**RESEARCH GROUP:**

**Project Title:** Evaluating the effectiveness of DNA methylation patterns for patient stratification in major depressive disorder.

**Supervisor(s):**
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**Level:** PhD

**Background to the project:**
Depression is the most commonly diagnosed mental health disorder worldwide and is characterised by low mood, anhedonia, and in severe cases suicidal behaviour. Northern Ireland has one of the highest rates of depression in Europe (1) and the highest incidence of suicide in the UK (2). Despite its prevalence, the pathophysiology of depression and risk factors for suicide are poorly understood.

Although antidepressants can be effective in treating depression there is a high rate of inter-individual variability in their efficacy. Only about 30% of patients achieve full remission following antidepressant treatment. An additional 30% of individuals are described as treatment resistant following failure to respond to two successive antidepressants (3). Treatment resistant depression is associated with increased symptom severity, increased rate of relapse, lower long-term quality of life and higher risk of suicide (4).

There is a clinical need to develop biomarkers to assist in diagnosis of depression and identify individuals at risk of treatment resistance and suicide. Genetic and environmental factors are linked to increased risk for depression and their interaction may lead to development of mental illness (5-7). Preliminary studies have identified DNA methylation patterns that are associated with severe depression, treatment resistance and suicidal behaviour. Depressive disorders and response to antidepressant have also been linked to inflammatory status. The aim of this study is to identify epigenetic and inflammatory markers that can be used to assist in diagnosis and treatment selection for individuals with depression.

**Objectives of the research project:**

**Hypothesis:** That response to antidepressant treatment will be linked to quantifiable changes in epigenetic markers. To test this, we Aim to measure methylation levels in adults with severe depression and matched controls and compare the two. Our specific Objectives are therefore to:

1. Continue recruiting participants as part of an ongoing study to identify biomarkers for depression (ORECN 16/NI/0133)
2. Conduct depression severity analysis and collect blood and saliva samples from individuals who meet the criteria for depression and matched healthy controls
3. Quantify global DNA methylation levels in carefully defined antidepressant treatment response cohorts and associated controls.
4. Identify differentially methylated regions (DMR) to act as biomarkers using locus-specific pyroassays.
5. FACS analyses and sorting/collection of T- and B- and other cell populations from a subset of patents (baseline naïve to treatment, responders and non-responders with associated controls).
6. Comparison of (a) candidate gene methylation status (e.g. FOXP3 upstream enhancer) and genes exhibiting differential methylation in selected T-cell (CD4+,CD25+ T-Reg; T effector cells) and B-cell populations from (5) above related to patients with an inflammatory aetiology putatively associated with depression.
7. Measurement of inflammatory markers (pro-inflammatory cytokines) and correlation with methylation status of candidate genes from specific T-cell and B-cell populations from selected patients

Methods to be used:

**Population:** This project will involve recruitment of patients in the following cohorts: treatment naïve, patients responding to antidepressant medications, treatment resistant individuals and healthy controls. Treatment resistance is clinically defined as failure to respond to successive treatment with two antidepressant medications. Participants will be asked to attend one appointment where they will provide informed consent to take part in the study, complete validated depression diagnostic and symptom severity assessments, lifestyle and medications adherence questionnaires.

**Sample Size calculations:** Analysis of samples (n=40/group, allowing for some failing QC, with matched controls) will allow us to detect genome-wide differences in methylation of 12% or locus-specific differences of approx. 5% with 80% confidence (8) though significant differences have been successfully detected using smaller cohorts of clinical samples (9-10).

**Methodologies:** DNA will be extracted from blood samples and processed for global methylation analysis using Illumina 850K array. DNA methylation in the long interspersed nucleotide element 1 (LINE-1), a second indicator of global DNA methylation levels, will be determined using pyroassay. Bisulphite-treated DNA will be amplified by PCR and pyrosequencing assays or methylation specific PCR will be used to determine methylation patterns of gene specific loci. Statistical significance between groups will be analysed using t-test, ANOVA and logistic regression.

**Outcomes:** Identification of significant alterations in DNA methylation between treatment-resistant depressed subjects and controls will:
1. Provide a diagnostic panel to better diagnose/sub-stratify patients with depression.
2. Provide quantifiable, reproducible markers for treatment resistant depression which could be assessed in patients using PCR-based assays
3. May identify gene networks which are novel drug targets for treating depression

**Skills required of applicant:**
Good knowledge of biomedical science and molecular biology. Good oral and written communication skills. facilitating professional interaction with patients and medical professionals.
Experience of of good organisational skills and working effectively as a member of a team.
Evidence of ability to undertake scientific writing. Good presentation skills essential. Willingness to travel between campuses and other locations relevant to the project.

**References:**
1. Bunting et al. (2012) Psychological medicine, 42(8), pp. 1727-1739
2. NISRA, Office of National Statistics (2016); reported by BBC News - [www.bbc.co.uk/news-northern-ireland-35491402](http://www.bbc.co.uk/news-northern-ireland-35491402)


