Reviewer's report

Title: Toll-Like Receptor Gene Polymorphism is Associated with Susceptibility to Graves' Ophthalmopathy in Taiwan Males

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Reviewer: Holger Kirsten

Reviewer's report:

The authors investigate association of SNPs and haplotypes in TLR4 and TLR9 with Grave’s disease (GD) and Grave’s Ophthalmopathy (GO). They compare allele frequencies of GD-patients with reported frequencies from the literature and allele and haplotype frequencies of GD patients with GO with GD patients without GO. The authors report nominal association (not corrected for multiple testing) for SNPs and haplotypes of the gene TLR-9 in the subgroup of males having GD and GO. They provide additional evidence why the association might be found in males only.

Major Compulsory Revisions:

There is a discrepancy between the abstract and the manuscript: In the abstract, the authors report that the aim of the work is identifying genetic markers for GO, whereas in the manuscript they write that the aim is to identify an association of SNPs with GD. Within the manuscript, they do actually both, finding no association when comparing GD with controls and a nominal allelic association in a subgroup of patients having GO and GD. The authors should clarify the aim.

The authors show information, how phenotypic parameters are distributed between Males and Females in Table 1. However, as they found association TLR-SNPs in male GD-patients with GO compared with male GD-patients without GO it is also very important to show how phenotypic parameters are distributed between these two groups. As the authors quantified proptosis, they should quantify proptosis in this table.

In addition, known information, how other anatomic sites were affected (as shortly mentioned in Material and Methods) should be added.

The authors should provide a power calculation for the study (incl. subgroup analysis) and compare the detectable effect sizes with effect sizes of TLR4 and TLR9 SNPs previously found in other studies.

As proptosis was quantified, the authors should perform a genetic association analysis using proptosis as quantitative trait. In this analysis, the authors can also quantify, how much of this quantitative trait is explained by the analyzed SNPs in males.

The authors should comment on correcting p-values. This has implications on the conclusion of the study. They conclude: “In conclusion, our findings showed that
TLR-9 gene polymorphisms were significantly associated with susceptibility to GO in the male GD patients in Taiwan.” As they did not correct for multiple testing, this conclusion is much to strong for the data they provide.

As the authors performed case-control analysis using published allele frequencies, they should 1. Provide references which papers they used from PubMed, as they only write that frequencies are extracted from PubMed 2. Discuss issues of ethnical confounding. 3. Discuss how many unknown cases of GD/GO might be included in this control cohort.

Minor Essential Revisions:

It would be of value for the reader to provide a number and a reference to an estimate of the genetic contribution to Graves' disease / Graves' ophthalmopathy.

The author should comment on reported genetic association with GO.

In introduction, the authors report that TLRs have been previously associated with autoimmune thyroiditis. They should provide a reference to this.

The authors provide report on 11 human TLRs. In one of the given reference, O'Neil LA, Immunol Rev. 2008 Dec;226:10-8. only 10 are mentioned. Unfortunately, I do not have access to the second reference. As I am aware of only 10 expressed human TLRs I would ask to check this number.

The authors should provide how they did SNP -tagging - which parameter did they use? How efficient was the tagging compared with known Hapmap-variants?

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

**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