

A normal genetic variation modulates synaptic MMP-9 protein levels and the severity of schizophrenia symptoms

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

Editor: Céline Carret

1st Editorial Decision

04 March 2017

Thank you for the submission of your manuscript to EMBO Molecular Medicine and for your patience while the manuscript was being peer-reviewed. We have now heard back from the three referees whom we asked to evaluate your manuscript.

As you will see from the comments below, the three referees are enthusiastic about the study and do have suggestions and recommendations to further improve conclusiveness and clarity.

We would like to give you the opportunity to revise your manuscript, with the understanding that the referees concerns must be fully addressed. Please note EMBO Molecular Medicine encourages one single round of revision and that, as acceptance or rejection of the manuscript may depend on another round of review, your responses should be as complete as possible.

EMBO Molecular Medicine has a "scooping protection" policy, whereby similar findings that are published by others during review or revision are not a criterion for rejection. Should you decide to submit a revised version, I do ask that you get in touch after three months if you have not completed it, to update us on the status.

Please also contact us as soon as possible if similar work is published elsewhere. If other work is published we may not be able to extend the revision period beyond three months.

Please read below for important editorial formatting and consult our author's guidelines for proper formatting of your revised article for EMBO Molecular Medicine.

I look forward to receiving your revised manuscript.

***** Reviewer's comments *****

Referee #1 (Remarks):

This is an interesting study that discusses the apparent impact of a MMP-9 variant in schizophrenic subjects on severity of delusions. The following issues need to be clarified: 1-the generation of a delusional subscore which has not been verified by prior literature is of concern as it is based on a mixture of both positive and negative symptoms and can no longer be called delusions, 2-the details of demographic data such as medication history, handedness, history of drug or cigarette use etc should be discussed, 3-As levels of FMRP in brains and blood of subjects with schizophrenia maybe lower, authors need to discuss the impact of this factor on binding of MMP-9

Referee #2 (Remarks):

Employing the deep-phenotype data of schizophrenic patients from their GWAS previously published, the authors report a significant association between chronic delusion in these patients and a SNP in 3'untranslated region of MMP-9. Following up on this finding, they next performed and report a remarkably thorough investigation into the structural, morphological and functional consequences of this polymorphism. Based on their results, the authors propose that MMP-9 modulates the severity of schizophrenia symptoms by its influence on the morphology of dendritic spines causing a decrease in the efficacy of excitatory NMDA synapses. Finally, the authors employed an animal model of schizophrenia to test their "hypoglutaminergic hypothesis". The results were in line with the predictions.

Suggested minor changes: The importance of deep phenotyping should be addressed.

Recommendation: The authors present novel and important findings that deserve to be published in EMBO Molecular Medicine. I recommend the publication without any hesitation.

Referee #3 (Remarks):

In the studies authors identified new SNP in 3'UTR of MMP9 mRNA. Although there was no significant difference in occurrence of CC/CT polymorphic variants between schizophrenia patients and control subjects, a significant correlation was noted between a chronic delusion composite score and occurrence of this SNP in schizophrenia patients. The findings are very interesting and suggest that reduced synaptic levels of MMP9 may exacerbate the behaviors in schizophrenia patients. While MMP9 was targeted for treatment of schizophrenia and increased plasma levels of MMP9 were reported in the patients, current studies suggest that decreased levels of MMP9 in synapses may contribute to behaviors associated with schizophrenia. Overall experiments are executed well. However, there are some minor concerns regarding analysis and presentation of the results. The paper is suitable for publication with minor revisions.

Minor concerns:

1. How MMP9-C and MMP9-T overexpression levels were quantified in the transfected hippocampal neuron cultures?
2. Was the density of mushroom spines also lower in MMP-T expressing neurons or only percent? Was overall spine density different between MMP9-C and MMP9-T expressing neurons?
3. Did two-way ANOVA show differences in the proportion of mushroom/thin spines or spine head size at 15 or 40 min after cLTP induction as compared to 0 min in neurons expressing MMP9-T or

MMP9-C (fig4)? This should be discussed.

4. Fig S3 shows only pro-forms of MMP9 and MMP2, but figure legends indicates that it shows "levels of active MMP-9" This should be clarified.

5. Two-way ANOVA should be used for statistical analysis of mouse behavior (genotype, WT versus Het and treatment condition, saline versus MK-801). Same mice were tested in the open field before and after injections of NMDAR antagonists. Was this taking into consideration during statistical analysis?

1st Revision - authors' response

22 April 2017

Please find enclosed a revised version of our manuscript entitled "A normal genetic variation modulates synaptic MMP-9 protein levels and the severity of schizophrenia symptoms". We thank Reviewers for their comments and insightful questions. We have tried to address all of the comments as carefully as we could.

