Author's response to reviews

**Title:** The rs1990760 polymorphism within the IFIH1 locus is not associated with Graves' disease, Hashimoto's thyroiditis and Addison's disease

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**Version:** 2  **Date:** 28 September 2009

**Author's response to reviews:** see over
To the EDITOR IN CHIEF OF
*BMC Medical Genetics*
Dr. Melissa Norton

Dear editor,

Please find enclosed our revised manuscript entitled “The rs1990760 polymorphism within the IFIH1 locus is not associated with Graves’ disease, Hashimoto’s thyroiditis and Addison’s disease”. We think that the thorough revision according to the advises of the reviewer lead to a significant improvement of our manuscript. In the following you will find our reply to the reviewer’s comments.

I look forward to your favorable consideration.

Sincerely yours,

Marissa Penna-Martinez, Ph.D.
BMC Medical Genetics - Decision on Manuscript ID 9519174882933291

Title: The rs1990760 polymorphism within the IFIH1 locus is not associated with Graves' disease, Hashimoto's thyroiditis and Addison's disease

Authors: Marissa Penna-Martinez, Elizabeth Ramos-Lopez, Inka Robbers, Heinrich Kahles, Stefanie Hahner, Holger Willenberg, Nicole Reisch, Christian Seidl, Maria Segni and Klaus Badenhoop:

We thank Ajda Bicek for the suggestions to improve our manuscript

Comments and answers

Minor comments:

1) Penna-Martinez et al report on no significant differences comparing allele and genotype frequencies in patients and controls. They could not confirm association between endocrine autoimmune diseases and IFIH1 polymorphism as it was found in previous study (Sutherland et al., 2007). Authors report that IFIH1 distribution in controls was not different from previous published results (Sutherland et al., 2007). Maybe they should mention comparison of their allele and genotype frequencies in patients with other published frequencies in patients in similar populations. Not only size of sample even change in allele frequency in patients could be the reason for lack of association.

We agree with the reviewer that the size of our sample is not the only reason for the lack of association. Studies for example dealing with IFIH1 polymorphism in type 1 diabetes (T1D) in Georgia and Colorado Causacian populations showed different results (Liu et al.2009). While in the Georgia population the allele A of IFIH1 polymorphism in T1D patients (66.7%) is more frequent compared to the controls (60.4%), in the Colorado population no difference was observed (T1D: allele A 63.3 %, control: Allele A 60.8%). On the basis of this finding other explanation for the lack of the association at rs1990760 with the investigated autoimmune disease could be possible:

1) The rs1990760 polymorphism is in linkage disequilibrium (LD) with other IFIH1 variants for instance rs2068330, rs2111485 and rs984971 and could be different due to population heterogeneity. Therefore the different patterns of LD across studies and populations could explain the discrepancy of the results.

This comment is now included in the discussion (page 10, line 9)

2) Although they performed analysis of stratifying polymorphism rs1990760 IFIH1 for different parameters, they do not give the exact results. Even if results are not significant they would be appreciated.
It would be helpful to describe formation of subgroups for stratifying analysis and discuss how sample/subgroup size could affect analysis. For instance, when patients were divided into subgroups (strata) based on factors (gender, HLA marker and antibody production) that are thought to be related to development of disease, subgroups may became rather small in size to detect association with polymorphism (19 men with Hashimoto’s thyroiditis and 51 men with Graves’ disease further divided in two subgroups regarding the HLA marker?).

In order to evaluate the influence of the determined HLA-marker on IFIH1 polymorphism in patients with GD HT and AD, patients were grouped according to patients which carry the human leukocyte antigen (HLA) risk haplotypes DQ2 (DQA*0501-DQB*0201) DQ8 (DQA*0301-DQB*0302) and patients which carry non-risk haplotypes (neither DQ2 nor DQ8)] and compared to their corresponding control group. 51 males and 207 females in GD, the 19 males and 87 females in HT as well as 57 males and 138 females in AD were again compared separately to their respective controls regarding the HLA marker but no difference was observed (data not shown) Also the 51 males with GD and the 19 males with HT were compared together however we obtained no significant difference.

We agree with this reviewer that an explanation of group formation was necessary. The group formation is now explained in the results section (page 7, line 6 and page 8, line 4).

Also the corrected p (pc) values are now presented in all tables.

3) Authors should check cited link:
http://www.rfcgr.mrc.ac.uk/~fdudbrid/software/unphased/
we check the address and change to
http://www.rfcgr.mrc.ac.uk/~fdudbrid/software/unphased/.

