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Peripheral blood gene expression of acute phase proteins in people with first episode psychosis

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ABSTRACT

Background: There is a growing interest in the association between schizophrenia and the activation of inflammatory system with signs of acute phase (AP) response. Majority of such studies had focused on C-reactive protein (CRP). The aims of the present study were (i) to examine the gene expression profiles of other acute phase proteins (APP), namely haptoglobin (HP), alpha-1 antitrypsin (A1T), and alpha-2 macroglobulin (A2M) in patients with first episode psychosis (FEP) over a period of three months and (ii) to explore the association between APP levels and severity of symptoms.

Methods: In this study, HP, A1T and A2M gene expression levels from whole blood were measured at recruitment, 1- and 3-month follow-up visits using quantitative PCR (qPCR) in 43 patients with FEP and in 57 healthy controls. Diagnoses was ascertained on the Structured Clinical Interview for DSM-IV-TR. Severity of symptoms in patients was assessed on the Positive and Negative Syndrome Scale (PANSS) and a previously validated 5-factor PANSS structure was applied in the subsequent analyses.

Results: The FEP sample comprised of 28 (65.1%) individuals with schizophrenia, 12 (27.9%) with schizophreniform disorder and 3 (7%) with schizoaffective disorder. HP gene expression level was noted to be significantly higher in patients than controls at all three time points: recruitment ($P = 0.049$), 1-month follow up ($P = 0.002$) and 3-month follow up ($P = 0.005$). PANSS positive, depression, and excitement symptom factors showed significant associations with HP ($P = 0.002$), A1T ($P = 0.016$) and A2M ($P = 0.034$), respectively. These findings remained significant after controlling for age, gender, smoking status and accumulated chlorpromazine dosage.

Conclusion: The current study provides information on HP, A1T and A2M gene expression profiles in FEP patients and their associations with psychopathology. This provides support for the hypothesis that inflammation is related to schizophrenia and further encourages studies on immune-inflammatory markers to understand the relationship between inflammation and schizophrenia.

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1. Introduction

Macrophages and T-lymphocytes were hypothesised to be activated in psychosis with an accompanying acute phase (AP) response (Smith and Maes, 1995). An acute phase response is characterised by: (1) an increase in serum levels of positive acute phase proteins (APP), examples of positive APPs are C-reactive protein (CRP), haptoglobin (HP), alpha-1 antitrypsin (A1T), alpha-2 macroglobulin (A2M), ceruloplasmin and complement factors; (2) a decrease in serum levels of negative APPs such as albumin, transferrin, transthyretin, transcortin and insulin like growth factor I (Maes et al., 1997b).

The functions of most APPs and their interactions have not been fully understood. Positive APPs are thought to have general functions in opsonization and trapping of micro-organisms and their products, in activating complement, in binding cellular remnants like nuclear fractions, in neutralizing enzymes, scavenging free haemoglobin and radicals and in modulating the host's immune system (Gruys et al., 2005), From the limited literature and in clinical practice, HP is used to screen for and monitor intravascular haemolytic anaemia. It binds strongly to haemoglobin, preventing iron loss and renal damage. HP also has antibacterial property and binds to receptors on cell membranes of leukocytes (Wassell, 2000). A1T, which is considered to be the most prominent serpin,

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is involved in minimizing damage caused by activated neutrophil granulocytes and elastase which breaks down connective tissue fibre, elastin (Gettins, 2002). A2M, a protease inhibitor, was studied to be able to bind to protease involved in coagulation, fibrinolysis, inflammation and proteases from pathogens. In animal models, A2M was observed to augment the phagocytosis of sheep red blood cells by mouse macrophages (Sottrup-Jensen, 1989).

There have been reports on the alterations of APP levels in various mental disorders; for instance, in acute psychosis, schizophrenia, depression, bipolar disorders, and mania (Maes et al., 1997a; Morera et al., 2006; Wan et al., 2007; Wong et al., 1996). CRP is used in clinical practice as a non-specific marker of inflammation, therefore its elevated protein levels has received attention in psychosis (Dickerson et al., 2007; Fan et al., 2007; Fawzi et al., 2011; Johnsen et al., 2016; Lin et al., 2013; Maes et al., 1997a; Wong et al., 1996). However, studies on other APPs were limited, inconsistent and sometimes lacking a comparison group. Studies have also reported inconsistent changes in other APPs such as HP, A1T, A2M, ceruloplasmin, complement C3, C4, and transferrin protein levels in different stages of schizophrenia, and in acute psychosis (Bock et al., 1971; Morera et al., 2006; Seal and Eist, 1966; Wan et al., 2007; Wong et al., 1996; Yang et al., 2006).

