Title: Analyzing the Energy Expression of Genes related to Major Depression Disorder

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Abstract

It is clear the genetic to increase risk in Major Depression Disorder (MDD). Both from a genetic and epigenetic point of view, biological markers are a factor that help improve diagnosis and therapy. The aim of the study was to determine how these genes related to major depression could be expressed in the whole brain. We analyzed 18 MDD-related genes that were previously reported in meta-analysis studies and determined the expression of these genes in the donors in the database of Allen Brain Human Atlas (2010). After determining the expression profiles, an analysis of coexpression networks was used to determine the relation between the expression of this group in the data generated by the Mouse Allen Brain Atlas (2004) in order to corroborate that there was no relationship in another model also used for the genetic study of psychiatric disorders. Qualitatively no relationship was found between the genes reported in MDD which would indicate a multiplicity of markers and correspond to the difference between individuals. Open access tools are an important source of evaluation of previous hypotheses and validation of prior consent that could lead to further research.

Keywords: Gene expression, Brain expression, Neuroanatomy genetic, Major Depression Disorder.

Introduction

Mental disorders has a neuronal correlate that has been studied from genetic markers [1,2]. Some studies has shown that the genetic to increase risk in Major Depression (MDD) suggesting a role in inheritance of disorder [3–5]. This without ignoring the contribution and enormous advance in the investigation on epigenetics and the factors of interaction that could influence in the development of the disorder, as well as its diminution from environmental interactions [6–9]. However, the inheritance of predisposing traits is a large field of study that has been approached in isolation by identifying specific and particular genes. The study of large samples with extensive screening, in genetic mapping studies (genome-wide association studies [GWAS]), also called genetic epidemiology, could indicate variants of genes that are related to the pathology [3,10–12].

This interest results from two major aspects. On the one hand, the high prevalence of the disease that has been reported and the great cost for health systems. Second, the great advances in the tracing of genetic markers that could allow a more effective intervention, pharmacologically or enhance some protection factors. Although there has been strong research on pharmacological interventions or psychotherapy, finding report that it is not possible to apply the same treatments in all cases, suggesting biological differences between individuals [13–16]. It has been argued that these differences are the particular genes that are called markers and it is clear that the study of these genetic markers has allowed to identify the risk in patients. It is such as recently Flint and Kendler (2014) signaling several genetic aspects related to depression and indicating the importance of genes directly related to the disorder. Their position is related to the classification of several subtypes of the disease that could correspond to specific markers, which could be related to other genetic markers [17].

On the other hand, the efforts to trace broad-spectrum genetic characteristics at the neural level have been very useful for the validation of previous knowledge [18–22], as well as the reconstruction of information from new discoveries [23]. These tools have allowed to validate studies in animal models compared with information in humans [24]. The potential

of the information increases if it is possible to explore this information through the use of open access tools, which may facilitate the exploration of characteristics such as genetic markers in donors from Human Allen Brain Atlas (2010). Additionally, the assessment of the information could increase it if databases used allows show the information in animal models traditionally used in the analysis of neuropsychiatric pathologies such as the mouse CBL57/6J from Mouse Allen Brain Atlas (2004).

The present article had two objectives. First, to explore the expression of genes at the brain level related to alterations of depression in the donors of the Human Allen Brain Atlas (2010). Second, comparing the expression of such genes in the mouse CBL576/J using co-expression networks to compare them with the distribution of the first objective. With the before, we can on the one hand to trace the expression of genes in humans at the level of the whole brain and on the other by qualitatively comparing said organization with the expression of genes in the brain of mice CBL57/6J this due of its wide use as a model of neuropsychiatric diseases.

Method and Procedure

The genes were selected from reported in MDD meta-analysis studies that consistently report alteration with significance in the study population mentioned by Flint and Kendler (2014) (Tabla 1).

Gene expression

Once identified the genes were searched manually and the expression energy values were downloaded from the complete set of data from the six human donors of the Allen Human Brain Atlas (http://human.brain-map.org/) (Table S1). We analyzed human gene expression data, obtained using the microarray technique, through a histological analysis and a microarray profile of more than 900 structures in six donors [25]. The procedure implies that they collected "approximately 500 anatomically discrete samples from cortex, subcortex, cerebellum and brainstem of each brain and profiled for genome-wide gene expression using a custom Agilent 8 × 60K cDNA array chip" [26]. More information about collect and extraction information in http://help.brain-map.org/display/humanbrain/documentation/. Each gene of table 1 was searched and download in R, after a matrix with values was done. The missing values (NAs) were removed and a heatmap with dendograms were make for

