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Gene expression analysis reveals schizophrenia-associated dysregulation of immune pathways in peripheral blood mononuclear cells

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ABSTRACT

Peripheral blood mononuclear cells (PBMCs) represent an accessible tissue source for gene expression profiling in schizophrenia that could provide insight into the molecular basis of the disorder. This study used the Illumina HT_12 microarray platform and quantitative real time PCR (QPCR) to perform mRNA expression profiling on 114 patients with schizophrenia or schizoaffective disorder and 80 non-psychiatric controls from the Australian Schizophrenia Research Bank (ASRB). Differential expression analysis revealed altered expression of 164 genes (59 up-regulated and 105 down-regulated) in the PBMCs from patients with schizophrenia compared to controls. Bioinformatic analysis indicated significant enrichment of differentially expressed genes known to be involved or associated with immune function and regulating the immune response. The differential expression of 6 genes, *EIF2C2* (*Ago 2*), *MEF2D*, *EVL*, *PI3*, *S100A12* and *DEFA4* was confirmed by QPCR. Genome-wide expression analysis of PBMCs from individuals with schizophrenia was characterized by the alteration of genes with immune system function, supporting the hypothesis that the disorder has a significant immunological component in its etiology.

1. Introduction

Schizophrenia is a heterogeneous disorder not characterized by a single gene or possibly even a single biological pathway and likely belongs within a “spectrum of psychosis” (Tienari et al., 2003). The existence of a continuum of psychiatric illness encompassing disorders displaying psychotic symptoms, such as schizophrenia and bipolar disorder, could manifest from altered gene expression, controlled at both transcriptional and post-transcriptional levels. Gene expression profiling may be useful for addressing the issue of heterogeneity by identifying genes related to the underlying biology of schizophrenia.

Gene expression studies have been conducted in several brain regions using post-mortem tissue (see Sequeira et al., 2012 for a recent review), but large sample sizes of post-mortem brain tissue are difficult to collect and gene expression profiling in brain tissue is impractical in living patients. Alternatively, the investigation of gene expression in peripheral blood mononuclear cells (PBMCs) that are easily obtained, offer the possibility of longitudinal follow-up, ensure excellent RNA quality and show a considerable degree of heritability and stability in gene expression levels (Meaburn et al., 2009) is a more feasible approach. Indeed, brain expressed genes are expressed in PBMCs and some show co-expression or similar expression levels in the same individuals, supporting their use as a surrogate tissue for gene expression profiling in schizophrenia (Bowden et al., 2006; Gladkevich et al., 2004; Liew et al., 2006; Rollins et al., 2010; Sullivan et al., 2006). In this regard, other groups have sought to identify blood-based expression profiles or develop panels of genes in small cohorts which may be useful for identifying functionally significant genetic and epigenetic changes in individuals with schizophrenia compared to healthy controls or even other

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related psychiatric disorders (Kurian et al., 2009; Maschietto et al., 2012; Middleton et al., 2005; Takahashi et al., 2010; Tsuang et al., 2005).

Gene expression profiling in PBMCs could also be used to identify functionally relevant pathways in schizophrenia as the lymphocyte constituents may act as a point of communication between the immune and nervous systems (Gladkevich et al., 2004; Sullivan et al., 2006). These cells have been shown to express a number of brain associated proteins including receptors for brain derived neurotrophic factor (*BDNF*), glucocorticoids, catecholamines, serotonin, dopamine and acetylcholine, and conversely many neurons express receptors for signaling molecules of the immune system (e.g. cytokines) (Guyon et al., 2008; Kronfol and Remick, 2000; McKenna et al., 2002; Muller and Ackenheil, 1998). That there may be an immunological link to the pathophysiology of schizophrenia is not a new concept. Linkage and GWAS support an association of a broad section of markers in the major histocompatability complex (MHC) region at 6p21.33 with schizophrenia (Consortium, 2008; Lewis et al., 2003; Li et al., 2010; O'Donovan et al., 2008; Ripke et al., 2011; Shi et al., 2009; Stefansson et al., 2009) and other reports suggest this locus is biologically relevant to schizophrenia (Laumbacher et al., 2003; Singh et al., 2008; Straub et al., 2002). Immune-associated genes, genetic variants and haplotypes are also implicated in schizophrenia (Lencz et al., 2007; Ozbey et al., 2009; Paul-Samojedny et al., 2011). A recent study combining Gene Set Enrichment Analysis (GSEA) and hypergeometric analysis of GWAS data showed pathways relating to apoptosis, inflammation or immunity were over-represented in schizophrenia (Jia et al., 2010).

Activation of the immune system is suggested by studies reporting altered levels of pro- and anti-inflammatory cytokines, acute phase proteins, complement components, antibodies and lymphocyte subset numbers, ratios, proliferation, activation and function in several tissue sources (serum, whole blood, cerebrospinal fluid (CSF)) from patients with schizophrenia (Maxeiner et al., 2009; Meyer et al., 2011; Miller et al., 2011; Potvin et al., 2008). Moreover, signs of inflammation and activation or increased densities of microglia have been observed in post-mortem brains and CSF from patients with schizophrenia (Doorduin et al., 2009; Drexhage et al., 2010; Monji et al., 2011; van Berckel et al., 2008). A wealth of evidence suggests diverse environmental risk factors impacting on immune function may play a role in schizophrenia including maternal infection and birth in the peak infection seasons of Winter/Spring (Brown and Derkits, 2010; Byrne et al., 2007; Meyer et al., 2006; Zuckerman and Weiner, 2005), maternal and neonatal deficiency in vitamin D which is critical for immunocompetency (McGrath et al., 2010; Schwalfenberg, 2011), malnutrition and psychological stress (reviewed in Markham and Koenig, 2011). Indeed, rodent models of maternal immune challenge results in schizophrenia-associated behavioral changes and cognitive deficits (Nawa and Takei, 2006; Shi et al., 2003).

In support of an immunological manifestation of the disorder we recently identified a microRNA (miRNA) signature associated with immune function in PBMCs from individuals with schizophrenia (Gardiner et al., 2011). Thus it is plausible that PBMCs reflect changes in the genome of patients with schizophrenia that define its character and etiology in those individuals. To further develop gene expression signatures that characterize the molecular background in individuals with schizophrenia we conducted gene or mRNA expression profiling in the largest PBMC cohort to date using the Australian Schizophrenia Research Bank (ASRB). The ASRB is a well characterized cohort of participants with a diagnosis of schizophrenia and carefully screened non-psychiatric controls (Loughland et al., 2010). Here we report differential expression of a large number of genes involved in the immune system.

2. Materials and methods

2.1. Participant recruitment and clinical assessment protocol

This study utilized participants, the majority of whom identified as Caucasian, from the Australian Schizophrenia Research Bank (ASRB) and the Hunter DNA Bank (HDB) (describe previously by Gardiner et al., 2011 and Loughland et al., 2010). Ethics approval was obtained from the Hunter Area Health Services Human Research Ethics Committee and written informed consent obtained from all participants. In this study, the cohort consisted of 114 participants with a lifetime diagnosis of schizophrenia or schizoaffective disorder (cases) as diagnosed by the World Health Organization's ICD-10 criteria and 80 non-psychiatric controls. Demographic and clinical variables of the cohort are summarized in Table 1 and detailed in Supplementary Table 1. There was a significant difference in the mean age of cases and controls (mean age cases 42.3 years, controls 38.7 years, 2-tailed t test $p = 0.0498$) that is of a small magnitude. Similarly, there was a difference in mean age between schizophrenia and schizoaffective cases (mean age schizophrenia 41 years, mean age schizoaffective disorder 46 years, 2-tailed t test $p = 0.03$). This small difference in age in mature adults was considered to be unlikely to have significantly impacted upon gene expression. There was a significant difference in gender distribution of cases and controls with more males in the patient group compared to the controls (60% male cases, 42% male controls, 2-tailed Pearson Chi-square $p = 0.013$) although this is similar to the gender distribution in population samples. There was no difference in gender distribution between schizophrenia and schizoaffective cases (Pearson Chi-square $p = 0.286$).

