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

ADDENDUM

REFLECTING ON IMPACT

This thesis:

- Shows a new approach in ophthalmology to synthesize current knowledge on the pathogenesis of eye diseases and to identify the involved genes and molecular processes. It provides a solid genetic foundation to further investigate the molecular processes in healthy TM, POAG and a corticosteroid-induced increase in IOP.

- Brings the identification of new interventions from all existing drugs close to new clinical applications in ophthalmology. It opens opportunities for the Maastricht University to patent these new applications.

- Contains the largest GWAS study on corticosteroid-induced increase in IOP conducted until now and shows new insights into its genetic mechanisms.

- It's data and new biomaterial give an interesting and promising foundation for future research and is available for other researchers from the Maastricht University or somewhere else.

- Opens the doors for individualized treatment. The study of individualized molecular mechanisms, especially in those with a corticosteroid-induced increase in IOP with specific SNP's, can be based on the development of TM from lymphocytes derived stem cells. The impact of exposure to steroids in specifically changed molecular processes can then be tested. Bringing this to the clinic it is then possible to target the treatment on the molecular mechanisms of individuals.

Research

What is the main objective of the research described in the thesis and what are the most important results and conclusions?

POAG is a chronic neurodegenerative disease of the optic nerve in which there is a progressive loss of retinal ganglion cells. POAG leads to a specific type of visual field loss and may eventually lead to blindness. It is the second leading cause of visual impairment and blindness worldwide.[1] The IOP is the most important risk factor. Treating an increased IOP leads to a slower progression of the loss of retinal ganglion cells and related visual field loss. An increased IOP can also be caused by topical corticosteroids, also known as a corticosteroid response. This occurs in approximately 18%-36% of patients and this percentage can be as high as 92% in patients with POAG.[2] A corticosteroid response may also occur when corticosteroids are used systemically, intranasal or after application on the skin around the eye. A sustained increase in IOP caused by the use of these drugs can also lead to visual field loss and eventually blindness.

POAG and a corticosteroid response are likely caused by molecular changes in the TM which increase the outflow resistance, thereby causing an increased IOP.[3-6] The precise molecular pathogenesis of both diseases is still largely unknown. One of the main objectives of the research described in this manuscript is therefore to further unravel their molecular pathogenesis.

In chapters two to four we described our approach and results. In more detail, we re-used, integrated and re-analyzed all publicly available gene expression data on healthy TM tissue, TM tissue exposed to corticosteroids and TM tissue of patients with POAG or a corticosteroid response. This allowed us to obtain an overview of the signature genes and molecular pathways of the functioning of the healthy TM, the molecular processes involved in the pathogenesis of POAG and a corticosteroid response.

In chapter five, we illustrated how the data retrieved in chapters two to four can be used to identify possible drug targets. The methods to identify new medical treatments allow to focus the search for drugs that are related to the identified genes and molecular processes that are known to be involved in the pathogenesis. This approach has the advantage that also already registered drugs are identified. Their adverse events are known and medical specialist have experience with these registered drugs. Three different methods to identify relevant drugs were shown, all specifically selected to modulate the disease outcome.

In chapter six, we report the results of a systematic review. This study was designed to systematically collect and summarize the current evidence on risk factors for the development of an increase in IOP after keratoplasty and followed a semi-quantitative method which allowed the identification of highly likely associated risk factors. These risk factors could be possible confounders which were taken into account in the identification of corticosteroid responders in chapter seven.

In chapter seven, we report about our GWAS study which was performed to identify which genetic variants are linked to a corticosteroid response. In order to do so, the study population was carefully selected as the long exposure to corticosteroids and the criteria used to identify a corticosteroid responder both lowered the risk to misclassify non-responder as responders. We compared responders vs. non-responders and we made a distinction between high and low responders.

Another objective of the thesis was to identify new treatment options that target the identified molecular processes that causes POAG and a corticosteroid response. In chapter seven, multiple of the identified genes are involved in the rho-kinase pathway, suggesting that rho-kinase inhibitors are highly suitable to treat a corticosteroid response. In chapter four, we also identified clues that rho-kinase inhibitors might be effective to treat a corticosteroid-induced increase in IOP. Therefore, rho-kinase inhibitors warrant further investigation as treatment for a corticosteroid-induced increase in eye pressure, with potential target genes identified in both high and low responders. A recently published study found that ripasudil (a rho-kinase inhibitor) significantly lowered the IOP in patients with a corticosteroid-induced increase in IOP.[7]

Relevance

What is the (potential) contribution of the results from this research to science, and, if applicable, to social sectors and social challenges?