each donor using the d3heatmap package [26]. First, the data were pre-processed by software packages included in the R-project (http://www.R-project.org) and Bioconductor (http://www.bioconductor.org). An unsupervised hierarchical clustering analysis was made of the rows of data matrix. So, the data was re-ordered according to the hierarchical clustering result by the matrix analysis in dendograms. Thus, the results putting similar observations close to each other. The blocks of 'high'and 'low' expression are adjacent in the data matrix and visually inspected. The method of measuring distances was Euclidean. Before cluster analysis, the matrix data were standardized using the function *scale()*, other packages used were *cluster(), factoextra()* and *ggplot()*.The cluster analysis was realized over standardized data of each matrices. This function for data standardized was definited as

$$\frac{x_i - center\ (x)}{scale\ (x)}$$

Energy expression

Additionally, the data from mice was downloaded from Allen Mouse Brain Atlas (2004) (http://mouse.brain-map.org) that was obtained mediating In Situ Hybridation proceduce (ISH). For this ISH patterns of gene expression, mice of 8-week old male C57BL/6J were used, briefly using a semi-automated process in which the brain was divided into 25 µm sections at intervals of 100 µm to 200 µm [27,28]. The slices were after hybridized with digoxigenin (DIG)-labeled riboprobes. Finally, a camera with 0,95µm/pixel resolution information was used, then analyzed and quantified using software for measuring signal intensity [27]. Despite the limitations of this method for identifying expression, it can be assumed to be reliable thanks to the control procedures and similar corroboration techniques [29].

The energy expression was defined as the sum of expression pixel intensity for each gene divided by the sum of all pixels in division. The energy expression (*E*) for each gene is represented in a voxel where Grange et al (2012) definite as "weighted sum of the greyscale-value intensities *I* evaluated at the pixels *p* intersecting the voxel:

$$E\ (v, g) = \frac{\sum_{p \in v} M\ (p)\ I\ (p)}{\sum_{p \in v} 1}$$

Where, *v* is voxel, *g* is gene *M(p)* is a Boolean mask that equals 1 if the gene is expressed at pixel p and 0 if it is not " [30]. Expression energy was then computed for each brain structure delineated in the Allen Mouse Brain Atlas (2004).

Results

The heatmaps show several particularities of gene expression. The division groups in the dendrograms vary according to the patients (Figure 1). The division made in the dendograms of 4 groups was established according to the optimum cluster methods, but the grouping of each of the individuals does not correspond neither to structural activation, that is to say, by anatomical profile of expression, nor by groups of genes that would be expected with those of similar function. It is also interesting that is not homogeneous distribution of expression levels between subjects. Although there are constant expressions, for example, the 5HTR2A gene usually has the same pattern of expression between individuals, the overall expression profiles was different. No homogeneous expressions were found that responded to possible anatomically localizable functions so that it could be a reflection of the variety of genes and functional groups from which each one proceeds, for example serotonin most but also of other types, which could explain heterogeneity.

Although not conclusive could be according to two perspectives. On the one hand the provision of some of the profiles presented may correspond with a greater predisposition to depression, on the other, taking into account that the diagnosis and expression is particular to each subject could be expected configurations as long as there is no further information from the subjects. It is also not possible to make a gender comparison because there is only one female among the donors. Although the result appears negative in the cluster analysis, it may be favorable as it is not being analyzed a population with pathology apparently. Since finding high patterns of expression and organization could be configured in an argument against participation in specific pathologies. In order to evaluate the correlation between these genes, we also sought to compare this group of genes in expression in the murine model.

Thus, in murine model the results are shown if Figure 2. From visual inspection was found that according to the cdf function the genes related to MDD present a lower expression to the function that is given by chance which would indicate non grouping of coexpression between

these genes. It does not find a difference outside the standard deviations of the cdf function, which does not clearly identify this difference in the co-expression network. It is possible due to that are genes implied in different functions but this is similar to find in human analysis by cluster analysis. Another important factor could be amount of genes because were found only 18 genes that is small.

Discussion

One area that has benefited greatly from technological advances in genetics and sequencing equipment is genetic epidemiology through genome-wide association studies (GWASs) which have suggested candidate genes as well as population variants related with MDD and their differential distribution between men and women [1,31].

The development of this type of research seems to validate studies with isolated genes that are related to some mental disorders [5,32]. In this study, no relationship was found in the anatomical expression of genes related to depression, which would seem to support the hypothesis of being a multisymptomatic condition and that such as suggested by some authors should be classified to patients with MDD in different subtypes [17]. Two important aspects in the identification of genes come from the GWAS as determinants of marker prevalence in the disease that has been suggested might not necessarily identify the loci genes. Second, less frequent variations of genes and that could contribute to the disease, could also contribute to non-homogeneity of expression and pathology. Thus, a complexity has been found in a number of genetic loci that could contribute to disease susceptibility.

