

Quinquennial Review

September 2016

Chronic Disease Research Fund

**Executive Summary**

Here at the CDRF we fund a wide range of ground breaking research programmes, which aim to discover the cause of common age-related diseases through the study of identical and non-identical twins.

Arthritis, migraine, asthma, dementia, heart disease, melanoma, deafness are just a few of the diseases that can benefit from our research.

We have funded a number of successful projects at the Department of Twin Research at Kings College where through the use of data gathered from over 12,000 twin volunteers researchers have been able to identify the contribution of genetic and environmental factors to many chronic diseases.

We aim to support the talented scientists and researchers conducting these studies and expand on current findings and maximise the research output. Where possible we aim to pump prime projects that will attract further main stream funding so getting maximum value for money from the limited funds.

Throughout the last 5 years the CDRF has receive d £981,220.59 in revenue and has paid or committed £756,335.18 as grants for medical research.

**Overview**

**Equipment**

2016

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**Grant awarded: £1,707**

**Timescale: one off**

**The CDRF has provided funds towards the cost of the DTR’s DEXA machine.**

A DEXA scan is a special type of X-ray that measures bone mineral density (BMD). DEXA stands for "dual energy X-ray absorptiometry". DEXA scans are often used to diagnose or assess someone's risk of osteoporosis; a condition that weakens bones and makes them more likely to break. As well as being quick and painless, a DEXA scan is more effective than normal X-rays in identifying low bone mineral density.

**Project Grants**

2016-ongoing

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**Grant awarded: £488,040**

**Timescale: 3 years**

**The Urinary Microbiome and its Relation to Morbidity in Older People**

Urinary symptoms and signs in the elderly are closely associated to frailty, delirium and distress, but our understanding of the relationship of the urinary flora and morbidity is currently very limited, leading to overtreatment with antibiotics. This study has the potential to re-write text books, which have propagated a false dogma that the urine is sterile, and create the first step to understanding the implications for the host of different urinary patterns of microbes. First cataloguing the populations of microbes in older people is essential at this stage of the science, to generate hypotheses that would be testable experimentally. In the shorter term, easy-to-obtain mid stream urine analysis could also be used to stratify management of older adults.

2014-2017

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**Grant awarded: £315,000**

**Timescale: 3 years**

**The role of the gut microbiome in cognitive decline and risk for dementia.**

The aim of this three-year project is to explore patterns of human faecal microbiome with cognitive performance and decline in older adults, utitizing up to 900 older twins studied longitudinally to control for host and family influences and explore metabolic mechanisms.

**Pilot Studies**

2016-ongoing

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**Grant awarded: £9,917.14**

**Timescale: 3 years**

**Biotwincot – Biology of twin from conception to toddler**

Foetal programming – the adaptive responses of the foetus to a variety of environmental cues, and consequences of mismatch between the prenatal and postnatal environments – can permanently shape the body’s structure, function, and metabolism and contribute to adult disease. The influence of the microbiome – the microorganisms living in and on a mammalian host - and how it is acquired in humans is poorly understood.

This project addresses this deficiency by studying the human microbiome longitudinally during early development, from the earliest possible time during pregnancy, using nature’s controlled experiment, twins. The project aims to pilot collection of microbiome and additional biological samples from 10 mothers and twin-pairs at four time-points during the first year of life, to assess the feasibility to support a major project of this kind.

2015-2016

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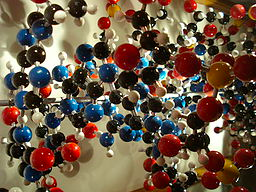
**Grant awarded: £7,013.30**

**Timescale: 3 years**

**Investigation of microbiome in rheumatoid arthritis discordant monozygotic twins**

There is good evidence that rheumatoid arthritis (RA) is influenced, and perhaps even caused, by alterations in the gut microbiome. The microbiome is, in turn, influenced by the host organism genotype. Thus twins are the perfect experimental model in which to study the influence of gut microbiome in RA.

2013-ongoing

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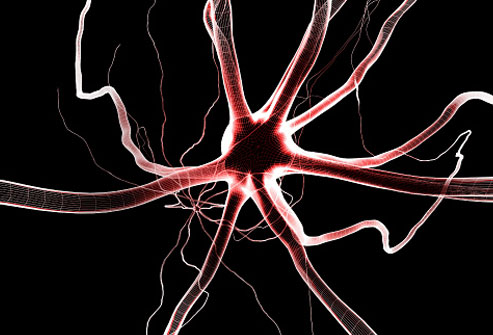
**Grant awarded: £9,695.50**

**Timescale: 4 years**

**Funding for twin recruitment and DNA Collection, in Rheumatoid Arthritis.**

Recruit and examine new twin pairs with at least one member of each pair having RA, in collaboration with patients associations and clinical rheumatologists in the UK, to perform DNA and RNA extraction and store blood samples for study of RA.

2012-ongoing

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**Grant awarded: £5,000**

**Timescale: 4 years**

**Funding for DNA collection in fibromyalgia.**

Establish a DNA resource from 2000 people having chronic widespread pain (CWP) will be constructed and then genomic/epigenomic pain candidates identified in twins will be validated in patients.

2011-2012

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**Grant awarded: £18,217.74**

**Timescale: 6 months**

**Understanding the genetic predisposition to migraine-a meta-analysis approach.**

Migraine is the most common brain disorder; however the molecular mechanisms giving rise to them are poorly understood. The migraine consortium involved research into the genetic determinants of migraine and its subsets, migraine with aura and migraine without aura.

Final reports and publications

**Equipment**

2016

**DEXA MACHINE**

**Grant awarded: £1,707**

**Timescale: one off**

A DEXA scan is a special type of X-ray that measures bone mineral density (BMD). DEXA stands for

"dual energy X-ray absorptiometry". DEXA scans are often used to diagnose or assess someone's

risk of osteoporosis, a condition that weakens bones and makes them more likely to break. As well

as being quick and painless, a DEXA scan is more effective than normal X-rays in identifying low

bone mineral density.

The CDRF has provided funds towards the cost of the DTR’s DEXA machine. The DEXA machine is a

crucial part of the twin visit, and the results from it are always shared with twin volunteers. This

information has been clinically useful for a number of twins in alerting them to low bone mineral

density and enabling them to seek early treatment.

The DEXA image data that is collected is of great value nationally and internationally, with a large

consortium based in The Netherlands (GEFOS) continuing to use the data to find genetic variants

associated with the control of bone mineral density (BMD) and fragility fractures. In addition, the

data is being used to study the relationship between BMD and intervertebral disc degeneration,

one of the major causes of back pain, in collaboration with Hong Kong University and builds on a

previous published research (see reference below).

The data from the DEXA machine is also playing a critical role in our studies of metabolic syndrome

based on our knowledge that the way in which fat is distributed around the body influences its risk

to health. The DEXA results have also been used to study the metabolic disturbance seen in

chronic widespread pain, which is associated with raised body mass index. Using DEXA results, DTR

researchers showed for the first time that the most highly associated measure with pain was fat

mass over height squared, proving that it is indeed fat that plays a role rather than other tissues.

Researchers have also shown that increased weight precedes the onset of chronic widespread

pain, so we can use this information to consider fat as a true risk factor for pain, and are now

trying to ascertain why.

