Nationwide prospective study on community-acquired bacterial meningitis: from genetics to therapy
(MeninGene)

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General Study Characteristics	
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SUMMARY

Background

Meningitis is an inflammation of the membranes covering the brain and spinal cord (the meninges). Each year 35,000 European patients suffer from bacterial meningitis, leaving 7000 deaths and 7000 disabled.[1,2] In developing countries, the burden of disease is up to 20 times higher.[3,4] *Streptococcus pneumoniae* and *Neisseria meningitidis* are the most common etiologic agents accounting for 85% of total cases.[1] Differences in susceptibility and outcome between individuals and populations are poorly understood but genetics of host and pathogen are considered crucial in this host-pathogen interaction.[5,6] Recent studies have shown that both host and pathogen genetic characteristics influence the risk of acquiring meningitis, the response to treatment in bacterial meningitis patients, and the risk of unfavourable outcome.[7–11] All of these studies used a candidate single-nucleotide polymorphism approach. Furthermore, genetic factors of the pathogen, which are thought to influence the virulence of the bacterium and thereby disease severity have not been studied. New adjunctive therapies are urgently needed to improve prognosis of bacterial meningitis.[12] Genetic studies can unravel which mechanisms contribute to unfavourable outcome in bacterial meningitis and can be used to identify new treatments.

Objective

The main objective of MeninGene is to identify genetic risk factors in host and pathogen influencing susceptibility to bacterial meningitis, and the rate of complications, unfavourable outcome and death.

Study-design

We will conduct a prospective observational nation-wide genetic association study in which we will performed massive parallel whole genome sequencing of causative bacteria and sequencing of host genes involved in the immune response in bacterial meningitis. DNA sequences of patients will be compared to healthy controls. Functionality of genes will be determined in leftover cerebrospinal fluid from the diagnostic lumbar puncture.

Study-population

All adult patients with community-acquired bacterial meningitis proven by culture or PCR of cerebrospinal fluid are eligible for the study. Controls consist of patients' partners of proxies living in the same dwelling, to ensure similar exposure to bacteria, similar age, ethnicity and socio-economic background.

European meningitis database

Data will be entered in a pseudonymized fashion in a European meningitis database and will be made available to European bacterial meningitis researchers upon request if scientific sound and free of direct commercial interest.

Nature and extend of the burden

For the patient and controls, participation will mean that 14 ml of blood will be withdrawn for DNA isolation, preferably together with a scheduled blood withdrawal for diagnostic purposes in the patient.

1. INTRODUCTION AND RATIONALE

Meningitis is an inflammation of the membranes covering the brain and spinal cord (the meninges). Each year 35,000 European patients suffer from bacterial meningitis, leaving 7000 deaths and 7000 disabled.[1,2] In developing countries, the burden of disease is up to 20 times higher.[3,4] *Streptococcus pneumoniae* and *Neisseria meningitidis* are the most common etiologic agents accounting for 85% of total cases.[1] Although these bacteria are common inhabitants of the human upper respiratory tract, in some individuals they spread to the bloodstream, slip through the blood-brain barrier and cause meningitis, with devastating consequences. Differences in susceptibility and outcome between individuals and populations are poorly understood but genetics of host and pathogen are considered crucial in this host-pathogen interaction.[5,6] Recent studies have shown that both host and pathogen genetic characteristics influence the risk of acquiring meningitis, the response to treatment in bacterial meningitis patients and the risk of unfavourable outcome.[7–11,13] However, all of these studies used a candidate single-nucleotide polymorphism approach, which is unable to detect novel genetic variants influencing the disease and is likely to overlook the most important genetic risk factors. In the recent years high throughput sequencing methods have enabled massive parallel sequencing of genes and whole genomes.

