How do peripheral and central vascular markers relate to cognitive decline?
Start date: 1 Aug 2015 **Completion date: 1 Jul 2018**

Executive Summary of Project

This pilot exploratory study aimed to investigate statistical relations between measures of plasma lipidomics and lipoproteins and cognitive and neurovascular imaging parameters. The main hypothesis being addressed was that the lipidomic/lipoprotein markers will correlate with, and allow the stratification of, declines in the cognitive and neurovascular parameters.

The work progressed satisfactorily although there was there was the need to alter the experimental plan to deal with updates to the literature. The work is likely to yield to 2 to 3 initial publications and data sets that will be available for other researchers to analyse.

We have used penalized regression models (LASSO) to identify sets of metabolites in the Biocrates, B.I.-LISA, untargeted UPLC-MS and untargeted NMR Spectroscopy data sets that can be used to predict general cognitive ability in LBC1936. Splitting the data into a training set and a test set, we predicted up to 9% of the variance in general cognitive ability, in the test set. Results for the MRI brain variables will be available in January. Elastic-net models are also being used to identify specific metabolites associated with general cognitive ability and MRI brain variables. These analyses will be completed shortly. Longitudinal analyses to investigate if plasmid lipid biomarkers predict cognitive decline in LBC1936 are also planned.

DPUK enabled many thousands of metabolites to be measured in the Plasma of LBC1936 participants, which is allowing us to investigate their association with cognitive function, cognitive decline and MRI brain variables.

Team members

John Starr*, Ian Deary, Joanna Wardlaw, Derek Hill, Paul Wren

*We are saddened to report that John Starr died in early December 2018.

Location:

Edinburgh University
Imperial College London
Ixico

Overall work package objectives:

An exploratory study which will investigate statistical relations between measures of plasma lipidomics and lipoproteins and cognitive and neurovascular imaging parameters. The main hypothesis is that the lipidomic/lipoprotein markers will correlate with, and allow the stratification of, declines in the cognitive and neurovascular parameters. Contingent on its results, this pilot may permit the consideration of larger strategic bids to complete plasma assessments from the full range of ages at which data have been collected on the LBC1936. It may also encourage further assessments in other DPUK cohorts to qualify findings at scale.

1. Determine the individual Lipidomic/Lipoprotein/Biocrates profiles that may represent fit to cognitive performance.
2. Determine the individual Lipidomic/Lipoprotein/Biocrates profiles that may directly associate with intermediary imaging markers of vascular disease burden including MRI variables: visible markers as individual (WMH, lacunes, PVS, global and regional atrophy) and combined SVD features (SVD burden score); and subvisible markers (MD, FA, T1, MTR in normal appearing white and deep grey matter).
3. Determine the individual Lipidomic/Lipoprotein/Biocrates profiles that may represent fit to cognitive performance in presence or absence of markers of cerebrovascular burden.

Dependencies to and from other work packages, networks and themes

4. To specifically test the 10 lipid panel identified by Mapstone et al, 2014 as to whether it may represent fit to cognitive performance with evidence of cerebrovascular burden. 5. To specifically test whether PC 16:0/20.4(5), PC16:0/22:6 & PC18:0/22:6 profiles may represent fit to cognitive performance with evidence of cerebrovascular burden to further enhance the King's Group established findings.	

Lessons Learnt

- The assays were all performed successfully although there was a delay to us receiving the data for the Lipoprotein profiling using NMR spectroscopy, because the National Phenome Centre had to optimise the assay to work on a low volume of plasma.
- Writing scripts to analyse such large data sets took longer than expected, therefore the initial publication will include only cross-sectional data. Longitudinal analyses will be included in a follow-up paper.
- We decided not to proceed with initial plans to analyse the 10 lipid panel identified by Mapstone et al, 2014 as other studies failed to replicate results from this paper whilst we were acquiring our data.

