Protocol EUCLIDS-study

PROTOCOL TITLE ***The genetic basis of meningococcal and other life threatening bacterial infections of childhood***

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PROTOCOL SIGNATURE SHEET

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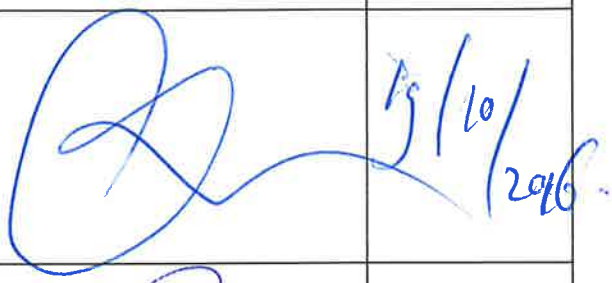
For non-commercial research, Head of Department: ***Prof. Dr. K. Noordam***		19/10/2016
Coordinating Investigator/Project leader/Principal Investigator: ***Prof dr. R. de Groot***		19-10-2016

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)**
AE	**Adverse Event**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
Wbp	**Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: Bacterial infection is the major cause of disability and death in children worldwide. We will use meningococcal disease (MD) as a model to understand genetic factors underlying susceptibility and severity of childhood bacterial infection.
In addition to MD, we will study infections caused by the following eight bacterial pathogens often causing severe disease: Streptococcus pneumoniae, Staphylococcus aureus, Group A Streptococcus, Salmonella species, Bordetella pertussis, Group B Streptococcus, Haemophilus influenzae and Escherichia coli. We have already undertaken a genome wide study (GWAS) to identify genes causing susceptibility to meningococcal disease in a European cohorts. In this study we identified complement factor H (fH) and fH-related (fHr) genes controlling MD susceptibility. (Davila et al Nature Genetics 2010;42 (9) 772-6) In addition we found SNP's in other genomic regions and in other pathways, which may also control susceptibility for MD.
Based on this study Professor Levin from Imperial College London together with researchers from Spain, Austria, and The Netherlands, who were involved in the previous study an interdisciplinary multinational team was formed including experts on paediatric infectious diseases, but also experts on immunogenetics, bioinformatics, molecular microbiology, vaccinology, and Research and Development in the area of diagnostics development, vaccine development, and genome analysis.
Currently materials available within the consortium (> 5.000 DNA samples of children with severe bacterial infections and ± 3500 DNA samples of children vaccinated with an experimental vaccine against meningococcal serogroup C or B) are analysed in the Genome Institute in Singapore to identify genetic factors that control severity of disease.
The previous findings related to factor H (fH) and fH-related (fHr) genes controlling MD susceptibility are fundamental to prevention as novel vaccines containing the MD fH receptor protein as a component are currently undergoing trials.
In the presented study we will use next generation sequencing to identify the causal variants within the fH/fHr region and other regions and pathways that control susceptibility and severity of meningococcal disease, pneumococcal disease, staphylococcal disease, Group A Streptococcal disease,Salmonella species disease, Bordetella pertussis disease, Group B streptococcus disease, Haemophilus influenzae disease and Eschericha coli disease. We will study the interaction between bacterial and host genetic variation. Hereto we will apply next generation sequencing, RNA expression analysis, functional analyses and animal models. We expect to identify Mendelian defects and rare mutations as well as copy number variation and epi-genetic effects.

The study will contribute to the identification of mechanisms underlying susceptibility, and may provide new targets for treatment and prevention.

Objective:
Primary Objectives:

1. To identify mechanisms underlying susceptibility to meningococcal, pneumococcal, staphylococcal, Group A streptococcal,Salmonella species, Bordetella pertussis, Group B Streptococcus, Haemophilus influenzae and Escherichia coli infection,with a focus on bacterial meningitis and S. aureus bacteremia
2. To identify mechanisms underlying severity of meningococcal, pneumococcal, staphylococcal, Group A streptococcal,Salmonella, Bordetella pertussis, Group B Streptococcus, Haemophilus influenzae and Escherichia coli infection, with a focus on bacterial meningitis and S. aureus bacteremia.

