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Altered neural signaling and immune pathways in peripheral blood mononuclear cells of schizophrenia patients with cognitive impairment: A transcriptome analysis

Jing Qin Wu [a,b,c], Melissa J. Green [b,d], Erin J. Gardiner [a,b,c], Paul A. Tooney [a,b], Rodney J. Scott [a,b,c], Vaughan J. Carr [b,d], Murray J. Cairns [a,b,c,*]

[a] School of Biomedical Sciences and Pharmacy, Faculty of Health, The University of Newcastle, University Drive, Callaghan, NSW 2308, Australia
[b] Schizophrenia Research Institute, Sydney, Australia
[c] Centre for Translational Neuroscience and Mental Health, Hunter Medical Research Institute, Newcastle, NSW 2305, Australia
[d] School of Psychiatry, University of New South Wales, Sydney, NSW, Australia

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ABSTRACT

Cognitive deficits are a core feature of schizophrenia and contribute significantly to functional disability. We investigated the molecular pathways associated with schizophrenia (SZ; $n = 47$) cases representing both 'cognitive deficit' (CD; $n = 22$) and 'cognitively spared' (CS; $n = 25$) subtypes of schizophrenia (based on latent class analysis of 9 cognitive performance indicators), compared with 49 healthy controls displaying 'normal' cognition. This was accomplished using gene-set analysis of transcriptome data derived from peripheral blood mononuclear cells (PBMCs). We detected 27 significantly altered pathways (19 pathways up-regulated and 8 down-regulated) in the combined SZ group and a further 6 pathways up-regulated in the CS group and 5 altered pathways (4 down-regulated and 1 up-regulated) in the CD group. The transcriptome profiling in SZ and cognitive subtypes were characterized by the up-regulated pathways involved in immune dysfunction (e.g., antigen presentation in SZ), energy metabolism (e.g., oxidative phosphorylation), and down-regulation of the pathways involved in neuronal signaling (e.g., WNT in SZ/CD and ERBB in SZ). When we looked for pathways that differentiated the two cognitive subtypes we found that the WNT signaling was significantly down-regulated (FDR < 0.05) in the CD group in accordance with the combined SZ cohort, whereas it was unaffected in the CS group. This suggested suppression of WNT signaling was a defining feature of cognitive decline in schizophrenia. The WNT pathway plays a role in both the development/function of the central nervous system and peripheral tissues, therefore its alteration in PBMCs may be indicative of an important genomic axis relevant to cognition in the neuropathology of schizophrenia.

1. Introduction

Schizophrenia is a severely debilitating psychiatric disorder characterized by delusions, hallucinations, and deficits in cognitive function. Recent genome-wide association (GWA) studies have identified a growing number of genetic loci associated with schizophrenia (Purcell et al., 2009; Ripke et al., 2011, 2013, 2014; Shi et al., 2009; Stefansson et al., 2009) which are implicated in biological processes relating to calcium signaling, immune system function (e.g., many variants in the extended major histocompatibility complex [MHC] region), and long intergenic noncoding RNAs. We have previously shown that one recent genetic variant regulating miR-137 (Green et al., 2013) is associated with a cognitive deficit subtype of schizophrenia. However, the precise functional implication of associated variants remains to be determined, and requires investigation of gene expression regulation, at both transcriptional and post-transcriptional levels. In this study we explore the biological pathways associated with cognitive subtypes of schizophrenia using gene-set analysis of high throughput expression profiles from patient derived PBMCs.

Gene expression changes are evident in several brain regions from post-mortem tissues, and have also been reported in studies using peripheral blood mononuclear cells (PBMCs; for a recent review, see (Sequeira et al., 2012). Findings from both types of

* Corresponding author at: School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, The University of Newcastle, University Drive, Callaghan, NSW 2308, Australia.
E-mail address: Murray.Cairns@newcastle.edu.au (M.J. Cairns).

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study converge on biological processes involved in cell cycle, intracellular signaling, oxidative stress and metabolism, phosphatidylinositol signaling, and ubiquitin proteasome system dysregulation (Bousman et al., 2010; Craddock et al., 2007; McCurdy et al., 2006). Briefly, transcriptome profiling of both fresh PBMCs and lymphoblastoid cell lines have demonstrated unique gene expression signatures related to these processes that discriminate schizophrenia from bipolar disorder, and healthy control groups (Tsuang et al., 2005); more recently, candidate blood biomarkers for two key psychotic symptoms in schizophrenia (hallucinations and delusions) were identified (Kurian et al., 2011), alongside the detection of blood-based gene expression signature using a supervised classifier in association with diagnosis of schizophrenia (Takahashi et al., 2010). Notably, PBMCs play important roles in the immune system, which is increasingly acknowledged in contributing to higher cognitive functions (Wolf et al., 2009). PBMCs express a number of brain associated proteins including receptors for brain derived neurotrophic factor, glucocorticoids, catecholamines, serotonin, dopamine and acetylcholine (McKenna et al., 2002), and many neurons express receptors for signaling molecules of the immune system such as cytokines (Guyon et al., 2008; Kronfol and Remick, 2000; Muller and Ackenheil, 1998).

