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Influence of correlation between HLA-G polymorphism and Interleukin-6 (IL6) gene expression on the risk of schizophrenia

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ABSTRACT

Converging evidence suggests important implications of immuno-inflammatory pathway in the risk and progression of schizophrenia. Prenatal infection resulting in maternal immune activation and developmental neuroinflammation reportedly increases the risk of schizophrenia in the offspring by generating pro-inflammatory cytokines including IL-6. However, it is not known how prenatal infection can induce immuno-inflammatory responses despite the presence of immuno-inhibitory Human Leukocyte Antigen-G (HLA-G) molecules. To address this, the present study was aimed at examining the correlation between 14 bp Insertion/Deletion (INDEL) polymorphism of HLA-G and IL-6 gene expression in schizophrenia patients. The 14 bp INDEL polymorphism was studied by PCR amplification/direct sequencing and IL-6 gene expression was quantified by using real-time RT-PCR in 56 schizophrenia patients and 99 healthy controls.

We observed significantly low IL6 gene expression in the peripheral mononuclear cells (PBMCs) of schizophrenia patients ($t = 3.8$, $p = .004$) compared to the controls. In addition, schizophrenia patients carrying Del/Del genotype of HLA-G 14 bp INDEL exhibited significantly lower IL6 gene expression ($t = 3.1$; $p = .004$) than the Del/Ins as well as Ins/Ins carriers. Our findings suggest that presence of "high-expressor" HLA-G 14 bp Del/Del genotype in schizophrenia patients could attenuate IL-6 mediated inflammation in schizophrenia. Based on these findings it can be assumed that HLA-G and cytokine interactions might play an important role in the immunological underpinnings of schizophrenia.

1. Introduction

Accumulating data from the recent genome wide association studies (GWAS) suggest contribution of common polygenic variation to the risk of schizophrenia [1,2]. In addition to genetic variation, risk of schizophrenia due to exposures to environmental adversities is widely appreciated [3,4]. This is evident from various gene-environment studies implicating immune related genes that play a crucial role in gene-environment interaction. This understanding has been supported by a largest ever GWAS that identified 108 schizophrenia associated genetic loci, of which majority have immune-related functions [2]. Amongst the immunity genes, the Major Histocompatibility Complex (MHC) as well as cytokine genes have been studied extensively in schizophrenia [5–7]. A recent study demonstrated that the functional alleles that were strongly implicated by schizophrenia GWAS was indeed represented by genes within MHC locus of chromosome 6 [8]. The MHC and cytokine molecules have been demonstrated to regulate fundamental events of brain development such as neurite outgrowth, synapse formation, and plasticity [9–11]. However, altered expressions of these immune molecules were shown to have deleterious effects on the developing brain and subsequently increasing the risk of schizophrenia in the offspring [11–13]. Based on this understanding recently, immune-mediated neurodevelopmental origin of schizophrenia has been postulated as one of the predominant research paradigms of schizophrenia pathogenesis [14,15].

The human leukocyte antigen-G (HLA-G) is a non-classical MHC class-I molecule which predominantly possesses tolerogenic and anti-inflammatory functions. It primarily suppresses the functioning of natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) [16,17]. With its predominant expression at the feto-maternal interface, HLA-G

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plays important immuno-modulatory role in the outcome of pregnancy. It is noteworthy that HLA-G expression also influences cytokine production as well as polarization during pregnancy [18–21]. The crucial role of HLA-G in influencing Th1/Th2 balance has been reported in physiological and various pathological conditions. HLA-G favouring Th2 dominance during normal pregnancy and promoting Th1 response in preeclampsia was also demonstrated in some studies [19,22]. It was previously hypothesized that perturbation of HLA-G expression due to environmental adversities in early pregnancy might enhance the risk of schizophrenia in the offspring through cytokine-mediated neurodevelopmental abnormality [23]. In our recent studies, we have shown association of soluble HLA-G (sHLA-G) and HLA-G genotypes with the core features of schizophrenia [24,25]. In addition, we have also reported the influence of IL10 genotype on sHLA-G levels and their association with the risk of schizophrenia [26].

However, the impact of functional interactions between HLA-G and pro-inflammatory cytokines on the risk of schizophrenia is currently not known. Amongst the pro-inflammatory cytokines, IL-6 plays crucial roles in the central nervous system development and function [27]. Higher expression of IL6 mRNA was found to alter fetal brain development during the early phases of pregnancy in rodents [28,29]. Data obtained from clinical studies exhibit elevated serum and cerebrospinal fluid (CSF) levels and increased mRNA expression of IL-6 in dorsolateral prefrontal cortex (DLPFC) in schizophrenia patients [30–32]. Notably, elevated plasma IL-6 levels were found to increase the risk for subsequent decline in cognitive function [33,34]. In addition to this, altered IL-6 levels and IL6 polymorphisms were found to be associated with psychopathology, severity of the illness, cognition, brain morphometry etc. in schizophrenia [4,35,36]. Taken together, a significant role of IL-6 in the immunopathogenetic risk of schizophrenia is a consistent finding; however, the impact of IL6 on schizophrenia risk in correlation to immuno-dampening HLA-G molecules has not been explored so far. To address this gap of knowledge, in this exploratory investigation, we have examined HLA-G 14 bp Insertion/ Deletion (INDEL) polymorphism and IL6 gene expression to understand whether HLA-G gene variants have any role in determining IL6 mediated risk of schizophrenia.