Secondary Objectives:

1. To provide new targets for treatment and prevention of meningococcal, pneumococcal, staphylococcal, Group A streptococcalSalmonella species, Bordetella pertussis, Group B Streptococcus, Haemophilus influenzae and Escherichia coli infections, with a focus on bacterial meningitis and S. aureus bacteremia To develop tools to identify patients at risk of disease or poor outcome

In order to accomplish these objectives the EUCLIDS clinical Working Party has established an international clinical network and biobank.
In this extension of the EUCLIDS-study we want to include more patients to achieve the above mentioned objectives. Prospectively we have narrowed the inclusion criteria to patients with bacterial meningitis and S. aureus bacteremia. For retrospective inclusions the criteria stay the same.

Study design: Observational Paediatric Cohort study using patient material (blood, saliva)

Study population: 300 children in the Netherlands aged 1 month -18 years with bacterial meningitis or S. aureus bacteremia or with a history of a severe meningococcal, pneumococcal, Group A Streptococcal, Staphylococcus aureus, Bordetella pertussis, Group B Streptococcus, Haemophilus influenzae or Escherichia coli infection and are/were admitted at one of the general hospitals or University Medical Centers participating in the PeD –BIG Network coordinated by the Radboud University Nijmegen Medical Center (prof dr R de Groot/ dr. M. van der Flier).

Main study parameters/endpoints: With the use of a novel pathway
based analysis methodology of GWAS developed by the Imperial College group

(Eleftherohorinou et al. 2009) biological pathways associated with disease can be identified by assessing the cumulative association of genetic variation within multiple genes in the same pathway. This will identify genes determining susceptibility and severity of bacterial meningitis, S. aureus bacteremia and other infections caused by N. meningitidis, S. pneumoniae, Group A Streptococcus, Staphylococcus aureus, Bordetella pertussis, Group B Streptococcus, Haemophilus influenzae and Escherichia coli

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: In none of the included patients extra vena punctures will performed, as all blood samples are only taken at times of routine blood sampling following the collection of diagnostic samples.
In patients not admitted to the paediatric intensive care one blood sample will be collected with a maximum of 8 mL. In patients admitted to the Paediatric Intensive Care Unit 3 sequential blood samples will be collected at t=0, t=24 hours and t=72 hours.with a maximum of 8 mL at each time point. In young infants < 12 months of age this blood volume will be reduced to 4 mL at each timepoint. If cerebrospinal fluid/blood was obtained at diagnosis surplus material will be collected from the laboratory. Optionally, an additional blood sample at convalescence will be obtained during a routine blood sampling. In materials collected from retrospective patients saliva will be collected for DNA analysis and no blood sampling is required. DNA analysis will be performed anonimized. The participants will not be informed on their own genetic code (DNA). Thus the DNA analysis will not have juridical impact (when gaining a mortgage or life insurance). Following the above, the study causes minimal burden and is without risks for patients included in the study.
The group relatedness is clear since the risk on a severe course of a bacterial infection in a child is much greater than in adults. This is caused by a combination of a –still- immature immunesystem and genetic factors influencing the immunity. In adults other factors such as secondary immunodeficiency are more frequent. An example is alcoholism or chronic disease as a risk factor for pneumococcal infections. Life threatening bacterial infections in childhood remain an important cause of morbidity and mortality despite current availability of antibiotics and vaccines. A better understanding of genetic determinants may provide new leads to improve therapy and prevention and to identify risk groups for increased susceptibility and unfavourable outcome.

1. INTRODUCTION AND RATIONALE

Bacterial infection remains the major cause of childhood critical illness, disability and death in children worldwide. Although the availability and application of antibiotics have reduced mortality and morbidity, and vaccines against bacteria such as Haemophilus influenzae type B and Streptococcus pneumoniae have decreased the incidence of disease in countries that have introduced these vaccines, severe bacterial infections such as bacterial meningitis, remain major problems in children in Europe. In developing countries bacterial infections are major causes of early childhood deaths. Reducing the burden of bacterial infection in childhood is key to achieving the global development aims of reduced childhood mortality by 2015.