**PUBLICATIONS**

Livshits G, Macgregor AJ, Gieger C, Malkin I, Moayyeri A, Grallert H, Emeny RT, Spector T,

Kastenmüller G, **Williams FM**. An omics investigation into chronic widespread musculoskeletal pain

reveals epiandrosterone sulfate as a potential biomarker. **Pain**. 2015 Epub ahead PMID:25915148

Livshits G, Ermakov S, Popham M, MacGregor AJ, Sambrook PN, Spector TD, Williams FMK.

Evidence that bone mineral density plays a role in degenerative disc disease: The UK Twin Spine

Study. Ann Rheum Dis, 69(12):2102-6, 2010.

**Project Grants**

2016-ongoing

**THE URINARY MICROBIOME AND ITS RELATION TO MORBIDITY IN OLDER PEOPLE**

**Grant awarded: £488,040**

**Timescale: 3 years**

The grant for this research project was approved in May 2016. Claire Steves, the project lead, is currently on Maternity Leave thus the project will begin as soon as she returns in September 2016. There are therefore no reports for this project at this time.

2014-2017

**THE ROLE OF THE GUT MICROBIOME IN COGNITIVE DECTINE AND RISK FOR DEMENTIA**

**Grant awarded: £315,000**

**Timescale: 3 years**

The human gut contains almost 100 trillion bacteria whose collective genome has been termed the microbiome. This plethora of different organisms significantly enrich the metabolism of the human ‘superorganism’1, comprising 150 times more microbial genes than the human gene complement alone 2. The microbiome of older adults appears to vary dependent on level of health dependency – with patients in continuing care having the least diverse microbial community.

Dietary factors appear to drive differences in microbiome profiles6. As well as effects on nutrient processing, and vitamin biosynthesis, microbial gut commensals have local and systemic effects on the immune system of the host 7. A number of studies, both animal and human, have demonstrated a relationship between the gut microbiome and risk of obesity8,9. Although causality in many studies is unclear, faecal transplantation has been shown to improve insulin sensitivity in a randomized controlled trial10 and transplants from human twins to germ-free mice have led to changes in obesity and health 11. In addition, ‘probiotic’ cultures have varying degrees of evidence for effectiveness in preventing antibiotic induced diarrhoea, upper respiratory chest infections, and allergy 8.

Microbiota are also thought to contribute to the gut-brain axis, which refers to the mutual cross-talk between gastrointestinal and mental health. In animal studies, the microbiota has been shown to alter brain biochemistry (hippocampal BDNF levels) 12, function (hypothalamic-pituitary axis) 13, and behaviour (anxiety) 14. Changes in microbiota associated with dietary changes were also temporally associated with changes in memory and learning in male mice 15.

Human studies have supported extrapolations from the animal literature that the microbiome is influential in human behaviours – such as appetite regulation, satiety 16, and pain responses 17, and is associated with autism spectrum disorders 18. *Coprococcus, Barnsiella* and *Actinomyces* have all been linked with autism 19-21, while *Faecalibacterium prausnitzii* and the Enterobacteriaceae family have been linked with cognitive function in hepatic encephalopathy22. There is also some evidence that probiotics improve mood/anxiety in humans 23 particularly with those with relatively low mood to begin with 24. However, this latter small study also reported deleterious effects of probiotics on memory.

Despite the known relationship between mood disorder in older adults and cognitive decline, very few studies in humans have reported on association of the gastrointestinal microbiome patterns and memory and cognition. An association between the gut microbiome and cognitive impairment as measured by the Mini-Mental State Examination (MMSE) 6 has been reported, but this may partly be accounted for by overall frailty, dependency and altered diet in later stages of disease. In view of the substantial prodrome before dementia is realized, it is important that studies of the effect of the microbiome on cognition and risk of dementia start early enough to establish the direction of any temporal association.

There are compelling reasons why a causal association between the microbiome and cognitive decline in later life might be observed. The inflammatory response, augmented or dampened by an individual’s microbial pattern, may have direct influence on the development of cognitive impairment, delerium and dementia. Important neurotransmitter metabolic pathways, such as tryptophan metabolism are known to be influenced by the microbiome, and microbial metabolites have central nervous system effects 17. Moreover, diet has been identified to be a significant factor in cognitive ageing, and may exert its influence via the microbiome. Clearly, if suggestive temporal associations are found between specific microbial patterns and subsequent cognitive decline, this may be readily translatable into interventions – either via probiotics or dietary changes – to reduce cognitive ageing and dementia.

During this grant period we have conducted a number of studies to investigate the link between the human gut microbiota and cognition. Below you will find an outline of each project, a summary of results and published papers.

**Ongoing Projects**

**Cognitive Function**

This study aims to investigate study the link between cognitive function and the human gut microbiome within a sample of up to 900 community twin volunteers.

In this initial analysis, three exclusively cognitive phenotypes were selected as markers of cognition; Deary-Liewald Reaction Time (DLRT), Mini-Mental State Examination (MMSE) and Verbal Fluency.

*Verbal Fluency*

111 OTU associations surpassed nominal significance, although none surpassed a multiple testing threshold. A number of the top hits were composed of negative associations between OTUs in Enterobacteriaceae, suggesting an increase of these bacteria in individuals with a lower verbal fluency score.

Enterobacteriaceae has previously been found to be increased in individuals showing lower cognition in hepatic encephalopathy22. The Enterobacteriaceae are an interesting group due to the high number of pathogens within this group.

Given the lack of power to detect associations at the OTU level, we collapsed the OTUs to the species, genus and family level and ran associations between these and verbal fluency. No associations at any collapsed level surpassed a multiple testing threshold. 4 associations at the family level surpassed nominal significance, 15 at the genus level and 19 at the species level. The top association at the species level was with *Dysgomonas gadei,* and was the most significant association of all the results (P=0.00017, beta=-0.211). Little is currently known about this species of bacteria. One of the top hits at the genus level was with *Barnsiella* (p=0.002, beta=-0.17) that has previously been linked with austim.

*DLRT*  
  
In the DLRT dataset there were 840 individuals that possessed both DLRT data and microbiome data. For this analysis, the choice-reaction time test was used, in particular the mean time taken to respond correctly. There were 24 nominally significant OTU associations (p<0.05), however no associations surpass a multiple testing threshold when considering false discovery rate. The top association was with *Oscillospira*, a prevalent member of the human microbiome. The second association was with *Faecalibacterium prausnitzii*, which as mentioned above has been previously linked with cognition in hepatic encephalopathy.

There were 3 nominally significant associations with the collapsed genus’ Oscillospira, Lachnobacterium and an unknown genus within the Clostridiales. 1 order, Lactobacillales was also nominally significantly associated with DLRT (p=0.03, beta=-0.05).

*MMSE*  
   
TwinsUK is a healthy cohort, and while the dataset was fairly large with 978 individuals, only 20 of had MMSE < 26. At this stage, the ceiling effect of this test precludes its analysis. More data has recently been received and as such all the above cognitive function analyses, including MMSE, will be repeated with an increased sample size.

*Future of the project*

With the addition of batch 3 microbial data, sample sizes can be extended. Therefore, the coming months will focus on re-running all past analyses with a larger sample size. In addition to this, we will use the larger sample size to determine microbial functional alterations in cognitively impaired individuals using the program PICRUSt.

