Visual pathway white matter alterations in glaucoma

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CHAPTER 6

Discussion
The main aim of my thesis was to advance our understanding of the underpinnings of visual pathway degeneration in primary open-angle glaucoma (POAG) patients. To this end, I utilized novel diffusion-weighted imaging (DWI) techniques to analyze visual pathway white matter (WM) changes in POAG, revealing new insights into the structural nature of these WM changes. Furthermore, by performing the first longitudinal study of glaucomatous WM degeneration, I investigated the source and pattern of spread of this degeneration. Additionally, I explored the potential of novel DWI techniques to serve as alternative approaches for the assessment of glaucoma.

6.1 PROGRESSION OF VISUAL PATHWAY WM DEGENERATION IN POAG

While the presence of widespread visual pathway degeneration in POAG patients has been established over a decade ago, the progression of this degeneration over time had never been studied before. This hindered our understanding of the underlying mechanisms of this degeneration. Therefore, by re-inviting POAG patients who participated in prior cross-sectional DWI studies, we aimed to perform the first longitudinal study of glaucomatous visual pathway WM degeneration (see Chapter 4). By doing so, we were able to verify the source of this degeneration and advance our understanding of its pattern of spread over time.

6.1.1 PRIMARY SOURCE AND POSSIBLE MECHANISM OF SPREAD OF WM DEGENERATION IN POAG

WM changes in the pre-geniculate optic tracts (OTs) and post-geniculate optic radiations (ORs) of POAG patients were investigated over two time-points using fixel-based analysis (FBA). We found evidence of OT axonal loss at both time-points, and OR axonal loss at the second time-point only. This indicates that glaucomatous degeneration starts in the pre-geniculate pathway and then spreads to the post-geniculate one. As the OTs are essentially formed of the axons of retinal ganglion cells (RGCs), the reported OT axonal loss is most likely a direct result of the death of RGCs in POAG patients. The spread of this degenerative process across the lateral geniculate nucleus (LGN) to reach the post-geniculate ORs can be attributed to anterograde trans-synaptic degeneration. Therefore, our findings affirm the conventional view of POAG as primarily a degenerative disease of the RGCs, and that the glaucomatous degeneration found throughout the visual pathway of POAG patients is a secondary manifestation of RGC degeneration.

6.1.2 TRANS-SYNAPTIC SPREAD OF GLAUCOMATOUS DEGENERATION CONTINUES IN THE ABSENCE OF PRE-GENICULATE PATHWAY DEGENERATIVE PROGRESSION

By comparing visual pathway WM degeneration in POAG patients to natural, age-related visual pathway degeneration occurring in healthy individuals, we found evidence suggesting
that post-geniculate glaucomatous degeneration continues progressing in the absence of detectable pre-geniculate degenerative progression. This demonstrates that the trans-synaptic spread of glaucomatous degeneration is a prolonged process which continues even after RGC degeneration is halted, which is usually the main goal of POAG treatment. A similar pattern of prolonged trans-synaptic spread has been previously reported in studies of both antero- and retrograde trans-synaptic degeneration of the visual pathway.\textsuperscript{1–3} A longitudinal investigation of multiple sclerosis patients who experienced an episode of optic neuritis reported evidence of OR degeneration for up to at least a year after the inflammatory episode.\textsuperscript{2} Another study of patients with homonymous hemianopia due to post-geniculate lesions found evidence of retrograde trans-synaptic degeneration in the form of RGC loss for up to ten years in some of the studied patients.\textsuperscript{1}

