Summary and General discussion

This thesis aims to provide more insight in factors explaining parts of the heterogeneity in MS, in an attempt to increase the accuracy of the prognosis and diagnosis of individual MS patients. We therefore assessed the following two main issues: 1) genetic role in phenotypic aspects of MS (especially disability (chapter 2) and lesion distribution (chapter 3)); and 2) the role of spinal cord findings in diagnosis and prognosis of MS (chapter 4).

In this final chapter, the main findings of this thesis will be discussed and placed in a broader perspective, and finally directions for future research will be discussed.

Genetic susceptibility studies in MS

Since 2007, when the first Genome Wide Association Study (GWAS) aiming to find genes related to MS-susceptibility was performed\(^1\), the understanding of the genetic background in MS susceptibility has increased enormously. Since then, multiple confirmation studies based on extensive international collaboration have been performed\(^2\)-\(^5\). Recently, in a large cohort of patients (involving almost ten thousand MS patients and more than 17,000 controls), 24 loci have been replicated\(^6\) and 29 new susceptibility loci have been identified.

But what are the clinical implications of these findings from genetic studies? Can they contribute to unraveling the complexity of MS? Indeed, these studies do increase our knowledge on the etiologic background of MS. Information from susceptibility studies can provide us with information on what processes are involved in starting the disease, or who is at risk for developing MS. This may confirm certain hypotheses on the pathological basis of MS (i.e. whether it is a primary or secondary neurodegenerative disease). However, the current strategy (Genome Wide Association Studies) is now facing the limits of its capacities in detecting genes related to MS susceptibility, because the expected effect sizes are too small to detect in medium or even large groups of patients. The most recent study included ten thousand patients from several countries. In my opinion it is not to be expected that further increasing the number of patients will result in many immediately relevant genes. Effect sizes are expected to be so small (OR below 1.1), such that no determining influence on disease susceptibility is to be expected. The likelihood of discovering a new focus for treatment with these low ORs is also expected to be low. In addition, the costs of performing a study of this magnitude require huge funding. Moreover, this amount of data will also lead to a higher complexity in analyzing it (i.e. increased heterogeneity due to genetic population differences). Challenges for the future are to incorporate the current findings from these genetic susceptibility studies into more functional or clinical studies leading to further understanding of MS pathology and hopefully ultimately leading to new treatment options.
Chapter 5

Genotype-Phenotype associations in MS

In the past decade the emphasis in MS literature has been placed on these susceptibility studies. In this thesis, however, we focused on finding genotype-phenotype associations. It is not likely that gene identification in MS will lead to genetic counseling or prenatal diagnosis, as even full knowledge of the genetic background on MS susceptibility will allow “only” 30% accuracy in predicting if a person will develop MS during his lifetime. It is expected that the rest of a person’s susceptibility is determined by (mostly unknown) environmental factors. Moreover, in the last ten years, several Disease Modifying Therapies (DMTs) have been proven helpful in reducing the number of relapses and the number of new MRI lesions in the brain, but no cure for MS is to be expected within the near future. Currently, knowledge of a possibly increased risk of developing MS during the lifetime of a healthy person (derived from already available commercial DNA-arrays) will not lead to the start of disease modifying treatment. I expect therefore, that it is only in the way that risk genes reveal details of disease triggering events, or that severity genes influence mechanisms driving the tissue damage, that genetic information (derived from genotype-phenotype-association studies) has the potential to more readily lead to clinical implications (or even therapeutical options). We aimed to predict more accurately MS diagnosis and prognosis using genetic variables and spinal cord MR findings. Initially, we focused on genotype-phenotype associations to predict disease course. We started by taking clinical disability measures as the primary outcome parameter (Chapters 2.1, 2.2 and 2.3). Assessing clinical disability remains the most important factor in treating MS patients, although clear correlations with the pathological processes involved are often lacking. When trying to accurately assess the genetic role in disability development (by assessing genotype-phenotype associations), these clinical parameters seem to be unsatisfactory. These clinical outcome measures are considered less sensitive in reflecting the ongoing MS pathology as can be demonstrated on MRI scans. It has been proven that lesion location within the brain and spinal cord lesions have an impact on disability. Due to these characteristics, we also took these imaging variables as outcome-parameters (Chapters 3.1 and 3.2).

To improve our understanding of genotype-phenotype associations in MS we used several strategies. In chapter 2.1 we found that a combination of genes could explain part of the disease progression and disability accumulation, while individual effects of the genes were not detected (when controlled for multiple testing). We included several outcome parameters for disability to increase our confidence in the results (preferably relevant genes were associated to more than 1 outcome parameter). Our hypothesis was based on the assumption that disability accumulation in MS is determined by several genes with small effect sizes. Although the statistical method on combining several genes in predicting disease severity seems a promising method, our results need to be confirmed first, ideally
Summary, Discussion and Future Perspectives

using information from the most recent GWAS and recent pathway studies or other candidate gene studies on MS phenotype. Our study demonstrates the possible advantage of a predictive model over assessing individual genetic effects.

