Chapter 1

Introduction

This thesis reports research on the changes in metabolism in the brain of patients with neurodegenerative diseases. Energy metabolism is of great importance for the functioning of the brain. It is known that energy metabolism is appreciably diminished in Alzheimer's disease and Parkinson's disease. This is based on measurements of glucose and oxygen uptake, which are among the most important materials that are used by the brain's metabolic network. Internally metabolism consists of a complex network of connected biochemical reactions. Little is known how the fluxes (flows, biochemical reactions) of metabolism are redistributed in this network during the development of neurodegenerative diseases. On the other hand, there is a wealth of information on how gene expression is changed in the brain of patients suffering from neurodegenerative diseases. The aim of this thesis project is to use gene expression changes to predict how the fluxes in the metabolic network are redistributed during neurodegenerative diseases. To this end computational modelling methods are applied.

Neurodegenerative diseases

It is estimated that the current world population is at least 7.3 billion and the number is expected to reach 8 billion by the year 2024 (Worldometers.info 2015). As people age, they have a higher chance to be affected by neurodegenerative diseases- neurological disorders that cause disabilities in movement (Parkinson's disease) or mental functioning (dementia). Neurodegeneration is characterised by progressive loss of neuronal structure and function and even neuronal cell death. There are several kinds of neurodegenerative diseases affecting millions of people worldwide such as Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Alzheimer's disease (AD), and Parkinson's disease (PD); in which AD and PD being the most prevalent. The exact causes are still being studied. Some factors are known to contribute to neurodegeneration, which among others include genetic mutations, increased oxidative stress, impaired mitochondrial function, deposition of aggregated proteins, inflammation, activation of neuronal apoptosis and altered intracellular signalling and gene expression (Solanki et al. 2015). Despite the significant advances in neurobiology research, there is still a lack of understanding on the processes and mechanisms of the above-mentioned disorders, which need further studies. Although there are treatments that may help relieve some of the physical or mental symptoms, no cure or way to slow the disease progression is yet available. Understanding of what causes neurodegeneration is therefore critical to help develop new treatment and prevention methods.

Alzheimer's disease

Alzheimer's disease is the most common type of dementia, accounting for 60-80% of the cases. Over 46 million people worldwide are estimated to be living with AD or related

dementia in 2015 (World Alzheimer's Report 2015). AD is a progressive age-related neurodegenerative disease with an insidious onset. 90% of AD cases are diagnosed in people over 65 years of age. Earlier onset ages are rare, and are usually linked to genetic mutations. One of the initial symptoms of AD is a short-term memory deficit, and as the disease progresses, other deficits such as impaired language capabilities (aphasia), inability to perform motor tasks (apraxia), inability to interpret signals from the five senses (agnosia), visuospatial difficulties and executive dysfunction start to appear (Scheltens 2009). Current treatment is symptomatic and at present there are no treatments available to stop the deterioration of brain cells in AD. Apart from neuronal and synaptic loss and volume reduction in specific brain areas, AD pathology is usually characterized by accumulation of amyloid beta (Aβ) plaques and neurofibrillary tangles (NFT) (Hardy & Selkoe 2002; Braak & Braak 1991). These proteins are found mainly in the brain regions involved in learning, memory and emotional behaviour, such as the hippocampus, entorhinal cortex, amygdala and forebrain. Substantial evidence has been found that impairment in brain glucose metabolism is an important pathophysiological feature in AD (Mosconi 2013).

