Investigation of changes in the expression of human blood plasma proteins in major depressive disorder patients associated to an effective antidepressant response

1. Introduction

Depression (MDD) is a multifactorial psychiatric disorder with potentially debilitating consequences. It is treated through the use of antidepressants combined with psychological assistance. Despite that, up to 30% of the treated patients present adverse reactions, which can lead to the necessity of switching medications or combining different medications. Due to that, comprehending the blood plasma biochemical differences between good and poor responders may give us an insight into the molecular mechanisms related to a good response and consequently the proteins which can be used as response prediction markers. Through the use of immunoaffinity and liquid chromatography techniques combined with label free shotgun mass spectrometry, sets of differentially expressed proteins between good and poor responders(GR and PR), before(T0) and six(T6) weeks into treatment, were obtained. A total of 10.474 peptides, corresponding to 540 proteins were found. After the exclusion of keratins and the remnant high abundance proteins which weren't excluded by the immunoaffinity chromatography, 15 proteins were found to be differentially expressed ($p<0,05$) at T0 between GR and PR; 13 at T6 between GR and PR; 22 between T0 and T6 of GR and 42 between T0 and T6 of PR, through the variance analysis (ANOVA).

2. Results

The analysis of the principal components of the 15 differentially expressed proteins between GR and PR at T0 (Image 1), shows that they can, potentially, aid in the prediction of which patients will respond well or poorly to treatment, which can also be seen in the hierarchical grouping of the up and down regulated proteins in GR and PR shown in Image 2.

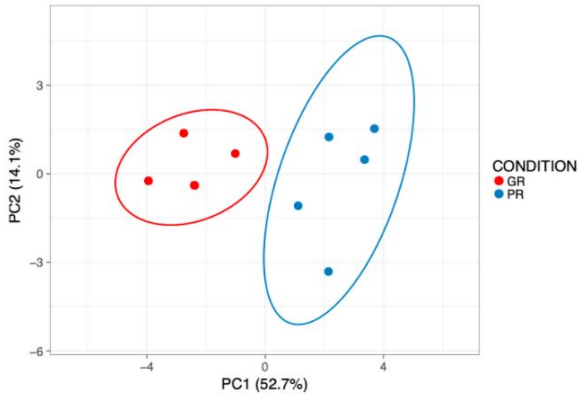

Image 1: Analysis of main components between good and poor responders at T0. Axes X and Y represent main components 1 and 2, which explain 14,1% and 52,7% of the total variance respectively. The prediction ellipses work in a way that there is a 0,95 chance that, in a new observation, the same group will be within the ellipse.

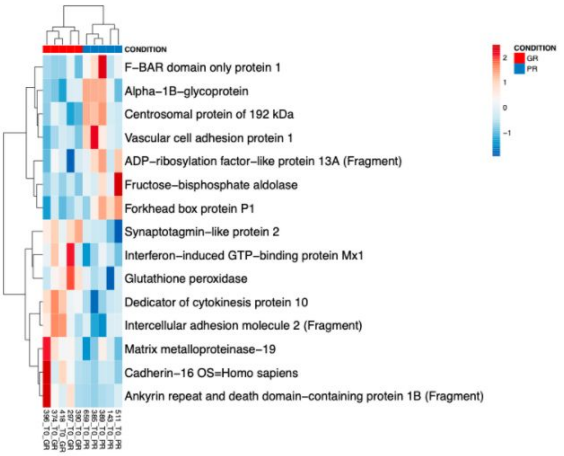

Image 2: Hierarchical grouping of the altered proteins between good and poor responders at T0.

The analysis of the principal components of the 13 differentially expressed proteins between GR and PR at T6 (Image 3), shows that they can, potentially, give us an insight into which pathways are differentially expressed in response to treatment, which can also be seen in the hierarchical grouping of the up and down regulated proteins in GR and PR shown in Image 4.

When analyzing the principal components of the 22 significantly differentially altered proteins between T0 and T6 in GRs (Image 5), we can see that these proteins make it possible to distinguish between GRs at T0 and T6. The hierarchical grouping in Image 6, on the other hand, shows one of the T6 samples grouped with the T0 samples. This can be due to a possible longer response time by the individual sampled as, despite this grouping, many of the proteins are expressed similarly to the T6 samples.

Finally, when analyzing the principal components of the 42 significantly differentially altered proteins between T0 and T6 in PRs (Image 7), contrary to the previously mentioned cases, there is an overlap between them, despite them still being considerably separate. This can be an indication of the less effective response to treatment in PRs. On the other hand, the hierarchical grouping of the proteins (Image 8) showed that the proteomic profiles are coherent with the T0 and T6 classifications. This can help in comprehending the PRs response to treatment, as the well-grouped proteins may show us which alterations, consequent to an antidepressant treatment, might lead to poor responses.

