Cell Type Specific Gene Interaction between Microbiota and Antidepressant Drugs

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Abstract: Major depression is one of the typical psychiatric diseases. When psychiatric disease occurs, the function of brain cells may be influenced by some intestinal microbiota, which provides a pathway for the development of antidepressant drugs. The research on the correlation between microbiota and antidepressant has become a key issue in the treatment of major depression. This work explores the cell type specific gene interaction between microbiota and antidepressant drugs and isolates some potentially important genes that can be targets of further investigation in the mechanisms of how depressive related behaviours occur and act. Our analysis provides a deeper demonstration of the interaction between antidepressant and microbiota compared to original study, by means of an effective genetic matrix and PLS model.

Key words: Genetic correlation, microbiota, antidepressant drugs, PLS model.

1. Introduction

Over the last decade, there has been an increasing understanding how intestinal microbiota influences different aspects of brain development, function, and behavior [1]. Some studies have shown gut microbiota affect the function of brain cells, thus resulting in anxiety and depressive behaviour and corresponding psychiatric diseases [2].

Major depression is one of the most common psychiatric illness, it can be treated by antidepressant drugs such as Prozac and Duloxetine, which are serotonin reuptake inhibitor (SSRI) [3]. These drugs can inhibit the reuptake of neurotransmitters such as serotonin, lead to increased synaptic concentrations, which may have therapeutic effects such as promoting neural plasticity and adapting downstream signaling pathway [4].

Thinking about important role of microbiota in major depression, it is interesting to investigate whether antidepressant drugs have influence on microbiota. In fact, some studies have shown antidepressants have antimicrobial effects against some microbiome through inhibiting motility of bacteria and production of slime [5]. This may result from the genetic interaction between microbiota and antidepressants. Therefore, it is of great interests to look at how they influence each other from molecular biological perspective.

In fact, there are some studies investigating genetic interaction between microbiota and antidepressant drugs. In the experiment in the former research [1], mice were treated with antidepressants, their microbiota was analysed using RNA sequencing in the medial prefrontal cortex (mPFC) and their depressive behaviour were assessed. The study did comparison on individual genes using DEG analysis and clustering based on correlation and GO analysis. They also show treating mice with microbiota such as R. flavefaciens can

attenuate anti-depressive effect of drugs.

However, in their study, the mRNA dataset analyses carried out were straight forward and they did not isolate genes underpin interaction between microbiota and depression. The bulk tissue sequencing means the cells are mixed before sending to sequencing [6]. Therefore, there are cells that are exclusively expressed in one type of cells. However, by looking at specific cell types, it is easier to get idea that if there is any change, which cell type does it occur to the largest extend. Thus, it is necessary to have cell type categorization. The most typical cells in the central nervous systems are neurons, microglia, and astrocyte. It is known that antidepressants such as SSRI acts through synaptic transmission which happens in neurons [7]and some studies show gut microbiota have more effects on microglia than other cell types [8], [9]. Thus, one can hypothesize antidepressant will have more effect on gene expression in neurons, whilst microbiota affects more on microglia.

Here, we extend the former work [1] and move on. Firstly, we compare the genetic correlation of mice across drug-treated, microbiota-treated, and double-treated groups to see the general trend. Then, we use an existed Barre's data base [10] to categorize the genes that is cell specific. Moreover, we use partial-least square regression (PLS) to look deeper how microbiota interacts with antidepressant drugs within 3 cell types, and we isolate these overlapped genes, which can be novel therapeutic target to understand the molecular mechanisms how microbiota affects efficiency of antidepressants acts on central nervous system.

2. Method

2.1. Resource

Dataset source is from [1], which is bulk tissue mRNA sequencing (mPFC) from mice treated with different conditions - Sample 1-6 is vehicle group, 7-12 is antidepressant treated group (DUL), 13-18 is microbiota treated group (RUM), 19-24 is double treated group (DUL+RUM).