Parkinson's disease

Parkinson's disease is a movement disorder, and symptoms are shaking at rest (resting tremor), muscle rigidity, slowed movement (bradykinesia) and impaired coordination. It affects 1-2% of people over 65 years of age. Pathologically, PD is described by the loss of neurons in the substantia nigra pars compacta (SNc) of the brain which use dopamine as neurotransmitter, and the formation of Lewy bodies, an intracytoplasmic inclusion mainly composed of alpha-synuclein (Abou-Sleiman et al. 2006). Mitochondrial dysfunction and oxidative stress are also associated with PD pathogenesis. Most PD cases are idiopathic having no known cause, but in some atypical cases, the cause is genetic. Several genes that are linked to familial cases of PD are associated with the mitochondria. Examples of such genes are DJ-1, PARKIN, PINK1, SNCA and LRRK2 (Schapira 2008). Evidence was found that exposure to 1- methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), an inhibitor of complex I of the mitochondrial electron transport chain, causes a PD-like syndrome in humans (Langston et al. 1983). Although there are methods to relieve the principle symptoms of motor dysfunction, there is currently no effective treatment that can prevent the disease progression. Current treatments include administration of drugs such as L-dopa to regulate dopamine level in the striatum, deep brain stimulation surgery to help control movement symptoms (Dostrovsky et al. 2002) and therapies to manage everyday symptoms.

Brain energy metabolism in Parkinson's disease and Alzheimer's disease

The human brain has high-energy requirements, approximately 20% of oxygen and 25% of glucose in people at rest is consumed by the brain although it constitutes only

2% of the total body weight (Attwell & Laughlin 2001). The brain requires a constant supply of glucose and oxygen. Under normal physiological conditions, glucose is the main energy substrate of the brain. In the presence of oxygen, glucose is fully oxidized to CO_2 and H_2O, via glycolysis in the cytosol, and the TCA cycle and oxidative phosphorylation in the mitochondria. This results in the production of energy in the form of ATP (Clarke & Sokoloff 1999). Most of the energy consumed is used for excitatory neurotransmission, while the rest is used to maintain the resting potential of the neuron and glia cells (Attwell & Laughlin 2001). Because of the continuous high-energy demand, disturbances in brain energy metabolism can have a direct consequence on brain function as demonstrated by the fast collapse of function when blood flow or oxygen supply to the brain is interrupted.

There is substantial evidence that impaired glucose metabolism occurs in Alzheimer's disease (reviewed in Mosconi 2013). Positron emission tomography studies using ^{18}F-fluorodeoxyglucose (FDG-PET) have demonstrated reduced cerebral metabolic rate of glucose (CMRglc) in AD patients, which may occur earlier than clinical symptoms (Minoshima et al. 1997). Besides glucose, oxygen consumption is also affected (Ishii et al. 1997). Reduction in glucose metabolism can have an impact on the downstream metabolism in the mitochondria. In the mitochondria, reduced cytochrome oxidase activity, an enzyme in the oxidative phosphorylation mechanism, has been reported in AD patients (Kish et al. 1992). Reduction in other mitochondrial energy related proteins including pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase, and isocitrate dehydrogenase has been documented in post-mortem brain and fibroblasts of AD patients (Bubber et al. 2005). Disturbed mitochondrial respiratory function can lead to production of reaction oxygen species (ROS) and oxidative damage.

The development of PD is often associated with disturbed mitochondrial function in the neurons in the substantia nigra pars compacta (SNc), which is the most affected area of the disease. Statistical analysis of gene expression suggests that mitochondrial electron transport and glucose metabolism pathways in the SNc and other brain regions are affected (Zheng et al, 2010). A study of mitochondrial complex activities in post mortem PD patients shows that there is a significant reduction of complex I activity (Schapira et al. 1989) in the SNc and frontal cortex (Parker et al. 2008). Alterations in other electron transport chain complexes, namely complex II, III and IV in substantia nigra and muscles have also been reported (Banerjee et al. 2009; Zhu & Chu 2010). Reductions of glucose and oxygen uptake have been described in PD patient in FDG-PET studies and lactate accumulation has been measured with NMR spectroscopy (Borghammer et al. 2010; Powers et al. 2008; Henchcliffe et al. 2008).

In brief, striking decreases in glucose and oxygen uptake and in mitochondrial enzyme activity have been reported for both Alzheimer's and Parkinson's disease. In contrast, little is known about the specific changes of the patterns of biochemical

reaction rates (fluxes) inside the metabolic network in the brain cells. These metabolic flux distributions are studied in a relatively new branch of science, systems biology.

