Author's response to reviews

Title: Hypertension after preeclampsia and relation to the C1114G polymorphism (rs4606) in RGS2: data from the Norwegian HUNT2 study

Authors:

Anne Stine Kvehaugen (akvehaugen@yahoo.no)
Øyvind Melien (oyvind.melien@helsedir.no)
Oddgeir L Holmen (oddgeir.linaas.holmen@gmail.com)
Hannele Laivuori (hannele.laivuori@helsinki.fi)
Ralf Dechend (ralf.dechend@charite.de)
Anne Cathrine Staff (UXNNAF@ous-hf.no)

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Author's response to reviews: see over
Dear Editors,

we hereby submit our revised research article entitled “Hypertension after preeclampsia and relation to the C1114G polymorphism (rs4606) in RGS2: data from the Norwegian HUNT2 study” by Anne Stine Kvehaugen, Øyvind Melien, Oddgeir L. Holmen, Hannele Laivuori, Ralf Dechend and Anne Cathrine Staff.

We appreciate the positive and thorough review of our paper. The reviewers’ comments and our responses are given in the following. The revisions in the manuscript and tables are shown as “visible differences”.

Reviewer 1.

Reviewer's report:

The study submitted by Kvehaugen et al is interesting and focuses on an important association between preeclampsia and later hypertension that previously has been investigated in depth only in few studies. Especially interesting is the aim to investigate how the different factors preeclampsia and rs4606 polymorphisms affect later risk of hypertension. The main strengths are the population-based design and the high number of subjects in subgroups, as well as the very good research question that is described in the introduction.

Major compulsory revisions

1. My main concern is that the statistical analyses are presented in a somewhat disorganized manner and may have been overinterpreted. My comments 2-4 go in more depth on this comment.

2. The authors use several different endpoints (systolic blood pressure above 140/160 and/or use of antihypertensive medications or blood pressure above 140/90 or 160/100 and/or use of antihypertensive medications). The paper would benefit from a clearer strategy on what endpoint that actually was used in the abstract, in tables and in the conclusion.

3. The findings in Table 2 that the rs4606 polymorphisms neither were associated with later hypertension in the control nor in the preeclamptic women, leave me wondering whether the findings in Table 3 are based on large enough numbers of women or whether these findings are mainly coincidental. Supporting my skepticism is the impression that the endpoint used in Table 3 was one third as common as the endpoint in Table 2 (based on numbers reported in Table 1 for number of subjects with systolic blood pressure above 160 vs 140 mmHg). When results for Table 2 were presented, low numbers in subgroups were used as a reason for not finding statistical significance. Adjusted analyses in general demand larger numbers in subgroups, the results in Table 3 are however based on smaller numbers. For the main results in Table 3, the authors should present number of subjects in subgroups (for example how many women with preeclampsia had hypertension and the different genotypes) and unadjusted results (or minimally adjusted for current age only) should be presented before the adjusted results. An argument for minimally adjusted analyses with adjustments...
for current age (and possibly also BMI) could be made, as these variables are clear confounders of any association with hypertension. I appreciate the authors’ argument on lines 302-304 on top of page 14 for the multivariate analysis with preeclampsia and genotype in the same model as the main analysis, but unadjusted (or minimally adjusted analyses) should also be presented in tables.

4. The authors have chosen to focus on severe hypertension (defined as systolic blood pressure >160 and/or use of antihypertensive medications) in the abstract and conclusions. The negative findings for the normal definition of hypertension (blood pressure >140/90 and/or use of antihypertensive medications) are more briefly commented upon. In my opinion, the authors should focus on the results for the normal definition of hypertension and that these results should be presented in a table similar to Table 3 (but including N’s). I appreciate the thorough description in the results text, but these results should be presented in a table. Results for severe hypertension should be presented in addition.

Authors’ response:

Comments 1-4: We agree that the statistical analyses and the results may appear somewhat disorganized. We recognize that the results in Table 2 may be easily misinterpreted (see our response to the comment by reviewer 3 below), and we have therefore now presented the results, both unadjusted and minimally adjusted, for the “normal” definition of hypertension (blood pressure ≥140/90 mmHg and/or use of antihypertensive drugs) in a revised, and comprehensive, Table 2.

The reviewer is correctly commenting on the negative findings for an association between the normal definition of hypertension and the polymorphism in the preeclampsia group and the control group separately. However, in the total study population (history of preeclampsia + control group), minimally adjusted analyses show a significant association between the normal definition of hypertension and the GG genotype. These results were previously given in the text, but are now presented in Table 2, and therefore easier to compare with the other findings. This association is also marginally significant for the group of women with a history of preeclampsia, but findings persist also when adjusting for a history of preeclampsia (see the revised manuscript with visible alterations on page 11, lines 245-248).

