Preliminary investigation of miRNA expression in individuals at high familial risk of bipolar disorder

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ABSTRACT

Bipolar disorder (BD) is a highly heritable psychiatric disorder characterised by recurrent episodes of mania and depression. Many studies have reported altered gene expression in BD, some of which may be attributable to the dysregulated expression of miRNAs. Studies carried out to date have largely studied medicated patients, so it is possible that observed changes in miRNA expression might be a consequence of clinical illness or of its treatment. We sought to establish whether altered miRNA expression might play a causative role in the development of BD by studying young, unmedicated relatives of individuals with BD, who are at a higher genetic risk of developing BD themselves (high-risk individuals). The expression of 20 miRNAs previously implicated in either BD or schizophrenia was measured by qRT-PCR in whole-blood samples from 34 high-risk and 46 control individuals. Three miRNAs, miR-15b, miR-132 and miR-652 were up-regulated in the high-risk individuals, consistent with previous reports of increased expression of these miRNAs in patients with schizophrenia. Our findings suggest that the altered expression of these miRNAs might represent a mechanism of genetic susceptibility for BD. Moreover, our observation of altered miRNA expression in the blood prior to the onset of illness provides hope that one day blood-based tests may aid in the risk-stratification and treatment of BD.

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1. Introduction

Bipolar disorder (BD) is a severe psychiatric disorder characterised by recurrent episodes of mania and depression, which occurs with a lifetime prevalence of approximately 0.6%

(Merikangas et al., 2011). The heritability of BD is estimated to be around 0.85 (McGuffin et al., 2003) and first-degree relatives of individuals with BD have a seven-fold risk of developing BD compared to the general population (Lichtenstein et al., 2009), indicating a substantial genetic component to the aetiology of this disorder. In addition to being at an elevated risk of developing BD, the first-degree relatives of BD patients are also at an increased risk of developing schizophrenia and major depressive disorder (MDD) (Lichtenstein et al., 2009; Rasic et al., 2014), indicating a shared component to the aetiology of these psychiatric disorders.

Altered gene expression has been identified in multiple studies of the blood and post-mortem brains of individuals with psychiatric disorders (Kumarasinghe et al., 2012; Seifuddin et al., 2013). Gene ontology analysis of these genes has implicated biological processes with apparent relevance to the pathogenesis of psychiatric illness such as synaptic function. More recently, altered microRNA

(miRNA) expression has been identified as a putative pathogenic mechanism for both BD and schizophrenia (Maffioletti et al., 2014). Multiple miRNAs have been found to show dysregulated expression in individuals with these conditions both in the brain (Banigan et al., 2013; Beveridge et al., 2010; Miller et al., 2012; Moreau et al., 2011; Perkins et al., 2007; Santarelli et al., 2011; Smalheiser et al., 2014) and in the periphery (Gardiner et al., 2012; Lai et al., 2011). Consistent with these changes, increased expression of components of the miRNA processing pathway has been detected in the brains of schizophrenic patients (Beveridge et al., 2010; Santarelli et al., 2011). Additionally, the expression of Ago2, which is involved in effecting miRNA-induced silencing (Cenik and Zamore, 2011), as well as the regulation of mature miRNA expression and miRNA processing (Diederichs and Haber, 2007), has been found to be down-regulated in peripheral blood mononuclear cells in schizophrenic patients (Gardiner et al., 2013). miRNAs are short (~22 nt) RNA sequences that can each target hundreds of mRNAs by complementarity to the 3′ untranslated region, generally resulting in the suppression of gene expression either by affecting mRNA stability or translation (Valencia-Sanchez et al., 2006). As such, changes in miRNA expression might underlie some of the observed changes in mRNA and protein expression observed in BD and schizophrenia.

Studies reporting altered miRNA expression in BD and schizophrenia have almost exclusively been carried out on individuals who have been ill for several years and are taking medication. It is, therefore, often not possible to determine whether observed changes in miRNA expression are a primary causative event, occur as a consequence of illness progression, or are induced by medication. Studies in primary neuronal cultures, cell lines and animal models have demonstrated that several miRNAs show altered expression following treatment with mood-stabilisers and/or antipsychotics (Chen et al., 2009; Gardiner et al., 2014; Hunsberger et al., 2013; Santarelli et al., 2013; Zhou et al., 2009), further supporting the need to study unmedicated individuals.

