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Association of HLA Locus Alleles with Posttraumatic Stress Disorder

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Abstract

**Background:** Immune dysregulation has been widely observed in those with posttraumatic stress disorder (PTSD). An individual’s immune response is shaped, in part, by the highly polymorphic Human Leukocyte Antigen (HLA) locus that is associated with major psychiatric disorders such as schizophrenia, major depression and bipolar disorder. The aim of the current study was to investigate the association between common HLA alleles and PTSD.

**Methods:** Genome-wide association data was used to predict alleles of 7 classical polymorphic HLA genes (A, B, C, DRB1, DQA1, DQB1, DPB1) in 403 lifetime PTSD cases and 369 trauma exposed controls of African ancestry. Association of HLA allelic variations with lifetime PTSD was analyzed using logistic regression, controlling for ancestry, sex and multiple comparisons. The effect of HLA alleles on gene expression was assessed by weighted correlation network analysis (WGCNA), using 353 subjects with available expression data. Enrichment analysis was performed using anRichet to identify associated pathways of each module.

**Results:** HLA-B*58:01 (p= 0.035), HLA-C*07:01 (p= 0.035), HLA-DQA1*01:01 (p= 0.003), HLA-DQB1*05:01 (p= 0.009) and HLA-DPB1*17:01 (p= 0.017) were more common in PTSD cases, while HLA-A*02:01 (p= 0.026), HLA-DQA1*05:05 (p= 0.011) and HLA-DRB1*11:01 (p< 0.001) were more frequent in controls. WGCNA was used to explore expression patterns of the PTSD related alleles. Gene expression modules of PTSD-related HLA alleles were enriched in various pathways, including pathways related to immune and neural activity.

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Conclusions: To the best of our knowledge, this is the first study to report an association of HLA alleles with PTSD. Altogether, our results support the link between the immune system, brain and PTSD.

1. Introduction

Posttraumatic stress disorder (PTSD) develops in those who have experienced extreme, life threatening stress or trauma exposure. Following a trauma, immune system activation is triggered as part of the body's natural fight-or-flight response. Normally, the acute inflammatory phase is temporary and gradually resolves. However, in some individuals, the immune response is dysregulated and persists. Immune dysfunction is suggested as one of the main mediators of psychiatric disorders, including PTSD (Michopoulos et al., 2015). Current studies empirically support the hypothesis of immune-related or inflammatory etiology for PTSD and suggest that inflammation may be a preexisting vulnerability factor for the development of PTSD (Segman and Stein, 2015). Studies also report high comorbidity between PTSD and inflammatory or auto-immune diseases, which may be explained by significant pleiotropy observed between PTSD and immune-related disorders such as type 2 diabetes (Lukaschek et al., 2013), rheumatoid arthritis, and psoriasis (Stein et al., 2016).

An individual's immunogenetic background plays an important role in shaping their response to stress or trauma exposure. One of the main elements of the immunogenetic background is the highly polymorphic Human Leukocyte Antigen (HLA) locus. HLA locus involves highly polymorphic classical HLA genes (HLA-A, -B, -C, -DQ, -DR, -DP) and non-classical genes (e.g. HLA-E, -F, -G) exhibiting limited polymorphism. Classical HLA alleles are combination of different SNPs (i.e. haplotypes) that results in functionally distinct proteins. For example, nucleotide polymorphisms that change amino acid sequences in an HLA gene (e.g. HLA-A) makes a functionally unique HLA allele (e.g. HLA-A*01:02) with distinct antigen presentation properties. This extensive variability of HLA alleles is the base of individual immune responses. Given the structural complexity and extensive polymorphism of the region, interpretation of the HLA locus with GWAS, which examine individual SNPs, is challenging, and HLA imputation tools are required to predict functional HLA alleles from SNP markers (de Bakker et al., 2006).

Multiple studies investigating the association between imputed HLA alleles and schizophrenia reported decreased frequency of HLA-B*08:01, HLA-C*07:01, HLA-DRB1*03:01, HLA-DQA1*05:01 and HLA-DQB1*02:01 alleles in schizophrenia patients (Andreassen et al., 2015; Bergen et al., 2012; Purcell et al., 2009). Even though, a recent study from the PTSD Workgroup of The Psychiatric Genomics Consortium (PGC-PTSD) identified a significant GWAS hit for PTSD in African American males in the HLA-B locus (Nievergelt et al., 2018), no study evaluated the association between PTSD and functional HLA alleles.