The association between the polymorphism and the hypertensive endpoint used in Table 3 (systolic blood pressure ≥160 mmHg and/or use of antihypertensive drugs) is based on the total study population (total number of subjects included in the regression analyses are now given in Table 3, whereas the number of affected women are rather given in text to avoid a too comprehensive table. Thus, total number of included women, and number of affected women, is higher than for the subgroups of preeclampsia (i.e. early-onset preeclampsia) given in Table 2. Although this hypertension endpoint is “more severe”, reducing the number of affected women in our HUNT-based study as compared to the normal definition of hypertension (n=159 (16.8%) vs. n=352 (37.7%) in the history of preeclampsia group and n=170 (8.3%) vs. n=436 (21.7%) in the group of women with previous normotensive pregnancy), and thereby reducing statistical power, we cannot exclude a true relationship between the genotype and more severe form of hypertension. The blood pressure reading in the HUNT2 study is based on readings at one study time point only. It is not regular clinical practice to suggest starting antihypertensive medication following registration of one (or several during same consultation) elevated blood pressure of 140-160 mmHg in systolic BP. As a routine, such a patient would be called back for control of BP; as it may vary over days and even represent a “white collar” hypertension. Therefore, we believe a cut-off of ≥140/90 mmHg may represent a less reliable measure of “true hypertension” than the more severely elevated blood pressure of systolic BP ≥160 mmHg. Our study
could suggest that the polymorphism may be more strongly associated with a more severely elevated blood pressure as compared to milder forms of hypertension. However, for the endpoint used in Table 3, we found a significant relationship to the polymorphism only after multivariate adjustment, including adjustment for physical activity. The negative finding between the polymorphism and the outcome in Table 3 in unadjusted analysis was previously described in the text. However, following the reviewer’s suggestion, the unadjusted and minimally adjusted results are now given in Table 3. If the editor finds the present Table too comprehensive, we can readjust and simplify the Table 3 by presenting the information in the text, as previously.

As to comment #4, we have now altered the abstract and conclusion, in line with the comment, with a stronger focus on the “normal” definition of hypertension.

Minor essential revisions 1. The discussion section is very long with 6 pages. It would benefit from shortening with a stronger focus on the main findings.

**Authors’ response:** We believe that most of the discussion elements are important for other researchers that could test the genotype in their population in order to test whether the association to hypertension remains across populations. Our discussion might facilitate the comparison of population characteristics and to consider stronger and weaker parts of the various study designs. We have however deleted some lines both in the results and the discussion part, following the recommendation of the reviewer.

Discretionary revisions 1. The authors use 34 weeks as cut-off for early-onset preeclampsia in Table 2 but very few women in this subgroup limit further analyses. I would suggest attempting 37 weeks as cut-off instead.

**Authors’ response:**

Although pre-term delivery is clinically defined as delivery prior to gestational week 37, the cut-off of 34 weeks gestation is used in order to distinguish better between the more severe and less severe forms of preeclampsia (PE). A cutoff of 34 weeks is more reliably defining the women with highest risk of remote cardiovascular disease; namely the group of women with early-onset PE (and delivered before week 34), as we have reviewed previously (1). Also, a recurrent PE is more common for this early-onset PE group. Our pathophysiological concepts are limited, but it is currently believed that the early-onset group of PE represents the group of dysfunctional placenta remodeling, whereas the late onset PE group is more often representing the “maternal type” of Preeclampsia, although many cases may be a mix of the two.

We therefore prefer to keep this classification of PE sub-groups (34 weeks as the cut-off); as do many other studies (And week 34 also is clinically in line with the cut-off for need of steroidtherapy to the mother in order to promote fetal lung maturation, in cases of premature delivery before week 34.

5. The authors do not discuss the significance of which pregnancy was affected by preeclampsia. Preeclampsia in the first pregnancy is more common than in later pregnancies, and preeclampsia in later pregnancies may be more difficult to separate from chronic hypertension or renal disease as well as more strongly associated with later outcomes. The proportion of women with a preeclampsia in first vs later pregnancies should be given, and the association of preeclampsia with later hypertension should be tested separately in women with preeclampsia in first vs later pregnancies.
Authors’ response:

We agree that preeclampsia in later pregnancies may be more difficult to separate from chronic hypertension or renal disease in general retrospective studies. However, our exclusion criteria means that no women that is registered in the MBRN with any pre-pregnancy registered chronic hypertension, renal disease, diabetes mellitus (or GDM) or heart disease were included in our study, and thus not genotyped. We have therefore included a very healthy selected group in our analyses. As we have argued in the discussion section, this selection might actually have reduced the strength of the association between the genotype and hypertension after pregnancy.

