Published in final edited form as:

Schizophr Res. 2016 January ; 170(1): 115–122. doi:10.1016/j.schres.2015.12.001.

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

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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

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Contributors:

Paulo Lizano contributed to study design, data analysis and interpretation, drafting of the article, revising the article, and final approval.

Matcheri Keshavan contributed to study design, data analysis, drafting of the article, revising, and final approval.

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Conflict of Interest

The authors declare no conflict of interest.

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 (IFN γ) 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.

Keywords

high risk; first episode psychosis; soluble fms-like tyrosine kinase; angiogenesis; inflammation; immune

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-1 β , 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 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; Almed *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-IV¹ 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-1 β , 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 greater than 75% of values reported (IL-2 and GM-CSF were excluded for failing these thresholds). Of the remaining analytes, 7 did not require imputation, 2 required more than 15%, and 2 required less than 15% imputation (supplementary Table 1). Analytes with QNS were imputed to the median of the sample, while analytes below the LLOD were imputed to the manufacture's median LLOD. Since drug and alcohol use can confound differences in analyte expression (Kirkpatrick and Miller, 2013), we performed the Wilcoxon sign-ranked test on each analyte across the whole sample and there was no significant difference observed (see supplementary Table 2). As a result, no further analytes were excluded from the analysis. We performed a parametric Gaussian survival regression model utilizing survival with left censoring if values were below the LLOD (Hayes *et al.*, 2014). Survival regression was performed to better account for differences in analytes that were actually at the LLOD versus those that were below the LLOD. The QNS values were not included in the regression model. We explored the normality of the distribution using histograms and Shapiro-Wilk normality test ($p < 0.05$) and the data was log transformed to stabilize the variance. An ANOVA ($\lambda = 0.7$) between diagnoses (HC vs FHR; HC vs FEP) was performed on the survival model. Multiple comparison correction was evaluated using q -value conversion to control for false discovery rate, and a q -value < 0.05 was considered significant. Median, mean, interquartile range (IQR), and standard deviation (SD) were calculated. Box-whisker plots show the median and IQR.

General linear model (GLM) analyses were applied to examine both the main effect of diagnosis (FHR vs FEP vs HC) and the interaction effects of age and sex. When the effect of age, sex, interaction term of diagnosis*sex and diagnosis*age was significant ($p < 0.05$), two-sample t-tests were conducted to compare the analyte between the diagnostic groups.

2.7. NodeXL network diagrams

We used NodeXL to build within group analyte network diagrams (Hornig *et al.*, 2015; Smith *et al.*, 2010). Network analysis was utilized to understand the relationships between analytes and identify connection patterns. Pearson correlations using log-transformed analytes, followed by Benjamini and Hochberg (1995) adjustment for multiple comparisons ($p < 0.01$), were conducted between pairwise analytes for each group. Only significantly correlated analyte pairs were used to produce the network diagram. The nodes represent

analytes and black lines connect significantly correlated analytes with the correlation coefficient embedded in the lines.

3. Results

3.1. Participant characteristics

Table 1 provides the demographic features of the study groups. The laboratory and clinical data for HC subjects were selected from the FHR and FEP cohorts. As anticipated, subjects in the FHR group were significantly younger (16.5 ± 3.7 years) in comparison to controls (25.1 ± 6.3 years, $p < 0.0001$). The subjects did not significantly differ with gender ($p = 0.08$) or ethnicity ($p = 0.1$). All subjects were antipsychotic-naïve at the start of the study. With regards to substance use, two subjects in the FHR group and 7 in the FEP group had either a comorbid alcohol or cannabis use disorder. Chapman schizotypy scale and Scale of Prodromal Symptoms (SOPS) sub-scores in the FHR group were below the attenuated psychosis threshold (Miller *et al.*, 2002). Also, the FHR subjects were followed for up to three years with three subjects converting to psychosis (CHR).

3.2. Comparison of molecular analytes among FHR, FEP, and control subjects

We compared HC, FHR, and FEP groups separately, since they are distinct groups at different stages of illness. We also took into account age, sex, interaction between diagnosis by age and diagnosis by sex, which showed that IL-10 and IL-1 β demonstrated an age effect, while IL-1 β and TNF α showed an age by diagnosis effect (supplementary Table 3). To address the effect of age on analyte expressions, we split the HC group by age and no significant ($p = 0.63$) analyte differences were detected between younger (age ≤ 21 , $n = 13$) and older (age > 21 , $n = 26$) HC subjects.