In the current study, we investigated the association between lifetime PTSD and HLA alleles. To our knowledge, this is the first study to evaluate the involvement of HLA alleles in PTSD.
2. Methods

2.1. Sample collection

The samples used in the current study are part of Grady Trauma Project (GTP), a large, primarily African American cohort recruited from a publicly funded hospital that serves a low socioeconomic, inner-city population of Atlanta, GA, USA (Gillespie et al., 2009). Screening interviews and saliva collection were completed on the same day. Study participants completing the initial interview were invited to participate in a secondary phase of the study in which additional detailed self-report measures and structured clinical interviews, including the Clinician-Administered PTSD Scale (CAPS), were administered, and blood samples were collected. Lifetime PTSD diagnosis was assessed based on CAPS for DSM-IV, a clinician administered interview with excellent psychometric properties (Weathers et al., 2001). Trauma burden was assessed with Traumatic Events Inventory (TEI), a fourteen-item self-report measure that asks participants if they have ever experienced a series of potential traumatic incidents (Schwartz et al., 2006). Only controls that have experienced trauma were included in this study. All study procedures were approved by the Emory University Institutional Review Board and the Grady Health Systems Research Oversight Committee.

2.2. Genotyping and HLA imputation

We leveraged available genotype data of 772 African American individuals with completed secondary phase study data including CAPS interviews, 403 of which were diagnosed with lifetime PTSD and 369 of which were trauma-exposed controls (Table 1).

The GWAS has been described by Almli et al. (2017). Briefly, genotyping was performed using Illumina Omni-Quad 1M, and genotypes were called in Illumina’s GenomeStudio. Genotype data quality control was performed using PLINK (Purcell et al., 2007) as described previously, and principal components (PCs) for ancestry were calculated according to the PGC guidelines (Nievergelt et al., 2018).

The HIBAG software package for R is used for prediction of 4-digit alleles of 7 available classical HLA genes (-A, -B, -C, -DQA1, -DQB1, -DRB1, -DPB1) from genotype data (Zheng et al., 2014). For the current study, we used published parameter estimates for multi-ethnic populations, which gave the highest Bayesian posterior probabilities after imputation, using recommended parameters (0.5 call threshold). The average posterior probabilities of our data (80–90%) were consistent with the HIBAG authors and studies that used HIBAG imputation (Kuniholm et al., 2016; Parks et al., 2018). A cutoff of 5% minor allele frequency (MAF) was applied to determine ‘common’ alleles in 772 subjects.

2.3. Statistical analysis

50 common HLA alleles were coded as dichotomous variables for the subsequent analysis under a dominant genetic model. Association of HLA alleles with PTSD was evaluated using permutation test for inference in logistic regression by resampling 1000 times. Sex and the first two PCs from GWAS for ancestry were added as covariates to the model. We
addressed multiple testing using permutation tests, consistent with the approach by Georgopoulos and colleagues (Georgopoulos et al., 2016).

We performed a post hoc power analysis using GAS Power calculator (http://csg.sph.umich.edu/abecasis/gas_power_calculator) to assess the power to detect a genotype relative risk ratio (GRR) of 1.3, equivalent to that reported in our top hit. The trait prevalence was set at 46.2%, as previously reported by Gillespie et al. (2009) in this cohort. Assuming these parameters, our study of n = 772 would have over 80% power to detect a GRR of 1.3 at a significance level of 0.05 for common alleles (MAF > 0.05).

Since PTSD symptoms are known to vary with trauma burden (Schwartz et al., 2005), we conducted sensitivity analysis for significant HLA alleles, including trauma burden as a covariate.

2.4. Gene expression, gene network construction and module detection

The gene expression dataset consists of 353 subjects who also have genotype data (Table 1). The methodology used for gene expression analysis has been previously described (Mehta et al., 2013). Briefly, RNA from whole blood was interrogated using Illumina HT-12 version 3.0 arrays. The data were transformed and normalized using the variance-stabilizing normalization. A total of 13,933 transcripts expressed in blood (detection p value <.01 in 5% of the individuals) were used for subsequent analysis.

Weighted correlation network analysis (WGCNA) (Langfelder and Horvath, 2008) was performed to build un-signed co-expression networks associated with allelic variants of HLA genes by using samples with both HLA and expression data. Consistent with the recommendations for WGCNA, sample clustering was performed to detect outlier samples in both networks and five samples in the HLA-C and one sample in HLA-DRB1 gene networks were removed as outliers. We detected network modules (clusters of highly co-expressed genes) correlated with HLA alleles by using default parameters of WGCNA. A different color is assigned for each module, with the grey module representing background noise.

Enrichment of modules was carried out by anRichment package, using internal collection compiled by Miller et al. (2011). An FDR corrected p-value <0.05 was used to determine significant enrichments.

3. Results

3.1. HLA allelic associations with PTSD

Allele frequencies of the common HLA alleles observed in this study are shown in Table S1. Eight HLA alleles associated with PTSD (Table 2). Of them, five alleles were more frequent in PTSD cases; while three alleles were less frequent in PTSD cases than in trauma-exposed controls.