A small number of studies have been carried out to explore the associations between APP levels and psychosis. While Johnsen et al. and Dickerson et al. indicated no relationship between CRP and severity of symptoms in both acute and chronic psychotic patients, Fan et al. and a recent *meta*-analysis by Fernandes et al. reported significant associations between CRP and symptoms in patients with schizophrenia (Dickerson et al., 2007; Fan et al., 2007; Fernandes et al., 2016; Johnsen et al., 2016). Up to now, there is only one report on significant associations between other APPs (C3, C4 and ceruloplasmin) and symptoms – negative and general psychopathology – in patients with schizophrenia (Wan et al., 2007).

While these reports had described changes in plasma and serum levels of APPs in psychosis, transcriptomic data on these APPs was lacking. The transcriptomic data is important for the understanding of functional elements of genome, unfolding of molecular constituents of cells and tissues, as well as to study the development of disorder (Wang et al., 2009). We had previously reported peripheral blood gene expression profiles of patients with FEP which revealed an up-regulation of HP gene expression (Lee et al., 2012). Therefore, the objectives of the current study were: (1) to extend the findings of differential HP gene expression levels, along with A1T and A2M in FEP to a larger sample group over a period of 3 months, and (2) to explore the associations between HP, A1T and A2M gene expression levels and severity of symptoms.

2. Methods and materials

2.1. Study sample

The current case control study was conducted at the institute of Mental Health, Singapore. Cases were individuals diagnosed with FEP who have had less than 4 weeks of antipsychotic treatment. Only individuals with a diagnosis of schizophrenia, schizophreniform or schizoaffective disorder at 3-month follow-up visit were recruited for this study. Three-month of follow up was chosen as this period should be adequate to identify antipsychotic response. Controls were healthy individuals with no history of mental illness, substance use, intellectual disability and neurological disorders. Controls were matched to cases for age, ethnicity and gender. Socio-demographic data such as age, gender, ethnicity and smoking status were obtained from all study participants. Prescription information for antipsychotics was obtained from medical records. Antipsychotic doses were converted into chlorpromazine (CPZ) equivalents and aggregated amount from initiation of pharmacotherapy to recruitment was computed (accumulated CPZ in mg). Ethics approval for the study was provided by the Domain Specific Review Board of the National Healthcare Group, Singapore.

2.2. Assessments

All participants were assessed using the Structured Clinical Interview for DSM-IV-TR (SCID-I). Clinical symptoms were assessed on the Positive and Negative Syndrome Scale (PANSS) by trained raters with established inter-rater reliability at >0.8. Controls were assessed on the SCID-I to determine history of mental illness at recruitment.

2.3. Sample collection and RNA extraction

A sample of venous whole blood was collected from all subjects at recruitment, 1- and 3-month follow-up visits into Tempus™ Blood RNA Tube (ThermoFisher Scientific, Foster City, CA, USA), and stored at −80 °C. The Tempus™ Blood RNA Tube contains Stabilizing Reagent, which lyses blood cells, inactivates cellular RNases and selectively precipitates RNA. Total RNA was then extracted with the Tempus™ Spin RNA Isolation Kit (ThermoFisher, Foster City, CA, USA) according to manufacturer's protocol. Quality and concentration of extracted RNA was measured with NanoDrop™ (Thermo Scientific, Wilmington, DE, USA) and all samples obtained an A260/A280 ratio of more than 2. Integrity of RNA was assessed via gel electrophoresis; only samples with clearly defined ribosomal peaks were used in the study.

2.4. Quantitative real time-polymerase chain reaction (qPCR)

Total RNA from all three time points was then converted to complementary DNA (cDNA) using iScript™ Reverse Transcription Supermix (Bio-Rad, Hercules, California, USA). HP, A1T and A2M gene sequences were obtained from NCBI gene bank and primers specific for respective genes designed using PrimerQuest (Integrated DNA Technologies, Coralville, Iowa, USA). GADPH was used as a reference for relative quantification of APP genes using ΔΔCt method. All samples were ran in triplicates; each reaction consisting 100 ng cDNA, 10 μM of forward and reverse primers and 2X iQ™ SYBR® Green Supermix (Bio-Rad, Hercules, California, USA). All reactions were programmed to run at a hold of 50 °C for 2 min, denaturation of 95 °C for 3 min, 40 cycles of denaturation at 95 °C for 10 s, annealing at 65 °C for 20 s, and elongation at 72 °C for 30 s, followed by a final elongation at 72 °C for 1 min on CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, California, USA). Melting curves and cycle threshold (CT) values were generated upon completion of qPCR cycle. Each sample was assayed in triplicates and each target gene (HP, A1T, and A2M) was normalised to the expression of a reference gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH). A second normalization against inter-run calibrator was performed to remove run-to-run difference. Relative quantitation using the $2^{\Delta\Delta}-Ct$ formula which derives fold change corresponding to gene expression was performed to analyse the results obtained from all the real-time PCR runs.

2.5. Statistical analyses

Categorical and continuous data were analysed using chi-squared test and Mann-Whitney *U* test respectively to compare the characteristics of patients and healthy controls. Linear regression model was used to investigate the association between APP

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