We integrated all current gene expression data and developed a model for the molecular pathogenesis of POAG and corticosteroid induced ocular hypertension. The relevance of this for science is that it helps other researchers to build on this model of current evidence for new research. It is the reference to specifically investigate genes or molecular processes of interest for POAG or a corticosteroid response to further unravel the pathogenesis of both diseases. In addition, it is the reference to find diagnostic or prognostic markers, new drugs or markers for effect modification of treatments.

In chapter two, we present an overview of the processes and genes that are involved in the functioning of the healthy TM which can be used as a future reference to study physiological processes of the TM. We showed that processes related to ECM, elastic fiber formation and actin crosslinking are important for the functioning of the healthy TM. In addition, multiple highly likely TM specific genes have been suggested which can be used for further functional investigation and might be of special interest for drug targeting of their encoded proteins. Furthermore, the complete lists with housekeeping genes and pathways and the investigated genes in the TM with their average gene expression are provided. This allows other researchers to use this data as a reference for investigating molecular mechanisms. Several identified molecular processes in the healthy TM have shown concordance with molecular processes in patients with POAG.

In chapter three we provide a comprehensive overview of the processes involved in the molecular pathogenesis of POAG. We identified multiple pathways which were clustered into five functional categories: ECM, inflammation, complement activation, senescence, and Rho GTPase. These categories were combined into a network of connecting genes, showing overlap between the categories and the central position of the inflammation cluster. Also, the genes present in at least three categories were visualized within the network. The additional Gene Ontology analysis on our pre-processed and quality controlled dataset showed that the significantly changed genes were involved in ECM, inflammation and cell adhesion, which we already found in the pathway analysis. Additionally, development and corticosteroid related clusters were found. The identified clusters and the genes involved in these clusters can be used by other researchers to further unravel the pathogenesis of POAG.

In chapter four we found the functional processes cell cycle and senescence to be highly likely involved in the pathogenesis of corticosteroid-induced increase in IOP. Other processes such as collagen, ECM, adhesion and WNT-signaling behave differently between responders and non-responders as well. However, as these differences are mainly based on differences in intensities of gene expression rather than opposites, further investigation of these processes is needed. These pathways and their involved genes, and maybe especially the genes shared between the identified processes after comparing responders and non-responders, are of interest for further investigation. Fibronectin 1 was shared by the largest numbers of clusters, however, as discussed in chapter four, the precise role of this gene in het pathogenesis of a corticosteroid-induced increase in IOP needs further investigation. This fuels the research initiatives of other researchers.

In chapter five, we showed how the obtained gene expression data can be used for the identification of new treatment possibilities for primary open angle glaucoma. As an example, the results of chapter three were used as input for three different methods for drug repurposing. 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 were previously found to be involved in the pathogenesis. Some drugs that are known to influence the trabecular meshwork and the eye pressure, such as rho kinase inhibitors, corticosteroids and statins were identified.

In chapter six we performed a review on the risk factors for the development of an increased IOP after keratoplasty and the level of evidence that is available for each risk factor. Based on the evidence tables, factors with a definitive and probable association with an increased risk for IOP elevation have been established. This can help to identify patients at risk and to individualize patient care concerning the choice of therapy, postoperative treatment and follow-up. However, we have also shown that many risk factors still lack sufficient evidence to determine its association and need further investigation.

In chapter seven we performed a GWAS study to identify which genetic variants are linked to a corticosteroid response. Comparing responders vs. non-responders, revealed 172 SNPs and 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*) might determine whether a patient develops a corticosteroid-induced increase in IOP or not. 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. We also found that several of the identified genes are involved in the rho-kinase pathway, suggesting that rho-kinase inhibitors are highly suitable to treat a corticosteroid response. A recently published study confirmed our hypothesis that ripasudil significantly lowered the IOP in patients with a corticosteroid-induced increase in IOP, however, they only included a small study cohort.[7] This gives a clue for ophthalmologists to effectively treat patients with a steroid response.