As for the comparison with the network of co-expression in mice, neither co-expression is presented again indicating what was reported in the cluster analysis. It is important to indicate that this correspondence could suggest a good way to corroborate from the genetic models the possible or not relation [24]. In this sense, one of the objectives of the text was to facilitate and encourage the consultation of open access data for the understanding and studies of pathological phenomena with open access tools and developed with very high quality standards [30,33–35].

It is clear that studies of expression of the complete genome allow to identify characteristics that are unknown in isolated studies. The importance of identifying and conducting studies on open access data, such as Allen Institute for Brain Science, allow the indirect validity of traditional research in the field of behavioral genetics.

The importance of psychiatry or genetic psychology is such that it is possible to intervene in potentially harmful behaviors for patients and society. Thus, some studies have attempted to find suicide risk for their obvious intervention and prevention [36]. From the point of view of functional factors, cognitive abilities have been linked to genetic markers that would potentially help to understand the effects of therapy or recovery, as well as the difference in psychological processes [37–39].

One of the important limitations of the study is the limited number of donors, as well as the absence in the medical history, which does not allow to relate the genetic profile of each one with possible behavioral alterations. The disparity in these profiles could be one way of understanding the difference in environmental interactions.

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common variants. J. Psychiatry Neurosci. Canadian Medical Association; 2009;34:361–6.

Table 1. List of genes related with MDD considered in this study

Gene-id	Gene Symbol	Gene Name	Entrez-id	Chromosome
1624	ACE	angiotensin I converting enzyme (peptidyl-dipeptidase A) 1	1636	17
624	BDNF	brain-derived neurotrophic factor	627	11
9433	CLOCK	clock homolog (mouse)	9575	4
1303	COMT	catechol-O-methyltransferase	1312	22
1802	DRD3	dopamine receptor D3	1814	3
1803	DRD4	dopamine receptor D4	1815	11
2540	GABRA3	gamma-aminobutyric acid (GABA) A receptor, alpha 3	2556	X
2767	GNB3	guanine nucleotide binding protein (G protein), beta polypeptide 3	2784	12
3330	HTR1A	5-hydroxytryptamine (serotonin) receptor 1A, G protein-coupled	3350	5
3331	HTR1B	5-hydroxytryptamine (serotonin) receptor 1B, G protein-coupled	3351	6
3338	HTR2C	5-hydroxytryptamine (serotonin) receptor 2C, G protein-coupled	3358	X
3336	HTR2A	5-hydroxytryptamine (serotonin) receptor 2A, G protein-coupled	3356	13
3342	HTR6	5-hydroxytryptamine (serotonin) receptor 6, G protein-coupled	3362	1
6496	SLC6A4	solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	6532	17
4103	MAOA	monoamine oxidase A	4128	X
4498	MTHFR	methylenetetrahydrofolate reductase (NAD(P)H)	4524	1
6494	SLC6A2	solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2	6530	16
6495	SLC6A3	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	6531	5
7123	TPH1	tryptophan hydroxylase 1	7166	11

Note: list of gene searched take of Flint and Kentler (2014)

