Pain Relief Foundation Research Grant

'Novel long-acting analgesic for chronic pain 'A Pilot project seeking genetic predispositions to fibromyalgia'

Award of £23,140 to;

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Background

Clinical

Fibromyalgia (FM) is a syndrome of persistent widespread pain, stiffness, fatigue, disrupted and unrefreshing sleep, and cognitive difficulties. Clinical management is challenging. Evidence is limited for the efficacy for drugs and regular use of analgesics, particularly opioids, are considered harmful. Hence, psychological and physical therapies are required to manage the consequences of living with illness.

FM is also a heterogenous disorder, defined based on symptoms that are 'medically unexplained', or persists despite disease remission. The condition is increasingly considered as a disorder of the nervous system, in which there is signal amplification or sensitisation in the nervous system, particularly sensory pathways. However, causative mechanisms at the molecular level remain unclear. Mitochondria are small elements in every cell of our body that have the major function of producing usable energy in the form of molecules, e.g. ATP; many researchers have previously suggested that mitochondrial function underlies FM, but have been unable to prove this.

The initiating event for FM is less clear though qualitative studies is associated with an early major stressor (infection, trauma), which may well prime or sensitise the nervous system. FM, as defined by the ACR 2010, has a prevalence of 5% in questionnaire-based surveys. Cases verified by a clinician are likely to be lower, particularly those, where repeated or extensive investigations have proven entirely negative for connective tissue or neurological diseases. These cases of 'primary fibromyalgia' are therefore more unusual and extreme sub-set of the condition. Cohorts enriched with the phenotype are likely to yield genetic variants that are over- or under-represented in the general population.

Genetics

Molecular genetic approaches have proved extremely powerful to discover and dissect the genetic components underlying many human diseases. Clear examples are, the discovery of highly penetrant, but often very rare, pathogenic mutations causing Mendelian diseases; Association Studies using SNPs with common rare allele frequencies able to discover haplotypes causing small phenotypic effects, which have revealed many disease processes, and in cancer where somatic mutation detection en masse are delineating hitherto unrecognized tumour types and leading to personalized therapies.

Genetic association studies have been attempted in FM. Most are candidate gene studies, but there are at least 2 notable GWAS studies to date. None have provided novel or potentially druggable targets. However, methods are limited by the possibility that FM is more than one disease and that very large numbers of people with FM are needed, typically >1000.

We have described a further genetic approach; functional single nucleotide polymorphism discovery (fSNPd). This was designed to detect genetic components of clinically important disorders that require an environmental trigger to occur, e.g. severe drug reactions, and susceptibility to infection. Our approach optimises discovery of novel genetic variants that could be associated with a disease/phenotype. It

requires typically <100 people, can work despite a condition having more than one cause, and the method detects changes in proteins that can be tested in the laboratory and quickly used to assess treatments.

As examples.

1) We have undertaken fSNPd in a cohort of women having their first delivery who required no analgesic for term spontaneous labour. In that context, birth served as the natural stimulus or trigger for the pain(less) phenotype. The fSNPd is efficient, allowing for discovery of functional rare variants with relatively small cohorts (n<100), providing the phenotype is sufficiently unusual. This led to the discovery of a gene previously unknown to control labour pain, KCNG4 (see, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7383234/). Our work immediately suggests KCNG4 as a target for a novel labour pain analgesic. A second gene discovery from that work awaits publication.
2) A smaller discovery cohort (n<74) was used in a subsequent study in complex regional pain syndrome, (CRPS) where the triggering event (usually a bone fracture) is trivial relative to the severity of the resulting condition. We found changes in four genes, which were all linked to the same inflammation pathway. We then obtain a grant from the UKRI APDP initiative to investigate these findings in more detail to lead to being able to predict which already-available drugs should be used in CRPS.

