CHAPTER 7

Summary, General Discussion and Future Perspectives
7. SUMMARY, GENERAL DISCUSSION AND FUTURE PERSPECTIVES

The general objective of this thesis was to better visualize and characterize tissue abnormalities in MS with MRI, and to find genetic or clinical correlates of changes observed histopathologically or with advanced MRI. The main research questions were:

- Do specific sequences and/or ultra-high field strength (7T) improve lesion detection?
- Can different stages of WM lesions and different types of GM lesions be distinguished using advanced MRI sequences? At standard and (ultra-)high field strength?
- Can histological variations in demyelination and inflammation in MS patients be explained by carriage of HLA-DRB1*1501?
- Can MRI distinguish clinical deficits between MS phenotypes?

Through these questions, this thesis is drawing a proverbial translational ‘red line’ connecting genetic, histopathological, MRI and clinical findings in MS patients. The following sections will summarize the results from the previous chapters, answer and discuss the above mentioned research questions, and look at future perspectives in these research areas and possible applications for clinical purposes.

7.1. MRI VISUALIZATION OF CORTICAL GRAY MATTER LESIONS

7.1.1 Increasing field strength, increasing cortical lesion detection?

MRI is the most sensitive tool to detect focal and diffuse changes in the MS brain and spinal cord and has been incorporated in the diagnostic criteria for MS. Furthermore, MRI has important prognostic value in predicting the conversion of clinically isolated syndrome (CIS) to clinically definite MS. Since the introduction of MRI, WM lesions have been visualized fairly easily and accurately, although the number of detected WM lesions increased with increasing field strength. In contrast, detecting cortical lesions has always been challenging, even though they are a key component of the disease process. In a post-mortem study at 1.5T, a staggering 63% of cortical lesions were retrospectively (i.e. with histopathological knowledge of lesion location) still missed. The reason for this difficulty in detecting cortical lesions could be the small size of lesions, a relative absence of inflammation (compared to WM lesions) and partial volume effects from adjacent CSF or WM. Improvements to detect cortical lesions have been made by developing specific pulse sequences (as discussed in the next paragraph) and by moving to high and ultra-high field MRI systems.

Increasing the field strength increases the signal to noise ratio (SNR), and often improves spatial resolution and image contrast. The SNR increases approximately linearly; doubling the field strength, doubles the signal. This increase in signal can then be used to reduce scan time or increase spatial resolution. In post-mortem studies, the latter approach is most often used. By increasing the spatial resolution, in a few of our studies up to 100 µm x 100 µm, even the smaller (often type II) lesions may become visible. The increase in image contrast may also be used to
detect subtle differences in the contrast in more sparsely myelinated outer layers of the cortex, in an attempt to detect the frequently overlooked subpial lesions (see chapter 4.1).

In the post-mortem setting, previous studies have compared MRI sequences within the boundaries of a certain field strength (discussed in more detail below), and as such, sensitivities between sequences across field strengths cannot be adequately addressed. To address this specifically, the first aim of the study undertaken in chapter 2.1 was to determine and compare sensitivities of a multi-contrast protocol at 3T and 7T MRI for the detection of cortical lesions in MS, by directly comparing MR images to histopathology. Histology is seen as the gold standard to which MRI lesion detection was compared.

In chapter 2.1, coronal hemispheric slices of MS patients were scanned and processed histopathologically. Lesions were detected both microscopically and on MRI. After matching, sensitivity of lesion detection was calculated. Results show that prospectively, regardless of pulse sequence, 7T MRI detected 59% more cortical lesions than 3T MRI. However, mean sensitivity for cortical lesion detection was 30% at 7T and 15% at 3T, both not a very high percentage of lesions detected (a challenge we will discuss below). Even though all pulse sequences found more cortical lesions at 7T, this was only significant for 7T FLAIR (detected 225% more cortical and 340% more intracortical lesions compared to 3T FLAIR) and 7T T2* (detected 200% more cortical and 250% more intracortical lesions compared to 3T T2*). Retrospectively, when lesion location was revealed to the MR reader, 7T MRI still detected 18% more cortical lesions than 3T MRI (a significant difference). DIR, FLAIR, T2* and T2 all found more cortical lesions at 7T, although this was only significant for T2*.

The results described above indicate that aside from a large improvement in (cortical) lesion detection when moving from 1.5T to 3T, a further improvement is observed when moving from 3T to 7T. Nevertheless, only two out of five sequences (FLAIR and T2*) prospectively benefited from this increase in field strength. Retrospectively, only T2* had a statistical advantage of higher field strength to detect cortical lesions.