6.1.3 CLINICAL IMPLICATIONS

We demonstrated that visual pathway degeneration in POAG starts at the pre-geniculate pathway, which supports the current approach to POAG management, where the end goal is to halt the progression of RGC death. However, the reported progression of post-geniculate pathway degeneration in the absence of pre-geniculate degenerative progression challenges our current view of what constitutes progressive and non-progressive glaucoma. Therefore, the status of post-geniculate glaucomatous activity should not be assumed based on the status of pre-geniculate activity. This could be of particular importance to the implementation of novel glaucoma therapies such as RGC transplantation and other stem-cell based treatments, as such approaches would not be ideal if the post-geniculate pathway is still experiencing degenerative progression. Furthermore, the identified time lag between pre- and post-geniculate pathway degeneration indicates the presence of a time window within which the post-geniculate fibers could still be salvaged before they are afflicted by the trans-synaptic spread of pre-geniculate degeneration. Such a time window could be exploited by novel neuroprotective therapies to halt the trans-synaptic spread of glaucomatous degeneration.

6.2 NEW INSIGHTS INTO THE STRUCTURAL NATURE OF VISUAL PATHWAY WM CHANGES IN POAG

Numerous diffusion tensor imaging (DTI) studies have demonstrated evidence of widespread visual pathway WM degeneration in POAG patients.\textsuperscript{4,5} However, due to the lack of direct biological interpretability of DTI measures, the structural nature of these degenerative changes has remained ambiguous. Recently, novel DWI techniques which overcome this limitation by producing biologically meaningful measures of WM changes have been developed. I presented the first application of two such novel DWI techniques to study glaucomatous WM changes in this thesis, namely fixel-based analysis (FBA) in Chapter 2
and neurite orientation dispersion and density imaging (NODDI) in Chapter 5. By utilizing these novel DWI techniques, we revealed new insights into the structural nature of visual pathway WM changes in POAG.

6.2.1 WHAT FBA REVEALED
FBA is a framework which enables the assessment of differently oriented WM fiber populations within the same voxel, and by doing so it addresses the “crossing fibers problem” encountered with DWI of WM fibers. FBA metrics include fiber density (FD; an estimate of WM axonal density) and fiber bundle cross-section (FC; a measure of changes in WM bundle cross-sectional area). Using FBA, we detected a loss of FD in the OTs and ORs of a group of POAG patients, and a loss of FC in their OTs only. This indicates that the pre-geniculate visual pathway of the studied POAG patients exhibited axonal loss and gross bundle atrophy, while their post-geniculate pathway exhibited axonal loss only. As glaucomatous WM degeneration generally starts with an axonal loss which eventually leads to gross WM bundle atrophy, our findings suggest that the pre-geniculate pathway of POAG patients expresses signs of later stages of WM degeneration compared to the post-geniculate pathway. This interpretation is in line with the notion that glaucomatous degeneration starts at the pre-geniculate pathway and then spreads through anterograde trans-synaptic degeneration to the post-geniculate pathway. Furthermore, our findings corroborate the presence of a time lag between pre- and post-geniculate pathway degeneration, which we observed in the aforementioned longitudinal study.

6.2.2 WHAT NODDI REVEALED
NODDI is a multicompartment diffusion model which produces metrics such as neurite density index (NDI; a measure of axonal density) and orientation dispersion index (ODI; a measure of axonal coherency). By using NODDI to study microstructural visual pathway WM changes in POAG, we found that the OTs of a group of POAG patients exhibited a lower NDI and a higher ODI compared to healthy controls, while their ORs exhibited a higher ODI only. This indicates that the pre-geniculate pathway of the POAG patients exhibited a loss of both axonal density and axonal coherence, while their post-geniculate pathway exhibited a loss of axonal coherence and a preserved axonal density. Given the anterograde direction of glaucomatous trans-synaptic spread, we proposed that a loss of axonal coherence precedes the loss of axonal density in the post-geniculate pathway of POAG patients, and hence can be used as a marker of early trans-synaptic spread. A similar observation was made in a longitudinal animal study of anterograde trans-synaptic degeneration following optic nerve axotomy, where a loss of axonal coherence of the post-geniculate pathway WM was found to precede axonal density loss.