Were all Milestones completed - No
Please see report below

Deliverables	Milestones	Milestone deadline	Work package dependencies	Person(s) responsible
Objective 1:				
D1.1The individual Lipidomic/Lipoprotein/Biocrates profiles that may represent fit to cognitive performance.	None	M1.1 Complete	None	JS/ID
Objective 2:				
D1.2 The individual Lipidomic/Lipoprotein/Biocrates profiles that may directly associate with intermediary imaging markers of vascular disease burden including MRI variables: visible markers as individual (WMH, lacunes, PVS, global and regional atrophy) and combined SVD features (SVD burden score); and subvisible markers (MD, FA, T1, MTR in normal appearing white and deep grey matter).	None	M1.2 Complete	None	JS/ID
Objective 3:				
D1.3 The individual Lipidomic/Lipoprotein/Biocrates profiles that may represent fit to cognitive performance in presence or absence of markers of cerebrovascular burden	None	M1.3 Ongoing	None	JS/ID
Objective 4:				
D1.4 To specifically test the 10 lipid panel identified by Mapstone et al, 2014 as to whether it may represent fit to cognitive performance with evidence of cerebrovascular burden	None	M1.4.1 Closed No longer appropriate.	None	JS/ID
Objective 5:				
D1.5 To specifically test whether PC 16:0/20.4(5), PC16:0/22:6 & PC18:0/22:6 profiles may represent fit to cognitive performance with evidence of cerebrovascular burden to further enhance the King's Group established findings	None	M1.5.1 Ongoing	None	JS/ID
D1.6 Aim to replicate the finding that sphingolipids (Biocrates) are associated with white matter microstructure in older adults (Gonzalez et al., Neurobiol Aging 2016 43, 156-163) and will investigate their association with cognitive change in later life.	None	M1.6.1 Complete		
D1.7 We will investigate the dimensionality of the NMR Bi-LISA data set and associations between individual and combined lipoproteins and cognitive function, cognitive decline and MRI variables.	None	M1.7.1 Complete		

WHAT IS THE MOST SUCCESSFUL OUTCOME AND WHAT DOES IT MEAN TO DEMENTIA RESEARCH

We have used penalized regression models (LASSO) to identify sets of metabolites in lipidomic data sets that can be used to predict up to 9% of the variance in general cognitive ability in relatively healthy 73-year olds.

Outputs

PUBLICATIONS

- A first paper is current being drafted. Its working title is "Plasma lipid biomarkers in LBC1936: Do they predict general cognitive function and brain structure?", the authors will be Sarah E Harris, Stuart J Ritchie, Chloe Fawns-Ritchie, John M Starr, Derek Hill, Paul Wren, Joanna M Wardlaw and Ian J Deary and it is anticipated to be submitted to Molecular Psychiatry.

We anticipate publications in the following areas.

1) Further investigation of the function of specific metabolites that are associated with general cognitive function and the structure of the brain.

2) Longitidinal analyses to investigate if plasmid lipid biomarkers predict cognitive decline and structural brain changes in LBC1936.

ENGAGEMENT

- Stuart J Ritchie, Sarah E Harris, Chloe Fawns-Ritchie, John M Starr, Derek Hill, Paul Wren, Joanna M Wardlaw and Ian J Deary. Plasma sphingolipid biomarkers in the Lothian Birth Cohort 1936: Towards associations with lifetime cognitive function, Alzheimer's Association International Conference, July 2017, poster presentation.
- Sarah E Harris. The Lothian Birth Cohorts: OMICS, Medical Genetics Section Talk, IGMM, University of Edinburgh, 7th February 2018.

Final Project Report (Max Word Count 10,000)

- **Introduction**

Testing potential biomarkers of cognitive ability in late life is of interest for both theory and practice in dementia research. Not only might such biomarkers be informative about the causes of age-related cognitive decline, they may also be predictive of phenoconversion to dementia; they may thus be used in the prodromal phase of the disorder to distinguish those who will experience pathological cognitive ageing from those who will age more healthily. In this proposal, we examine one set of possible cognitive ability level and change biomarkers: lipidomics and lipoproteins.

Evidence suggests that diverse lipidomic profiles exist in dementia, and that these may predict phenoconversion (http://www.ncbi.nlm.nih.gov/pubmed/24608097 & http://www.ncbi.nlm.nih.gov/pubmed/24041970). Differential lipidomic profiles in diverse patient populations across brain white and gray matter have also been suggested (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4099521/; see http://www.ncbi.nlm.nih.gov/pubmed/24568356 for a recent review). Lipidomic profiles may therefore be highly relevant to profiles of change in brain and cognitive ageing, including conversion to dementia.

Brain White Matter Hyperintensities (WMH), detected on magnetic resonance imaging scans, may be linked to lipidomic profiles. WMH are indicative of cerebral small vessel disease, and are a common feature of ageing, but are also related to neurodegenerative diseases such as AD and VCI, poorer cognitive and functional outcomes (http://www.ncbi.nlm.nih.gov/pubmed/24190781). Their presence is associated with increased risk of stroke, dementia, and mortality. They are part of small vessel disease burden (including lacunes, perivascular space enlargement and microbleeds; http://www.ncbi.nlm.nih.gov/pubmed/25165388), and are a marker of diffuse damage and loss of integrity throughout 'normal appearing' white matter (http://www.ncbi.nlm.nih.gov/pubmed/25457555). Such relationships may be moderated by genetic variants such as *ApoE* e4, the presence of which has been linked to the extent of cerebrovascular disease as indicated by WMH (http://www.ncbi.nlm.nih.gov/pubmed/23858411).