Table 2 showed that FHR subjects had elevated levels of IL-10 ($p = 0.046$), VEGF ($p = 0.025$), and sFlt-1 ($p = 0.002$), with sFlt-1 surviving multiple comparisons ($q = 0.02$). Since age was significantly different between HC and FHR subjects (Table 1), we age-matched the groups ($p = 0.99$) and found that sFlt-1 continued to be significantly ($p = 0.005$, $q = 0.03$) higher in FHR (199 ± 98 pg/mL, 19.28 ± 0.94 years, $n = 9$) than in HC (100 ± 52 pg/mL, 19.28 ± 0.62 years, $n = 13$) subjects.

In the FEP group, ANOVAs showed that subjects had reduced levels of IFN γ ($p = 0.026$), but did not survive multiple comparisons (Representative analytes with a p value < 0.05 are shown in Fig. 1). The 3 converters to psychosis (CHR) in the FHR group had higher mean values (between 2 to 7 fold higher) for IL-10, IL-12, PIGF, VEGF, bFGF, and sFlt-1 compared to HC, FHR and FEP (Table 3). All ANOVAs and q -values are listed in Table 2. Additionally, Pearson correlations performed between the 12 analytes and baseline clinical status in the FHR (Chapman and SOPS) and FEP (SAPS and SANS) groups did not show significant association that survived multiple comparisons (data not shown).

3.3. Network Associations

The network analysis demonstrated a differential association among analytes in the FHR subjects that were different from the FEP and HC groups (Fig. 2). In the FHR group there

was a reduction in the number of regulatory components with positive correlations between sFlt-1-VEGF, IL1 β -TNF α , and IL-10 with IL-12 and IL-6. In the HC group, IL-10 was related to more analytes than in the FHR group, with an absence in sFlt-1-VEGF correlation. In the FEP group, the sFlt-1-VEGF co-expression persisted and there was an increase in inter-analyte correlation with IL-1 β , IL-6, and IL-8 driving many of these interactions. While there were negative regulatory differences, none reached significance.

4. Discussion

To the best of our knowledge this is the first study to demonstrate altered plasma immune and angiogenesis signatures in antipsychotic-naïve, non-psychotic, familial high-risk subjects. Analyses revealed that the FHR group showed significant increases of anti-inflammatory, angiogenesis, and anti-angiogenesis analytes, as well as a dissociation of characteristic inter-analyte regulatory networks. The three converters to psychosis showed higher levels of several analytes compared to HC and FHR (non-converters). Furthermore, FEP subjects showed a significant reduction in IFN- γ (down trending in FHR), and increased association of angiogenesis and inflammatory analytes. Previous studies of immune, inflammatory, oxidative stress and infectious disturbances in HR and FEP subjects may have missed these findings because they were collected from a heterogeneous population, confounded by substance use, treatment with medications, attenuated psychotic state, brief intermittent psychotic symptoms, and functional decline (Huang *et al.*, 2007; Stojanovic *et al.*, 2014; Hayes *et al.*, 2014; Perkins *et al.*, 2015).

The observed alteration in sFlt-1, an anti-angiogenesis factor, in FHR and FEP subjects is consistent for a disruption in the angiogenesis cascade. Angiogenesis is a dynamic process between pro- and anti-angiogenesis that is critical to health, and an imbalance can result in various pathological conditions (Wu *et al.*, 2010). Biologically, the expression of sFlt-1 is promoted by hypoxia via HIF1- α and VEGF (Wu *et al.*, 2010; Fan *et al.*, 2014). A splice variant of the VEGF receptor (VEGFR), sFlt-1, has two proposed mechanisms; 1) ligand trapping of VEGF and PlGF, i.e. lowering effective concentrations and 2) heterodimerization with VEGFRs to form dominant-negative complexes, i.e. lowering the potential of unoccupied VEGFR to be activated (Kendall *et al.*, 1996). Additionally, sFlt-1 sensitizes endothelial cells to inflammatory cytokines by antagonizing VEGF receptor mediated signaling (Cindrova-Davies *et al.*, 2011). Thus, while sFlt-1 expression increases in response to hypoxia, its attenuation of neutrophilic factors and sensitization to inflammatory signals can make cells susceptible to damage (Bergmann *et al.*, 2010).