In regards to cortical lesion types, our study in chapter 2.1 was unfortunately not sufficiently powered to statistically assess prospective differences in cortical lesion detection between 7T and 3T per lesion type. This was mostly due to the very low numbers of MR visible lesions. Retrospectively, where numbers of MR visible lesions were higher, an advantage was found for the T2* pulse sequence in detection of subpial type III lesions (7T T2* found 54% more type III lesions than 3T T2*). This is an interesting observation, as subpial lesions are difficult to visualize, but still show a clear clinical relevance: patients who are believed to have more subpial demyelination, have higher physical disability and worse cognitive performance. Nevertheless, this correlation may not come as a surprise considering that extensive subpial demyelination is predominant in the progressive stages of the disease, stages which inherently have higher physical disability and worse cognitive performance.

In summary, moving to 7T MRI in general increases cortical lesion detection compared to 3T, although not for all pulse sequences equally so. From this, another question may arise - does a specific sequence at 7T MRI show a particular advantage above other sequences at 7T?
7.1.2 Which sequence is best at 7T?

In the previous paragraph, the advantage of ultra-high field MRI above high field MRI in the detection of cortical lesions was discussed. In this section, I will discuss whether specific pulse sequences at ultra-high field should be chosen over others when the aim is to maximize cortical lesion detection.

Previous in vivo studies found a superiority of DIR over T2-w and FLAIR pulse sequences in detecting cortical lesions at standard 3T field strength.\textsuperscript{13,14} However, at 7T, FLAIR outperformed DIR, while DIR and T2-w showed nearly identical lesion detection.\textsuperscript{15} This indicates that sequences may perform optimally (in terms of cortical lesion detection) at one field-strength, but lose their advantage at another. For the DIR sequence this would not be surprising; due to the increase in field strength, the T1 and T2 relaxation times come closer together, making it more difficult to give a selective inversion pulse suppressing one tissue type (in this case WM). In turn, a decrease in contrast between GM/WM is observed and (especially) mixed lesions will be less visible, something DIR was superior in detecting. Without this superiority, perhaps other sequences may be more useful in detecting cortical lesions.

Therefore, we compared pulse sequences at the same (ultra-)high field strength. In chapter 2.1, we compared five pulse sequences (DIR, FLAIR, T2*, T1 and T2) and found that none of the sequences was superior in detecting cortical lesions at a specific field strength. Not at 3T, nor at 7T. Nevertheless, when looking at the difficult-to-detect subpial type III lesions, the 7T T2* sequence showed descriptively (not statistically) a better lesion detection sensitivity than other sequences (ranging from 9% better compared to FLAIR to 25% better compared to DIR). This increase in subpial lesion detection with T2* compared to other sequences confirms the results from a previous 7T MRI study.\textsuperscript{10} Based on this, it has been suggested that this T2* sequence should be used as the new gold standard for GM lesion detection.\textsuperscript{16} As we could not find the statistical support for this recommendation in our study, we took a closer look (with statistically more power through fewer comparisons) and compared a standard T2-w sequence and a T2* sequence in chapter 2.2.

In this second study, we could not confirm the previously mentioned significant difference in cortical lesion detection between the two sequences. If anything, the T2-w sequence detected slightly more cortical lesions than the T2* sequence (T2-w prospective detection sensitivity 28%, T2* detection sensitivity 16%). Retrospectively, when histological lesion location was revealed to the MR reader, this difference between T2-w and T2* became even smaller (83% and 84% respectively). Similar to the study in chapter 2.1, the low number of MR visible lesions prohibited a comparative analysis between T2-w and T2* per cortical lesion type. Descriptively, the largest difference was found for type IV lesions, T2-w detected 44% of type IV lesions, while T2* detected only 16% of type IV lesions.

In summary, even though certain sequences detect more cortical lesions at 7T compared to 3T MRI, there is not a single pulse sequence at 7T (or 3T) that has a clear advantage over other pulse sequences. Nevertheless, it appears that an MRI observer may find it ‘easier’ to detect cortical lesions with a more frequently used standard T2 sequence (most cortical lesions are detected with this sequence in chapter 2.1 and chapter 2.2).
7.1.3 Challenges and future directions for (ultra-)high field strength MRI and sequence development in MS

In the paragraphs above, it was shown that 7T MRI is more apt at detecting cortical MS lesions than 3T, but that there is no specific 7T MRI sequence that outperforms the others.

One of the main challenges that came forward in both chapters is the (very) low prospective detection rate, while retrospective detection rate dramatically improved (compared to studies at lower field strength). Increasing the field strength has therefore increased the possibility to detect cortical lesions (if the MR reader knows where the cortical lesions are, they can often be found retrospectively). Not only the proverbial “tip” but also most of the “iceberg” has been uncovered. However, the opportunity to detect/uncover cortical lesions has not been fully seized yet. Although lesion detection is increased at 7T when compared to lower field-strengths, a substantial number of cortical lesions still remain prospectively undetected. Rather than further increasing the field strength it would be more interesting to investigate the effects of better and continued observer training on prospective lesion detection. This could be achieved by creating teaching files of matched MRI and histology sections. Radiologists are generally only involved in prospective in vivo lesion detection, therefore, gaining awareness of the many (small) lesions that could potentially be missed by receiving histopathological feedback, would not only improve post-mortem MRI research, but also clinical practice.

