Reviewer’s report

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

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Reviewer: Marieke Emonts

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Review of
Toll-like receptor 9 polymorphisms are associated with severity variables in a cohort of meningococcal meningitis survivors
MS Sanders et al.

Major Comments

1. In the abstract the authors state that they relate TLR9 polymorphisms to predictors of death and severity in survivors. Studying death predictors in a cohort of survivors will give a skewed picture. In the methods section it is however only stated that these are severity markers, while nothing is stated about mortality.

2. Why are continuous variables dichotomized? This will result in a loss of statistical power. In the results section, the continuous outcome variables are presented as both continuous and dichotomised. Advise to limit this to continuous variables. (this also limits the number of tests, added to the already 13 for both SNPs). Why use two dichotomous variables for CSF leukocyte count limits 600 and 1000)?

3. A p value of <0.05 is considered significant. However, 13 outcome variables were tested. A correction for multiple testing is in place, which would result in no remaining significant results.

4. Genotype distributions were checked for deviations of HWE. In a previous study the TLR9 2848 polymorphism was observed to be associated with susceptibility to MM in this cohort. This implies automatically a deviation. Was this checked in a control population?

5. TT is a wild type genotype, not allele (results page 10). Please check use of the terms ‘carrier’, ‘allele’ and ‘genotype’ throughout the text.

6. Table 2. As bacterial meningitis is associated with decrease CSF/blood glucose ratio, it is surprising that the association observed for the TLR9 polymorphism is associated with decreased numbers of positive blood cultures as well as a decreased CSF/blood glucose ratio. Please explain.

7. What is the effect of not only increased (in silico) binding of TLR9 -1237 C variant to NF#B, but also to RelA, and STAT3? In other words: what do you expect when not NF#B binds, but preferably RelA or STAT3?
8. The authors state that (page 14) TLR9 polymorphisms have a small but important contribution to warrant the balance between beneficiary and injurious effects of inflammation in the CNS. This was not shown from the results. I would then have expected an association with neurological outcome, which was not demonstrated.

9. Conclusions: The remark that these SNPs result in a lower chance of developing meningitis when colonized with N. meningitidis should be limited to TLR9 +2824 SNP. (9page 16).

Minor comments
1. Where all individuals CSF cultures positive for N. Meningitides?
2. CRP > 100 is considered to be related to more severe disease. This is in contrast to meningococcal sepsis in which a low CRP at presentation is associated with more severe disease and mortality.
3. Surprisingly CRP is missing for many of the individuals while it is usually a parameter that is checked in ill children. Can you please explain?
4. Table 3: please describe TLR9 haplotypes, reference only is not sufficient.
5. Discussion page 13 and 14: The authors suggest a protective effect of TLR9 polymorphism, preventing bacteremia and increase leukocyte influx in the CNS. Did the individuuals with positive bloodculture but no clinical signs of sepsis have increased CNS leukocyte influx compared to the patients with both positive bloodculture and clinical signs of sepsis?

Minor issues not for publication
1. Both in the abstract and methods section (page 8) CSF/blood glucose ratio instead of CSF blood/glucose ratio.
2. How was controlled for pre-existing hearing loss?
3. Reference numbers are listed from 1-21 and then 0-3, please adjust.
4. Table 1 CSF leukocytes (/ µL or µL-1)
5. Rephrase sentence page 13: TLR9 -1237 was....TLR9 -1237 C variant (figure 4). Now it says: TLR9 -1237 was associated with significantly increased binging of NF#B, RelA, and STAT3 to the TLR9 -1237 C variant (figure 4). Do you mean: The TLR9 -1237 C variant was associated with significantly increased binging of NF#B, RelA, and STAT3 (figure 4). ?
6. Suggest to omit statement on Public health genomics, bottom page 14. It does not add to the content of the manuscript.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published
**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I have worked with some of the authors on this paper. This was however in no way related to the work presented here, nor does it influence my review on this paper.