Genetic and molecular pathogenesis of primary open angle glaucoma and corticosteroid-induced glaucoma

ADDENDUM

SUMMARY

The subjects of this thesis are the pathogenesis of an increase in intraocular pressure (IOP) in primary open angle glaucoma (POAG) and a corticosteroid-induced increase in IOP. A sustained increase in eye pressure causes damage to the optic nerve, also called glaucoma. This results in visual field loss which slowly progresses over time and eventually causes blindness. Glaucoma is the second leading cause of visual impairment and blindness worldwide. The increase in eye pressure is the only manageable risk factor and since the precise pathogenesis of an increased IOP is largely unknown, current treatment is focused on lowering the eye pressure.

A small part of the eye, the trabecular meshwork (TM) tissue, is thought to play an important role in the pathogenesis of an increase in eye pressure. In the eye, a specific type of fluid (aqueous humor) is produced. The eye pressure is regulated by a balance between the production and outflow of this fluid. The outflow is regulated by the TM as this is the part of the eye through which this fluid leaves the eye. There is substantial evidence that the TM plays an important role in increasing the IOP .

An increase in eye pressure may also be caused by corticosteroids. These drugs are often used by ophthalmologists for the treatment of several eye diseases or to prevent inflammation after eye surgery. It is known that approximately 3 out of 10 patients who use corticosteroid eye drops develop an increase in eye pressure. This is called a steroid response. When this response takes place over a prolonged period of time, the sustained elevation of eye pressure will damage the optic nerve. When this occurs, this is called corticosteroid-induced glaucoma. As in other forms of an increased eye pressure, changes in the TM are thought to be the cause of a corticosteroid-induced increase in eye pressure, however, the precise pathogenesis is unknown. At this time, the only final way to lower the eye pressure is to stop the treatment with corticosteroids. However, the corticosteroids are prescribed for a reason and stopping them is not always possible due the underlying disease of the patient. In addition, conventional treatment of an increased IOP may be ineffective or invasive. If there is no alternative for corticosteroids, the development of an increased eye pressure upon the use of corticosteroids can create a difficult dilemma for the treating ophthalmologist.

In this thesis we describe how we obtained a better insight into the molecular pathogenesis of an increased IOP in primary open angle glaucoma, a corticosteroid-induced increase in IOP and the molecular mechanisms in the healthy TM tissue.

Throughout the years several studies have been performed, generating large amounts of biological data, among which, omics data. However, due to lack of analytical techniques, these data were not always explored to its full extent. The techniques are now available

and are summarized by the term 'bioinformatics'. Bioinformatics is the science that enriches the biological knowledge by applying methods from informatics to the biological data. These analyses create the opportunity to integrate the existing data and to use it to its full potential.

In chapter two to four, we build on the shoulders of previously performed research by integrating and re-analyzing publicly available datasets by means of bioinformatics analysis. The type of data that we used is gene expression data as it is known that the level of the expression of genes is different between diseased tissue and healthy control tissue. Within these studies, we did not only re-use data which allowed to save resources and costs, we also used freely accessible tools. Therefore, the methods used in this thesis can easily be applied by other researchers and for other diseases.

The first step towards a better understanding of how an increase in IOP develops and the role of the TM in it, is to obtain knowledge about the functioning of the healthy trabecular meshwork. Therefore, in **chapter two**, we performed a systematic search and collected all gene expression data (micro-array) on healthy TM tissue. After a check of the quality of the data, 18 datasets containing gene expression data of the healthy TM of 44 different deceased individuals remained. These data were integrated by means of bioinformatics analysis. In this study, we investigated which genes are overall highly expressed in the TM and therefore form the signature genes of this tissue. This study led to the identification of 1882 genes. However, a gene is only a small part of a complete molecular process in which it participates. For the human body, these molecular processes are presented as pathway diagrams of interacting molecules. Therefore, we also investigated in which pathways or molecular processes the identified signature genes of the healthy TM are involved. Pathway analysis revealed multiple molecular processes, including some that were already known to be active in the trabecular meshwork, for example extracellular matrix, elastic fiber formation, elastin crosslinking and focal adhesion. Also, 46 candidate TM-specific genes were identified. These consist mainly of pseudogenes or novel transcripts of which the function is unknown. The signature genes and processes identified in this study can be used as a reference to compare diseased TM tissue. The candidate TM-specific genes are of interest for further investigation and characterization.

In **chapter three**, we compared the gene expression of the TM tissue in patients with and without POAG. The data consisted of 14 patients with POAG and 13 control cases, all derived from one previously published and publicly available study. We identified the genes that are differently expressed between the two groups. Following, we investigated in which molecular processes these genes are involved. These processes could be

grouped in five large clusters: extracellular matrix, inflammation, complement activation, senescence, and Rho GTPase signaling. Some of these clusters were already known to play a role in POAG, for the others, a clear path of involvement could be hypothesized. Of special interest is Rho GTPase as Rho kinase inhibitors are known to lower the eye pressure. In addition, to obtain a better insight in how these genes in the retrieved pathway are working together and how they are interconnected, we made a network of connecting genes. This showed a central role for inflammation. Furthermore, some genes such as *TGF-β1*, *CD44*, *COL3A1* and *COL1A1*, *SERPINE1* and *PLAU* were shared by multiple clusters and could be linked to known biochemical processes causing POAG, confirming a possible important role in the pathogenesis of the disease.

