children and the elderly.

Progress – DNA extracted from blood samples from BMHA members is currently being analysed for mutations in the RYR1 gene, the major MH susceptibility gene. Mutations are being detected using a new analysis process. Independent of the lottery funding, the BMHA have contributed to the purchase of a new machine to allow this analysis to be performed more efficiently.

DETAILS

The BMHA, together with the Leeds MH Investigation Unit, worked for many months to formulate a research project entitled “The advancement of Malignant Hyperthermia (MH) testing by DNA analysis”. This was submitted to the Lottery Research Grant programme in 2002, but unfortunately was turned down.

The BMHA Management Committee decided to have another attempt at obtaining lottery funds for this project. In late 2003, after many more months of work, we were approved. The grant awarded was ~£150,000. We were warned that it would take at least 6 months before we would be able to begin the project, but in the end, for various reasons beyond our control, this turned out to be over a year!

The following are the main areas of work as submitted in our bid.

What is the overall aim of the research project?

Anyone suspected of having had an MH reaction needs to be tested at the Leeds MH Investigation Unit for a definitive diagnosis. Until recently, this involved a muscle biopsy. Guidelines for DNA testing were published in 2001. An initial study by the Leeds MH Unit indicated that DNA diagnosis according to these guidelines is possible for approximately 25% of families. This leaves 75% of families who would not benefit.

The overall aim of the project is to be able to offer blood tests to all families not just the 25% who can benefit at present. This will involve contacting families,

Progress & Final Results

The Big Lottery Fund (BLF) Project - Final Year

The project was put on hold at the end of June 2007 as Dr Rachel Robinson left to take up a post as Head of Cancer Genetics at the Yorkshire DNA laboratory. In conjunction with the BLF, we discussed whether the project should be terminated at this stage or whether we should delay the start of year 3 so that we could see it through as planned. Around August 2007, it was agreed that we should continue.

The final year therefore got underway in November 2007 with Dr Danielle Carpenter taking over as clinical scientist.

Final year results

From a total of 655 MH susceptible families, we were able to build up a resource of stored DNA from 500 families, some of whom had been included in DNA studies prior to this project. We were unable to contact the remaining 155 families.

151 independent UK families were included in screening. Changes in the RYR1 gene (mutations) are known to contribute to an increased risk of susceptibility to MH and these changes were identified in 83 (54%) families. Eighteen of these families can already benefit from diagnostic testing, as they carry a known causative mutation, and 36 families carry as yet uncharacterised mutations, including 4 RYR1 mutations that are new to the UK - 3 are actually new globally too. These mutations, once shown to have a causative or functional effect (characterised) should eventually be included in the diagnostic list. In 29 families no mutations (both causative and uncharacterised) in either RYR1 or another gene possibly involved in MH (CACNA1S) were identified, which makes them an exclusive panel of patients to be investigated in other projects.

That left 95 families still requiring further investigation using an alternative approach. To screen for new mutations in these families two methods were used, one of which required the use of DNA extracted from frozen muscle samples. We were able to investigate 52 families with this method and the remainder using DNA from blood. After completion of these studies, 11 new mutations, 4 suitable for diagnosis and 11 already known but not yet characterised, were identified.

Thus, seventy percent of UK MH patients are now accounted for by changes in the RYR1 sequence, and 41% can now benefit from DNA diagnostic testing, compared to 25% at the start of the project. Further work to characterise the new RYR1 mutations so they can be included in the diagnostic list is ongoing at the MH Unit in Leeds which should result in greater than 50% of patients benefiting from DNA testing. In addition the 29 families in whom both RYR1 and CACNA1S

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RYR1 mutations so they can be included in the diagnostic list is ongoing at the MH Unit in Leeds which should result in greater than 50% of patients benefiting from DNA testing. In addition the 29 families in whom both RYR1 and CACNA1S have been excluded are being investigated in detail for alternative candidate genes for this disorder.

Was the project a success?

At the beginning of the project it was predicted that MH is due to changes in the RYR1 gene in 90% of cases in the UK. However, it has become apparent during this project that the RYR1 gene plays a more conservative role, accounting for 60-70% of MH cases in the UK.

The project has highlighted the complexity of the MH condition. By identifying that RYR1 plays a less than predicted role, something else must be playing a part for around 30% of MH families in the UK and we now have a panel of patients that can be entered into other projects to screen for these alternatives.

The aim of the project has been successfully achieved in that there has been a significant increase in the characterisation of MH families in the UK and therefore increased availability of a genetic test for patients. Interestingly, it has also thrown up another line of work for the future.

A summary of the progress made in the previous two years is below

Year two progress

At the end of year two 153 independent MH families have ongoing genetic investigations being conducted. The patient rebleeding program is largely complete. Response to rebleed request letters sent to BMHA members was not as high as expected. However, the Leeds MH unit has many patient samples already stored which still require genetic analysis to be performed so these were latterly included in the study. Nevertheless, 57% of families included were affiliated with the BMHA through current or previous membership.

Progress with the laboratory work has been good with analysis of 68/153 patient samples almost complete for targeted screening of the RYR1 gene which is associated with MH in the majority of cases. Approximately one third of the analysis is presently complete for the remaining 83 patients being studied. Work has been conducted using a combination of MELT and sequence analysis methods. So far mutations have been detected in 33 families. In 7 families a mutation which may be used in genetic diagnosis of MH has been detected – the Leeds MH Unit is now in the process of contacting these families who will immediately benefit from these findings. In the remaining families 13 mutations

previously reported in the literature have been detected and a further 10 novel mutations have been identified.

These findings were recently reported by the research group at the XXVI Annual European MH Meeting held in Siena, Italy at the end of May.

Unfortunately, late last year, the technician ceased working on the project due to an unrelated incident. The MH Unit seconded another member of staff part time (Chris) and the University provided a technician (Nikla) so that the momentum built up would be affected as little as possible. It is a great testament to the commitment of those involved with this project that the above work has been achieved despite the difficulties.

We have also just heard that Rachel will be leaving the unit as she has secured a high-level promotion within the Yorkshire Regional DNA laboratory. A proposal is currently with the Big Lottery Fund to delay the start of year 3 and we will keep you updated when we have more news.

Year one progress

One of the tests originally proposed for searching for mutations in the patient DNA samples was superseded by a new MELT analysis process. This works on the basis that when a stretch of DNA is heated, a fragment with a mutation will 'melt' at a different rate compared to the same fragment without the mutation. If such a difference is observed the DNA requires further analysis to properly characterise the mutation. This is good method of analysis and will provide the researchers with new work for publication. However, it meant that an additional machine was needed by the laboratory for DNA amplification (PCR machine). This was funded mainly by BMHA, independent of this project.

At the end of year one of the project, progress met expected targets. The MELT analysis process had been successfully introduced into the laboratory after much careful optimisation. A database was developed to record and co-ordinate all results for the project. The first group of BMHA members donated blood samples for analysis. The first batch of patient DNA samples was analysed for areas of the RYR1 gene, which from previous investigations appear to be hot-spots for MH related mutations. Some mutations were detected and further characterised.