Computational modelling and analysis of brain metabolism in neurodegenerative diseases

Supandi, F.B.

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Chapter 6

General Discussion

Neurodegenerative diseases have been extensively studied. Nevertheless the actual causes and mechanism underlying these diseases have only been partly clarified. Many studies on gene expression in the brain of neurodegenerative disease patients have been done but information on brain metabolism during the development of these diseases is scarce. There is however convincing evidence that uptake of oxygen and glucose in the brain is substantially diminished, suggesting that energy metabolism is strongly affected. This is often found early in the disease process, before clinical symptoms develop. Understanding the details of the changes of biochemical reactions and the metabolic flux distribution in affected brain areas is of high interest. In this thesis project this was attempted through data analysis of gene expression in combination with computational modelling and simulation approaches with the aim to deduce how the metabolic network in the patient's brain is affected during disease.

Flux balance analysis (FBA) is the most widely used constraint-based metabolic modelling approach because of its efficiency to calculate metabolic fluxes from limited data. In this thesis, we developed a model for brain central carbon metabolism. We further developed a method to integrate FBA with gene expression measurements to predict metabolism during a disease process. This method, termed Lsei-FBA is used to predict metabolic fluxes in two of the most common neurodegenerative diseases, i.e. Alzheimer's disease and Parkinson's disease.

Core model of brain human metabolism

A number of computational models of brain metabolism exist in the literature (Cakir et al. 2007; Occhipinti et al. 2007; Occhipinti et al. 2009; Occhipinti et al. 2010; Cloutier et al. 2009; Aubert & Costalat 2005; Lewis et al. 2010). Most of these models are multi-compartment models that describe the interaction between astrocytes and neurons, and the reactions are compartmentalized in the respective cytosolic and mitochondrial compartments of each of these. In some models only the core of brain metabolism is accounted for with every reaction modelled in detail, while in another model (Patel et al. 2004) some intermediate reactions are lumped together. This is done to maintain a balance between the complexity of the model and the aspects for which experimental data are available. Nonetheless, leaving out details, 'coarse graining' and abstraction are inevitable parts of the computational modelling process and, if well designed, do not diminish the value of an integrated model capable of capturing the essential aspects of the system (Occhipinti et al. 2007). When constructing large models (Lewis et al. 2010) careful manual curation is needed to make sure that the reconstruction is correct and complete, which is difficult because examination of the complexity and size of the network require a lot of time and effort, and reactions may have been missed during a genome annotation procedure. Large reconstructions of human metabolism are known to contain errors and omissions. In this thesis project, we chose to study a model that contains the quantitatively important reactions representing central carbon metabolism, comprising 69 reactions, which occur in astrocytes and neurons. Our

model was not designed to distinguish between cell types because the available microarray gene expression measurements are in most cases taken from whole tissue samples of the brain and gene expression for neurons and astrocytes is represented by one lumped measurement. Because of the manageable size of the model, we could carefully inspect and curate the reactions.

Assumptions for the objective function

The human brain consumes a large amount of energy. About 20% of the total energy turnover of the human body at rest takes place in the brain. Thus, a reasonable assumption can be made that ATP synthesis is maximized in order to fuel energy metabolism at a certain uptake of nutrients. Our medium-sized model provides a reasonable subset of the metabolic system to represent the reactions involved in ATP synthesis. For a given quantified nutrient uptake, the ATP synthesis is maximized. In this case, we opted to concentrate on ATP synthesis since the brain is a highly energetically active organ, which does not grow or produce substances on a large scale. For the human brain at rest we therefore concentrated on the total ATP turnover. Cakir et al. (2007) has discussed several possible other objective functions for models of metabolism in brain (see Supplementary Table 2 in Cakir et al. (2007)). In their paper, Cakir et al. considered maximal ATP production as one of the objective functions, and suggested that the disadvantage of maximal ATP production is the predicted inactivity of the pentose-phosphate pathway (PPP) flux and zero flux in the GABA cycles. To deal with these drawbacks, we implemented constraints on the PPP and GABA shunt fluxes in addition to optimizing ATP synthesis as the objective functions. The lack of lactate release flux, as remarked by Cakir et al. (2007), is in good accordance with the measurements of the control group of elderly healthy people, which we used for the resting state. Maximization of the glutamine/glutamate/GABA cycle has also been considered by Cakir et al. (2007) as an objective function for brain metabolism. The question is whether this is a reasonable idea, because cycling of the neurotransmitters glutamate and GABA should adapt to the environment and to variable needs of signalling in the brain, and is doubtful a priori whether such cycling should be maximized. We did also prefer not to focus on these cycles, which involve transfer between cell types and enzymes with cell-type specific localization, because our present model is designed for the analysis of gene expression measurement on whole brain tissue and does not contain compartments for different cell types. Nevertheless, maximizing ATP turnover in our model of brain metabolism is compatible with maximizing the glutamine/glutamate cycle in the multiple cell type model, considering that the glutamate and GABA cycles are stoichiometrically linked to major ATP consumption.

