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MicroRNA expression profiling in the prefrontal cortex of individuals affected with schizophrenia and bipolar disorders

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ABSTRACT

MicroRNAs (miRNAs) are a large family of small non-coding RNAs which negatively control gene expression at both the mRNA and protein levels. The number of miRNAs identified is growing rapidly and approximately one-third is expressed in the brain where they have been shown to affect neuronal differentiation, synaptosomal complex localization and synapse plasticity, all functions thought to be disrupted in schizophrenia. Here we investigated the expression of 667 miRNAs (miRBase v.13) in the prefrontal cortex of individuals with schizophrenia (SZ, N = 35) and bipolar disorder (BP, N = 35) using a real-time PCR-based Taqman Low Density Array (TLDA). After extensive QC steps, 441 miRNAs were included in the final analyses. At a FDR of 10%, 22 miRNAs were identified as being differentially expressed between cases and controls, 7 dysregulated in SZ and 15 in BP. Using in silico target gene prediction programs, the 22miRNAs were found to target brain specific genes contained within networks overrepresented for neurodevelopment, behavior, and SZ and BP disease development.

In an initial attempt to corroborate some of these predictions, we investigated the extent of correlation between the expressions of hsa-mir-34a, -132 and -212 and their predicted gene targets. mRNA expression of tyrosine hydroxylase (TH), phosphogluconate dehydrogenase (PGD) and metabotropic glutamate receptor 3 (GRM3) was measured in the SMRI sample. Hsa-miR-132 and -212 were negatively correlated with TH ($p = 0.0001$ and 0.0017) and with PGD ($p = 0.0054$ and 0.017, respectively).

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1. Introduction

Both schizophrenia (SZ, MIM 181500) and bipolar disorder (BP, MIM 125480) are common and debilitating psychiatric illnesses with a lifetime prevalence of 0.5–1% and 0.8–2.6% respectively. The etiology of SZ and BP is currently unknown, however in conjunction with environmental and developmental factors (Jablensky et al., 1992; Kendler et al., 1994; Kendler and Diehl, 1993), consistent evidence for a substantial genetic component (Kato et al., 2005) has been shown, with some shared between both diseases (Berrettini, 2003; Purcell et al., 2009a).

Protein-coding genes have long been the focus of research in disease genetics, but efforts to identify replicable protein-coding risk loci in SZ (Shi et al., 2009a; Stefansson et al., 2009b) and BP (Sklar et al., 2008) remain elusive. Current

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GWAS studies have also been unable to establish a uniform set of protein-coding genes exhibiting a clear etiology in disease (Purcell et al., 2009b; Shi et al., 2009b; Stefansson et al., 2009a). Additionally, genetic association does not appear to reflect DNA variants with any obvious effect on protein structure (Bray, 2008) for several of the best supported SZ susceptibility genes identified to date (e.g. DTNBP1 and NRG1).

MiRNAs are a large family of small non-coding RNAs which negatively control gene expression at both the mRNA and protein levels (Lai, 2002). Most miRNAs are processed from a Pol II transcript called a primary miRNA (pri-miRNA), which is further processed to a smaller hairpin-like structure called a precursor miRNA (pre-miRNA). The terminal loop of the pre-miRNA is then cleaved, generating a miRNA/miRNA* duplex and one strand is preferentially loaded into the RNA induced silencing complex (RISC) (Filipowicz et al., 2008) leading to mRNA degradation and/or translational repression. MiRNAs control mRNA stability and translation by binding to sequence motifs in the 3′-UTR of target mRNAs (Valencia-Sanchez et al., 2006). This binding does not require complete complementarity except for nucleotides 2–8 (the "seed region") at the miRNA's 5′ end. Although the majority of miRNA/ mRNA interactions require perfect complementarity at the seed region, there are some exceptions to this rule, which complicates miRNA target predictions (Lai, 2004).

Microarray studies have revealed that individual miRNAs can affect the expression of multiple genes (Krichevsky et al., 2006), suggesting that miRNAs can have pleiotropic effects on cellular processes. A growing number of miRNAs are discovered every year (Griffiths-Jones et al., 2006) and approximately one-third is expressed in the brain (Sempere et al., 2004) where they have been shown to be involved in maintaining brain function (Fiore et al., 2008). In vitro studies demonstrate that miRNAs are localized to the synaptosomal complex (Lugli et al., 2005) where they affect neuronal differentiation (Vo et al., 2005) and modulate synapse plasticity (Schratt et al., 2006), both of which have been implicated in SZ and BP (Eastwood and Harrison, 2001; Vawter et al., 2002). Thus, miRNAs are likely candidates in neurodegenerative disorders (Kuss and Chen, 2008) and recent studies have attempted to evaluate the impact of miRNAs in SZ etiology at both genetic (Rogaev, 2005; Hansen et al., 2007; Burmistrova et al., 2007) and expression (Burmistrova et al., 2007; Beveridge et al., 2008; Perkins et al., 2007) levels.

