

Investigating molecular mechanisms of progression of Parkinson's Disease in human brain

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Research question and background

Our project aimed to investigate the molecular basis of pathological spread in Parkinson's disease (PD) human brain. To do this we sourced post mortem tissue from Braak stage 3/4 stage Parkinson's disease brains, including tissue from the Netherlands Brain Bank. We specifically collected cases with limited pathology to gain an insight into the early stages of PD progression. We selected nine different consensus regions that are representative of the different stages of PD. This allowed us to investigate molecular changes that occur with increasing lewy body pathology. Using proteomic and biochemical techniques we looked for new markers of pathological progression in our tissue.

Methods and tissues used

A total of 66 control and 66 Braak stage 3/4 cases were selected, matched for age, post-mortem delay and pH. Microdissection from 9 regions (Substantia nigra, caudate, putamen, temporal cortex, parahippocampus, cingulate cortex, frontal cortex, parietal cortex and the cerebellum) was performed. Proteins were extracted, digested and expression changes investigated using quantitative label-free mass spectrometry based proteomics. Validation was performed using multiple reaction monitoring and functional mitochondrial assays for Complex I-IV.

Results and conclusion

Our method provides in-depth coverage of the human brain proteome from post-mortem brain, detecting 1147 unique proteins. We identified candidate proteins whose expression changed significantly in at least 5/9 of the affected regions in PD brain compared to control. Further to this, proteins that had a >5 fold change in expression in one region were also identified. The majority of the top preliminary candidates are mitochondrial constituents, and include proteins involved in the TCA cycle, mitochondrial metabolism and respiratory chain function. Preliminary results from functional assays show reduced complex I activity in frontal cortex of early PD compared to control. Both application of mass-spectrometry based proteomics and the complex I assay to our unique collection of early stage Parkinson's disease cases, indicate that mitochondrial dysfunction is apparent in both unaffected regions and affected regions early in PD. This may reflect an early pathogenic process in sporadic PD.

Conference talks and posters

March 2014 – 115th meeting of the British Neuropathological Society (talk – published)

Alpha-synuclein expression in early stage post-mortem Parkinson's disease brain

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Research question and background

Parkinson's disease (PD) is the second most common neurodegenerative disease with the main clinical features being tremor, rigidity and slowness of movement. The pathological hallmark lesions of PD are Lewy bodies, the main component of which is the alpha-synuclein (a-syn) protein. Pathological changes in the disease appear to spread in a characteristic manner in most cases (1). We have analysed a-syn expression in different brain regions in early-stage post-mortem PD cases to test the hypothesis that a-syn expression is altered early in the disease process.

Methods and tissues used

We collected control and Braak stage 3/4 PD brain samples from 6 brain banks and the following regions were microdissected: putamen (n=5); caudate (n=4); parahippocampus (n=5); temporal neocortex (n=10); cingulate cortex (n=11); parietal cortex (n=5); frontal cortex (n=8) and cerebellum (n=8). All PD cases were pathologically classified as Braak stage 3/4, and were matched to controls for age and post-mortem delay. RNA was extracted and quantitative Real Time PCR was performed using Taqman probes.

Results: Similar variation in a-syn expression across brain regions was observed in normal brain and early PD brain. The highest expression levels were found in the cortical areas, and the lowest expression levels in the striatum and cerebellum. There was no significant difference in a-syn expression between PD cases and control cases across any of the brain regions tested.

Results and conclusion

We have generated a map of the a-syn expression throughout the brain regions affected in PD and we show interesting and significant variation in a-syn levels in normal brain. In early PD the a-syn expression does not change significantly compared to controls, either in areas with Lewy body pathology or in areas without Lewy body pathology. This data suggests that alterations in a-syn expression do not occur early in PD, and do not precede the development of Lewy bodies.

References:

Braak et al. *Neurobiol Aging* 2003; **24**:197–211

April 2014 - "The Spreading Pathology of Neurodegenerative disorders" The John Van Geest Centre for Brain Repair Spring School 2014 (poster)

Proteomic investigation into early markers of pathological progression in Parkinson's Disease

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In Parkinson's disease (PD), pathological progression is believed to start in one vulnerable neuronal group, and spreads throughout the brain in a predictable sequence (Braak et al., 2003). By examining early stage PD cases (Braak 3/4) we can compare pathologically affected regions with pathologically unaffected regions that are predicted to develop PD pathology later in the disease course. This enables us to investigate the earliest molecular changes that arise during the spread of disease. Specific regions were microdissected from controls and Braak stage 3/4 PD cases matched for age, post-mortem interval, and pH. Proteins were extracted, digested and expression changes were investigated using quantitative label-free mass spectrometry based proteomics. Our method provides in-depth coverage of the human brain proteome from post-mortem brain: we detected a total of 1147 unique proteins of which 1004 were in the soluble fraction and 531 were insoluble. We found that the expression of insoluble alpha-synuclein was significantly higher in PD brain compared to control brain in the following pattern: 9.1 fold in the substantia nigra; 1.96 fold in putamen, 1.22 fold in the parahippocampus, 1.14 fold in the temporal cortex and 1.09 fold in the parietal cortex. Therefore the level of insoluble alpha-synuclein detected by proteomics reflects the lewy body pathology and Braak stage. We identified candidate proteins with change in expression across at least 5 regions with at least one region >2 fold change. Differentially expressed proteins were analysed for over-represented GO biological processes. A significant number of our preliminary top candidates, and processes with the most significant changes throughout the brain in early PD, are located in the mitochondria, and include involvement of the TCA cycle (e.g. aconitate hydratase), alterations in metabolism (e.g. monoamine oxidase) and respiratory chain function (e.g. ATP synthase). Proteomic techniques offer a powerful resource to explore global protein expression. Application of mass-spectrometry based proteomics to our unique collection of early stage PD cases has revealed a number of candidate proteins and pathways which may be involved in the pathological progression of PD. These results indicate that mitochondrial dysfunction may play an early role in sporadic PD.