By sequencing whole genes in patients and whole genomes of bacteria we will be able to

- Determine genetic risk factors for susceptibility to meningitis
- Determine genetic risk factors for unfavourable outcome, cerebrovascular complications and death
- Determine virulence factors in the bacterial genomes

This may improve patient's care in the following ways:

- By determining genetic risks factor for increased susceptibility to bacterial meningitis protective measures such as vaccination and patient education on early symptoms can be implemented. This will especially be useful patients with recurrent meningitis and families with high incidence of meningitis, in which genetic counselling may be given.
- By determining genetic risk factors for complications or unfavourable outcome new treatments can be devised which counteract the pathophysiological mechanism that leads to the increased disease severity.
- Genotypes may be used to identify patients at high risk for specific complications. Physicians may, in the future, be able to use genetic information to tailor immune-based therapy to modulate the response in a given patient. Future therapeutic trials are likely to be designed to target specific genotypes and associated cellular responses, thereby maximizing clinical response and patient safety.[14]
- Identification of genetic variants in bacteria causing increased virulence could lead to development of new vaccines that includes antigens specific for virulent bacteria. This may result in vaccines preventing severe cases of bacterial meningitis.

2. OBJECTIVE

The main objective of the MeninGene study is to identify genetic risk factors in host and pathogen influencing susceptibility to bacterial meningitis, and the rate of complications, unfavourable outcome and death.

3. STUDY DESIGN

We will conduct a prospective observational nation-wide cohort study in which we will perform massive parallel sequencing of causative bacteria and genes in patients and controls involved in the immune response in bacterial meningitis.

4. STUDY POPULATION

4.1 Population

All adult patients with proven bacterial meningitis acquired in the community are eligible for the study.

4.2 Inclusion criteria

1. Age ≥ 16 yr
2. Bacterial meningitis defined by positive bacterial culture or PCR of cerebrospinal fluid

4.3 Exclusion criteria

1. Neurosurgical operation in the month previous to the meningitis episode
2. Head trauma in the month previous to the meningitis episode
3. Presence of neurosurgical devices in the central nervous system such as cerebrospinal fluid catheters or deep brain neurostimulator.

4.4 Sample-size calculation

For susceptibility research, with mean number of transcripts of 0.02 in the control group and 0.05 in the experimental group (2.5 fold increase), and 1000 patients and 1000 controls (20 times a specific sequence order in controls and 50 times in cases), using a significance level of 0.05, a total number of tags of 100.000, a percentage of tags truly differentially expressed of 0.10%, and a significance level used in the false discovery rate approximation of 0.001, will result in a study power of 0.94 and a false discovery rate of 21%. For evaluating the role transcripts on outcome, we will compare patients with unfavourable outcome (assuming overall-event rate 40%, n=400) to patients with favourable outcome (n=600). For outcome research we will reduce the level of significance§ so that we will have a false discovery rate of <21%.

DNA is expected to be available for 66% of included patients.[8] Therefore the total sample size is set at 1500 patients and controls. This can be achieved by including bacterial meningitis patients in all Dutch hospitals, which has been previously done for several bacterial meningitis study coordinated by the principal investigator.[1,8,16,18] In a previous cohort study with similar design we have included 1400 patients, from 2006-2012. Data of the previous cohort will also be used to validate genetic associations.

5. METHODS

5.1. Study procedures

5.1.1. Inclusion procedures

Patients can be included by two procedures:

1. The Netherlands Reference Laboratory for Bacterial Meningitis will provide daily updates of names of hospitals where patients with bacterial meningitis have been admitted in the preceding two to four days. The Reference laboratory receives cerebrospinal fluid (CSF) and blood isolates from approximately 85% of all patients with bacterial meningitis in the Netherlands. Physicians are informed about the study by telephone. Subsequently, patients or their legal representatives and controls receive written information and are asked to give written informed consent for participation.
2. A 24-hour telephone service for participating physicians is provided. Physicians can consult one of our meningitis researchers 24/7 to include a patient. Subsequently, patients or their legal representatives and controls receive written information and are asked for written informed consent for participation.

Controls will be selected from the same population as cases. Controls for exposure/susceptibility will be patients' partners or their non-related proxies living in the same dwelling. This will ensure that cases and controls have similar socioeconomic characteristics, ethnic backgrounds, and bacterial exposure. Controls receive written information and are asked to give written informed consent for participation.

