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Potential involvement of the interleukin-18 pathway in schizophrenia

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ABSTRACT

Objective: Accumulating evidence implicates inflammatory cytokines in the development of psychiatric disorders, including schizophrenia (SZ). IL-18 is one of cytokines that plays a crucial role in immune response and neurodevelopment. We aimed to investigate potential genetic alterations of the cytokine system underpinning SZ.

Methods: We tested the association of genetic variants within the cytokine–cytokine receptor interaction (CCRI) pathway with SZ, using GWAS-derived data involving 768 adult SZ patients and 1348 controls, and replicated the association of IL18R1 rs1035130 with SZ in an independent sample of 1957 adult patients and 1509 controls. We compared expression levels of IL18, IL18R1 and IL18RAP in peripheral blood of a cohort of adolescent participants (<18 years), including 14 early-onset SZ patients and 13 healthy controls. Furthermore, we carried out a cis-eQTL (expression Quantitative Trait Loci) and a cis-mQTL (Methylation Quantitative Trait Loci) analysis for IL18R1 rs1035130.

Results: In the discovery stage, we detected association signals within two IL18 pathway genes, IL18R1 and IL18RAP, with the most significant marker being IL18R1 rs1035130 ($P = 1.84E\text{-}7$, $OR = 0.70$). In the validation stage, we found rs1035130 was associated with SZ ($P = 0.028$, $OR = 0.89$). Expressions of IL18 and IL18R1 were altered in blood of SZ patients compared with 13 controls. Furthermore, cis-QTL analyses indicated that rs1035130 was associated with an eQTL and 5 mQTLs.

Conclusion: Our findings suggest the alteration of IL18 pathway may contribute to the psychopathology of SZ.

1. Introduction

Schizophrenia (SZ) is a common yet disabling mental disorder characterized by profound disruption in cognition and emotion, affecting the most fundamental human attributes including language, thought, perception, affect, and diminished self-affection or self-presence. The onset of the illness is typically beginning in late adolescence or early adulthood.

When the disease manifests before age 18, it is categorized as early-onset SZ (EOS), a subgroup of SZ linked with more familial vulnerability and poor outcomes (Clemmensen et al., 2012). Epidemiological research showed that a variety of pre- and perinatal insults are associated with increased vulnerability to SZ (Messias et al., 2007; Rapoport et al., 2012).

The neurodevelopmental hypothesis postulates that SZ may root in disturbed development of the nervous system during early brain development, long before the full-blown of the illness (Murray and Lewis, 1987; Weinberger, 1987). It was further hypothesized that immune systems may modulate normal neurodevelopment and there is increasing evidence for altered inflammatory factors in the etiology and pathophysiology of SZ (Meyer, 2013).

The cytokine–cytokine receptor interaction (CCRI) pathway (KEGG: 04060, Supplementary Fig. 1) consists of cytokines and their receptors, involving 211 genes. Examples of cytokines include

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interleukins (IL), interferons (IFNs), tumor necrosis factors (TNFs), transforming growth factors (TGFs), and chemokines. Cytokines are produced by peripheral immunocompetent cells, glial cells, and neurons (Woodroofe, 1995). They mediate signal communications among various immune and neuronal cells during the immune response and play pleiotropic roles in the central nervous system (CNS), including roles in synaptic plasticity (Ben Achour and Pascual, 2010), neurotransmission (Dunn et al., 1999), nuclear signal transduction (Song et al., 2009), and neurogenesis (Monje et al., 2003), as well as inflammatory responses. Various pro-inflammatory cytokines exert both neuroprotective and neurotoxic actions based on the complexity of the signals occurring in their microenvironment.

Numerous studies have documented changes in cytokines and cytokine receptors in schizophrenic patients (Miller et al., 2011; Na et al., 2014). Cytokines such as IL-1β, IL-6, and tumor necrosis factor α (TNF-α) participate in regulating normal brain development and have been implicated in abnormal corticogenesis (Yirmiya and Goshen, 2011). Two recent meta-analyses supported the cytokine imbalance and activated macrophage hypothesis of SZ (Miller et al., 2011; Potvin et al., 2008).

Given the plausible link between cytokines and SZ, we hypothesized that some genetic variants within the CCRI pathway may influence the genesis of SZ. In this work, we evaluated the potential roles of genetic variants within the CCRI pathway in SZ using a two-stage study design. In the discovery stage, we derived the data from our own GWAS dataset (Yue et al., 2011), involving 5012 SNPs in 768 SZ cases and 1348 healthy controls. In the validation stage, we replicated the top ranked SNP, rs1035130 within the IL18R1 gene, in 1957 cases and 1509 controls. We then compared the mRNA levels of the IL18 pathway genes in 14 EOS patients with those in 13 controls.