A second strategy, which we applied in chapter 2.2 is to combine genetic studies with studies on the mRNA level to gain more functional information, thereby increasing our understanding of how genetic polymorphisms exert their effect on the disease. It is generally accepted that disease progression and tissue damage are continuously or intermittently supported by several mechanisms (which are assumed to be predominantly pro-inflammatory). By gaining insight into the functional consequences of a genetic polymorphism supporting these tissue damaging mechanisms, we hope to find new possibilities in halting these mechanisms. In chapter 2.2 we applied this strategy for the IL7RA risk allele (rs6897932-T), that was found to be related to susceptibility to MS. Of this SNP within the IL7R gene, it was known from the literature that it had direct consequences on the mRNA level and also on protein functioning. In light of the function of this protein in inflammatory processes and the consequences of the polymorphism on protein functioning, we expected that this would be a promising gene to affect disease progression. Unfortunately, we could not demonstrate this effect neither at the gene-level, nor at the mRNA-level. Our observation that this susceptibility SNP within the IL7R gene was not associated with disability parameters, was confirmed by several (GWAS) studies. These studies on disease phenotype showed that disability was associated with genes relatively new to MS literature. Our study and others illustrate that in MS, disease-modifying genes are not necessarily identical to disease susceptibility genes. In other diseases this has been shown (e.g. the effect of the ApoE4-genotype on Alzheimer’s susceptibility and progression. Probably, genes that are related to susceptibility to MS provide information on mechanisms involved in disease initiation and are not directly related to mechanisms that are involved in ongoing tissue damage. Another possibility is that the genetic effects on disease phenotype are too small to detect. However, IL7RA remains an intriguing gene (within a relevant pathway) of which more and more functional data is becoming available. The relevance of IL7R in MS susceptibility was confirmed by another study in MS patients in 2010, using an IL7/IL7RA pathway approach. Recently, in HIV, polymorphisms in the IL7RA gene were found associated with rapid progression to AIDS by using an extreme of outcome strategy. This study analyzed several non-synonymous SNPs and haplotype analysis, demonstrating that different SNPs (on the same haplotype) may cause opposing effects on inflammation via mRNA. This elegant approach has not yet been applied in assessing the relationship with MS phenotype. However, it is worth assessing this to definitively exclude an effect of the IL7R gene on MS phenotype.

In chapter 2.3 we started from the function of the gene and correlated this to a specific clinical phenotype. We assessed whether the risk allele of KIF1B (rs10492972) corresponded
with the expected functioning of the gene. KIF1B is responsible for axonal transport of mitochondria and synaptic vesicle precursors and is related to neurodegeneration. Rs10492972 was reported as the first MS susceptibility gene with a known function in neurodegeneration (instead of inflammation) in a large study\(^1^4\). We hypothesized that carriers of the risk allele might exhibit a disease course with a more neurodegenerative phenotype (clinically, and using MRI characteristics). We found no evidence for a determining influence of the risk allele on clinical and MRI measures related to neurodegeneration. Our findings further strengthen the hypothesis that susceptibility genes need not necessarily be related to phenotypic appearances of MS. Our findings are furthermore supported by a recent large genotype-phenotype study on MS, that showed that most genes identified as related to MS are related to immune system function (overrepresentation of genes that influence T-cell maturation) and a relative absence was noted of genes related to neurodegeneration independent from inflammation\(^6\). Based on these results it was strongly suggested that the pathogenesis of MS is mostly related to dysregulation of the immune system. This conclusion favours the “primary inflammatory hypothesis” (i.e. auto-immunity). However, this hypothesis is still heavily debated based on pathological findings in early MS comprising not only early inflammatory, but also meningeal and cortical gray matter pathology\(^1^5\).

Although replication of the association of KIF1B-gene to MS susceptibility is still awaited, the KIF-family already is an interesting set of genes and proteins. Both in MS and in other auto-immune diseases, other members of the KIF-family have been found to be associated with susceptibility\(^1^6-2^0\). These new studies strengthen the idea that the KIF-family is involved in MS, possibly by influencing axonal transport and thereby increasing susceptibility to axonal damage, however, the exact mechanism is not clear and should be studied. Possibly a pathway approach would be able to provide insight into the relevant mechanisms.

