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# Altered MHC class I expression in dorsolateral prefrontal cortex of nonsmoker patients with schizophrenia

Shin-ichi Kano[a,1], Evaristus Nwulia[b,1], Minae Niwa[a], Yian Chen[a], Akira Sawa[a,*], Nicola Cascella[a,**]

[a] Department of Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, 600N. Wolfe Street, Meyer 3-166, Baltimore, MD 21287, USA
[b] Department of Psychiatry, Howard University College of Medicine, 520W Street, NW, Washington, DC 20059, USA

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ABSTRACT

Schizophrenia (SZ) is a psychiatric disease with plausible neurodevelopmental etiology. Although genetic studies show significant association of immune molecules loci such as major histocompatibility complex (MHC) class I with SZ, it is not clear whether these immune molecules are involved in the pathology observed in SZ brains. MHC class I and the classical pathway components of complement system (C1q and C3) have been shown to regulate brain neuronal maturation and function. We have examined the expression of MHC class I and complement protein C3 in two frontal cortical regions of postmortem brains of SZ patients. Since cigarette smoking may modulate MHC class I protein expression and a higher rate of smoking is observed in SZ patients, we studied the expression of MHC class I and C3 in relation to the presence of smoking. We found that MHC class I protein expression is reduced in the dorsolateral prefrontal cortex (DLPFC) but not in the orbitofrontal cortex (OFC) of nonsmoker SZ patients. We did not observe SZ-associated changes in C3 mRNA expression. Our exploratory research suggests a potential involvement of MHC class I in SZ and implies that smoking might modulate its expression.


## 1. Introduction

Schizophrenia (SZ) is a psychiatric disease with plausible neurodevelopmental etiology (Insel, 2010; Harrison and Weinberger, 2005; Jaaro-Peled et al., 2009). Multiple lines of evidence suggest the involvement of altered immunity in SZ including the possibility of histocompatibility complex (MHC) region as disease susceptibility locus (Heath and Krupp, 1967; Kirch, 1993; Wright et al., 2001). Recent genome-wide association studies (GWAS) have provided further supportive evidence by revealing several markers spanning MHC region on chromosome 6p21.3-22.1 to be associated with SZ (Purcell et al., 2009; Shi et al., 2009; Ophoff et al., 2009). Moreover, microarray studies of postmortem prefrontal cortex (PFC) from SZ patients have highlighted altered expression of immune/inflammatory molecules such as a serine protease inhibitor (SERPINA3), heat shock proteins (HSPB1, HSPA1B, and HSPA1A), interferon induced transmembrane proteins (IFITM2 and IFITM3), and an interferon-gamma-inducible human guanylate binding protein 1 (GBP1) in SZ (Arion et al., 2007; Saetre et al., 2007). Nonetheless, it is not clear whether altered expression of these immune molecules is involved in the pathological changes of SZ brains. This is partly because there is no obvious evidence of immune/inflammatory responses in the brains of SZ patients, and thus it is difficult to link altered expression of these molecules to specific neuronal changes.

Recent rodent studies have provided evidence on the role of MHC class I and classical pathway components of complement system (C1q and C3) in brain development and function (Fourgeaud et al., 2010; Glynn et al., 2011; Needleman et al., 2010; Corriveau et al., 1998; Huh et al., 2000; Goddard et al., 2007; Stevens et al., 2007; Shatz, 2009). These immune molecules are expressed in dendrites or synapses of developing pyramidal neurons, and regulate synapse formation and neural activity (Glynn et al., 2011; Needleman et al., 2010; Corriveau et al., 1998; Huh et al., 2000). MHC class I proteins are also expressed in normal adult cerebral cortex and seem to contribute to functional plasticity in mature synapses (Fourgeaud et al., 2010; Corriveau et al., 1998; Goddard et al., 2007). Interestingly, MHC class I deficient mice show enlarged ventricles, a phenotype reminiscent of neuroimaging findings in SZ patients even at the beginning of the disease (Degreef et al., 1992; Huh et al., 2000). These results suggest a possible role for MHC class I and complement proteins in altered brain neurocircuitry function observed in SZ.

Cigarette smoking is an important confounding factor in the studies of molecular changes in SZ postmortem brains because

* Corresponding author at: 600N. Wolfe Street, Meyer 3-166B, Baltimore, MD 21287, USA. Tel.: +1 410 955 4726; fax: +1 410 614 1792.
** Corresponding author at: 600N. Wolfe Street, Meyer 3-166B, Baltimore, MD 21287, USA. Tel.: +1 410 502 2643; fax: +1 410 614 1792.
*E-mail addresses:* asawa1@jhmi.edu (A. Sawa), cascella@jhmi.edu (N. Cascella).
[1] Contributed equally to this work.

