Gene Expression Profiling of the xMHC Region Reveals 9 Candidate Genes in Schizophrenia

To the Editor: Strong association of the extended major histocompatibility complex (xMHC) region on human chromosome 6 with schizophrenia has been supported by a number of genome-wide association studies.[1] However, since the xMHC region is featured by numerous polymorphisms, dense gene clusters, and strong linkage disequilibrium, it is difficult to attribute the association to specific genes. A targeted scrutinization of gene expression in the xMHC region can provide valuable candidate genes for future validation. Here we utilize 2 real-time polymerase chain reaction (PCR)–based platforms and 2 sample sets to investigate protein-coding gene expressions in the xMHC region in peripheral blood leukocytes to identify schizophrenia candidate genes.

Methods. The 2 sample sets include 1 discovery sample set (24 patients and 24 healthy controls) and 1 validation sample set (41 patients and 37 controls) (Supplementary eTable 1). All patients were first-episode, drug-naïve, and diagnosed with schizophrenia according to the Fourth Edition of the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-IV*). All patients and controls were recruited after informed consent and institutional review board approval. Blood samples were collected in the morning after overnight fasting (10–12 hours). Leukocytes were isolated, and RNA was extracted and reverse transcribed into complementary DNA. First, we applied real-time PCR–based TaqMan low-density arrays (TLDAs) (Thermo Fisher Scientific) to screen differential gene expressions in the discovery sample set. Expressions of 162 protein-coding genes located in the xMHC region were investigated, with expression of *ACTB* (actin, beta) as an internal standard. Second, we reprepared RNA samples in the discovery set and reassayed previous positive genes by 384-well plate TaqMan real-time PCR. Third, we evaluated the result in the independent validation sample set by 384-well plate TaqMan real-time PCR.

Results. In the TLDA experiment, 127 of the 162 protein-coding genes were above the limit of detection. Sixty genes reached nominal significance in Student *t* tests (unadjusted *P* values <.05), while 11 of them survived Bonferroni correction for multiple comparisons and thus were regarded as differentially expressed genes in this step (Figure 1A). As shown in Figure 1B, expression of the 11 genes were all significantly decreased in schizophrenia with prominent fold changes (*P* values: 9.78×10^{-6} to 3.74×10^{-4}, fold changes: 0.61 to 0.77). This result was successfully confirmed with the 384-well plate TaqMan real-time PCR method, which supported even greater changes (*P* values: 3.32×10^{-7} to 2.29×10^{-3}, fold changes: 0.45 to 0.76). In the independent validation sample set, the 11 genes consistently exhibited decreased trend of expressions in schizophrenia, among which 9 genes with statistical significance were finally identified as candidate genes (*P* values: 5.27×10^{-11} to 3.58×10^{-3} [after adjustment for smoking status], fold changes: 0.61 to 0.83).

In the 9 validated genes,* there were 3 genes that encode zinc-binding proteins, ie, *ZNF184*, *ZSCAN12*, and *ZNRD1*. Zinc-binding proteins usually regulate transcriptions. It deserves future investigation to determine whether and how these genes would affect gene expressions in schizophrenia. Genes *HIST1H4I* and *HIST1H4C* encode histones. Strong evidence of associations was observed within and around histone genes in schizophrenia.[2] Abnormal expression of genes related to nucleosome and histone structure and function has also been found in both schizophrenia patients and their siblings.[3] *MRPS18B* encodes ribosomal protein that helps in mitochondrial protein synthesis. *TUBB* encodes tubulin that is the major constituent of microtubules. Altered expression of *TUBB* has been reported in a previous microarray study in schizophrenia patients.[4] *ABCF1* and *BTN3A3* are immune-related genes. *BTN3A3* is involved in T-cell activity in adaptive immune response. *ABCF1* enhances protein synthesis and promotes inflammation. Decreased expression of *ABCF1* had been found in the whole blood of patients with schizophrenia and identified as a hub gene in a gene expressional subnetwork.[5] Decreased expression of *ABCF1* and *BTN3A3* genes implies malfunctioning of inflammation processes in schizophrenia. Although changes in peripheral blood have limited power to directly reflect changes in brain tissues due to blood-brain barrier or tissue specificity, disturbances in the main neurotransmitter and hormonal systems in the central nervous system are concomitant with altered function of blood immune cells.[6] Moreover, a recent study[7] reported the discovery of the lymphatic vessel system in the central nervous system, which suggests gene expression changes in blood leukocytes, might affect normal inflammation process in brains and cause psychiatric symptoms.