When trauma burden was included as a covariate, the effect of HLA-C*07:01 (OR= 1.75, CI= 1.07 – 2.85, p= 0.025), -DQA1*05:05 (OR= 0.57, CI= 0.38 – 0.86, p= 0.008), -
DQA1*01:01 (OR= 1.79, CI= 1.08 – 2.96, p= 0.023), -DQB1*05:01 (OR= 1.70, CI= 1.14 – 2.54, p= 0.009), -DRB1*11:01 (OR= 0.45, CI= 0.25 – 0.80, p= 0.0067) and -DPB1*17:01 (OR= 1.78, CI= 1.05 – 3.019, p= 0.032) remained consistent.

3.3. Associations between HLA alleles and gene expression modules

To evaluate the degree to which these genotypes can influence gene expression, unsigned gene co-expression network modules for PTSD-associated HLA alleles were constructed by WGCNA package (Langfelder and Horvath, 2008). The objective of using WGCNA in our analysis was to determine the effect of HLA alleles on gene expression patterns and functional pathways. HLA-A*02:01, HLA-B*58:01, HLA-C*07:01, HLA-DQA1*01:01, HLA-DQB1*05:01 and HLA-DPB1*17:01 were each associated with gene expression modules (p<0.05), some of which demonstrated biological pathway enrichment (FDR<0.05) (Table S2). For most HLA alleles, the enriched pathways were blood cell types and immune pathways. The most intriguing modules was MEblue of HLA-C*07:01 that was enriched for pathways relevant for neural activity.

4. Discussion

This study—the first to evaluate association between PTSD and HLA functional alleles—supports the immunopsychiatric hypothesis that suggests immune dysregulation may precede the onset of psychiatric disorders and contribute to symptom manifestation in these disorders (Leboyer et al., 2016). We found eight significant HLA alleles associated with PTSD. HLA-B*58:01, HLA-C*07:01, HLA-DQA1*01:01, HLA-DQB1*05:01 and HLA-DPB1*17:01 alleles were more frequent in PTSD cases than controls, suggesting that individuals carrying these alleles may have a higher risk of developing PTSD. On the contrary, HLA-A*02:01, HLA-DQA1*05:05 and HLA-DRB1*11:01 alleles were frequent in trauma exposed controls, indicating protective effect of the allele. The association between other psychiatric disorders and HLA-C*07:01 and HLA-DPB1*17:01 was previously implicated in HLA PheWAS database (Karnes et al., 2017). Although previous studies reported negative association of HLA-C*07:01 with schizophrenia (Andreassen et al., 2015; Purcell et al., 2009), we observed a positive association between this allele and PTSD.

In the current study, we also investigated the high-order systematic associations of HLA alleles with gene expression modules (Langfelder and Horvath, 2008). Most of the identified gene expression modules were enriched in immune pathways or blood cell types. Since HLA alleles are key players in the immune system, we expected that HLA alleles would primarily associate with expression modules enriched in immune pathway. Among blood cell type enrichments, the correlation found between modules most enriched for genes in blood platelets and HLA-C*07:01 (MElightyellow, FDR= 1.53E-04) and HLA-B*58:01 (MEblack, FDR= 7.02E-05) are the most interesting. A recent study reported direct correlation of blood platelet count with PTSD symptom severity and inflammation in individuals with PTSD (Lindqvist et al., 2017). This supports the idea that the presence of HLA-C*07:01 in PTSD subjects may result in an aberrant immune response, which may also contribute to dysregulation of hematopoietic cells in PTSD.
HLA-DQA1*01:01 and HLA-DQB1*05:01 were correlated with gene modules that were enriched in rhodopsin-like G-protein-coupled receptors (GPCRs). This rich family includes proteins ranging from neurotransmitters, hormonal receptors to chemokine receptors. The role of GPCRs are implicated in various psychiatric disorders (Komatsu, 2015). Hence, it is possible that HLA alleles may alter expression of GPCRs that are involved in PTSD.

Another intriguing finding from our network analysis was the correlation between HLA-C*07:01 and a module enriched for expression in PTSD-relevant brain region including the dentate gyrus, central gray matter of midbrain, caudate nucleus, nucleus accumbens (NAcc) and amygdala (Hayes et al., 2017; Kuo et al., 2012). Though gene expression patterns are typically tissue-specific, Qi et al. (2018) report that expression patterns that associate with genotypes, termed eQTLs, are more likely to be consistently detected in both blood and brain. Thus, it is possible that HLA-C*07:01, which associates with distinct expression modules in the blood, may influence expression of similar genes in the brain.

