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Innate immune response is differentially dysregulated between bipolar disease and schizophrenia

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ABSTRACT

Schizophrenia (SZ) and bipolar disorder (BD) are severe psychiatric conditions with a neurodevelopmental component. Genetic findings indicate the existence of an overlap in genetic susceptibility across the disorders. Also, image studies provide evidence for a shared neurobiological basis, contributing to a dimensional diagnostic approach. This study aimed to identify the molecular mechanisms that differentiate SZ and BD patients from health controls but also that distinguish both from health individuals. Comparison of gene expression profiling in post-mortem brains of both disorders and health controls (30 cases), followed by a further comparison between 29 BD and 29 SZ revealed 28 differentially expressed genes. These genes were used in co-expression analysesthat revealed the pairs *CCR1/SERPINA1*, *CCR5/HCST*, *C1QA/CD68*, *CCR5/S100A11* and *SERPINA1/TLR1* as presenting the most significant difference in co-expression between SZ and BD. Next, a protein-protein interaction (PPI) network using the 28 differentially expressed genes as seeds revealed *CASP4*, *TYROBP*, *CCR1*, *SERPINA1*, *CCR5* and *C1QA* as having a central role in the diseases manifestation. Both co-expression and network topological analyses pointed to genes related to microglia functions. Based on this data, we suggest that differences between SZ and BP are due to genes involved with response to stimulus, defense response, immune system process and response to stress biological processes, all having a role in the communication of environmental factors to the cells and associated to microglia.

1. Introduction

Schizophrenia (SZ) and bipolar disorder (BD) are severe psychiatric conditions, with a lifetime prevalence of about 1% (Merikangas et al., 2007; Alaerts and Del-Favero, 2009; Doherty et al., 2012). Both disorders have a neurodevelopment component, with onset of symptoms occurring most frequently during late adolescence or early adulthood (Maier et al., 2006; Doherty et al., 2012). Family studies demonstrate that the recurrence risk in families of SZ patients is 8-12% and the recurrence risk in BD families is approximately 10% (Barnett and Smoller, 2009; Ivleva et al., 2010). The estimates of heritability range between 40 - 80% for both diseases (Sullivan et al., 2003; Bienvenu et al., 2011) with genetic findings indicating an overlap in familial-genetic susceptibility across the diseases (O'Donovan et al., 2008; Lichtenstein et al., 2009; Ivleva et al., 2010). In addition, chromosomal regions, including risk variants show linkage to both BD and SZ (Barnett and Smoller, 2009; Moskvina et al., 2009; Williams et al., 2011a,b). Global gene expression analyses revealed common genes for SZ and BD, which were associated with synapse, neuronal and glial functions, metabolism, cellular and mitochondrial function, nervous system development, immune system development and response, and cell death (Iwamoto et al., 2005; Choi et al., 2008; Shao and Vawter, 2008; Lin et al., 2012).

Due to the similarities between both disorders, gene expression profiling of BD and SZ were first compared as one entity to controls to identify common alterations. Further, genes from this comparison were analyzed in co-expression and protein-protein interaction (PPI) networks contexts allowing the identification of changes in expression

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and biological processes potentially involved in the different clinical phenotypes observed in SZ and BD.

2. Material and methods

2.1. Sample

RNA samples were obtained from the frontal cortex of 104 subjects from the Stanley Neuropathology Consortium. Potential donors for the brain collection were identified by the pathologist who contacts the family of the deceased, request permission for donation, make a preliminary diagnosis and require psychiatric records; if necessary a psychiatrist contacts one or more family members and make a telephone call to clarify the symptoms. All records are reviewedfor DSM-IV psychiatric diagnosis independently by two senior psychiatrists. For normal controls, a structured telephone interview with a first-degree family member was carried out in all cases. Detailed sample collection is available in (Torrey, 2000). RNA concentration was determined by spectrophotometry (Nanodrop, Thermo Scientific, US), and integrity was accessed using the 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany). Due to low RNA integrity, 16 samples were discarded. The final samples included 30 non-psychiatric controls, 29 bipolar patients and 29 schizophrenic patients. A summary of subject characteristics is shown in Table 1.

The study protocol was approved by the ethics committee of A.C.Camargo Cancer Center and was performed in accordance with the Declaration of Helsinki.