**SCFA (Faecal Acquisition Study)**

Short-chain fatty acids (SCFAs) are primarily produced in the colon via microbial fermentation and are important not only for colonic health, but may also influence obesity, diabetes and cardiovascular disease30. Furthermore, some SCFA such as n-butyrate, acetate and proprionate are known neuroactive compounds31 that by interacting with G protein-coupled receptors32,33 can influence memory decline34 and autism35. For this reason, we were interested to measure SCFA concentrations in faecal samples held in storage at TwinsUK. Due to the volatile nature of SCFA we wished to test whether or not we could extract SCFA from samples that had undergone our collection procedure, i.e. refrigerated for up to 3 days prior to freezing at -80. We conducted a pilot study where we collected 2 samples from the same faeces from 5 individuals, freezing one sample immediately and the other after 2 days of refrigeration. Samples were then homogenised and subjected to gas chromatography for measurement, resulting in final concentrations.

Samples that underwent refrigeration prior to freezing showed an increase in overall SCFA concentration suggesting that refrigeration is not enough to stop microbial fermentation. The SCFA profile however remains largely stable, but there does seem to be significant alterations in levels of butyrate during refrigeration. This is a very limited sample size however making it difficult to extrapolate to a larger population and as such we have decided to broaden this study and collect more samples to investigate this effect further. This we have named the Faecal Acquisition Study (FAQS). In addition to refrigeration effect, we will also collect Bristol Stool Chart scores, and measure faecal water content to determine if these factors affect SCFA content in stool.

**Microbiome Sampling Review**

It came to our attention recently that while there is an abundance of literature that discusses various sequencing methods and techniques in microbiome studies, there is a lack of literature which considers sampling and storage biases in microbiome research. We performed some analysis in our own TwinsUK cohort to explore the effect of length of time spent refrigerated prior to freezing, as well as length of time spent in frozen storage on faecal microbiome profiles. These results, as well as a review of current literature have been written up in to a review article. The manuscript will shortly be submitted to PLoS One.

**Completed Projects**

**Benchmarking of methodology used to summarise gut microbiome data sets**

The gut microbiome is often characterised by sequencing of the 16S rRNA genes within DNA extracted from stool samples. This gene has high variability between bacterial species enabling the quantification of different bacteria within samples, which can then be used to investigate associations between bacteria and disease.

A number of different methods can be used to summarise the complex 16S sequencing read data into a manageable format for data analysis. The standard approach collapses sequences based on their similarity into units called operational taxonomic units (OTUs). We have carried out a large comparison of different computational tools to create OTUs from sequencing data. To do this we used the power of the twin structure of our cohort to find which methods produced the most heritable OTUs. This is the first application of heritability in methodological comparisons and allows us to gauge the biological relevance of the OTUs generated by a method.

This work enables us to determine which is the best method to use to generate OTUs that accurately represent bacteria, and should improve the quality of the data we are using in further investigations of microbiome and disease interactions. This project has also recently been accepted for publication, and we hope its dissemination will provide guidance to other researchers investigating similar human microbiome associations (see Publications list).

**PPI Use**

Proton pump inhibitors (PPIs) are drugs to treat GI disorders such as gastric reflux and peptic ulcers. Considered to be low-risk medication, they are distributed quite widely, particularly to elderly and frail populations and emerging research has shown that PPIs may not be as benign as originally thought27,28. In a sample of 1827 twins, PPI usage was associated with a significant decrease in microbial diversity (p<0.05) and a significant increase in Streptococcaceae (q<10-6, β=0.46). This association with Streptococcaceae, as well as 7 others, was further replicated in an independent study of drug intervention29. Given the significance of these findings, caution should be used in prescribing PPIs unless absolutely necessary. This work has been published in *Gut* (see Publications list).

**Frailty**

While not a direct measure of cognitive function, there is a well recognized association between cognitive frailty and generalized frailty as measured using the Rockwood Frailty index, a finding which is also found in the TwinsUK dataset (Negative association between frailty and verbal fluency: Beta= -1.87 p= 2.2x10-16). We were therefore interested to characterise the association between frailty and the human gut microbiota. In the TwinsUK cohort, 728 female twins possessed both microbiome and frailty data. The cohort has a relatively low level of frailty (14% considered pre-frail). Frailty was found to associate with the microbe *Faecalibacterium prausnitzii* negatively, suggesting a reduction of this microbe in frailer individuals, consistent with previous research 25,26. After adjustment for microbial alpha diversity (within sample diversity), three microbes, *Eubacterium dolichum*, *Eggerthella lenta* and *Faecalibacterium prausnitzii*, remained significantly associated with frailty, independently of PPI and analgesic use (q = 0.013, q = 0.048 & q = 0.027 respectively). This finding was replicated within the Eldermet cohort, a cohort of frailer individuals in Ireland. These three microbes may make therapeutic targets for reducing the rate of cognitive decline, or they may even be useful as probiotics to help slow down the rate of cognitive decline. This work has been published in *Genome Medicine* (see Publications List).

**Microbiome and Musculoskeletal Conditions of Aging**

Recent understanding of the microbiome is highlighting the importance and effect of the human gut flora on the adaptive and innate immune system. A number of autoimmune diseases and inflammatory diseases are now being linked with changes in the human gut microbiome and given the potential therapeutic target that the microbiota presents, this developing knowledge could be vital in providing new treatment options for patients. We outline this recent literature in a review, covering the conditions: Frailty, sarcopenia, osteoporosis and osteoarthritis, rheumatoid arthritis, gout and drug interactions. This work was published in American Society for Bone and Mineral Research (see Publications list).

**PUBLICATIONS**

Beaumont M, Martin T, Spector TD, Steves CJ. (In preparation, 2016) **Perspective on faecal sample collection and storage for microbiome studies.**

Jackson MA, Bell JT, Spector TD, Steves CJ. (Forthcoming, 2016) **A heritability-based comparison of methods used to cluster 16S rRNA gene sequences into operational taxonomic units.** *PeerJ*

[Steves CJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Steves%20CJ%5BAuthor%5D&cauthor=true&cauthor_uid=26676797), [Bird S](http://www.ncbi.nlm.nih.gov/pubmed/?term=Bird%20S%5BAuthor%5D&cauthor=true&cauthor_uid=26676797), [Williams FM](http://www.ncbi.nlm.nih.gov/pubmed/?term=Williams%20FM%5BAuthor%5D&cauthor=true&cauthor_uid=26676797), [Spector TD](http://www.ncbi.nlm.nih.gov/pubmed/?term=Spector%20TD%5BAuthor%5D&cauthor=true&cauthor_uid=26676797). (2016) **The Microbiome and Musculoskeletal Conditions of Aging: A Review of Evidence for Impact and Potential Therapeutics.** [*J Bone Miner Res.*](http://www.ncbi.nlm.nih.gov/pubmed/26676797)

Jackson MA, Goodrich JK, Maxan ME, Freedberg DE, Abrams JA, Poole AC et al. (2015). **Proton pump inhibitors alter the composition of the gut microbiota**. *Gut*

Jackson MA, Jeffery IB, Beaumont M, Bell JT, Clark AG, Ley RE et al. (2016). **Signatures of early frailty in the gut microbiota.** *Genome medicine, 8*(1), 8-8.

