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ARAŞTIRMA / RESEARCH

Relationship between human leukocyte antigen (HLA) alleles and pediatric autoimmune neuropsychiatric disorders associated with Streptococcal infections (PANDAS)

Streptokok enfeksiyonu ile ilişkili pediatrik otoimmün nöropsikiyatrik hastalık (PANDAS) ile insan lökoksit antijen (HLA) ilişkisi

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Abstract

Purpose: The aim of this study is to examine the relationship between Pediatric autoimmune neuropsychiatric disorders associated with Streptococcal infections (PANDAS) and Human Leukocyte Antigen (HLA) alleles.

Materials and Methods: HLA alleles of 41 children patients, whom are diagnosed with rapid onset of obsessive compulsive disorder (OCD), and 88 healthy children are analyzed by using PCR. Results are evaluated by using univariate and multivariate logistic regression analysis.

Results: DRB8, DRB5.2 and DQ5 alleles increase risk of disease while A1, B18 and B35 alleles decrease risk of disease.

Conclusion: Findings of this study will help researchers to examine related genes in PANDAS and the effects of gene products on development of the disease. Presentation of exogenic antigens to T-helper cells by HLA class II loci is determined in different autoimmune diseases. Similarity of these findings with PANDAS etiology and risk increasing alleles found in this study being HLA class II is remarkable.

Keywords: PANDAS, HLA, OCD, neuropsychiatric disorders

Öz

Amaç: Bu çalışmada Streptokok enfeksiyonları ile ilişkili pediyatrik otoimmün nöropsikiyatrik bozukluklar (PANDAS) ve İnsan Lökosit Antijen (HLA) allelleri arasındaki ilişkinin incelenmesi amaçlanmıştır.

Gereç ve Yöntem: Ani başlangıçlı obsesif kompulsif bozukluğu (OKB) olan 41 çocuk hastanın ve 88 sağlıklı çocuğun HLA alelleri PCR kullanılarak analiz edildi. Sonuçlar univariate ve multivariate lojistik regresyon analizi kullanılarak değerlendirildi.

Bulgular: A1, B18 ve B35 allellerinin hastalık riskini azaltırken, DRB8, DRB5.2 ve DQ5 allellerinin hastalık riskini artırdığı saptanmıştır.

Sonuç: Bu çalışmanın bulguları, araştırmacıların PANDAS'taki ilgili genleri ve gen ürünlerinin hastalığın gelişimine olan etkilerini incelemelerine yardımcı olacaktır. HLA sınıf II lokusları tarafından T-yardımcı hücrelere ekzojenik antijenlerin tanıtımı farklı otoimmün hastalıklarda belirlenmiştir. Bu bulguların PANDAS etiyolojisi ile benzerliği ve bu çalışmada bulunan hastalık riskini artıran allellerin, HLA sınıf II'de bulunması dikkat çekicidir.

Anahtar kelimeler: PANDAS, HLA, OKB, nöropsikiyatrik bozukluklar

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INTRODUCTION

Pediatric autoimmune neuropsychiatric disorders associated with Streptococcal infections (PANDAS) is a hypothesis claiming that rapid onset of obsessive compulsive disorder (OCD) or tic disorders are caused by an autoimmune reaction triggered by group A beta-hemolytic streptococcal (GABHS) infections[1]. Initial autoimmune reaction to GABHS infections is thought to produce antibodies that interfere with basal ganglia function and to cause these disorders[2,3]. Although PANDAS is not listed as a diagnosis by the Diagnostic and Statistical Manual of Mental Disorders (DSM), there is supportive evidence for the relationship between *streptococcus* infections and OCD[4-6].

Immune system recognizes self-antigens of the body and distinguishes them from foreign antigens. Autoimmune diseases arise when an alteration resulting in the failure of this recognition occurs[7].