When it comes to pulse sequences for post-mortem cortical lesion detection at ultra-high field, there does not seem to be a “gold standard” sequence as previously proposed by Mainiero and colleagues. FLAIR and T2* at 7T perform better than their 3T counterparts, but at the same field strength, they are only minimally different from one another. Nevertheless, a follow-up study including more cortical lesions (we only had approximately half the number of lesions compared to Mainiero and colleagues), more a priori observer training and scan sequences that are clinically feasible in terms of scan time, would help to perhaps finalize this “gold standard” debate.

An interesting observation in the two studies are the “false positives”; hyperintensities detected on MRI, but not verified as lesions histopathologically. Histopathologically these false positives appeared to be incomplete demyelination or partial remyelination (both showing an abnormal signal on MRI). The MRI pulse sequences used are sensitive to detect tissue abnormalities, but not specific enough to differentiate lesions from incomplete demyelination or remyelination. Nevertheless, to be able to make this differentiation would be of great value in the clinical setting, for instance in the monitoring of treatment effects. If ‘conventional’ sequences (all of those used in chapter 2), cannot make this distinction, perhaps quantitative MRI (qMRI), which is more pathologically specific, could be a useful tool. I will briefly touch upon this in the next section, which discusses quantitative MRI more in depth.
CHAPTER 7

7.2 MRI CHARACTERIZATION OF LESIONS

7.2.1 Standard field strength is insufficient to differentiate lesion types

Conventional MRI techniques are sensitive to detect WM lesions, however, there is a lack of correlation between conventional MRI findings (e.g. T2 lesions load) and clinical outcome measures such as disease severity or burden.\textsuperscript{17} This is also called the clinico-radiological dissociation or paradox.\textsuperscript{18} In an attempt to solve this paradox, various quantitative MRI (qMRI) sequences were developed such as T1 relaxation time (T1-RT) mapping, magnetization transfer imaging (MTI) and diffusion tensor imaging (DTI). These qMRI measures have not only shown to be sensitive, but are also more pathologically specific.\textsuperscript{19-21} For this reason we undertook several studies in an attempt to gain more insights into the usefulness of various qMRI techniques to make pathological distinctions in the WM and GM, at standard and at high field strength.

In \textit{chapter 3.1} and \textit{chapter 3.2} we started with standard (1.5T) field strength and sought to investigate if we could distinguish the pathological heterogeneity of WM lesions. This heterogeneity can microscopically be distinguished into four distinct lesion stages (pre-active, active, chronic active and chronic inactive) depending on their degree of microglia, adaptive immune response and demyelination. In our studies we aimed to distinguish these stages by means of qMRI, e.g. T1-RT mapping and T2-w texture analysis, in two independent studies on the same lesion sample.

The results of these studies were very similar. In \textit{chapter 3.1}, T1-RT mapping could not make a full statistical distinction between all four lesion stages. However, T1-RT did increase consistently when moving from normal appearing white matter (NAWM) to preactive and active lesions to chronic lesions. When lesion types were grouped into three distinct groups, e.g. NAWM/pre-active, active and chronic (including chronic active and chronic inactive lesions), all three groups differed significantly from each other. This means that in this study, there was additional value in distinguishing active from chronic lesions with T1-RT at 1.5T, but not more.

In \textit{chapter 3.2}, T2-w texture analysis was used which works on the premises that greater tissue damage gives rise to more heterogeneous organization of MRI signal intensity.\textsuperscript{22} The study showed that myelin content was the driving force for differences in MRI texture; demyelinating lesions (both active and inactive) were more heterogeneous than lesions with intact myelin (NAWM and pre-active lesions). In addition, this study briefly touched upon remyelinating lesions and found that its texture was similar to NAWM but different from demyelinating lesions. This suggests that it could be beneficial to further explore remyelination by means of T2 MRI texture distribution.

In summary, although some WM lesion differentiation could be achieved at standard 1.5T field strength with T1-RT mapping and T2-w texture analysis, this field strength does not appear to be sufficient to make a full (possibly clinically relevant) distinction. Therefore, it would be of interest to pursue similar studies at higher field strength, with better signal-to-noise ratio and spatial resolution.
7.2.2 Quantitative MRI characterization of GM lesions at ultra high field strength