2.2. Amplification and labeling of RNA

Whole blood was collected, followed by PBMC isolation, RNA extraction and integrity assessment as described previously (Gardiner et al., 2011). The mean (SD) RQI for this cohort was 9.1 (0.8) and the RQIs 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

Table 1
Summary of demographic and clinical characteristics of the participants.

Demographic/clinical variable	Summary statistics
Non-psychiatric control	80
Mean age (years)	38.7
Gender: M/F	34/46
Mean RQI	9.0
Cases[a]	114
Schizophrenia	77
Schizoaffective disorder (manic type)	16
Schizoaffective disorder (depressed type)	13
Schizoaffective disorder (bipolar type)	8
Mean age (years)	42.4
Gender: M/F	69/45
Mean RQI	9.1
Mean age at onset of illness (years)	23.96
Family history schizophrenia (present/none/unknown)	46/67/1
Family history other psychosis (present/none/unknown)	67/46/1
Mean duration of illness (years)	18.16

M – male; F – female; RQI – RNA quality indicator.
[a] ICD-10 diagnosed schizophrenia or schizoaffective disorder (depressed, bipolar or manic subtype); family history other psychosis – reports any other psychiatric mental illness in any first or second degree relative.

TotalPrep Amplification kit (Ambion, ABI, CA, USA) according to the manufacturer's protocol.

2.3. Differential gene expression profiling

Labeled RNA (750 ng) was hybridized to Illumina HT-12_V3 beadchips (~48,000 probes) according to the manufacturer's protocol. Expression data underwent quality control analysis and background subtraction in GenomeStudio V3.0 (Illumina, CA, USA) and expression data was exported into R. Further quality control was conducted using the R with lumi packages (www.bioconductor.org) (Du et al., 2008), where the variance-stabilizing transformation (VST) (Lin et al., 2008) was applied. An average of 9624 transcripts were detected for the cohort representing 20% of the total number of transcripts present on the array (detection p value <0.05, before normalisation/background subtraction). Robust Quantile Normalization (RSN) was then applied to expression values for genes considered to be expressed (determined using the detection p value <0.05), followed by differential expression analysis using a linear empirical Bayes model (Smyth, 2004). Significantly differentially expressed genes were identified after p value correction for multiple testing by the Benjamini and Hochberg method. Initial analysis indicated 307 transcripts were differentially expressed in schizophrenia compared to non-psychiatric controls (Supplementary Table 2), which was refined to 164 altered transcripts after exclusion of genes with <10% fold change and discontinued or poorly annotated NCBI Entrez Gene Database records.

2.4. Quantitative real-time reverse transcription PCR (Q-PCR)

Q-PCR validation of differentially expressed mRNA was performed on a subset of the cohort (83 participants: 48 schizophrenia or schizoaffective patients, 35 non-psychiatric controls) as described previously (Santarelli et al., 2011). 10 genes were selected for Q-PCR validation based on strong differential expression of the array and/or biological significance. Both *MEF2D* (myocyte enhancer factor 2D) and *EIF2C2* (or argonaute 2; *AGO2*) (eukaryotic translation initiation factor 2C, 2) are implicated in miRNA biogenesis which we have previously shown to be altered in post-mortem brain (Beveridge et al., 2010) as well as in PBMCs (Gardiner et al., 2011). In addition, MEF2D was altered in neuroblast culture in response to retinoic acid-induced differentiation suggesting it may be involved in neuronal differentiation, a process that is biologically relevant to schizophrenia. Several genes were also chosen for their involvement in immune function: *EVL* (Enah/Vasp-like), *DEFA4* (defensin α4), *PI3* (peptidase inhibitor 3, skin-derived), *S100A12* (S100 calcium binding protein A12), *CCR7* (Chemokine (C–C motif) receptor 7), *CD6* molecule and *HMHA1* (histocompatibility (minor) HA-1). *VAMP5* (vesicle-associated membrane protein 5 (myobrevin)) was chosen, as it was one of the most strongly up-regulated genes. Primers were designed in Oligo Explorer V1.5 (Gene Link, NY, USA) (primer sequences are listed in Supplementary Table 3). Relative mRNA expression was calculated as the ratio of the gene and the geometric mean of the most stable and efficient housekeeping genes hydroxymethylbilane synthase (*HMBS*) and 18S ribosomal RNA (*18S*). Outliers (expression >3 standard deviations from the mean) were excluded from further analysis. Statistical significance of differential mRNA expression between schizophrenia and control groups was determined by Student's t-test (one-tailed $p < 0.05$).

2.5. Effects of demographic variables on gene expression

The effect of demographic variables was tested by correlation analysis where Pearson's Correlation was used for normally distributed data and Spearman Correlation was used for data that did not follow a normal distribution. Expression values from the microarray (for all 194 differentially expressed genes) and Q-PCR (validated genes) were tested for correlation with age. Additionally, for the validated genes, microarray and Q-PCR expression were analyzed for covariance with gender, RQI and diagnosis (schizophrenia compared to schizoaffective disorder) using a 2-tailed Mann–Whitney-*U* test and one-way ANOVA.

2.6. Bioinformatic functional analyses

To determine the most significant biological functions and pathways represented by the differentially expressed genes, a list of these genes and their corresponding fold changes were uploaded into Ingenuity Pathway Analysis (IPA) knowledge base v6.3 (Ingenuity Systems, USA, www.ingenuity.com). Of the 166 differentially expressed genes, 164 unique transcripts mapped to annotated gene IDs of which 140 were included in network analysis and 118 were eligible for functions annotation and pathways analysis. Functional Annotation Analysis of the differentially expressed genes was applied to determine the significant Biological Functions and Functions Annotation ($p < 0.05$ after Benjamini–Hochberg correction for multiple testing) with at least 2 or more genes representing each annotation. Networks showing relationships and interactions between differentially expressed genes and others that functionally interact with them, were generated and ranked in terms of their relevance i.e. the number of participating genes, degree of connectivity and size relative to the total number of network eligible genes.

IPA also allowed the integration of mRNA expression data with miRNA expression data previously collected in an overlapping cohort in which 134 participants were common to both studies (61 controls and 73 cases) (Gardiner et al., 2011). Expression data for the 83 miRNA that were identified as significantly differentially expressed in schizophrenia (FDR <5) was uploaded to IPA where 60 had target prediction information. IPA identifies putative mRNA targets for the miRNA using experimentally validated interactions (TarBase and miRecords) as well as predicted miRNA–mRNA interactions (TargetScan Human Release 6.2; http://www.targetscan.org/; Lewis et al., 2005) and miRNA-related findings from the peer-reviewed literature. The putative miRNA:mRNA pairs were then filtered with respect to fold change to identify inverse miRNA:mRNA target pairs (where the expression of the miRNA is the opposite of it's mRNA target).