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the prevalence of smoking remains high in the mentally ill subjects, particularly in those with SZ (McClave et al., 2007; Chapman et al., 2009). Indeed, at least one previous postmortem hippocampus study of SZ patients has shown interactive effects of smoking status and SZ diagnosis on the expression of several sets of mRNAs such as those constituting NMDA–PSD complex (Mexal et al., 2005). MHC class I mRNA expression was also affected by smoking in SZ. While these data suggest a negative effect of smoking on MHC class I mRNA expression in SZ hippocampus, it remains to be examined whether a similar effect is observed in other brain regions relevant to the pathophysiology of SZ including PFC areas such as dorsolateral prefrontal cortex (DLPFC) and orbitofrontal cortex (OFC) (Glantz and Lewis, 1997; Garey et al., 1998; Selemon and Goldman-Rakic, 1999; Glantz and Lewis, 2000; Kalus et al., 2000; Broadbelt et al., 2002; Black et al., 2004).

In order to clarify whether MHC class I protein expression is altered in postmortem SZ PFC, we examined MHC class I protein expression in two distinct areas of the frontal cortex: DLPFC and OFC. We also evaluated complement protein C3 expression as an index of activation of complement system because C3 plays a central role in the complement cascade (Spech et al., 2008). We addressed the interactive effects of smoking and SZ diagnosis on MHC class I and C3 expression by comparing smokers to non-smokers SZ patients and normal controls.

## 2. Materials and methods

### 2.1. Postmortem brain samples

Protein lysates, total RNA, and cDNA from DLPFC and OFC were obtained from the Stanley Medical Research Institute's Array Collection (35 SZ and 35 normal control). Diagnoses were made using DSM-IV. Smoking status for each group was as follows. SZ group: 23 smokers, 4 non-smokers and 8 unknown; normal control group: 9 smokers, 9 non-smokers and 17 unknown. Demographic details about the sample of this study are available in Supplementary Table 1.

### 2.2. Western blotting

Equal amounts of protein lysates were loaded onto NuPAGE 4–12% Bis–Tris Gels (Invitrogen). Eight gels were used to run 35 × 3 samples. One control sample from the same protein lysates were loaded on each gel for data normalization. Protein lysates from human lymphoblastoid cell lines were also loaded as positive control. Electrophoresis was performed at 200 V and the proteins were transferred to Immobilon-P PVDF membrane (Millipore) at 200 mA. Following overnight blocking in PBS 0.1% Tween solution containing 5% (w/v) milk at 4 °C, membranes were incubated at 4 °C overnight with anti-MHC class I heavy chain antibody (3B10.7, a kind gift from Dr. Peter Cresswell) (1:1380 dilution). After washing, membranes were incubated for 1 h at room temperature with secondary antibody in PBS 0.1% Tween solution. Blots were developed with ECL kit (GE Healthcare) and exposed to film. Membranes were blotted again with anti-β tubulin antibody (Sigma) (1:1000 dilution) after incubation in Restore Western Blot stripping buffer (ThermoScientific). Film images were scanned, converted into TIFF files and analyzed with Image J software (NIH). MHC class I expression level was calculated in relation to β-tubulin expression.

### 2.3. Quantitative real-time RT-PCR

Total RNA was isolated using RNEasy Mini kit (Qiagen) with on-column or post hoc DNase I treatment, and first-strand cDNA was synthesized with use of random hexamer primer and SuperScript III Reverse Transcriptase (both from Invitrogen). Quantitative real-time PCR was carried out with SYBR GreenER reagent (Invitrogen) and gene-specific primers using cDNA as template. PCR reaction and measurement was performed with ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems). PCR was done with two 50 °C, 2 min; 95 °C, 10 min; (95 °C, 15 s; 60 °C, 1 min for 40 cycles) including dissociation curve step at the last step to verify single amplicon in the reaction. Data were normalized by GAPDH expression level. The following primer sets were used: GAPDH, 5′-TCCCCTCCTCACAGTTGCC-3′ (forward) and 5′-CTAGGCCCCTCCCCTCTTC-3′ (reverse); C3, 5′-TGAAGCACCTCATTGTGACCC-3′ (forward) and 5′-CGATCATGTTCTGTTCCCCG-3′ (reverse).