As noted in the previous paragraph, standard field strength may not be sufficient to fully capture pathological subtleties such as WM lesion differentiation or remyelination versus (partial) demyelination. Similarly, chapter 2.1 and chapter 2.2 show that standard field strength may not be sufficient to (even retrospectively) detect most GM lesions, but that ultra-high field strength is already a dramatic improvement. A similar need for (ultra-)high field has been found in quantifying differences between cortical lesions and NAGM; where a study at 1.5T found no qMRI differences between lesional and non-lesional GM tissue, studies at 3T\(^2\)\(^3\), 7T\(^2\)\(^5\) and 9.4T\(^2\)\(^6\) did. Nevertheless, a previous attempt to find qMRI differences between the various GM lesion types was unsuccessful, possibly due to a lack of power.\(^2\)\(^6\) However, it would still be of interest to do so, as it has been shown that different GM lesion types may have a differential effect on physical or cognitive disability.\(^1\)\(^1\)

In chapter 4.1 we used the lesions retrospectively found in chapter 2.2, and co-registered them to qMRI (MTR and qR2*) maps to obtain mean values for each lesion. In addition, ROIs of NAGM were selected. Subsequently, cortical lesion groups were compared to myelin density matched NAGM. This matching is important as myelin content varies across cortical layers; low myelin density is observed in the superficial layers closer to the pial surface, and higher myelin density is observed in layers closer to the white matter. Results from this study showed that intracortical lesions had lower MTR and qR2* values than NAGM and type I lesions showed lower MTR than type III lesions. Furthermore, type III lesions showed lower MTR than matched NAGM, and type I and IV lesions showed lower qR2* than matched NAGM. These results indicate that MTR/qR2* has added value to conventional MRI measures such as T2 and T2*, with which type III lesions are generally difficult to detect. In summary, qMRI at 7T can provide additional information on extent of cortical pathology, especially concerning subpial lesions.

In chapter 4.2 we looked at DTI measures of GM lesions in relation to NAGM. Due to the lower spatial resolution (more similar to the in vivo setting), we did not look to differentiate GM lesion types among each other. Instead we looked at possible FA and MD changes in lesional and non-lesional areas. This was ignited by the literature, where an increase in FA was observed in cortical GM lesions compared to NAGM in vivo.\(^2\)\(^7\)\(^2\)\(^8\) However, as seen in chapters 2.1 and chapter 2.2, many cortical lesions are prospectively still missed and NAGM could therefore be ‘contaminated’ with lesions and influence FA values. Therefore, a post-mortem verification study with histopathologically verified lesions and NAGM was in order. Further to this, in the in vivo studies, an explanation was given for the increase in FA, which consisted of cortical inflammation and presence of (activated) microglia causing anisotropy of water diffusion. However, again, this theory was not verified in a post-mortem setting. For that reason, we not only aimed to observe (directional) change in FA and MD, but also what the underlying pathological substrate could be, if a difference was observed. Our results are the first to histopathologically verify an increase in FA in GM lesions compared to NAGM, while no change in MD was observed. This means that GM tissue in lesional areas becomes more anisotropic than in NAGM. Subsequent histological analysis found no difference in activated microglia/macrophages in GM lesions compared to NAGM to explain for this increase in FA. However, our results show that the observed FA increase
in cortical GM lesions may possibly be due to an increase in neuronal density without a difference in neuronal size (i.e. compaction of tissue). Nevertheless, other possibilities will also need to be further explored, such as synaptic, axonal and dendritic density, which were not included in the current analysis.

In summary, the two studies discussed in chapter 4.1 and chapter 4.2 shed some light on the characteristics of GM lesions by means of qMRI. MTR and qR2* differences have been found between some lesion types and matched NAGM, and FA differences have been observed between GM lesions and NAGM which could possibly be explained by compaction of tissue by means of cell density.

7.2.3 Challenges and future directions of characterizing WM and GM lesions with qMRI

Visualizing lesions is a dichotomy: an area of interests contains a lesion, or not. Characterizing lesions is more continuous and could ideally distinguish different lesion stages or types, which may give additional pathological information that could be clinically relevant. Compared to visualization, studies in relation to characterization of WM and GM tissue are still limited and much work still needs to be done, as will be clear from the following paragraphs.

At standard field strength, the major overlap in T1-RT values for the various lesion stages in chapter 3.1 made it difficult to assign non-overlapping T1-RT value ranges to particular lesion stages. Nevertheless, distinguishing lesion stages and determining which lesion types are predominant in which patients and how these correlate to various measures of clinical disability would be of great clinical value. Therefore, it is important to set up experiments similar to those in chapter 3.1 and chapter 3.2, in which qMRI was used to differentiate WM lesion stages, at (ultra-)high field to assess if better characterization is possible at higher field strength. If lesion stages can be distinguished in the (ultra-)high field post-mortem setting, translation to the clinic would be the next step.

One way to make this translation is through the following steps and can include T1-RT mapping, T2-w texture analysis, MTR, qR2* or other quantitative method.