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**Pilot Studies**

2016-ongoing

**BIOTWINOT: BIOLOGY OF TWINS FROM CONCEPTION TO TODDLER**

**Grant awarded: £9,917.14**

**Timescale: 3 years**

Investigators: ***Dr J Bell,*** Dr D Patsupathy, Dr D Hart, Prof T Spector

**Summary:** Fetal programming – the adaptive responses of the foetus to a variety of environmental cues, and consequences of mismatch between the prenatal and postnatal environments – can permanently shape the body’s structure, function, and metabolism and contribute to adult disease. The influence of the microbiome – the microorganisms living in and on a mammalian host - and how it is acquired in humans is poorly understood. This project addresses this deficiency by studying the human microbiome longitudinally during early development, from the earliest possible time during pregnancy, using nature’s controlled experiment, twins. The project aims to pilot collection of microbiome and additional biological samples from 10 mothers and twin-pairs at four time-points during the first year of life, to assess the feasibility to support a major project of this kind.

**Aim: *The proposal aimed to conduct a pilot study to assess the feasibility of a large-scale funding application for a major project of this kind.*** The overall objective of the project is to create a clinical database and biobank of twin pregnancies and infants, which will ultimately include microbiome, genetic, phenotypic and clinical data to address the aims of this study. We would specifically prospectively collect demographic information; ultrasound scan images, volumes, and measurements; pregnancy (maternal/foetal), delivery, and neonatal data; blood results; and collect and store biological samples for both current and future research.

**Study Update**

***Aim:*** The funded proposal created ***a multi-disciplinary research network*** comprising experts in twin research, neonatal medicine, genomics and epigenetics, and microbiome research, who hold regular scientific meetings. As a result of the scientific discussions we now have concrete plans to submit a large-scale funding application to the Wellcome Trust.

***Ethical approval:*** We have experienced a slight delay with the study ethics application and approval process. We submitted an application to obtain ethical approval for the study to the London Bridge Research Ethics Committee. The application was approved pending several amendments, which somewhat delayed us. While we have now obtained ethical approval for the study, we are currently awaiting for the final approvals to be set in place at the local level, in order for the sampling to commence. The local R&D approval process was undergoing changes at the administrative level at the time of application and we were advised to await submission until these changes took place. The application for local approval is now being reviewed and we anticipate a positive outcome within the next weeks.

***Methods:*** The study sampling protocols have been drafted and are now confirmed at the clinical level. These protocols are now in place for sampling to begin. All of the consumables have been purchased and are also now in place. A laboratory database has been set up for this particular study. The database covers sample collection, processing, and storage. It also has the potential to be expanded to incorporate information on future sample handling, processing, and shipment.

***Staff:*** The investigators with help from administrative staff have prepared and submitted the study ethical approval application. As soon as the final local ethical approvals are in place we will begin sampling. The staff costs that we requested on the grant will be allocated towards collection and processing of the samples.

**PUBLICATIONS**

There are currently no projects linked to this project.

2015-2016

**INVESTIGATION OF MICROBIOME IN RHEUMATOID ARTHRITIS DISCORDANT MONOZYGOTIC TWINS**

**Grant awarded: £7,013.30**

**Timescale: 3 years**

**2015 - current**

Frances MK Williams, Reader and Hon Consultant

Dept of Twin Research and Genetic Epidemiology, King's College London

**Summary**

There is good evidence that rheumatoid arthritis (RA) is influenced, and perhaps even caused, by alterations in the gut microbiome. The microbiome is, in turn, influenced by the host organism genotype. Thus twins are the perfect experimental model in which to study the influence of gut microbiome in RA. In this study we will identify twin pairs in TwinsUK in which one member has a diagnosis of RA. We will invite the pair for a twin visit and provide, prior to the visit, a stool collection kit. Twins attending the visit will undergo physical examination, demographic and antirheumatic drug information, and venous blood draw. Stool will be analysed for microbial content and microbial "richness" will be analysed with presence/absence of RA and correlated with disease activity. The results of this study will provide pilot data for a larger application to a grant funding body to explore in greater depth the microbial species implicated in RA pathogenesis and other rheumatic conditions.

**Background**

The microbial population of bacterial species which reside in a mammal's gut has been shown to be intimately linked to host health and disease. This has been found not only for disorders of the gut and liver [[1](#_ENREF_1)], but also for spondylarthritis [[2](#_ENREF_2)]and other conditions influencing sites far removed from the GI tract [[3](#_ENREF_3)]. Rheumatoid arthritis (RA) is an autoimmune condition. Besides genetic predisposition a number of other risk factors have been identified, but their precise mechanism of action leading to RA remains unclear. Smoking and periodontal disease are two such risk factors and their effect on RA risk may be mediated by alterations in the microbiome [[4](#_ENREF_4)]. Small studies have investigated the microbiome in unrelated RA patients and controls, however there is known to be an interaction between host and microbial genomes: that is, the metagenome is itself heritable [[5](#_ENREF_5)]. Thus it is difficult to know whether RA predisposes to microbial constituents or vice versa. Identical twins are the perfect experimental design to answer questions of direction of causation, as they are completely matched for genetic factors. Thus twin pairs in which one member has RA, allows us to investigate in a controlled fashion, the association between metagenome and RA.

We have already identified twins within the TwinsUK registry having RA and recruited them recently to an epigenetic study of RA. In the present small study, five pairs of RA discordant MZ pairs will be invited to attend DTR for a clinical visit. In that visit further information about RA would be collected, blood taken and stool sent for analysis of the microbiome. Ethics committee approval has been obtained.

**Aims**

The aims of this study are to determine if :-

1. microbial differences can be detected in twins having RA compared to their unaffected co-twins

2. microbial species richness correlates with RA disease activity

The pilot data generated were used to support a much larger grant application to Arthritis Research UK to perform similar studies in a larger number of twins and a wider variety of arthritic conditions. The increased sample size would provide the power to determine whether particular microbiome species predispose to the development or severity of RA.

**Progress**

We were approached for collaboration by immunology professor Daniel Altman of Imperial College and agreed to perform a small pilot study together which would provide data in support of a larger Arthritis Research UK proposal. The charity had recently visited the Dept of Twin Research, at FW’s suggestion, and had been shown around the new department and given up to date information on all the rheumatological studies that had been performed using TwinsUK since its inception in 1992, which was funded by the charity. At that meeting it became apparent that microbiome studies were going to be a major plank of future funding in rheumatic disease research. With the existing microbiome interest and sample collection and analysis pipeline already in place in the DTR we were well placed to apply for grant monies.

Five twin pairs identified has having a member affected by RA were invited to St Thomas' Hospital for a twin visit during a single week in June 2015. They were sent stool sample collection packs prior to the visit and asked to bring in fresh stool. During the visit they were examined for RA by a trained research assistant, demographic data collected and venous blood drawn. This will be used for DNA collection and omics technologies in the future. In addition, our collaborators from Immunology at Imperial arrange for collection of hot blood which goes straight to Imperial for same day PBMC separation and analysis of T cell subtypes by flow cytometry. Serum was sent to the immunology laboratory at St Thomas' Hospital (Viapath) if twins have not already been tested for rheumatoid factor and anti-citrullinated proteins. Stool was sent to our collaborator Ruth Ley in Cornell University, USA, for analysis of the microbiome.