Interestingly, while we found no evidence of axonal density loss in the ORs of POAG patients using NODDI, we did find evidence of it using FBA in the form of FD loss. This incongruency
is most likely the result of NODDI’s inability to resolve the crossing WM fibers of the ORs as opposed to FBA’s ability to do so, which makes FBA more sensitive to axonal density changes in WM regions with crossing fibers.

6.2.3 CLINICAL IMPLICATIONS
While utilizing FBA and NODDI advanced our fundamental understanding of the structural nature of visual pathway degeneration in POAG, our findings suggest that they could also be of benefit to glaucoma therapeutics. Specifically, they could provide biomarkers for early trans-synaptic spread of glaucomatous degeneration, which could play a crucial role in developing and implementing novel glaucoma treatments. For example, such biomarkers could be used to assess whether or not a glaucoma patient is a good candidate for therapies such as RGC transplantation, where determining the status of the post-geniculate pathway would be essential. Furthermore, the efficacy of novel neuroprotective agents which aim to stop glaucomatous degeneration of the visual pathway would need to be assessed at both the pre- and post-geniculate level, the latter of which could be accomplished through the use of such biomarkers.

6.3 POAG: A BRAIN DISEASE?

Some DWI studies of POAG have found evidence of WM degeneration outside the visual system, bringing into question the involvement of the whole brain in the pathophysiology of POAG. These findings can be interpreted in two possible ways: 1) POAG is a global degenerative disorder of the brain, and the degenerative changes exhibited by the RGCs are secondary to a degenerative process occurring downstream in the visual pathway; or 2) POAG is primarily a degenerative disorder of the RGCs, and the spread of this degeneration extends beyond the post-geniculate visual pathway to reach non-vision-related regions of brain. However, the findings presented in this thesis contradict both of these possible explanations. Firstly, the longitudinal study presented in Chapter 4 demonstrated that the pre-geniculate visual pathway is the primary source of WM degeneration in POAG, and that this degeneration spreads in an anterograde direction to affect the post-geniculate pathway. Secondly, an explorative whole-brain FBA of POAG patients found no evidence of WM changes outside the visual system (see Appendix 1), which contradicts previous reports of the presence of glaucomatous WM changes outside the visual system.

It should be noted that the studies which reported WM changes outside the visual system used somewhat lenient statistical approaches to reach their results. For example, Frezzotti et al. and Giorgio et al. used tract-based spatial statistics (TBSS) to perform whole-brain analysis without correction of the voxel-wise p-values for multiple comparisons as recommended. In fact, Frezzotti et al. found that “no results survived at multiple comparisons
Another TBSS study by Boucard et al. performed the recommended correction for multiple comparisons, but only found WM changes outside the visual pathway after using “a more lenient statistical threshold of $p < 0.09$.” Furthermore, similar TBSS studies of WM changes in POAG which did perform the recommended family-wise error correction for multiple comparisons found no evidence of WM changes outside the visual system.\textsuperscript{12,13}

For the above-mentioned reasons, I do not believe that enough evidence exists to make the claim that POAG is a degenerative disorder of the brain as some have suggested.

### 6.4 FBA AS A CLINICAL TOOL FOR ASSESSING OPTIC NERVE DEGENERATION IN GLAUCOMA

In Chapter 3, we investigated both micro- and macrostructural degenerative changes of glaucomatous optic nerves (ONs) using FBA. We found evidence of axonal loss and gross atrophy throughout their length, which is in line with both postmortem\textsuperscript{14} and animal studies\textsuperscript{15,16} of glaucomatous ONs. Furthermore, we found a strong correlation between the results of established structural and functional clinical tests of glaucoma and our FBA results, which demonstrates the potential of FBA to serve as an alternative clinical tool for assessing glaucomatous ON degeneration. Such a tool would be particularly useful in scenarios where current clinical tests of structural glaucomatous degeneration, such as optical coherence tomography (OCT), fail to perform adequately. For example, the presence of ocular media opacities such as dense cataracts renders OCT measurements unreliable,\textsuperscript{17} a limitation which could be overcome by using FBA. Furthermore, OCT measurements exhibit a so-called “floor effect,” which signifies a point after which a loss of retinal thickness is no longer measurable,\textsuperscript{18} making the assessment of glaucomatous progression difficult after this point is reached. Such a “floor effect” may not be present in FBA measures of ON degeneration, allowing FBA to assess the progression of structural glaucomatous degeneration at later disease stages compared to OCT. In Appendix 2, we propose a framework which enables the use of FBA to examine glaucomatous ON degeneration on an individual basis, and we explore the viability of the framework by applying it to examine the ONs of a glaucoma patient.