- Lesions (either WM lesion stages, or GM lesion types) need to be distinguishable in the post-mortem setting by finding quantitative measures (e.g. a range of values) unique to a certain stage/type.
- This ability to distinguish stages/types then requires testing in an independent sample, determining sensitivity and specificity of correctly classifying lesions according to their stage/type.
- Subsequently, an in vivo study with parameters similar to those used in the post-mortem setting need to be undertaken, to see how qMRI values change between the post-mortem and in vivo setting and how this affects classification.
- Eventually, lesional changes may be studied in vivo and in relation to measures of clinical disability or in response to treatment.

Similar studies can be done in regards to demyelination/remyelination. Here too, it would be of great clinical value to be able to distinguish them quantitatively and relate them to the
monitoring of disease progression and treatment effects. Several myelin-relevant MRI techniques have been developed and are demonstrating promising results, but tissue verification is needed. Therefore, a project looking at quantifying incomplete demyelination/remyelination in the (ultra-)high field PA-MRI setting would be of great interest in the near future.

Overall, a comparative study as done in chapter 2.1, where we compared five sequences at two field strengths in regards to cortical lesion detection, should be done to assess optimal usability of qMRI in characterizing tissue pathology. We have shown that standard field strength is not sufficient to differentiate WM pathology, but will high field strength be sufficient or is ultra-high field strength required? Therefore, in a future study, include two field strengths (3T and 7T) and several qMRI measures (e.g. T1-RT mapping, T2 texture analysis, MTR, DTI, qR2*) and compare their abilities at characterizing/distinguishing WM lesion stages, GM lesion types and remyelinating lesions, all with histopathological verification. This way questions in regards to usefulness of higher field strength and which qMRI technique will be a good candidate for further analysis in vivo can be explored in direct comparison.

A better understanding of which underlying pathology is driving certain imaging techniques would also be beneficial. We briefly looked at the underlying pathology of increased DTI FA in lesions compared to NAGM, but only took into account activated microglia and cell density. Subsequent studies could also take axon density, oligodendrocytes and gliosis into account. Which pathological processes could be driving their changes in WM and GM pathology, can then be further understood. These histological verification studies can also be done for newer qMRI techniques, such as diffusion kurtosis imaging (DKI).

There have been a few studies already looking at the applicability of qMRI characterization at ultra-high field strength in vivo. For instance, T1-RT mapping of NAWM at 1.5T and 3T found differences between MS patients and healthy controls. Nevertheless, only at 7T it became clear that the observed differences were possibly due to the influence of Virchow Robin spaces (VRS), something that was not feasible to be differentiated at lower field strength. Other qMRI techniques are also benefiting from an increase in field strength. MR spectroscopy (MRS) has a better spectral separation at 3T compared to 1.5T which resulted in a more accurate and reproducible quantification of metabolites. Advantages of MRS at ultra-high field are still being investigated, but preliminary results suggest an improved spectral resolution and increased SNR for low concentration compounds.

A recent study has been looking into qMRI through T2*-RT rates in an attempt to map the spatial distribution of intracortical pathology. MS patients showed widespread increases in T2* compared to controls, indicating myelin and iron loss. Furthermore, a correlation was found between disability and laminar quantitative T2* changes (see figure 1).

These studies show how ultra-high field and qMRI can be used to better characterize tissue abnormalities in MS. We will touch upon the clinical aspects of in vivo characterization again in chapter 7.4. Nevertheless, longitudinal studies are needed to further examine pathological changes and their relation to clinical outcome measures.
FiGure 1 | From “A gradient in cortical pathology in multiple sclerosis by in vivo quantitative 7 T imaging.” by Mainero et al (2015).36 Quantitative T2* differences between multiple sclerosis patients and controls independent from cortical thickness. Overlay of the GLM significance maps (P < 0.05 corrected for multiple comparisons) on the average pial surface showing in early multiple sclerosis (MS), RRMS and SPMS, clusters of increased T2* relaxation time relative to healthy controls at 25%, 50% and 75% depth from the pial surface, after including in the GLM cortical thickness at the vertex level as a covariate of no interest, along with age. WM = white matter.

7.2.4 Opportunities and future directions of post-mortem research in MS

Post-mortem MRI and histopathology correlations have been a crucial method to investigate the nature and extent of MS pathology. The previous paragraphs have shown various ways in which the post-mortem setting has aided our understanding of MRI visibility, and in turn characterization, of WM and GM pathology.

A discussion worth mentioning is the future of post-mortem MRI studies. In a previous paragraph it has been mentioned that it is better to emphasize on observer training than going for studies at even higher field strengths (> 7T). Although there is still room for improving lesion detection (we are still not at 100% detection), previous research has shown that the number of MRI visible cortical lesions correlate well with the actual number of lesions that are confirmed histopathologically.23 Meaning, we might already have a fair reflection of the cortical lesion burden that lies beneath. Therefore, it is of more fundamental importance to (i) correlate post-mortem MRI to clinical outcome measures and (ii) use the post-mortem setting for larger scale studies. I will shortly elaborate on these two aspects.

