Author's response to reviews

Title: ErbB3 mRNA leukocyte levels as a biomarker for Major Depressive Disorder

Authors:

Elena Milanesi (emilanesi@fatebenefratelli.it)
Alessandra Minelli (aminelli@fatebenefratelli.it)
Nadia Cattane (ncattane@fatebenefratelli.it)
Annamaria Cattaneo (cattaneo_annamaria@libero.it)
Cristina Mora (cristina.mora@med.unibs.it)
Alessandro Barbon (barbon@med.unibs.it)
Alessandra Mallei (alessandra.mallei@unimi.it)
Maurizio Popoli (maurizio.popoli@unimi.it)
Vincenzo Florio (vincenzo.florio@asbz.it)
Andreas Conca (andreas.conca@asbz.it)
Stefano Bignotti (sbignotti@fatebenefratelli.it)
Massimo Gennarelli (gennarelli@fatebenefratelli.it)

Version: 3 Date: 9 July 2012

Author's response to reviews: see over
Dear Prof. Deesha Majithia,

Thank you very much for your kind letter and for having give us the opportunity to present a revised version of our manuscript. We have carefully considered the reviewers’ comments and changed the manuscript accordingly (please see response to reviewers). All corrections were written in the manuscript in bold.

Once again we would like to thank you and the reviewers for their suggestions that have allowed us to improve the quality of our manuscript. We hope that the manuscript can be now suitable for publication.

Your sincerely

Massimo Gennarelli

Responses to reviewers: Revision MS: 1890581912682290

Reviewer #1

1) Although the authors corrected the age using logistic regression, a significant difference in age between patients and healthy subjects is problematic. Erbb3 expression level is known to show the age-related change in the brain (Colantuoni C et al. Brain Struct Funct. 2008 Sep;213(1-2):255-71). Thus, the authors should reconfirm these results using age-and-sex matched controls.

Regarding gender the two groups (MDD and controls) did not differ for this variable (p=0.28, Controls 89% female; 77% MDD patients). Furthermore, no correlation was found between ErbB3 T0 mRNA levels and gender in the whole cohort (p=0.17), in the controls (p=0.47) and MDD (p=0.37) samples, and with ErbB3 mRNA levels changes (p=0.42). Thus, we can consider that gender did not affect our data.

Regarding the age no correlation was found between ErbB3 T0 mRNA levels in the whole cohort (p=0.76), controls (p=0.52), and with ErbB3 mRNA levels changes (p=0.50). However, we obtained a correlation between ErbB3 T0 mRNA levels and age in MDD sample (p=0.004) where older people had higher levels and, as indicated in the manuscript the two groups (MDD and controls) differed for this variable (p=0.01). For this reason we corrected the data using logistic regression.

In this ANOVA no variables were significant apart the variable “group”. Indeed, the variable age was not significant (p=0.11) and the Partial Eta Squared Indice estimated the effect size was very low (p=0.06). On the contrary the Observed Power of the variable “group” was high (p=0.93). We have inserted this sentence for better clarify this issue.

In order to better clarify the results we fully modified the results section.

The Calantuoni’s paper showed that ErbB3 expression levels change with age before and after age 30 years in the controls group. No significant correlation was found with age in our healthy group.
2) Although the improvement of MDD symptoms assessed by MADRS correlated significantly and positively with the increase of ErbB3 mRNA levels at T12, there was no change in ErbB3 mRNA levels during antidepressant treatment. The authors should discuss about this discrepancy. I suspect that the only two patients whose delta ErbB3 mRNA levels% were increased to 120% and 240% made a significant impact on this correlation. As you suggested, we carried out the non-parametric Wilcoxon signed rank test without these two patients and the effect remained significant p<0.001. We have included this sentence in the manuscript. In order to better clarify the results we fully modified the results section.

3) The authors should show the data from experiments using rats.
We have inserted data from experiment using rats, as you suggested (see table 3).

Reviewer #2
1) It is better to show only the data of 26 patients with MDD. There are no reasons to analyze their samples with MDD separately (17 and 26 patients) and their sample size is not large to separate. For examples, mRNA expression levels of ERBB3 and FGFR1 genes in leukocytes from 26 patients with MDD and 19 control subjects were measured using a quantitative real-time PCR method. Expression levels from 17 patients were followed up after 12 weeks - treatments. We have better explained the subject section in order to avoid confusion in the readers. In order to better clarify the results we fully modified the results section.

2) It is better to show one table for samples’ information in methods (at Subjects), not in Results.
Done

3) They used three housekeeping genes. However, they showed only one data and it is unclear which ones they showed in results. They should show all data done by other two housekeeping genes using a supplemental table.
We have corrected the sentence in this way: “Target genes mRNA levels have been normalized on the arithmetic mean of β2 microglobulin (B2M; Hs99999907_m1), cytochrome c1 (Cycl; Hs00357717_m1) and ATP synthase, H+ transporting mitochondrial F1 complex β subunit (Atpb5; Hs00969569)” (page 10, lines 11-12) (methodological article: Larinov A et al., 2005. “A standard curve based method for relative real time PCR data processing”).

4) Is it really possible to calculate delta ERBB3? Their mRNA levels are relative expression, not absolute expression.
We agree with the reviewer that Delta is computed generally on absolute values. For this reason, we have expressed changes in ErbB3 mRNA levels as Fold Change using 2^-ΔΔCT method, as reported also in other similar studies (i.e. Belzeaux R. et al., 2010). Correlation remained significant. We modified the result section and Fig.2 has been modified accordingly.

5) P13 L7. They should change o=0.004 to p=0.004.
Done

6) In figure 2, they should write comments of numbers (controls and patients) analyzed and mean values of mRNA expression.
We have changed both figures and their legends.
7) Were mRNA levels of ERBB3 at T12 also significantly decreased compared with that of controls? ErbB3 mRNA levels in patients after 12 weeks of antidepressant treatment were still significantly reduced compared to T0 levels of controls (p=0.02). However, running the analysis including only patients who obtained a relevant amelioration of depressive symptomatology (ΔMADRS > 60%) the difference between patients (T12) and controls was lost (p=0.17). We could suppose an increase of ErbB3 levels when patients will be fully remitted. We have include this result in our paper.

8) They should show animal data in detail although they wrote data not shown. We have inserted data from experiment using rats, as you suggested (see table 3).

9) They concluded that ERBB3 could be considered a biomarker of depressive status in discussion. However, mRNA levels of ERBB3 were not changed before and after treatment (both decreased) and average MADRS scores after treatment looked low (6.53±4.58). So some patients who were not depressive status at T12 might show decreased ERBB3 levels. These suggest that ERBB3 may be a trait marker for MDD, not a biomarker of depressive status. Following your suggestion at point 7, we run the analysis including only patients who obtained a relevant amelioration of depressive symptomatology. On the basis of this new data, we partially modified the discussion sustaining the role of ErbB3 as biomarker of depressive status. However, the sample size is too small and further studies are necessary to confirm our finding.