Physiologically, the short variant of sFlt-1 has been associated with increased sFlt-1 expression and low birth weight in first-time mothers (Muehlenbachs *et al.*, 2008), while low levels of sFlt-1 were protective against low birth weight (Davila *et al.*, 2010). Also, pathological elevations of sFlt-1 have been well characterized and highly implicated in pre-eclampsia (McMahon *et al.*, 2014; Levine *et al.*, 2004).^{2,3} Interestingly, pre-eclampsia and other pregnancy complications have been associated with an increased risk for schizophrenia in adults, and can have additive effects to genetic factors (Dalman *et al.*, 1999, 2001; Cannon *et al.*, 2000). Additionally, it was demonstrated using an animal model that offspring from pre-eclamptic mothers with adenoviral elevated sFlt-1, had a sex-specific

reduction in brain volume that was prevented with prenatal pravastatin treatment (Carver *et al.*, 2014). When taken together, this evidence supports a model for genetic vulnerability and obstetrical complication having an additive effect that may result in the development of psychosis.

Disruption of the angiogenesis cascade can help us to understand the neuropathological findings in SZ. Studies have demonstrated that ultra-structural damage of capillaries result in region-specific capillary deficiency that can contribute to lowered blood flow, reduced glucose metabolism, and hypoactivation in the prefrontal cortex of SZ patients (Uranova *et al.*, 2010, 2013).^{4,5} A recent randomized control trial demonstrated that vasodilators, such as sodium nitroprusside, might have a therapeutic effect in SZ by increasing blood flow to low flow regions (Hallak *et al.*, 2013). Also, clinical studies have reported that sorafenib, a VEGFR inhibitor used in the treatment of cancer, results in psychosis (Kuo *et al.*, 2014; Kunene and Porfiri, 2011; Schellekens *et al.*, 2011). Thus, we hypothesize that sFlt-1 may mediate anti-angiogenesis, inflammatory, and developmental effects culminating in psychosis and contributing to a neurofunctional decline in a subgroup of patients with SZ.

Our observation that VEGF was increased in FHR and associated with sFlt-1 expression in the FHR and FEP group, further implicates the role of angiogenesis in psychosis risk. VEGF is central to survival, migration, proliferation, and vascular permeability of vessels (Wu *et al.*, 2010). It has been reported that VEGF is expressed by endothelial, astroglia (Krum *et al.*, 2008), microglia (Ryu *et al.*, 2009), and T-cells (Mor *et al.*, 2004). Also, VEGF receptors are present in the hippocampus (Wang *et al.*, 2005). Investigations correlating either genetic, gene expression or peripheral markers to brain structural changes have been described. For example, VEGF polymorphisms in HCs have been associated with hippocampal size (Blumberg *et al.*, 2008). In SZ, post mortem brain analysis demonstrated that dysregulated VEGF signaling occurs in both the dorsolateral prefrontal cortex (Fulzele and Pillai, 2009) and thalamus (Chu *et al.*, 2009). Recently, Pillai *et al.* demonstrated that serum VEGF levels were associated with prefrontal cortical volume (Pillai *et al.*, 2015). These studies demonstrate that an imbalance of angiogenesis regulation can have detrimental effects. In further support, mouse studies have shown that VEGF regulates neurogenesis (an effect that is blocked by VEGF RNA interference (During and Cao, 2006) or adenoviral expression of sFlt-1 (Fabel *et al.*, 2003) and rescues hippocampal neurons from glutamate-induced toxicity (Matsuzaki *et al.*, 2001), but at the same time can augment proinflammatory T-cell differentiation (Mor *et al.*, 2004). Thus, increased expression of VEGF in FHR subjects could be compensatory to the deleterious effects of sFlt-1, but could also be a driver for sFlt-1 expression (Fan *et al.*, 2014). Therefore, future studies will need to evaluate the cause of sFlt-1 upregulation in FHR subjects and how it relates to hippocampal volume and prefrontal cortical thickness.

In FEP, we describe a reduction of IFN- γ (down-trending in FHR) and an increased association with inflammatory cytokines (IL1 β , IL-6, and IL-8). IFN- γ has multiple biological functions including anti-viral and immune-stimulatory effects that exacerbate inflammation in autoimmune disorders (Schroder *et al.*, 2004). Studies have shown that SZ is not only associated with autoimmune disease (Eaton *et al.*, 2006), but that IFN- γ is reduced in acute psychosis (Arolt *et al.*, 2000), which is consistent with immunologic

abnormalities seen in other autoimmune disorders. Also, increased association of pro-inflammatory cytokines (IL1 β , IL-6, and IL-8) in the FEP group, but not FHR suggests that immune-inflammatory cascades are activated during acute exacerbations. Thus, our data suggests that decreased IFN- γ , starting in FHR and persisting into the FEP group, support the idea that immunological activity plays a role in psychosis pathogenesis.

