Final Report

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Title of Project: **Identifying the transcriptional cause of multiple system atrophy**

Summary
Multiple system atrophy (MSA) is defined as a sporadic neurodegenerative disease, with an onset in adult life characterized by progressive development with etiology of an underdetermined nature. It is clinically characterized by varying degrees of the features of Parkinson's disease (PD) such as shaking, rigidity, slowness of movement, and difficulty with walking, and autonomic disorders of the genitourinary system and cortex. MSA equally affects both men and women, primarily in their 50s; however, disease onset as early as age 30 has been diagnosed. The progression of disease is rapid, and patients are confined to bed within 5 years of symptom onset with death resulting within an average of 9 years. With 3 cases per 100,000 individuals, MSA is considered rare; however, its prevalence is similar to multiple sclerosis (MS) (2.5 per 100,000) and motor neuron disease (1.5-2 per 100,000).

We hypothesized that compared with PD, MSA has a fundamental dysregulation of transcription causing increased expression of α-synuclein protein. Therefore the aim of our proposal was to determine global transcriptome pattern in distinct regions of MSA brains using next-generation sequencing and to validate expression patterns of genes determined as specific to the molecular pathophysiology of MSA.

We performed transcriptome sequencing of the brain tissue samples representing the superior frontal cortex derived from the multiple system atrophy (MSA) donors. We separately analysed grey (GM) and white matter (WM), of which major cellular components are neurons and oligodendrocytes, respectively. Since MSA-specific pathology is primary localized within the WM, affecting oligodendrocytes, through this sample selection we were able to assign changes in transcriptome profiles to the specific structures of the human cortex relevant to the disease pathology. We conducted the comparative transcriptome analysis in two dimensions. First, we considered the role of oligodendrocytes in MSA pathology and analyzed differences between the GM and WM transcriptomes within the MSA sample set. Second, we performed a pairwise analysis of GM and WM between MSA and control samples to capture fundamental changes in gene expression patterns resulting from neurodegenerative processes in each tissue type.

Differential, genome-wide gene expression analysis revealed that MSA WM shows strongly elevated expression levels of haemoglobin genes (*HBA1*, *HBA2* and *HBB*). Haemoglobin is the largest source of peripheral iron in the human body, and it may play a role in iron homeostasis throughout the brain. Disrupted iron homeostasis has long been associated with various neurodegenerative disorders. It has been demonstrated that high levels of iron correlate with regions of neurodegeneration in

both MSA and PD. High iron levels may also promote oxidative stress, alter myelin synthesis, increase the aggregation of α-synuclein and cause neuronal death. The selection of frontal WM with limited MSA-specific damage in our study further supports that such increases in haemoglobin levels may occur prior to neuronal loss. The correlation among iron, haemoglobin and neurodegeneration is further supported by the finding that high levels of α-synuclein are present in blood, specifically in red blood cells.

Further, human histocompatibility complex class I genes including *HLA-A*, *HLA-B* and *HLA-C*, that relate to immune function, were all expressed at higher levels in control WM compared with MSA WM. These genes encode cell surface proteins that are constitutively expressed on microglia and endothelial cells in the brain and can be up-regulated in all brain cells in response to an immune challenge. Such reduced expression of HLA cell surface proteins in MSA WM may suggest a reduction in WM-specific immune function that could relate to the documented reduced involvement of inflammatory microglia in the progression of MSA degeneration.

We also discovered a number of novel long intervening non-coding RNAs (lincRNAs) which were differentially expressed between GM and WM in MSA cortical tissue. Interestingly, the top 10 up-regulated genes in GM included 4 un-annotated genes that satisfied the criteria for lincRNAs. The top 10 up-regulated genes in WM included 9 un-annotated genes that fulfilled the criteria for lincRNAs. Thus differential expression of lincRNAs suggests a strong regulatory component is involved in MSA-specific neurodegeneration.

Taken together the results of this project contribute to our further understanding of the MSA pathology, in particular in the early stages of the neurodegenerative process. Of note, the outcomes of this project have been presented in three recent publications from Dr Janitz's laboratory (Mills et al. 2014; Chen et al. 2015; Mills et al. 2015).

Hypothesis vs Findings

As we determined using RNA-Seq technology, the levels of α-synuclein gene (*SNCA*) expression were only moderately elevated in the MSA tissues. In contrast to our working hypothesis we conclude that despite an accumulation of α-synuclein aggregates, the levels of *SNCA* transcript remain largely unchanged in MSA. However utilization of a genome-wide approach to investigate the transcriptome perturbation in the course of MSA led us to discovery of new biological pathways which are affected in early stages of the disease progression. We have also been surprised by a high number of differentially expressed lincRNAs in the MSA brain.

Unanswered Questions

Transcriptome analysis provides a comprehensive and unbiased view on majority of genes which are expressed at any given time in a cell. Yet our discovery of haemoglobins or histocompatibility complex class I genes, that are differentially expressed in MSA requires further validation on a protein level. Further, functional analysis of these genes in α-synuclein overexpressing cell lines, representing cellular components of the human cortex, such as oligodendrocytes and astrocytes,

will be in focus of future studies. Moreover, there is an urgent need for functional characterization of novel lincRNAs which seem to be specific for the MSA pathology.

What these research outcomes mean

There are two key aspects of this research. Firstly, thanks to utilization of the RNA-Seq technique, we created transcriptome profiles of MSA brain with unprecedented resolution in terms of the gene expression and alternative splicing. This transcriptional repository, currently publicly available, will serve as an important reference set in MSA research. Secondly, the outcomes of this project pave the way for new avenues of MSA research, in particular in respect to exploration of link between ion metabolism and α-synuclein aggregation as well as investigation of non-protein-coding transcripts of which expression is perturbed in MSA.

References

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