Author’s response to reviews

Title: The role of MMP-12 gene polymorphism -82 A-to-G (rs2276109) in immunopathology of COPD in Polish patients: a case control study

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Author’s response to reviews:

Dear Editors

BMC Medical Genetics

Dear Professors,

Please find enclosed our revised manuscript MGTC-D-18-00382R1 entitled “The role of MMP-12 gene polymorphism -82 A-to-G (rs2276109) in immunopathology of COPD in Polish patients: a case control study”.

We hope that the revised manuscript will be acceptable for publication in BMC Medical Genetics.
Please find below point-by-point response to the comments.

Editor Comments:

This study found an association of a functional promoter variant in the MMP-12 gene with risk of COPD. Higher levels of MMP-12 protein were seen in COPD, though not related with the variant genotype. These data provides useful information in understanding COPD genetics, though the association is not conclusive and mechanism is unclear.

Besides the comments from reviewers, these points need to be addressed:

1. Abstract modify “rs2276109 protected against COPD development” to e.g. associated with reduced risk of COPD.

Response: we modified this sentence to “(…) SNP rs2276109 was associated with reduced risk of COPD”. Thank you for this suggestion.

2. Abstract modify “rs2276109 were significantly higher in controls than in COPD patient (16.9% 51 vs 12.5%,” to put COPD first then ctrl, as you did in the results.

Response: thank you for this comment, this sentence has been re-described in accordance with your suggestion.

3. L44: Remove “; genotyped using pre-design TaqMan Genotyping Assay”

Response: thank you for this comment, it has been removed.

4. Table 4. list p values inside the table; maybe add a raw for p value at the bottom and use ns for copd and ctrl column. avoid using * for non-significance. Similar for table 5

Response: p values have been listed in the table 5. Table 4 was replaced by Figure 3.

5. Table 4, 5. List n for each genotype.

Response: n for each genotype have been listed in the table 5. Table 4 was replaced by Figure 3.
6. Consider whether to use figures to replace some tables.

Response: thank you for this comment. Now, the tables 2, 3 and 4 have been replaced using figures (#1, 2, and 3, respectively).

7. L214: "To the best of our knowledge, this is the first study to analyze the relationship between rs2276109 and MMP-12 protein level in Poles." Any previous study in other populations? if so, discuss them.

Response: in careful and accurate analysis of published papers we found that in paper by Boumiza et al. (Disease Markers: Volume 2017, Article ID 6198526) both factors (-82A/G variant and MMP-12 level in plasma) were analyzed in obesity-related phenotypes in a Tunisian Population. In this work, both factors seem to be independent. Now, this paper has been included to the references, and we changed the statement “To the best of our knowledge, this is the first study to analyze the relationship between rs2276109 and MMP-12 protein level in Poles." to “……in COPD”.

BMC Medical Genetics operates a policy of open peer review, which means that you will be able to see the names of the reviewers who provided the reports via the online peer review system. We encourage you to also view the reports there, via the action links on the left-hand side of the page, to see the names of the reviewers.

Reviewer reports:

Xiang Zhao (Reviewer 2): As a revised manuscript, I have read through it very carefully. The research has analyzed the COPD patients’ -82A-to-G SNP frequency in the promotor of MMP-12 together with the control groups. By the genotyping results, the author claimed that COPD patients group has lower -82A-to-G frequency than control group. Then the author thought the higher frequency of -82 A-to-G in normal control group indicates the protective effects of this SNP to COPD. And the author showed there is part of the COPD patients (60 out of 335 or 355, not sure) has significant higher serum MMP-12 expression than controls.

This paper is full of contradictions and mistakes. Only from the abstract and background, I would list out several apparent ones as follow. First, it is hard to understand why the control smokers has even higher -82 A-to-G frequency than non-smokers. If the author’s statement “-82 G SNP protect against COPD development” is true, I would doubt here does it mean that smoking can help to protect against COPD? It is not reasonable. Secondly, I don’t understand the
meaning of the p value in the abstract result part. Is it p of t-tests? Why did the author make t-test with two different allele numbers? For sure it would be lower than 0.05, but what’s the meaning of it? It is not the right way for using t-test to compare two groups of numbers at all. Third, the authors said that the COPD patients had higher MMP-12 expression in serum than controls, which is independently on -82 A-to-G SNP. How did the author get this kind of conclusion? Interestingly, in row 102-106 page 5, the author has written the sentences which expressed clearly that the -82 A-to-G SNP is directly related to MMP-12 expression decrease. How to explain that?