Referee #1 (Remarks):

1. The generation of a delusional subscore which has not been verified by prior literature is of concern as it is based on a mixture of both positive and negative symptoms and can no longer be called delusions,

We agree with the reviewer that the **chronic delusion composite score** is new as subscore of the well-established PANSS, but we still feel that it most adequately reflects the behavioral phenotype that is influenced by the risk genotype (C allele). It would certainly have been easy for us to just use the PANSS P1 item which appears to take the 'lead' as far as significance is concerned ($p=0.0005$). See Figure 1D. Adding the other (borderline significant) PANSS signals to the composite results in 'only' $p=0.0003$. Nevertheless, together with the other PANSS signals, the **clinical picture of risk allele carriers** is much better and more adequately described. PANSS P1 alone does not distinguish between acute or chronic delusion. The clinical picture of C carriers, however, is associated with social and emotional withdrawal, somatic concern, unusual thought content and preoccupation which together form the composite score used here for our PGAS study. This is presented in a completely transparent fashion. The reader can clearly see not only the score composition (Figure 1B), but also the relative statistical weight of its parts (Figure 1D). Since this is the overall result we obtained, we would not really want to skip any of them. We now slightly modified the respective paragraph page 7/8.

2. The details of demographic data such as medication history, handedness, history of drug or cigarette use etc should be discussed,

We have now added a table on the requested information (sociodemographic and disease-related parameters) which will be added to the manuscript. Please note that there are no significant differences between risk allele (CC/CT) and TT carriers. The Results and Materials & Methods sections have been updated accordingly (page 7/8 and 21/22, respectively).

3. As levels of FMRP in brains and blood of subjects with schizophrenia maybe lower, authors need to discuss the impact of this factor on binding of MMP-9

We thank the Reviewer for the very interesting comment. The findings of lower levels of FMRP in the brain and blood of subjects with schizophrenia have now been discussed on page 18.

Referee #2 (Remarks):

Suggested minor changes:

The importance of deep phenotyping should be addressed.

We thank the Reviewer for the very positive and stimulating feedback. The importance of deep phenotyping has now been further addressed, see modified paragraph in the discussion, page 15.

Referee #3 (Remarks):

Minor concerns:

1. How MMP9-C and MMP9-T overexpression levels were quantified in the transfected hippocampal neuron cultures?

As suggested by the Reviewer, the appropriate description of the procedures has been added to the revised manuscript in Materials & Methods, on page 28.

2. Was the density of mushroom spines also lower in MMP-T expressing neurons or only percent? Was overall spine density different between MMP9-C and MMP9-T expressing neurons?

To comply with this request, the results from the spine density analysis have been added to the revised manuscript. The Results (page 12) and Materials & Methods (page 31) sections have been updated accordingly.

3. Did two-way ANOVA show differences in the proportion of mushroom/thin spines or spine head size at 15 or 40 min after cLTP induction as compared to 0 min in neurons expressing MMP9-T or MMP9-C (fig4)? This should be discussed.

A two-way ANOVA with post-hoc analysis by Tukey's multiple comparisons was performed to assess the effect of polymorphic variant and cLTP stimulation on the proportion of spine types as well as on the mushroom spines' head size. To comply with the Reviewer's request, the results on the effect of stimulation for each polymorphic variant have been added to the revised manuscript in the Results section (page 12/13) and in the Discussion, on page 17.

4. Fig S3 shows only pro-forms of MMP9 and MMP2, but figure legends indicates that it shows "levels of active MMP-9" This should be clarified.

Please note that due to the reformatting of the manuscript, the order and naming of Supplementary Figures has been changed. Fig S3 is now Fig. EV2 with figure legend in the Supplementary Information, page 1, which has been corrected appropriately.

5. Two-way ANOVA should be used for statistical analysis of mouse behavior (genotype, WT versus Het and treatment condition, saline versus MK-801). Same mice were tested in the open field before and after injections of NMDAR antagonists. Was this taking into consideration during statistical analysis?

To comply to this request, results from two-way repeated measures ANOVA have been added to the revised manuscript in the Results section, page 14 and in Materials & Methods, on page 33.

We appreciate your careful evaluation of our work that helped us to improve the quality of the paper. We hope that this revision meets with your approval. We have included the revised manuscript version that highlights in yellow the changes from the original submission.

Thank you again for your interest in our work. We await your review of our revised manuscript.