6. The abstract should be modified to have a stronger focus on the findings for the normal definition of hypertension to give a more balanced presentation of the findings

Authors’ response: The abstract is now revised, according to the comment. Please see also our responses above to comments 1-4.

Reviewer 2.

Reviewer's report

Kvehaugen et al present data that associates the C1114G rs4606 polymorphism in RGS2 with later hypertension in women from a Norwegian study. This paper extends their previous observation of a higher incidence of the GG and CG polymorphism in women with preeclampsia. In the current study the differences in genotype between groups relates to a 4-5% drop in CC carriers with a matched increase in CG carriers in the preeclampsia group. Interestingly, the relevance to higher blood pressure seems to be largely in the GG carriers. An association is reported between severe hypertension and the GG polymorphism, independent of a history of preeclampsia, and that the association between preeclampsia and hypertension is independent of the polymorphism. It strikes me the key message is of an association between this polymorphism and the later development of severe hypertension independent of pregnancy history or a history of preeclampsia? To strengthen the paper I think it would be useful to consider the following.

Discretionary revisions

1. The title could be adjusted to reflect the concept that the association between polymorphism and blood pressure is independent of pregnancy history or that preeclampsia is associated with higher blood pressure independent of the polymorphism.

Authors’ response:

We agree that the title can be adjusted, but we would like to emphasize that the study is not primarily studying the association between PE and later hypertension (as this is epidemiologically shown before), but assessing association between the genotype and later hypertension in women that were normotensive before pregnancy. We hope the current title alteration is acceptable:

The title is now altered slightly from
2. As the results are informative about genetic associations with blood pressure it will be of value to put the results into the context of results from large scale Hypertension GWAS studies that have been reported in the last few years.

Authors’ response:

Our group, which includes the experienced medical geneticist (and obstetrician) Hannele Laivuori, finds that the results from the GWAS studies so far may not be easy to translate in relation to our findings. Also, as it has been commented on above, the discussion is already too long, therefore we hope that it is acceptable not go into this broad GWAS field in the current paper.

3. The section on interaction with physical activity is interesting but the study group is probably underpowered to do this interaction analysis and the results are lost in the detailed statistical analysis related to pregnancy history. There is only a very brief comment made in the abstract. I wonder whether this interaction with genotype should be removed and perhaps explored in more detail in a separate study with additional data?

Authors’ response:

We agree that the low registered numbers of physically active women with hypertension within each of the genotype groups weakens the reliability of the results. However, we find this interaction very interesting, and, as we do not have access to additional data to explore this further, we think this is important to report. By exposing our data, other researchers may in the future investigate this particular issue in larger epidemiological studies or in intervention studies.

Reviewer 3.

Reviewer’s report:

In the paper titled as “Hypertension after preeclampsia in relation to the C1114G polymorphism (rs4606) in RGS2: data from the Norwegian HUNT2 study”, the authors described a significant association between the GG genotype of rs4606 and severe hypertension later in life, defined as systolic blood pressure greater than 160 mmHg. However, the polymorphism was not associated with diastolic hypertension alone. In this study, the question to be addressed was important, the numbers of studied populations were sufficient- 934 cases and 2011 controls, the technology of SNP genotyping was reliable, the methods of statistical analyses were standard, and some associations were significant. However, the summary of results, especially in Table 2, was not clear enough to convince readers.

- Major Compulsory Revisions
The results in Table 2 are the essence of this study. However, I found Table 2 to be confusing to understand. For instance, I expected the sum of % of CC, CG and GG to be 100% for each row of Table 2, but they were not: 65.3% for Controls, 120.6% for preeclampsia... etc.. For each row of this Table, “what was denominator and what was numerator” should be explicitly clear to understand, but they were not. Therefore, the following analyses in this paper using the data of Table 2 lost their credibility.

Authors’ response:

The table gives the prevalence of hypertension among women with the different genotypes. As the table did not present the distribution of genotypes, the sum of % of CC, CG and GG will not be 100% for each row of Table 2. We recognize however, that the results in Table 2 may be easily misinterpreted. We have therefore revised the table to rather include odds ratios with 95% CIs for the risk of hypertension with the CG or GG genotypes as compared to CC genotype. This is now also presented for the total study population in addition to each of the “obstetric history groups”.

All authors have accepted the last revised version of the manuscript, and have contributed to the revision and responses.

We sincerely hope the revised manuscript is acceptable for publication in BMC Medical Genetics.

Kind regards, on behalf of the authors,

Anne Cathrine Staff,
Department of Obstetrics and Department of Gynecology,
Oslo University Hospital, Ulleval, Norway.
UXNNAF@ous-hf.no

Reference List