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Full-length Article

Clinically proven drug targets differentially expressed in the prefrontal cortex of schizophrenia patients

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ABSTRACT

Background: Due to the heterogeneous nature of schizophrenia, understanding the genetic risk for the disease is a complex task. Gene expression studies have proven to be more reliable than association studies as they are consistently replicated in a tissue specific manner.
Methods: Using RNA-Seq we analysed gene expression in the frontal cortex of 24 individuals with schizophrenia and 25 unaffected controls.
Results: We identified 1146 genes that were differentially expressed in schizophrenia, approximately 60% of which were up-regulated and 366 of 1146 (32%) also have aberrant DNA methylation ($p = 2.46 \times 10^{-39}$). The differentially expressed genes were significantly overrepresented in several pathways including inflammatory ($p = 8.7 \times 10^{-3}$) and nitric oxide pathways ($p = 9.2 \times 10^{-4}$). Moreover, these genes were significantly enriched for those with a druggable genome ($p = 0.04$). We identified a number of genes that are significantly up-regulated in schizophrenia as confirmed in other gene expression studies using different brain tissues. Of the 349 genes associated with schizophrenia from the Psychiatric Genomics Consortium we identified 16 genes that are significant from our list of differentially expressed genes.
Conclusions: Our results identified biological functional genes that are differentially expressed in schizophrenia. A subset of these genes are clinically proven drug targets. We also found a strong pattern of differentially expressed immune response genes that may reflect an underlying defect in schizophrenia.

1. Introduction

Schizophrenia is a debilitating disorder that is still poorly understood due to the complexity of the relationship between genetic and environmental risk factors (van Os et al., 2014). Schizophrenia has a high genetic heritability and has been researched extensively with the hope of producing improved treatment options. Due to the heterogeneous nature of the disorder and the hundreds of small effect genetic variants associated with schizophrenia, a large proportion of the genetic risk is unknown. Gene expression studies performed in functional brain tissue show much stronger schizophrenia association even when performed with limited sample sizes (Horvath and Mirnics, 2015). Identifying the molecular mechanism of the functional pathways involved and the associated genetic markers may provide more clues to a more complete understanding of schizophrenia.

With the advent of next generation sequencing and high throughput techniques, new pathways have been identified including those involved in the immune system. A study of the prefrontal cortex of individuals with schizophrenia revealed an increase in inflammatory mRNA expression and identified a number of differentially expressed cytokines and immune modulators including *IL-6*, *IL-8*, *IL-1β* and *SERPINA3* (Fillman et al., 2013). There has also been schizophrenia RNA-Seq studies performed in other regions of the brain including the hippocampus. Like the study performed in the frontal cortex, genes were over-represented from pathways involved in immune/inflammation response (Hwang et al., 2013).

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Genetic association studies have also identified the human leukocyte antigen (HLA) locus and interleukin-1 gene complex as risk factors for schizophrenia (Ripke et al., 2013; Xu and He, 2010). A recent molecular pathway analysis study identified genes in the toll-like receptor family and innate immunity to play a significant role in a number of pathways that are disrupted in schizophrenia (Crisafulli et al., 2015). Epigenetic mechanisms such as DNA methylation also effect the expression of genes and therefore it is important to investigate both gene expression and epigenetics if we are to understand the molecular mechanism of schizophrenia. In a whole genome methylation analysis of schizophrenia we identified a number of differentially methylated genes that were previously associated with schizophrenia including *NOS1*, *AKT1*, *DTNBP1*, *DNMT1*, *PPP3CC* and *SOX10* (Wockner et al., 2014). In a further schizophrenia analysis we identified seven regions that were consistently differentially methylated in three separate cortex data sets including regions near genes *CERS3*, *DPPA5*, *PRDM9, DDX43, REC8*, *LY6G5C* and a region on chromosome ten (Wockner et al., 2015). Lymphocyte antigen 6 complex (*LY6G5C*) belongs to a cluster of leukocyte antigen-6 (LY6) genes located in the major histocompatibility complex (MHC) class III region (Mallya et al., 2006). MHC encodes 400 genes critical to immune system function and is strongly associated with schizophrenia susceptibility (Corvin and Morris, 2014).

In this study high throughput next-generation sequencing (RNA-Seq) was used to analyse the brain transcriptome of schizophrenia patients and controls. We aimed to identify candidate genes that were differentially expressed and identify genes that are both differentially expressed and differentially methylated in the same sample set. We also verified the differentially expressed genes in an independent sample set from brain cortex using PD-NGSAtlas. A functional annotation and gene set enrichment was performed to identify statistically significantly enriched biological processes in the differentially expressed genes. Finally, we further examined the association of our differentially expressed genes with GWAS data from the Schizophrenia Working Group of the Psychiatric Genomics Consortium. By understanding the genetic and epigenetic regulatory mechanisms involved in schizophrenia we hope to gain a more complete understanding of the relationship between brain function and schizophrenia.