2.2. cDNA microarray experiments

The Agilent 4x44K human oligonucleotide microarray assay was used (Agilent 4112 F; Agilent Technologies, CA) for microarray analyses. Slides were scanned with the Agilent Bundle Microarray Scanner System (Agilent Technologies) and data were processed using the Feature Extraction 10.7.3.1 software (Agilent Technologies). Among the 45,015 spots present in each array, only those with none flag for quality control (i.e. low intensity, saturation, controls, etc.) were selected for analysis. For analysis of replicate spots, the average intensity after background correction was calculated, normalization was performed using locally weighted linear regression (LOWESS) with $\alpha = 0.2$ within slides using the R software version 2.11.1 (R Development Core Team, 2010). For statistical analyses only transcripts that were presented in at least 24 cases of each group were considered. In total, 22,639 transcripts were analyzed. Gene expression data described in this study are available from GEO with accession ID GSE6210.

2.3. Statistical analysis

For analysis of genes related to pathological changes, patients with either BD or SZ (PSY) were compared to individuals without pathology (CON). From this list of differentially expressed genes, BD and SZ patients were compared to identify possible disease-specific genes. Analyses using Multiple Significant Analysis of Microarray (SAM) identified differentially expressed genes. Five hundred permutations were performed using a False Discovery Ratio (FDR) of 1% for both the PSY vs. CON and BD vs. SZ analyses. To assess similarity patterns, Pearson correlation and complete linkage were used for non-supervised hierarchical clustering, and reliability was assessed by bootstrapping using multiExperiment Viewer (MeV) software (Saeed et al., 2003).

Differentially expressed genes were annotated using biological process categories in the Gene Ontology Database (GO) and a hypergeometric test with multiple test adjustments was applied to find over-represented chromosome regions with WebGestalt, using the human genome as reference, p-value <0.05 and Benjamin Hochnerg adjustment (Zhang et al., 2005).

To assess differences in network organization between individuals with BD and SZ, co-expression of pairs of genes were evaluated as previously described (Silva et al., 2012). Briefly, the Pearson Correlation Coefficient (PCC) for each gene and partners in each group was calculated. PCC absolute difference between groups was used to identify genes whose co-expression was different between BD and SZ. To identify genes that were significantly different between patient groups, an empirical p-value distribution was created as follows: cases were randomly assigned to two groups and the PCC was calculated for each group and a difference ranking was calculated. These analyses were repeated 1,000 times to create a random distribution of PCC's difference rankings. Real PCC differences for genes between patient groups were compared to the random distribution to generate p-values (Supplemental table 1) defining a network of genes whose co-expression was significantly different ($p \leq 0.05$) between SZ and BD. The network was visualized using Cytoscape (Cline et al., 2007).

To identify additional properties potentially associated with the differentially expressed genes between SZ and BD, a Protein-Protein Interaction (PPI) networkwas used. By querying three human interactome databases: HPRD (Keshava Prasad et al., 2009), MINT (Licata et al., 2012) and IntAct (Kerrien et al., 2012), a network starting with differentially expressed genes (seeds) and their first neighbors (genes with direct interaction in the interactome databases) and genes that connected first neighbors from seed was constructed. To identify broker (i.e., a gene that connects different genes that do not connect directly with each other) and bridge (i.e., a gene that has only a few connections but connects broker genes and their associated partners (i.e., hubs)) genes, previously published algorithms (Cai et al., 2010) were used in the Interactome Graph website (http://bioinfo.lbhc.hcancer.org.br/interactomegraph/). Top 5% genes were selected. Using the entire set of genes available in the three banks (14,276), the probability of a node appearing in a random network was estimated by generating 1,000 networks using a random collection of 25 genes (an equivalent number of genes differentially expressed found in this study and present at the PPI data). For each gene in the original network, the number of times it appeared in the 1,000 networks was computed. Genes that appeared more than 20% times (5th percentile) were considered random, because the gene has a high probability of appearing in any human PPI.

Table 1
Summary of subject characteristics.

	Bipolar patients	Schizophrenic patients	Non-psychiatric controls
Number of Samples	29	29	30
Age	44,46	42,17	44,43
Gender	52% Male	79% Male	76% Male
Race	93% White	96% White	100% White
PMI	36,89 ± 18,26	31,40 ± 16,93	29,97 ± 12,71
Brain pH	6,49 ± 0,25	6,45 ± 0,25	6,61 ± 0,28

For each variable, mean ± standard error or percentage value is reported. PMI: post-mortem interval.

3. Results

3.1. Characterization of differentially expressed genes between BD and SZ

Gene expression profile analyses of PSY (i.e., BD and SZ) versus CON identified 1,264 differentially expressed genes. These genes were involved with nervous system, vasculature and ectoderm development, regulation of metabolism and the immune system biological processes.

Of the 1,264 genes, 28 were differentially expressed when comparing individuals with BD and SZ (FDR < 1%), all having a higher expression in BD. These genes are involved with immune system response, immune system regulation, and response to stimulus (Table 2). Non-