A key assumption in FBA is that the concentrations of metabolites inside the organ or cell do not change. The fluxes (metabolic rates) into and out of each internal metabolite pool are assumed to be exactly balanced. This may be a good approximation

for a slowly developing disease, but the assumption may not be valid under acute conditions, for instance if blood supply to the brain is acutely interrupted, glucose and oxygen are depleted and lactate accumulates. Similarly, the adult brain is (unfortunately) not renewing itself, and therefore the emphasis on biomass components is quite different than in metabolic models of microorganisms.

To produce a reasonable baseline flux distribution, not only maximization of ATP synthesis, but several other constraints were taken into account: the pentose phosphate pathway flux and the GABA shunt flux which also reflect metabolic functions necessary in brain cells were constrained to values (as a fraction of glucose uptake) that are representative of measurements in normal brain cells. We considered ATP the limiting commodity, and use additional constraints to take other aspects of brain metabolism into account.

Application of transcriptomics technologies in genome-scale metabolic models

The advances in high-throughput 'omics' technology nowadays have allowed scientists to collect large-scale biological data at various levels, thus enabling us to study biological systems as a whole. Despite these advances, the information on protein expression and regulation of enzyme activity in cells is often lacking. Transcriptomics is usually studied genome-wide, but it does not give an accurate picture of the cellular environment at the protein level, because all mRNAs may not be translated equally efficiently and proteins are subject to post-translational modifications and are degraded. Proteomics analysis provides additional information compared to transcriptomics analysis, however usually only a small percentage of the complete proteome is measured and the accuracy varies across samples and preparation method. Metabolomics provides information on cellular metabolite level and fluxes can be determined from metabolite exchange between cells and their environment. The latter has the disadvantage that the measurements are often difficult and only a limited number of fluxes can be measured. Therefore integration of 'omics' data on genome scale metabolic networks can increase their predictive power and add to direct metabolite and flux measurements.

In this thesis, we integrate metabolic flux analysis (FBA) in a metabolic network with gene expression data to predict fluxes during a disease (Chapter 3), developing a method which we termed Lsei-FBA. The key assumption of Lsei-FBA is that the fluxes in a metabolic reaction tend to change proportionally to the changes in expression of the gene(s) connected to the enzyme. While this is probably not a good assumption for individual enzyme-catalyzed reactions, we hypothesize that it may be predictive if the flux distribution in an entire metabolic network is considered. For the human brain, detailed and comprehensive information is lacking on enzyme activity levels and the kinetic parameters that describe the affinity for substrates and products. For an

accurate and detailed model it should also be taken into account which isoforms of the enzymes are expressed and how protein function is affected by binding to other proteins and influenced by the densely populated intracellular environment. Importantly, also too little is known on the processes that regulate the enzymes. Details of the metabolic interactions between the neurons and other cell types in the brain are also incompletely known. We therefore chose for a hypothesis that allows a first, rough approximation. We are presently in a similar position as scientists who set long-range weather prediction as a goal (although their results may have improved in recent years): we cannot expect that our predictions will be very accurate but we hope that our predictions are useful. Given the potential importance of changed metabolism for neurodegenerative disease, we consider that predictions of changes of metabolism during disease, even if they give only direction and order of magnitude of the changes, are of sufficient interest. Glucose and oxygen uptake can be measured in patients and were shown to be substantially decreased, in Parkinson's disease and even more strongly in Alzheimer's disease. Based on our method further details of the changes inside the brain metabolic network are predicted which can in general not be measured in patients.

There are several other applications of gene expression studies in a large genome-scale or pathway-scale meta-analysis that have shown mitochondrial and energy metabolism to be affected in neurodegenerative diseases. A genome wide expression study (GWES) on a pathway-scale model for a Parkinson's disease datasets (Zheng et al. 2010) identifies several gene sets associated with PD, which among others includes defects in mitochondrial electron transport, glucose utilization and glucose sensing. In this analysis the flux distribution was not predicted, although effects on the coarse-grained pathway level were analysed. Lewis et al., (2010) identified significantly differentially expressed pathways in AD gene expression datasets, which suggest significant decreases in glycolysis, TCA cycle pathways, oxidative phosphorylation and malate-aspartate shuttle pathway in the hippocampus, middle temporal gyrus and posterior cingulate cortex region of the brain. While Lewis predicted flux changes during AD based on biochemical activity measurements of one enzyme, alpha-ketoglutarate dehydrogenase, flux changes were not predicted based on gene expression. Instead, an ensemble of feasible flux distribution points is computed using a Monte Carlo sampling algorithm, with the upper and lower boundary of the exchange metabolites constrained to the measurements in AD brains and the flux across alpha-ketoglutarate dehydrogenase limited to a maximum guessed from the biochemical assay value.

