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Differential expression of brain immune genes and schizophrenia-related behavior in C57BL/6N and DBA/2J female mice

Li Ma [a], Natalia Kulesskaya [a], Vootele Võikar [a], Li Tian [a,b,*]

[a] Neuroscience Center, University of Helsinki, Helsinki, Finland
[b] Psychiatry Research Center, Beijing Huilongguan Hospital, Beijing, China

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ABSTRACT

Mounting evidence suggests the association of immune genes with complex neuropsychiatric diseases, such as schizophrenia. However, immune gene expression in the brain and their involvement in schizophrenia-related behavior in animal models have not been well studied so far. We analyzed the social (resident–intruder) and sensorimotor gating (pre-pulse inhibition (PPI) of acoustic startle) behaviors, and expression profiles of several brain immune genes in adult C57BL/6N and DBA/2J female mice. Compared to C57BL/6N mice, DBA/2J mice exhibited less social interaction in the resident–intruder test and reduced pre-pulse inhibition. The mRNA levels of *Il1b* and *Il6* genes were significantly higher in the cortex and hypothalamus, while the mRNA level of *C1qb* was lower in the cortex, hippocampus and hypothalamus of DBA/2J mice compared to C57BL/6N mice. Furthermore, *Tnfsf13b* was up-regulated in the cortex and hippocampus, and so did *Cd47* in the hippocampus, while *Cx3cl1* was down-regulated in the cortex of DBA/2J mice. Our study demonstrates the differential expression of several immune genes in C57BL/6N and DBA/2J strains and more importantly provides clues on their potential importance in regulating schizophrenia-related endophenotypes in animal models.

1. Introduction

Schizophrenia is a complex psychiatric disorder that clinically expresses several classes of symptoms, which are commonly referred to as positive, negative, and cognitive symptoms (Keefe and Harvey, 2012). Negative and cognitive impairments are in most cases resistant to anti-psychotic treatment, and are the major debilitating factors for patients and society alike (Keefe and Harvey, 2012). A plethora of previous epidemiological and pre-clinical data has implied a link of immunological processes and schizophrenia (Muller and Schwarz, 2010). Interestingly, a recent genetic association study, which is the largest of its kind up to date, has provided a convincing support on the existence of such link (Schizophrenia Working Group of the Psychiatric Genomics C, 2014).

Cytokines, such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF), and interferon gamma, play a key role in orchestrating the adaptive reactions of the brain towards physiological or emotional stressors under normal conditions (Dantzer et al., 2008). However, when overactive and/or combined with other risk factors, they may also disturb neurogenesis, neural migration, neurotransmitter metabolism, and synaptic plasticity, and are closely associated with the inception/progression of schizophrenia and autism spectrum disorder in humans (Meyer et al., 2011; Beumer et al., 2012). A plethora of other immune-related genes, including major histocompatibility complex (MHC) molecules, complement and their receptors, innate and adaptive immune receptors, have also been found to be expressed in the brain and regulate neuronal structural and functional plasticity, either directly or indirectly by controlling microglial activation (Perry and O'Connor, 2008; Tian et al., 2009; McAllister, 2014). Dysfunctions of microglia, an important cellular source of immune genes in the brain, have recently been suggested to play a role in neurocognitive disorders, albeit by still obscure mechanisms (Bilimoria and Stevens, 2014; Skaper et al., 2014). Thus, understanding the underlying mechanisms of immune activation in the brain and finding a proper way to control it warrant more thorough research, and may provide a better strategy to tackle these diseases.

C57BL/6 and DBA/2 strains belong to the most extensively studied inbred strains and differ in many behavioral tasks, such as spatial learning and memory (Singer et al., 2009), sensorimotor gating (Olivier et al., 2001) and sociability (Moy et al., 2008). Differences in these behaviors are dependent on genetic backgrounds together with environmental factors, which are also impacting components for the pathogenesis of schizophrenia.