In addition to our findings, our data and biomaterial can also be used by other researchers. We therefore described two new research proposals in chapter eight to illustrate how the results, data and biomaterial collected for this thesis may form the foundation for further research. We presented that the collected blood samples for the GWAS studies contain peripheral blood mononuclear cells (PBMCs) which can be used to generate TM-like stem cells. As TM cells are rather difficult to obtain in vivo this can be a way to study the molecular pathways in TM cells of individual patients, especially in those patients with specific genetic changes. In addition, we would be able to investigate how the gene expression changes after exposing these cells to new treatment possibilities which would also facilitate an individualized approach. Developing cells and tissue from stem cells can be seen as the biopsy of the twenty-first century.

The blood samples for the GWAS study were collected from patients who underwent a corneal transplantation mainly due to Fuchs endothelial dystrophy and keratoconus. Therefore, the data from our GWAS study can also be used by other researcher to study the genetics of these corneal diseases. The obtained samples and data also create the opportunity to join international collaborations.

The societal impact of our results is not only based on the opportunities to influence the outcome of glaucoma but also on the opportunities to influence the cost of glaucoma. Besides an impact on quality of life, visual impairment and blindness also have a significant impact on economic costs of which a substantial part is related to caretaking and home-help.[8-10] This economic impact will grow on the population level due to the high prevalence and increasing number of elderly people affected by glaucoma. It has already been shown that decreasing the IOP prevents the occurrence of blindness and reduces the costs of glaucoma for society.[10] A better understanding of the pathogenesis and the development of effective drugs, with fewer or no side effects, will not only improve quality of life but also reduce costs of blindness.

Target group

To whom are the research results interesting and/or relevant? And why?

The results are relevant for ophthalmologists and glaucoma specialists. The pathways and networks in the thesis show how multiple processes interfere with each other, increasing the understanding of the pathogenesis and emphasizing which processes eventually have to be targeted. It also shows ophthalmologists that there is a promising future for the medical treatment of glaucoma as multiple opportunities for new drugs have been proposed in this manuscript. More specifically, we have shown that Rho-kinase is involved in the pathogenesis of a corticosteroid-induced increase in IOP. Therefore, Rho-kinase inhibitors should be introduced in the Netherlands urgently. In addition, in chapter six, we identified the risk factors for developing an increased IOP after corneal transplantation. This can help clinicians to select corneal transplant patients at risk of developing an increased IOP and adjust their follow-up and treatment accordingly.

The results are also of interest for other researcher in the glaucoma field who want to compare their results to ours or can use our databases as a reference or starting point. As already mentioned above, in chapters two to four we laid the foundations for applied research to develop new treatment options for glaucoma.

As mentioned above, researchers in the field of Fuchs endothelial dystrophy or keratoconus may also benefit from our findings and data.

Lastly, omics research is booming. This type of research creates large amounts data which contain a lot of information. For example, it allows us to obtain information about the gene expression of thousands of genes within one single tissue of one single patient. Therefore, we need a way to integrate and present the results in a (clinically) meaningful way. Bioinformatics analyses are the key to do this. All the information we need is available online, even the data. This creates new possibilities for researchers to build on already obtained data and knowledge and therefore: "The World Wide Web is our lab". The methods presented in chapters two to four and chapter eight of this manuscript are based on freely available tools. Therefore, researchers in other fields can apply our methods to explore other gene expression datasets for other diseases as well.

Activity

In what way can these target groups be involved in and informed about the research results, so that the knowledge gained can be used in the future?

Our data are the final summary and overview of the known molecular pathways based on all publicly available omics data. Other researchers can use our articles and data to develop new hypothesis and to start their investigations.

The articles have been published in, or has been submitted to, peer-reviewed journals which target researchers in the same field. The findings have also been presented at multiple national and international conferences such as the European Glaucoma Society Conference (EGS) and the Association for Research in Vision and Ophthalmology (ARVO). In addition, the methods have been presented on national bioinformatics conferences among which the Netherlands Bioinformatics and Systems Biology research school (BioSB) and the Maastricht Centre for Systems Biology (MaCSBio) Science Days.

Moreover, the opportunities for drug repurposing, and in the future possible patents, that follows form our results have been discussed with the head of the Department for Toxogenomics, professor Kleinjans, at the University of Maastricht.

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