3. Results

3.1. Gene expression profiling

mRNA expression was measured in 114 participants with schizophrenia or schizoaffective disorder (cases) compared to 80 non-psychiatric controls. A total of 164 genes displayed differential expression with changes ≥10% and $p < 0.05$; 105 were down-regulated and 59 up-regulated in the cases compared to controls (Fig. 1, Table 2). Ten genes were chosen for Q-PCR validation based on strong differential expression on the array and/or biological significance. Six genes were confirmed to have significant alterations in expression in the cases; myocyte enhancer factor 2D (*MEF2D*), eukaryotic translation initiation factor 2C, 2 (*EIF2C2*; or argonaute 2 (*AGO2*)) and Enah/Vasp-like (*EVL*) were down-regulated and defensin α4 (*DEFA4*), peptidase inhibitor 3, skin-derived (*PI3*) and S100 calcium binding protein A12 (*S100A12*) were up-regulated, validating the results of the microarray (Fig. 2 and Table 3). Validation of four additional genes was conducted. Chemokine (C–C motif) receptor 7 (*CCR7*; $p = 0.054$) and *CD6*

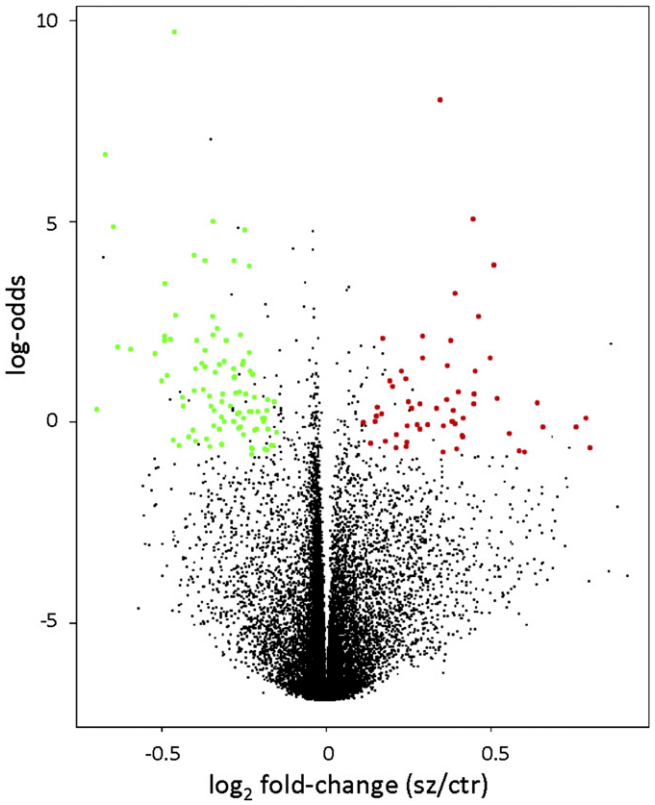


Fig. 1. Volcano plot of differentially expressed genes. A scatter-plot of the log odds (probability) against the $\log_2$ fold change in expression in PBMCs (schizophrenia/control). Genes with statistically significant differential expression in schizophrenia (Benjamini–Hochberg corrected $p < 0.05$, fold change >10% up or down-regulation) with current NCBI Entrez gene records are depicted in the upper quadrants; 105 genes down-regulated on the left (green) and 59 genes up-regulated on the right (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

molecule ($p = 0.053$), showed a strong trend toward down-regulation in the cases, whilst histocompatibility (minor) HA-1 (*HMHA1*; $p = 0.089$) and vesicle-associated membrane protein 5 (myobrevin) (*VAMP5*; $p = 0.101$) showed general trends toward down and up-regulation respectively (Fig. 2A and B), all of which were consistent with the microarray analysis. The fold changes detected by the microarray and Q-PCR were significantly correlated (Pearson $r = 0.976$, $p < 0.0001$, data not shown) and in all but one instance, fold changes detected by Q-PCR were of greater magnitude compared to those detected on the microarray.

3.2. Effects of demographic variables on gene expression

Neither microarray nor Q-PCR data for *MEF2D*, *EIF2C2* (*AGO2*), *EVL*, *PI3*, *S100A12* and *DEFA4* showed a correlation with age or RQI (microarray: 2 tailed Spearman $p > 0.086$; Q-PCR: Pearson correlation, $p > 0.304$). There was no difference in expression of these genes between males and females (2-tailed Mann–Whitney-*U* test $p > 0.154$) with the exception of *DEFA4* where the expression was greater in males than females by microarray (2-tailed Mann–Whitney-*U* test, $p = 0.001$). Indeed, this up-regulation of *DEFA4* was significant in male cases compared to male controls but was also up-regulated in female cases compared to female controls (2-tailed Mann–Whitney-*U* test $p = 0.003$ and $p = 0.004$ respectively) and this trend was confirmed by the Q-PCR data (not shown). One-way ANOVA revealed no difference in expression between schizophrenia and schizoaffective cases with the exception of *PI3*, in which mean expression in schizophrenia was higher than schizoaffective disorder on the microarray ($p = 0.013$) that was not supported by the Q-PCR data ($p = 0.485$). There was a significant correlation between the expression of *CCR7* (microarray data) and age (Spearman $r = -0.2792$, two-tailed $p = 0.0001$).

3.3. Functional annotation and bioinformatic analysis

The list of differentially expressed genes and fold changes was submitted for Ingenuity Pathways Analysis (IPA). This analysis revealed the strong presence of genes involved in various aspects of immune function with ~37% of the total functional annotations categorized as being immune/inflammation-associated (Fig. 3). Top ranked biological functions included Infectious Disease, Inflammatory Response, Antigen Presentation, Immune Cell Trafficking and Cell-mediated Immune Response (Table 3). Immune/Inflammation related Functional Annotations included Severe Acute Respiratory Syndrome, Chemotaxis/Recruitment of various immune cells, Replication of a Virus, Respiratory/Infectious Disorder, Antimicrobial Response, Inflammatory/Immune Response (Table 4). The full Functional Annotation Analysis is provided in Supplementary Table 4. IPA identifies molecular relationships of differentially expressed genes in the context of biological pathways and indicated an enrichment of differentially expressed Immune/Inflammation-related genes in top scoring networks and canonical pathways (Supplementary Tables 5 and 6). Network 1, enriched with functions including Cell-to-Cell Signaling and Interaction, Infectious Disease and Respiratory Disease is illustrated in Supplementary Fig. 1.

Comparison of the mRNA expression data with the previously described miRNA expression data (Gardiner et al., 2011) revealed 102 miRNA:mRNA pairings (in all cases the miRNA was down-regulated while the mRNA was up-regulated), consisting of 42 unique miRNA targeting 37 unique mRNA (Supplementary Table 7).

4. Discussion

In this study, we conducted differential mRNA expression profiling in the largest cohort of patients with schizophrenia or schizoaffective disorder compared with non-psychiatric controls reported to date. This revealed 164 differentially expressed genes (≥10%) after correction for multiple testing, supported by Q-PCR analysis of gene expression. Bioinformatic analysis of differentially expressed genes indicated enrichment of immune/inflammation-related functions providing supporting evidence for immune dysfunction in schizophrenia.