A major limitation of current post-mortem studies is the lack of clinical information to connect the post-mortem setting to the clinic. A few studies by our group have attempted to make
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this translation by connecting histopathological findings to (the limited) ante-mortem clinical information that was available.\textsuperscript{37,38} It would be very beneficial to have better clinical (physical, but especially cognitive) assessment of MS patients, not long before death, so a post-mortem MRI to clinical translation can be made. Regular (for instance every 5 years), clinical and cognitive assessment of patients who are enrolled with the Netherlands Brain Bank, would be an ideal situation.

Using the post-mortem setting for larger scale studies, such as network and (functional or structural) connectivity analysis, is a very interesting future perspective. In vivo studies in MS have moved towards the use of even more advanced imaging techniques, such as magnetoencephalography (MEG) or (task or resting-state) functional MRI (fMRI). However, the pathological basis of these techniques is still largely unknown. To use functional connectivity as an example for the remainder of this section, questions such as “which pathological processes underlie functional connectivity changes that may lead to cognitive impairment in MS patients?” may give us valuable additional insights into the pathology of the MS brain. However, translating functional connectivity measures to (histo)pathology is challenging, if not impossible; changing connectivity patterns as measured with fMRI are inherently linked to the in vivo setting. Therefore, any pathological assessment is limited to indirect observations. Nevertheless, if “changes in functional connectivity” relate to more conventional in vivo measures such as “atrophy”, the post-mortem setting can be, by investigating the (histo)pathology of atrophy, extremely helpful in elucidating the possible relationships between connectivity and pathology. This will be illustrated with examples in the next paragraphs.

Various links between connectivity, conventional MRI measures, the post-mortem setting and clinical measures have already been made by our group (see figure 2), although in different studies and for different structures. For instance, decreased hippocampal functional connectivity was more pronounced in MS patients with hippocampal atrophy, linking connectivity and conventional MRI.\textsuperscript{39} Additionally, hippocampal connectivity\textsuperscript{40} and thalamic volume and functional connectivity\textsuperscript{41} have been related to cognitive impairment, linking connectivity and conventional MRI to clinical outcome measures in vivo (as shown in figure 2). Other studies are linking conventional MRI, clinical outcome measures and pathology (also shown in figure 2); MRI measures of cortical volume have been associated with pathological measures of axonal density and neuronal density and size,\textsuperscript{42} and pathological measures of demyelination and inflammation have been associated with worse disease outcome.\textsuperscript{38}

If these studies can be combined for the same structure, for instance the thalamus or hippocampus, in two related studies (one in vivo and one post-mortem), perhaps a (cautious) link may be made between pathology and (functional) connectivity analysis. The in vivo study could relate thalamic/hippocampal connectivity to thalamic/hippocampal volume and clinical outcome measures (e.g. loss of connectivity is related to thalamic/hippocampal atrophy and worse clinical outcome). The post-mortem study could in turn relate thalamic/hippocampal volume to ante-mortem clinical outcome measures and underlying pathological processes (e.g. thalamic/hippocampal atrophy is related to worse clinical outcome and to neuronal and axonal loss). This way we may be able to bring “connectivity” and “pathology” closer together (e.g. loss
of connectivity may be related to neuronal and axonal loss). Nevertheless, we need to keep in mind it is not a direct relation and particular care needs to be taken in matching of the two study populations, MRI and clinical measurements. This is what lies at the heart of translational neuroscience: being conscious of the bridges between areas of investigation.

To summarize, future directions in post-mortem research include a better ante-mortem clinical characterization of patients, and more integrated research (proposals) connecting advances in in vivo research to the post-mortem setting.

**Figure 2** Schematic representation of different modalities and translations. Several studies have made various translations between different modalities; connectivity and conventional MRI, connectivity and clinical outcome, conventional MRI and clinical outcome, conventional MRI and post-mortem, and post-mortem and clinical outcome. A study combining these modalities could indirectly elucidate the relationship between connectivity and post-mortem findings.

### 7.3 Genetic Characterization: Associations between Histology and Genetics

#### 7.3.1 Histological changes and HLA-DRB1*1501

In the previous chapters I have tried to relate histological differences, e.g. WM lesion stages or GM lesion types, to differences in qMRI values. Ideally, to eventually be able to relate qMRI measures, without histological verification, to clinical outcome measures. In other words, to use qMRI as an endophenotype. Endophenotypes are biological variations that are quantifiable and indicators of vulnerability to disease. Suitable endophenotypes have indications of relationships between measurement levels (genetic/histology/MRI/clinic) of disease. For instance, directly relating...
histological findings to what was known about clinical outcome measures ante-mortem, and see if there are any smaller scale measures explaining this histological stage. An example would be genetic markers that can explain or shed light on disease severity though histopathology.