The presence of a specific abnormal angiogenic and immune profile early in the course of SZ has important implications for diagnostic and predictive markers. First, we defined a distinctive angiogenic signature that differed from that of healthy controls. Integration of these angiogenic markers with clinical findings can provide clinicians with a stronger framework for establishing an earlier diagnosis. It also expands on the clinical heterogeneity observed in schizophrenia beyond inflammatory and stress associated phenotypes. Second, the pattern of worsening IFN- γ disturbances from FHR to FEP, suggests a dysregulation of immunologic functioning with opportunities for intervention beginning prior to the onset of illness. Testing therapeutic strategies targeting abnormalities found in analyses of FHR and FEP subjects in the early course of illness and following their trajectory over time will be necessary to elucidate the clinical significance of these findings. It is possible that delineation of analyte signatures in well-characterized subjects at familial, clinical, and attenuated risk can provide a unique tool for the establishment of clinically relevant phenotypes and discovery of novel treatment targets. If replicated in longitudinal studies, these data may provide a basis for targeted interventions to prevent long-term complication of schizophrenia. Current therapeutic avenues that decrease the expression of sFlt-1 [antipsychotics (Buckley *et al.*, 2007), sulfasalazine (Brownfoot *et al.*, 2015b), aspirin (Li *et al.*, 2015), statins (Brownfoot *et al.*, 2015a), proton pump inhibitors (Onda *et al.*, 2015), and choline (Jiang *et al.*, 2013)] and modulate autoimmune disorders are already in existence.

In SZ, the unspecific, “innate” immune system appears to be over-activated as evident by an increase of monocytes and gamma/delta cells, resulting in an increased release of IL-6 (see review by Yao and Keshavan, 2011). However, data published so far have been inconsistent, which may be the result of differences in assay methodology, sample size, sample handling, diagnostic criteria, and comparison groups (Yao and van Kammen, 2003). Nevertheless, in a meta-analysis of 62 cross-sectional studies involving 2298 SZ patients and 1858 healthy volunteers, Potvin *et al.*, (2008) concluded that SZ patients have increased levels of *in vivo* IL-1 receptor antagonist, soluble IL-2 receptor, and IL-6, and decreased levels of *in vitro* IL-2, establishing an inflammatory syndrome in SZ. More recently, Miller *et al.* (2011) also concluded that there are significant increases for IL-6, IL-12, TNF- α , IL-1 β , IL-8, TGF- β , IL-1 RA, IFN- γ , and sIL-2R, whereas IL-10 is significantly reduced in patients with first-episode psychosis. While there is a general agreement that plasma IL-6 levels are increased in patients with schizophrenia, some studies have shown no significant difference in IL-6 in antipsychotic-naïve FEP subjects (Borovcanin *et al.*, 2013; Brinholi *et al.*, 2015). Additionally, there are a limited number of studies looking at IL-6 in the clinical and ultra-high risk for psychosis population and the results have been inconsistent, with only one study demonstrating a significant difference in IL-6 between at risk subjects and healthy controls (Hayes *et al.*, 2014; Perkins *et al.*, 2015; Stojanovic *et al.*, 2014).

The role of neurotrophic disruption in SZ, including BDNF, has been demonstrated previously (Buckley *et al.*, 2007). In general, FEP and chronic schizophrenia patients have reduced serum levels of circulatory BDNF, however, results published to date have been inconsistent and confounded by stage of illness, medication status, and substance use (Ahmed *et al.*, 2015). Several studies have demonstrated that there is no significant difference in BDNF levels between HC and antipsychotic-naïve FEP subjects (Huang and Lee, 2006; Theleritis *et al.*, 2014). In the high risk for psychosis population, there is only one study (Perkins *et al.*, 2015) that showed no significant difference in BDNF. In the present study, BDNF was not significantly different among FEP, FHR, and HC, and this finding is consistent with the literature.

