Schizophrenia Research 170 (2016) 115–122

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Angiogenic and immune signatures in plasma of young relatives at familial high-risk for psychosis and first-episode patients: A preliminary study

Paulo L. Lizano [a,b], Matcheri S. Keshavan [a,b,c,d], Neeraj Tandon [a,e], Ian T. Mathew [a], Suraj Sarvode Mothi [b], Debra M. Montrose [d], Jeffrey K. Yao [d,f,g,*]

[a] Department of Psychiatry, Beth Israel Deaconess Medical Center, Boston, MA, United States
[b] Division of Public Psychiatry, Massachusetts Mental Health Center, Boston, MA, United States
[c] Department of Psychiatry, Harvard Medical School, Boston, MA, United States
[d] Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA, United States
[e] Baylor College of Medicine, Houston, TX, United States
[f] VA Pittsburgh Healthcare System, Medical Research Service, Pittsburgh, PA, United States
[g] Department of Pharmaceutical Sciences, University of Pittsburgh School of Pharmacy, Pittsburgh, PA, United States

ARTICLE INFO

Article history:
Received 21 October 2015
Received in revised form 27 November 2015
Accepted 2 December 2015
Available online 11 December 2015

Keywords:
High risk
First episode psychosis
Soluble fms-like tyrosine kinase
Angiogenesis
Inflammation/immune

ABSTRACT

Schizophrenia (SZ) is a heterogeneous disorder that presents in adolescence, persists into adulthood, and has many clinical features. Recent evidence suggests that abnormalities in inflammatory, neurotrophic, and angiogenic processes may play a role in the etiology of SZ. The identification of molecular biomarkers early in the course of disease is crucial to transforming diagnostic and therapeutic avenues. We investigated 14 molecular analytes focusing on inflammatory, neurotrophic and angiogenic pathways from the plasma of antipsychotic-naïve familial high risk for SZ (FHR; n = 35) and first-episode psychosis (FEP; n = 45) subjects, in comparison to healthy controls (HC, n = 39). We identified distinct alterations in molecular signatures in young relatives at FHR for SZ prior to psychosis onset and FEP subjects. Firstly, the expression of soluble fms-like tyrosine kinase (sFlt-1), an anti-angiogenic factor that binds vascular endothelial growth factor (VEGF), was significantly increased in the FHR group compared to HC, but not in FEP. Secondly, interferon gamma (IFNγ) was significantly reduced in the FEP group compared to HC. Thirdly, network analysis revealed a positive correlation between sFlt-1 and VEGF, suggesting an activation of the angiogenic cascade in the FHR group, which persists in FEP. Our results indicate an angiogenesis and immunological dysfunction early in the course of disease, shifting the balance towards anti-angiogenesis and inflammation.

Published by Elsevier B.V.

1. Introduction

Schizophrenia (SZ) is a complex disorder with clinical features that typically present in adolescence, persist into adulthood, and have a considerable impact on morbidity, mortality, and cost (Whiteford et al., 2015; Lee et al., 2014). SZ diagnosis is primarily based on an experienced clinician's ability to recognize signs and symptoms of the disorder (AP Association, 2013). Treatment mostly consists of antipsychotic medications, with growing evidence suggesting the effectiveness of anti-inflammatory and vascular agents as augmentation strategies (Fond et al., 2014; Hallak et al., 2013). The absence of laboratory measures for guiding diagnosis and treatment underlines the need for plasma biomarkers to aid in identifying biological mechanisms and predicting conversion to psychosis, resulting in targeted or preventative treatment for those at elevated risk. To date, plasma biomarker studies have been investigated in chronic SZ (Potvin et al., 2008), first episode psychosis (FEP) (Miller et al., 2011), and prodromal patients (Huang et al., 2007; Stojanovic et al., 2014; Hayes et al., 2014; Perkins et al., 2015).

A distinguishing feature of SZ is its heterogeneity, with many genetic and environmental factors impacting disease risk (McGrath et al., 2013). Evidence from several genome-wide association studies (Shi et al., 2009; SZ Psychiatric Genome-Wide Association Study C, 2011; de Jong et al., 2012), including the recent study involving ~37,000 SZ participants and 113,075 controls has identified immune related genes that may play a role in the etiology of SZ (SZ Working Group of the Psychiatric Genomics C, 2014). A meta-analysis has strongly implicated inflammation (IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12, TNF-α, IFN-γ) in SZ, with cytokine concentrations varying with clinical status; *e.g.* trait and state markers (Miller et al., 2011). Additionally, there is growing

* Corresponding author at: VA Pittsburg Healthcare System, B-1-2E-140, University Drive C, Pittsburgh, PA 15240, United States.
E-mail address: jkyao@pitt.edu (J.K. Yao).

http://dx.doi.org/10.1016/j.schres.2015.12.001
0920-9964/Published by Elsevier B.V.

evidence for neurotrophic and angiogenic factors (brain-derived neurotrophic factor, BDNF; vascular endothelial growth factor, VEGF; and basic fibroblast growth factor, bFGF) contributing to the pathophysiology of SZ (Buckley et al., 2007; Ahmed et al., 2015; Lopes et al., 2015). There are also micro- and macro-vascular abnormalities that are consistently reported in SZ pathology, and angiogenesis may provide a novel biological framework for understanding these changes (Lopes et al., 2015). Given the genetic regulation of these immune, angiogenic, and neurotrophic factors, and due to substantive heritability of SZ, it is important to examine changes in young relatives at familial risk for SZ. Only a few studies have applied multiplex biological screens to the plasma and CSF of symptomatic ultra-high risk for psychosis subjects and found changes consistent with the inflammatory hypothesis (Huang et al., 2007; Stojanovic et al., 2014; Hayes et al., 2014; Perkins et al., 2015). To the best of our knowledge, multiplex screens targeting the inflammatory, neurotrophic, and angiogenic cascades have not been applied to asymptomatic untreated familial high-risk (FHR) subjects. Additionally, there is a scarcity of literature comparing biological signatures in FHR and comparing them to FEP patients.