Abbreviations: IL, interleukin; TNF, tumor necrosis factor; PPI, pre-pulse inhibition

* Corresponding author at: Neuroscience Center, University of Helsinki, Viikinkaari 4, FIN-00014 Helsinki, Finland. Tel.: +358 294157613; fax: +358 294157620.
E-mail address: li.tian@helsinki.fi (L. Tian).

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Due to the specific schizophrenia-like behavioral features of DBA/2 mice, they have been extensively studied as an animal model for schizophrenia and have been successfully used for preclinical development of several potential new drugs to treat the sensory processing deficit in schizophrenic patients (Willott et al., 2003; Olincy et al., 2006; Bortolato et al., 2007; Ng et al., 2007; Crowley et al., 2012).

A major drawback in the preclinical research of schizophrenia, however, is that a great majority of previous studies have focused only on male animals, with females rarely touched so far (Willott et al., 2003; Olincy et al., 2006; Bortolato et al., 2007; Ng et al., 2007; Crowley et al., 2012). Recently, we have reported significant differences in cognitive and emotional behaviors of female C57BL/6N and DBA/2J female mice toward social stress, which may provide a novel channel on drug screening and therapeutic development for neuropsychiatric disorders (Kulesskaya et al., 2014). Our aim here was to further characterize the differences in schizophrenia-related behavior between C57BL/6N and DBA/2J female mice. More specifically, we applied resident–intruder and pre-pulse inhibition (PPI) tests that are commonly utilized to evaluate psychiatric phenotypes in animal models. Moreover, we compared their cortical, hippocampal and hypothalamic immune gene expressions to explore molecular mechanisms underlying the behavioral differences.

2. Materials and Methods

2.1. Animals

Seven-week-old female from two inbred strains, female C57BL/6N ($n=14$), female DBA/2J ($n=14$), was purchased from a single supplier, Harlan Laboratories (The Netherlands), to avoid a possible source of variation. Female mice were group-housed in standard cages with aspen chips bedding and nesting materials, food and water available ad libitum and under a 12 h light/dark cycle (lights on 6.00–18.00). Animals were acclimated to the environment for 3 weeks before experiments. The research was performed with permission by the National Animal Experiment Board of Finland.

2.2. Modified resident–intruder test

This test is modified from the classical resident–intruder test performed in home cage (Crawley, 2007). In this study, for assessment of reciprocal social behavior in group-housed animals, tested mice (residents) were placed in single cages provided with bedding material for a 30-min adaptation period (Kulesskaya et al., 2013). Then a gender- and age-matched unfamiliar mouse of the same strain (intruder) was placed into the resident's cage and mice interaction was videotaped for 5 min. Time spent by the residents in social behavior (sniffing, chasing, following, and heterogrooming) was measured.

Table 1
Forward and reverse primers used for target genes in RT-qPCR.

Gene	Primers sequence
Il1b	For: 5′-GGT CAA AGG TTT GGA AGC AG-3′ Rev: 5′-TGT GAA ATG CCA CCT TTT GA-3′
Il6	For: 5′-TCT GAA GGA CTC TGG CTT TG-3′ Rev: 5′-GAT GGA TGC TAC CAA ACT GGA-3′
Tnf	For: 5′-AGG GTC TGG GCC ATA GAA CT-3′ Rev: 5′-CCA CCA CGC TCT TCT GTC TAC-3′
Tnfsf13b	For: 5′-GAC TGT CTG CAG CTG ATT GC-3′ Rev: 5′-CCT CCA AGG CAT TTC CTC TT-3′
Cd47	For: 5′-AGG AGG AGA AAG GAG GTT GC-3′ Rev: 5′-CCA AAC TTT CCC CAG AAC AG-3′
Cx3cl1	For: 5′-CGC GTT CTT CCA TTT GTG TA-3′ Rev: 5′-TGG GAT TCG TGA GGT CAT CT-3′
C1qb	For: 5′-TCT GGG AAT CCA CTG CTG TC-3′ Rev: 5′-AGA CCT CAC CCC ACT GTG TC-3′
Gapdh	For: 5′-TGT TCC TAC CCC CAA TGT GT-3′ Rev: 5′-TGT GAG GGA GAT GCT CAG TG-3′