The emerging studies of PBMCs in schizophrenia have reported dysregulated gene expression of actin assembly factor DAAM2 (Kuzman et al., 2009), a splice variant of NRG1, involved in neurological function and higher levels of sensory and motor neuron derived factor (SMDF) (Petryshen et al., 2005); other implicated genes are involved in processes of neurotransmission and presynaptic function (e.g., dopamine receptor D2 [DRD2]), the inwardly rectifying potassium channel (Kir2.3), and neuropeptide Y (NPY1R) (Middleton et al., 2005a; Vawter et al., 2004a; Zvara et al., 2005). More recently, our study using the largest PBMC cohort to date has reported differential expression of a substantial number of genes involved in the immune system (Gardiner et al., 2013), consistent with a recent mRNA sequencing study implicating a number of immune pathways in schizophrenia, such as antigen presentation and chemokine signaling (Xu et al., 2012).

In this context, there has not yet been a systematic investigation of gene expression profiles associated with putative cognitive subtypes of schizophrenia. While cognitive deficits are a core feature of schizophrenia, there remains substantial heterogeneity among schizophrenia patients in the severity of cognitive impairments. The potential to detect specific biological associations with cognitive subtypes of schizophrenia has been demonstrated in two independent cohorts (Green et al., 2013; Hallmayer et al., 2005; Morar et al., 2011), and in a similar vein, it has been recognised that better use of phenotypic information can increase the power of GWA studies considerably (van der Sluis et al., 2013). In this study we explore the biological pathways associated with cognitive subtypes of schizophrenia using gene-set analysis of high throughput expression profiles from patient derived PBMCs.

2. Materials and methods

2.1. Participants

Participant data was obtained from the Australian Schizophrenia Research Bank (ASRB), an established register of participants and research data collected by scientific collaborators across five Australian states and territories, with written informed consent obtained from all participants (Loughland et al., 2010). Ethical approval for this project was obtained from the Hunter Area Health Services Human Research Ethics Committee. ASRB participation required that participants be fluent in English (essential for the neurocognitive assessments) and aged 18–65 years. Exclusion criteria included having an organic brain disorder, brain injury with greater than 24 h post-traumatic amnesia, mental retardation (IQ < 70), movement disorders, current diagnosis of substance dependence, and electroconvulsive therapy received in the last 6 months. Controls with a personal or family history of any psychosis or bipolar 1 disorder were also excluded. For more detailed information regarding the sampling framework and consent procedures, see Loughland et al. (Loughland et al., 2010). Although none of the volunteers were hospitalized at the time of assessment, the majority of the SZ cases had anti-psychotic medication.

An initial set of ASRB participants (comprising 617 cases and 659 HCs) included clinical cases meeting ICD-10 criteria for schizophrenia or schizoaffective disorder, with diagnoses confirmed using the OPCRIT algorithm applied to interviewer ratings on the diagnostic interview for psychosis. These cases were subject to Grade of Membership (GoM) analyses applied to cognitive data available for this sample (see Additional file 1) to determine individuals within three subtypes representing Good Cognition (GC, mostly healthy controls), cognitive deficit (CD, clinical cases) and cognitively spared (CS, mostly clinical cases) abilities (Akaike information criterion = −13201.80). The GoM analysis used 9 cognitive performance indicators, including five subscales of the repeatable battery for the assessment of neuropsychological status (attention, language, visuo-spatial construction, immediate memory, and delayed memory), the controlled oral word association test (COWAT), the letter number sequencing (LNS) Test, and estimates of premorbid (WTAR) and current (WASI) intelligence quotient (IQ). In the entire ASRB sample ($n = 1276$), the cognitive performance of the GC group was superior to both the CD and CS subtypes on all cognitive tests used to discriminate subtypes (all $p < 0.001$; see Table 3 of Additional file 1), and the CS subtype was superior to CD on all cognitive domains (all $p < 0.001$; see Table 3 of Additional file 1). The CD subtype also showed greater severity of negative symptoms and reduced severity of positive symptoms (both $p < 0.001$) relative to the CS subtype (Table 3 of Additional file 1).

For the gene expression analyses reported here, we randomly selected a sub-sample of these ASRB participants who all identified as Caucasian, including 49 HCs (all deemed to have "Good Cognition" according to the GoM analysis outlined above) matched for age, sex, and RNA quality with 47 SZ cases (clinically stable outpatients, of which 22 belonged to the cognitive deficit [CD] subtype, and 25 belonged to the cognitively spared [CS] subtype). Demographic, clinical, and cognitive features of these three experimental groups (CS, CD and HC) were compared using analysis of variance (ANOVA) for continuous variables and chi-squared for categorical variables and Tukey's HSD test was used to perform post hoc comparisons (Table 1). Fisher's exact test was used for the comparison of antipsychotic types between CS and CD subtypes.

2.2. RNA preparation, microarray hybridization and scanning

Whole blood was collected from each subject after an overnight fast, followed by PBMC isolation, RNA extraction and integrity assessment as described previously (Gardiner et al., 2012). The mean RQIs for controls and cases were 8.6 ± 1.6 and 9.0 ± 1.5, respectively, which were considered to be within the range of acceptable RNA quality according to the manufacturer's instructions (Bio-Rad Laboratories). Contaminants including phenol–chloroform, salts and genomic DNA were removed from total RNA using the RNeasy minikit (Qiagen, VIC, Australia) according to the manufacturer's instructions. Each RNA sample was then amplified, biotinylated and column purified prior to hybridization to the array using the TotalPrep Amplification kit (Ambion, ABI, CA, USA) according to the manufacturer's protocol. Labeled RNA