Interestingly, when we considered up-regulated genes in this enriched group separately, the innate immune response in particular was featured. For example, Secretory Leukocyte Peptidase Inhibitor (*SLPI*) and Azurocidin 1 (*AZU1*) are chemotactic for cells of the innate immune system and modulate the inflammatory response (Sallenave, 2002; Soehnlein et al., 2008; Subramaniyam et al., 2011). Lipocalin 2 (*LCN2*) is up-regulated during inflammation and in the plasma of patients with mild cognitive impairment (Choi et al., 2011), and is induced by *IL-1β*, a protein elevated in the CSF of first episode schizophrenia patients (Cowland et al., 2003; Soderlund et al., 2009). Chemokine-like factor (*CKLF*), has roles in dendritic cell maturation (Han et al., 2001; Ke et al., 2002; Shao et al., 2010) and is chemotactic for immune cells and possibly has roles in brain development (Han et al., 2001; Wang et al., 2010). Two α-defensin family members, *DEFA4* (Q-PCR validated) and *DEFA1β* were up-regulated, consistent with increased α-defensin protein levels in T cell lysates from treated and antipsychotic free schizophrenia patients (Craddock et al., 2008). Defensins (endogenous peptide antibiotics) are abundant in neutrophil granules,

Table 2
Differentially expressed genes in PBMCs in schizophrenia compared to non-psychiatric controls.

Gene symbol	Gene name	Chromosomal location	*p* Value	Fold change
Up-regulated				
ASGR2	Asialoglycoprotein receptor 2	17p	0.0008	1.27
RNASE3	Ribonuclease, RNase A family, 3	14q24–q31	0.0049	1.36
RNASE2	Ribonuclease, RNase A family, 2	14q24–q31	0.0069	1.43
ARPC4	Actin related protein 2/3 complex, subunit 4	3p25.3	0.0106	1.31
TCN1	Transcobalamin I (vitamin B12 binding protein, R binder family)	11q11–q12	0.0147	1.38
SLPI	Secretory leukocyte peptidase inhibitor	20q12	0.0192	1.3
RPL34	Ribosomal protein L34	4q25	0.0192	1.23
ATP5S	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit S (factor B)	14q21.3	0.0192	1.13
C9ORF16	Chromosome 9 open reading frame 16	16p11.2	0.0229	1.23
VAMP5	Vesicle-associated membrane protein 5 (myobrevin)	2p11.2	0.0229	1.41
RNASE6	Ribonuclease, RNase A family, 6	14q11.2	0.0246	1.29
CKLF	Chemokine-like factor	16q21	0.0247	1.37
EXOSC1	Exosome component 1	10q24	0.0247	1.17
WLS	Wntless homolog (drosophila)	1p31.3	0.0247	1.13
GTF2H5	General transcription factor IIH, polypeptide 5	6q25.3	0.0255	1.18
CYBRD1	Cytochrome b reductase 1	2q31.1	0.0259	1.14
HIBADH	3-hydroxyisobutyrate dehydrogenase	7p15.2	0.0277	1.15
NTPCR	Nucleoside-triphosphatase, cancer-related	1q42.2	0.0290	1.2
CFP	Complement factor properdin	Xp11.4	0.0296	1.32
DYNLL1	Dynein, light chain, LC8-type 1	12q24.23	0.0297	1.37
CTSD	Cathepsin D	11p15.5	0.0303	1.44
PPPDE2	PPPDE peptidase domain containing 2	22q13.2	0.0309	1.29
ENTPD1	Ectonucleoside triphosphate diphosphohydrolase 1	10q24	0.0309	1.19
DEFA4	Defensin, alpha 4, corticostatin	8p23.1	0.0309	1.56
NME1	Non-metastatic cells 1, protein (NM23A) expressed in	17q21.3	0.0314	1.22
LSM1	LSM1 homolog, U6 small nuclear RNA associated (*S. cerevisiae*)	8p11.2	0.0314	1.37
TFF3	Trefoil factor 3 (intestinal)	21q22.3	0.0322	1.11
CSTB	Cystatin B (stefin B)	21q22.3	0.0326	1.31
RAB32	RAB32, member RAS oncogene family	6q24.3	0.0326	1.26
MRPS18C	Mitochondrial ribosomal protein S18C	4q21.23	0.0326	1.2
CNPY2	Canopy 2 homolog (zebrafish)	12q15	0.0326	1.19
UQCRB	Ubiquinol-cytochrome c reductase binding protein	8q22	0.0331	1.12
C18ORF10	Chromosome 18 open reading frame 10	18q12.2	0.0336	1.11
DEFA1B	Defensin, alpha 1B	8p23.1	0.0339	1.73
HIST1H2BK	Histone cluster 1, H2bk	6p21.33	0.0339	1.34
AZU1	Azurocidin 1	19p13.3	0.0348	1.31
ACPP	Acid phosphatase, prostate	3q21–q23	0.0348	1.11
RNF130	Ring finger protein 130	5q35.3	0.0354	1.31
LGALS3	Lectin, galactoside-binding, soluble, 3	14q22.3	0.0356	1.28
RAB5C	RAB5C, member RAS oncogene family	17q21.2	0.0356	1.24
HMGN2	High mobility group nucleosomal binding domain 2	1p36.1	0.0356	1.21
RPS4Y2	Ribosomal protein S4, Y-linked 2	Yq11.223	0.0356	1.19
S100A12	S100 calcium binding protein A12	1q21	0.0357	1.7
HBD	Hemoglobin, delta	11p15.5	0.0357	1.58
POLR2C	Polymerase (RNA) II (DNA directed) polypeptide C, 33 kDa	16q13–q21	0.0366	1.22
LCN2	Lipocalin 2	9q34	0.0392	1.47
MS4A3	Membrane-spanning 4-domains, subfamily A, member 3 (hematopoietic cell-specific)	11q12.1	0.0402	1.16
DBI	Diazepam binding inhibitor (GABA receptor modulator, acyl-CoA binding protein)	2q12–q21	0.0412	1.33
C19ORF70	Chromosome 19 open reading frame 70	19p13.3	0.0418	1.33
TM2D1	TM2 domain containing 1	1p31.3	0.0440	1.13
GMPR2	Guanosine monophosphate reductase 2	14q12	0.0448	1.19
CHMP3 (VPS24)	Vacuolar protein sorting 24 homolog (*S. cerevisiae*)	2p11.2	0.0453	1.1
CDC42SE1	CDC42 small effector 1	1q21.3	0.0454	1.18
MRPS33	Mitochondrial ribosomal protein S33	7q34	0.0454	1.16
PI3	Peptidase inhibitor 3, skin-derived	20q13.12	0.0457	1.74
PRDX5	Peroxiredoxin 5	11q13	0.0460	1.32
RPL23	Ribosomal protein L23	17q	0.0478	1.5
UBTD1	Ubiquitin domain containing 1	10q24.2	0.0487	1.28
RBX1	Ring-box 1, E3 ubiquitin protein ligase	22q13.2	0.0488	1.52
Down-regulated				
TRRAP	Transformation/transcription domain-associated protein	7q21.2–q22.1	0.0003	0.73
CCR7	Chemokine (C–C motif) receptor 7	17q12–q21.2	0.0017	0.63
EVL	Enah/Vasp-like	14q32.2	0.0049	0.64
ZNF827	Zinc finger protein 827	4q31.22	0.0049	0.79
BRAT1	BRCA1-associated ATM activator 1	7p22.3	0.0049	0.83
LEMD2	LEM domain containing 2	6p21.31	0.0049	0.84
C16ORF58	Chromosome 16 open reading frame 58	16p11.2	0.0067	0.76
E4F1	E4F transcription factor 1	16p13.3	0.0067	0.77
RNF216	Ring finger protein 216	7p22.1	0.0067	0.82
IL16	Interleukin 16 (lymphocyte chemoattractant factor)	15q26.3	0.0069	0.85
HMHA1	Histocompatibility (minor) HA-1	19p13.3	0.0097	0.71
CHTOP	Chromatin target of PRMT1	1q21.3	0.0133	0.88
IQSEC1	IQ motif and Sec7 domain 1	3p25.2	0.0147	0.73

(*continued on next page*)