Our findings may further drive studies of immunogenetic factors in PTSD for future clinical assessment of PTSD by HLA alleles, which is currently under consideration for schizophrenia (Laiyu et al., 2017). Discovering the relationship between immune dysregulation and PTSD will also likely contribute to research on potential anti-inflammatory treatments for individuals with PTSD (Michopoulos and Jovanovic, 2015), which may be achieved to some degree by the use of current first-line treatments for PTSD, such as SSRI antidepressants (Kao et al., 2016).

4.1. Limitations

The current study has some limitations, the most notable of which was the modest sample size. For this study, we had a total of 772 subjects, which is relatively small for genetic studies in general, though not inconsistent with studies of HLA (Wright et al., 2001). We did not have data that allowed us to test association between HLA alleles and co-morbid inflammatory or autoimmune disorders. Finally, the expression data was generated in blood. Although blood-based expression studies can capture part of the PTSD sequelae, they may not reflect specific gene expression changes in the brain regions relevant for PTSD.

4.2. Conclusions

As an initial study investigating the effects of HLA allelic variations on PTSD, the current study may have broad impact in psychiatric genetics and immunology. Our analyses identified two HLA alleles that may increase PTSD risk in traumatized individuals. These findings suggest that individuals carrying particular HLA alleles may have altered transcriptome, which may contribute to development of PTSD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.
References


Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, … Sham PC, 2007 PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81, 559–575. [PubMed: 17701901]


Highlights:

- HLA-C*07:01 and HLA-DQB1*05:01 are identified as PTSD risk alleles.
- Weighted correlation network analysis showed HLA alleles’ effect on gene expression.
- HLA-C*07:01 gene expression modules enriched in PTSD relevant brain regions.
Table 1:

Demographics of the study datasets

<table>
<thead>
<tr>
<th></th>
<th>Genetic Dataset</th>
<th>Gene Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (N=403)</td>
<td>Control (N=369)</td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>78.4%</td>
<td>66.7%</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>41.49 (11.53)</td>
<td>42.62 (12.52)</td>
</tr>
<tr>
<td>PTSD severity $^1$, mean (SD)</td>
<td>70.12 (20.76)</td>
<td>25.93 (17.12)</td>
</tr>
<tr>
<td>Trauma burden $^2$, mean (SD)</td>
<td>6.27 (3.31)</td>
<td>4.32 (2.71)</td>
</tr>
</tbody>
</table>

$^1$ Measured by Clinician Administered PTSD Scale (CAPS).

$^2$ Measured by Traumatic Events Inventory (TEI). Significance was assessed with a Student’s two-tailed t test for continuous variables and fisher’s exact test of proportions for binary variables.
Table 2:
Nominally significant alleles associated with PTSD

<table>
<thead>
<tr>
<th>HLA Alleles</th>
<th>Frequency in Cases</th>
<th>Frequency in Controls</th>
<th>LR</th>
<th>GRR</th>
<th>p_{perm}</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-A*02:01</td>
<td>10.3%</td>
<td>12.9%</td>
<td>4.552</td>
<td>0.908</td>
<td>0.026</td>
<td>0.005</td>
</tr>
<tr>
<td>HLA-B*58:01</td>
<td>6.1%</td>
<td>4.5%</td>
<td>4.618</td>
<td>1.292</td>
<td>0.035</td>
<td>0.006</td>
</tr>
<tr>
<td>HLA-C*07:01</td>
<td>14.8%</td>
<td>11.1%</td>
<td>4.478</td>
<td>1.317</td>
<td>0.035</td>
<td>0.006</td>
</tr>
<tr>
<td>HLA-DQA1*05:05</td>
<td>11.0%</td>
<td>15.0%</td>
<td>6.794</td>
<td>0.770</td>
<td>0.011</td>
<td>0.003</td>
</tr>
<tr>
<td>HLA-DQA1*01:01</td>
<td>8.8%</td>
<td>5.9%</td>
<td>8.278</td>
<td>1.258</td>
<td>0.003</td>
<td>0.002</td>
</tr>
<tr>
<td>HLA-DQB1*05:01</td>
<td>20.3%</td>
<td>14.2%</td>
<td>7.395</td>
<td>1.267</td>
<td>0.009</td>
<td>0.003</td>
</tr>
<tr>
<td>HLA-DRB1*11:01</td>
<td>6.1%</td>
<td>10.8%</td>
<td>8.693</td>
<td>0.680</td>
<td>\gt0.001</td>
<td>\gt0.001</td>
</tr>
<tr>
<td>HLA-DPB1*17:01</td>
<td>9.5%</td>
<td>5.8%</td>
<td>6.473</td>
<td>1.233</td>
<td>0.017</td>
<td>0.004</td>
</tr>
</tbody>
</table>

LR: likelihood ratio; GRR: genotype relative risk (probability of being affected for individuals with the allele / probability of being affected individuals with the allele); p_{perm}: permutation corrected p-value; estimated standard errors associated with permutation p-values.