The data have been returned to the DTR and are in the process of being QCed. Analysis of microbiome richness and intrapair difference in species will be performed, allowing inferences to be drawn about the influence of microbiome on the expression of RA. Furthermore, species richness will be correlated with RA disease activity.

**Outcomes**

The RA microbiome pilot study in collaboration with Imperial supported two applications to Arthritis Research UK both of which were awarded:

1. Pathfinder grant led by Prof Altman at Imperial College entitled ‘Towards functional correlates of microbiota, immune phenotype and RA’ with Frances Williams as co-PI: **£198,000**

The Pathfinder grant has started and a kick off meeting held at Imperial in June 2016.

2. Strategic award led by Frances Williams entitled “The role of the microbiome in rheumatoid arthritis” **£936,000**

The RA microbiome work in this strategic award has been expanded to include not only gut microbiome (stool sample) but also oral microbiome (saliva sample) because there is good evidence that periodontal disease is a significant trigger in RA. This will be novel, and few data exist at present on the oral microbiome. In addition to twins, patients are going to be recruited having new RA to see if the introduction of disease-modifying treatments influences the microbiome (at both sites). We are working with the consortium EUDiscotwin to obtain samples from twin pairs discordant for RA from all over Europe from whom we will collect samples by post.

This means that further RA microbiome studies can take place, and staff are currently being recruited to collect and analyse further microbiome data. The strategic award will start in the autumn, but recruitment to posts is taking place at present.

In addition to these awards, and leveraging off them:-

1. the Dean of KCL has provided MRC funding for a microbiome PhD student. This student is going to study the microbiome in chronic pain and chronic pain syndromes.
2. An application to King’s College Hospital Joint Medical committee was made to support a PhD student in RA microbiome studies

Interviews for both these studentships will take place next week.

**PUBLICATIONS**

Steves CJ, Bird S, **Williams FM**, Spector TD. The Microbiome and Musculoskeletal Conditions of Ageing: A Review of Evidence for Impact and Potential Therapeutics. **J Bone Miner Res**. 2015 Dec 16. doi: 10.1002/jbmr.2765. PMID: 26676797

**References**

1. Qin N, Yang F, Li A, Prifti E, Chen Y, et al. (2014) Alterations of the human gut microbiome in liver cirrhosis. Nature 513: 59-64.

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2013-ongoing

**TWIN RECRUITMENT AND DNA COLLECTION, IN RHEUMATOID ARTHRITIS: THE RA EPIGENETICS PROJECT**

**Grant awarded: £9,695.50**

**Timescale: 4 years**

**2013 - current**

Dr. Flore Zufferey (Swiss Rheumatology Fellow) and Dr. Frances Williams (Reader and Honarary Consultant)

**King’s College London**

**Abstract** Rheumatoid arthritis (RA) is the most common inflammatory arthritis. Both genetic and environmental factors participate in the disease mechanisms of RA pathogenesis. Although RA is known to be heritable, the disease concordance rate between monozygotic twins (MZ) is low, around 12-15%. Identifying how non-genetic variation, including epigenetic factors, could influence complex disease aetiology is now a leading research area. Various study designs are emerging in the exploration of genome wide epigenetic changes for epigenome-wide association studies (EWAS). Among these, MZ twins discordant for the disease represent a very useful resource. In this project, we investigated methylation of DNA in MZ twins discordant for RA, as a potential disease-causing epigenetic factor.

**Funding was requested**

1. to recruit and examine new twin pairs with at least one member of each pair having RA, in collaboration with patients associations and clinical rheumatologists in the UK
2. to perform DNA and RNA extraction and store blood samples for study of RA.

**Background**

RA is a chronic, systemic, inflammatory disorder. The disease predominantly affects the joints but it is also a multi-systemic condition with extra-articular involvement (which may be life threatening) in about 40% of patients over a lifetime of disease. The disease constitutes a worldwide major health problem associated with high morbidity, significant healthcare costs and increased mortality.

RA is an immune-mediated disease, of still unknown aetiology. Criteria - including clinical, serological and biological markers (2010 ACR/EULAR) - assist clinicians in the diagnosis of RA. Nowadays, two autoantibodies of clinical utility (RF, i.e rheumatoid factor and anti-CCP, i.e anti-cyclic citrullinated peptide) provide diagnostic and prognostic values in a large sub-group of patients. Genomics has advanced our understanding of RA susceptibility, pinpointing markers inside and outside the MHC, among which the *HLA-DRB1* genedrives the strongest association. Studies also suggest different genetic susceptibility between anti-citrullinated protein antibodies (ACPA)-positive and ACPA-negative patients. Cumulatively, genetic markers identified to date explain only 50% of RA heritability9. Disease modifying antirheumatic drugs (DMARDs) have been developed in the last years, including biological agents which target various cytokines and components of the immune systems. Identifying new pathways involved in the pathogenesis of the disease may allow better diagnostic classification, prognostic value and target therapeutic intervention.

The aims of this project were:-

1. **to expand the existing TwinsUK RA resource**
2. **to collect serum samples relating to RA immunology during clinical visit or via mail (RF, anti-CCP, FBC, ESR)**
3. **to perform DNA and RNA extraction from blood**
4. **to test for DMRs in the DNA of RA discordant MZ twin pairs**

**Methods**

Compelling evidence suggest influences of genetic, environmental and stochastic factors on epigenetic variation19-21. Twins provide a unique opportunity to study DNA methylation, because they are perfectly age matched as well as controls for nearly all genetic variants and many environmental factors5,22. MZ disease-discordant study design has yet been efficiently applied in a few EWAS23-24.

Among the various epigenetic mechanisms, DNA methylation is the most studied and readily measurable using high throughput technique12-13. Methylation of DNA cytosine residues at the carbon 5 position, generating 5-methylcytosine (5mC), occurs primarily in the context of cytosine-guanine dinucleotides (CpGs). Methylation target sequences, which are regions of the genome with high CpGs content, termed CpG islands, are distributed in a non-uniform manner across the genome. CpGs islands are enriched in promoters in vicinity of transcriptional start sites and are often hypomethylated when associated with the promoter regions of actively transcribed genes, whereas CpG DNA methylation is increased in the gene bodies of actively transcribed genes in mammals14-15. If DNA methylation is altered at multiple adjacent CpG sites, this is refereed to as a differentially methylated region (DMR)5. The delineation of regional DNA methylation patterns, and broader DNA methylation profiles, has important implications for understanding how epigenetic changes might enable aberrant expression patterns and disease5,12.

Various experimental methods have been developed for genome-wide methylation mapping, each having its pro and cons.Methylation data have been generated in TwinsUK using methylation arrays (Illumina HumanMethylation450 BeadChip) or MeDIP-sequencing at Wellcome Trust Sanger Institute, England and Beijing Genomics Institute (BGI), China. Twin Research has long-term previous and current collaborations with these institutes through several other projects. The costs of this analysis will be met by other projects, so were not requested.

