Genetics in Meningococcal Disease: One Step Beyond

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(See the article by Darton et al. on pages 587–94)

Neisseria meningitidis is the leading cause of meningitis and septicemia among young adults [1]. The incidence of meningococcal disease for high-income countries is 0.9–1.5 cases per 100,000 population [1]. For low-income countries, incidences are estimated to be much higher, with the highest burden occurring in sub-Saharan Africa (“the Meningitis Belt”), with rates of up to 1000 cases per 100,000 population [1]. The World Health Organization is committed to eliminating this important and preventable health problem [2].

Several risk factors for meningococcal disease have been identified, but the cause of basic differences in susceptibility and outcome between individuals and populations remains unknown. In the 1980s, studies of adopted children and twins revealed that genetics are major determinants of susceptibility to infectious diseases [3, 4]. Recently, several other studies further suggested that genetics play a role in susceptibility and outcome of meningococcal disease [5–7].

New techniques in genetic research emerged in the 1990s, creating useful tools for meningococcal disease. The standard for epidemiologic research has become multilocus sequence typing. Multilocus sequence typing analysis determines genetic variation in 7 housekeeping genes of the meningococcus and has shown that most cases of disease are caused by a few clonal complexes of related sequence types, the hypervirulent lineages [8, 9].

As a diagnostics tool, PCR has shown to be a highly sensitive test in meningococcal disease [10]. Meningococcal DNA detection by PCR has been used in Great Britain since 1996 and can be regarded as a routine diagnostic test in patients with suspected meningococcal disease. Quantitative PCR to determine bacterial load has not been broadly implemented, and its clinical value still has yet to be assessed.

New genetic research techniques have also identified host genetic polymorphisms that influence severity and outcome in meningococcal disease. A clear association between mortality and meningococcal disease was found for polymorphisms in the IL-1 (IL-1), IL-1 receptor antagonist (IL-1RA) genes (figure 1) [5–7, 11–13]. For these genes, 8 different polymorphisms have been described in 6 studies, which included a total of 2534 patients. The median mortality rate among patients included in these studies was 7% (range, 1%–19%). Diagnosis was microbiologically confirmed in 4 of 6 studies; 2 studies also included patients with a clinical picture of meningococcal disease without microbiological confirmation. We performed a meta-analysis of available studies and reported that the polymorphisms PAI 4G/5G, IL1-RA +2018C/T, and IL-1B -511C/T were related to death, with ORs of 2.3 (95% CI, 1.5–3.5), 1.9 (95% CI, 1.3–2.9), and 1.8 (95% CI, 1.1–3.1), respectively.

In this issue of Clinical Infectious Diseases, Darton et al. [14] go one step beyond and include both host and pathogen factors in a stratified analysis. With use of a subset of patients detected by excellent surveillance of the Meningococcal Reference Unit for England and Wales, they correlated bacterial DNA load, host factors (including genetic polymorphisms), and bacterial serogroup with clinical outcome. During a 3-year period, 1910 patients received a diagnosis of meningococcal disease, and 1645 had positive PCR results. The bacterial load was successfully determined in 1045 patients’ samples, and complete data were available for 504 patients. Of these patients, 327 had samples obtained ≤48 h after hospital admission and were included in a multivariate analysis. A high bacterial load was associated with an increase in the mortality rate (OR, 2.04 per 1–log10 copy/mL increase in the bacterial load). Other important factors determining outcome were age, serogroup C, and presence of the IL-1RN +2018C/T polymorphism. With this study, Darton et al. [14] have shown the direction in which genetic research of meningococcal disease
should be going, and they should be applauded for that.

Nonetheless, “one step beyond” may not be completely straightforward. The study by Darton et al. [14] has many methodological shortcomings common to genetic case-control studies. The interaction between host and bacterial genotype is complex, and much larger numbers of patients are needed to assess the influence of all factors on prognosis. No power analysis was performed by the authors. Of 1910 samples that had been obtained, quantitative PCR was performed for 1645; 248 were not quantifiable, and 352 were not available, leaving 1045 samples. Complete data were available for 504, of which only 327 were included in the analysis. Thus, only a small portion of the available samples could be included in the final analysis. It is also unclear whether this selected group of patients was representative for the cohort. Second, the study included patients whose blood samples were received by the Meningococcal Reference Unit during the period 1999–2001, but clinical data were retrospectively collected using questionnaires 1–3 years later, potentially leading to recall bias. As a result, limited clinical data were collected, leaving potential important clinical characteristics unassessed. Previous studies revealed a clear relation between disease type (sepsis vs. meningitis) and signs of systemic compromise on outcome [8]. Therefore, the interaction of the bacterial characteristics and both clinical and genetic features of the patients can not be determined in the current study. Finally, the performed analysis does not take into account whether the variables are effect mediators or confounders.

A good genetic association study needs a clear definition of cases, microbiological confirmation, careful selection of controls, and a sample size and power calculation. Control subjects should be selected from the same source population as case patients, to be representative. For susceptibility research, control subjects should be equivalently exposed to nasopharyngeal carriage. Detailed phenotypic and severity information needs to be collected if genetic influence on severity and outcome are to be assessed. Furthermore, genotyping accuracy should be stated, and quality control measures (e.g., internal validation, test failure rate, blinding of laboratory personnel, and concurrence with the Hardy-Weinberg equilibrium of the control population) should be specified [15]. Relevant factors should be integrated into a statistical effect model to see whether possible prognostic factors are really effect modifiers or confounders. Group stratification with the use of two $2 \times 2$ tables and consecutive testing for the ORs’ homogeneity or a logistic regression model with multiple variable interaction are ways of detecting true associations.

Future research in meningococcal dis-

Figure 1. Meta-analysis of applicable genetic association studies of the outcome in meningococcal disease. The meta-analysis was performed with the Mantel-Haenszel fixed-effect model [15]. ORs and 95% CIs are shown. Values $<1.0$ indicate decreased mortality, and values $>1.0$ indicate increased mortality.
ease is likely to determine which host and bacterial factors are important in susceptibility and outcome. Recent research related to tuberculous meningitis has shown evidence of this [16]. Results will lead to new insights in pathophysiology and improved understanding of meningococcal infection. This can lead to the development of new adjunctive therapies, resulting in decreased mortality. Research groups should work together, because unraveling the interaction between host and bacterial genetic factors will require large numbers of patients and controls [15]. Data from negative studies should either be published or gathered on an internet-base register to prevent publication bias. Pooled analyses of available gene–disease association studies are preferable to meta-analyses, because they compare data instead of results. However, because a pooled analysis can be time consuming and expensive, it is not always better, per se, than standard meta-analysis. Biobanks should be established to collect DNA and clinical data from various study group. In the near future, however, the most important benefit will come from vaccination, which can prevent outbreaks of infection in areas of endemicity. Nevertheless, genetic research will be important in determining the future of the meningococcus: here we go!

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References