Response: thank you for these comments. We carefully read our manuscript and re-described some parts (highlighted) for limit of contradictions and mistakes.

First, “the control smokers has even higher -82 A-to-G frequency than non-smokers” – in our opinion, mentioned phenomenon could be explained by the possibility that the presence of -82G allele in healthy smokers may reduce a COPD development in response to tobacco smoke, and they still possess normal lung function. It is considered that a major risk factor of COPD is an exposure to cigarette smoke, but it is postulated that the knowledge about the reason why some persons exposed to cigarette smoke develop COPD whereas others do not is insufficient. Nevertheless, in smokers controls group are some individuals with a “protective” genetic profile (-82G allele), who smoke but do not develop COPD. We apologize for suggesting that smoking can help to protect against COPD and we hope that, now, it has been clearly discussed in the text.

Second, we did not use t-test in any our analyses. P-values have been added to the abstract for clear indication of significant differences – on based on your comment, the adequate statistics, which seem to help to identification of statistic tools, were added to the abstract.

Third, thank you for this comment. We re-described this part of the text and hope that it is clear now. Both factors (MMP-12 level and analyzed SNP) seem to be independent due to non-significant differences in MMP-12 level in patients as well as controls subgroups divided accordingly to alleles/genotypes carrying (intragroup analyses). In accordance with this, you are perfectly right that “After checking the result from table 4, and table 5, it is clearly shown that the -82 A-to-G SNP has no positive relevance to the expression of MMP-12 (…)” (point #6). We discussed mentioned by you phenomenon as possible impact of rigorous system of MMPs expression, but not only -82 A-to-G mutation (lines #245-249; “It is most likely that, besides promoter polymorphism, MMPs levels depend on the transcriptional control includes epigenetic mechanisms such as DNA methylation and histone acetylation, and post-transcriptional regulation is by cytosolic mRNA stability. Final MMP-12 concentration in blood seems to be a results of this rigorous control system as well as e.g. number of the cells which secrete the enzyme.”).
There are some other places hard to be understand, I have chosen several one as below:

1. In row 74/page 3, I have checked the reference quoted here. It has not described anything about emphysema directly.

Response: thank you for this comment. Unfortunately, we made a mistake in reference managing; now, the reference #3 (Abboud RT and Vimalanathan S. Int J Tuberc Lung Dis. 2008;12:361–7) was cited for description of protease-antiprotease imbalance-related emphysema and reference #4 (Brew K and Nagase H. Bioch Bioph Acta. 2010;1803:55–7) for TIMPs role in this phenomenon.

2. In row 102/page 5, the function of the common polymorphism within the MMP-12 gene promoter (an A-to-G substitution at position -82) has been reported by Jormsjö S et al., 2000. Please include it in the reference. I would like to emphasize here that it is -82 A-to-G substitution for this SNP, it is much clearer to label as that than as “-82 A/G”. The author should add original paper and reports in the reference to show they do have studied previous cases. And we all should respect the original works by using the right references. It is short of responsibility for the authors to just list some review papers there. The reference is important part of the paper for offering background in detail.

Response: thank you for this comment. In accordance to your suggestion, we added the reference #16 by Jormsjö S et al. (Circ Res. 2000;86:998-1003) at lines #102 and #242. You are perfectly right that the using of “-82A/G” code is confused, especially due to the fact that this code (“/”) is not recommended for DNA variations description (http://varnomen.hgvs.org). Nevertheless, for DNA substitution a recommended code is “>” (http://varnomen.hgvs.org/recommendations/DNA/variant/substitution/) – in our opinion it could be much clearer than “/” and recommended “>” code has been now used in shorten description (e.g. in tables) of this SNP. On the other hand, we used “-82 A-to-G” in unsimplified description in the text.

3. In row 106 page 5, the reference 17 is wrong. As it has no relationship to the sentence. I have no time to check all the reference in the paper. But I do wonder about if the authors had cited right reference carefully.

