# Gene expression profile associated with major depression and with antidepressant response

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## ABSTRACT

Despite the increasing variety of antidepressant (AD) drugs currently available, only a third of depressed patients respond adequately to the treatment, and up to half of them relapse within one year. Therefore, there is a pressing need to identify predictive biomarkers of treatment outcome.
With the aim to identify predictive biomarkers we focused the attention on 15 genes involved in HPA axis functionality , inflammation and neuroplasticity in leukocytes of depressed patients (n = 74) and controls (n = 49) prior to and after 8 weeks of AD treatment with escitalopram or nortryptiline (GENDEP study).
We found increased levels of MIF (F = 0.247, p < 0.0001), IL-1β (F = 117.925, p < 0.0001), TNF-α (F = 87.739, p < 0.0001), IL-6 (F = 86.279, p < 0.0001), a reduction in IL-4 levels (F = 5.580, p = 0.020), BDNF (F = 46.447, p < 0.0001), VGF (F = 37.333, p < 0.0001) and p11 (F = 12.086, p = 0.001) in depressed patients as compared to controls. Furthermore, in the group of depressed patients we also observed reduced GR expression (F = 63.161, p < 0.0001) and increased FKBP-5 levels (F = 69.389, p < 0.0001), but no differences in FKBP-4 (F = 0.148, p = 0.701).
Interestingly, when we compared the baseline gene expression levels in patients who responded versus patients who didn't respond to the treatment, we found that MIF, IL-1β and TNF-α were higher in non responder patients as compared to responder ($\eta 2$ = 0.656, F = 137.460, p < 0.0001 for MIF, $\eta 2$ = 0.333, F = 35.919, p < 0.0001 for TNF-α, $\eta 2$ = 0.431, F = 54.474, p < 0.0001 for IL-1β). Moreover, although each cytokine correlated with the treatment response, the best predictive model was when we added all the three cytokines together(adjusted R2 = 0.459, F = 21.686, P < 0.0001).
These results demonstrate that the baseline levels of MIF, IL-1β and TNF-α are able to predict the response to the pharmacological treatment and suggest that the development of new drugs blocking their effect could contribute to get a better response to the treatment.

## BACKGROUND

✧Despite the increasing variety of antidepressants currently available, only a third of depressed patients respond adequately to treatment, and up to half of them relapse within one year. Unfortunately, we still cannot predict the likelihood of response of an individual patient to a specific drug. Therefore, there is a pressing need to identify biomarkers that, assessed before starting treatment, predict future response, as well as biomarkers that are targeted by antidepressants and change longitudinally during antidepressant treatment.

✧Based on the current conceptualization of this disorder, we suggest that hypothesis-driven blood-based biomarkers analysis should focus on the biological systems that have been more consistently described as abnormal in depression: the glucocorticoid receptor (GR) complex, inflammation and neuroplasticity (Chopra et al., 2011).

✧One of the most consistent biological findings in depression is an hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis: a multitude of studies describing high levels of cortisol, the main HPA axis hormone, in the context of a reduced function of the GR, the cortisol receptor primarily involved in HPA axis regulation during stress. Moreover, this reduced GR function, or glucocorticoid resistance, is particularly evident in patients with treatment-resistant depression (Juruena et al., 2009), and indeed persistent glucocorticoid resistance during antidepressant treatment is associated with early relapse (Ribeiro et al., 1993).

✧A second biological system involved in the antidepressant response is inflammation. Pro-inflammatory cytokines, and in particular interleukin IL-1β, IL-6, and TNF-α, are increased in depressed patients as compared to controls; in turn, antidepressants have been shown to have anti-inflammatory effects, and anti-inflammatory drugs, such as celecoxib and TNF-α antagonists, have been shown to have antidepressants properties (Haroon 2012).

✧Finally, one of the potential mechanisms by which excessive HPA axis activity and inflammatory responses may contribute to the pathogenesis of depression is through inhibition of neurotrophic factors and hence disturbance of neuroplasticity. Indeed expression levels of neuroplastic molecules like BDNF have been found reduced in depressed patients (Cattaneo et al., 2010).

## METHODS

✧ We have analysed the expression levels of genes involved in HPA axis functionality (GR, FKBP-4 and FKBP-5), inflammation (IL1-α, IL-1β, IL-4, IL-6, IL-7, IL-8, IL-10, MIF and TNF-α) and neuroplasticity (BDNF, VGF and p11) in 74 depressed patients and 49 controls. Depressive symptoms were assessed by weekly administration of three established measures of depression severity: the clinician-rated 10-item Montgomery–Asberg Depression Rating Scale (MADRS), the 17-item Hamilton Rating Scale for Depression (HRSD–17) and the Beck Depression Inventory (BDI). The response to antidepressant medication was quantified as percentage reduction in MADRS score from baseline to week 12, and responder patients were identified as patients. Controls were screened using the Psychosis Screening Questionnaire (PSQ), and excluded if they met criteria for a present or past psychotic disorder. Controls were also excluded if taking any kind of hormonal treatment.

