EMBO *reports*

Mitofusin gain and loss of function drive pathogenesis in *Drosophila* models of CMT2A neuropathy

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Report:

Authors contact the journal 27 December 2019

I am contacting you regarding the paper "Mitofusin gain and loss of function drive pathogenesis in Drosophila models of CMT2A neuropathy" by El Fissi *et al.* published in EMBO reports in June 2018 and for which you were the editor.

While pursuing our research in this field of study, we have unfortunately discovered an error in the sequence of the vector used in our manuscript to express the MFN2-R364W allele in mouse embryonic fibroblasts (MEFs).

Indeed, an inexcusable mistake in the MFN2 mutagenesis process led us to generate an R364T substitution. Consequently, in Figures 3B-F, the MFN1-MFN2 double knock out MEFs were not transfected with a MFN2-R364W vector but rather expressed the erroneous R364T mutation.

We have reanalysed the rest of the work and confirmed that the error is restricted to the experiments performed with human MFN2-R364W (Fig. 3B-F) and does not affect the results obtained in MEFs with the other MFN2-mutants (L76P, R94Q and T105M). The experiments performed in the *Drosophila* model system and their conclusions are not concerned.

We are aware of the gravity of such mistake and we sincerely apologize to you and to the readers of EMBO reports.

We are currently conducting the experiments described in Fig. 3 with a vector containing the correct R364W substitution. We seek to know to which extent the MFN2-R364W mutant will phenotypically differ from the MFN2-R364T mutant characterized in our publication.

Meanwhile, we will follow all your guidelines in order to communicate about our mistake to the readers in the way you will consider the most appropriate. Also, if you have any requests or advices please contact me, I will be very grateful.

Finally, I would like to emphasize that I am entirely responsible of this error and that none of the other authors of our manuscript should be blame for it.

Journal's response 2 January 2020

Thank you for your mail and for alerting us to the mistake in Figure 3B-F.

I will discuss the issue further with our head of publications and get back to you. We will certainly have to retract the corresponding figure panels but I can only tell you more once we looked into the case in more detail.

Journal correspondence 22 January 2020

Thank you again for alerting us to the mistake in your paper "Mitofusin gain and loss of function drive pathogenesis in Drosophila models of CMT2A neuropathy". I have now carefully looked into the case and discussed it with our head of publication.

As I understand from your letter, you have reanalysed all other human and Drosophila alleles used and confirmed that the error is restricted to the human MFN2-R364W allele, correct?
You also indicated that you are currently testing a vector containing the correct R364W substitution.

After discussing the matter, we have decided to publish an Editorial Note for now on the paper to alert the reader to the fact that the experiments described in Fig. 3B-F and Fig. EV4D do not report on the human MFN2-R364W allele but on a 364T mutation instead. This editorial note will be superseeded by a formal corrective measure informed by the outcome of the experiment.

Please keep us updated on the progress and outcome of the ongoing experiments.

Author correspondence 5 February 2020

Thank you for having carefully looked into this issue and discussed with the head of publication. Following your email, I contacted all the authors and discussed the ongoing work performed in MEFs by our collaborators in Bordeaux.

First, we all agree that the publication of an editorial note seems much appropriated before a more formal corrective measure take place.

Second, I do confirm that the mutagenesis error is restricted to the hMFN2-R364W mutant expressed in mouse embryonic fibroblasts (MEFs). The other hMFN2 mutants studied in MEFs (hMFN2 L76P, R94Q, T105M), as well the *marf* alleles in transgenic drosophila have been verified and are not concerned by this mutagenesis error.

As mentioned in my previous email, we have generated the correct hMFN2-R364W vector for expression in MEFs. This construct has been transfected in MEFs knock out for both MFN1 and MFN2 as described in Fig. 3.

We have now transfected twice the hMFN2-R364W in MEFs. As a comparison, hMFN2WT, the (erroneously studied) hMFN2-R364T and the GTPase mutant hMFN2-R94Q were also transfected in parallel. For each hMFN2 alleles, MEFs were categorised based on mitochondrial morphology: "filamentous", "fragmented", "aggregated". Expression level of the hMFN2 alleles was not determined at this stage but a GFP vector was co-transfected.

Consistent with Fig. 3 of our manuscript, the expression of hMFN2-WT and hMFN2-R364T in MFN1/MFN2 knock-out MEFs restore the formation of mitochondrial tubules (cell distribution: 65% "filamentous", 12% "fragmented", 23% "aggregated"), whereas the R94Q mutant does not (cell distribution: 6% "filamentous", 32% "fragmented", 62% "aggregated").