The most remarkable feature of many infections is the variability of response to infection among individuals within the population. If we consider common childhood bacterial infections by for example *S. pneumoniae, H. Influenzae, S. aureus* and *N. meningitidis*, the majority of the population appears to be innately resistant to (serious) disease, and these organisms behave as harmless commensals, being carried intermittently or long term throughout life without development of disease.

There is now clear evidence that genetic factors are major determinants of both susceptibility and outcome (severity) of infectious diseases. Identification of the genes responsible is a powerful method to understand disease pathogenesis, identify susceptible individuals, elucidate the biological pathways involved, and to identify new therapeutic interventions. Genetic approaches may also help to identify those at risk of vaccine failure or adverse effects.

Meningococcal infection is not only one of the most important life threatening infections in children, but also forms a unique model through which the genetic basis of infectious diseases can be studied, as it is easily recognizable by its clinical features such as by the characteristic rash, is notifiable to health authorities and is common.
The disease is a major public health concern in the EU and also in developing countries, specifically in sub-Saharan Africa, where epidemics of Group A meningococcal disease affect thousands of individuals annually.
There is profound interest and research in the development of vaccines against the Group B meningococcus. However, effective vaccination against Group B Meningococcus is not yet implemented.

Meningococcal disease is a unique model through which to identify the genetic basis of childhood infection. Our previous GWAS (Davila, et al. Nat Gen 010) has identified gene variation in the factor H (fH) region as the key gene determining susceptibility to meningococcal disease. Of great biological importance is the fact that the meningococcus expresses a factor H binding protein (fHbp) and is known to have genetic variation within the fHbp gene. Many of the genes controlling susceptibility and severity of meningococcal

infection are also likely to be important in other serious bacterial infections in childhood. For example, several other pathogens including pneumococcus have Factor H Binding Proteins (FHBPs) on their outer surface to regulate factor H (fH) or factor H Receptor (fHR) binding in order to evade complement mediated damage. Human genetic variation in the fH-fHR region is thus likely to be important in evasion of complement mediated killing in a range of infections. Similarly, genes controlling the intensity of the inflammatory response, which influence disease severity may control outcome of a range of bacterial infections.

OBJECTIVES

Primary Objectives:

1 To identify mechanisms underlying susceptibility to meningococcal, pneumococcal, staphylococcal, Group A streptococcal, Salmonella species, Bordetella pertussis, Group B Streptococcus, Haemophilus influenzae and Escherichia coli infection with a focus on bacterial meningitis and S. aureus bacteremia
2 To identify mechanisms underlying severity of meningococcal, pneumococcal, staphylococcal, Group A streptococcal, Salmonella, Bordetella pertussis, Group B Streptococcus, Haemophilus influenzae and Escherichia coli infection, with a focus on bacterial meningitis and S. aureus bacteremia

Secondary Objectives:

1 To provide new targets for treatment and prevention of meningococcal, pneumococcal, staphylococcal, Group A streptococcal, Salmonella species, Bordetella pertussis, Group B Streptococcus, Haemophilus influenzae and Escherichia coli infections, with a focus on bacterial meningitis and S. aureus bacteremia
2 To develop tools to identify patients at risk of disease or poor outcome

In order to accomplish these objectives the EUCLIDS clinical Working Party will establish an international clinical network and sample collection. The consortium already holds the most important meningococcal disease (MD) sample collection in the world, with more than 5000 DNA samples and clinical records of children with severe bacterial infections and 3500 DNA samples of children vaccinated against meningococcal serogroup C or B (located at Oxford Vaccine Centre Biobank).
In this extension of the EUCLIDS-study we want to include more patients to achieve the above mentioned objectives. Prospectively we have narrowed the inclusion criteria to patients with bacterial meningitis, because this is one of the most severe infections and easily recognized, and S. aureus bacteremia. For retrospective inclusions the criteria stay the same.

In the Netherlands ± 300 paediatric patients will be recruited including retrospective cases. In general, any patient from 1 month to 18 years presenting with a bacterial meningits or S. aureus bacteremia will be eligible for the study. Also retrospective cases of severe bacterial

infection with N. meningitidis, S. pneumoniae, S. aureus, Group A streptococcus, Salmonella, Bordetella pertussis, Group B Streptococcus, Haemophilus influenzae and Escherichia coli will be recruited.