On the other hand, our study has several limitations. First, the small sample sizes make our observations preliminary, and type I errors cannot be confidently ruled out. Second, our assays used plasma samples and correlations with cerebrospinal fluid would have enabled greater insights into immune, angiogenic and neurotrophic activation in the CNS. Third, age was significantly different between HC and FHR subjects. Several studies have shown that there is no correlation between analyte level and age in healthy controls except for a few cytokines; sCD40L, TGF α , GM-CSF, G-CSF, MCP-1 (Kim *et al.*, 2011). VEGF could be thought to have an age related effect between HC and FHR, however a study by Kleiner *et al.*, 2013 in healthy human subjects demonstrated that there is no significant difference in serum levels of VEGF between ages 1–6, 7–17, and >18 (median 36, range = 21–86). Additionally, sFlt-1, VEGF, and IFN- γ were not associated with age, thus this was not considered to be a significant confounder in either FHR or FEP group. To further address the age difference we performed an age-matched analysis between HC and FHR and found that sFlt-1 remained significantly elevated in the FHR group. Also, we split the HC group by age (younger versus older) and did not find any significant differences between analytes. The findings from this study are preliminary, and future studies are needed to determine their clinical utility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors are grateful to P. Cheng, C. Korbanic and J. Haflett for their technical assistance and to Diana Mermon for her help in clinical assessments of FHR subjects, and Jean Miewald for her help in data management. We thank the clinical core staff of the Center for the Neuroscience of Mental Disorders (MH45156, MH084053, David Lewis MD, Director) for their assistance in diagnostic and psychopathological assessments. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The contents of this article do not represent the views of the Department of Veterans Affairs, the United States Government, Harvard Catalyst, Harvard University and its affiliated academic healthcare centers, or the National Institutes of Health.

Role of funding source

This work was supported in part by Department of Veterans Affairs [Merit Reviews (JKY) and Senior Research Career Scientist Award (JKY)], the VA Pittsburgh Healthcare System, National Institute of Health [MH58141 (JKY), MH64023 (MSK), KO2 MH 01180 (MSK), MH45156 (MSK), c UL1 RR024153, and NIH/NCRR/GCRC Grant M01 RR00056]. Biostatistician consultation was supported by Harvard Catalyst, The Harvard Clinical and Translational Science Center (National Center for Research Resources and the National Center for Advancing

Translational Sciences, National Institutes of Health Award UL1 TR001102) and financial contributions from Harvard University and its affiliated academic healthcare centers.

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Fig. 1.

Comparison of plasma analyte levels in HC, FHR, and FEP subjects. Figures show median $\pm$ IQR for each analyte. Only analytes meeting significance criteria ($p < 0.05$) in ANOVA analyses are represented. Group distributions are depicted by shapes, HC (●), FHR (■), 3 converters to psychosis (CHR, +), and FEP (▲). (A) sFlt-1, an anti-angiogenesis factor was significantly elevated ($p = 0.002$) in the FHR versus HC group and survived multiple comparisons ($q = 0.021$); (B) VEGF, an angiogenic factor was significantly elevated ($p = 0.025$) in the FHR versus HC group; (C) The anti-inflammatory analyte, IL-10 was significantly increased ($p = 0.045$) in FHR versus HC; (D) IFN γ , a pro-inflammatory analyte was down trending in FHR and significantly decreased ($p = 0.026$) in FEP versus HC. ANOVA, analysis of variance; HC, healthy control; FHR, familial high risk; FEP, first episode psychosis.

Fig. 2.

Network inter-analyte associations differ for HC, FHR, and FEP subjects. Network analysis for HC subjects (n = 39), FHR (n = 35), and FEP (n = 45). Network diagrams of the 12 analytes were created in NodeXL (<http://nodexl.codeplex.com>) using a 0.01 family-wise false discovery rate (FDR) to adjust for multiple comparisons. Black lines indicate positive correlations (correlation coefficients depicted) with *p*-values (< 0.0001) that fall below the

FDR-corrected p -value (< 0.01) criterion for each group. HC, healthy control; FHR, familial high risk; FEP, first episode psychosis.

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Table 1

Demographic Information for HC, FHR, and FEP subjects

Demographical Features	HC	FHR	FEP	Test	p-value ^a
Number	39	35	45	-	-
Age ^b (years)	25.1±1.0	16.5±0.6*	25.5±1.4	ANOVA	< 0.0001
Gender (M/F)	25/14	15/20	30/15	Fisher's Exact	0.08
Ethnicity ^c	24/10/3/1/1	15/20/0/0/0	22/17/4/1/1	Fisher's Exact	0.10

Abbreviations: HC, healthy controls; FHR, familial high-risk; FEP, first-episode psychosis; M, male; F, female.