This approach has been explored in chapter 5.1. A previous study by our group found cortical lesions with a rim of activated microglia which was related to more active WM inflammation, a younger age at death and shorter disease duration. Since patients who carry HLA-DRB1*1501, the main susceptibility gene in MS, have an earlier age at disease onset and increased disease severity, we wondered if there was a relationship between HLA-DRB1*1501 and our histopathological findings of demyelination and inflammation (as assessed by microglia activation). The results of our study indicate that HLA-DRB1*1501 had no influence on the presence and extent of independent measures of cortical demyelination and inflammation. Nevertheless, in combination, extent of demyelination and extent of inflammation showed an association for patients carrying the HLA-DRB1*1501 allele, which was not found in patients not carrying HLA-DRB1*1501. This means that in patients carrying HLA-DRB1*1501, more cortical demyelination was related to more inflammation, while this relation was not present in patients not carrying HLA-DRB1*1501. Nevertheless, this relation is not fully understood yet, and type I or II error are possibly present. Therefore, observation in a larger sample is required. This highlights the complexity of MS etiopathogenesis; the same clinical outcome (a less favorable disease course) can be caused by non-related genetic (HLA-DRB1*1501) and molecular (demyelination and inflammation) mechanisms, which warrants further explorations.

7.3.2 Challenges and future directions of genetic studies in MS

The abovementioned study is an example of genetic characterization rather than characterization by qMRI. Although the study had a negative message (HLA-DRB1*1501 has no influence on cortical demyelination or inflammation), it makes room for other histology-genetic studies. Perhaps not the main susceptibility gene, but other genes may be significantly involved in demyelination and inflammation (genes that could possibly be used as therapeutic targets in the future). For example, a recent study compared transcript levels of myelin and inflammation related genes in different types of white matter lesions (active, inactive and remyelination). It turned out that fibroblast growth factor (FGF) 1 showed higher expression levels in remyelinated lesions. It would be interesting to explore this gene and its effect on remyelination further, both in WM and GM lesions.

With advances made in post-mortem MRI studies, and certain (quantitative) MRI signatures becoming good phenotypic reflections of histological processes, histology-genetic studies could become MRI-genetic studies. If a qMRI measure is able to distinguish remyelination from demyelination and NAGM, this measure could be used to distinguish patients with much remyelination from patients with little remyelination in the brain (stratifying patients based on MRI rather than clinical outcome measures such as EDSS). Subsequently, these groups of patients can be genotyped to see if there is anything in their genetic make-up that could explain the difference in pathological processes occurring in their brains. This could then be explored as possible therapeutic targets.
Other MRI-genetic studies could include genetic-network analysis. Schoonheim and colleagues have proposed a hypothesis of network collapse as a cause for developing cognitive impairment in MS (see figure 3); in early MS, structural damage is low, leaving network efficiency relatively high. As the structural damage accumulates over time, network efficiency levels drop, inducing a network collapse. After this, the network is unable to function normally and cognitive impairment develops. The reason for this network collapse is unknown and difficult to predict, however, it is likely that genetic predispositions play a role.

For instance, an animal study has shown that brain-derived neurotrophin factor (BDNF) enhances oligodendrocyte differentiation to myelin protein expressing cells. In turn, in vivo studies have shown that BDNF secretion is associated with WM volume and that the Val66Met SNP of BDNF may be associated with lower GM atrophy in MS. Additionally, a recent study found an influence of the Val66Met SNP on functional connectivity of the brain in healthy volunteers. It would be interesting to see if this BDNF gene or Val66Met SNP also has an influence on brain network activity and connectivity in MS patients.

It is hopefully starting to become clear how genetics, histology and MRI can not only be used separately to investigate pathological processes, but also in combination to make translations between these fields and elucidate and connect processes from a microscopic to a macroscopic scale. The next step is to tie clinical outcome measure into the combination.

**Figure 3** From “Network collapse and cognitive impairment in multiple sclerosis” by Schoonheim et al (2015). A hypothesis of network collapse as a cause for developing cognitive impairment in MS. Being able to identify possible genetic predispositions leading to network changes may lead to further research into possible new treatment targets aiming to stall the onset of collapse or to promote network reorganization.
7.4 in vivo characterization: MRI-cognition correlates

7.4.1 MRI changes and cognition

The previous chapters have continuously emphasized the need for studies in the in vivo setting to verify results obtained in the post-mortem setting to ascertain their usability in predicting clinical outcome. Some steps have already been made as mentioned in paragraph 7.2.3. To conclude this thesis, we will end with two in vivo studies to show how MRI can distinguish clinical deficits between MS phenotypes and how qMRI can be used to assess cognitive dysfunction in vivo. Although this is not a direct translation from the pathological to MRI to in vivo setting, it does show that MRI measures that have previously been pathologically verified, such as measures of atrophy, can in turn be used to explain differences in clinical disease course.

