SUMMARY OF THE THESIS

Host genetic variants in susceptibility, severity, and outcome of bacterial meningitis

Introduction

Although incidence of bacterial meningitis (BM) is decreasing in developed countries, it is still one of the top ten infectious causes of death worldwide. Meningitis is an infectious disease of the central nervous system (CNS) involving the dura mater, the arachnoid, the pia mater and the interposed cerebrospinal fluid (CSF). It is one of the most feared infectious diseases in children and remains a serious threat to global health. Despite the introduction of antibiotics, introduction of universal immunization strategies, and continuing improvement of supportive care, mortality remains four to 21 percent. Up to a quarter of survivors develop neurological sequelae ranging from subtle learning and behavioral disorders, to seizures and severe encephalopathy. Hearing loss is the most common type of sequela. It became evident that the host immune response to the pathogen, rather than the pathogen itself is largely responsible for this neuronal damage.

*S. pneumoniae* and *N. meningitidis* have become the most common causes of BM in the world. Faced with two of the most frequent nasopharyngeal colonizers in children and young adults, the host immune response is assumed to play an important role in preventing the development from colonization to invasive disease and meningitis. Only a limited fraction of those who become infected will develop a clinical disease with infection of the meninges. In case of meningitis, outcome of disease differs from complete recovery to severe life-long sequelae or even death. The question rises: how can these differences in the course of disease as a result of infection with the same pathogen be explained and moreover be predicted?

Differences in clinical course of BM depend on the causative microorganism, epidemiological-, clinical, environmental factors and host factors (*e.g.* immunogenetics). Several studies showed that single nucleotide polymorphisms (SNPs) in innate immunity genes are associated with susceptibility and severity of pneumococcal and meningococcal infections, but little studies focused on meningitis exclusively. In this thesis it was hypothesized that SNPs in the pathogen recognition receptors and signal transduction peptides affect the susceptibility to- and severity of BM.

Chapter 1 provides an overview of all studied polymorphisms involved in meningococcal and pneumococcal infections, with a special focus on meningitis. The genes are described conform the sequential steps in pathogenesis of BM including colonization, adhesion to
epithelial surfaces, pathogen recognition, bacteremia, complement activation, passage of the blood brain barrier and cytokine responses in the circulation as well as the CNS.

**Polymorphisms in the susceptibility to meningitis**

In order to study susceptibility to BM, in buccal DNA from survivors of pneumococcal- or meningococcal meningitis in childhood, 11 SNPs in 7 genes encoding for pathogen recognition receptors including: *Toll-like receptors* (*TLR2, TLR4 and TLR9*) and *Nucleotide oligomerisation domain proteins* (*NOD 1 and NOD2*) and signal transduction peptides (*i.e.* *caspase-1*, and *tumour necrosis factor related apoptosis inducing ligand (TRAIL)*) were determined by real-time PCR. Genotype distributions were compared to those in healthy controls.

In chapter 2, *TLR9* SNPs and haplotypes are studied to discover an association with susceptibility to BM by comparing *TLR9* SNPs between 471 BM patients and 392 controls. The *TLR9*+2848 SNP was associated with a decreased susceptibility (protection) against meningococcal meningitis (MM). *In silico* analysis of pathogen genomes showed a very strong immune inhibitory potential for *N. meningitidis* CpG DNA. We hypothesized that *TLR9* SNPs result in an increased activation by meningococcal CpG, abolishing its strong immune-inhibitory effect, leading to increased immune activation. Chapter 3 describes whether five other SNPs are associated with BM, by comparing 473 patients with 1141 healthy controls. It was found for the first time that *TLR4* and *NOD2* SNPs are associated with susceptibility to MM. Combined carriage of variant alleles of both SNPs significantly increased the risk to develop BM. The proposed mechanism, as supported by other functional studies, is a decreased immune response upon TLR4 and NOD2 activation in carriers of variant alleles and interactions between both genes.

**Polymorphisms in the severity of meningitis**

To study severity of disease, patients were subdivided into groups with a severe versus a less severe course of disease based on different clinical parameters that are relevant in severity of meningitis. In chapter 4 we studied whether *TLR9* SNPs are associated with severity of MM. The *TLR9* -1237 C variant allele is associated with protection against bacteremia, increased CSF leukocyte counts and decreased CSF/blood glucose ratios. These associations may indicate an increased TLR9 mediated immune activity in carriers of *TLR9* variants, as supported by recent functional studies. Moreover this increased TLR9 activity may help to explain the association with protection against BM in chapter 2.

In chapter 5 associations are studied between 11 SNPs and severity of meningitis in 393 BM patients and a multigene analysis was performed to study interactions between SNPs. *TLR4* was associated with an increased risk to develop hearing loss (HL) after BM and combined carriage with *TLR2* or *TLR9* variants significantly increased this risk.
SUMMARY

Prediction of sequelae in survivors of meningitis
It was studied whether two existing prediction models for hearing loss and academic and behavioral limitations after BM could be validated in an external cohort. With these models it is possible to identify children at risk for these sequelae by determination of easy obtainable clinical variables at admission. Further we investigated whether performance of these models could be improved by addition of the aforementioned high-risk SNPs.

Chapter 6 shows that the prediction model for post-meningitis hearing loss was successfully validated in an external Dutch cohort of survivors of BM in childhood. Theoretically this prediction rule could be used as an additional helpful tool for clinical decisions and lead to improved identification, early diagnosis and treatment of children at risk for post meningitis hearing loss.

In chapter 7 this methodology has been applied to another prediction model for academic and behavioral limitations after childhood meningitis. This model could not be validated since discrimination in the validation cohort was poor. There may be several causes for this including differences in a subjective outcome variable or in characteristics between both cohorts such as gender. For potential use in clinical practice this model should be newly developed.

In chapter 8 it is shown that addition of aforementioned high-risk genes to the prediction model for HL resulted in a good prediction model, but did not significantly increase the original clinical model. However, we agree that the concept of addition of genetic markers to clinical prediction rules is promising and further studies on this subject are in progress. More (combinations of) genes in larger patient cohorts will be included.

Conclusions and perspectives
In this thesis it was shown that polymorphisms in the pathogen recognition system are associated with susceptibility to and severity of BM. The exact mechanisms how these SNPs may result in clinical consequences need to be studied in functional studies. The concept of integration of genetic markers in clinical prediction models is promising and needs to be explored further. It should be mentioned that the effect of a single SNP is limited and that combinations of several SNPs, environmental- and pathogen factors are also involved in the course of meningitis.

The relevance of pathogen recognition receptors, especially TLRs in the pathogenesis of BM and other infectious diseases is continuously increasing. Genetic variation is likely to play an important role in functionality of this complex recognition system. In the future
this information may be used for identification of patients at high risk for meningitis and sequelae, to improve understanding of the immune system in the CNS during BM and to modulate specific elements of the immune system, without compromising an adequate immune response.