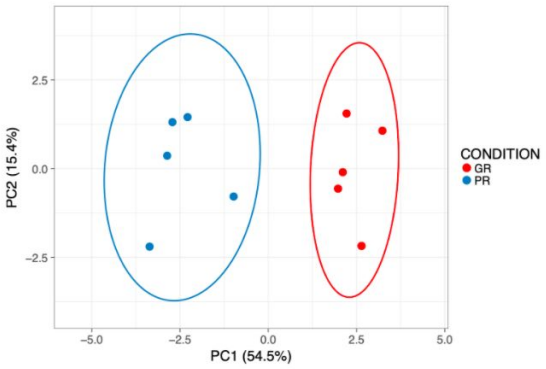

Image 3: Analysis of main components between good and poor responders at T6. Axes X and Y represent main components 1 and 2, which explain 15,4% and 54,5% of the total variance respectively. The prediction ellipses work in a way that there is a 0,95 chance that, in a new observation, the same group will be within the ellipse.

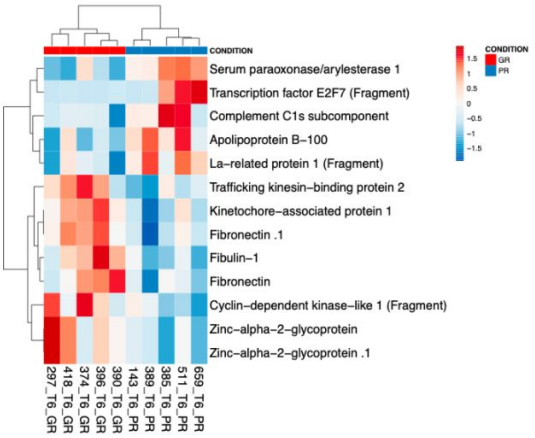

Image 4: Hierarchical grouping of the altered proteins between good and poor responders at T6.

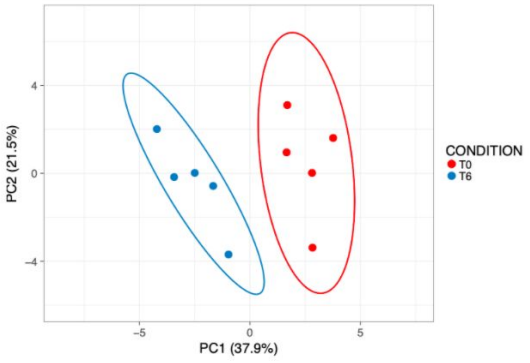

Image 5: Analysis of main components between T0 and T6 in good responders. Axes X and Y represent main components 1 and 2, which explain 21,5% and 37,9% of the total variance respectively. The prediction ellipses work in a way that there is a 0,95 chance that, in a new observation, the same group will be within the ellipse.

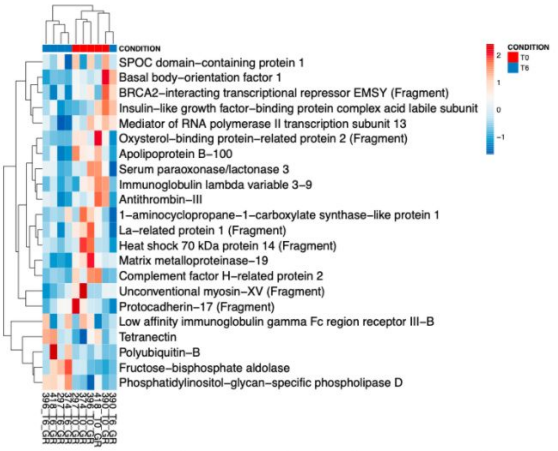

Image 6: Hierarchical grouping of the altered proteins at T0 and T6 in good responders.

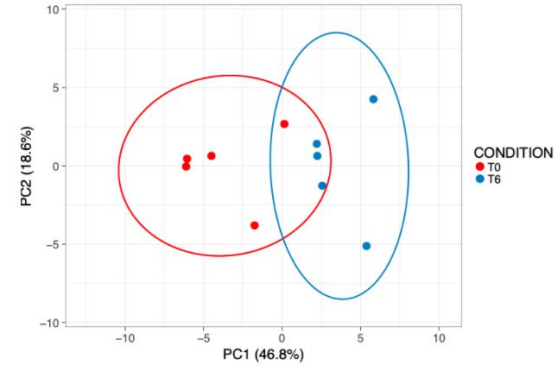

Image 7: Analysis of main components between T0 and T6 in poor responders. Axes X and Y represent main components 1 and 2, which explain 18,6% and 46,8% of the total variance respectively. The prediction ellipses work in a way that there is a 0,95 chance that, in a new observation, the same group will be within the ellipse.