Postmortem brain tissue has been successfully used to identify transcriptional dysregulation of candidate genes in SZ and BP (Harrison and Weinberger, 2005). Here, we evaluated the expression of 667 miRNAs in the postmortem prefrontal cortex of patients with SZ and BP disorder.

2. Materials and methods

2.1. Postmortem brain tissue

The Stanley Medical Research Institute (SMRI) provided 200 mg of postmortem brain tissue from the dorsolateral prefrontal cortex (DLPFC, Brodmann's area 46) (Torrey et al., 2000). The sample demographics are described in Suppl. Table 1. Exclusion criteria included: (1) brain pathology, (2) history of pre-existing CNS disease, (3) poor RNA quality, (4) IQ<70, (5) age<30 and (6) substance abuse within one year of death.

2.2. RNA isolation and quantification

Total RNA containing the small RNA fraction was isolated from 100 mg of tissue using the miRvana-Paris Kit (Ambion, Austin, Texas) following manufacturer's recommendations. RNA quality and concentration were measured on the 21000 Bioanalyzer (Agilent, Santa Clara, CA). Average RNA integrity number (RIN) was 7.2. The tissue for one BP sample was not received and 1 CON sample gave poor RNA quality, both of which were excluded from further analyses.

2.3. MiRNA reverse transcription

cDNA synthesis for TLDA was performed according to manufacturer's recommendation (ABI). Briefly, RNA was reverse transcribed using the MicroRNA reverse transcription kit (ABI) in combination with the stem-loop Megaplex primer pool (ABI). 3 μl of total RNA (33.3 ng/μl) was combined with 0.8 μl RT primer mix (10×), 0.8 μl RT buffer (10×), 1.5 μl MultiScribe Reverse Transcriptase (10 U/μl), 0.2 μl dNTPs with dTTP (0.5 mM each), 0.9 μl MgCl2 (3 mM) and 0.1 μl RNase inhibitor (0.25 U/μl) in a total reaction volume of 7.5 μl. Reactions were run on an Eppendorf Mastercycler (Eppendorf, Westbury, NY) in a 384-well plate for 40 cycles at 16 °C for 2 min, 42 °C for 1 min, 50 °C for 1 s, and 85 °C for 5 min.

cDNA synthesis for the single tube miRNA reactions was performed using the TaqMan MicroRNA reverse transcription kit (ABI). 10 ng of RNA (2 ng/μl) was combined with 3.0 μl 5X RT primer, 0.15 μl 100 mM dNTPs, 1.0 μl MultiScribe Reverse Transcriptase (50 U/μl), 1.5 μl 10X RT buffer and 0.19 μl RNase inhibitor (20 U/μl) in a total reaction volume of 15 μl. Reactions were run on an Eppendorf Mastercycler (Eppendorf) in a 96-well plate for 40 cycles at 16 °C for 30 min, 42 °C for 30 min and 85 °C for 5 min.

2.4. MiRNA expression detection

Prior to detecting miRNA expression, the cDNA for the TLDA array was pre-amplified following ABI's recommendations, allowing for increased detection sensitivity while preserving the miRNA (Cogswell et al., 2008b; Mestdagh et al., 2008; Tang et al., 2006) expression profile (Suppl. Fig. 1). The reactions were diluted with 75 μl of 0.1X TBE and stored at −20 °C. 9 μl of product and 450 μl of TaqMan PCR Master Mix, was combined with 441 μl nuclease free water, mixed and centrifuged for 30 s. 100 μl was loaded into each port of the appropriate 384 well TLDA array, centrifuged twice at 1200 rpm in a Sorvall Legend centrifuge (Thermo Scientific, USA), and sealed with a micro-fluidic card staker (ABI). The arrays were run on the 7900HT Real-Time PCR System according to manufacturer's protocol. Raw Cq values (RDML guidelines, http://www.rdml.org(Lefever et al., 2009)) were calculated using the SDS software v.2.3 with automatic baseline settings at a threshold of 0.2.

The single tube miRNA expressions were detected using TaqMan chemistry according to the manufacturer's protocol (ABI). 1.33 μl of cDNA (diluted 1:15) was combined with