Spot the difference

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English summary

Summary

Alzheimer's Disease (AD) and Parkinson's Disease (PD) are the two most common neurodegenerative diseases. The disease course for both AD and PD is progressive: over time, patients become increasingly ill. Age is a major risk factor for both diseases. For example, at an age of 70, roughly 1 out of 100 people is diagnosed with AD. This number increases dramatically to 1 out of every 3 individuals of 85 years and older. In a population that is growing older and older, it is therefore difficult to underestimate the impact of these diseases, both on a personal and socio-economic level. In particular, the absence of options to effectively treat, or even slow down the progression of these disorders means that individuals diagnosed with AD or PD face a long and debilitating disease with an inevitably negative outcome.

During the last 20 years, substantial progress has been made in identifying the biological processes potentially involved in these diseases. Notably, the field of clinical genetics has contributed invaluable knowledge by the discovery of gene mutations that cause rare, familial forms of PD and AD. Furthermore, several toxins have been identified that cause Parkinson-like alterations in the human brain. Both the gene mutations and toxins have been fundamental in the development of cellular and animal models that allow researchers to study certain aspects of AD and PD.

The currently known gene mutations underlying familial forms of AD and PD are very rare, and account for less that 5% of the disease cases. Thus, 95% of disease cases are of sporadic origin, and are not caused by any known gene mutations. A valid question therefore is whether genetic and sporadic forms of AD are caused by exactly the same biological mechanisms. This is very likely not the case. The current hypothesis is that sporadic AD and PD are not caused by a single alteration in the brain, but by a combination of unknown biological and environmental factors.

One of the ways in which molecular biologists study disease processes, is by measuring changes in the activity of genes. The human genome contains around 20.000 genes. Unfortunately, until recently researchers were only able to study the activity of tens of genes at a time. As it is unknown which genes are more or less active in AD or PD, it is not feasible to gain a complete picture of changes biological processes in these diseases using 'classical' molecular biological techniques. A recently developed technique however does allow for the parallel measurement of gene activity of thousands of genes at one time. This is accomplished by microarrays technology: glass slides containing microscopically small detectors for virtually all genes. Such an experiment can therefore provide a global overview of all the changes in gene activity, and thus the biological processes, in the brain of a patient. Microarray technology therefore is highly suitable for studying complex, multifactorial disorders such as AD and PD.

The main research question this thesis addresses is: which alterations in gene activity occur in the brains of AD and PD patients? The application of microarray technology plays a central role to address this question in this thesis. Furthermore, the use of human postmortem brain tissue represents a unique and essential aspect of the research presented in this thesis, as no cell culture or animal model can provide a complete picture of the diseases as they occur in the human brain. We are particularly interested in gene activity changes that occur early in the disease process, at a point where intervention (for example in the form of medication) might be able to prevent the disease process from spreading throughout the entire brain.

In **Chapter 3** we present alterations in gene activity, as determined by microarray analysis, in the brains of PD patients compared with control donors. We have studies three brain areas: the substantia nigra, the caudate nucleus and the putamen. We have chosen the substantia nigra, as this brain region is most severely affected in PD: around 80% of the nerve cells are lost during the disease course. The degenerating nerve cells project their extensions towards the caudate nucleus and putamen. One of the current treatment strategies is therefore based on compensating the loss of nerve cell contacts in the caudate nucleus and putamen by administering L-dopa.

Our research shows that the activity of only very few genes is changed in the caudate nucleus and putamen. This is most likely due to the fact that patients were treated with L-dopa and the lack of neurodegeneration in the striatum. In the substantia nigra however we have successfully identified a group of 287 genes for which the activities are significantly changed in PD. The majority of these genes were less active. As we have investigated particular parts of the substantia nigra where the loss of nerve cells was relatively mild, the reduction of gene activity could not be explained by the loss of nerve cells alone for the majority of genes, but represents actual changes in activity. An extensive analysis of the data using bio-informatics demonstrated that the reduced gene activity is most profound in genes that are involved in the transfer of nerve signals, the energy supply of cells and the clearance of damaged proteins. Some of these changes have been reported before, but we for the first time demonstrate that the loss of activity of these genes already occurs in nerve cells that are still alive.

Furthermore, we have identified a number of previously undiscovered changes in gene activity that point to a reduction of neurotrophic support (the maintenance of nerve cells by growth factors) and alterations in signals that regulate the outgrowth of and contacts between nerve cells. When the communication between nerve cells is reduced, and essential growth and maintenance signals are reduced, nerve cells are at risk of degenerating. We therefore hypothesize that these changes might causally contribute to the loss of nerve cells in PD.

In **Chapter 4** we describe an alternative method for the analysis of datasets that are generated using so-called two-color microarrays. This kind of microarrays is characterized by the simultaneous measurement of gene activity in two samples, one of which is labeled with a green fluorescent label, the other with a red fluorescent label. If the green sample is derived from for example an AD patient, and the red sample from a control donor, the relative amounts of green and red signal directly determine the relative gene activity between the patient and the control donor. Thus, two-color microarrays are very well suited for comparing two different groups, and are robust against variations between experiments. A drawback of dual-color microarrays is that the comparison between multiple groups is more difficult, as only the ratio between two samples can be measured at one time. We have therefore explored the possibility of using the separate intensities of the red and green signal for data analysis, opposed to the standard ratio between the two colors. In this manner, the measurements are treated separately, which allows for the comparison between samples that are not measured on the same microarray.