Other measures of the structure of the brain's white matter are also related to cognitive and health outcomes. Measures derived from diffusion-tensor MRI (DT-MRI), such as the magnetization transfer ratio (MTR), longitudinal relaxation time (T1), fractional anisotropy (FA) and mean diffusivity (MD) have been found to associate with cognitive ability in later life (http://www.ncbi.nlm.nih.gov/pubmed/25309438, http://www.ncbi.nlm.nih.gov/pubmed/24561387, http://www.ncbi.nlm.nih.gov/pubmed/25247594). Such measures may therefore provide complementary information about links between lipidomic profiles, cognitive decline and phenoconversion.

Recent developments in technology allow hundreds to thousands of lipids to be analysed simultaneously. Mass spectrometry-based lipidomic platforms are increasingly popular. They may be targeted to measure specific well-defined metabolites or untargeted whereby they detect thousands of features, with those showing a positive association with the trait of interest potentially being annotated using databases post analysis. Ultra-performance liquid chromatography (UPLC) mass spectrometry is a highly sensitive method of quantifying a wide range of biologically relevant small molecules http://www.ncbi.nlm.nih.gov/pubmed/27479709. In nuclear magnetic resonance (NMR) spectroscopy, the area of a resonance is directly related to the number of nuclei generating the signal. This provides a highly quantitative method for detecting small molecules in plasma and other biofluids (http://www.ncbi.nlm.nih.gov/pubmed/25691689).

Goal of the study: To investigate the potential links between plasma lipidomics and lipoproteins and cognitive and neurovascular imaging parameters that index variation in age-related decline. The project will test whether these lipidomic parameters could be used to stratify subjects relevant to diverse populations with varied risk of decline in cognitive performance, vascular health and lifestyle measures. The results would provide data in a pre-competitive framework that could support potential therapeutic strategies for vascular causes of cognitive impairment and dementia.

Scope of the study
To design and conduct a scientifically robust pilot study with new analysis of plasma samples and existing cognitive and imaging data derived from the Lothian Birth Cohort 1936 (LBC1936).

Cost to DPUK: £91,000

Additional funds from elsewhere: None.

RESULTS:
Lothian Birth Cohort 1936 (LBC1936) plasma samples were delivered to the National Phenome Centre (NPC) in April 2016. A problem was identified with some of the samples and further samples were sent in July 2016. We received the first batch of data from the National Phenome Centre in November 2016. Final data sets were received from the National Phenome Centre in March 2017. Four data sets were received: 1) Ultra-Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS) untargeted data set; 2) The Biocrates AbsoluteIDQ® p180 (Biocrates Life Sciences AG, Innsbruck, Austria) kit provided absolute quantification for 53 compounds, and semi-quantitative measurements for a further 135 compounds. Biocrates data were acquired on Waters TQ-S instruments; 3) Nuclear Magnetic Resonance (NMR) Spectroscopy untargeted data set; 4) Specific lipoproteins were measured using the Bruker B.I.-Lisa platform (Bruker IVDr Lipoprotein Subclass Analysis). The lipoproteins measured were, cholesterol, free cholesterol, phospholipids, triglycerides, apolipoproteins A1, A2, B and particle numbers for the primary plasma and serum lipoproteins and their subclasses.

EM 1 End report

Initial analyses to investigate the underlying structure of the Biocrates-derived sphingolipid markers was performed using exploratory factor analysis. One strong general factor explained ~72% of the variance across all 14 sphingolipids. A small correlation was identified between general cognitive ability and general sphingolipid level (r=0.12, p=0.008).

We decided not to test the 10 lipid panel identified by Mapstone et al., 2014 (http://www.ncbi.nlm.nih.gov/pubmed/24608097) as to whether it may represent fit to cognitive performance with evidence of cerebrovascular burden. Larger studies suggest that it is not a good predictor of Alzheimer's Disease (http://www.ncbi.nlm.nih.gov/pubmed/26617567 & http://www.ncbi.nlm.nih.gov/pubmed/26806385.

We have used penalized regression models (LASSO) to identify sets of metabolites in the Biocrates, B.I.-LISA, untargeted UPLC-MS and untargeted NMR spectroscopy data sets that can be used to predict general cognitive ability in LBC1936. Splitting the data into a training set and a test set, we predicted up ~ 9% of the variance in general cognitive ability using the UPLC-MS dataset, in the test set. The other three data sets did not predict variance in cognitive ability in LBC1936. Models are currently running to identify the percentage of the variance in MRI brain variables that can be predicted by the four data sets. Elastic-net models are also being used to identify specific metabolites associated with general cognitive ability and MRI brain variables. These analyses will be completed shortly. Longitudinal analyses to investigate if plasmid lipid biomarkers predict cognitive decline in LBC1936 are also planned.

The analyses were performed by Drs Sarah Harris and Stuart Ritchie, who will prepare the manuscripts for peer-reviewed publication.

- **Conclusion**

Lipid biomarkers can be used to predict a small amount of the variance in general cognitive function in members of LBC1936 at age ~73 years.

- **Recommendations** None

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