2nd Editorial Decision

09 May 2017

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. We have now received the enclosed reports from the referees that were asked to re-assess it. As you will see the reviewers are now supportive and I am pleased to inform you that we will be able to accept your manuscript pending the following final editorial amendments:

1) Animal ethics details: in the paragraph about neuronal culture of rats brains, you have provided no details about the animals. Please do so: age, gender, background (wild type?) etc.

2) Genotype deposition: we duly note that you did not obtain explicit consent to deposit the clinical data into EGA (or else). However I am afraid that you must do so. EGA allows strict access control of datasets should you need it. Please see below:

It is possible to submit information to the EGA while still continuing to manage access via a Data Access Committee (DAC): <https://www.ebi.ac.uk/ega/home>

It's important to stress that the Data Access Committee - which one would need to allow access to the raw data in some way - would remain unchanged. Many studies, each with managed access, do this (see: <https://www.ebi.ac.uk/ega/datasets>).

Be warned that it often takes quite a bit of time for submission. This can be fast tracked but more like 3 or 4 weeks rather than 3 or 4 days. This is because one's data access committee needs to be set up, documentation submitted around it, etc.

Please think of updating the author's checklist as you get the accession number.

TEXT FROM EGA:

Who controls access to this dataset?

For each dataset that requires access control, there is a corresponding Data Access Committee (DAC) who determines access permissions. Data access requests are reviewed by the relevant DAC, not by the EGA.

The text within the study could look like this: "Our datasets were obtained from subjects who have consented to the use of their individual genetic data for biomedical research, but not for unlimited public data release. Therefore, we submitted it to the European Genome-phenome Archive, through which researchers can apply for access of the raw data."

I look forward to reading a new revised version of your manuscript as soon as possible.

***** Reviewer's comments *****

Referee #1 (Remarks):

The revision is acceptable to me. I recommend publication.

Referee #3 (Remarks):

Revisions have addressed the reviewer's concerns and the paper is appropriate for publication in its present form.

2nd Revision - authors' response

15 May 2017

Authors made the requested editorial changes.

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Leszek Kaczmarek
 Journal Submitted to: EMBO Molecular Medicine
 Manuscript Number: EMM-2017-07723-V2

Reporting Checklist For Life Sciences Articles (Rev. July 2015)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures**1. Data****The data shown in figures should satisfy the following conditions:**

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if $n < 5$, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions**Each figure caption should contain the following information, for each panel where they are relevant:**

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/ varied/ perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
 - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ^2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
 - are tests one-sided or two-sided?
 - are there adjustments for multiple comparisons?
 - exact statistical test results, e.g., P values = x but not P values < x;
 - definition of 'center values' as median or average;
 - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Sample sizes were estimated taking into account expected possible effects of interest (estimated from previous own and literature data), availability of the resources and statistical requirements (demonstration of statistical significance with assuring the appropriate statistical power of the test). Page 33
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	The sample size was designed to give statistical power and estimated taking into account expected possible effects of interest (estimated from previous own and literature data). Page 33
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Page 32
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	There was no issue of randomization because all available heterozygous and wildtype animals were taken for the experiment.
For animal studies, include a statement about randomization even if no randomization was used.	There was no issue of randomization because all available heterozygous and wildtype animals were taken for the experiment.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	Page 32
4.b. For animal studies, include a statement about blinding even if no blinding was done	Page 32
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes, page 32
Is there an estimate of variation within each group of data?	Yes, estimated from previous own and literature data
Is the variance similar between the groups that are being statistically compared?	Yes

C- Reagents**USEFUL LINKS FOR COMPLETING THIS FORM**

<http://www.antibodypedia.com>
<http://1degreebio.org>
<http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo>
<http://grants.nih.gov/grants/olaw/olaw.htm>
<http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm>
<http://ClinicalTrials.gov>
<http://www.consort-statement.org>
<http://www.consort-statement.org/checklists/view/32-consort/66-title>
<http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun>
<http://datadryad.org>
<http://figshare.com>
<http://www.ncbi.nlm.nih.gov/gap>
<http://www.ebi.ac.uk/ega>
<http://biomodels.net/>
<http://biomodels.net/miriam/>
<http://ijb.biochem.sun.ac.za>
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
<http://www.selectagents.gov/>

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	NA
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Page 32
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Page 33
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We confirm the compliance.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	Page 20
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Page 20
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	NA
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	NA
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	We encourage individuals interested in information as derivable from the GRAS database to contact us in the form of a collaboration request. The data themselves cannot be given out of our hands due to very strict ethical requirements.
21. As far as possible, primary and referenced data should be formally cited in a Data Availability section. Please state whether you have included this section. Examples: Primary Data Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in <i>Shewanella oneidensis</i> MR-1. Gene Expression Omnibus GSE39462 Referenced Data Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	NA
22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomedel (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

23. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA
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