In 2011 a huge GWAS (7000 MS patients) on MS phenotype did not find a genome-wide-significant association of any gene with disease phenotype (disability, age at onset, MS subtype)\(^6\). Despite the extremely high number of patients in this study, no strong correlation with a particular gene could be found, indicating that it is not to be expected that common variations within the genome will be identified with a large effect on disability accumulation. However, this negative study does not imply that disease progression is not genetically influenced. One must consider the many difficulties researchers have been faced with in their search for genetic predictive markers for disease phenotype in the last decade. Not only is it expected (based on familial phenotype studies) that effect-sizes on disability accumulation of genes are small, but also, several interaction within genes (gene-gene), and between genes and environment (gene-environment and gene-gene-environment) have been described, that should ideally be controlled for\(^2^1,2^2\). Moreover, epistasis and epigenetic effects (micro-RNA’s, methylation, etc) and rare variants have not been taken into account.
in GWAS studies using SNPs. These genetic variants may play an important role in mediating genetic effects in MS\textsuperscript{23,24}. GWAS are not able to assess these effects and other genetic methods should be used to assess these effects (i.e. sequencing).

Furthermore, an important complicating factor in genotype-phenotype associations is the enormous heterogeneity in MS (variety in subtypes, neurological complaints, rate of disability accumulation and lesion distribution throughout the brain and spinal cord). Often the contrasts of the outcome parameters (phenotype) are insufficient, thereby decreasing the power of the results. The conservative statistical approaches that have been used often, are not capable of detecting these subtle differences. This problem highlights the urgent call for new statistical approaches that aim to keep adequate power but do not increase the number of false positives findings.

Clinical outcome parameters are often considered less sensitive than certain MRI parameters in reflecting the ongoing MS pathology. Clinical measures may underestimate the extent of damage to the brain due to functional reorganization, while this can be seen in an earlier phase on MRI. It is not well understood whether this variability in MRI parameters reflects genetic subgroups of MS patients. In this thesis we aimed to study the genetic role in lesion distribution (a uniformly assessed outcome parameter) that may be a clinically relevant\textsuperscript{25}. In chapter 3.1 we assessed whether the lesion distribution between the brain and spinal cord was influenced by genetic factors, while in chapter 3.2 we assessed whether lesion distribution within the brain was associated with a set of candidate genes in MS. We expected a genetic role on lesion distribution based on certain demographic hallmarks. The prevalence of spinal cord lesions differs between distinct populations (Asian MS patients have more frequently spinal cord involvement than “Western-type” MS)\textsuperscript{26,27} and between patients (for instance some patients exhibit repetitive spinal cord involvement with less brain involvement)\textsuperscript{28,29}.

In chapter 3.1 we showed a genetic role on lesion distribution. In our cohort, spinal cord lesion number and volume were associated with the HLA-DRB1*1501 genotype (the most important susceptibility gene in MS that is possibly related to MS disability features). Our findings were recently more thoroughly investigated by an Australian group of researchers using high resolution HLA typing (4-digit). They showed a correlation between HLA-DRB1 alleles (not HLA-DRB1*1501, but DRB1*1104 and DRB1*0701) and lesion numbers in different parts of the spinal cord (cervical vs thoracic regions). They also hypothesize that regional differences in the levels of expression of MHC Class II antigens and the presentation of myelin antigens to the immune system may underlie these effects\textsuperscript{30}. Previously, differences in Myelin Basic Protein (MBP) and PLP levels between brain and spinal cord were found in healthy controls\textsuperscript{31}. However, this has not been demonstrated in MS patients. Two mice EAE models with different MHC strains reported different lesion distribution patterns in the central nervous system (spinal cord vs brain parenchyma)\textsuperscript{32}. This was found to be mediated via different preferential MOG epitope presentation and ultimately via alternative Th17/Th1
ratio\textsuperscript{22}. This study indicates a different mechanism of lesion formation in the brain versus spinal cord with a possible indirect role of the MHC class II genes. However, the exact role of the MHC class II genes on this mechanism is still unclear and future pathological studies of spinal cord lesions and brain lesions, including assessment of local expression profiles of MHC Class II molecules, could possibly help us to understand the lesion developing mechanisms in the brain and spinal cord and any differences between them.

In chapter 3.2 we assessed whether lesion location within the brain is genetically influenced. We suspected a genetic role because of clinical observations that show a tendency of individual patients and relatives to develop relapses, related to pathology in the same location\textsuperscript{28,29,31}. Our results suggest that such a genetic influence may indeed be present. The most significant association was found for rs2227139. This SNP is located in MHC class II region, which is involved in self versus non-self immune recognition. Another recent study (in MS patients) showed that carriers of HLA-DR4, DR7 and DR13 have a higher incidence of brainstem and cerebellar lesions (and a higher reactivity to the PLP 184-209 region (a component of myelin))\textsuperscript{34}. Some studies (in healthy controls) show that there are natural spatial variations in myelin content, which may in turn reflect areas with variable predilection for damage in demyelinating disease\textsuperscript{35}. Moreover, in other white matter diseases (Krabbe’s disease, late infantile metachromatic leukodystrophy) several predilection sites have been identified\textsuperscript{36-38}. Unfortunately, MS lesions can reflect different pathological processes (edema, de- and remyelination, gliosis, etc), that can not be distinguished in detail on regular T2-weighted MRI sequences. Therefore, after this hypothesis-free study, a more focused approach using different MRI techniques that are able to detect specific pathologically determined lesion subtypes (for instance: T1 black holes (more related to axonal loss) and cortical lesions), may increase our understanding of the pathophysiological mechanisms involved in lesion distribution and its relationship to (HLA)genes. Careful interpretation of our results is furthermore warranted because our results were found in a clinical heterogeneous population and should therefore first be confirmed in different MS populations.