### 2.4. Data analysis

All statistical analyses were conducted in STATA 11 statistical software (StatCorp, 2009). Raw data on relative expression levels of MHC class I proteins, as well as relative levels of complement component C3 were log (to base 10) transformed to approximate normal values (see Supplementary Fig. 1 showing distribution curves of raw data on relative expression levels of MHC class I proteins). To examine the independent associations of schizophrenia and smoking with MHC and C3, as well as the moderating effect of smoking on the association between schizophrenia and these molecules, we specified multiple (i.e. multivariate) linear models with main and interaction variables for the respective parameters of interest. Here, we specified the MHC or C3 for each unit as:

$$Y_i = b_0 + b_{sz} + b_{smk} + b_{sz\_smk} + b_k + e_i;$$

where $b_0$, sample mean of MHC or C3; $b_{sz}$, mean effect of schizophrenia, or the change in mean level of molecules comparing non-smoking schizophrenia to controls, accounting for differences in other variables; $b_{smk}$, change in mean levels comparing control smokers to control non-smokers, accounting for differences in other variables; $b_{sz\,smk}$, change in mean levels comparing schizophrenia smokers to schizophrenia non-smokers, accounting for differences in other variables; $b_k$, effect of other variables that could potentially confound the association between the variables of interest (i.e. schizophrenia and smoking) and molecular levels. In this analysis, we adjusted for age (centered at the mean age), sex, smoking status, lifetime treatment with neuroleptics, alcohol use and brain pH, using generalized linear parameterizations (McCullagh and Nelder, 1989), depicted in the mathematical model above. Although, these variables compensated for in this model did not affect the outcome (MHC or C3 levels), we still included it because other investigators have postulated possible relationships between these covariates and expression of immune molecules (see Supplementary Table 2). It is noteworthy at this juncture, to state that parallel analyses without inclusion of sex, neuroleptics, alcohol use or brain PH did not change the results of our findings.

Because of the small number of non-smoker SZ patients ($n = 4$), we did not assume that the asymptotic $P$ value derived from this multivariate model would be valid, therefore we will present both asymptotic $P$ values and empirically derived $P$ values from bootstrapped standard errors. Briefly, bootstrapping is a computer-based approach, involving repeated sampling of the observed dataset, for assigning measures of accuracy to sample estimates (Efron and Tibshirani, 1994). This approach was implemented in our case, by constructing 1000 re-samples of equal sizes of the observed dataset (each obtained by random sampling with replacement from the original dataset), with multivariate statistics estimated from each resample. The distribution of the estimated means from this approach is then used to obtain the empirical $P$ values. In the analysis, we only included the samples whose smoking status was known.

## 3. Results

As shown in Fig. 1 and Table 1, we found reduced expression levels of MHC class I heavy chain proteins in the DLPFC of non-smokers SZ patients ($P < 0.008$). We noted that the presence of smoking habit significantly increased the expression level of MHC class I heavy chain proteins in DLPFC ($P < 0.03$) only in SZ patients. No other variable had a statistically significant effect. On the other hand, we did not see changes in MHC class I expression in the OFC of SZ patients, even in non-smokers. Contrary to the changes in MHC class I protein expression, complement C3 mRNA expression did not show any SZ-specific changes either in DLPFC or OFC (Fig. 2 and Table 1).

## 4. Discussion

Our exploratory approach revealed that MHC class I protein expression is reduced in DLPFC of nonsmoker SZ patients. In contrast, we did not observe any significant changes in MHC class I protein in OFC. Notably, one previous study shows that MHC class I mRNA expression in postmortem hippocampus of SZ patients is significantly upregulated in non-smokers compared to controls (Mexal et al., 2005). In view of these findings, our results raise the possibility that region-specific changes in MHC class I expression might be associated with SZ. SZ has been repeatedly associated with a hypoactive or dysfunctional DLPFC using functional in vivo imaging techniques and an overactive hippocampus has been associated with psychosis in SZ (Schobel et al., 2009). Our findings seem to agree with these previous imaging data because MHC class I expression is regulated by neural activity in mature CNS and lower MHC class I expression may reflect hypoactive or dysfunctional DLPFC (Corriveau et al., 1998).

Multiple MHC class I molecules were shown to be expressed in distinct brain regions in rodents (Huh et al., 2000), and it is intrigu-