5 antidepressants were used, diluted in PBS and the doses are shown as following: fluoxetine 10mg/kg, escitalopram 10mg/kg, venlafaxine 10mg/kg, duloxetine 10mg/kg and desipramine 20mg/kg, they were delivered every day in 8ml/kg fashion. The control group had same volume in PBS. Mice all treated with antidepressants 21days before they collected stools.

Bacteria Ruminococcus flavefacien and Adlercreutzia equolifaciens were got and cultured in anaerobic condition. Each mice colony forming units were suspended in PBS and delivered suing gavage. Control group, of course gavaged with similar volume of PBS. Mice were all treated with microbiota each day for first 3 days, then twice each week until the end of experiment, they the behaviour test were done.

2.2. Genetic Association

Firstly, we compare genetic correlation o samples to see general trend. Correlation matrices were generated using MATLAB.

2.3. Cell Type Categorization

Using Barre's database [10] to get a list of gene expression in different cell types, we find astrocyte specific, neuronal specific and microglia specific gene list by setting threshold of differential expression. Use the threshold so that the expression of gene in dedicated cell type is at least 5 times higher than maximal expression of rest cell types.

In MATLAB create a normal variable from 0/1 flags which corresponds to gene name. To know the index of cell-type specific genes in original data, we 1. create a raw_gene_name variable 2. Past gene names from raw data into it 3. Create cell-type flags 4. Fill in the cell-type flags 5. Do the same for 2nd and 3rd columns of the gene name. Then we can find shared genes for astrocyte (156), neuron (283) and microglia (360).

2.4. Look at Cell-Specific Expression Change

Look at changes between control and treated groups in different cell types since different treatment may preferentially affect one type of cells.

1) Generate change ratio between conditions, take a mean of each gene and calculate a ratio between two groups.

2) Generate violin plots to show distribution using violin plot package.

2.5. Partial Least-Square Regression (PLS) to Cell-Specific Genes

To see whether microbiota and antidepressant are interacted, it is important to find the direction of change in high-dimensional space. Run PLS with neuronal genes, astrocyte genes and microglia genes along no treatment vs. dul treatment data to see if antidepressant treatment has a distinct expression pattern that is consistent across samples. Then, do the same for rum group and finally look at the interaction with double treated group.

From the PLS, we can see Rum changes the expression to some direction, Dul changes expression to some other direction, but how similar are these directions and how do they interact is still unclear. We solve this by projecting Rum+Dul group to Rum or Dul only and see the direction. We contrast the changes we see in Rum+Dul with changes with Rum and Dul only to look at whether the data agree with a pure additive effect between Rum and Dul, or the interaction has some unexpected result. Finally, we inspect PLS weights to find what are the genes underly all these changes and we use graph to see their relationship.

2.6. Projecting the Rum+Dul Group to Rum or Dul Only

Since Rum changes expression to some direction, Dul changes expression to some other direction, how similar are these directions and how do they interact is unclear.

Since PLS is constructing new directions in the data that maximally correlated with the assigned direction through linear combinations, one can predict new data points into the new directions and see where they locate in the new space. Then based their projected coordinates, one can see which group the new data points are closer to in the designated direction.

We project RUM+DUL group to DUL/CON direction with neuronal, astrocyte and microglia genes. By projecting Rum+ Dul group to Rum or Dul only and see the direction.

To formally quantify the closeness, mahal command in matlab can be done. Then, one can see whether the data agree with a pure additive effect between Rum and Dul, or the interaction has any other results by contrasting the changes seen in Rum+ Dul with the changes with Rum and Dul only.

If the projected points (RUM+DUL) are not closer to CON, it is likely that 1) RUM and DUL treatment changes expression in the same pattern, so combined group stays the same pattern. 2) The changes by RUM are orthogonal to changes by DUL so that combined group show no interaction.

If the projections are closer to CON, this would suggest that RUM and DUL treatment have some common but differentially regulated genes, so that the combined group show changes from the single treatment clouds. Tsne needs to be done to make sure not fooling we by looking at the first 2 PLS dimensions, however, it is more likely that the second scenario is true if it is consistent with ^[1] reported.