2. Methods and materials

2.1. Samples

Frontal cortex post-mortem brain tissue from individuals with Diagnostic and Statistical Manual of Mental Disorders, 4th Edition-diagnosed schizophrenia (n = 24) and controls (n = 25) was provided by the Human Brain and Spinal Fluid Resource Centre, California (courtesy of James Riehl). Four samples failed quality control (QC) following RNA extraction and or library preparation with 45 samples remaining for final analysis (22 schizophrenia and 23 controls). Each sample consisted of a coronal section (7 mm thick) that had been quick frozen and a section of frontal cortex was dissected from each frozen section sample weighing (0.4–1.0 g). 38 samples were taken from Brodmann's area 10 and 11 samples were taken from Brodmann's area 46. Demographic data including age, post-mortem interval (PMI) and gender are summarized in Supplementary Table 1. There were significant differences in age and PMI between cases and controls. These known confounds as well as unknown variables were adjusted for in the analysis. PMI in our study is defined as the time between death and when the brain section is quick frozen. The mean (±SD) time between death and post-mortem refrigeration was 4.48 ± 3.86 h. All but two of the participants with schizophrenia were known to be receiving antipsychotic medication at time of death. The cause of death of five schizophrenia patients was suicide.

Ethics approval for the project was obtained from the Human Research Ethics Committee of the Queensland University of Technology.

2.2. RNA sequencing

Extraction of RNA was performed at the UCLA Clinical Microarray Core Laboratory using the Roche MagNa Pure Compact.

Quality of RNA samples was assessed by electrophoresis using the Agilent Bioanalyzer RNA 6000 Nano assay. The percentage of RNA fragments >200 nucleotides was assessed to determine the appropriate input. Samples with <30% of RNA >200 nucleotides were excluded. Forty-six samples passed the initial QC. Following sample QC, samples were processed with the TruSeq RNA Access Sample Preparation Kit as per the manufacturer's instructions (Illumina, San Diego, CA, USA). Briefly, the protocol follows the following steps: cDNA Synthesis; DNA library preparation; library validation (libraries were assessed using by electrophoresis using Agilent TapeStation D1K TapeScreen assay and quantified by PicoGreen fluorometry); coding exon enrichment; captured library validation (one sample failed to generate enough library to proceed to capture hybridisation and was excluded from the final analysis).

Two captured (eight samples) libraries were pooled for sequencing. Each pool of libraries was clustered on the Illumina cBot system using HiSeq PE Cluster Kit v4 reagents followed by sequencing on the Illumina HiSeq 2500 system with HiSeq SBS Kit v4 reagents with 159 cycles (75 base pair pared end reads). Illumina RTA 1.18.61 software was used for base calling and bcl2fastq pipeline 1.8.4 was used for quality scoring, de-multiplexing and FASTQ file generation.

2.3. Statistical analysis and read normalisation

Reads were mapped to the NCBI hg38 reference genome using Tophat 2.0.13, following the protocol described by Trapnell et al. (2012). The tool featureCounts from the SubRead package 1.4.6p5 was used to assign raw read counts to exons and genes (Liao et al., 2014).

Non-normalised read counts were used for the edgeR package 3.12.0 (Robinson et al., 2010; McCarthy et al., 2012) to perform differential gene expression analysis after quality control and normalisation. The edgeR package normalises read counts on each gene using the TMM method (Robinson and Oshlack, 2010) and then tests for differential expression by fitting a model to the negative binomial distribution. Surrogate variable analyses (SVA) was used to correct for known confounds and hidden variables. The significance of differential gene expression was tested using a likelihood-ratio statistic.

Using the same tissue samples used in this study, 19 582 differentially methylated regions, associated with 7833 genes were identified from a previous methylation study by our group (Wockner et al., 2014). Set intersection was performed and the hypergeometric test was to test for the enrichment, this was performed in R.

2.4. Validation of RNA–Seq using PD-NGSAtlas, GWAS Loci, and Existing disease annotations

Using the PD-NGSAtlas web application, we downloaded differential gene analysis results in brain region BA9 between 5 schizophrenia and 6 control patients. We selected "edgeR, TMM method" as the closest equivalent of our statistical analysis. For Hwang et al. and Qin Wu et al., we compared their differentially expressed lists with our list.