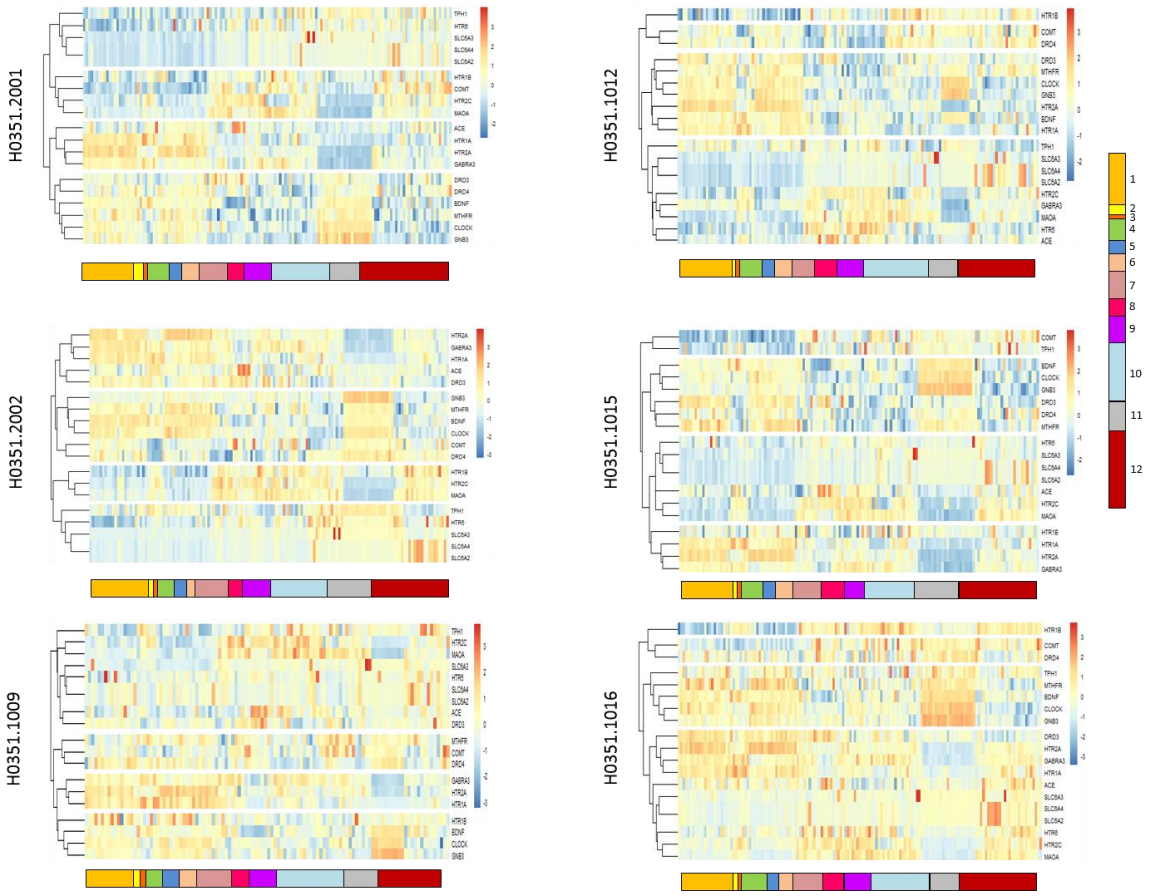

Figure 1. Heatmaps of gene expression related to MDD in donors. Below each heatmap a color representation of brain structures. The orginal heatmap for each donor in supplementary images. The column of right have the convention of colors: 1. Frontal Lobe; 2. Insula; 3. Limbic Lobe; 4. Hippocampal formation; 5. Occipital Lobe; 6. Parietal Lobe; 7. Temporal Lobe; 8. Amygdala; 9. Basal Ganglia; 10. Diencephalon; 11. Mesencephalon; 12. Hindbrain

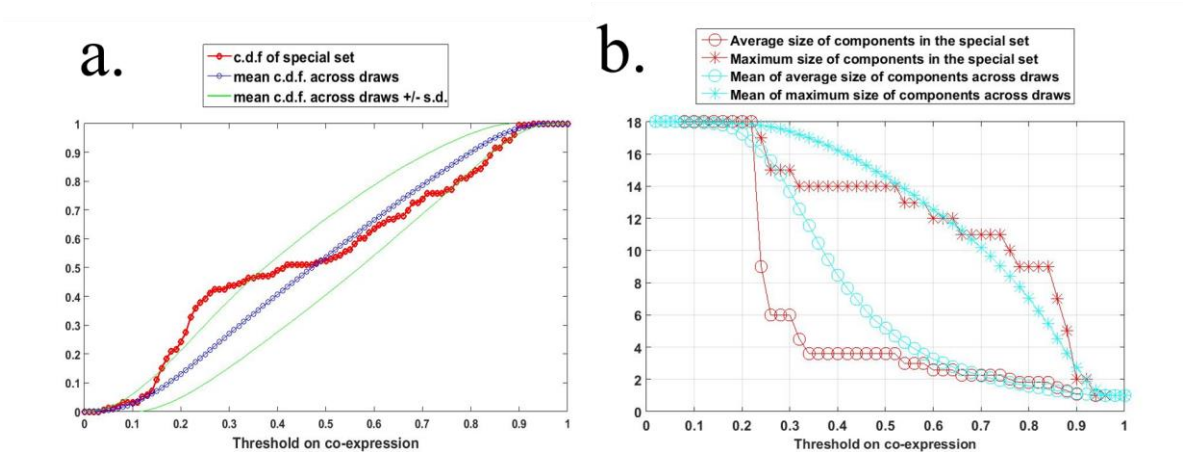

Figure 2. Co-expression of gene related to MDD in C57BL/6J. Distributions of co-expresion are depicted for the matrix network (red) and the 3041 genes in the mouse Allen Mouse Brain Atlas (Blue), showing cumulative distribution functions.

Table S1. Summary patient characteristics from Allen Human Brain Atlas (2010) (http://human.brain-map.org).

Donor	Age (years)	Sex	Etnicity	Postmortem interval (hours)
H0351.1009	57	M	White or Caucasian	26
H0351.1012	31	M	White or Caucasian	17
H0351.1015	49	F	Hispanic	30
H0351.1016	55	M	White or Caucasian	18
H0351.2001	24	M	Black of African American	23
H0351.2002	39	M	Black of African American	10

Details of qualitative and description of procedure and donors profile in: http://help.brain-map.org/download/attachments/2818165/CaseQual_and_DonorProfiles.pdf?version=1&modificationDate=1382051848013