5.1.2. Data collection and outcome score

Online case-record forms will be used to collect data on patients' history, symptoms and signs on admission, laboratory findings at admission, treatment (including adjunctive treatment), clinical course, outcome and neurological findings at discharge. Severity and clinical deterioration will be evaluated. Data on complications will be collected according to predefined criteria. Results of neuroimaging will be collected. At discharge, all patients undergo a neurological examination performed by a neurologist, and the outcome is graded according to the Glasgow outcome scale. A score of 1 on this scale indicates death; a score of 2, a vegetative state (the patient is unable to interact with the environment); a score of 3, severe disability (the patient is unable to live independently but can follow commands); a score of 4, moderate disability (the patient is capable of living independently but unable to return to work or school); and a score of 5, mild or no disability (the patient is able to return to work or school). A favourable outcome will be defined as a score of 5, and an unfavourable outcome as a score of 1 through 4. The Glasgow outcome scale is a well-validated instrument with good inter-observer agreement.[19]

5.2. Collection and storage of patient and control specimens

5.2.1. DNA

After the patient and control have given informed consent 14 ml of EDTA-blood with be drawn in both patient and control. In patients this withdrawal will combined with a regular blood withdrawal for diagnostic purposes if possible. Specimens of patients and controls will be coded and send to the AMC Genetics Core Facility. In the AMC Genetics Core Facility, DNA will be isolated with the Gentra Puregene DNA purification kit and stored at +4° Celsius until final analysis. Quality control procedures for DNA extraction will be performed to determine the yield of isolation.

5.2.2. Cerebrospinal fluid (CSF)

Functionality of genes will be evaluated by protein measurements in leftover CSF (waste material) from the diagnostic puncture if available. No extra procedures will be performed. CSF from the first diagnostic lumbar puncture will be locally stored for study purposes. CSF will be centrifuged to separate CSF and cells; both specimens will be stored at -80° Celsius. Local laboratory protocols with this purpose have been implemented in each hospital. Twice a year researchers from the AMC Neurology department will visit the laboratories in which CSF is stored and transport the samples to the AMC in dry ice, after which it will be stored until the analysis at -80° Celsius.

5.2.3. Collection of bacterial isolates

The Netherlands Reference Laboratory for Bacterial Meningitis is a collaboration of the Academic Medical Center and the National Institute of Public Health and the Environment. All clinical microbiology laboratories in the Netherlands collaborate by sending bacterial isolates and/or CSF samples from patients with meningitis.

5.3 Storage of data, blood, CSF and DNA in MeninGene biobank

All patient samples and data will be stored in the MeninGene biobank, which is located in the AMC, Amsterdam, the Netherlands in the AMC Genetics Core Facility (DNA), and the Netherlands Reference Laboratory for Bacterial Meningitis (CSF and blood). All patient samples and data will be stored in the Biobank and will only be used for research on bacterial meningitis. As research on epidemiology, pathophysiology and changes in management requires large number of patients and samples, all samples will be stored for a period of 50 years. Further information is available in the "*Biobank Protocol MeninGene studie*".

5.4. European Meningitis Database

We assure a safe management of the data. There will be two databases. The first database is the MeninGene database. This database will include coded data; this dataset will not be accessible for other researchers. Prof. Dr. D. van de Beek and Dr. M.C. Brouwer will have access to this biobank.

The second database will be an European Meningitis Biobank. In this database an pseudonymized limited dataset will be included. There will be no date of birth (age in years only), hospital, etc in the biobank. Data will be coded by case record form numbers.

The European database will be made available to European bacterial meningitis researchers through a web-based application procedure. Requests for information will be evaluated by a committee consisting of Prof. Dr. D. van de Beek (neurologist, principle investigator), Dr. A. van der Ende (head Netherlands Reference Laboratory for Bacterial Meningitis), Prof. Dr. F. Baas (head AMC Genetics Core Facility), Prof. Dr. A.H. Zwinderman (biostatistician, Department of Clinical Epidemiology and Biostatistics, AMC), and the chair of the ESCMID Meningitis Study Group (Prof. Dr. S.L. Leib, Institute for Infectious Diseases, Bern, Switzerland). Requests will be granted if scientifically sound. For each proposal a review by an ethical committee is required. Information about institution, background, aim, expected results, publication, storage of data, safety of computers used, will be required; this will be formalized in a contract with the European meningitis biobank. If results of the project are not published in a peer-reviewed journal, they must be published in a European meningitis biobank database. In addition, all published work from these projects will be noted on our website. We are currently working on this procedure. The Ethics Board of this project will also review this procedure (see below)..