2.7. Finding Genes That under All These Changes

We will inspect PLS weight to find these genes and use graph to see their relationships. To get better handle of affected genes, it is important to look at genes with high weights in the PLS models. The selection of high weights was done by plotting histogram, decide a threshold in each PLS model about RUM vs CON and DUL vs CON. The higher the number, the higher the weights in the model. Then combine 2 dimensions together, compare the indices for each cell types between the RUM/CON and DUL/CON models.

Alternatively, one can show the similarity/difference between the PLS directions between treatments, with one model one cell type by plotting the ranking of genes.

Finally, names of overlap genes and expression level plot were done using software of MATLAB.

3. Result and Discussion

3.1. Gross Correlation Matrices between Samples

24 samples, each has 15456 genes were compared. The results are shown in Fig. 1. Yellow represents for high correlation while blue is low correlation. DUL group has only a few changes in their correlation. By contrast, RUM and RUM+DUL group gene expression pattern become obviously less correlated.



Fig. 1. Gross correlation matrices between 24 samples.

3.2. Looking at Cell-Specific Expression Change across Different Conditions

After getting cell-specific (astrocyte, neuronal, microglia) genes from Barr's dataset, we use violin plot and generate the cell type specific gene expression pattern. From the Fig. 2, microbiota changes preferentially affect microglia since rum_vs_con is much higher than dul_vs_con. However, for neurons and astrocyte, although microbiota also has more effect than antidepressant, it is not obvious.



Fig. 2. Fold change for different cell type.

3.3. PLS with Neuronal Genes, Astrocyte genes and Microglia Genes along No Treatment vs. Dul Treatment Data

We want to see if antidepressant has different expression pattern that is consistent across samples. Accoding to the Fig. 3, we can see the change of expression pattern is consistent across individuals so we can find some common directions in the data to split them - they all have some sort of expression footprint due to Rum or Dul treatment.



Fig. 3. PLS with 3 cell type genes along treatment and control groups.

3.4. Projecting the RUM+DUL Groups to the DUL/CON and RUM/CON Direction for All Three Cell Types

We project Rum+Dul group to Rum or Dul only to see the direction of change and interaction. We use the mahal command to quantify the closeness and it shows projected points (Rum+Dul) are closer to CON for neuron and microglia cells than astrocyte, as shown in Fig. 4. This means the changes by RUM are orthogonal to changes by DUL so that combined group show no interaction only in astrocytes, but for neurons and microglia, there RUM and DUL treatment has interactions. The projection data would suggest that neurons and microglia will have some overlap but astrocyte not.



Fig. 4. Projecting DUL+RUM groups to DUL/CON and RUM/CON directions for 3 cell types.

3.5. Look at Genes with High Weights in the PLS Model and Their Expression Pattern

We show the similarity/difference between PLS directions between treatments by plot the ranking the shared genes, as shown in Fig. 5.

A small script was written that one can generate a null distribution and give the plot a p-value. For example, in the astrocyte plot, when x=8, y=2, it means when we choose top 8 from both models, there are 2 overlapped ones. The astrocyte genes have in 156 totally in our model, so this script simulating 1000 times, picking random 8 from a total of 156 twice and plot the probability of getting overlapped numbers.

If we simulate picking random 8 from total of 156 (Astrocyte) twice and the probability of getting overlapped numbers is >0.005. But picking 8 from 283 (neuron) and getting 3 overlap over has 0.0003 chance. And 8 from 360 (microglia) and getting 6 overlap has about 0.0001 chance. It strongly indicates that RUM and DUL has some overlapped but differential modulation on neuronal and microglia genes rather than astrocyte.



Fig. 5. Plot Ranking of Shared Genes for 3 Cell Type.

