Progress Report

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Title of Project: Micro-RNA expression profiling in patients with cerebral vasospasm after aneurysmal subarachnoid haemorrhage

Summary: Subarachnoid haemorrhage from a ruptured brain aneurysm is a major cause of mortality and morbidity. Cerebral vasospasm often complicates subarachnoid haemorrhage and can lead to stroke or death. The precise mechanisms contributing to the development of vasospasm are not well understood but it is thought that blood breakdown products induce a cascade of events including inflammation, the release of free radicals and vasosactive substances that result in sustained cerebral vasoconstriction, cerebral ischaemia and ultimately brain infarction. Vasospasm most often occurs 7-10 days after the onset of haemorrhage. It is currently not possible to accurately predict which patients will go on to develop vasospasm and prophylactic treatments are often ineffective.

Over the last 4 years we have been collecting cerebrospinal fluid from patients after aneurysmal subarachnoid haemorrhage and established a database of biochemical, radiological and clinical information. We have performed proteomic analysis of CSF samples using gel electrophoresis and mass spectrometry, aiming to identify proteins implicated in the pathogenesis of vasospasm. As part of this work we have identified Haptoglobin, amongst other proteins, as being protective against vasospasm. Haptoglobin is an acute phase protein and its primary function is to bind and clear free haemoglobin released in the subarachnoid space after red blood cell breakdown following subarachnoid haemorrhage. Free haemoglobin is highly toxic and contributes to oxidative stress, acute inflammation, Nitric Oxide depletion,and ultimately vasospasm and delayed ischaemic neurologic deficit. Intrathecal/ intraventricular Haptoglobin administration in these patients may have a protective effect against vasospasm. An alternative to exogenous Haptoglobin administration is the upregulation of endogenous Haptoglobin production which may potentially be achieved using gene therapy.

Micro-RNAs (miRNAs) are small, non-coding, single stranded RNA molecules involved in the regulation of gene expression at a post-transcriptional level. MiRNA's have been shown to be involved in the regulation of multiple cellular processes including differentiation, proliferation, and apoptosis in both health and disease. Circulating miRNAs, packaged in microvesicles, have been detected in human serum and cerebrospinal fluid.

The aim of this study is to quantify cerebrospinal fluid haptoglobin levels in patients with and without vasospasm and delayed ischaemic neurologic deficit after aneurysmal subarachnoid haemorrhage and to correlate with individual Micro-RNA's in the same sample to ultimately identify Micro-RNA's that may be responsible for up- or down-regulation of Haptoglobin expression.

Hypothesis
Haptoglobin levels will be higher in patients who do not develop vasospasm compared to those that do and corresponding Individual Micro-RNA's responsible for Haptoglobin upregulation will be differentially over-expressed.

Unanswered Questions
Which are the individual Micro-RNA's responsible for regulation of Haptoglobin expression?

What these research outcomes mean
Identification of Micro-RNA's responsible for regulation of Haptoglobin expression may allow prophylactic upregulation of Haptoglobin expression and prevention of vasospasm using gene therapy in the form of RNA interference

Progress to date and timeframes for completion of study
Haptoglobin identification has already been performed in cerebrospinal fluid samples from patients with subarachnoid haemorrhage with and without vasospasm and delayed ischaemic neurologic deficit using proteomics (gel electrophoresis and mass spectrometry). Normal control cerebrospinal fluid samples were obtained via lumbar puncture from patients who presented with sudden onset severe headache for investigation and were found not to have a subarachnoid haemorrhage. Micro-RNA from corresponding samples has been extracted and purified. Quantification of Micro-RNA is still pending. This will be analyzed on an nCounter instrument using the Human V3 miRNA assay kit. The data will then imported into the nSolver

software package for differential miRNA expression analysis. Correlations between differentially expressed Micro-RNA's and haptoglobin levels in individual samples will then be performed. We anticipate this work to be completed by the end of calendar year 2016.