The EUCLIDS international clinical network constitutes a key factor for the EUCLIDS project as a whole and for each of the different participating workpackages.

Specifically, the objectives of EUCLIDS clinical WP are:

- To maintain and conduct the international clinical network including European and African partners
- To create the network workspace and database
- To organize recruitment of patients with different severe bacterial infections across all sites, as well as healthy controls and meningococcal vaccine recipients.
- To provide the necessary new patient cohorts for the validation of the results of the different work packages in a timely manner
- To organize and regulate the consortium sample collection.
- To regulate, collect and record all the clinical data to be obtained in order to describe accurate clinical phenotypes

2. STUDY DESIGN

Design:
Observational Paediatric Cohort study using patient material (blood, saliva)

Duration:
Patient recruitment to take place over 60 months.

Setting:
Recruitment of patients will be done in the United Kingdom, Austria, Spain, Germany, Italy, Gambia and The Netherlands. The recruitment situation within the consortium is described within the EUCLIDS Project CLINICAL PROTOCOL.The recruitment situation in the Netherlands is described below.
The Dutch clinical network for the EUCLIDS Project is named Pediatric Dutch Bacterial Infection Genetics network (PeD-BIG; Coordinator Prof Dr. R. de Groot/Dr. M. van der Flier). This network consists of ~30 general hospitals. The data will be collected by the coordinating centre Radboud University Nijmegen Medical Centre, RUNMC; Ronald de Groot/ Michiel van der Flier. The Ped-BIG will have a core committee which consists of Ronald de Groot (Chair), Michiel van der Flier, Marceline van Furth, Arie van der Ende (from the Netherlands Reference Laboratory Bacterial Meningitis Amsterdam) Lieke Sanders from the University Medical Centre Utrecht/RIVM, Diederik van de Beek from the Department of Neurology Academic Medical Centre Amsterdam, and Gertjan Driessen from the Erasmus Medical Centre Rotterdam.

All these sub-investigators and centres will be centrally coordinated by Radboud University Nijmegen Medical Centre. The arrangement of the coordinating centre with the collaborator centres is strictly scientific. The collaborating investigators or centres are not receiving any kind of payment-reimbursement for their work. The coordinating centre directly provides to each centre any specific material for sampling-collection that might be needed for the project. The shipment of the samples (for DNA) from the collaborating centres to the coordinating centres is organized and paid by the coordinating centre.

The collaborating centres and sub-investigators will be listed under the Pediatric Dutch Bacterial Infection Genetics (PeD-BIG) research team in the publications derived from the study. The PeD-BIG network will closely collaborate with The Netherlands Reference Laboratory for Bacterial Meningitis of the AMC and with the existing adult Bacterial Meningitis Study Group of the AMC (Diederik van de Beek; www.meningitisamc.nl) which performs successful studies on adult bacterial meningitis patients in the Netherlands and has collected a large cohort of adult meningitis patients.

Procedures

Clinical characteristics will be recorded on a clinical report form (CRF) for all patients included in the study. Data collected from the medical records will include complaints at presentation, course of disease, data on the infecting organisms and severity of illness scoring as well as disease phenotype and outcomes (results of hearing assessment etc.). Research samples would be taken for both host and bacterial characterization.
All patients will not have any additional vena punctures, as blood sample collection will be combined with routine blood sampling.
In non-PICU patients there will be 1 blood sample with a maximum of 8 mL. Optionally a convalescence blood sample will be obtained.
In PICU patients there will be 3 sequential blood samples each with a maximum of 8 mL at t=0, t=24-48 hours and t=72 hours/at discharge and an optional sample at convalescence.
In young infants < 12 months volume for each sample will be reduced to maximum 4 mL.
If cerebrospinal fluid / blood was obtained at diagnosis surplus material will be collected from the laboratory.

All patients will not have any additional vena punctures during hospitalisation, as blood sample collection will be combined with routine blood sampling. An exception is the convalescence blood sample. If this can not be combined with routine blood sampling, a vena puncture will be needed. Parents and children always have the possibility to refuse this.