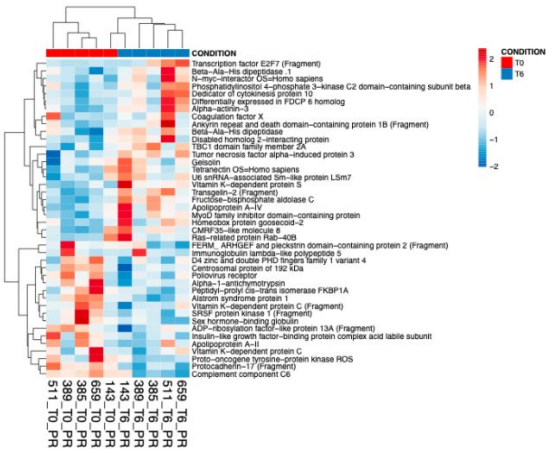

Image 8: Hierarchical grouping of the altered proteins at T0 and T6 in poor responders.

3. Discussion and conclusion

3.1 Differences between good and poor responders (GR x PR) before the onset of treatment (T0)

Many pathways can be seen altered between GR and PR at T0 when using the reactome tool (https://reactome.org), especially those related to cellular division, as well as pathways, such as the loss of proteins necessary for the organization of microtubules during interphase, loss of Nlp of mitotic centrosomes, AURKA activation by TPX2, centrosome maturation, among others. Other pathways, related to immune responses, were also shown to be altered, such as cell surface integrins interactions, interleukins 4 and 13 signaling, platelet degranulation and interferon gamma signaling.

Changes in the mitotic process can lead to cell death[1], which can trigger an immune response[2]. Taking into account the heterogeneous and complex characteristics of depression[3], diverse factors can lead to the different symptoms found in MDD. Therefore, there is a chance that the causes of the depressive symptoms in PRs are different from those in GRs. Two proteins which spiked particular interest were vascular cell adhesion molecule 1 (VCAM1), super expressed in PRs, and intercellular adhesion molecule 2 (ICAM2), super expressed in GRs. VCAM1 is frequently related to inflammation[4], which can be accumulated as a consequence of autophagy induced by tumor necrosis factor α (TNF-α) and Interleukin-1-beta, facilitating the adhesion of lymphocytes and endothelial cells[5,6]. It is also present in the cerebrospinal fluid and has been shown to be correlated with inflammatory mediators[7]. The slightest variation in ICAM2 concentrations can not only regulate cellular proliferation and survival[8,9], which, when dysregulated, can lead to apoptosis and consequently higher inflammatory responses, but also interfere in the inflammatory response as a whole[10–12]. It has also been described as a target of p53 in cancer cells, regulating their migration and invasion potentials[13].

3.2 Differences between good and poor responders (GR x PR) six weeks into treatment (T6)

Through the use of the reactome tool, the main pathways found to be altered between GR and PR at T6 are related to the extracellular matrix (ECM), integrins, cellular signaling and motility. Integrins, which are cellular surface adhesion molecules[14], can be activated by platelet aggregation[15,16]. One of the platelet functions, when activated, is to secrete the immune regulation Interleukin 10(IL-10)[17], which can lead to a reduction in inflammatory levels. Another pathway which caught our attention was an inflammatory pathway related to the creation of C4 and C2 activators, which are part of the complement signaling pathway[18]. Various inflammatory pathways are differentially activated between GR and PR, which can help us understand the differences between the responses, such as pathways related to apoptosis and inflammation, which were less expressed in GRs.

3.3 Differences between good responders (GR) throughout treatment (T0XT6)

Throughout treatment, through the use of the reactome, we can see that the main altered pathways were related to protein metabolism. Among the differentially expressed proteins, the two that caught our attention were paraoxonase 3 (PON3), found to be more expressed at T0 and specific glycan phosphatidylinositol phospholipase D (GLPD1), more expressed at T6. PON3 circulates in the plasma attached to the high density lipoprotein (HDL)[19,20], potentially having anti inflammatory effects aside from reducing the oxidation of low density lipoproteins (LDL) and oxidative stress[21–23]. When taking into account the fact that the brain can become more vulnerable to reactive oxygen species due to low oxidative enzymes activities and high concentrations of oxidative lipids[24] and that PON3 levels were lower at T6, this alteration can be a consequence of a reduction in inflammatory and/or oxidative stress levels. Some studies have found a correlation between low levels of GLPD1 in the cerebrospinal fluid and blood plasma, and neurodegenerative disorders[25]. Therefore, its higher expression at T6 in good responses could be related to a reduction of neurodegeneration and myelin loss.