4) Method of correction of p value in Tables 2 and 3 should be described.

The correction of the results was calculated using the Bonferroni-correction. This is now described in the statistic section (page 7, line 2).
BMC Medical Genetics - Decision on Manuscript ID 9519174882933291

Title: The rs1990760 polymorphism within the IFIH1 locus is not associated with Graves' disease, Hashimoto's thyroiditis and Addison's disease

Authors: Marissa Penna-Martinez, Elizabeth Ramos-Lopez, Inka Robbers, Heinrich Kahles, Stefanie Hahner, Holger Willenberg, Nicole Reisch, Christian Seidl, Maria Segni and Klaus Badenhoop:

Comments and answers

Reviewer: yongju zhao

We thank the reviewer yongju zhao for the feedback and look forward to the consideration of our manuscript for publication.

- Minor Essential Revisions
The author can be trusted to make these. For example, missing labels on figures, the wrong use of a term, spelling mistakes.

1. Key words: IFIH1 is just abbreviation of “interferon induced helicase”. They shouldn’t be regarded as two key words.
interferon induced helicase was deleted from the key words (see old title page)

2. In the abstract, 54 GD families included 162 individuals, but in method, comprising 177 subjects, why?
Thank you very much for the precise observation
We change the number of the individuals in method from 177 to 162. However, after revision of the family number, the family number was corrected to 55 and thus the individuals number reach 165. Family number in table 3 (see file: result Table 3 deleted) was corrected in the legend. The number of individuals was changed to 165 in abstract (page 2, line 9) and subjects/methods (page 5, line 3, line 4, line 11).

3. The discussion: abbreviation of “interferon induced helicase” is IFIH1, but it had been rewritten as “IFH1” in some place.
we replace IFH1 to IFIH1

“The association between IFIH1 rs1990760 polymorphism and T1D was not only confirmed by other authors but also reported in other autoimmune diseases including Grave’s disease.” this sentence should be corrected . Addison disease don’t should be included in ATD (autoimmune thyroid disease).
We corrected this sentence in the discussion (page 9, line 4) to “the association between IFIH1 rs1990760 polymorphism and T1D was not only confirmed by other authors but also reported in other autoimmune diseases”.

4. Tables 2 and 3: corrected p values should be in tables.
- Discretionary Revisions
The corrected p (pc) values are now given in the tables.

These are recommendations for improvement which the author can choose to ignore. For example clarifications, data that would be useful but not essential.

1. The allele frequencies in GD patients were different from that in affected offspring of GD families, why not analyze them together.

Exactly, the allele frequencies in the GD patients are different from that in affected offspring of GD families because the GD patient group is composed of GD affected members of the GD family (for example the 55 affected, table 3) and other GD patients without samples of a family.

2. In some other studies, allele A increased the risk of Graves’ disease. In this manuscript, the German GD families showed that parents and preferentially mothers with the haplotype DQ2/DQ8 tended to overtransmitted the allele G to affected offspring, and in group 4 of HT family, overtransmitted the allele A of rs1990760 IFIH1 to their HT affected offspring. Authors can give their apprehension in discussion.

Since only DQ2 but not DQ8 influence the susceptibility for development of Graves’ disease and Hashimoto thyroiditis we performed a new transmission analysis for the two diseases and no statistical significance in transmission from IFIH1 depended on risk haplotype DQ2 and non risk haplotype (neither DQ2) was found. We estimate that the analysis in table 3 is not specific therefore the table 3 was deleted from the result section. Following sections were changed: abstract (page 2, line 10, line 18), Background (page 4, line 21), statistical analysis (page 6, line 6), results (page 7, line 6 and page 8, line 7).

The sentence “The group 4 of HT family, overtransmitted the allele A of rs1990760 IFIH1 to their HT affected offspring” is now shown in the discussion (page 9, line 24).

3. In this manuscript, the distribution of rs1990760 polymorphism in controls was different from that in UK controls. The Hardy-Weinburg balance should be proved in selected controls.

The Hardy–Weinberg equilibrium (HWE) was tested separately in cases and controls using a chi-square test. A threshold of p < 0.05 was used to indicate departure from HWE. The Allele and genotype frequencies for IFIH1 rs1990760 polymorphism were in HWE in cases (GD p(HWG) = 0.7, Ha p(HWG) = 0.7, AD p(HWG) = 0.8 and controls (p(HWG) = 0.8).