Table 2 (*continued*)

Gene symbol	Gene name	Chromosomal location	*p* Value	Fold change
CBLB	Cas-Br-M (murine) ecotropic retroviral transforming sequence b	3q13.11	0.0147	0.79
LRP5L	Low density lipoprotein receptor-related protein 5-like	22q11.23	0.0189	0.79
SAFB	Scaffold attachment factor B	19p13.3–p13.2	0.0192	0.71
PLA2G4B	Phospholipase A2, group IVB (cytosolic)	15q11.2–q21.3	0.0192	0.71
SGSM2	Small G protein signaling modulator 2	17p13.3	0.0192	0.72
SEC16A	SEC16 homolog A (*S. cerevisiae*)	9q34.3	0.0192	0.76
LONP1	Lon peptidase 1, mitochondrial	19p13.2	0.0192	0.79
SEC24C	SEC24 family, member C (*S. cerevisiae*)	10q22.2	0.0192	0.81
MTMR14	Myotubularin related protein 14	3p26	0.0192	0.84
CD6	CD6 molecule	11q13	0.0205	0.64
TYK2	Tyrosine kinase 2	19p13.2	0.0211	0.66
CBX6	Chromobox homolog 6	22q13.1	0.0212	0.77
RASAL3	RAS protein activator like 3	19p13.12	0.0212	0.7
STK10	Serine/threonine kinase 10	5q35.1	0.0212	0.85
FAM153B	Family with sequence similarity 153, member B	5q35.2	0.0237	0.81
C7ORF54	Chromosome 7 open reading frame 54	7q31	0.0237	0.84
CLUAP1	Clusterin associated protein 1	16p13.3	0.0241	0.77
BTBD11	BTB (POZ) domain containing 11	12q23.3	0.0241	0.8
P2RY11	Purinergic receptor P2Y, G-protein coupled, 11	19p13.2	0.0241	0.84
TJAP1	Tight junction associated protein 1 (peripheral)	6p21.1	0.0246	0.77
ITPR3	Inositol 1,4,5-trisphosphate receptor, type 3	6p21	0.0247	0.72
TMEM175	Transmembrane protein 175	4p16.3	0.0247	0.76
TGFBRAP1	Transforming growth factor, beta receptor associated protein 1	2q12.1	0.0247	0.79
SURF6	Surfeit 6	9q34.2	0.0247	0.82
NOP2	NOP2 nucleolar protein homolog (yeast)	12p13	0.0247	0.82
PMPCA	Peptidase (mitochondrial processing) alpha	9q34.3	0.0247	0.83
CTC1	CTS telomere maintenance complex component 1	17p13.1	0.0247	0.83
ZNF212	Zinc finger protein 212	7q36.1	0.0247	0.85
ASXL1	Additional sex combs like 1 (drosophila)	20q11.1	0.0247	0.86
CDK12	Cyclin-dependent kinase 12	17q12	0.0247	0.88
MEF2D	Myocyte enhancer factor 2D	1q12–q23	0.0259	0.71
COX19	COX19 cytochrome c oxidase assembly homolog (*S. cerevisiae*)	7p22.3	0.0290	0.77
UBN1	Ubinuclein 1	16p13.3	0.0295	0.76
SNX29	Sorting nexin 29	16p13.13–p13.12	0.0296	0.73
REC8	REC8 homolog (yeast)	14q11.2–q12	0.0297	0.8
RHBDF2	Rhomboid 5 homolog 2 (drosophila)	17q25.1	0.0297	0.83
MOV10	Mov10, moloney leukemia virus 10, homolog (mouse)	1p13.2	0.0297	0.83
QSOX2	Quiescin Q6 sulfhydryl oxidase 2	9q34.3	0.0297	0.84
URGCP	Upregulator of cell proliferation	7p13	0.0297	0.88
C19ORF6	Chromosome 19 open reading frame 6	19p13.3	0.0297	0.78
NAT10	N-acetyltransferase 10 (GCN5-related)	11p13	0.0297	0.74
YY1AP1	YY1 associated protein 1	1q22	0.0297	0.86
EIF2C2 (AGO2)	Eukaryotic translation initiation factor 2C, 2	8q24	0.0303	0.72
PPP1R3E	Protein phosphatase 1, regulatory (inhibitor) subunit 3E	14q11.2	0.0304	0.88
INTS1	Integrator complex subunit 1	7p22.3	0.0309	0.8
ISYNA1	Inositol-3-phosphate synthase 1	19p13.11	0.0309	0.9
ANP32A-IT1	ANP32A intronic transcript 1 (non-protein coding)	15q23	0.0309	0.89
FCGBP	Fc fragment of IgG binding protein	19q13.1	0.0319	0.79
SBF1	SET binding factor 1	22q13.33	0.0319	0.82
SPG7	Spastic paraplegia 7 (pure and complicated autosomal recessive)	16q24.3	0.0322	0.74
EDC4	Enhancer of mRNA decapping 4	16q22.1	0.0326	0.62
REXO1	REX1, RNA exonuclease 1 homolog (*S. cerevisiae*)	19p13.3	0.0326	0.79
METTL16	Methyltransferase like 16	17p13.3	0.0326	0.82
C9ORF91	Chromosome 9 open reading frame 91	9q32	0.0326	0.85
ZSCAN18	Zinc finger and SCAN domain containing 18	19q13.43	0.0326	0.83
CACNA1I	Calcium channel, voltage-dependent, T type, alpha 1I subunit	22q13.1	0.0326	0.85
HIC2	Hypermethylated in cancer 2	22q11.21	0.0326	0.87
RASA3	RAS p21 protein activator 3	13q34	0.0326	0.88
BCL11B	B-cell CLL/lymphoma 11B (zinc finger protein)	14q32.2	0.0331	0.83
HDC	Histidine decarboxylase	15q21–q22	0.0339	0.8
TSHZ1	Teashirt zinc finger homeobox 1	18q22.3	0.0339	0.84
MED29	Mediator complex subunit 29	19q13.2	0.0339	0.87
FBXO32	F-box protein 32	8q24.13	0.0339	0.88
LRWD1	Leucine-rich repeats and WD repeat domain containing 1	7q22.1	0.0348	0.81
SFMBT2	Scm-like with four mbt domains 2	10p14	0.0348	0.82
ATF5	Activating transcription factor 5	19q13.3	0.0348	0.88
TNPO2	Transportin 2	19p13.2	0.0356	0.79
CREBBP	CREB binding protein	16p13.3	0.0357	0.84
CTRL	Chymotrypsin-like	16q22.1	0.0360	0.83
C17ORF63	Chromosome 17 open reading frame 63	17q11.2	0.0366	0.86
ZNF672	Zinc finger protein 672	1q44	0.0367	0.8
POM121	POM121 membrane glycoprotein	7q11.23	0.0367	0.88
ULK1	Unc-51-like kinase 1 (*C. elegans*)	12q24.3	0.0370	0.76
TELO2	TEL2, telomere maintenance 2, homolog (*S. cerevisiae*)	16p13.3	0.0370	0.86
ZNF446	Zinc finger protein 446	19q13.43	0.0384	0.9
ELP2	Elongation protein 2 homolog (*S. cerevisiae*)	18q12.2	0.0406	0.84

Table 2 (*continued*)