The hMFN2-R364W led to low transfection efficiency compare to the other constructs. However, mitochondrial morphology could be visualised in about 500 cells in total. In these experiments, the hMFN2-R364W does not efficiently restore fusion (cell distribution: 17% "filamentous", 35% "fragmented", 48% "aggregated") in contrast to hMFN2-R364T or WT. Of note, hMFN2-R364W cells categorised as "filamentous" have generally shorter mitochondrial tubules than cells expressing hMFN2-WT or hMFN2-R364T. Therefore, these experiments suggest that the human MFN2-R364W mutant is not/very poorly fusion competent in MEFs and they contradict the conclusion of Fig. 3B, C, D and Fig. EV4 D.

Regarding the overall manuscript, these data do not affect the conclusions that the R364W homologous *marf* allele (R404W) increases fusion when expressed in drosophila motor neurons and that both increased and defective fusion can lead to similar alterations in neurons. Indeed, these conclusions are supported solely by the observations made in drosophila (as indicated by title and abstract). 1) Enlargement of mitochondria (Fig. 2). 2) Restoration of fusion activity in *marf* knock out neurons (Fig. 3A). 3) Aberrant mitochondrial size and shape typical of unbalanced fusion (also found upon increased WT marf expression or decreased DRP1 expression) (Fig. 4a), 4) Rescue of mitochondrial shape (and related phenotypes: locomotion, synaptic mitochondria…) by over-expressing DRP1. Of note, we also showed that a second allele (L118P), homologous to L76P, behave similarly in flies.

In our manuscript, increased fusion was not corroborated by the experiments in MEFs with L76P. Our recent experiments show that fusion is not restored in MEFs expressing the correct R364W allele. So far, we do not know the origin of these discrepancies between human mutant MFN2 expressed in mouse fibroblasts and Marf alleles expressed in drosophila neurons. They can come from differences between cell types (as discussed in our manuscript), between model organisms, or to non-conserved molecular properties between Marf and hMFN2 proteins.

I sincerely apologize for this inexcusable mistake and to take your time to solve this issue. We are awaiting for your opinion regarding the formal corrective procedures you will find the most appropriated in order to communicate to the readers about the points I have described and discussed above. Meanwhile, do not hesitate to require any further precisions.

Journal correspondence 14 February 2020

Thank you very much for the update on the experiments with the correct MFN2-R364W mutation.

I have discussed the matter again with our head of publication, Bernd Pulverer, and our Data Integrity Analyst, Erica Boxheimer (cced here).
We note thus that the conclusions regarding the fusion activity of human MFN2-R364W cannot be maintained.

Since the data and conclusions based on the work in Drosophila, which constitute a major body of the study, remain valid, we have decided to correct your manuscript with a " Partial withdrawal" of the data in Figure 3 and Figure EV4. The "Note of Editorial Concern" appears to be obsolete now.

We propose to withdraw panels B-D of Figure 3 completely since the data on R364W are not valid and the data on the fusion activity of R94Q not important anymore in this context. Moreover, the MFN2-R94Q allele is known to be fusion-incompetent (e.g. Detmer and Chan, JCB 2007). For panel (B) the pictures showing Hs-MFN2 and non-transfected control could be kept, since they serve as control for the data shown in Figure EV4
In Figure EV4 panel D will be withdrawn.

Could you please prepare a text for the Withdrawal note explaining what happened, maybe including a discussion on the observed discrepancies between Drosophila and human alleles expressed in fibroblasts?

We further note that the relevance to human CMT2A pathogenesis has been significantly weakened now, since the relevance of the findings for patients carrying the frequent R364W allele is unclear at this stage. We therefore strongly recommend withdrawing the conclusions made at the end of the Abstract and the Discussion section.

We suggest to quote the two statements below and to state that you herewith withdraw these conclusions. We strongly recommend a withdrawal of these sentences rather than a rephrasing.

" … and propose for the first time that excessive mitochondrial fusion drives CMT2A pathogenesis in a large number of patients"

" Our data also indicate that anti-fission or pro-fusion drugs, envisioned as treatments for CMT2A or neurodegenerative disease, could be detrimental for patients with R364W and L76P alleles that would rather benefit from the development of pro-fission or anti-fusion molecules"

Please let me know if you have further questions or need more information.