In comparison with case control studies, the MZ discordant twin strategy using sufficient sample size and increasing methylation coverage should allow the capture of smaller effects and increase the signal to noise ratio. It is well recognised that the power of this type of approach to detect disease-related DNA differential methylation effects depends on the coverage of the methylation status and sample size31. Regarding study scale and power, based on previous studies for other phenotypes with a similar approach, a minimum of 25 MZ discordant twin pairs are necessary to have sufficient power to identify DMRs that replicate in independent samples32-33. In this project we aimed to recruit 50 pairs MZ pairs to optimise our chances of discovery.

**Progress**

Dr Zufferey used existing TwinsUK questionnaires to identify twin pairs in which one twin member seemed likely to have RA while the co-twin was unaffected. Those pairs in whom there was ambiguity of diagnosis and who were will to attend were invited for a clinical visit at the Dept of Twin Research, St-Thomas Hospital. Dr Zufferey examined the patients and recorded joint and medication details as well as taking peripheral blood for analysis. Where this is not possible, blood was collected by mail, a method of which we have great experience. Blood is a known relevant tissue for several reasons: 1) diagnosis of RA is made through the presence of rheumatoid factor and anti-CCP 2) whole blood methylation is correlated with other tissues.

In addition we sought new twins with RA from other sources including the rheumatology clinic (asking patients with RA if they happened to have a twin – 1 in 80 people is a twin). We also asked contacts at the National Rheumatoid Arthritis Association (NRAS) charity and support group if they would advertise the project on their website. This they kindly did in April 2013 and several twin pairs got in touch with DTR as a result of seeing the publicity. As a result we requested NRAS do the same again in July 2014 and they also mentioned the research as part of their enews newsletter.

In late 2013 we approached Manchester University for a collaboration on the RA epigenetics project. We had seen an abstract of theirs showing interest in epigenetics of RA and they have the largest collection of twins having RA in the UK. We could offer in-house expertise in epigenetics and greater knowledge of the technologies available. This was formalised with an application by Manchester to form a DTR collaboration in January 2014.

Twin attendance at DTR

52 individuals completed the RA study, 50 individuals were seen between 07/06/2013 and 17/12/2013 and 1 twin pair completed the study on 17/01/2014. In addition

* 7 postal bloods were drawn by twins’ GPs and were sent to the department by August 2013
* other twins already had provided blood samples on previous visits

The importance of seeing twins is to ensure the diagnosis of RA is correct. Thus a number of twins were invited to attend based on their previous reports of having RA were found to have osteoarthritis, psoriatic arthritis or gout. These individuals became ineligible for the RA study.

Thus 34 DNA samples were considered to come from RA discordant twin pair who had consented to be in the study. These samples were were sent to Beijing Genome Institute in Sept 2013 for MeDIPseq analysis.

* MeDIP data were returned to DTR in 2014
* results of 17 discordant pairs (34 methylomes) were sent to Manchester (February 2014) to provide a total study sample of 78 twin pairs.

**2016**

The collaborative project has been running almost 3 years now. We have shared our MeDIPseq with Manchester who analysed the total sample of 78 pairs. Unfortunately this type of epigenetic measurement (MeDIPseq) has been found to have poor signal to noise ratio and we were not convinced of any signals in the rheumatoid epigenome. This work was written up and submitted for publication in Annals of Rheumatic Diseases (November 2015) and Arthritis and Rheumatism (January 2016) but because the findings are negative it was accepted at neither.

While all **four aims of this grant have been met**, it is disappointing not to have identified any new methylation signals in RA. However, this technology is in its infancy and improvements are made all the time. On review of the data with our Manchester collaborators, it has been decided to re-analyse the data using different methods to see if this reveals any RA-associated signals. Depending on the results this work will be resubmitted for publication before the end of 2016.

**PUBLICATIONS**

Zufferey F, **Williams FM**, Spector TD. Epigenetics and methylation in the rheumatic diseases. *Semin Arthritis Rheum,* 43(5):692-700, 2014

2012-2015

**DNA COLLECTION IN FIBROMYALGIA – THE FIBROGENE PROJECT**

**Grant awarded: £5,000**

**Timescale: 4 years**

**2013 - current**

**Frances MK Williams, Reader and Honorary Consultant, King’ s College London**

**Background**

Chronic widespread pain (CWP, when severe referred to as fibromyalgia) and low back pain (LBP) are a major problem in the UK and US with prevalence 5-10% but in the elderly as high as 50%. Indeed, the cost of chronic pain to society as a whole is considered by some to be grossly underestimated. CWP is a prominent presenting complaint in Rheumatology in which, along with sleep disturbance and seen in isolation is labelled “primary fibromyalgia”, or “secondary” to other rheumatic conditions. It is well recognised that social and psychological factors play a large role in the manifestation of pain and its severity, and there is an overlap with chronic depression.

**Abstract**

Chronic widespread pain (CWP) is major medical and social problems in Western countries. They are increasingly considered part of an affective spectrum disorder (comprising headache, irritable bowel and pelvic pain amongst others). CWP is known to be heritable but few genetic variants have been identified. This proposal builds on an existing programme of work on pain in TwinsUK: 2,500 twins have undergone quantitative sensory testing (QST) for pain sensitivity. Most twins have genome-wide association (GWA) data imputed to 2.5 million SNPs, many have exome sequence and expression arrays; and methylation is also already funded and becoming available. This project will translate the genomic findings from twin volunteers to patients.

**Funding from CDRF**

Was requested to establish a DNA resource from 2000 people having CWP and to examine genetic pain candidate genes that had been identified in twins and validate them in patients.

**Methods**

We collected, by secure mail delivery boxes, samples of DNA from people with fibromyalgia/chronic widespread pain, identified through membership of the Fibromyalgia UK, along with information on individuals’ demographics and two pain questionnaires. The purpose of these will be confirm the presence of widespread musculoskeletal pain, to detect evidence of a subtype of pain called neuropathic pain [1](#_ENREF_1) and ultimately to form a chronic pain resource on which genetic studies could be performed.

**Progress**

Because the collection of samples through NHS rheumatology clinics had been so limited we used social media to access the enormous numbers of people living in the UK with chronic pain. In particular we had the support of the charity Fibromyalgia UK, and so asked them to place an advertisement on their website. We began recruiting individuals from all over the country to this project in mid 2013.

Patients with fibromyalgia are usually exceptionally keen to volunteer for research. Many have struggled over many years to obtain a diagnosis and have been dismissed as mad. At present there is little in the way of satisfactory treatment for chronic pain and many live disabled by symptoms for many years. Where GPs refused to take their sample, we worked with the volunteers and sought alternative methods of obtaining samples such as visiting the GSTT Clinical Research Facility where patients lived locally.

In late 2013 we had a second initiative using FMA UK to increase recruitment and obtained a further batch of samples. In addition, to ease the administrative burden and to take advantage of opportunities being presented but the NIHR BRC BioResource at GSTT/KCL, it was decided that the Fibrogene project should become one of the flagship BioResource projects. This meant that where possible samples were, with a second consent provided by the volunteers, taken into the BioResource.

**Intern support**

In August 2013 we had an sixth form intern attend the Dept of Twin Research who helped on the Fibrogene project by telephoning volunteers who had queries and wrote a small project for submission to a school science writing competition. Felix is now a Biomedical Sciences undergraduate at Imperial College.