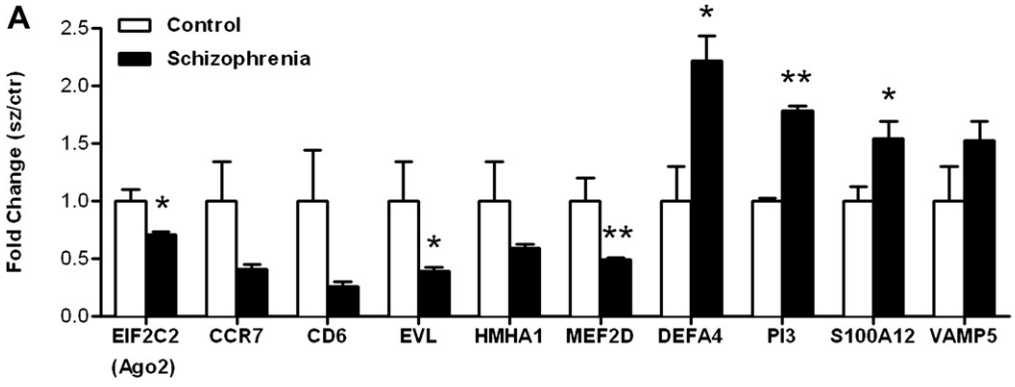
Gene symbol	Gene name	Chromosomal location	*p* Value	Fold change
ZNF746	Zinc finger protein 746	7q36.1	0.0416	0.75
ZNF395	Zinc finger protein 395	8p21.1	0.0429	0.78
TRABD	TraB domain containing	22q13.33	0.0435	0.72
CCDC130	Coiled-coil domain containing 130	19p13.2	0.0436	0.76
MAP7D1	MAP7 domain containing 1	1p34.3	0.0453	0.76
TNK2	Tyrosine kinase, non-receptor, 2	3q29	0.0453	0.8
ZNF828	Zinc finger protein 828	13q34	0.0453	0.8
EIF2B5	Eukaryotic translation initiation factor 2B, subunit 5 epsilon, 82 kDa	3q27.1	0.0454	0.89
EML3	Echinoderm microtubule associated protein like 3	11q12.3	0.0454	0.73
ABCF1	ATP-binding cassette, sub-family F (GCN20), member 1	6p21.33	0.0454	0.78
CCNT1	Cyclin T1	12q13.11	0.0454	0.89
MYO9B	Myosin IXB	19p13.1	0.0457	0.85
ZNF362	Zinc finger protein 362	1p35.1	0.0460	0.86
ZSWIM4	Zinc finger, SWIM-type containing 4	19p13.13	0.0460	0.88
KIAA0182	KIAA0182	16q24.1	0.0461	0.88
DMAP1	DNA methyltransferase 1 associated protein 1	1p34	0.0497	0.85

Gene symbols for significantly up-regulated ($n = 59$) and down-regulated ($n = 105$) genes are shown (p value represents the corrected p value, after Benjamini–Hochberg multiple testing correction <0.05).

natural killer cells and T lymphocyte subsets and act as immunomodulatory factors regulating acute inflammation, phagocytosis, cell migration/maturation and cytokine secretion (Klotman and Chang, 2006; Rodriguez-Garcia et al., 2007; Selsted and Ouellette, 2005). *DEFA4* inhibits synthesis of anti-inflammatory glucocorticoids known to have significant influence on developing synaptic structure and function in the adult brain (Owen et al., 2005). Many α-defensin genes cluster at 8p23, near a schizophrenia linkage site (Fallin et al., 2011; Suarez et al., 2006) known to be a hot spot for copy number variation (CNV) in normal individuals (Aldred et al., 2005).

Alternatively, when we considered the down-regulated genes, we observed a more even distribution of genes with function in both innate and cell-mediated immunity. Indeed, Surfeit 6 (*SURF6*), a marker of lymphocyte activation for proliferation (Moraleva et al., 2009) and Interleukin 16, a dendritic cell chemo-attractant and modulator of T cell activation and inflammation (Cruikshank and Little, 2008; Kaser et al., 1999) were down-regulated in schizophrenia. *CCR7* controls memory T cell migration to sites of inflammation and stimulates dendritic cell maturation (Dieu et al., 1998; Forster et al., 1999). Down-regulation of *CD6*, a gene involved in T-cell activation promoting their commitment to a Th1 subtype and enhancing their sensitivity to the pro-inflammatory *IL-2* (Nair et al., 2010), might be expected to have anti-inflammatory consequences.

This immune-related gene expression signature is in agreement with other blood-based studies in schizophrenia. Glatt et al. (2005) identified several down-regulated genes from the MHC region in PBMCs and we identified changes to *ABCF1*, *LEMD2*, *TJAP1*, *ITPR3* and



B

Gene	Fold Change Array	Array p-value	Fold Change QPCR	QPCR p value (1-tailed)
EIF2C2 (Ago2)	-1.39	0.03	-1.41	0.014
CCR7	-1.59	0.002	-2.66	0.054
CD6	-1.56	0.021	-3.16	0.053
EVL	-1.56	0.005	-2.57	0.043
HMHA1	-1.41	0.01	-1.68	0.089
MEF2D	-1.4	0.026	-2.01	0.008
DEFA4	1.56	0.031	2.21	0.011
PI3	1.74	0.046	1.78	<0.001
S100A12	1.7	0.036	1.54	0.037
VAMP5	1.41	0.023	1.53	0.101

Fig. 2. **A** QPCR validation of differentially expressed genes. The expression of ten genes highlighted by the microarray was analyzed by QPCR. Bars indicate mean fold change + SEM for 83 participants: 48 schizophrenia or schizoaffective patients and 35 non-psychiatric controls. The control cohort is set at 1. Differential expression of EIF2C2, EVL, DEFA4, S100A12 (*$p < 0.05$) and PI3 and MEF2D (**$p < 0.01$) was validated, with a further two genes (CCR7 $p = 0.054$; CD6 $p = 0.053$) showing a non-significant trend in the same direction as the microarray data. **B** Fold changes and p values for each gene on the microarray and the QPCR (one tailed student's t-test) are shown.

Table 3
Top biological functions enriched for differentially expressed genes in schizophrenia using IPA (Benjamini–Hochberg corrected *p* value range).

	p-Value range	# Molecules
Diseases and disorders		
Infectious disease	7.02E-08–3.35E-02	22
Respiratory disease	7.02E-08–3.35E-02	12
Inflammatory response	3.52E-05–4.63E-02	18
Dermatological diseases and conditions	1.13E-04–4.72E-02	20
Genetic disorder	5.46E-04–4.98E-02	24
Molecular and cellular functions		
Antigen presentation	3.52E-05–4.98E-02	8
Cellular movement	3.52E-05–4.98E-02	14
Cell–cell signaling and interaction	7.13E-05–4.98E-02	15
RNA damage and repair	9.34E-05–1.69E-02	3
Cell death	1.29E-04–4.98E-02	15
Physiological system development and function		
Hematological system development and function	3.52E-05–4.98E-02	10
Immune cell trafficking	3.52E-05–4.98E-02	8
Tissue development	2.94E-03–4.17E-02	6
Cell-mediated immune response	8.48E-03–4.98E-02	4
Connective tissue development and function	8.48E-03–4.98E-02	4

HIST1H2BK that reside at or near this locus. Interestingly, the major histocompatibility complex, class II, DR beta 1 (*HLA-DRB1*) was also down-regulated in the prefrontal cortex (Glatt et al., 2005). Three genes up-regulated in our study and in Glatt's study include the Galectin family member lectin, galactoside-binding, soluble 3 (*LGALS3*), a negative regulator of T lymphocyte activation (Yang et al., 2008); the antiprotease, antibacterial and possibly anti-inflammatory Peptidase inhibitor 3, skin-derived (*PI3*) (Sallenave, 2002) and the pro-inflammatory S100 calcium binding protein A12, calgranulin C (*S100A12*) (Glatt et al., 2005). *S100A12* was also up-regulated in leukocytes obtained from discordant schizophrenic sibling pairs with known linkage to 5q (Middleton et al., 2005). Related S100 genes (*S1200A1* and *S100A6*) were also up-regulated in our post-mortem study of the superior temporal gyrus (STG) in schizophrenia (Bowden et al., 2008) and *S100A9* was up-regulated in whole blood from schizophrenia patients (Tsuang et al., 2005).