Finally, dysfunction of the mitochondria has been frequently suggested to be a cause of FM. Each mitochondria in our body has a circular piece of DNA which encodes 37 genes which work exclusively in mitochondria. (Other genes in the nucleus of the cell also contribute to mitochondrial function). Mitochondrial DNA is difficult to sequence /read and to interpret the results. However, a program MToolBox has been written which can use exome (nearly all of the genes in a person nucleus) and genome (all of a person's nucleus DNA) results. Some mitochondrial DNA changes are known to reduce the activity of mitochondria, and two of these have been previously linked to FM.

Aims

1) Ascertain/find 100 people with FM (who have had an expert work-up) who are willing to give us a blood sample for DNA research. For inclusion in our study each person with FM must - fulfil the ACR2016 and are diagnosed following specialist (rheumatology) consultation, and - have inflammatory or connective tissue diseases excluded either by serology or musculoskeletal imaging.
2) Use Next-Gen sequencing to produce an "exome" from each person's DNA – which contains details of the sequence/spelling of each of their nucleus genes.
3) Analyse the exome result with fSNPd – which will detect if any SNP (single nucleotide polymorphism, i.e. "relatively common DNA changes") have statistically unusual results.
4) Confirm any potential findings by Sanger DNA sequencing/reading.
5) Analyse the exome result with MToolBox – which will detect any changes in the mitochondrial DNA SNP that are statistically unusual results.
6) Confirm any potential findings by Sanger DNA sequencing/reading.

Delays

Firstly, the two clinicians' involved (ML and AG) both found that it took longer than expected to ascertain patients with chronic fibromyalgia that met the strict criteria for diagnosis we agree was required. Not all ascertained cases then progressed to be able to participate, and consented to give a blood sample for DNA analysis. In reflexion, we should have expected that this part of the project would take nearer to three years than one to complete. The result of this activity was the ascertainment of 100 patients with unexplained (i.e. known diagnoses excluded), chronic (greater than 6 months, but actually over a year), classical fibromyalgia (e.g. PMID: 34850195 criteria).

Secondly, was the genetic analysis studies. The genomic fSNPd analysis relied on a program written in the CIMR called fSNPd, but this program was found to have stopped working. This was due to upgrades to the PEARL, R and PYTHON programming languages which led to the need for rewriting of sections of fSNPd. The CIMR expert who was going to reprogram fSNPd became ill during COVID and has since passed away.

The MToolBox program was in use by Cambridge colleagues , but they had lost their programmer and their analysis was of mouse single cells. So they could not fulfil their previously offered analysis – and when we installed MToolBox, although fit was free to download, did not work.

We finally found a programmer who has got both fSNPd and MToolBox working over a six month period – which required frequent checks that the expected results were being produced at each stage of these two programs.

Results

Clinical

Two established pain clinics were recruitment sites, Liverpool (49 ascertained, 47 donated DNA) and Cambridge (51 ascertained, 26 donated DNA). So, our clinical research series is of 100 patients, and genetic research series of 73

Genetics – nuclear gene analysis

We have the data from 73 exomes, each from a unique individual with chronic Fibromylagia.
We have checked that the quality of data is satisfactory and that the sex assigned to each person agrees with their genetic sex – so there were no sample mix-ups.
For the whole cohort/group we found no significant changes. For a small, easily definable sub-group we found changes in three genes linked to energy production by mitochondria (these were all genes in the cell nucleus). However, the small number in the sub-group means the results need checking in a larger sample before we will believe them to be correct/ convince clinical and scientific colleagues that the results are correct.

Genetics – mitochondrial gene analysis

There were no significant mitochondrial genome changes found. In particular, a previously reported mitochondrial change associated with FM was not found.

Conclusion

Our results are exciting as they a definable sub-group of FM may have a different disease drivers compare to most. And, recent results from David Andersson at KCL provides strong data supporting a role for autoantibodies causing FM. So maybe most people with FM have autoantibodies, but our sub-group needs both autoantibodies and genetic changes to give them FM. This been said, our genetic results have to be assessed in mote people in our sub-group of FM before we can be sure – and we will need this data before we can raise further funds to take this work further and into therapeutics.

Our mitochondrial results strongly suggest that mitochondrial genome variants/changes are only a rare cause of FM.