In conclusion, this study revealed down-regulation of 9 xMHC-region gene expressions in first-episode and drug-naïve schizophrenia patients, which was consolidated by 2 real-time PCR–based experimental platforms and 2 independent sample sets. This result suggests a decreased transcriptional activation in the xMHC region and highlights potential malfunctioning of immune cells in schizophrenia. Compounds that can normalize the expressions of certain genes may have therapeutic effects. Although our sample sizes are relatively small, given the strictly controlled sample background and the consistency in 2 subsets, the candidate genes we identified may serve as genetic markers for schizophrenia. Future validation in other larger populations is suggested.

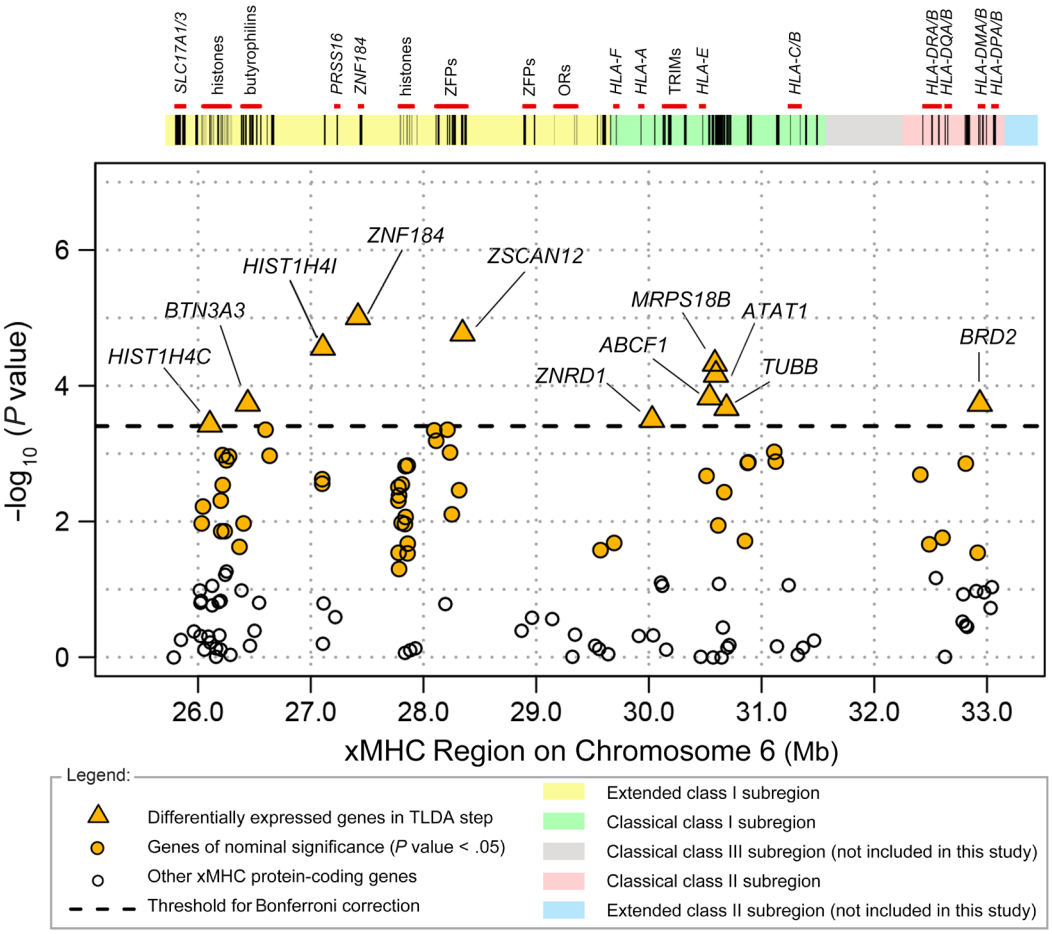
**Definitions for the genes appear in the Figure 1 footnote.*