The current study compared inflammatory, neurotrophic, and angiogenic signatures in the plasma of antipsychotic naïve subjects with FEP, nonpsychotic FHR for SZ, and HCs using a 14 analyte multiplex screen. Our specific hypotheses were that molecular changes would be present early in the disease course and intermediary to patients in the acute phase of the psychotic illness.

2. Methods

2.1. Study population

The study population comprised of FHR (n = 35), FEP (n = 45, subjects diagnosed with SZ, schizoaffective or schizophreniform), and HC (n = 39) subjects who contributed plasma samples. The recruitment and assessment methods were described previously (Keshavan et al., 2008; Gilbert et al., 2001). The study was approved by the University of Pittsburgh and VA Pittsburgh Healthcare System (VAPHS) institutional review board and each subject provided consent; in the case of minors, the participants provided assent, with a parent or guardian also providing informed consent.

The FHR participants were identified at the Pittsburgh Western Psychiatric Institute Clinic by approaching patients with schizophrenia with eligible offspring. Psychopathology of the ill relative was determined using the SCID. FHR participants were evaluated using the Schedule for Affective Disorders and Schizophrenia for Children (K-SADS), behavioral and anxiety disorders sections, and the Structural Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (First et al., 2012), and diagnoses were confirmed by consensus. All FHR individuals had either a first- or second-degree relative diagnosed with schizophrenia or schizoaffective disorder. Exclusion criteria included subjects diagnosed with mental retardation, lifetime evidence of a psychotic disorder, prior antipsychotic exposure, or significant neurological/medical conditions (Keshavan et al., 2008).

The FEP subjects were diagnosed according to DSM-IV criteria and confirmed *via* consensus by senior diagnosticians within 1 month of study entry. Exclusion criteria included subjects with significant head injury, neurological/medical illness, prior antipsychotic exposure, or mental retardation. HCs were recruited simultaneously from both FHR and FEP studies, there was no history of mental illness, and they were recruited within the same geographic area. Additionally, the FHR and FEP groups are independent and they are not genetically related.

2.2. Clinical assessments

In FHR subjects, schizotypy scores were obtained from the Chapman psychosis proneness scales (Chapman et al., 1994), which have predictive value for future psychosis (Keshavan et al., 2008). The Chapman scales are true–false self-report questionnaires that measure positive (magical ideation and perceptual aberration) and negative schizotypy (social and physical anhedonia). In the FEP group, the Scale for the Assessment of Positive Symptoms (SAPS), Negative Symptoms (SANS), and Global Assessment of Functioning (GAF) were obtained to measure positive symptoms, negative symptoms, and global functioning, respectively (Andreasen, 1990). The FHR participants were naturalistically followed for up to 3 years, outcome was assessed by interim medical/psychiatric histories and annual SCID interviews by the same clinicians who assessed them at baseline. The overall FHR sample contained approximately 100 subjects and 15% converted to psychosis. Thirty-five FHR subjects had serum samples collected at baseline. In this smaller sample, three of the thirty-five subjects converted to psychosis during the 3 year follow up period.

2.3. Plasma collection

Blood samples were obtained from consented subjects at the baseline visit after overnight fasting. Samples were de-identified and plasma aliquots frozen at −80 °C until use in immunoassays to avoid freeze/thaw cycles. All laboratory analyzes were conducted in Dr. Yao's laboratory at VAPHS.

2.4. Plasma assay

The plasma concentrations of inflammatory, neurotrophic, and angiogenic molecules were determined using MESO SCALE DISCOVERY'S (MSD) MULTI-ARRAY® Technology to measure biomarkers utilizing next generation electrochemiluminescent detection. MSD is a multiplex immunoassay system with specific Capture Antibodies for analytes that are coated in arrays, within each well of a 96-well carbon electrode plate. The detection system uses patented SULFO-TAG™ labels, which emit light upon electrochemical stimulation initiated at the electrode surfaces of the MULTI-ARRAY and MULTI-SPOT® plates. The electrical stimulation is decoupled from the output signal, which is light, to generate assays with minimal background. MSD labels can be conveniently conjugated to biological molecules, are stable and non-radioactive.

MSD human growth factor panel I assay kit provided quantifications of basic fibroblast growth factor (bFGF), sFlt-1, placental growth factor (PlGF) and VEGF, and plasma BDNF levels were measured by MSD multi-array BDNF assay kit. These analytes were chosen based on two recent review literatures (Buckley et al., 2007; Lopes et al., 2015). The human proinflammatory 9-plex assay kit measured 9 cytokines and chemokines including GM-CSF, IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, and TNF-α in plasma samples, which were chosen based on their implication in schizophrenia from a recent meta-analysis (Miller et al., 2011).

Assays were developed, validated, and raw intensities converted to absolute concentrations by comparison with a standard curve. Final data were reported as the absolute concentrations in the plasma, lower limit of detection (LLOD), and quantity not sufficient (QNS). Technicians ran assays without knowledge of clinical status of the subjects.

2.5. Statistical analysis

All statistical analyses were performed using the R statistical analysis software (version 3.1.1). Descriptive statistics were computed for demographic variables across diagnostic groups. Differences in mean age across groups were examined using analysis of variance (ANOVA), followed by pairwise t-tests (Table 1). Differences across groups in gender and ethnicity were analyzed using Fisher's exact test (Table 1).

2.6. Analyte analysis

Among the 14 analytes examined, 12 were selected for further analysis based on the following criterion: less than 5% with QNS values and