✧ Blood sample collection have been performed using PaxGene Tubes that were stored at –80 ℃ until their processing. RNA isolation was performed using the PAXGene Blood RNA Kit and the RNA quantity and quality was assessed by using a Nanodrop spectrometer and Agilent Bioanalyzer. Two micrograms were processed for Real Time PCR analyses using HOT FIREPol® EvaGreen® qPCR Mix (Solis BioDyne, Tartu, Estonia) according to the SYBR Green method. Pfaffl Method was used to determine relative target gene expression. Data are normalized to the geometric mean of all three reference genes and expressed as fold change from the vehicle treated control condition.

✧ Categorical variables were tested by means of Chi-square and Fisher's tests. Univariate analysis of variance was used for comparing the mean values of the mRNA levels of the genes of interest in patients vs. controls and in responders vs. non responders, while changes over time were analyzed using the General Linear Model (GLM) according to a repeated-measures design, with time (T0, T8) and response (yes/no) as a within-subjects factors.

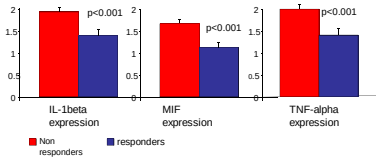
## RESULTS

### Biomarkers differences between patients at baseline, and controls

✧Depressed patients, as compared to controls, had higher FKBP-5 mRNA levels (+27%, F=69.4, p<0.0001) and lower GR mRNA levels (-18%, F=63.2, p<0.0001). Moreover, they had higher mRNA levels of the pro-inflammatory cytokines, IL-1β, (+48%, F=117.9, p<0.0001), IL-6 (+24%, F=86.3, p<0.0001), MIF (+32%, F=34.8, p<0.0001), and TNF-α (+58%, F=87.7, p< 0.0001), and lower levels of IL-4 (-9%, F=5.6, p=0.02). Finally, depressed patients had lower mRNA levels of the neuroplastic molecules, BDNF (-24%, F=46.5, p<0.0001), p11 (-16%, F=12.1, p=0.001) and VGF (-36%, F=37.3, p<0.0001).

### Baseline differences in biomarkers between responders and non-responders ("predictors")

Based on the percentage change in MADRS score, in our cohort of depressed patients we had 51 responders and 23 non-responders.



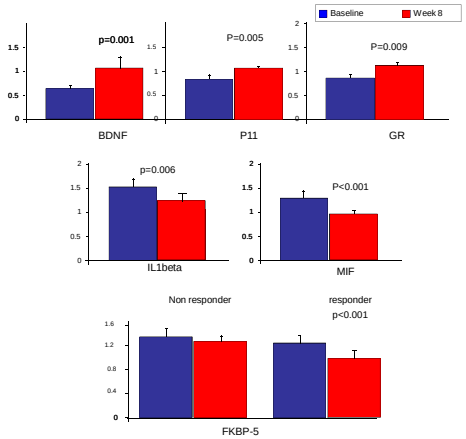

| | ΔMADRS t12 |
|---|---|
| IL-1beta | R= -0.56 p<0.001 |
| MIF | R= -0.62 p<0.001 |
| TNF-alpha | R= -0.44 p<0.001 |

✧Non-responders had higher mRNA levels of the three pro-inflammatory cytokines, IL-1β (+33%, F=55.9, p<0.0001), MIF (+48%, F=14.6, p<0.0001) and TNF-α (+39%, F=39.4, p<0.0001). Indeed the expression levels of each cytokine correlated with the treatment outcome.

✧When we examined the relative contributions of the three cytokines in predicting treatment response as a linear outcome by a linear regression model

| | adjusted R2 | pvalue |
|---|---|---|
| IL-1beta | 31% | <0.001 |
| MIF | 37% | <0.001 |
| TNF-alpha | 19% | <0.001 |
| IL1beta, MIF, TNF-alpha | 46% | <0.001 |

### Change in biomarkers ("targets") during antidepressant treatment




## CONCLUSIONS

✧Our main finding is that the expression levels of three pro-inflammatory cytokines, namely IL-1β, TNF-α and MIF predict the treatment outcome. In particular we found that the best predictive model was when we included all the three cytokines in the linear regression, suggesting that they tap in both similar and different molecular mechanisms. Moreover, we find that some genes are both abnormal in depressed patients (vs. controls) *and* are normalized by antidepressant treatment, but not in connection with antidepressant response (at least within the 8 weeks time-frame), suggesting that some biological abnormalities in depression are targeted by antidepressants pharmacological action but also in patients who are not improving.
Finally, a normalization of FKBP5 levels in depressed patients only suggest that depression is characterized by the coexistence of higher FKBP-5 and lower GR, leading to GR resistance, and that antidepressant treatment requires normalization of GR function via normalization of both genes.

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