In chapter 6.1 we aimed to compare RRMS and PPMS patients in terms of cognitive performance, and investigated the MRI correlates of cognitive impairment in the two groups using measures of brain volume and cortical thickness. The extent of GM involvement in MS patients became clear; compared to healthy controls, MS patients had more GM volume loss (while no significant difference in WM volume was found) and showed widespread cortical thinning and subcortical atrophy. Furthermore, PPMS patients scored lower than RRMS patients on most neuropsychological tests, in absence of any specific pattern. Although there was no significant difference in MRI correlates of cognitive impairment, there was a prevalent association with MRI measures of cortical GM injury in RRMS patients, and with subcortical GM injury in PPMS patients. Where brain MRI of RRMS patients are often characterized by accrual of inflammatory/demyelinating WM lesions and brain MRI of PPMS patients generally characterized by fewer WM lesions, and more extensive pathological involvement of gray matter, we suggest that cortical and subcortical GM injury may play a different role, depending on disease course.

In chapter 6.2 we characterized WM and GM by means of T1-RT qMRI mapping at high (3T) field strength and related these characterizations to clinical outcome measures. We found subtle GM damage in the cortex and thalamus of MS patients (as measured by increased T1-RT skewness). Furthermore, in contrast to the subtle and local damage found in cortical and thalamic GM, tissue damage in the NAWM appeared to be more widespread and diffuse. Lastly, increased T1-RT skewness was an independent predictor for cognitive dysfunction.

In summary, whereas conventional MRI is insufficiently sensitive to subtle GM damage, qMRI characterization is a promising perspective to detect subtle differences that are clinically relevant.

7.4.2 Future directions of clinical studies

It is outside the scope of this thesis to go into the numerous possible studies that can be done in the clinical setting to visualize and characterize tissue abnormalities in MS. Several suggestings for using (q)MRI in the clinical setting to improve our understanding of tissue damage have already been mentioned throughout the discussion of this thesis. To summarize; in relation to the methods used in this thesis, the clinical research setting can be used to (i) validate studies of advanced MRI techniques (T1-RT, T2 texture, MTR, DTI, qR2*) in their ability to differentiate WM lesion stages, GM lesion types or other pathology, (ii) to translate from gene to network and (iii) to better characterize patients in various stages of their disease and their response to treatment.
7.5 Concluding remarks

This thesis has shown that:

1. in the visualization of cortical lesions,
   - $T_7$ MRI increases cortical lesion detection;
   - sequences perform similarly in detecting MS cortical lesions within the boundaries of one field-strength (3T or 7T);
   - increasing field strength has improved the possibility to detect cortical lesions, but the opportunity to detect more lesions has not been seized yet;
   - rather than increasing the field strength, observer training should receive more attention.

2. In the characterization of lesions,
   - some WM lesion differentiation can be made with qMRI at 1.5T, but it would be fruitful to, using similar techniques, look at (ultra-)high field with better SNR and spatial resolution;
   - GM lesions are not only different with respect to their location, but also reflect MTR/qR2* differences at 7T, indicating that there might be varying degrees of pathology;
   - an increase in FA in GM lesions at 7T is possibly explained by compaction of tissue, but not by microglia.

3. As for genetic characterization of histology,
   - the main susceptibility gene in MS, HLA-DRB1*1501, is not associated with extent of demyelination or microglia activation in cortical lesions.

4. As for in vivo characterization; MRI and cognition,
   - association between cognitive impairment and MRI measures of cortical GM injury were found in RR-MS patients, and with MRI measures of subcortical GM injury in PP-MS patients.
   - T1-RT skewness is a predictor for cognitive impairment in long standing MS.
Important future perspectives:

1. In the visualization of cortical lesions,
   - better and continued observer training on prospective lesion detection;
   - to achieve this, create teaching files of matched MRI and histology data;
   - perform a follow-up study at 7T, including more cortical lesions, more a priori observer training and scan sequences that are clinically feasible in terms of scan time to assess if there is a ‘gold standard’ sequence for visualization.

2. In the characterization of lesions,
   - understanding which underlying pathology is driving certain qMRI techniques;
   - conduct a large comparative post-mortem MRI study including different field strengths and different qMRI sequences;
   - determining sensitivity and specificity of correctly classifying WM lesion stages, GM lesion types or remyelination to eventually study lesional changes in vivo and in relation to measures of clinical disability;

3. As for future post-mortem research,
   - obtain clinical information of patients ante-mortem to be able to make a better translation from the post-mortem setting to the clinic.
   - connecting in vivo and post-mortem research of specific structures to indirectly study the pathological processes underlying network changes.

4. As for genetic characterization,
   - use genotypes/gene expression profiles to characterize a patients’ genetic make-up/expression that could explain for differences in pathological processes;

5. As for in vivo characterization,
   - use the clinical research setting to validate studies of advanced MRI techniques in their ability to differentiate WM lesion stages, GM lesion types or other pathology, to translate from gene to network and to better characterize patients in various stages of their disease and their response to treatment.
REFERENCES