2.3. Pre-pulse inhibition of acoustic startle reflex (PPI)

PPI test for assessment of sensorimotor gating was performed in Med Associates (St. Albans, VT) apparatus as described previously (Kulesskaya et al., 2013). Briefly, test was performed in three blocks of trials preceded by a 5-min adaptation period. Animals were enclosed in a transparent acrylic holder fixed on a platform with transducing amplifier to measure the startle reaction to acoustic startle stimulus (SS, 40 ms, 120 dB white noise). The first and third blocks, each consisting of five SS trials, were used to assess the baseline and habituation levels of the startle reaction. Block 2 consisted of 50 trials that were divided into five different types of stimulus (10 trials per type): SS alone or SS preceded by a pre-pulse stimulus (PPS, 20 ms, 68 dB, 72 dB, 76 dB, or 80 dB white noise burst), which were given in a pseudo-random order. The startle response was averaged over 10 trials for each trial type. The inhibition for each pre-pulse stimulus was calculated by using the following formula: $100-[(\text{startle response on PPS+SS trials/startle response on SS trials})\times 100]$.

2.4. Total RNA isolation and real-time quantitative polymerase chain reaction (RT-qPCR)

Several immune genes and cytokines – *Tnfsf13b*, *Cd47*, *Cx3cl1*, *C1qb*, *Il1b*, *Il6* and *Tnf* – were selected and their expressions in the cortex, hypothalamus and hippocampus of C57BL/6N and DBA/2J mice were compared by RT-qPCR. Mice were deeply anesthetized with pentobarbital (Orion Pharma) for about 5 min; the cortex, hypothalamus and hippocampus were dissected after intracardial perfusion with ice-cold PBS, immediately frozen in liquid nitrogen and kept at −80 °C. Total RNAs from the tissues were extracted by using GeneJET RNA Purification Kit (Thermo Scientific), and reversely transcribed (1 μg) with RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). RT-qPCR was performed by using corresponding primers and Maxima SYBR Green Master Mixes (Thermo Scientific) on a CFX384 Real-Time PCR Detection System (Bio-Rad) according to the manufacturer's instructions. Forward and reverse primers used for target genes were listed in Table 1. Relative fold change was calculated by first normalization with the reference gene *Gapdh* and then against the level in C57BL/6N, and presented as 2-ΔΔCT. No strain difference in *Gapdh* was observed.

2.5. Statistical analysis

Student's *t* test was used for RT-qPCR analysis of immune gene expression and resident–intruder test. PPI was analyzed by two-way repeated measures analysis of variance (ANOVA) for effects of the inter-subjects factor "strain" and the intra-subjects factor "stimulus intensity" with Bonferroni's post-hoc test. Results are presented as mean ± SEM. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

3. Results

3.1. DBA/2J mice exhibit social deficiency and impaired PPI as compared to C57BL/6N mice

Compared to C57BL/6N female mice, DBA/2J female mice displayed less time in social interaction (sniffing, chasing, following, and heterogrooming) with intruders in the resident–intruder test (Fig. 1A, $p=0.0022$), and reduced PPI (Fig. 1B, strain effect: $F(1, 26)=17.507$, $p=0.0003$; stimulus intensity effect: $F(3, 78)=2.116$, $p=0.1050$; strain × stimulus intensity interaction: $F(3, 78)=1.275$, $p=0.2887$). These findings suggest the expression of schizophrenia-related symptoms in the DBA/2J strain, and endows it an appropriate model for us to further pursue genetic mechanism that may be involved in this disease.

3.2. DBA/2J mice produce more canonical proinflammatory cytokines than C57BL/6N mice in the cortex and hypothalamus

We found that, similar to our previous observation in male mice (Li et al., 2014), female DBA/2J mice expressed significantly higher mRNA levels of *Il6* in the cortex ($p=0.0015$) and hypothalamus ($p=0.006$) (Fig. 2A and C). In addition, *Il1b* mRNA level was higher in the DBA/2J cortex ($p=0.0415$, Fig. 2A). However, no significant difference in the hippocampus was discovered here (Fig. 2B).