References

1. Corvin A, Morris DW. Genome-wide association studies: findings at the major histocompatibility complex locus in psychosis. *Biol Psychiatry*. 2014;75(4):276–283.
2. Shi J, Levinson DF, Duan J, et al. Common variants on chromosome 6p22.1 are associated with schizophrenia. *Nature*. 2009;460(7256):753–757.
3. Glatt SJ, Stone WS, Nossova N, et al. Similarities and differences in peripheral blood gene-expression signatures of individuals with schizophrenia and their first-degree biological relatives. *Am J Med Genet B Neuropsychiatr Genet*. 2011;156B(8):869–887.
4. de Jong S, van Eijk KR, Zeegers DW, et al. PGC Schizophrenia (GWAS) Consortium. Expression QTL analysis of top loci from GWAS meta-analysis highlights additional schizophrenia candidate genes. *Eur J Hum Genet*. 2012;20(9):1004–1008.
5. de Jong S, Boks MP, Fuller TF, et al. A gene co-expression network in whole blood of schizophrenia patients is independent of antipsychotic-use and enriched for brain-expressed genes. *PLoS ONE*. 2012;7(6):e39498.
6. Gladkevich A, Kauffman HF, Korf J. Lymphocytes as a neural probe: potential for studying psychiatric disorders. *Prog Neuropsychopharmacol Biol Psychiatry*. 2004;28(3):559–576.

Figure 1. Results of Gene Expression Profiling in the xMHC Region in Schizophrenia

A. Performance of the 127 Detectable Protein-Coding Gene Expressions in the TLDA Experiment[a]



B. Replication and Validation of Differentially Expressed Genes[b]

Gene Name	TLDA (Discovery Set) FC	TLDA (Discovery Set) *P* Value[c]	384-Well Plate RT PCR (Discovery Set) FC	384-Well Plate RT PCR (Discovery Set) *P* Value[c]	384-Well Plate RT PCR (Validation Set) FC	384-Well Plate RT PCR (Validation Set) *P* Value[c,d]
ABCF1	0.66	1.49 E-04**	0.69	1.63 E-04**	0.81	1.00 E-03**
ATAT1	0.61	6.90 E-05**	0.71	3.64 E-04**	0.88	1.80 E-02*
BRD2	0.63	1.85 E-04**	0.76	2.29 E-03**	0.96	2.83 E-01
BTN3A3	0.68	1.84 E-04**	0.63	4.12 E-04**	0.81	3.58 E-03**
HIST1H4C	0.72	3.74 E-04**	0.60	5.38 E-07**	0.73	5.02 E-07**
HIST1H4I	0.76	2.76 E-05**	0.45	3.32 E-07**	0.61	3.05 E-09**
MRPS18B	0.69	4.74 E-05**	0.54	8.30 E-07**	0.70	2.00 E-06**
TUBB	0.70	2.13 E-04**	0.67	3.38 E-05**	0.79	8.00 E-06**
ZNF184	0.71	9.78 E-06**	0.63	3.00 E-04**	0.83	1.41 E-03**
ZNRD1	0.77	3.19 E-04**	0.66	1.20 E-04**	0.74	5.27 E-11**
ZSCAN12	0.72	1.71 E-05**	0.57	1.58 E-05**	0.76	2.58 E-04**

