Reviewer's report

**Title:** Toll-like receptor 9 polymorphisms are associated with severity variables in a cohort of meningococcal meningitis survivors

**Version:** 4  **Date:** 4 January 2012

**Reviewer:** Uwe Koedel

**Reviewer's report:**

The authors investigated the genotype frequencies of two TLR9 single nucleotide polymorphisms (SNPs) and their association with 13 clinical variables in 390 children who survived meningococcal meningitis (MM). The TLR9 -1237 TC and CC allele were found to be associated with a decreased incidence of a positive blood culture as well as an increased cerebrospinal fluid (CSF) pleocytosis. The same holds true for the TLR9 +2848 AA mutant allele. All the other variables (e.g., hearing loss, ICU admission, vigilance level, and presence of convulsions at admission) did not differ between children carrying different alleles. These data suggest an important (albeit minor) role of TLR9 SNPs in the host defense against meningococcal infection of the CSF and are in line with recent findings from this group and others that showed [i] a reduced susceptibility to MM in children carrying the TLR9 +2848 A allele (Sanders et al., Clin Infect Dis 2011:52, 475-80) and [ii] an involvement of TLR9 in immunological recognition of Neisseria meningitidis in vitro and in vivo (Mogensen TH et al., J Leuko Biol 2006: 80, 267-77 and Sjolinder H et al., Infect Immun. 2008: 76:5421-8).

In general, the manuscript is well written and clearly structured. The data are interesting and provide further evidence for a role of TLR9 in the immune response to meningococcal infection in humans. A few alterations would improve the manuscript.

**Major Compulsory Revisions:**

[1] Recent experimental work has provided substantial evidence that [i] in meningitis, pathogens migrate from the blood to the CSF and vice versa, and [ii] high bacterial concentrations are key determinants for the development of both meningitis and secondary bacteremia. Accordingly, a protective effect of the TLR9 SNPs against primary meningococcemia - a prerequisite for the meningeal invasion, as stated by the authors – should result in a reduced frequency of meningitis (as previously shown by the authors) and/or a mild course of meningitis. The latter could be expected since the degree of CSF pleocytosis depends among others on the infectious dose. In my opinion, it seems to be more likely that the decreased incidence of positive blood cultures in children carrying TLR9 -1237 C and TLR9 +2848 AA alleles may represent a reduction in the occurrence of secondary bacteremia due to more pronounced (and efficient) host immune response in the CSF. The authors should include a discussion of this topic in their manuscript. Moreover, the authors could test for associations between the presence of positive blood cultures and both, the duration of clinical
illness and the start of antibiotic therapy before admission.

[2] The author did not detect any association between the TLR9 SNPs and important clinical severity variables including postmeningitic hearing loss, presence of convulsions at admission, and ICU admission. However, important severity measures are lacking, namely the length of the hospital stay and neuropsychological impairments. In previous studies (Koomen I et al., Acta Pediatr 2003 and Developmental Medicine & Child Neurology 2004), van Furth’s group investigated the frequency of neuropsychological sequelae in survivors of MM. There is a considerable overlap between the patient cohorts of this and the previous studies. Therefore, I tend to think that it should be doable to include these measures into the actual association analysis.

[3] The authors mention the results of a so-called in silico regulatory SNP detection method in the Discussion section. This method was already used and described in detail in their previous publication in Clin Infect Dis. The authors should either delete the figure or, if able to provide new information, describe the method and results in the respective sections.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests'