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A myelin gene causative of a catatonia-depression syndrome upon aging

Nora Hagemeyer, Sandra Goebbels, Sergi Papiol, Anne Kästner, Sabine Hofer, Martin Begemann, Ulrike C. Gerwig, Susann Boretius, Georg L. Wieser, Anja Ronnenberg, Artem Gurvich, Stephan H. Heckers, Jens Frahm, Klaus-Armin Nave and Hannelore Ehrenreich

Corresponding author: Hannelore Ehrenreich, Max Planck Institut of Experimental Medicine

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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.)

1st Editorial Decision 30 January 2012

Thank you for the submission of your manuscript "A myelin gene causative of a catatonia-depression syndrome upon aging" to EMBO Molecular Medicine. We have now heard back from the three referees whom we asked to evaluate your manuscript and they find the topic of your manuscript potentially interesting. However, they also raise significant concerns on the study, which should be addressed in a major revision of the manuscript.

You will see that reviewer #2 is rather positive about the study and only raises minor concerns, while reviewers #1 and #3 are much more reserved. In particular, reviewer #3 highlights that the statistical analysis has to be improved and the statistical significance, especially regarding the findings in humans, re-evaluated. In addition, Reviewer #1 points to an apparent inconsistency, which should be addressed.

Given the balance of these evaluations, we feel that we can consider a revision of your manuscript if you can convincingly address the issues that have been raised within the space and time constraints outlined below.

Revised manuscripts should be submitted within three months of a request for revision. They will otherwise be treated as new submissions, unless arranged differently with the editor.

I look forward to seeing a revised form of your manuscript as soon as possible.

Yours sincerely,

Editor
EMBO Molecular Medicine

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

Referee #1 (Comments on Novelty/Model System):

Technical quality: Combination of animal and human studies; application of quite different methods.
Novelty: Especially the catatonia-depression test battery for mice is new and valid.

Referee #1 (Other Remarks):

This is a well-written, methodically rigorous paper that combines findings from animal and human studies to show that CNP, the gene coding for the myelin-associated enzyme CNPase, is "causative of a catatonia-depression syndrome upon aging". In aging cnp +/- mice the authors observed increased anxiety and impaired social and exploratory behaviour, but normal motor activity. More importantly, these animals showed signs of catatonia and depression. These behavioural alterations were accompanied by anatomical changes (histochemical signs of inflammation, increase in immune cells, astrogliosis and axonal degeneration). The "human" part of the study compared individuals suffering from schizophrenia and control cases with regard to the question, if a mutation in the human CNP gene (SNP 207016, AA instead of GG) does really increase the risk of schizophrenia. Their clear answer is NO [unlike others (Peirce et al. 2006), but in accordance with, for example, Che et al. 2009]. Then Hagemeyer and colleagues attempted at revealing if schizophrenic carriers of the 207016 mutation (aged above 40 years) may present a characteristic catatonic-depressive behaviour. A depression-catatonia composite consisting of five phenotype domains was used to test this. It could be demonstrated that this is indeed the case. Besides, AA carriers show white matter structural abnormalities in MRI.

Concerns:
The paper has undoubtedly several strengths, but there remain certain inconsistencies which should be eliminated (or at least be plausibly explained).
1. No doubt, in cnp +/- mice the cerebral expression of CNPase is greatly reduced. This reduced enzyme expression (which is most probably accompanied by reduced enzyme activity) may generate the aforementioned behavioural and structural deviations. Further, there is evidence from several studies that CNPase expression is decreased in brain tissue of schizophrenics. However, the AA mutation 207016 does not further reduce the CNP expression in schizophrenics (absolutely no difference between GG and AA, and, ironically, even increased expression in GA carriers; human post-mortem studies, Iwamoto et al. 2008). In the present investigation schizophrenic individuals having or not having this mutation were studied, and only AA carriers showed the catatonia-depression problem. How do the authors explain this, once CNPase expression is unchanged in AA compared to GG schizophrenic carriers? Do they have own postmortem data showing a reduced CNPase in AA? If yes, please provide.
This makes it, by the way, also difficult to compare the results of the mice with those from humans.
2. Hagemeyer et al found that AA carriers appear in schizophrenia and controls with exactly the same frequency, which is in accordance with previous findings published by others (Peirce et al. 2006, Iwamoto et al. 2008; Che et al. 2009). But if AA is "causative" of catatonia-depression (and quite unrelated to schizophrenia), one must ask: what about controls carrying AA? As outlined in the MS, there are more than 1000 controls cases. My strong suggestion is to test at least some of them (GG against AA) for catatonia-depression. Otherwise it is not justified to write that CNP is "causative". Further, structural abnormalities were seen in AA schizophrenic carriers. Would we see the same alterations in healthy AA carriers?
3. What is the frequency of AA in identified individuals with major depression or bipolar disorder?
4. Minor point: please provide a list of abbreviations. Not all readers will know what, for example, IBA stands for.

Referee #2 (Comments on Novelty/Model System):

This is an important paper because it demonstrates that additive damage to oligodendrocytes and myelin is able to cause psychopathological syndromes which are part of major psychoses.

Although transcriptional, neuropathological and imaging studies had previously obtained evidence for myelin damage in these psychiatric disorders, the nature of this relationship remained elusive. The article provides convincing evidence for myelin disturbances as - at least partial - cause of major psychoses.

The authors describe their thorough investigations of the damaging effect of a genetic polymorphism (AA at CNP SNP rs2070106) and aging on myelin in animal models and schizophrenic patients. The results considerably improve our understanding of the pathogenesis of major psychiatric disorders and are most likely to be replicated.

Despite careful reading of the manuscript, I was unable to discover any major or minor errors. I unhesitatingly recommend the publication of this important article in "EMBO Molecular Medicine".

Referee #2 (Other Remarks):

On page 12 last para, it would be helpful if the authors could provide a list of catatonic signs used for the catatonia-depression composite since not all readers are familiar with the Cambridge Neurological Inventory.

Referee #3:

This is a potentially interesting manuscript that studies an animal model of a myelin gene, CNP, Cnp heterozygous mice, that have behaviors similar to those thought to occur in patients with schizophrenia with a genotype in the CNP gene thought to increase risk for the disease. The behaviors attributed to the Cnp heterozygous mice which are described in the manuscript are convincing for the most part. One exception is the measure for general locomotor activity and anxiety that is "significant " only to P=.096. Another, significant to .036, would probably not survive corrections for multiple testing. In addition, the refence in the Introduction to data from a manuscript in preparation is not acceptable.

The data on the patients has bigger problems. While the DTI data has a relatively small "n" it could be convincing but it is missing data on the heterozygotes with this genotype. Similarly, these subjects are not mentioned in the catatonia-depression data. Results from these subjects would either greatly enhance these findings or call the results into question. Similarly, it is unclear whether the depression-catatonia syndrome was arrived at post hoc or what the data looks like for the 5 phenotypes examined. While the catatonia-depression phenotype would probably survive correction combining the two after the first five are examined is a questionable statistical practive. Toward that end, I doubt the DTI datawould survive correction for multiple comparisons, the exact number which is unclear in the manmuscript. Lastly, although the CPZ equivalents may not be statistically different between the genotypically defined subgroups, the large variability in the CPZ equivalents make the finding of no difference between the subgroups less convincing and a potential source of a type II error. If one covaries for CPZ equivalents does the data still hold for catatonia depressionand DTI findings?

1st Revision - Authors' Response 02 February 2012

Point-to-point response to the reviewers' comments

The authors would like to thank the referees for their comments and suggestions, which have significantly contributed to the improvement of the manuscript. In the following, our answers are always presented in italics.

Referee #1 (Comments on Novelty/Model System):

Technical quality: Combination of animal and human studies; application of quite different methods.
Novelty: Especially the catatonia-depression test battery for mice is new and valid.

Referee #1 (Other Remarks):

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Concerns:

The paper has undoubtedly several strengths, but there remain certain inconsistencies, which should be eliminated (or at least be plausibly explained).

1. No doubt, in cnp +/- mice the cerebral expression of CNPase is greatly reduced. This reduced enzyme expression (which is most probably accompanied by reduced enzyme activity) may generate the aforementioned behavioural and structural deviations. Further, there is evidence from several studies that CNPase expression is decreased in brain tissue of schizophrenics. However, the AA mutation 207016 does not further reduce the CNP expression in schizophrenics (absolutely no difference between GG and AA, and, ironically, even increased expression in GA carriers; human post-mortem studies, Iwamoto et al. 2008). In the present investigation schizophrenic individuals having or not having this mutation were studied, and only AA carriers showed the catatonia-depression problem. How do the authors explain this, once CNPase expression is unchanged in AA compared to GG schizophrenic carriers? Do they have own postmortem data showing a reduced CNPase in AA? If yes, please provide. This makes it, by the way, also difficult to compare the results of the mice with those from humans.

Here the authors have to disagree with the reviewer. Please note that in the manuscript, 3 articles have been referenced regarding decreased CNP expression associated with the A-allele in post-mortem studies (Page 4, first paragraph). The first report (Peirce et al 2006), based on an allele-specific messenger RNA expression assay, showed in 25 subjects a lower expression of A-allele transcripts as compared to G-allele with a mean reduction of 24%. A second study (Mitkus et al 2008) involving 28 subjects was again able to show a decrease in CNP expression in A-allele carriers in comparison to GG subjects in the dorsolateral prefrontal cortex grey matter (qPCR). The study of Iwamoto, as cited by the reviewer, failed at first view to replicate this effect. However, when looking more closely, this study is much less convincing. Its conclusions are based on a very low sample size (some genotype groups contain only 2 subjects) and on array data that are less sensitive and reliable than qPCR. Therefore, solid evidence points to the A-allele being associated with a decrease in CNP expression. Unfortunately, we do not have the possibility at this point to test CNP expression in own post-mortem samples.

2. Hagemeyer et al found that AA carriers appear in schizophrenia and controls with exactly the same frequency, which is in accordance with previous findings published by others (Peirce et al. 2006, Iwamoto et al. 2008; Che et al. 2009). But if AA is "causative" of catatonia-depression (and quite unrelated to schizophrenia), one must ask: what about controls carrying AA? As outlined in the MS, there are more than 1000 controls cases. My strong suggestion is to test at least some of them (GG against AA) for catatonia-depression. Otherwise it is not justified to write that CNP is "causative".

In the manuscript, we stated that CNP loss-of-function genotypes are causative of catatonia-depression. This assumption is based both on findings in mouse and man. Without the mouse data, it would be difficult to justify this conclusion. In such a case, we would definitely need healthy control subjects to be tested for catatonia-depression as well. Since, luckily, we have loss-of-function genotypes in 2 species giving us similar phenotypical readouts, we think that information from healthy subjects is dispensable. Nevertheless, we agree with the reviewer that it would be very nice to have such a control database. But unfortunately, such information does not seem to be available anywhere in the world (we tried hard to find it). And our own healthy controls are not comprehensively phenotyped.

Further, structural abnormalities were seen in AA schizophrenic carriers. Would we see the same alterations in healthy AA carriers?

This is certainly an interesting question, which we cannot answer at this point. As already pointed out in the manuscript, it would be very interesting to also have information on respective genotype carriers in major depression or Alzheimer disease. For our study, however, the difference between AA and GG subjects within our particular disease population, schizophrenia, was the most important research question.

3. What is the frequency of AA in identified individuals with major depression or bipolar disorder?

We have not been able to find genetic association studies analyzing this genetic variant in the context of bipolar disorder or major depression.

4. Minor point: please provide a list of abbreviations. Not all readers will know what, for example, IBA stands for.

We thank the reviewer for his helpful comment. We have now integrated a list of abbreviations in the manuscript (page 17).

Referee #2 (Comments on Novelty/Model System):

This is an important paper because it demonstrates that additive damage to oligodendrocytes and myelin is able to cause psychopathological syndromes, which are part of major psychoses.

Although transcriptional, neuropathological and imaging studies had previously obtained evidence for myelin damage in these psychiatric disorders, the nature of this relationship remained elusive.

The article provides convincing evidence for myelin disturbances as - at least partial - cause of major psychoses.

The authors describe their thorough investigations of the damaging effect of a genetic polymorphism (AA at CNP SNP rs2070106) and aging on myelin in animal models and schizophrenic patients. The results considerably improve our understanding of the pathogenesis of major psychiatric disorders and are most likely to be replicated.

Despite careful reading of the manuscript, I was unable to discover any major or minor errors. I unhesitatingly recommend the publication of this important article in "EMBO Molecular Medicine".

We would like to thank this reviewer for his very stimulating statements.

Referee #2 (Other Remarks):

On page 12 last para, it would be helpful if the authors could provide a list of catatonic signs used for the catatonia-depression composite since not all readers are familiar with the Cambridge Neurological Inventory.

We thank this reviewer for his suggestion. The CNI items used were: gait mannerism, gegenhalten, mitgehen, imposed posture, abrupt or exaggerated spontaneous movements, iterative movements, automatic obedience and echopraxia. Now they have been included in the Materials and Methods section (page 12, paragraph 3 line 4-6).

Referee #3:

This is a potentially interesting manuscript that studies an animal model of a myelin gene, CNP, Cnp heterozygous mice, that have behaviours similar to those thought to occur in patients with schizophrenia with a genotype in the CNP gene thought to increase risk for the disease. The behaviours attributed to the Cnp heterozygous mice, which are described in the manuscript, are convincing for the most part. One exception is the measure for general locomotor activity and anxiety that is "significant " only to P=.096.

There seems to be a misunderstanding. We never talked about significance with respect to the measure for 'time in zones' in the open field. Since other tests point to significantly increased anxiety (Figure 2D & 2F), we mentioned the tendency in Figure 2A in this particular context. All three tests point into the same direction, i.e. anxiety. If the tendency in Figure 2A were the only 'sign of anxiety' we would not even have mentioned it.

Another, significant to .036, would probably not survive corrections for multiple testing.

For describing the phenotype of mice, we performed a comprehensive test battery that is required for making any firm conclusions with respect to the behavioural phenotype. Therefore, each test is needed as part of a whole set of tests which are prerequisite for a solid behavioural diagnosis. In such a constellation, multiple testing does not really apply.

In addition, the reference in the Introduction to data from a manuscript in preparation is not acceptable.

We actually adhered here to the instructions for authors of EMBO MolMed: "References to manuscripts in preparation or submitted, but not yet accepted, should be cited in the text as (C Lee and N Jones, in preparation), not as (C Lee and N Jones, submitted), and should not be included in the list of references." *To be completely correct, we now removed the word 'manuscript' (page 3, paragraph 2 line 8).*

The data on the patients has bigger problems. While the DTI data has a relatively small "n" it could be convincing but it is missing data on the heterozygotes with this genotype.

The DTI experiments were set up as a small follow-up study, investigating clear and well defined subgroups ('extremes') to test our hypothesis of axonal degeneration also detectable in humans carrying the partial 'loss-of-function' genotype. We purposely selected the best contrasting genotypes. Heterozygous individuals would not have added to this particular research question. As known from many other studies in different fields, we cannot always expect a gene 'dose-response effect'. Actually, the data displayed below (showing very similar catatonia-depression scores for GA and GG) strongly support this view.

Similarly, these subjects are not mentioned in the catatonia-depression data. Results from these subjects would either greatly enhance these findings or call the results into question.

As already pointed out above, we cannot necessarily expect an intermediate phenotype. This is why results from heterozygous individuals are unlikely to call any results into question. For the reviewer's interest, we are presenting below the descriptives and ANOVA results for all 3 genotypes. The Table below shows that heterozygous subjects have very similar composite scores to GG carriers; they do not show an intermediate phenotype.

Catatonia-depression composite score:

Descriptives

Composite_score_final

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
AA	40	,3794	,86258	,13639	,1036	,6553	-1,13	1,9
GA	188	,0497	,76584	,05585	-,0605	,1599	-1,20	2,7
GG	202	,0308	,74461	,05239	-,0725	,1341	-1,20	2,4
Total	430	,0715	,77000	,03713	-,0015	,1445	-1,20	2,7

ANOVA

Composite_score_final

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4,217	2	2,108	3,599	,028
Within Groups	250,138	427	,586		
Total	254,355	429			

Similarly, it is unclear whether the depression-catatonia syndrome was arrived at post hoc or what the data looks like for the 5 phenotypes examined.

The depression-catatonia syndrome, as described here in a composite score, was not arrived at post hoc, but derived from observations in our extensive mouse studies. In fact, a similar composite was created before in the mice. Based on these findings we searched for associations of the 'loss-of-function' genotype (AA) in humans with the respective readouts. A detailed presentation of the underlying 5 phenotypes has been given in Supporting Figure 1, including statistical information. For any of these phenotypes, a similar genotype-dependent profile across age groups is observed.

While the catatonia-depression phenotype would probably survive correction combining the two after the first five are examined is a questionable statistical practice.

As explained above, all association studies performed in the context of the catatonia-depression syndrome (5 phenotypes) were performed in a hypothesis-driven fashion based on our mouse data. All of these 5 phenotypes, not only 2 of them, were integrated into the score. This is clearly stated in the Material and Methods section (Page 12): Catatonia-depression composite.

Toward that end, I doubt the DTI data would survive correction for multiple comparisons, the exact number which is unclear in the manuscript.

Here we would like to clarify for the reviewer that the whole DTI sub-study of this manuscript was performed in a strictly hypothesis-driven fashion. Based on our behavioural and histological (axonal degeneration!) mouse data and the findings in the human population regarding the catatonia-depression syndrome, we expected to see differences (if any) between axonal integrity readouts in the frontal versus caudal regions of the corpus callosum. Therefore, we <u>pre</u>defined the frontal region as target and the caudal region of the corpus callosum as control area. The DTI readouts (Fractional Anisotropy, Axial Diffusivity, Radial Diffusivity and Apparent Diffusion Coefficient) belong to the 'package' required for comprehensively delineating the structural quality of fiber tracts.

Lastly, although the CPZ equivalents may not be statistically different between the genotypically defined subgroups, the large variability in the CPZ equivalents make the finding of no difference between the subgroups less convincing and a potential source of a type II error. If one covaries for CPZ equivalents does the data still hold for catatonia depression and DTI findings?

We thank this reviewer for this excellent suggestion. We have now followed his advice and added the CPZ equivalents as a covariate in our analysis. It turns out that without this addition, we even underestimated our results. The new, even higher significant results are now included in Table 1 (page 27) and Figure 3 as well as the Figure legend 3 (page 26) and the result section (page 8, paragraph 2 line13).

2nd Editorial Decision 09 February 2012

Thank you for the submission of your revised manuscript "A myelin gene causative of a catatonia-depression syndrome upon aging" to EMBO Molecular Medicine. We have now received the reports from the reviewers who were asked to re-review your manuscript.

You will be glad to see that the reviewers are now globally supportive and we can proceed with official acceptance of your manuscript pending the minor changes detailed below:

- Please include the data regarding GA heterozygotes in the manuscript as recommended by Reviewer #3.

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

Yours sincerely,

Editor
EMBO Molecular Medicine

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

Referee #1:

The corrections further improved the quality of the paper.
I have no concerns now.

Referee #3:

The authors have responded to all items not necessarily as I might have hoped, but I guess adequately. I would encourage them to include the data on the heterozygotes for GA, which looks similar to the GG genotype using an ANOVA with posthoc t tests.

2nd Revision - Authors' Response 09 February 2012

Thank you very much for your favourable decision regarding our manuscript ″A myelin gene causative of a catatonia-depression syndrome upon aging”.

As requested, we have now mentioned the heterozygotes' (GA) data in the paper and added them to the supporting information (new Supporting Table II).