Deferred consent procedure:
In order to study children with severe illness, it is important to look at patient samples taken before treatment has started. On admission a small amount of blood, urine and throat swab may already have been taken for this study at the same time as the other blood tests, before there was an opportunity to discuss the research with the parents/patient. We will use these materials only if the parents (and patient if >12 years old) have given consent to the study, otherwise we will discard it.

In patients retrospectively recruited for the study saliva will be collected for DNA isolation. In these patients no blood sampling will occur.

DNA analysis will be performed anonimized. The participants will not be informed on their own genetic code (DNA). Thus the DNA analysis will not have juridical impact (when gaining a mortgage or life insurance)

Bacterial isolates will be stored by the Netherlands Reference Laboratory for Bacterial Meningitis and in time transported to the relevant workpackage.

Blood will be processed and stored at the site and then batched and transferred to the laboratory performing the relevant work package including genome wide association study (GWAS), next generation sequencing, RNA expression analysis, "Beyond-State-Of-The-Art" Genome studies.

In selected cases DNA analysis by GWAS will be extended with exome sequencing. In these cases we want to compare the DNA of the patient with the DNA of the parents. Therefore, in these cases parents will be asked for giving a blood sample.

Optionally, patients and parents will be approached for follow-up tests/questions, for example hearing assessment after enduring a meningitis.

STUDY POPULATION

2.1 Population (base)

In the Netherlands in this extension of the study we intend to include patients with bacterial meningitis and S. aureus bacteremia. The age of these patients will be between 1 month – 18 years. Ethnic background will reflect the population of the Netherlands, with ± 80% Caucasians (Statistics Netherlands CBS). We will also retrospectively include patients who have suffered a severe bacterial infection with N. meningitidis, S. pneumoniae, S. aureus, Group A streptococcus, Salmonella, Bordetella pertussis, Group B Streptococcus, Haemophilus influenzae and Escherichia coli .

2.2 Inclusion criteria

CLINICAL DEFINITIONS AND INCLUSION CRITERIA

Our aim is to include 300 patients.The following patients will be eligible for the study:

C.1. – Prospectively recruited bacterial meningitis and S. aureus bacteremia patients.

C.2.- Retrospective recruited patients after an infection with N. meningitidis, S. pneumoniae, S. aureus, Group A streptococcus, Salmonella, Bordetella pertussis, Group B Streptococcus, Haemophilus influenzae and Escherichia coli

C.1. INCLUSION CRITERIA for the PROSPECTIVE study

Any patient from **1 month to 18 years** admitted to hospital with

a) **Bacterial meningitis** or suspected bacterial meningitis or S. aureus bacteremia will be eligible for the EUCLIDS study, **anytime**
   i. Informed consent is obtained
   ii. At least a DNA sample is obtained
   iii. At least the minimum mandatory data set is collected.

Meningitis patients will be included when they meet criteria that identify bacterial meningitis also in the absence of a positive culture. If patients meet minimal one of the criteria (Spanos et al.,JAMA 1989) mentioned below, there is more than 99% chance that it is a bacterial meningitis and then they can be included.

Criteria: * Cerebrospinal fluid (CSF) glucose level < 1.9 mmol/L.
* CSF-blood glucose ratio < 0.23.
* CSF totaal eiwit > 2.2 g/L
* CSF leukocyten > 2000 x 10^6 cells/L.
* CSF granulocyten > 1180 x 10^6 cells/L.

C.2. INCLUSION CRITERIA for the RETROSPECTIVE study:

Any **retrospectively identified** patient born **from 1970 to date** having suffered at **1 month-to-18 years** of age from an infection with N. meningitidis, S. pneumoniae, S. aureus, Group A streptococcus, Salmonella, Bordetella pertussis, Group B Streptococcus, Haemophilus influenzae and Escherichia coli, will be eligible as a **retrospective patient** for the EUCLIDS study, **anytime**

i. a DNA sample is obtained
ii. Informed consent is obtained
iii. In those retrospectively accessed DNA samples: informed consent is obtained and no specific rejection / contraindication to the use of the sample in a different study than the one that prompted the original sample collection exist
iv. the patient minimum mandatory data set for retrospective cases is available

2.3 Exclusion criteria

* The study blood sample volume has been chosen such as to exclude the risk that blood sampling for study purposes would result in a need for earlier blood transfusion. However, no children should be included in the study for whom it cannot be excluded that blood sampling for study purposes would result in an earlier need for blood transfusion.
* Bone marrow transplant patients
* Patients already recruited who are readmitted within the same illness.