[a]Genes are mapped in the plot according to chromosomal location (x-axis) and "$-\log_{10}$ (*P* value)" value (y-axis). The 11 differentially expressed genes in schizophrenia identified in the TLDA step are annotated by gene names.
[b]All *P* values presented here are unadjusted (for multiple testing). *P* values are from Student *t* tests. The final validated 9 candidate genes are in boldface.
[c]*P* values represents value times 10 to the negative power, eg, 6.90 E-05 = 6.90×10^{-5}.
[d]*P* values in validation set are after adjustment for smoking status.
*$P < .05$. **P values that could survive Bonferroni correction in TLDA or 384-well plate real-time PCR experiments, and only these *P* values are regarded as statistically significant.

Abbreviations: FC = fold change, RT PCR = real-time polymerase chain reaction, TLDA = TaqMan low-density array, xMHC = extended major histocompatibility complex.
Gene abbreviations:
ABCF1 = ATP binding cassette subfamily F member
ATAT1 = alpha tubulin acetyltransferase 1
BRD2 = bromodomain containing 2
BTN3A3 = butyrophilin subfamily 3 member A3
HISTIH4C = histone cluster 1, H4c
HIST1H4I = histone cluster 1, H4i
HLA = major histocompatibility complex
-*A* = class I, A
-*C/B* = class I, C/B
-*DMA/B* = class II, DM alpha/beta
-*DPA/B* = class II, DP alpha/beta
-*DQA/B* = class II, DQ alpha/beta
-*DRA/B* = class II, DR alpha/beta
-*E* = class I, E
-*F* = class I, F
MRPS18B = mitochondrial ribosomal protein S18B
ORs = olfactory receptor genes
PRSS16 = protease, serine 16
SLC17A1/3 = solute carrier family 17 member 1/3
TRIMs = tripartite motif-containing genes
TUBB = tubulin beta class I
ZFPs = zinc finger protein genes
ZNF184 = zinc finger protein 184
ZNRD1 = zinc ribbon domain containing 1
ZSCAN12 = zinc finger and SCAN domain containing 12

7. Louveau A, Smirnov I, Keyes TJ, et al. Structural and functional features of central nervous system lymphatic vessels. *Nature*. 2015;523(7560):337–341.

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Supplementary material: See accompanying pages.

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Supplementary Material

Article Title: Gene Expression Profiling of the xMHC Region Reveals 9 Candidate Genes in Schizophrenia

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List of Supplementary Material for the article

1. **eTable 1** Demographic data of the sample sets

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Supplementary eTable 1. Demographic data of the sample sets

Items	Sample set 1 (discovery set)		Sample set 2 (validation set)	
	Patients (n=24)	**Normal Controls (n=24)**	**Patients (n=41)**	**Normal Controls (n=37)**
Male / Female	12 / 12	12 / 12	15 / 26	19 / 18
Age (year, mean ± SEM)	25.17 ± 0.70	24.71 ± 0.34	27.59 ± 1.52	29.03 ± 1.06
Height (cm, mean ± SEM)	164.00 ± 1.41	163.79 ± 2.01	162.27 ± 1.22	165.78 ± 1.15
Weight (kg, mean ± SEM)	56.75 ± 2.17	56.94 ± 1.81	58.61 ± 2.02	64.2 ± 2.54
Waist circumference(cm, mean ± SEM)	77.38 ± 1.75	75.92 ± 1.73	78.07 ± 1.67	79.19 ± 1.31
BMI (kg/m^2, mean ± SEM)	21.02 ± 0.64	21.12 ± 0.35	22.13 ± 0.61	23.23 ± 0.82
Nonsmoker/Smoker	24 / 0	24 / 0	35 / 6	23 / 14
Location (city/village/rural)	7 / 2 /15	6 / 1 / 17	8 / 4 / 29	21 / 4 / 12