3.4 Differences between poor responders (GR) throughout treatment (T0XT6)

When comparing the differentially expressed proteins between poor responders through a 6 week period of treatment, we can find pathways related not only to ~~the~~ lipid metabolism, potentially related to myelination[26], but also cytoskeleton alterations and centrossome organization, alterations which can be related to apoptosis[27], as well as to the formation of amyloid fiber, potentially induced by inflammation and related to neurodegenerative disorders[28]. In PRs, we see alterations compatible with the hypothesis that enhanced inflammatory responses can indicate an inadequate response to treatment.

3.5 Differences between good and poor responders throughout treatment

The main differences between GR and PR throughout treatment can be seen in Image 9. In GRs, we could see alterations in pathways of both lipids and carboxylic acids processes, which might contribute to the better response to treatment. There is a correlation between lipid metabolism and depression not only in animal models[29] but also in patients[30]. The myelin sheath, which involves neurons, is formed by bi-layers of lipids[31] which, in animal models, are known to be continuously exchanged at different speeds throughout adult life[26]. Aside from that, some studies indicate a reduction of myelin in MDD patients[32,33], which can lead us to the conclusion that alterations in the lipid biosynthesis processes, lower at T6 in GR, can be a consequence of the reduction of demyelination and influence patient's response to treatment.

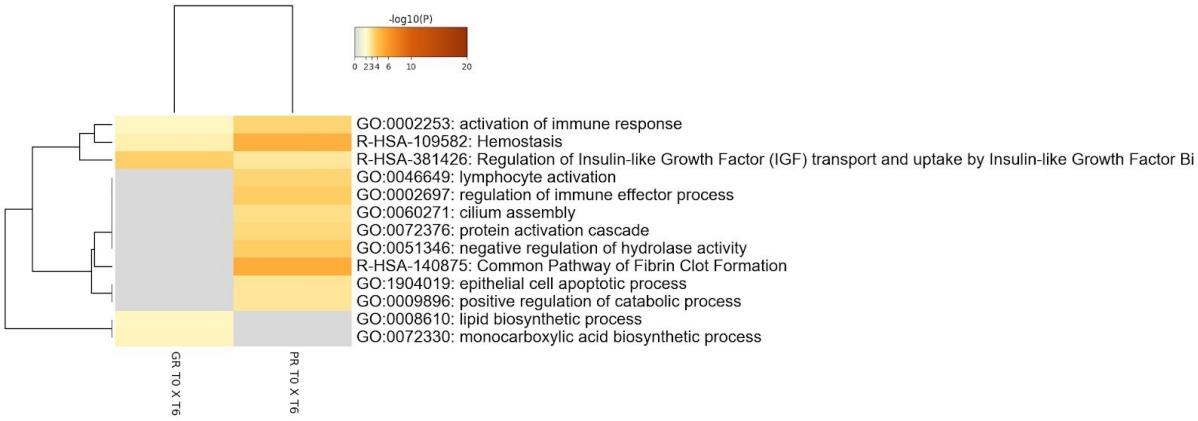

Image 9. Enriched terms between good and poor responders throughout treatment, showing the main differences.

When it comes to the PRs, reduction in other pathways such as fibrin coagulum formation, regulation of the immune effector system and lymphocyte activation were found. Fibrins coagulum can be formed after the proteolytic cleavage of fibrinogen[34], a coagulation factor which has already been pointed out as a potential response marker[35]. This factor can be deposited in the brain in cases of blood brain barrier (BBB) disruption[36], which can be caused by inflammatory processes[37]. Therefore, a higher effector process of the immune system, potentially disrupting the BBB, combined with a deposition of fibrins in the brain, could explain their poor response to treatment, despite the need for further studies to corroborate this hypothesis.

To sum up, alterations in the thrombotic states of the patients could be seen, data consistent with the fact that a hypo thrombotic state as well as differential expression of fibrinogen can be related to a depressive state[35,38]. Aside from that, PRs presented higher inflammatory levels and the activation of different apoptotic pathways, when compared to GRs. A common characteristic among MDD patients is cognitive and memory loss, which can be related to inflammation and synaptic elimination mediated by microglia[39]. In GRs, we mainly found pathways related to protein metabolism, which could be related to myelination, as well as a reduction in inflammation. Adding to that, some studies show that treating PRs with non-steroidal anti-inflammatories can lead to a better response[39]. Therefore, higher inflammation levels and apoptosis can act as a response prognosis, which can lead to a better adapted treatment for those patients.

4. References

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