Systems Biology

Systems biology is an emerging field in biological science. However, it is not a new research area since system-level understanding approach has been introduced as early as 1948 (Wiener 1949). The main reasons it is becoming of high interest throughout the life sciences nowadays are the advancements of high-throughput measurements, which allow us to assemble comprehensive data sets on system performance to gain information on the molecular level (Kitano 2002a). Systems biology ultimately aims at global measurement to understand a biological system as a whole, which is the contrary to the reductionist approach that analyse specific isolated parts to provide an understanding of a larger system. This new holistic approach has the goal to provide more understanding and insights of the complexity of biological systems.

Systems biology is an inter-disciplinary field combining, among others, knowledge and technology from molecular biology, chemistry, mathematics, bioinformatics, and computer science. It integrates data and measurements to study complex biological organization and processes. Since the data set of genome, transcriptome, proteome, and metabolome are large-scale and the interactions of cellular networks are complex, computational modelling has been applied to predict and characterize the complex biological processes and how they function together as a system. Moreover, these in vivo data are difficult to measure and are often incomplete. An integration of computational modelling and experimental data is promising to resolve the intrinsic complexity of biological systems (Kitano 2002b). One of the systems analysis approaches to investigate microarray data has been applied to post-mortem brain samples in neurodegenerative disease cases to identify meaningful biological differences between healthy and diseased state across the entire genome (Wood et al. 2015). Pathway analysis methods e.g. genome wide association studies (GWES) combined with gene ontology (GO) analysis have been used to identify gene expression changes at the pathway level to identify gene sets associated with PD (Zheng et al. 2010). Sometimes, computational models have sufficient explanatory power to provide understanding and knowledge on the studied system. To strengthen the confidence in the model hypothesis, experimental validation is needed and in case accurate experimental results and model predictions do not conform, we need to improve the computational model.

Computational methods to analyse metabolic networks

Metabolic networks

Cellular function is based on the interaction of chemical reactions in a complex network. A metabolic network typically comprises the chemical reactions of metabolism, which together form metabolic pathways. In addition to the chemical reactions there are powerful regulatory mechanisms that guide these interactions. A network reconstruction is a mathematical representation of this biological knowledge. Various databases and reconstructions of human metabolic network exist in public databases including the Kyoto Encyclopedia of Genes and Genomes (KEGG), the Edinburgh Human Metabolic Network (EHMN), HumanCyc, Reactome, Recon 1 (BiGG), and a recent effort by Ines Thiele, Neil Swainston and their team to combine several of these networks into one comprehensive human metabolic network reconstruction, known as Recon 2 (Thiele et al. 2013). One of the most important features of network reconstruction is to catalogue the gene-protein reaction (GPR) in a quantitative, structured and chemically consistent manner. The reconstruction can then provide the basis to construct the metabolic analysis of a system. Mathematical methods to analyse the metabolic reconstruction have also advanced in parallel.

Fluxomics

Metabolic fluxes in large networks are often difficult to measure, which makes computational flux prediction for a metabolic network particularly useful. Determination of the flux distribution in metabolic networks is termed fluxomics. One way to accomplish this is by using measurements of the distribution of stable isotopes. Another approach to fluxomics is to integrate in-vivo measurements of exchange of metabolites with the metabolic system with stoichiometric networks models to allow determination of absolute flux distributions in the network. One of the most widely used approaches is flux balance analysis (FBA) (Winter & Krömer 2013). FBA is one of the most common mathematical methods for simulating metabolism in genome-scale reconstructions of metabolic networks. FBA requires knowledge of the stoichiometry of the reaction network at steady state, and metabolic fluxes are calculated by optimizing an objective function subject to given constraints (Palsson 2006). The objective function reflects the biological function that the biochemical system is assumed to optimize, such as ATP production or biomass production. Decreases in energy metabolism, as discussed above, have been proposed to contribute causally to neurodegenerative diseases such as AD and PD. Although it is clear that glucose and oxygen uptakes are diminished, details of the changes in metabolic fluxes in the complex metabolic network in brain cells are unclear. It is therefore useful to use fluxomics as the model framework to study the systems biology of neurodegenerative diseases. Mathematical models can be used to represent energy and metabolite flow within the system and thus can be

used to understand cellular functions within a particular condition such as during a disease state.

