Author’s response to reviews

Title: Monitoring candidate gene expression variations before, during and after a first Major Depressive Episode in a 51-year-old man

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Author’s response to reviews:

Reviewer 1:

“The authors want to describe new biomarkers for personalized medicine. However, it is doubtful if a single case can provide enough data for biomarker retrieval. At least the authors should have a replication”

We agree that a single case do not provide enough data for biomarker description, as stated in the Conclusion section of the manuscript. Nevertheless, our case report describes the serendipitous development of an MDE in a healthy subject. To be able to follow-up in a relatively small time scale (< 1 year) at least another case of a first MDE onset, we would need to enrol a very large cohort (>100) of healthy subjects or individuals. Therefore, we feel that it is best to put forward our observation to allow further data from future investigators can be added for more debate/validation.

For clarity, we introduced an additional sentence in the Conclusion section. On p8: “Despite these new insights, the results presented here have some limitations. First of all, it is difficult to draw general conclusions based on an isolated case of MDE patient. Replications are warranted but finding similar case requires a very large prospective cohort within healthy subjects or individuals at risk for an MDE.”

“Several transcripts were analyzed, but I found no adjustment for multiple testing. After this correction, is there any significant regulation left?”

We have calculated the values of the false discovery rate (FDR) for all the correlation analyses involving the level of expression of the nine selected candidate genes. For the few of genes whose expression is highly correlated, we observed FDR values below 20%, which represents a common threshold for multiple testing evaluations in candidate gene approaches. Therefore, the text now reads on p 5-6 as follows:

“Of note, SLC6A4/5HTT expression was inversely correlated to IL10 expression (Spearman’s coefficient correlation #=-0.71, p=0.047, false discovery rate (FDR)=0.161). Conversely, S100A10 demonstrated variations in the opposite
direction compared to SLC6A4/5HTT, and was also strongly correlated to TNF variation ($\# = 0.86, p=0.007, \text{FDR}=0.067$), IL1B ($\# = 0.81, p=0.015, \text{FDR}=0.067$) and CCL2 ($\# = 0.83, p=0.010, \text{FDR}=0.067$). Finally, TNF, which demonstrated an opposite pattern of expression compared to PDLIM5, was strongly correlated to important mediators of inflammation such as IL1B ($\# = 0.93, p=0.001, \text{FDR}=0.036$), IL8 ($\# = 0.81, p=0.015, \text{FDR}=0.067$), and CCL2 ($\# = 0.81, p=0.015, \text{FDR}=0.067$).

“What was the antidepressant and the dosage?”

The antidepressant was Agomelatine and dosage was 25 mg. We have now added this indication in the Case presentation section.

On p5, first paragraph, it is now written: « He initiated oral antidepressant treatment at week 29 (Agomelatine, 25 mg/day) and recovered from his MDE at week 36.

We also specified the dosage within the figure legend.

“Why did the authors not analyse FKBP5, since FKBP5 mRNA expression was associated with major depression, HPA-axis dysregulation and treatment response”

As stated in the Background section, the genes tested in our case report were selected on the basis of previous published data obtained by our group and also by the group of Carmine Pariante, both groups being the first to investigate gene expression profile as a way to predict antidepressant response. As FKBP5 was not among our candidate gene list, in agreement to our previous genome-wide microarray experiment, we did not test this gene. However, we do not deny that FKBP5 might be an interesting marker to follow in agreement with the reviewer’s recent results. In fact, in our own data, we found that FKBP5 was significantly overexpressed in responder MDE patients at baseline compared to controls and that there was also a significant return to “normal” level of expression 8 weeks later. Therefore we have now added a sentence within the Discussion section and added 2 bibliographic references.

On p8, penultimate paragraph: “Finally, we favored a candidate gene approach and restricted our analysis to a few genes of interest. Of note, other candidate biomarker genes such as FKBP5 could also be informative in such a case and deserve further investigations [30, 31].”


“It was not clear to me why this subject was enrolled and in which study, this
should be clarified.”

The subject was indeed enrolled twice in the same protocol (Ref. no. AORC 2009-15), as a control for his first blood draws and then as an MDE subject for the following collected blood samples. To provide more precision in the manuscript, it has been edited as below in the Case presentation section.

On p5, first paragraph: “A 51 year-old male was enrolled as a healthy control in a previously published prospective study of gene expression [6]. Based on the French version of standardized interview validated for healthy control subjects (SCID-NP) [22], the patient did not have a history of psychiatric disorder or other notable medical conditions prior and at the time of his inclusion. Nevertheless, at week 20 after inclusion, he experienced a mild to moderate MDE, which was diagnosed based on the occurrence of depressive mood, anhedonia, psychomotor retardation, asthenia, and thoughts of death. He consented to stay in the study despite this medical event and was switched to the MDE group of the same study protocol.”

Reviewer 2:

“Figure 1- Did the author run technical replicates for the qPCR experiments? This should provide a standard error for the measurements at the different time points. Otherwise one may wonder whether changes in single time points for four genes out of 10-12 may just reflect normal technical variability.”

We agree that replicates in qPCR experiments are important. Indeed, we conducted all qPCR measurements in duplicate and used the mean value for threshold cycle (Ct) calculation. In fact, qPCR were run on the same batch of TaqMan low density arrays (tLDAs) used for the previously published study by Belzeaux et al (reference 6 of the manuscript) and herein the coefficient of variation (CV%) of our replicates (quadriplicates) for all analyzed genes were very low. Remarkably, more than half of the analyzed genes, with mean Ct under 30, exhibited a CV inferior to 1 % while the other genes show a CV between 1 and 3%. Thus, there was a very low intra-assay variation, making it unlikely that the changes observed for the 4 genes represented in Figure 1 reflect technical variability instead of biological variations. We have included in the present manuscript the following sentence within the Figure legend section : « All qPCR experiments were conducted in duplicate and the mean Ct value was used for FC calculation. »

“The conclusion of the abstract is very generic and does not take into consideration the actual results of the current report”

We modified the abstract’s conclusion as follows: “Conclusion: This case demonstrated the applicability of peripheral mRNA expression as a way to monitor the natural history of MDE.”

“PBMC needs to be spelled out in the first time it is used.”

We have now spelled out the acronym in p4: “Peripheral mononuclear blood cells (PBMCs) were isolated from the blood by Ficoll density centrifugation.” We also added the acronym within the list of abbreviations.
“Please, explain what is “recurrent depressive cognition”?"
We modified the sentence for clarity which now reads within the Case presentation section on p5, first paragraph: “Following the MDE, the subject retrospectively reported soft depressive signs, such as isolated feelings of worthlessness and hopelessness, that first appeared at week 14 of the study.”

“Quality of written English: Needs some language corrections before being published”
We edited the current manuscript with the help of a native English reader.