We include the following sentence in the results section “Allele and genotype frequencies for IFIH1 rs1990760 polymorphism in all subjects did not deviate from Hardy-Weinberg equilibrium (HWE; p > 0.05) (page 7, line 1).
4. In table 3, why the number about mothers or fathers were 49?
This discrepancy results from the fact that only the informative parents can be used for the analysis. This means that those families in which all members are heterozygous could not be included for the evaluation, because a clear assignment of transmission is not possible. Therefore usually a lower number of women/men than the whole family number were used for the separate analysis.
The rs1990760 polymorphism within the IFIH1 locus is not associated with Graves' disease, Hashimoto's thyroiditis and Addison's disease


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Short title: IFIH1 rs1990760 polymorphism

Keywords: IFIH1, rs1990760 polymorphism, Graves' disease, Hashimoto's thyroiditis and Addison's disease
Table 3: Transmission (T) and non transmission (NT) of IFIH1 rs1990760 polymorphism from parents to offspring according to human leukocyte antigen (HLA) haplotype in 55 Graves’ disease families

<table>
<thead>
<tr>
<th></th>
<th>Allele</th>
<th>F (%)</th>
<th>T (%)</th>
<th>NT (%)</th>
<th>p</th>
<th>pc</th>
<th>Allele</th>
<th>F (%)</th>
<th>T (%)</th>
<th>NT (%)</th>
<th>p</th>
<th>pc</th>
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<tr>
<td>Non-HLA high-risk DQ x/x (n = 21)</td>
<td>A</td>
<td>63.1</td>
<td>25 (47.2)</td>
<td>28 (52.8)</td>
<td>0.50</td>
<td>1.0</td>
<td>A</td>
<td>69.1</td>
<td>42 (44.7)</td>
<td>52 (55.3)</td>
<td>0.06</td>
<td>0.12</td>
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<tr>
<td></td>
<td>G</td>
<td>36.9</td>
<td>17 (54.8)</td>
<td>14 (45.2)</td>
<td></td>
<td></td>
<td>G</td>
<td>30.9</td>
<td>26 (61.9)</td>
<td>16 (38.1)</td>
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<tr>
<td>HLA high-risk DQ2/DQ8 (n = 34)</td>
<td>A</td>
<td>69.1</td>
<td>42 (44.7)</td>
<td>52 (55.3)</td>
<td>0.06</td>
<td>0.12</td>
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<td>Non-HLA high-risk DQ x/x (n = 19)</td>
<td>A</td>
<td>57.9</td>
<td>10 (45.5)</td>
<td>12 (54.5)</td>
<td>0.50</td>
<td>1.0</td>
<td>A</td>
<td>73.3</td>
<td>19 (43.2)</td>
<td>25 (56.8)</td>
<td>0.08</td>
<td>0.16</td>
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<tr>
<td></td>
<td>G</td>
<td>42.1</td>
<td>9 (56.3)</td>
<td>7 (43.8)</td>
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<td></td>
<td>G</td>
<td>26.7</td>
<td>11 (68.8)</td>
<td>5 (31.3)</td>
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<tr>
<td>HLA high-risk DQ2/DQ8 (n = 30)</td>
<td>A</td>
<td>73.3</td>
<td>19 (43.2)</td>
<td>25 (56.8)</td>
<td>0.08</td>
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<tr>
<td>Non-HLA high-risk DQ x/x (n = 19)</td>
<td>A</td>
<td>71.1</td>
<td>13 (48.1)</td>
<td>14 (51.9)</td>
<td>0.7</td>
<td>1.4</td>
<td>A</td>
<td>70.0</td>
<td>19 (45.2)</td>
<td>23 (54.8)</td>
<td>0.25</td>
<td>0.5</td>
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<td></td>
<td>G</td>
<td>28.9</td>
<td>6 (54.5)</td>
<td>5 (45.5)</td>
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<td></td>
<td>G</td>
<td>30.0</td>
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</table>

DQ2/DQ8 = DQ2 (DQA1*0501-DQB1*0201) and DQ8 (DQA1*0301-DQB1*0302) highest risk group
DQx/x = Neither DQ2 (DQA1*0501-DQB1*0201) nor DQ8 (DQA1*0301-DQB1*0302) low risk group

F = Frequency

pc = p corrected

*OR = odds ratio; (95% CI) = 95% confidence interval.
parents: allele G OR = 2.01 (0.96-4.23) and allele A OR = 0.5 (0.24-1.05)
p = 0.06
mother: allele G OR 2.89 (0.86-9.74) and allele A OR 0.35 (0.1-1.16)
p = 0.08