A second intern approached us in summer 2015 and helped with the project by phoning volunteers. It so happened that the same week my research assistant resigned and Helena is now working on the EU FP7 Pain\_omics project. In September she leaves that post to become an Action on Hearing Loss funded PhD student, examining the genetic variants for hearing in the UKBiobank dataset as part of a collaborative project with UCL and Manchester University.

**Current status – Fibrogene project**

The sample size at present stands at n=1500 Fibromyalgia volunteers. We have managed to obtain both genome-wide association scans and serum metabolomic screening on approximately half of the the Fibrogene samples. This means that they will soon (once the data have been QCed) be suitable for analysis as part of larger, likely international studies. We will also be able to test the genetic variants identified in twins (both chronic pain variants [2](#_ENREF_2) and those identified using QST[3](#_ENREF_3)) in patients with fibromyalgia. We have published on the metabolomics of chronic pain in twins [4](#_ENREF_4) and will be able to attempt replication of this in Fibrogene samples in late 2016.

If funding allows we will aim to have a third wave of collection in 2017. This will take our sample to in excess of 2017. We are also exploring recruitment from the dedicated Fibromyalgia clinic at GSTT, in collaboration with the BioResource team.

There is a new research associate funded by Arthritis Research UK being appointed this week who will start work in the autumn. The role of this individual will be omics analyses in chronic pain. In particular, they will examine both the twins and Fibrogene samples for genetic variants and shared metabolites which may provide biomarkers of CWP.

**Outcomes**

While there have not yet been publications from this collection having this DNA resource has been helpful in a number of ways.

1. It has supported the KCL/GSTT BioResource recruitment and output
2. Its contribution to the CWP research effort of the Department of Twin Research was helpful in securing grant funding from Arthritis Research UK: grant 20682 “'Epigenetic factors in fibromyalgia: an omics study' for £170,921.00.

PUBLICATIONS

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widespread pain: environmental and genetic influences. Pain. Jun 24 2015.

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widespread pain: evidence for involvement of the 5p15.2 region. Annals of the rheumatic diseases.

Sep 21 2012.

3. Williams FM, Scollen S, Cao D, et al. Genes contributing to pain sensitivity in the normal

population: an exome sequencing study. PLoS genetics. Dec 2012;8(12):e1003095.

4. Livshits G, Macgregor AJ, Gieger C, et al. An omics investigation into chronic widespread

musculoskeletal pain reveals epiandrosterone sulfate as a potential biomarker. Pain. Apr 22 2015.

2011-2012

**UNDERSTANDING THE GENETIC PREDISPOSITION TO MIGRAINE – A META-ANALYSIS APPROACH**

**Grant awarded: £18,217.74**

**Timescale: 6 months**

**2011 (6 months)**

Dr Lydia Quaye (post-doctoral research associate)

1. **Background and aims of the project**

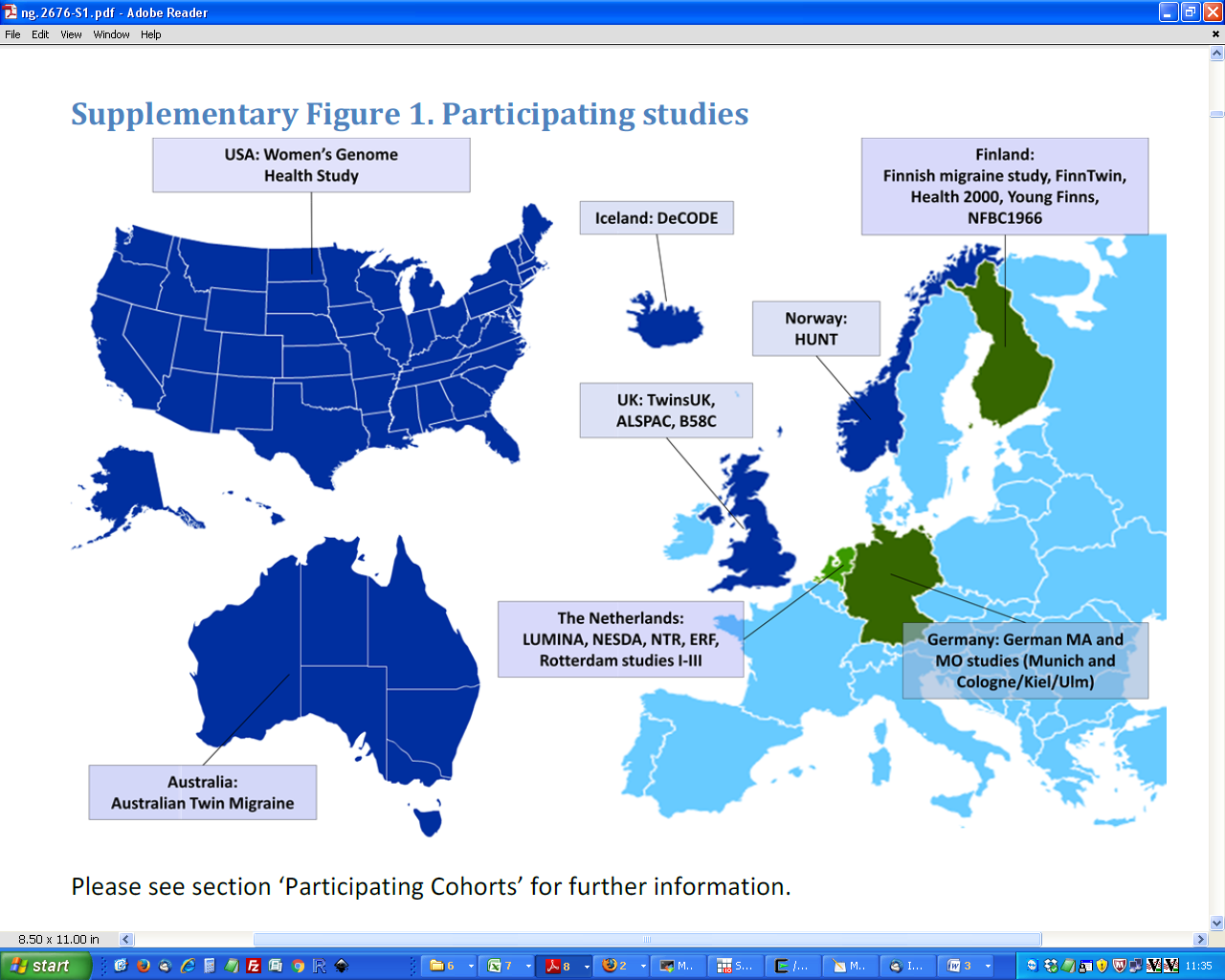
Migraine is the third most common disease worldwide, with a lifetime prevalence of 15–20%, affecting up to 1 billion people across the globe, but its molecular mechanisms are poorly understood. Family and twin studies estimated an heritability of 42% for migraine1, pointing to a genetic component of the disease. Despite this, genome-wide association studies (GWAS) of clinic-based migraine with aura2, migraine in the general population3,4 and clinic-based migraine without aura5 have uncovered relatively little about the molecular mechanisms that contribute to migraine's pathophysiology. Indeed, the identified loci explained only a minimal part of the phenotypic variance (the so called “missing heritability” problem6). A likely explanation for this phenomenon is the smaller effect of some of the genetic variants. Due to the cost and difficulty to collect brain phenotypes on large sample sets, the obvious next steps to identify migraine's susceptibility loci (which may well provide important new information on disease susceptibility and lead to new therapeutic targets) is to perform large GWA meta-analyses on dataset exceeding 10,000 samples.