2.4 Sample size calculation

Simplified, for susceptibility research, genotype frequencies in cases will be compared with controls; with 1500 patients sample size provides 80% power or more for a low frequent (5%) risk genotype with an odds ratio of 2.5 or more using significance-level of 0.001 to take account of the number of SNPs that we will evaluate. For evaluating the role of SNPs on outcome, we will compare patients with unfavorable outcome (assuming overall-event rate 15%, n=225 cases) to patients with favorable outcome (n=1275); sample size provide sufficient power (80%) when a risk-genotype has relative risk of 3.0 or more. Power on subgroup analyses for bacterial species will be sufficient for 300 patients; in a conservative power analysis and an event rate of 30% (90 cases and 210 controls), using a low frequent (5%) risk genotype, minimal 80% power will be provided if 21% of cases is carrier, using a significance-level of 0.001. The establishment of well characterised cohorts with patients collected following common diagnostic and management procedures will allow us to follow an approach in which initial genome-wide association study undertaken in one (discovery) cohort (for example the UK cohort: Davila, Nature Genetics 2010) which can then be validated on each of the other EU cohorts). Furthermore genes identified through the Meningococcal disease GWAS can be validated in our prospective cohorts of other bacterial species. The common clinical features of our patients allow meta analysis of the combined cohorts to increase the power to detect genes of lesser significance. Genes with significant associations in the region of $p=10^{-3}$-10^{-7} not meeting genome-wide significance on a single cohort, but which have consistent significance in each of the other cohorts, can be further studied in the meta-analysis.

Power calculations for our meningococcal diseases discovery cohorts estimate that we will have more than 80% power to detect a $P=5x10^{-7}$ of genome-wide significance with risk allele frequencies of 10% and relative risk around 2 in both cohorts. SNPs and/or genes identified to be associated with single SNP, severity or pathway analysis will be genotyped in a prospective cohort of meningococcal disease cases and other invasive bacterial infections (European and West African cohorts). New variants identified through the re-sequencing effort will be typed in other bacterial infections disease cohorts from Europe and the West Africa cohorts to establish risk variants associated with invasive bacterial infection, and those controlling severity and outcome. The final global sample size planned to be recruited through the EUCLIDS network results from the sum of all required samples to perform each of the more than 30 a priori planned trials under the different WPs configuring EUCLIDS. Our already existing biobank has been taken into account for this calculation.

For this specific extension of the study newly collected samples from bacterial meningitis and S. aureus bacteremia patients and retrospective patients who have suffered from an infection with N. meningitidis, S. pneumoniae, S. aureus, Group A streptococcus, Salmonella, Bordetella pertussis, Group B Streptococcus, Haemophilus influenzae and Escherichia coli, will be 150-300.

3. STATISTICAL ANALYSIS

The EUCLIDS proposal goes beyond the state-of-the-art through the use of a novel pathway

based analysis methodology of GWAS developed by the Imperial College group (Eleftherohorinou et al. 2009). We have shown that we can reproducibly identify biological pathways associated with disease by assessing the cumulative association of genetic variation within multiple genes in the same pathway. Application of this method to the Wellcome Trust study of 7 common diseases (WTCCC 2007) identified common highly significant pathway effects in inflammatory diseases. Furthermore, we developed a pathway-driven gene-selection methodology to identify the key genes within associated pathways using a Bayesian variable selection algorithm in the framework of stability selection. In this latest work, we showed that using both biologically driven pathway information and a robust gene stability selection methodology yields improved power and consistent results across two independent cohorts of the same disease. The pathways and genes that were identified in one study were also identified in the other, with a probability occurring by chance $P<10^{-20}$. These novel GWAS analytical strategies will be applied independently to the three MD GWAS to identify associated biological pathways and then the individual genes.