Our results indicate that intensity-based analysis of dual-color microarray data is indeed feasible. Even more so, the reproducibility of the results and the sensitivity for the detection of changes in gene activity both increase when choosing an intensity-based analysis method instead a ratio-based method. The improvements are most pronounced when the number of groups increases, and in the case of substantial within-group variation. We therefore chose to analyze the AD dataset in Chapter 5 with the intensity-based method.

In **Chapter 5** we describe the changes in gene activity during the development and progression of AD, again using microarray technology to measure gene activity. The microscopic changes that are associated with AD are known to appear in precisely defined brain areas during the course of AD. It is therefore possible to postmortem classify the brains of individuals into different phases of the disease, even those patients who are cognitively unaffected, but do show the first alterations associated with AD. The different stages of AD were first described by prof.dr. H. Braak, and are therefore called the Braak stages.

We have made use of the Braak stages for the selection of donors in our study. For each of the 6 Braak stages, we have included 7 patients. We also included a group of 7 patients without any AD-associated pathology. In this manner, we have been able to measure alterations in gene activity throughout the entire process of AD. The brain area we have used was the prefrontal cortex, an area that is affected relatively late in the disease process. This allowed us to discriminate between early, potentially causative changes in gene activity, and late changes that might be secondary to the disease process. This knowledge is of particular importance for the development of new drugs that might prevent the development of AD altogether, as opposed to drugs that only alleviate symptoms.

The most important finding of this study is the increased activity in a large group of genes during the earliest stages of AD, before the onset of the microscopic alterations and before any cognitive alterations are detectable. The main biological themes of represented by this group of genes are the transfer of nerve signals and the energy supply of nerve cells. We hypothesize that the changes in gene activity represent a population of more active nerve cells, as a compensatory response to the earliest, as of yet unknown alterations in AD. At the moment that AD-associated microscopic alterations become visible in the prefrontal cortex, the activities of these genes decreases dramatically, and continue to decrease towards the latest stages of the disease. Interestingly, a number of genes that are present in this gene group are directly involved in the formation and breakdown of the key component of the senile plaque, one of two microscopic alterations associated with AD. We therefore hypothesize that the changes in activity of these particular genes, possibly in combination with other unknown factors, might play a causal role in the development of the disease.

Even though data generated by microarray experiments are very reliable, independent confirmation of results when determining changes in gene activity remain important. This is especially true for experiments on human tissue, as these experiments have to deal with substantial variation between subjects. In **Chapter 6**, we therefore combine the results from the AD dataset in Chapter 5 with four previously published studies on gene activity changes in the human end-stage AD brain. This 'meta'-analysis resulted in a list of 69 genes, for which an altered activity was detected in at least 3 out of 5 studies. A number of these genes are involved in maintaining the calcium balance or the energy levels of nerve cells. We also observed evidence for changes in the capacity to repair damage to DNA, the regulation of cell division cycle and the breakdown of cholesterol in the brains of AD patients. These data provide leads for further research into the specific contribution of these genes and processes in AD.

It is a well known fact that women are at greater risk for developing AD, even after correction for the fact that women live to be older than men. One obvious difference between men and women are sex hormone levels. Recent studies have shown that sex hormones are also produced in the brain, and sex hormones such as progesterone and estradiol can provide protection to nerve cells. Sex hormones and derivatives can also affect the transfer of nerve signals by modulating the receptors for neurotransmitters. These observations suggest a potential relationship between differences in sex hormone levels and susceptibility to AD. In **Chapter 7** we describe a systematic search for alterations in the synthesis and breakdown of several sex hormones in AD, by measuring gene activities and localizing the corresponding proteins using immunohistochemical methods. The results from this study point to an increase in the production of sex hormones in the early stages of AD, which might indicate a compensatory mechanism to protect nerve cells against AD-associated changes.

In the general discussion of this thesis (**Chapter 8**) we summarize and discuss the studies as presented in the previous chapters. We discuss a number of interesting observations that warrant further investigation, such as the interaction between variations in the ApoE gene - the most important genetic risk factor for AD - and the gene activity alterations described in Chapter 5, the commonalities and differences between normal aging and AD, and the role of genetic variations in nerve cell outgrowth signals in the neurodegenerative events in PD. Furthermore, we discuss specific prerequisites - such as parameters that influence the quality of brain tissue - that are specifically associated with experiments on human postmortem brain tissue. In the final part of the discussion we propose future experiments. We argue that future research efforts should focus on the question what the specific functional consequences are of the alterations in gene activity for the development of AD and PD. We propose several bioinformatics, cell culture and animal experiments that should address these questions. We are currently engaged in several of these experiments.