Response: thank you for this comment. You are perfectly right because we have mistakenly replaced two papers by Hunninghate GM et al. Now, the paper Hunninghate et al. 2009 (N Engl J Med. 2009;361:2599-608) where the authors concluded that “The minor allele of a SNP in MMP12 (rs2276109) is associated with a positive effect on lung function in children with asthma
and in adults who smoke. This allele is also associated with a reduced risk of COPD in adult smokers.” was cited as reference #17 in line #105.

4. The authors summarized all results in tables. To be frank, except table 1, most other data would be more suitable to be shown in the format of statistic graphs. I do suggest the authors to transform those tables into statistic graph with columns or curves. It is very difficult to understand what the author would like to explain in table 2 and table 5.

Response: We transformed data from tables 2, 3 and 4 to graphs. Now, the data from table #2 (now, figure 1) and #5, in addition to Results section, were cited in the discussion for clear explanation of their meaning.

5. In table 2, the sum of the number of genotypes matched to the N number. But, the number of alleles doesn’t match. Where are those numbers from? No explains in figure legends. Also in the table, most OR values are less than 1. It means the factors are negatively related. According to the revision opinions, the previous reviews just would like to confirm about it. The author just calculated the OR, had not explained in results or at all. Even more, the p values in table 2 does not mean any significance or not at all. At the meanwhile, why the OR of genotype has not been added?

Response: thank you for this comment. In accordance to your suggestion, we re-calculated all alleles numbers in table 2 and, in our opinion, they are correct – the numbers of alleles (gene copies) are double count of genotypes e.g. 335 patients with COPD possess 670 alleles. You are perfectly right that most OR values are less than 1 – these results are associated with a possible protective effect of -82G allele for which the ORs were calculated. It is related to standard interpretation of OR: OR = 1 - no association; OR>1 - risk factor; OR<1 protective factor. We did not calculate the ORs for genotypes due to using 2x3 table in Fisher exact test. Now, based on your suggestion we added analyses using the multiple inheritance models (co-dominant, dominant, recessive, over-dominant and log-additive) in which ORs were calculated (additional results are available in an additional file 1). In accordance with your suggestion, we added OR results in the text (Discussion section, line #239).

6. After checking the result from table 4, and table 5, it is clearly shown that the -82 A-to-G SNP has no positive relevance to the expression of MMP-12, then would not have any directly protection against COPD.

Response: thank you for this comment. As it was pointed out above, both factors (MMP-12 level and analyzed SNP) seem to be independent – no significant difference in MMP-12 level in
patients as well as controls subgroups divided accordingly to alleles/genotypes carrying (intragroup analyses) was found. Due of this, you are perfectly right that Analyzed SNP do not play direct role in COPD development/protection. Our explanation od this phenomenon was showed in Discussion section (lines #245-249; “It is most likely that, besides promoter polymorphism, MMPs levels depend on the transcriptional control includes epigenetic mechanisms such as DNA methylation and histone acetylation, and post-transcriptional regulation is by cytosolic mRNA stability. Final MMP-12 concentration in blood seems to be a results of this rigorous control system as well as e.g. number of the cells which secrete the enzyme.”).

This paper is short of sufficient results to support the idea and conclusion. It is lacking enough convincing evidence here to claim that SNP is crucial for COPD.

Response: We are grateful for many valuable suggestions and your critical comments which helped us to improve our manuscript and hope that it is now suitable for publishing.

Robert J Trumbly (Reviewer 3): The authors studied groups of COPD patients and controls, with respect to a specific polymorphism in the promoter region of the MMP-12 gene. The found differences in the % of the different alleles in the COPD and normal subjects. The COPD subjects had higher levels of MMP-12 protein expression. However, they could not demonstrate an effect of the MMP-12 polymorphism on MMP-12 protein activity.

There are some important limitations to the study that the authors discuss at the end of the results section. A major limitation is that they measured MMP-12 levels in serum, not in the lungs.

One problem with their conclusion is that they state that the -82G allele protects against COPD. I think this is an over-interpretation, since they didn't find an effect of this allele on MMP-12 activity. They should stick to the observation that there were differences in allele frequencies between COPD and normal subjects, without concluding a causal relationship.

Response: Thank you for your comments. In accordance with your suggestion we softened our conclusion to “the -82G allele of SNP rs2276109 was more frequent in controls than in COPD patients group.”.

Sincerely yours,

Edyta M. Majorczyk