4. ETHICAL CONSIDERATIONS

4.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (Version Oct 2008) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts.

4.2 Recruitment and consent

Subjects or their legal representatives (parent/caretaker) will be approached by their doctor or by the investigator for recruitment for the study. Subjects and or their legal representatives will be informed about the study and will be asked for informed consent as applicable depending on the age of the subject. Subjects or their legal representitives will be given as much time as needed to decide on their consent. The patient information letter and informed consent form are attached separately.

Because the study involves further development of an international sample collection, an Ethics Steering Group will be established before or immediately after the project starts and will ensure that all partners comply with all aspects of the ethics.

4.3 Objection by minors or incapacitated subjects (if applicable)

It may occur that a subject does not cooperate during study procedures. In this situation the investigator will end the study immediately.

4.4 Benefits and risks assessment, group relatedness

In none of the included patients extra vena punctures will occur during hospitalisation, as all blood samples are only taken at times of routine blood sampling.

In patients not admitted to the paediatric intensive care 1 blood sample will be collected with a maximum of 8 mL. In patients admitted to the Paediatric intensive care unit 3 sequential blood samples will be collected at t=0, t=24-48 hours and t=72 hours/at discharge with a maximum of 8 mL at each time point. In young infants < 12 months of age this volume will be reduced to 4 mL at each timepoint.
Optionally a convalescence blood sample will be obtained. If this can not be combined with routine blood sampling, a vena puncture may be performed. Parents and children always have the possibility to refuse this.
In retrospective patients recruited for the study saliva will be collected for DNA analysis and no blood sampling is required. DNA analysis will be performed anonimized. The participants will not be informed on their own genetic code (DNA). Thus the DNA analysis will not have juridical impact (when gaining a mortgage or life insurance).
Following the above, the study causes minimal burden and is without risks for patients included in the study.
The group relatedness is clear since the risk on a severe course of a bacterial infection in a child is much greater than in adults. This is caused by a combination of a –still-immature immunesystem and genetic factors influencing the immunity. In adults other factors such as secondary immunodeficiency are more frequent. An example is alcoholism or chronic disease as a risk factor for pneumococcal infections. life threatening bacterial infections in childhood remain an important cause of morbidity and mortality despite current availability of antibiotics and vaccines. A better understanding of genetic determinants may provide new leads to improve therapy and prevention and to identify risk groups for increased susceptibility and unfavourable outcome.

4.5 Compensation for injury

Dispensation from the statutory obligation to provide insurance was granted by the METC, because participating in the study is without risks for the participants.

5. ADMINISTRATIVE ASPECTS AND PUBLICATION

5.1 Handling and storage of data and documents

Data will be collected on a paper CRF and put in a web based database which is built in accordance with all European Union requirements in regard to personal data protection and complies with the Dutch Personal Data Protection Act. Patients will receive an unique identification number. The code will be based on a number for the recruiting center and a number for the patient (see EUCLIDS CLINICAL PROTOCL V4 2011-10-28 p15). In each center the local sub investigator has access to the key for the code for that center. In each center the local sub investigator and a member of the study team will have access to the source documents for collecting the clinical data. Patient samples will receive the same unique patient number. Blood samples of the parents will receive the patient code with the addition of a P for the father and a M for the mother. Patient materials will not be destroyed at the end of the study but will be kept for 15 years if the patient or legal representative consents. The materials will be kept, because of the great interest for

scientific research of potential future use of body materials (DNA) sampled from children with disease.

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

5.2 Annual progress report

The sponsor/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, serious adverse events/ serious adverse reactions, other problems, and amendments.

5.3 End of study report

The investigator will notify the accredited METC after recruitment of the last patient within a period of 8 weeks.

In case the study is ended prematurely, the investigator will notify the accredited METC, 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. The end of the study will be two years after recruitment of the last patient, since all genetic analysis will start when the cohorts are completed.

5.4 Public disclosure and publication policy Publication/ disclosure and public report of the findings of the study have been explicitly agreed upon with the European Union in line with FP7 guidelines.

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Spanos A., et al. Differential diagnosis of acute meningitis. An analysis of the predictive value of initial observations. JAMA. 1989 Nov 17;262(19):2700-7.