Sertbas et al. (Sertbaş et al. 2014) conducted a 'reporter pathway' and 'reporter metabolite' analysis on six neurodegenerative diseases including PD and AD. Gene expression p-values of the diseased patients are used to identify metabolite 'hotspots' around which changes are conspicuous, and subsequently significantly affected pathways in a whole pathway network model were identified. From their analysis, they concluded that energy related metabolism including glycolysis, TCA cycle, and oxidative

phosphorylation, among others, are significantly changed in neurodegenerative pathways. However, we propose that the detailed prediction of flux changes based on the analysis of gene expression changes can play a useful role in the understanding of disease in addition to these pathway-level studies. Analysing gene expression measurements in a connected, balanced network allows to make such detailed predictions. The contribution of this thesis was to develop and apply a method for prediction of the flux distribution in a metabolic network. Where metabolic measurements in patients are often limited to exchange fluxes, in particular the uptake into the metabolic network by PET measurements of oxygen and glucose, our new method allows insight in changes inside the metabolic network based on widely available gene expression measurements in patients.

Energy metabolism in aging and in neurodegenerative diseases

Aging is a major risk factor for neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease and Huntington's disease. The risk of developing PD and AD rises significantly with age, particularly above 60 years. As the brain ages, it loses its ability to self-repair and it undergoes a gradual decline in energy metabolism, and because of their high-energy requirements, the neurons are vulnerable to degeneration. In both PD and AD, the pathways leading to neurodegeneration involve a cascade of events, many of which are parallel to aging, including oxidative stress, inflammation, accumulation of altered proteins, excitotoxicity, proapoptotic mechanisms and mitochondrial dysfunction (Hindle 2010; Chen & Zhong 2013).

Brain glucose hypometabolism has been considered one of the main phenomena accompanying neurodegeneration in both PD and AD. It has also been suggested that altered glucose metabolism occurs early in AD development and precedes or even contributes to neuropathology (Cunnane et al. 2011; Mosconi et al. 2008). Decreased glucose metabolism is sometimes considered the proximate cause of the clinical disabilities in Alzheimer's dementia (Blass 2002). It is also often said that clinical AD symptoms never happen without metabolic reductions, the degree of which correlates with the seriousness of cognitive impairment both in vivo and in vitro (Mosconi et al. 2008). Positron emission tomography (PET) measurements of glucose metabolism (CMRglc) in normal aging have shown mixed results. In some studies, reduced CMRglc is observed in elderly subjects with no known cognitive impairment, while other studies demonstrate no CMRglc decline with age (reviewed in Cunnane et al. 2011). On the other hand, the regional pattern of glucose hypometabolism in AD is different from that seen in normal aged brains. CMRglc studies using FDG PET have shown that the regions with the earliest changes in glucose metabolism in AD are the medial temporal lobe (MTL), entorhinal and perirhinal cortex and the hippocampus (Mosconi 2013). Contrary to AD, a study across age groups in normal aging has shown a mild decrease in glucose

metabolism in the frontal lobe (Loessner et al. 1995). Metabolism in parietal, temporal and occipital lobe varies considerably, while with advancing age, regions in the basal ganglia, hippocampal area, thalamus, cerebellum, posterior cingulate gyrus and visual cortex remained metabolically unchanged (Loessner et al. 1995).

Energy in the form of ATP is produced in the mitochondria through the process of oxidative phosphorylation. The mitochondria are downstream of glycolysis in the metabolic network and impaired glucose uptake may have severe consequences for mitochondrial function (Cunnane et al. 2011). We investigated in particular how metabolism is altered in brain cells during Parkinson's and Alzheimer's disease. A striking prediction is that the availability of ATP, which supplies the energy for cellular processes, is decreased much more on a relative basis than oxygen and glucose uptake. In both AD and PD the decreases in brain metabolism may have an effect in the disease pathology.

PD has been linked with mitochondrial dysfunction and the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) part of the brain. Decrease in complex I activity of the electron transport chain (ETC) has been found in the SNc of patients with PD (Schapira et al. 1989). A decrease in complex I activity has been shown to increase reaction oxygen species production which leads to increased oxidative stress (Hastings 2009). In addition, human exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), an inhibitor of complex I, creates Pakinsonian symptoms in humans through damage to neuronal mitochondria (reviewed in Zhu & Chu 2010). In this thesis it is shown that expression of the genes linked with many of the enzymes in central energy metabolism is modestly decreased. Based on our algorithm a decrease in uptake of oxygen and glucose is predicted that is of the same order of magnitude and direction as was measured in PD and AD patients. The relative decrease in cellular energy production in the form of ATP is shown to be even larger as the measured and predicted decrease in glucose and oxygen uptake. Consequently, it is possible that the decreased energy availability plays a role in the disease process during PD. This decrease in energy availability is even larger during AD.