**Spinal cord MRI in predicting diagnosis and prognosis of MS**

In chapter 4.1 and 4.2 we focus on the predictive capacities of spinal cord lesions and cervical spinal cord atrophy on diagnosis and prognosis of MS. In the past, the emphasis has been placed on brain MRI scanning in MS. However, we show in our studies that lesions on spinal cord MRI can both aid in the MS diagnosis and in the prognosis of a second relapse and are therefore of significant clinical relevance. Moreover, cervical spinal cord atrophy and diffuse abnormalities are associated with a higher disability (in our study even more associated to disability than brain parameters (atrophy or brain lesion volumes). Based on the results of our studies, we advise to more frequently perform an MRI scan of the spinal cord in order to contribute to a better diagnosis and prognosis of MS patients.
We show in our study in chapter 4.1 that presence of spinal cord lesions in CIS patients is an important factor in prognosing (time to) a relapse and therefore we advocate to perform an MRI scan of the spinal cord early after onset of symptoms especially in brain onset CIS patients that do not fulfill the diagnostic criteria. Not only does information of this scan aid in diagnosing MS early, but presence of spinal cord lesions is also associated with a short interval to the second relapse. Scanning of the spinal cord can be performed easily in most hospitals and the burden for patients is minimal (especially when combined with the brain MRI scan). Moreover we show in our study that by adding the results from the spinal cord MRI scan, an earlier diagnosis could be made. We had to perform a spinal cord MRI scan in seven patients to be able to diagnose one extra patient early. Although we think that the benefits of an early diagnosis outweigh the costs and burden to the patients, others may argue that a yield of 1 in 7 does not justify this extra scan (depending on the local health care system).

The diagnostic criteria39-41, were created to be able to early identify CIS patients that will convert to MS. An early diagnosis can reduce uncertainty for the patient and enables an early start of disease modifying therapy, that has shown to moderately reduce the number of relapses on the short term and may decrease disability accumulation in the long term. However, there is no standard test to confirm MS diagnosis. The diagnosis is made based on fulfilling the clinical and/or MRI criteria for dissemination in time and space. However, to my opinion, diagnosing MS should not only focus on fulfilling these important criteria but should also comprise a more accurate prognosis of future relapses and (preferably also) future disability. In light of these considerations, maybe the diagnostic criteria should be considered as a prerequisite to diagnose MS, however, this process should ideally be followed by an effort to provide the most accurate prognosis on disability accumulation and prediction of (time to) subsequent relapses. The uncertainty of the extent and timing of disability accumulation and relapses are highly relevant for the individual MS-patient. The range of disability accumulation and time to first relapse are extremely variable. We know that there is a subgroup of officially diagnosed MS patients, that have a “benign” course (none or little disability even after 15-20 years). Moreover, we also know that there is a subgroup of patients that will not experience a second relapse within 15 years, although the MRI has shown dissemination in time. We now can officially diagnose these patients with MS using the new diagnostic criteria41. However some of these patients will not benefit from the current disease modifying therapies (because no relapse would have occurred with or without DMT) and one could argue whether a diagnosis of MS has any consequences for the patients and whether the diagnosis increases their quality of life. Therefore, clinical, radiological or biomarker studies that focus on increasing the accuracy of prognosing the disease course of MS are equally or maybe even more important.
In chapter 4.2 we evaluated the clinical relevance of cervical spinal cord atrophy and other spinal cord and brain parameters on disability. We confirmed in a large multicenter study that atrophy of the spinal cord (assessed by mean upper cervical cord area (UCCA)) is associated with a higher clinical disability. This variable was found to be the most significant variable correlated with disability in addition to clinical parameters. Also other spinal cord parameters (extent of focal lesions in the spinal cord and presence of diffuse abnormalities) were found related to disability. This study highlights the clinical importance of spinal cord findings (especially atrophy and to a lesser extent focal lesions). It was already known that brain atrophy is more correlated to disability at the long term when compared to the extent of lesions in the brain. Moreover, we applied a technique enabling assessment of the volume of the cervical spinal cord using the brain MRI scan, without the necessity of performing an extra spinal cord scan. This technique therefore, does not put extra burden on MS patients and is therefore easy to apply in clinical practice.