Because this project is an ERC Starting Grant project we have formed an ethics board that will evaluate the ethical aspects of this European Database annually. This board will provide yearly reports to the ERC. Members will be:

1. Prof. R.C. Hennekam, Chair Ethics Board, Pediatrician and Clinical Geneticist, member of the Medical Ethical Committee, Academic Medical Center;
2. Mr. Dr. M.C. Ploem, Health law, Department of Social Medicine, member of the Medical Ethical Committee, Academic Medical Center;
3. Drs. A. Cahn, co-owner and Chief Technology Officer, Hippo, Amsterdam, member of the Apache Software Foundation;
4. W. Witkamp, president of the Netherlands Meningitis Foundation, the Dutch patients' organization for patients with meningitis.

5.5. Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences.

5.6. Participating centres

All hospitals throughout the Netherlands will be approached for participation following approval of the AMC Medical Ethical Committee.

6. ETHICAL CONSIDERATIONS

6.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (version of 2008, Seoul, South-Korea) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts. Data management, monitoring and reporting of the study will be performed in accordance with the ICH GCP guidelines. Technicians and data managers of the AMC Clinic Research Unit (CRU) will perform central data management. Internet-based remote data capture will be used for entering, managing and validating data from the investigative sites.

6.2 Recruitment and consent

When the selection criteria are fulfilled, the patient or, if necessary, the patients representative will be asked for written informed consent, in accordance with the guidelines of the local medical ethics

committee (METC). When the patient has diminished decision-making capacity as result of the meningitis e.g. due to aphasia or cognitive impairment informed consent will be obtained from the patient's representative. Exclusion of these non-communicative bacterial meningitis patients would lead to a selective patient sample. Information materials for patients and patients' relatives are attached separately. If patients with diminished decision-making capacity regain this capacity, they will be asked again if the agree with participation in the study. For patients of 16 and 17 years old, the parents or legal guardian will be asked to co-sign the informed consent form.

Provided informed consent procedures and privacy measures and safeguards are in accordance with the Dutch Personal Data Protection Act and the Medical Treatment Contract Act and the EU-Directive 95/46/EC on the protection of individuals with regard to the processing of personal data and on the free movement of such data.

6.3 Benefits and risks assessment
Patients do not have direct benefit from this study. Therapies identified as a result of this study may be beneficial to a future episode of meningitis in patients with recurrent meningitis (approximately 5%). The risks of the study are limited to those of a venous blood withdrawal, which are minor.

6.4 Compensation for injury
This study is exempt from insurance obligations as there are no significant risks attributable to participation to this study.

6.5 Incentives
Included patients will not receive any special incentives, compensation or treatment through participation in this study.

7. ADMINISTRATIVE ASPECTS AND PUBLICATION
7.1 Handling and storage of data and documents
The study will be conducted according to the principles of the Declaration of Helsinki (version of 2004) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts. Data management, monitoring and reporting of the study will be performed in accordance with the ICH GCP guidelines. Technicians and data managers of the AMC Clinic Research Unit (CRU) will perform central data management. Internet-based remote data capture will be used for entering, managing and validating data from the investigative sites.
When the study is finished, all essential documents (Case Record Forms, Informed Consent forms, patient files, radiological images) will be archived and stored for the next 15 years, in accordance to GCP-guidelines.

7.2 Coding of and access to data
Patient's data are coded with a unique number. This consists of four numbers, which will be used sequentially for included patients. The study code does not include data that may be used for identification of the patient such as date of birth, initials or codes for hospital of admission. The key to this code is known in the AMC; the coordinating researchers and research team will have entry to this code. This code will also be used to store DNA, leftover cerebrospinal fluid, radiological images and discharge letters.
The health-inspection (inspectie voor gezondheidszorg – IGZ), the METC, and audits will have access to source documents.

7.3 Amendments
A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the study;

- the scientific value of the study;
- the conduct or management of the study.

All substantial amendments will be notified to the METC and to the competent authority.
Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

7.4 Annual progress report

The investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, and amendments.

7.5 End of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the inclusion of the 1500th patient. In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 30 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

7.6 Public disclosure and publication policy

The coordinating investigators will have the responsibility for decisions regarding publication of data for scientific purposes.

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