To our knowledge, only one study has measured miRNA expression in unmedicated individuals diagnosed with BD. Rong et al. (2011) measured a single miRNA, miR-134, and demonstrated reduced plasma expression in drug-free manic patients with BD1, which increased in response to treatment with mood-stabilisers.

We sought to further understanding of the involvement of altered miRNA expression in the aetiology of BD by measuring miRNA expression in young, unaffected relatives of patients with BD who are at higher genetic risk of developing BD (henceforth referred to as high-risk individuals). The high-risk and control individuals were selected from a larger cohort recruited as part of the Edinburgh-based Bipolar Family Study (BFS). Individuals from this cohort have undergone structural and functional magnetic resonance imaging, which has revealed reduced white matter integrity and alterations in the activity of the amygdala and insula, brain structures previously implicated in the pathophysiology of psychiatric illness, in high-risk individuals (Sprooten et al., 2011; Whalley et al., 2011, 2013b). Additionally, high-risk individuals have been shown to carry more BD-associated variants than control subjects, as indicated by a significantly higher BD polygene score (Whalley et al., 2013a).

Here, we measured the expression of 20 miRNAs previously implicated in the pathogenesis of BD and/or schizophrenia in whole-blood samples from high-risk and control individuals. As such, we aimed to identify miRNAs whose expression is altered prior to illness onset and which, thus, may play a causative role in illness development.

2. Methods

2.1. Participants

We examined 34 unaffected individuals at higher genetic risk of developing a mood disorder and 46 control subjects. These individuals were recruited as part of The Bipolar Family Study (BFS), as described previously (Sprooten et al., 2011; Whalley et al., 2011). High-risk subjects met the following selection criteria: (i) at least one first-degree or two second-degree relatives suffering from BD1; (ii) no personal history of BD; and (iii) aged 16—25 at the time of recruitment. Control individuals of a similar age were selected on the basis that they did not have a personal history of BD or a family history of any mood disorder amongst their first-degree relatives. Exclusion criteria for both groups were: a history of major depression, mania, or hypomania, psychosis, generalized anxiety disorder, panic disorder, eating disorder, substance dependence, an IQ < 70 or clinical diagnosis of learning disability, any major neurological disorder or history of head injury that included loss of consciousness, and any contraindications to magnetic resonance imaging (MRI). Only unrelated individuals were included. All participants provided written informed consent and the study was approved by the Multicentre Research Ethics Committee for Scotland.

2.2. Selection of miRNAs for assessment

MicroRNAs were selected for assessment on the basis that they were either implicated in the pathogenesis of BD or schizophrenia through: (i) altered expression in patients or (ii) altered expression in response to treatment with a mood-stabilising drug. A literature search was carried out using PubMed using the terms "micro RNA", "bipolar disorder", "schizophrenia", "lithium", and "valproate", together with commonly used abbreviations, in August 2011.

2.3. Extraction of blood RNA

Blood for RNA extraction (3 ml) was collected in Tempus Blood RNA Tubes (Life Technologies). RNA was extracted using MagMAX for Stabilized Blood Tubes RNA Isolation Kit (Life Technologies), according to the manufacturer's instructions. RNA yield and quality were assessed using the Agilent Bioanalyzer.

2.4. Assessment of miRNA expression

Assessment of miRNA expression was carried out using TaqMan MicroRNA Assays (Life Technologies; SOM Table 1), following the manufacturer's protocol for custom reverse transcription and pre-amplification pools (https://tools.lifetechnologies.com/content/sfs/manuals/cms_094060.pdf). Briefly, for all genes of interest and reference genes, a custom reverse transcription (RT) pool was created by combining 10 ul of each 5× RT primer and a custom pre-amplification pool was created by combining 10 ul of each 20× TaqMan MicroRNA Assay. The RT reaction was performed using 100 ng RNA. A half volume reaction without the RT enzyme was also performed using 50 ng RNA (RT negative). cDNA was pre-

Table 1
Demographic characteristics of the sample.

	Control	High-risk	Test statistic	p-value
Age (SD)	26.2 (4.24)	24.9 (4.98)	−1.23	0.224
Gender				
Female (%)	26 (56.5)	18 (52.9)	NA	0.707
Male (%)	20 (43.5)	16 (47.1)		
RIN (SD)	7.05 (0.915)	7.09 (0.859)	0.211	0.833