### 6.5 FUTURE RESEARCH DIRECTIONS

#### 6.5.1 UNDERSTANDING THE UNDERLYING MECHANISMS OF VISUAL PATHWAY DEGENERATION IN POAG

While the findings presented in this thesis contributed to advancing our understanding of the underlying mechanisms of visual pathway degeneration in POAG, the exact pathophysiology
of this degeneration remains unclear. Therefore, further research is needed to unravel the underpinnings of glaucomatous visual pathway degeneration. Importantly, because most of the investigations presented in this thesis have not been undertaken before, confirmatory studies of adequate statistical power are still needed to verify our findings. Furthermore, to understand how the measures produced by novel DWI techniques (such as FBA and NODDI) are temporally related to visual pathway degeneration in POAG, prospectively planned longitudinal studies of early stage glaucoma patients, and perhaps glaucoma animal models, are needed. Additionally, combining novel DWI techniques with other advanced imaging modalities, such as myelin mapping, can give a more complete picture of the changes occurring in the visual pathway of POAG patients.

### 6.5.2 FBA AS A CLINICALLY FEASIBLE DIAGNOSTIC TOOL

In Chapter 3, we reported a strong correlation between FBA measures of ON degeneration and the results of established clinical tests of glaucoma, which demonstrates the potential of FBA to serve as an alternative clinical tool for assessing glaucoma. To render FBA a clinically feasible tool to examine the ONs, a DWI scanning protocol specifically optimized for imaging the ONs should be developed, aiming to acquire high-resolution images of the ONs at relatively short scan times. Such a scanning protocol would lower the patient burden without compromising image quality. Additionally, the use of microscopy surface coils\(^\text{19}\) should be considered to increase the signal-to-noise ratio, which is crucial for imaging relatively small structures such as the ONs. Furthermore, a normative database of FBA measures of healthy ONs would be needed to act as a reference against which FBA measures of the examined ONs can be compared.

### 6.6 CONCLUSIONS

The main aim of my thesis was to unravel the underpinnings of visual pathway degeneration in POAG. To do so, I utilized novel DWI techniques which produce biologically meaningful measures of WM changes to analyze glaucomatous visual pathway WM degeneration in a cross-sectional and a longitudinal manner.

The main findings of my thesis are:

- Visual pathway degeneration in POAG starts at the pre-geniculate pathway and eventually spreads downstream through anterograde trans-synaptic degeneration, affirming the conventional view of glaucoma as primarily a degenerative disease of the RGCs.
- Post-geniculate pathway degeneration appears to progress in the absence of detectable pre-geniculate pathway degenerative progression in POAG patients. This suggests the presence of a time lag between pre- and post-geniculate degeneration, and hence a
time window within which post-geniculate pathway fibers could be salvaged.

- The pattern of glaucomatous WM changes detected by the applied DWI techniques seems to differ between the pre- and post-geniculate visual pathways, which suggests that certain DWI measures could be utilized as biomarkers of early glaucomatous spread beyond the RGCs.
- The measures of glaucomatous ON degeneration produced by the utilized DWI techniques showed a strong correlation with the results of established clinical tests of glaucoma, demonstrating the potential of these DWI techniques to function as alternative approaches for the clinical assessment of glaucoma.
6.7 REFERENCES


