The impact of IL10 polymorphisms and sHLA-G levels on the risk of schizophrenia

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ABSTRACT

Early life immune aberrations have strongly been associated with the risk of schizophrenia. Amongst them, inflammation induced neurodevelopmental origin has emerged as one of the widely recognized underlying mechanisms. Interleukin-10 (IL-10) is an important anti-inflammatory and immunoregulatory cytokine. It modulates the expression of another immuno-inhibitory molecule, Human Leukocyte Antigen-G (HLA-G), predominantly expressed at the feto-maternal interface. Under physiological conditions, IL-10 and HLA-G molecules regulate the feto-maternal immune homeostasis by limiting the inflammatory states and influence the outcome of pregnancy. The aberrant expression of these molecules can cause pregnancy complications, which are known to confer strong risk to schizophrenia in the offspring. However, there is a considerable lack of information on the effect of the functional interactions between IL-10 and HLA-G on the risk of schizophrenia. We therefore examined the impact of possible correlation between IL-10 genetic variations and the plasma levels of soluble HLA-G (sHLA-G) on schizophrenia risk. Genotyping of IL10 (-592 C > A, -1082 A > G) single nucleotide polymorphisms (SNPs) was performed by PCR-RFLP method in 219 schizophrenia patients and 197 healthy subjects and levels of sHLA-G were estimated by ELISA in 46 patients and 44 healthy subjects. There was no significant difference in the genotype and allele frequencies between the groups for both the IL10 SNPs analyzed. However, we observed a correlation between IL10 genetic variation and plasma levels of sHLA-G in schizophrenia patients. Patients carrying CC genotype of IL10 −592C > A polymorphism had significantly lower sHLA-G levels compared to CA and AA genotypes. Our findings suggest the impact of possible correlation between IL-10 and HLA-G on schizophrenia risk.

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1. Introduction

Schizophrenia is a highly complex and debilitating neuropsychiatric disorder that affects approximately 1% of the global population (Saha et al., 2005). Converging evidences based on epigenetic, immunological and imaging studies point towards neurodevelopmental origin of schizophrenia(Frangou and Murray, 1996; Winter et al., 2009; Pidsley et al., 2014). Although the precise mechanistic basis of neurodevelopmental origin is yet to be elucidated, maternal immune activation (MIA) or maternal inflammation has emerged as a widely recognized mechanism (Smith et al., 2007; Canetta et al., 2014). Cytokines are known as the key modulators of inflammation (Billingham, 1987). Interestingly, cytokines also possess non-immune functions in the nervous system and their signaling plays critical roles during the development of the nervous system (Deverman and Patterson,

2009). Prenatal adverse events like maternal infections, hypoxia etc. can alter cytokine production and affect fetal brain development(Urakubo et al., 2001). Data obtained from human as well as animal studies indicate that MIA alters fetal brain development through cytokines like IL-6, IL-8 etc. (Brown et al., 2004; Smith et al., 2007). Contextually, imbalance of fetal brain pro-and anti-inflammatory cytokines following MIA has also been proposed to enhance the risk of schizophrenia in the offspring (Meyer et al., 2009). In a mouse model of prenatal immune activation, enhanced IL-10 production at the maternal-fetal interface in the absence of a discrete prenatal inflammatory stimulus was found to precipitate behavioral abnormalities in the adult offspring(Meyer et al., 2008). There is a growing recognition that prenatal or early life immune changes play pivotal role in shaping the immunological phenotype and modulating brain and behavior in offspring (Bakos et al., 2004; Mandal et al., 2013). Although there is a lack of quality data to support such a causal link in people with schizophrenia, altered cytokines levels and their association with the core features of schizophrenia are evident in multiple studies(Reale et al., 2011; Beumer et al., 2012; Fillman et al., 2015).

In this regard, one pertinent and unanswered question is that how prenatal adverse events can lead to MIA or prenatal inflammation, despite the presence of immunosuppressive molecules like IL-10 and HLA-G? IL-10 is a major anti-inflammatory cytokine that regulates inflammatory response by inhibiting pro-inflammatory cytokine production. Additionally, IL-10 plays crucial immunomodulatory role during normal pregnancy (Holmes et al., 2003). In experimental mice, IL-10 deficiency is shown to cause hypoxia induced preeclampsia (Lai et al., 2011). Further, IL-10 is known to regulate the expression of another anti-inflammatory and tolerogenic molecule, HLA-G (Moreau et al., 1999) which is expressed predominantly at the feto-maternal interface and aids in the orchestration of normal processes of pregnancy (Rouas-Freiss et al., 1997). In a study, IL-10 was found to selectively induce expression of HLA-G in human trophoblasts and monocytes (Moreau et al., 1999).

