Author's response to reviews

Title: Genomic NGFB variation and multiple sclerosis in a case control study

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Version: 4 Date: 24 September 2008

Author's response to reviews: see over
Dear Editor:

Please find enclosed the revised version of our manuscript for the article ‘Genomic NGFB variation and multiple sclerosis in a case control study’ as well as the supplementary file ‘Supplemental NGFB rev’ which we would like to submit for publication in ‘BMC Medical Genetics’.

All referee comments have been taken into account as far as ever possible. We hope that you will find everything in order for rapid acceptance and publication. Enclosed please find the comments, addressing points of the referees point by point.

Thank you and your referees for reviewing the manuscript so promptly. We look forward to learning about your decision.

Yours sincerely

Denis A. Akkad

Jörg T. Epplen

Bochum, den 24.09.08
Comments of the reviewers and authors’ answers

Reviewer's report
Title: Genomic NGFB variation and chronification in multiple sclerosis: case control study and expression analyses
Version: 2 Date: 30 August 2008
Reviewer: Daniela Galimberti

Reviewer's report:
Aims of this work are clear and methods sound. Results obtained add a substantial piece of knowledge about gender-related differences influencing the development of MS.

Regarding the association analysis, the population is large enough to get reliable results. However, whereas one SNP (rs6330) is likely a risk factor, another one (rs11102930) seems to act as a protective factor, raising the question whether NGFB overall effect is to increase or decrease the risk of developing MS in male population. This point should be better addressed.

- We thank the expert reviewer for this valuable comment which leads us to revise the manuscript and include minor changes throughout in order to underscore the modulatory properties of NGFB.

In addition, when stratifying genetic data according to MS course, Authors should take into account that patients with RR-MS could develop Secondary Progressive (SP)-MS in the future. To overcome this possibility, patients could be stratified in “bout onset” (RR+SP) and progressive onset (PP). Alternatively, the duration of the disease should be specified for each group, in order to have homogeneous RR and SP populations.

- Certainly, rr MS patients could develop into sp MS cases, both groups combined into “bout onset”. Creating such a cohort may bear the problem of mixing two different phenotypes with potentially different pathogenic processes and thus probably different genetic contributions. Clinical expertise tells that also patients with >15 years of rr course have a risk to develop sp MS. Therefore, we analyzed these MS patient groups as separate entities, especially also since the cohorts were exceptionally well characterized in all clinical aspects of MS.

In addition, I suggest some formal changes in order to help readers to get the key-points of the association analysis:
Table 1 should be split in two smaller Tables, the first regarding rs6330, the second on rs11102930.

- This suggestion of the reviewer is now realized in the manuscript.

P value, together with OR(CI) of these two SNPs, could be added in the text (“Results” section) to be more easily noticed at a first glance by readers.

- The suggestion of the reviewer is included in the manuscript in the appropriate locations.
Table 2 describes negative results, therefore could be deleted and included in the supplementary material.

- The suggestion of the reviewer is adopted in the manuscript by translocating former table 2 into the supplemental file.

Lastly, clinical criteria used for MS diagnosis should be mentioned when describing patients.

- This point of the reviewer is accepted in the revised version of the manuscript by explicitly mentioning the Poser criteria.

Concerning expression analysis, I would suggest more caution regarding conclusions described. First of all, the group of patients analyzed is quite small (23 males and 10 females) and no controls have been included.

- As requested, more caution was exerted in discussing the data.

In addition,
Authors should consider that the following variables could influence results obtained:
- were RR and SP patients having relapses (if any) in an acute or stable phase of the disease at time of sampling?
- It is now mentioned that the patients were in stable phase.
- were patients under treatment with an immunomodulatory agent?
- It is clarified now how many patients of the respective disease courses received treatment.

All these information should be added in the “Methods” section and results discussed on the basis of these data.

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
Reviewer's report

Title: Genomic NGFB variation and chronification in multiple sclerosis: case control study and expression analyses
Version: 2 Date: 11 September 2008
Reviewer: Antonio Alcina

The authors have studied the association of 10 SNPs from the NGFB gene with MS. Two of the SNPs genotyped in 1120 unrelated MS patients and 869 controls resulted in a positive association, the rest typed in 263 rr patients and 259 controls were negative. They also measure the expression of NGFB from PBMC in 23 males and 10 females patients.

1.- The data are interesting for the two SNPs found positively associated, mainly for the rs6330 considering the acceptable size of the cohort. The rs*30 was not associated in the whole MS cohort, though after stratification in sex and MS course it appeared associated with males and rr males. In this last case the sample size decreases so much that the power may be low and I would not draw the conclusions stated in the abstract and discussion.

- We thank also this reviewer for his insightful statement which led us to modify the manuscript in the respective parts.

Similarly, I think you do not have enough power to conclude that the other 8 SNPs found negatively associated are really not associated because of the small sample size.

- We admit that stratifying a relatively small sample reduces power. Hence, stratification in table 2 has been removed. Mentioning further stratification for smaller subgroups just is used to provide additional hints for further investigations. Based on the GPower calculations using the stated parameters we reach >80% power for the unstratified subgroups which is as a commonly used threshold.

So as a suggestion I would simplified the presentation of results mainly removing stratifications in Table 2 (and perhaps in table 1) and removing "expression analysis" from the title.

- The proposed changes are now realized in the manuscript by removing stratification in table 2 as well removing “expression analysis” from the title.

2.- Though is valuable the effort in doing the expression analysis using PBMC from a few ms patients, taking account the many factors affecting the expression of NGFB (sex, menstruation, perhaps treatment, tissue and others), I don’t think

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the sample numbers is enough large to get true significant results. So the conclusion stated in the Abstract and Discussion is in my opinion over interpreted, and should be modified.

- The abstract and discussion sections were modified accordingly, mentioning the small cohort size tested as well as the necessity of replication.

In relation with the rs*30, in the promoter region of NGFB, something specific to do to clarify its effect in the expression level of the gene would be to set up a in vitro-reporter assay. On the rs 6330, if it affects the binding to its receptor, this has to be shown. In absence of this data, the functional relevance of these two SNPs in the expression level of the gene and the functional activity of the product is quite speculative. In my opinion, the relationship between these two SNP and their affects in the gene is not supported by the data presented in this paper and therefore most of the conclusions are over interpreted.

- The reviewer correctly states that any functional relevance of the two sequence variations has to be clarified with adequate assays. This is mentioned in the manuscript including the speculative nature of our interpretation.

In summary, these are interesting and valuable data deserving publication but the results are presented with to much stratification and speculative conclusion.

- Stratification was reduced and emphasis on speculative conclusions has been toned down.

**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