In AD, apart from neuronal loss, the clinical neuropathology is characterized by accumulation of extracellular senile plaques composed of β-amyloid (Aβ) deposits and intracellular neurofibrillary tangles (NFT) (Hardy & Selkoe 2002; Braak & Braak 1991). Aβ is a protein fragment derived from amyloid precursor protein (APP). According to the traditional hypothesis in AD, the 'amyloid cascade hypothesis', Aβ formation and accumulation over time causes synaptic dysfunction, and accelerates the formation of NFT that eventually causes neuronal death (Hardy & Selkoe 2002; Hardy et al. 2014). The generation of abnormally cut species of Aβ that nucleate in oligomeric species is likely toxic. These fragments can be secreted and aggregate into plaques but also spread from cell to cell to initiate new seeds for aggregation. Apart from plaques and tangles, there is substantial evidence pointing to dysfunction in mitochondrial bioenergetics and oxidative stress in AD. Mitochondrial changes may occur earlier in AD brain than plaque

deposition and may promote the Aβ plaque deposition in mice (Praticò et al. 2001). However, Aβ may form toxic oligomeric species and plaque formation may already constitute a later stage. The potential early causal role of mitochondria in these processes in AD is not clearly established and requires further investigation. The relationship of AD to aging, a process which may involve mitochondrial function and integrity, has been invoked to argue that mitochondria may show a biochemical cascade of progressive dysfunction which affects amyloid precursor protein and plaque formation in late onset AD (Mosconi 2013) which may in turn feedback to progressive mitochondrial decline (Swerdlow et al. 2014). Although genes for APP and for enzymes that process APP are strongly linked to familial cases of AD that usually occur before old age, mitochondrial function may play an important role in late onset AD. Oxidative stress as a result of perturbed mitochondrial function may promote the processing of APP to amyloidogenic derivatives of Aβ itself (Mosconi 2013 and reference therein).

A deficient energy metabolism resulting from defective mitochondrial function and increase oxidative damage may change the overall oxidative microenvironment leading to neuronal dysfunction and death (Mosconi 2013). Synaptic loss and decreased neurotransmitter production has been proposed to inhibit mitochondrial enzymes, increase oxidative stress and thereby initiate synaptic dysfunction that further reduces glucose demand in affected brain regions in AD (Cunnane et al. 2011). These mechanisms combined thus create a vicious cycle between brain hypometabolism, and neuropathology in AD (Mosconi 2013; Cunnane et al. 2011). These theories underscore the potential importance of changes in brain metabolism for the development of AD.

Conclusion: Towards systems biology of neurodegenerative diseases

Neurodegenerative diseases are caused by complex mechanisms. Computational modelling approaches to integrate various levels of 'omic' measurements in the study of metabolic changes in neurodegeneration are becoming useful to help understand the complex dynamic mechanism. In the future such approaches may facilitate development of therapeutic treatment strategies.

There are a number of gene expression studies in neurodegenerative diseases, but to the best of our knowledge there were no fluxomics studies of these diseases that take into account gene expression measurements to predict metabolic fluxes. Therefore, in this thesis, we addressed the challenge to predict how internal metabolism is altered during the disease and among others calculated ATP production from changes in gene expression measurements and compared predictions with what has been reported in the literature about measurements of the exchange reactions of glucose and oxygen in the diseased state. This approach may be useful as model of prediction in neurodegenerative diseases which can also be applied in other studies. Where comparisons are possible with measurements, the predictions agree reasonably well

with the experimental data. Although these first results are promising, further comparisons with experimental data are highly desirable to assess the scope and accuracy of the prediction method developed in this thesis. It cannot yet be decided whether the present approach is applicable to other organs and diseases. Nevertheless, based on the results on neurodegenerative diseases in the brain, it is possible that the present approach is not only applicable to neurodegeneration, but also to other diseases affecting the brain and other organs.

The computational models could be extended in the future to include multiple pathways and regulatory mechanisms. Particularly, a whole cell model can be built that integrates cellular interactions at multiple levels, i.e. gene regulation, transcription factors, protein interactions, etc. (Andres 2012). In conclusion, computational models of brain energy metabolism provide understanding of neurodegenerative processes, and this can in turn serve as a basis to develop future treatment of neurodegenerative diseases.

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