There are several challenges when developing or working with network reconstructions. This includes differences in naming schemes for reactions or metabolites, and different annotation formats. Different pathway analysis tools may use different formats. The most common annotating format nowadays is the Systems Biology Markup Language, SBML. Several tools such as the COBRA toolbox, work in the MATLAB environment. There are other open source environments; most notably among them is the R computational environment, which is widely used in statistics and in the genomics community. It is therefore very useful to develop a software tool in this open source environment.

Several objective function principles in FBA have been proposed such as biomass, ATP and metabolic product maximization. However it is subject to debate what is a suitable objective function. An inappropriate objective function may lead to unreliable flux estimates obtained by FBA. Instead of using such objective functions, we would like to search for additional experimental data to improve flux estimates. A valuable data source for this purpose is gene expression data (van Berlo et al. 2011). In this thesis, we would like to take on an additional approach to traditional FBA, by integrating 'omics' data to increase the predictive power of flux prediction. For this purpose, we aimed to incorporate microarray gene expression data into metabolic fluxes. Gene expression data obtained in patients in disease conditions are amply available, especially for brain tissue samples obtained post-mortem from neurodegeneration patients.

Research Objectives and Thesis Outline

The general aim of the thesis is to establish how metabolism is changed in brain cells in Parkinson's and Alzheimer's disease. Specifically, our aim is to (1) develop computational models for metabolic fluxes in brain cells, (2) develop methods to predict changes in the flux distribution in metabolic networks from gene expression measurements (3) predict how the distribution of metabolic fluxes changes during diseases. The following section will describe the general outline of this thesis.

Chapter 1 presents an introduction about neurodegenerative diseases and computational approach to study metabolism.

As a first step, we studied brain metabolism in healthy subjects, at rest and during physical exertion when the brain shows substantially increased metabolism. This is part of a computational study of human metabolism during endurance sports event which is described in **Chapter 2**. The analysis of changes in brain metabolism during exercise was part of this demonstration of a 'virtual physiological human' modelling approach of human metabolism during exercise. Among others a whole body

model was developed. We investigate energy conversion and heat transport, applied to a cyclist during a mountain time-trial of the Tour de France, and the biochemical events during muscle contraction in the cyclist leg are predicted. As part of this study of exercise, a model of brain central energy metabolism is introduced in this chapter. We calculate brain metabolism during normal resting conditions and during maximal exercise when the uptake of carbon substrates, glucose and lactate, is appreciably increased. The calculations of brain metabolism during exercise and neurodegenerative disease make use of flux balance analysis (FBA), which is a mathematical method for simulating metabolism of metabolic networks at steady-state by optimizing an objective function subject to a given constraints.

To facilitate metabolic network construction and calculation of flux distribution based on the constraint-based modelling approach of FBA, we have developed a software package named BiGGR. BiGGR allows the assembly of metabolic models from, among others, the BiGG database which contains metabolic reconstructions of many organisms, among others humans. The package can be used for FBA computation and visualization of the computational result on the metabolic pathways model. An introduction to the first version of BiGGR is given in **Chapter 2**, and detailed explanation of an improved version of this software package as well as example of its application is given in **Chapter 3**. Availability and advances in other 'omics' field makes data available that can be integrated into FBA to improve flux estimates when the condition of the cell changes. Here we describe a new algorithm, named Lsei-FBA, which incorporates flux balance analysis and gene expression data to predict changes in metabolic fluxes in a diseased condition. The Lsei-FBA algorithm is also described in detail in **Chapter 3**.

The application of Lsei-FBA to Parkinson's disease and Alzheimer's disease is described in **Chapter 4** and **Chapter 5**, respectively.

Chapter 6 provides general discussion and perspective of this study. Finally we summarize this thesis in **Chapter 7**.

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