**The main aim of this project was:**

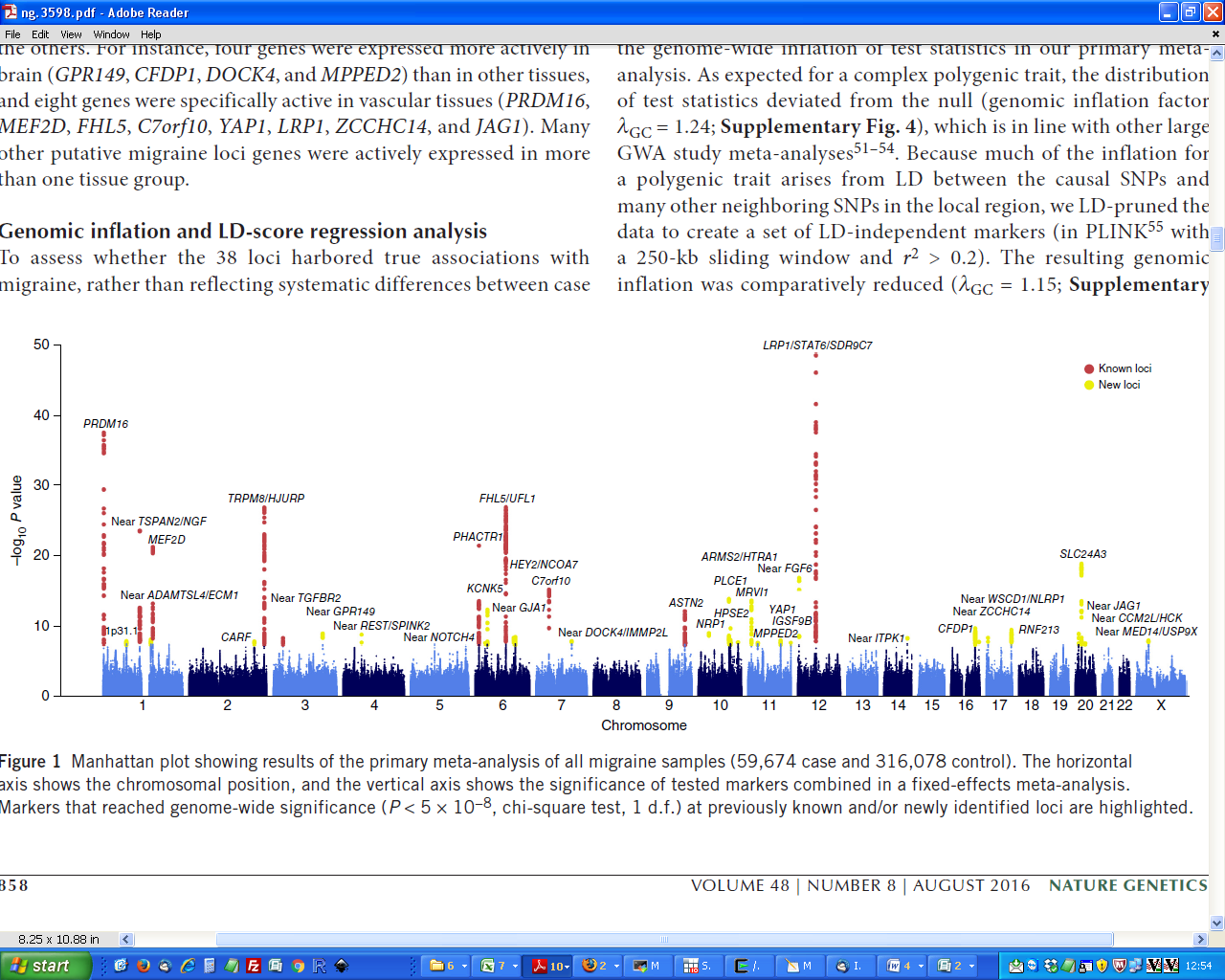
* To perform genome wide analysis of clinic-based migraine and its sub-phenotypes on TwinsUK dataset as part of a large international meta-analysis effort based on a total of 23,285 cases and 95,425 controls.

1. **Genome Wide Meta-analysis results**

TwinsUK genome wide summary results were included in a large meta-analysis across 29 genome-wide association studies, including a total of 23,285 individuals with migraine (cases) and 95,425 population-matched controls (Figure 1).



Results from the primary meta-analysis and for the 3 subgroups identified 142 SNPs, at a total of 12 loci, that were significantly associated with migraine susceptibility (*P* < 5 × 10−8) (Figure 2). Five of the 12 genome-wide significant loci were new (*AJAP1*, *TSPAN2*, *FHL5*, *C7orf10* and *MMP16*). The remaining seven loci confirmed previously reported loci associated with migraine (*PRDM16*3, *MEF2D*5, *TRPM8*2,3, *TGFBR2*5, *PHACTR1*5, *ASTN2*5 and *LRP1*3). All seven previously reported loci seen in this study remained significant after excluding the samples used in previous reports2,3,5.



Observed differences in the number of identified loci (and the strength of association) suggested that the genetic background of migraine with aura is considerably less influenced by common variants than that of migraine without aura, contrary to previous expectations. Although pathway analysis of the 146 loci showed no concentration of candidate causal genes in any particular pathway or tissue, 8 of the 12 identified loci are located in or immediately adjacent to genes with known functions in synaptic or neuronal regulation, and several exert regulation, including over one another.

A recent meta-analysis effort including a total of 59,674 affected subjects and 316,078 controls collected from six tertiary headache clinics and 27 population-based cohorts through the worldwide collaboration with the International Headache Genetics Consortium (IHGC), identified 44 independent single-nucleotide polymorphisms (SNPs) significantly associated with migraine risk (*P* < 5 × 10−8) that mapped to 38 distinct genomic loci, including 28 novel loci and the first chromosome X locus (Figure 2).

1. **Shared biological basis between migraine and coronary artery disease**

Recent studies indicate a similar risk increase for coronary artery disease (CAD), the most common vascular disorder; although the association is less certain than for stroke7-10. This raised the question of whether migraine and cardiovascular disease have a shared biological basis.

Comparing nominally significant SNPs from the migraine and CAD meta-analysis, it was observed an overlap of association signals in excess of what would be expected by chance. An overlap of signals was seen for SNPs at different p values thresholds. These findings were supported by permutation testing.

The significant sharing of risk loci between migraine and CAD may reflect that they involve some of the same biological processes. Indeed further experimental studies will clarify this and whether the shared risk loci can give information on vascular mechanisms involved in migraine pathogenesis.

**PUBLICATIONS**

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The Future

* *All Future projects depend on future donations and legacies*
* Aim to continue funding small pilots aiding future grants and projects. These projects may attract matching funding from government
* Project grants will fund innovative areas of research which are difficult to fund elsewhere
* If funds allow – CDRF plans to fund PHD students seen as good value for money.
* Discretionary funds up to £5000 pa can be used by the department without peer review for equipment or travel