Given the significant importance of prenatal immune activation mediated immunopathogenesis of schizophrenia, there is a pressing need to understand the impact of immuno-suppressive roles of IL-10 and HLA-G on the risk and progression of schizophrenia. A variety of data obtained from biochemical and genetic studies, as well as their meta-analyses indicate the involvement of IL-10 in schizophrenia (Miller et al., 2011; Gao et al., 2014). On the contrary, the precise role of HLA-G in schizophrenia is inadequately known. In a recent study, we have shown significantly low level of sHLA-G in schizophrenia patients and this was found to be associated with early age at onset in male patients (Rajasekaran et al., 2015). Taking all these into consideration, it is assumed that functional interaction between IL-10 and HLA-G may have significant implications in inflammation-mediated risk of schizophrenia. To gain further insight, the present study aimed at correlating the impact of two IL10 promoter polymorphisms (rs1800872 and rs1800876), which significantly influences the production of IL-10 (Kingo et al., 2005; Turner et al., 1997) and sHLA-G plasma levels on the risk of schizophrenia.

2. Methods

The study was carried out after obtaining the Institutional Ethics Committee approval. Subjects diagnosed with schizophrenia based on the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM IV) classification were considered for this study. A total of 219 patients, belonging to south Indian ethnicity (Males: 116; Females: 103) with a mean age of 30.4 ± 8.1 yrs, who attended clinical services of National Institute of Mental health and Neurosciences (NIMHANS), Bangalore, India were recruited.

Ethnicity (South Indian) matched healthy subjects (N = 197; Males: 119; Females: 78) with mean age of 26.5 ± 4.4 yrs were also recruited. Structured scales and proformas (Mini International Neuropsychiatric Interview (MINI) plus and comprehensive mental status examination) were employed to establish the diagnosis in patients and to rule out the presence of any psychiatric conditions in healthy controls. The patient's clinical examination was done using Scale for the Assessment of Negative Symptoms, SANS and Scale for the Assessment of Positive Symptoms, SAPS scales. Subjects with co-morbid conditions that can significantly impact immune system (recent history of high grade fever/ infection within the past 6 weeks, or any co-morbid medical disease that can potentially influence immune system; and substance abuse/dependence) were excluded. 10 mL of fasting blood samples were collected from all the subjects between 8–9 am and were processed within half an hour to obtain plasma and leukocyte layer. Plasma was stored at $-80\,^{\circ}$C, which was later utilized for the estimation of sHLA-G. All the plasma samples underwent only one freeze and thaw cycle. The leukocyte layer was utilized for genomic DNA isolation by spin column method (Qiagen, Inc, Limburg, Netherlands). Quality and quantity of extracted DNA was checked using UV spectrophotometer (Thermo Scientific, Waltham, USA) and Qubit® 2.0 Fluorometer (Life Technologies) and the concentration ranged between 50–100 ng/ μL. DNA was then stored at $-80\,^{\circ}$C for future use.

2.1. Genotyping

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) based assay was used for genotyping of IL 10−592 C > A (rs1800872) and IL10 -1082 A > G (rs1800896) SNPs, as previously described (Rad et al., 2004; Yu et al., 2004). Samples with indeterminate results (i.e. 10 samples) were repeated and confirmed by sequencing.

2.2. Measurement of sHLA-G levels in plasma

The data for plasma levels of sHLA-G were available for 46 patients (mean age = 26.6 ± 6.70 yrs) and 44 age matched healthy controls (25.95 ± 2.75 yrs) and were taken from our previous study (Rajasekaran et al., 2015).

sHLA-G level was measured using commercially available ELISA kits (BioVendor, Brno, Czech Republic). The ELISA assays were performed as per the manufacturer's instructions. Limit of detection of the ELISA kit was 0.6 U/mL. The calibrators of known concentration ranging between 3.91 U/mL to 125.00 U/mL, provided in the kit were used to construct the standard curve, which was used to determine the concentration of sHLA-G in each sample. 4-Parameter Logistic (4-PL) plotting was used to generate the standard curve. The samples were run in duplicates and those samples exceeding the Coefficient of Variance (CV) threshold of 15% were repeated. The colour intensity was measured within 5 minutes after stopping the reaction (as suggested in the kit insert), using Multiskan™ GO Microplate Spectrophotometer (Thermo Scientific, Waltham, USA) at 450 nm and a reference wavelength correction at 630 nm to correct for the optical imperfections of the microplate.

2.3. Statistical analyses

Hardy-Weinberg equilibrium (HWE) deviations in the genotype frequency distributions were checked using chi square analysis, which was also used to evaluate the difference in genotype and allelic frequencies between patients and controls. Haploview software was used to check for Haplotype association analysis. Shapiro-Wilk test was performed to evaluate if the level of sHLA-G