A quantitative trait locus (QTL) is a section of DNA (the locus) that correlates with variation in a phenotype (the quantitative trait). QTLs that map to the approximate location of their gene-of-origin are referred to as local eQTLs or cis-eQTLs. Expression QTLs (eQTLs) are genomic loci that contribute to variation in expression levels of mRNAs. Similarly, it has been revealed that DNA methylation at specific loci can be influenced by sequence variations, such that individual genotypes at a given locus may result in different patterns of DNA methylation due to allele-specific methylation (Bell et al., 2011; Gamazon et al., 2013; Shoemaker et al., 2010). These sites are called methylation QTLs (mQTLs) and can influence the methylation pattern across an extended genomic region (Bell et al., 2011). We carried out a cis-eQTL and a cis-mQTL analysis for rs1035130 using curated data. Finally, we explored the protein–protein interaction (PPI) network concerning the IL18 pathway and their regulation by miRNAs.

2. Material and methods

2.1. Subjects

All participants were unrelated Han Chinese recruited from the North of China. The initial GWAS sample consisted of 768 unrelated subjects with SZ (360 males and 408 females) and 1348 control subjects (658 males and 690 females) (Yue et al., 2011). For validation, an independent sample consisting of 1957 cases (1037 males and 920 females) and 1509 controls (360 males and 1149 females) was recruited from northern China (Beijing, Tianjin, Hebei province, Shandong province, and Henan province). The consensus diagnoses were made by two experienced psychiatrists according to the Diagnosis and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV) criteria for SZ. None of the patients enrolled in this study had severe medical complications. Healthy controls were recruited from communities in the same area with simple non-structured interview performed by psychiatrists, who excluded individuals with history of mental health and neurological diseases.

For the real-time quantitative PCR (RT-qPCR) analysis, we recruited 14 first-onset drug-naive SZ patients (7 males and 7 females, aged 14.4 ± 1.1 years, ranging from 12 to 16 years) and 13 healthy adolescent controls (7 males and 6 females, aged 13.5 ± 1.9 years, ranging from 12 to 17 years). The patients came from First Hospital of Shanxi Medical University. The consensus diagnoses were made by two experienced psychiatrists according to the DSM-IV criteria for SZ. Healthy teenager controls were recruited from several middle schools with an interview performed by psychiatrists; individuals with a history of mental health or neurological diseases were all excluded. The two groups matched in age and sex.

The study was approved by Medical Research Ethics Committee of First Hospital of Shanxi Medical University and Medical Research Ethics Committee of the Institute of Mental Health, Peking University. All participants enrolled in the study signed written informed consent.

2.2. Genotyping

In the discovery stage, we selected all SNPs within the CCRI pathway genes from our previous GWAS dataset (Yue et al., 2011). In the validation stage, peripheral blood samples were collected from all subjects. Genomic DNA was extracted using the Qiagen QIAamp DNA Mini Kit. The genotypes of IL18R1 rs1035130 was determined using the Sequenom MassARRAY system (Sequenom iPLEX). 1957 cases and 1509 controls were successfully genotyped, the calling rate being 99.89%.

2.3. RT-qPCR expression

Total RNA was isolated from peripheral blood cells using TRIzol (Invitrogen; USA) with on-column DNase I treatment as described by the manufacturer. A 3-mL peripheral blood sample was taken from each of 14 EOS patients and 13 healthy control subjects. cDNA was synthesized using High Capacity RNA-to-cDNA Kit (Invitrogen; USA) as described by the manufacturer. RT–qPCR was performed and a SYBR® Select Master Mix (Invitrogen; USA). PCR was performed using a 7900HT real-time PCR machine (Applied Biosystems; USA) for 2 min at 50 °C, 2 min at 95 °C, and then 40 cycles consisting of 15 s at 95 °C, 60 s at 60 °C, followed by a subsequent standard dissociation protocol to ensure that each amplicon was a single product. All quantifications were normalized to GAPDH. The RT–qPCRs were performed in triplicate for each of the three independent samples.

2.4. Statistics and bioinformatics analyses

Genetic association tests were analyzed using PLINK 1.07 (Purcell et al., 2007). Linkage disequilibrium (LD) among the markers was plotted with Haploview (Barrett et al., 2005). Genomic map of SNPs was plotted using R package mapsnp (Zhang et al., 2015). The differences in gene expression levels between the patient and control groups were analyzed by the Mann–Whitney U test using R and plotted using R package plot2groups (Xu et al., 2014). We performed the cis-mQTL using Genevar 3.3 (Yang et al., 2010) in the MuTHER dataset (Grundberg et al., 2013), with which we analyzed the association of rs1035130 genotypes with neighboring methylations within 100 Kb distance. Genevar is a platform of database and web services designed for data integration, analysis and visualization of SNP-gene associations in eQTL and mQTL studies. PPI network was built via STRING v9.1 using default parameters as shown in Supplementary Fig. 2 (Franceschini