June 2014 – UCL Neuroscience symposium 2014 (poster)

Proteomic investigation into early markers of pathological progression in Parkinson's Disease

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In Parkinson's disease (PD), pathological progression is believed to spread throughout the brain. By examining early stage PD cases (Braak 3/4) we can compare pathologically affected regions with pathologically unaffected regions that are predicted to develop PD pathology later in the disease. This enables us to investigate the earliest molecular changes that arise during the spread of disease. Specific regions were microdissected from controls and Braak stage 3/4 PD cases matched for age, post-mortem interval, and pH. Proteins were extracted, digested and expression changes were investigated using quantitative label-free mass spectrometry based proteomics. Our method provides in-depth coverage of the human brain proteome from post-mortem brain. We found that the expression of insoluble alpha-synuclein was significantly higher in PD brain compared to control brain in a pattern that reflects the Braak stage. A significant number of our preliminary top candidates, and processes with the most significant changes throughout the brain in early PD, are located in the mitochondria, and include involvement of the TCA cycle, alterations in metabolism and respiratory chain function. Application of mass-spectrometry based proteomics to our unique collection of early stage PD cases indicates that mitochondrial dysfunction may play an early role in sporadic PD.

November 2014 – Parkinson’s UK Research Conference 2014 (poster)

Detecting early markers of pathological progression in Parkinson’s disease using proteomics

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Objective: To investigate the molecular mechanisms that underlie pathological spread in early Parkinson’s disease using proteomic techniques.

Background: In Parkinson’s disease, pathological changes spread throughout the brain in a predictable sequence (Braak et al., 2003). By examining early stage Parkinson’s disease cases (Braak 3/4), we can compare pathologically affected regions with pathologically unaffected regions predicted to develop Parkinson’s disease pathology later in the disease, thus enabling us to dissect the earliest molecular changes that arise in disease.

Methods: A total of 66 controls and 66 Braak stage 3/4 cases were selected for age, post-mortem delay and pH. Microdissection from 9 regions (Substantia nigra, caudate, putamen, temporal cortex, parahippocampus, cingulate cortex, frontal cortex, parietal cortex and the cerebellum) was performed. Proteins were extracted, digested and expression changes investigated using quantitative label-free mass spectrometry based proteomics.

Results: Our method provides in-depth coverage of the human brain proteome from post-mortem brain, detecting 1147 unique proteins. We identified proteins whose expression changed significantly in at least 5/9 of the affected regions in PD brain compared to control. Interestingly, the majority of the top preliminary candidates are mitochondria constituents, and include proteins involved in the TCA cycle, mitochondrial metabolism and respiratory chain function.

Conclusions: Application of mass-spectrometry based proteomics to our unique collection of early stage Parkinson’s disease cases indicates that mitochondrial dysfunction is apparent in both unaffected regions and affected regions early in Parkinson’s disease, and therefore may reflect an early pathogenic process in sporadic Parkinson’s disease.

March 2015 – 116th meeting of the British Neuropathological society (talk - published)

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Mitochondrial dysfunction in Parkinson's disease: Is it the earliest feature?

Introduction: In Parkinson's disease (PD), pathological changes spread throughout the brain in a predictable sequence (Braak et al., 2003). By examining early stage PD cases (Braak 3/4), we can compare pathologically affected regions with pathologically unaffected regions predicted to develop PD pathology later in the disease, thus enabling investigation of the earliest molecular changes that arise in disease.

Materials and methods: A total of 66 control and 66 Braak stage 3/4 cases were selected, matched for age, post-mortem delay and pH. Microdissection from 9 regions (Substantia nigra, caudate, putamen, temporal cortex, parahippocampus, cingulate cortex, frontal cortex, parietal cortex and the cerebellum) was performed. Proteins were extracted, digested and expression changes investigated using quantitative label-free mass spectrometry based proteomics. Validation was performed using multiple reaction monitoring and functional mitochondrial assays for Complex I-IV.

Results: Our method provides in-depth coverage of the human brain proteome from post-mortem brain, detecting 1147 unique proteins. We identified candidate proteins whose expression changed significantly in at least 5/9 of the affected regions in PD brain compared to control. Further to this, proteins that had a >5 fold change in expression in one region were also identified. The majority of the top preliminary candidates are mitochondrial constituents, and include proteins involved in the TCA cycle, mitochondrial metabolism and respiratory chain function. Preliminary results from functional assays show reduced complex I activity in frontal cortex of early PD compared to control.

Conclusions: Both application of mass-spectrometry based proteomics and the complex I assay to our unique collection of early stage Parkinson's disease cases, indicate that mitochondrial dysfunction is apparent in both unaffected regions and affected regions early in PD. This may reflect an early pathogenic process in sporadic PD.

References:

Braak et al. *Neurobiol Aging* 2003; **24**:197–211