Summary

Multiple sclerosis (MS) is a chronic inflammatory demyelinating autoimmune disease of the central nervous system (CNS). Although the exact etiology of MS is unknown, environmental exposure and genetic susceptibility are involved. The pathological hallmark of MS is a complex process involving inflammation, demyelination, astrocytosis and axonal degeneration, eventually leading to the formation of sclerotic plaques in the CNS white matter. More recent evidence shows that, especially in progressive phases of MS, changes are not confined to the white matter lesions, but also involve normal appearing brain tissue and grey matter. Both demyelination and axonal loss in brain and spinal cord may cause impaired nerve conduction with temporary, (partially) reversible and/or gradually progressive neurological disability, expressing itself in relapsing remitting (RR), secondary progressive (SP), primary progressive (PP) or progressive relapsing (PR) MS. Conventional magnetic resonance imaging (MRI) can show the focal lesions, but has limited histopathological specificity and clinical correlation. Atrophy measures and non-conventional MRI provide information about the more diffuse abnormalities of the normal appearing brain tissue (NABT) and correlate better with disability. Disease course, prognosis and treatment responses are highly variable between patients. Current disease modifying treatment can reduce relapse rate, but probably not disease progression.
The heterogeneity in MS, clinically, radiologically, immunologically, pathologically as well as genetically, might indicate more than one pathogenic mechanism, for which different treatment approaches may be required. Many genetic, immunopathological and radiological investigations have been performed to further elucidate disease mechanisms and prognostic and therapeutic consequences. Ideally, this would lead to the ability to classify the individual patient. So far, the most obvious and commonly applied classification is based on clinical disease course. Although the clinical subtypes differ in respect to certain radiological, immunological and pathological properties on a group level, heterogeneity remains large. A more recently proposed pathological classification suggesting fundamentally different pathogenic mechanisms is not applicable *in vivo* in the vast majority of patients.
In this thesis several investigations are presented about various possibly useful bio-markers in an attempt to detect clinically relevant associations or subgroups and thereby order the heterogeneity to some extent.

Chapter 2 describes several studies assessing the influence of the apolipoprotein E (APOE=gene, apoE=protein) polymorphism, a genetic marker, on MS. The APOE gene is located on chromosome 19q13, one of the regions that may contain genes influencing MS susceptibility. The most common alleles ε2, ε3 and ε4 encode for the three major isoforms E2, E3 and E4. ApoE is involved in lipid and cholesterol transport and plays a role in growth and regeneration of neurons. Evidence is accumulating for a negative role of APOE ε4 in CNS diseases, although in MS conflicting results have been reported.
In **chapter 2.1**, the association of the APOE polymorphism with both disease susceptibility and disease course was assessed in a large group of MS patients, with subgroup analysis of MRI measures. In this cohort no association of the ApoE genotype with disease susceptibility nor clinical and MRI measures could be identified. However, combined analysis of published data could not definitely exclude the possibility of a minor negative role for ε2-carriership in susceptibility to MS.
In **chapter 2.2**, we examined the sex-specific association of the APOE polymorphism with disease severity in the same group of multiple sclerosis patients, in reaction to reported findings that in women ε2-carriership is associated with a more favorable disease course. In contrast, by applying likewise analysis we found a trend in the opposite direction. In women,

ε2-carriership was associated with a shorter time to EDSS 6 and a higher relative increase of T2 and T1 lesion loads.

Chapter 2.3 describes the results of extensive meta- and pooled analyses using large patient groups to determine whether APOE variation influences disease susceptibility or severity in MS. Meta-analysis of 22 studies (3299 MS patients and 2532 controls) showed no effect of ε2 or ε4 status on MS risk. Results obtained from analyses of APOE genotype in 1279 MS families were also negative. Pooled analyses of 4048 MS patients showed no association with disease course or severity. The findings do not support a role for APOE in multiple sclerosis.

Chapter 3 focusses on immunopathological markers of disease.

In **chapter 3.1**, linking on to the previous chapter, it is investigated whether CSF apoE concentration is associated with cross-sectional and longitudinal disease characteristics in MS. Mean CSF apoE concentration in MS patients (n=44) was lower than in controls (n=28), maybe indicative of increased utilization of apoE-lipid-complexes for repair processes in MS. However, subgroup analyses showed no association with APOE genotype or (changes in) clinical, neuropsychological or MRI characteristics.

Chapter 3.2 contains the results of a study assessing the prognostic value of immunological markers for the long-term progression of disability in 25 MS patients. Markers were selected because they represent the activity of pro- and anti-inflammatory cytokines, chemokine receptors and mediators of apoptosis, reflecting mechanisms that have been associated with short-term disease activity in previous studies. Baseline TNF-α, IL-12p35, IL-12p40, IL-4, IL-10, TGF-β1, CCR3, CXCR3, CCR5, Fas and FasL mRNA levels in peripheral blood mononuclear cells (PBMC) were analysed with respect to their correlation with the increase in disability over a period of 10 years. High levels of Fas mRNA in RR MS and high levels of FasL mRNA in SP MS were associated with a favorable disease course, suggesting that Fas-mediated apoptosis plays a major role in the mechanism underlying long-term disease progression in MS.

Chapter 4 deals with MRI measures.

In **chapter 4.1**, it is attempted to make a restricted classification of MS patients purely based on MRI characteristics and to test whether the resulting subgroups are associated with clinical and laboratory characteristics. MRI examinations of the brain and spinal cord of 50 patients were scored for 21 quantitative and qualitative characteristics. Latent class analysis revealed two subgroups that mainly differed in the extent of lesion confluency and MRI correlates of neuronal loss in the brain (atrophy, amount of 'black holes'). Demographics and disease characteristics were comparable except for cognitive deficits. It was concluded that latent class analysis offers a feasible approach for classifying subgroups of MS patients based on the presence of MRI characteristics, although the reproducibility, longitudinal evolution and further clinical or prognostic relevance of the observed classification will have to be explored in a larger and independent sample of patients.

Chapter 4.2 describes three cases with a clinical course and cerebrospinal fluid findings consistent with a diagnosis of primary progressive multiple sclerosis (PPMS). Extensive and repeated magnetic resonance imaging (MRI) examinations showed only diffuse abnormality in brain and spinal cord, but no focal lesions. From literature it is known that PP MS patients typically show less focal lesions on conventional MRI and that diffuse abnormalities are characteristic hallmarks of a (primary) progressive disease course. Therefore, it is proposed that these cases represent the most pure form of PP MS, even though according to currently applied criteria this diagnosis can not be made in the absence of focal lesions on MRI.

Conclusion

The studies presented in this thesis suggest that:

- APOE genotype has no influence MS susceptibility or severity;

- CSF apoE might be utilized for repair processes in MS;
- Fas-mediated apoptosis plays a major role in the mechanism underlying long-term disease progression in MS;
- latent class analysis offers a feasible approach for classifying subgroups of MS patients based on the presence of MRI characteristics;
- isolated diffuse abnormalities on conventional MRI may represent the most pure form of PP MS.