Similarly, Kurian et al. (2011) identified differentially expressed genes in whole blood associated with "high hallucination state" or "high delusion state" groups, with 'Inflammatory Response', 'IL-8 Signaling' and 'Chemokine Signalling' as well as 'IL-15 production' among the top Diseases/Disorders or canonical pathways (Kurian et al., 2011). Takahashi et al. (2010) reported up-regulated genes in whole blood from patients with schizophrenia using the supervised classifier Artificial Neural Networks that featured in Gene Ontology (GO) biological processes such as 'Inflammatory Response', 'Lymphocyte Homeostasis', 'Defense Response', 'Immune System Process', 'Cytokine Biosynthetic Process' and 'Cytokine Metabolic Process' (Takahashi et al., 2010).

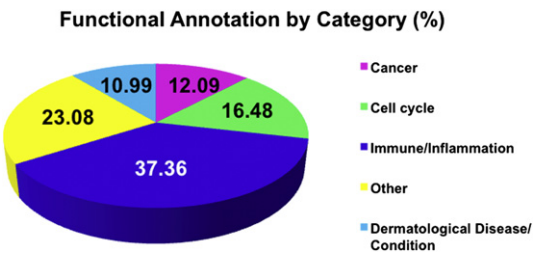


Fig. 3. Functional annotations of differentially expressed genes by category. Assortment of functional annotations by broad functional categories revealed over-representation of genes with immune and inflammation-related functions (~37%) in the list of genes differentially expressed between schizophrenia and controls.

The immune-associated mRNA expression signature also agrees with genes predicted to be targeted by a cluster of differentially expressed miRNA identified in an overlapping cohort to this study at chr14q32 that have roles in a range of immune-related pathways such as 'T Cell Receptor Signaling', 'Chemokine Signaling', 'Natural Killer Cell-Mediated Cytotoxicity' and 'Cytokine–Cytokine Receptor Interaction' (Gardiner et al., 2011). Down-regulation of *EVL* is interesting since it is a host gene for miR-342 (Grady et al., 2008), a member of the down-regulated 14q32 miRNA cluster. *EVL* is involved in remodeling of the actin cytoskeleton which is essential for processes in the CNS (such as axon guidance) and in the immune system (interaction between T lymphocytes and antigen-presenting cells, phagocytosis and chemotaxis of immune cells) (Krause et al., 2003). Down-regulation of *EIF2C2* (encoding the endonuclease argonaute 2; *AGO2*) which functions in small non-coding RNA mediated gene silencing (Cenik and Zamore, 2011) is interesting considering that members of the 14q32 miRNA cluster down-regulated in the cases in this cohort (Gardiner et al., 2011) were over-represented among miRNA that were down-regulated in dopaminergic neurons from the striatum of Ago2-deficient mice (Schaefer et al., 2010). Members of this cluster also contain structural features associated with dicer-independent/Ago2-slicer activity-dependent processing (Diederichs and Haber, 2007; Frank et al., 2010; O'Carroll et al., 2007). This suggests that *EIF2C2* is especially important for 14q32 cluster biogenesis and may be related to their down-regulation in PBMCs in these cases (Gardiner et al., 2011) and supports our previous observations of altered miRNA biogenesis in schizophrenia in the cortex (Beveridge et al., 2010). Similarly, down-regulation of *MEF2D*, a calcium-activated transcription factor, may also provide a mechanism driving the schizophrenia-associated down-regulation of the 14q32 miRNA cluster observed in this cohort since this transcription factor was shown to be a positive regulator of some of these miRNA in rat neurons (Fiore et al., 2009). Moreover, MEF2s have been shown to regulate immune cells (Aude-Garcia et al., 2010; Potthoff and Olson, 2007) and *MEF2D* has important roles in the brain in neuro-development, neuronal survival and synaptic plasticity (Heidenreich and Linseman, 2004; Lam and Chawla, 2007; She et al., 2011). In addition, we have previously reported up-regulation of *MEF2D* in response to retinoic acid-induced neuronal differentiation (Beveridge et al., 2009). The distinct roles this transcription factor has in immune function and in the brain makes it an attractive candidate for future investigation.

To further investigate the relationship between mRNA expression and differentially expressed miRNA reported in the overlapping cases (Gardiner et al., 2011), the expression data was integrated revealing 102 predicted inverse miRNA:mRNA target pairings (where the miRNA was down-regulated and would be expected to lead to de-repression of the expression of the target mRNA, which was up-regulated), suggesting that miRNA and post transcriptional gene silencing has a significant influence on schizophrenia-associated changes in gene expression and regulatory networks.

Could this schizophrenia-associated change in the expression of genes with immune function in PBMCs have implications for brain development or function? One possibility is that this peripheral immune-related expression signature could be reflective of immune-dysfunction that is also manifested in the brain and may be indicative of underlying neuropathology. Indeed, the peripheral expression signature we observed was consistent to some extent with gene expression in the CNS which also show aberrant expression of immune and inflammation-associated genes, proteins and pathways, suggestive of an immune component in schizophrenia

Table 4
Immune and inflammation related functions enriched with differentially expressed genes in schizophrenia using IPA (Benjamini–Hochberg corrected *p* value).