In **chapter four**, we investigated the gene expression data of TM tissue exposed to corticosteroids. For this study, we were able to include data from five publicly available studies. The data were integrated and re-analyzed by means of bioinformatics analysis. The differentially expressed genes were found to be involved in multiple processes such as collagen, extracellular matrix, adhesion and inflammation. These processes have been researched and hypothesized by other research groups to play a role in the development of a corticosteroid-induced increase in eye pressure. However, for patients included in this part of the study, it was unknown whether they developed a steroid response or not after exposure to corticosteroids. Therefore, it is not known whether the identified processes are causative for the development of a corticosteroid response or only represent the effect of corticosteroids on the TM tissue.

In the second part of the study presented in chapter four, we included data on bovine eyes for which it was known whether they developed a corticosteroid-induced increase in IOP or not. The processes we identified after exposure of the bovine TM to corticosteroids were identical to the process identified in the first part of the study. As this validated the suitability of the bovine data, we compared the gene expression in this TM tissue with and without a corticosteroid response. The same clusters as identified in the first part of the study were identified again. In addition, two other clusters were found to be highly involved as well: cell cycle and senescence. Further investigation showed that the gene expression of the genes involved in these two clusters are in the opposite direction for those with and those without a corticosteroid response. This finding of even an opposite response instead of a different quantitative response suggests that these two clusters play an important role in the pathogenesis of corticosteroid-induced increase in IOP.

In **chapter five**, we showed how gene expression data of chapters two to four can be used for the identification of new treatment possibilities for POAG. Three different methods were described that allowed the identification of new medical treatments for POAG by focusing the search for known drugs that are related to the identified genes

and molecular processes that are known to be involved in the pathogenesis. Some drugs that are known to influence the TM and the eye pressure, such as rho kinase inhibitors, corticosteroids and statins were identified. However, to further downsize the number of potentially effective drugs, additional research, among which a detailed investigation on the applied methods, combining the results with insights in biochemical processes and pharmacodynamics and eventually validation in animal experiments, is necessary.

After obtaining a better insight into the molecular processes involved in a corticosteroid-induced increased IOP (chapter four), we also aimed to obtain a better insight into the role of relevant genes in a different way. We performed a genome-wide association analysis (GWAS) in which we investigated single base-pair genetic variances, also called single nucleotide polymorphisms (SNPs), between patients with and without a steroid response (see further). As it was very important for this study to correctly identify which patients are steroid responders and which are not and to exclude other causes of an increase in IOP, we performed an additional study in **chapter six.** In this chapter we identified which risk factors are associated with an increase in eye pressure (other than the use of corticosteroids or in interaction with these drugs) after a corneal transplantation. We did this based on the results of 76 studies. All risk factors were identified and per risk factor, all the evidence was visualized in tables. These tables allowed to judge which risk factors are highly likely to cause an increase in eye pressure after corneal transplantation. The following risk factors were identified: pre-existing glaucoma, high eye pressure before the surgery, corneal transplantation combined with the removal or exchange of an intraocular lens, and aphakia and pseudophakia with the intraocular lens placed in the anterior or posterior chamber.

In **chapter seven**, we performed a GWAS in which small variances in the genomes between patients with and without a corticosteroid response were identified. These variances help to identify associated genes. In turn, these genes enable us to increase the understanding of the pathogenesis of a corticosteroid response and can be investigated as candidate target genes for new treatment options. We included a study cohort of 339 patients and collected their blood samples for genome-wide association analysis. All the included patients had undergone a corneal transplantation and had to use steroid eye drops, in a protocolled fashion, for at least a year after this procedure. Since most patients develop a corticosteroid-induced increase in IOP when corticosteroids are used between one and eight months, this group is the ideal study population to investigate the genetic components of a corticosteroid response. Two ophthalmology residents, and when needed a glaucoma specialist, independently identified the responders and non-responders based on strict criteria. In addition, a distinction between high and low responders was made. Within our study cohort, 39.5% developed a corticosteroid

response of which 27.6% were high responders. Genome-wide association analysis on all responders vs. non-responders revealed 172 SNPs which were assigned to 18 genes. These genes are involved in the expression of the glucocorticoid receptor, the development or functioning of the trabecular meshwork, or refer to molecular processes like the extracellular matrix and cell cycle. We also found that small genetic variances in one of the identified genes (*UBL5*) determine whether a patient develops a corticosteroid-induced increase in IOP. Multiple of the identified genes are targeted by rho-kinase inhibitors, indicating that these drugs might be an effective treatment by addressing its molecular pathogenesis. In addition, a distinction was made between high and low responders. This revealed that there are genetic differences between these groups, however, this requires more research.

In **chapter eight**, the general discussion, we reflect on the findings within this thesis. In addition, the future perspectives of this research are highlighted as we look forward to the use of peripheral blood mononuclear cells (PBMCs) to obtain a better insight into the individualized pathogenesis of a corticosteroid-induced increase in IOP.