3.6. Finding the Name and Expression Level of Overlapped Genes

For astrocytes, there are 4 overlapped genes which is Abr, Dgkh, Grin2a and Mgll. For neurons, they are Atp1b2, Cacnale, Dpys12, Gas7 and Kcna2 and for microglia they are Adgrl1, Gpr26, mt-Nd2, Parcl1, Slc12a5 and Sv2b, they have different expression levels across different conditions, as shown in Fig. 6.



Fig. 6. Name and expression level for overlapped genes.

In this study, we firstly created the genetic correlation matrices to see overall effects of gene expression of mice. Antidepressant treated groups express the similar patterns as control groups. However, when adding microbiota, the expression pattern become more distinct and the double treated groups how less distinct pattern, which means 1. Adding antidepressant has minimal effect to gene expression. 2. After treating microbiota to sample, their expression pattern becomes more different. 3. There are some interactions between microbiota and antidepressants with regards to gene expression, probably microbiota can attenuate the effects of antidepressants.

This may be due to mechanisms of antidepressants such as SSRI or SNRI, their action is achieved by inhibiting reuptake of neurotransmitter and increase the concentration within synaptic cleft and results in down-regulation of neural signalling pathway [11], therefore, their genetic change might be minimal. By contrast, some studies indicate microbiota might influence the production of serotonin through enterochromaffin cells in the gut [12], which may elucidate a potential signalling pathway.

Then, we did cell categorization, we got – neuronal, microglia and astrocyte specific genes. It is consistent with our original hypothesis that antidepressant treatment specifically affects neuronal genes. Interestingly, microbiota treatment has much larger footprint on gene expression overall, regardless of cell type. It is contradicted to our hypothesis since microbiota influenced all types of cells, not only microglia.

Next, to see whether microbiota and antidepressants are interacted, we ran PLS model for 3 cell types of gene. We found that due to drug or microbiota treatment, antidepressant changes expression to one direction and microbiota changes expression to the other direction, however, there are some common directions in the data and we want to confirm how similar are these directions and how do they interact within each other.

By projecting the data in PLS models, we found for astrocyte, the change of antidepressant and microbiota treatment is orthogonal, therefore no interaction. However, for microglia and neuron, antidepressant and microbiota treatment have some common but differentially regulated genes, so that the combined group show changes from the single treatment clouds.

Also, by the statistical methods, that is hypothesis testing through random simulation, we conclude that microbiota and antidepressants has some overlapped but differential modulation on neuronal and microglia genes rather than astrocyte. It strongly indicates there are overlapped but differential modulation on neuronal and microglia genes.

Therefore, we found these genes by ranking their weights within 3 cell types and finally we sorted out their names and expression level across different treatment – for neurons, there are 5 overlapped genes and microglia has 6 overlapped genes. After the treatment, some of their expression significantly increased and some decreased, showing different regulation pathways. For example, for Atp1b2, it is upregulated when treating with antidepressant and/or microbiota. These candidate genes are important for further investigation in mechanisms of depressive behaviours or possibility to be a potential therapeutic target.

4. Conclusion and Perspective

This analysis provides a more compelling demonstration of the interaction between antidepressant and microbiota compared to original study, by means of an effective genetic matrix and concise experiment design. We isolated some potentially important genes that can be targets of further investigation in the mechanisms of how depressive related behaviours occur as well as the interaction between microbiota and central nervous system.

However, there are several limitations for our current study. First, the experiments are done by mice and it may not be recapitulated in human. We expect the corresponding result appears in human vivo experiment after some safe improvement. Next, we only investigated SSRI and SNRI types of antidepressants, the microbiota in our study only includes Ruminococcus flavefacien and Adlercreutzia equolifaciens. Since

different antidepressants as well as microbiota have different mechanisms, therefore this study is less representative and further research needs to be done in other types of psychotropic drugs. Finally, we only isolate the overlapped gene names and expression level, which leaves empty room for further researches on the functional importance of these candidate genes.

Conflict of Interest

There is no conflict of interest in this paper. The author hereby declare that I have never received any thirdparty funding or services for this research. There is no economic relationship between the author and other units or enterprises. There are no other relationships or activities that may affect or potentially affect the content of this article.

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