^a $p < 0.05$ in HC vs FHR.

^b Mean ± SEM.

^c Caucasian, African American, Asian, Hispanic, other.

Table 2Comparison of plasma analytes among HC, FHR, and FEP groups^a

Analytes	FHR vs. HC		FEP vs. HC	
	p-value	q-value	p-value	q-value
IFNγ	0.291	0.702	0.026	0.311
IL-10	0.046	0.183	0.847	0.966
IL-12	0.882	0.967	0.620	0.966
IL-1 β	0.338	0.702	0.540	0.966
IL-6	0.966	0.967	0.813	0.966
IL-8	0.967	0.967	0.882	0.966
TNF α	0.351	0.702	0.966	0.966
PlGF	0.416	0.714	0.251	0.966
VEGF	0.025	0.152	0.338	0.966
bFGF	0.967	0.967	0.230	0.966
sFlt-1	0.002	0.021	0.624	0.966
BDNF	0.941	0.967	0.947	0.966

Abbreviations: HC, healthy controls; FHR, familial high-risk; FEP, first-episode psychosis; IFN- γ , interferon- γ ; IL, interleukin; TNF- α , Tumor Necrosis Factor- α ; PlGF, placental growth factor; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; sFlt-1, soluble fms-like tyrosine kinase 1; BDNF, brain-derived neurotrophic factor.

^aOne-way ANOVA with independent variable of diagnosis (HC, FHR, and FEP).

Table 3

Comparison of mean (SEM) and median (IQR) among HC, FHR, and FEP groups

Analyte ^a	HC	FHR	FEP	CHR	HC	HR	FEP	CHR
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
IFN γ	1.0 (0.8)	0.8 (0.7)	0.5 (0.8)	0.6 (0.3)	1.1 (0.7)	1.0 (1.1)	0.83 (0.8)	0.5 (0.3)
IL-10	2.1 (1.8)	2.6 (2.7)	2.0 (2.0)	4.3 (17.6)	2.2 (1.4)	4.4 (6.4)	2.5 (2.3)	13.7 (19.3)
IL-12	0.9 (1.1)	0.9 (1.1)	0.9 (1.0)	2.1 (21)	1.3 (1.2)	3.1 (7.7)	1.4 (2.0)	14.9 (23.6)
IL-1 β	0.3 (0.4)	0.5 (0.6)	0.4 (0.7)	0.2 (0.2)	0.5 (0.3)	0.6 (0.6)	0.7 (1.0)	0.3 (0.2)
IL-6	0.6 (0.7)	0.5 (0.5)	0.5 (0.6)	0.8 (1.0)	0.6 (0.5)	0.6 (0.6)	0.8 (1.3)	1.0 (0.9)
IL-8	2.8 (2.4)	2.8 (2.3)	2.6 (3.7)	3.1 (1.9)	3.5 (2.5)	3.2 (1.8)	3.6 (2.7)	3.1 (2.0)
TNF α	4.8 (3.1)	5.1 (3.4)	4.8 (3.1)	2.6 (2.1)	5.4 (2.6)	5.1 (3.4)	6.1 (5.4)	3.2 (2.1)
PIGF	9.5 (4.5)	11.6 (5.4)	11.4 (7.2)	21.2 (10.9)	9.8 (3.8)	11.9 (6.2)	11.2 (5.1)	16.3 (11.7)
VEGF	180 (171)	217 (158)	171 (199)	346 (285)	198 (123)	291 (207)	251 (213)	505 (408)
bFGF	72 (87)	70.3 (91)	90.5 (72)	106 (125)	85 (67)	84 (70)	95 (66)	158 (133)
sFlt-1	105 (54)	156 (95)	112 (57)	180 (70)	106 (48)	182 (113)	119 (71)	213 (75)
BDNF	11.5 (8.0)	11.6 (10)	11.6 (8)	12.5 (9.4)	12.7 (5.4)	13.3 (7.0)	12.6 (5.4)	16.1 (9.8)

Abbreviations: Healthy control (HC), familial high risk (FHR), converted high risk (CHR), first episode psychosis (FEP).

^aUnits in pg/mL except for BDNF which was measured in ng/mL.