Category	Functions annotation	*p*-Value	# Molecules	Genes
Infectious disease; respiratory disease	Severe acute respiratory syndrome	<0.001	10	CCR7, DEFA1 (includes others), DEFA4, HIST1H2BJ/HIST1H2BK, LCN2, RAB32, RNASE2, S100A12, SLPI, TCN1
Antigen presentation; cellular movement; hematological system development and function; immune cell trafficking; inflammatory response	Chemotaxis of antigen presenting cells	<0.001	6	AZU1, CCR7, CKLF, DEFA1 (includes others), IL16, RNASE2
Cell death	Killing of cells	<0.001	6	AZU1, CTSD, DEFA1 (includes others), LGALS3, PI3, SLPI
Antigen presentation; cellular movement; hematological system development and function; immune cell trafficking; inflammatory response	Chemotaxis of phagocytes	<0.001	7	AZU1, CCR7, CKLF, DEFA1 (includes others), IL16, RNASE2, SLPI
Infection mechanism	Replication of HIV	0.00121	5	CCNT1, DEFA1 (includes others), IL16, MOV10, S100A12
Inflammatory response	Inflammation of tissue	0.0015	3	AZU1, CTSD, SLPI
Antigen presentation; cellular movement; hematological system development and function; immune cell trafficking; inflammatory response	Chemotaxis of macrophages	0.00319	3	AZU1, CKLF, DEFA1 (includes others)
Infection mechanism	Replication of RNA virus	0.00443	10	CCNT1, DEFA1 (includes others), DMAP1, EIF2C2, IL16, LONP1, MOV10, S100A12, SAFB, TNK2
Inflammatory response	Immune response	0.00451	17	ABCF1, AZU1, CCR7, CFP, CKLF, CTSD, DEFA1 (includes others), ENTPD1, IL16, IQSEC1, LCN2, PRDX5, RNASE2, RNF216, S100A12, SLPI, TYK2
Infection mechanism	Replication of HIV-1	0.00474	4	CCNT1, DEFA1 (includes others), MOV10, S100A12
Cellular movement; hematological system development and function; immune cell trafficking	Cell movement of leukocytes	0.00612	8	AZU1, CCR7, CKLF, DEFA1 (includes others), IL16, LGALS3, RNASE2, SLPI
Antigen presentation; cellular movement; hematological system development and function; immune cell trafficking; inflammatory response	Chemotaxis of dendritic cells	0.00779	3	CCR7, IL16, RNASE2
Cellular movement	Chemotaxis of eukaryotic cells	0.00784	8	AZU1, CCR7, CKLF, DEFA1 (includes others), IL16, RNASE2, SLPI, TFF3
Antimicrobial response	Inhibition of HIV	0.00892	2	DEFA1 (includes others), SLPI
Cellular movement; hematological system development and function; immune cell trafficking	Homing of mononuclear leukocytes	0.01	5	AZU1, CCR7, CKLF, DEFA1 (includes others), IL16
Inflammatory response; antimicrobial response	Antimicrobial response	0.0107	4	AZU1, CFP, RNF216, S100A12
Cellular movement; hematological system development and function; immune cell trafficking	Homing of lymphocytes	0.0118	4	CCR7, CKLF, DEFA1 (includes others), IL16
Inflammatory response	Inflammatory response	0.0124	9	ABCF1, AZU1, CCR7, CKLF, DEFA1 (includes others), IL16, PRDX5, RNASE2, SLPI
Infection mechanism	Production of virus	0.0125	3	CREBBP, MOV10, ULK1
Inflammatory response	Inflammation	0.0129	5	AZU1, CTSD, ENTPD1, S100A12, SLPI
Cellular movement; immune cell trafficking	Migration of leukocytes	0.0163	8	AZU1, CCR7, CKLF, DEFA1 (includes others), IL16, LGALS3, RNASE2, SLPI
Cellular movement; hematological system development and function; immune cell trafficking	Chemotaxis of leukocyte cell lines	0.0188	2	CCR7, DEFA1 (includes others)
Inflammatory response; antimicrobial response	Antibacterial response of organism	0.0233	2	AZU1, CFP
Infectious disease	Infectious disorder	0.0252	22	ABCF1, ASGR2, CBLB, CCNT1, CCR7, DEFA1 (includes others), DEFA4, EIF2B5, ELP2, HIST1H2BJ/HIST1H2BK, IL16, LCN2, POLR2C, RAB32, RNASE2, RNASE3, RNF216, S100A12, SLPI, SPG7, TCN1, TYK2
Cellular movement; hematological system development and function; immune cell trafficking; cell-mediated immune response	Homing of T lymphocytes	0.0258	3	CCR7, DEFA1 (includes others), IL16
Cellular movement; hematological system development and function; immune cell trafficking	Cell rolling of leukocytes	0.0282	2	CCR7, LGALS3
Cellular movement; immune cell trafficking	Migration of antigen presenting cells	0.0286	3	CCR7, IL16, LGALS3
Antigen presentation; cellular movement; hematological system development and function; immune cell trafficking; inflammatory response; lymphoid tissue structure and development	Chemotaxis of neutrophils	0.0335	3	AZU1, CKLF, SLPI
Immunological disease; hematological disease	Hypereosinophilia	0.0354	2	RNASE2, RNASE3
Cellular movement; hematological system development and function; immune cell trafficking; inflammatory response	Chemotaxis of mononuclear leukocytes	0.038	4	AZU1, CKLF, DEFA1 (includes others), IL16
Cellular movement; hematological system development and function; immune cell trafficking	Cell movement of granulocytes	0.0404	4	AZU1, CKLF, LGALS3, SLPI
Infection mechanism	Binding of virus	0.0431	2	ASGR2, CCNT1
Cellular movement; immune cell trafficking; inflammatory response	Migration of phagocytes	0.0463	4	CCR7, IL16, LGALS3, SLPI
Cell-to-cell signaling and interaction	Recruitment of cells	0.0481	3	CCR7, ENTPD1, SLPI

(Arion et al., 2007; Fillman et al., 2012; Levin et al., 2009; Martins-de-Souza et al., 2010; Matigian et al., 2010; Mistry et al., 2012; Saetre et al., 2007; Schmitt et al., 2011).

Despite these connections, the biological significance of altered expression of genes associated with immune function in schizophrenia is unknown. Therefore it is difficult to determine whether the immune-related expression signature reflects abnormality central and specific to the underlying pathogenesis of schizophrenia or is an indirect consequence of its pathophysiology or comorbid environmental factor(s). The immune-related signature could reflect the *state* of illness. Narayan et al. (2008) report that differentially expressed genes in post mortem brain from schizophrenia subjects are involved in inflammation, stimulus-response and immune-related pathways and were more strongly associated with long-term chronic schizophrenia (Narayan et al., 2008). The immune-related expression signature in our cohort, perhaps more representative of chronic schizophrenia is consistent with this observation. The molecular basis of the disorder may change with duration of illness and therefore whether these immune signatures are persistent through exacerbation and remission requires longitudinal studies. Additionally, whether these changes can be detected at the onset of illness could be assessed using first-episode psychosis cohorts. Another possibility is that medications are contributing toward the immune-associated gene expression signature. Indeed, a number of antipsychotics and antidepressants have been shown to display immunosuppressive and anti-inflammatory effects (Chen et al., 2012; Drzyzga et al., 2006; Schmitt et al., 2005; Tynan et al., 2012). Nevertheless, the influence of antipsychotic medication on gene expression could not be determined due to the nature of this information being self-reported by the participants with schizophrenia rather than through medical records. Similarly, obesity could also foster a pro-inflammatory state and may be affecting gene expression. However, since measures of current weight status e.g. body mass index (BMI) or waist circumference were not available, this possibility could not be further investigated.

Alternatively, the immune-related expression pattern may be indicative of a generalized immune disturbance apparent in many psychiatric and neurological disorders. In support of this, immune-related changes or abnormalities have been identified in schizophrenia and bipolar disorder (Bousman et al., 2010; Shao and Vawter, 2008), depression (Maes et al., 2009; Wager-Smith and Markou, 2011), anxiety (Hou and Baldwin, 2012) and Alzheimer's disease (Deretic, 2005; Horesh et al., 2011). This hints at some common elements and suggests the immune system is vitally important for the development and function of the brain.

In summary, we have conducted a large genome-wide survey of gene expression in PBMCs from individuals with schizophrenia and schizoaffective disorder and identified a significant over-representation of genes associated with the immune system. While this has immediate functional implications for the relationship between the brain and the immune system, it may also be reflecting genetic, environmental or developmentally significant insults that are relevant to the pathogenesis of the disorder (Bilbo and Schwarz, 2009; Kinney et al., 2010). In other words the immune expression signature in blood may be a residual image of this disturbance and provide insight into the etiopathogenesis of schizophrenia.

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Contributors

E. Gardiner contributed to the study design, experimental work, data analysis and preparation of the manuscript.

M. Cairns contributed to the study design, manuscript preparation and sourcing of funding.

B. Liu contributed to data analysis.

N. Beveridge contributed to data analysis.

V. Carr contributed to the study design, manuscript preparation and sourcing of funding.

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R. Scott contributed to the study design, manuscript preparation and sourcing of funding.

P. Tooney contributed to the study design, manuscript preparation and sourcing of funding.

Conflict of interest

None of the authors have conflicts of interest regarding this manuscript.

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Appendix A. Supplementary material

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