2. Materials and methods

2.1. Subjects

A total of 154 schizophrenia (DSM-IV) patients (age range: 18–45 years) attending the clinical services at National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India were recruited for the study. Amongst them, 56 patients were antipsychotic-naïve/antipsychotic-free; 43 patients were never treated, antipsychotic-naive and presenting to the hospital for the first time & 13 patients were antipsychotic-free and without receiving any treatment for at least 3 months. This study was approved by the Institutional ethics committee. Patients and healthy volunteers were explained about the study in their language and a written informed consent was obtained. The diagnosis was established using the Mini International Neuropsychiatric Interview Plus [37], which was confirmed independently by a qualified psychiatrist. The patients were assessed for their demographic and clinical characteristics (history of presenting illness as well as any other medical illness, family & personal history as well as antipsychotic-naïve/antipsychotic-free status) with reliable information as ascertained by a first-degree relative. The Scale for Assessment of Positive Symptoms (SAPS) [38] and the Scale for Assessment of Negative Symptoms (SANS) [39] Likert-type scales were used to assess the clinical symptoms of patients. Healthy controls (N = 170) were recruited purely on voluntary basis and were screened using MINI plus to rule out any psychiatric diagnosis. A comprehensive mental status examination was done.

None of the controls had family history of psychiatric disorder in first-degree relatives or had co-morbid substance abuse/dependence. History was elicited carefully to rule out any recent high grade fever/infection within the past six weeks or any co-morbid medical condition that could influence immune system in all the subjects (patient/control).

2.2. Genotyping

From all the consenting participants, peripheral blood (10 mL) was drawn from the median cubital vein into EDTA-coated vacutainers (BD Vacutainer® tubes, Becton & Dickinson, NJ, USA) under aseptic conditions. 5 mL blood was processed for separation of plasma and leukocyte layer and the remaining 5 mL was used for separation of PBMCs. The leukocyte suspension was utilized for genomic DNA extraction. Genomic DNA was isolated by spin column method (Qiagen, Inc, Limburg, Netherlands). Genotyping of HLA-G 14 bp INDEL (located in Chr6: 29798582) was done by PCR amplification (Applied Biosystems VeritiTM) in 154 Schizophrenia patients and 170 healthy controls. For PCR amplification, 1 μL of genomic DNA (100 ng) was added to a 30 μL reaction mix containing 18 μL of Applied Biosystems True Allele PCR Premix (Applied Biosystems, USA), 1μL of each primer (10 μM), and 9μL of RNase free water. The forward and reverse primers were 5′ GTGATGGGCTGTTTAAAGTGTCACC 3′; 5′ GGAAGGAATGCAGTTCAG CATGA 3′, respectively. The mixture was then initially subjected to denaturation for 12 min at 95 °C, followed by 30 cycles of denaturation for 30 s at 94 °C, annealing for 60 s at 64 °C, extension for 120 s at 72 °C, and final extension for 10 min at 72 °C. The amplified products were separated by electrophoresis on 3% agarose gel, containing ethidium bromide (0.5 μg/mL). The gel was visualized under ultraviolet illumination using Gel Documentation system (VilberLourmat, France). The genotype profile of the 14 bp INDEL polymorphism was further validated by direct sequencing using Applied Biosystems 3730xI DNA analyzer in a subset of subjects.

2.3. IL6 gene expression

The gene expression profile of IL6 was examined in 56 drug naïve/drug free schizophrenia patients and 99 healthy subjects. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque Plus (GE Healthcare Bio-Sciences AB, Sweden), based on the principle of differential migration of cells during centrifugation. Total RNA isolation was carried out using commercial spin column method (Qiagen, Inc, Limburg, Netherlands) from the isolated PBMCs. The extracted RNA was reverse transcribed to single-stranded cDNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA). qPCR was performed with a commercially available TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, California) containing-AmpliTaq Gold® DNA polymerase. IL-6 TaqMan probes and primers were pre-formulated and designed by the same manufacturer, with primers spanning exon boundaries to avoid non-specific amplification of gDNA due to cDNA contamination. The PCR amplification of IL-6 in each sample was normalized to ACTB endogenous control (Assay id: 4326315E). Multiplex Relative Standard Curve method [RSCM] was applied for relative gene expression quantification. Five points of twofold dilution series was used as the standard curve in every plate. qPCR was performed on StepOnePlus™ Real-Time PCR Systems (Applied Biosystems, Foster City, California) in a 96-well format. Reactions were performed in a final volume of 10 μL containing, TaqMan Universal PCR Master Mix, probes (final concentration of 250 nM) and primers for IL-6 (final concentration of 900 nM) and ACTB (final concentration of 150 nM) as well as 1μL of RT reaction. The assay for every sample was run in quadruples, including the standards, and no-template control. Standard PCR conditions of 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C was followed.

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