Human Leukocyte Antigen (HLA) genes are first defined in white blood cells and are misnamed after it. However, after the determination of these antigenic structures in all tissues, they are named as Major Histocompatibility Complex (MHC)[7]. MHC gene region, which encodes tissue antigens for the immune system to distinguish between self-antigens and foreign antigens, is an important element of autoimmune response. Exogenous antigens that have molecular similarities to tissue antigens are known to cause autoimmune response by molecular mimicry[8]. Also, some haplotypes of MHC system are known to trigger autoimmune response in targeted tissues/organs by apoptosis and to cause cell loss[9].

There are personal differences in susceptibility to autoimmune diseases, allergies, infections or various malign diseases that are seen in childhood. This situation is related to genetic diversity based on HLA polymorphism alongside with difference of environmental factors[10].

There are not many studies about the relationship between psychiatric disorders and HLA genes. Few studies report about the relationship between Tourette Syndrome (TS) and HLA genes in which A11, A26 alleles are found as risk factors and A13, A24 alleles are found as protective[11]. Psychiatric disorders and their relationship with HLA genes are mostly studied in schizophrenia. DR1 allele is determined to be seen more frequently in Japanese and Turkish patients diagnosed with schizophrenia[12]. According to a meta-analysis, alleles related to schizophrenia are A9, A10, A24, A28, DRB1 and protective alleles are DRB1-4, DQB1-6[13]. In this study, determination of the relationship between PANDAS and HLA alleles is aimed.

MATERIALS AND METHODS

This study includes 41 children patients (age 9±2.2, 55.6% male, 44.4% female), who applied to Cukurova University Medical Faculty, Department of Child Psychiatry and are diagnosed with rapid onset of OCD or tics, together with 88 healthy children (age 8.1±2.5, 47.4% male, 52.6% female). All children included in the study are aged between 4-12. Children that have frequent upper respiratory tract infection, acute rheumatic fever, rheumatic heart disease, OCD or TS are not included in control group. Study is approved by the human studies ethical committee of the Cukurova University (date:21.05.2009, #:5) and informed consent is taken from all subjects and their parents.

Psychiatric diagnoses of the children are made by psychiatry specialists according to the DSM-IV by using Washington University in St. Louis Kiddie Schedule for Affective Disorders and Schizophrenia (WASH-U-KSADS). WASH-U-KSADS is a semi-structured interview form used to identify psychiatric diagnoses in children and adolescents. The form was developed by Kaufman et al.[14] in 1997 according to DSM-IV diagnostic criteria and allows the diagnosis to be made in a certain standard.

OCD symptom scale is determined by using Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS). It is a semi-structured interview form that is commonly used all over the world to identify OCD and symptom severity. It was developed in 1997 by Scahill et al.[15].

PANDAS diagnosis is done according to the criteria which are; Presence of obsessive-compulsive disorder and/or a tic disorder, pediatric onset of symptoms, a positive throat culture for streptococcal infection, physical hyperactivity, or unusual, jerky movements that are not in the child's control and very abrupt onset of symptoms.

Ages, obsession/compulsion scores, severity scores and ASO values of patients (at the beginning of the

diagnostic procedure) diagnosed with PANDAS are given in Table 1. Aggravation of OCD symptoms was observed in all patients.

DNA of the subjects are isolated from blood by using DNA isolation kit and following the protocol of the supplier (Vivantis Inc. GF-1 Blood DNA Extraction Kit). HLA alleles are amplified by using PCR (HLA typing kit, HLAA, -B,-Cw,-DQB1,-DRB1,-DRB345, One lambda) and are read by LABScan™ 100 equipment. Results are evaluated by using univariate analysis and multivariate logistic regression analysis.

Table 1. Ages, obsession/compulsion scores, severity scores and Anti-streptolysin O (ASO) values of patients

Patient #	Age	Compulsion Score	Obsession Score	Severity Score	ASO (mg/dl)
1	10	10	13	3	372
2	12	12	13	3	208
3	9	17	16	4	498
4	8	5	5	2	497
5	6	17	16	5	550
6	7	15	13	4	180
7	9	10	8	2	415
8	12	10	13	4	415
9	11	9	6	2	462
10	11	12	10	3	750
11	12	6	7	2	452
12	11	10	10	3	450
13	9	18	16	4	731
14	12	13	9	3	250
15	10	14	12	4	450
16	6	12	12	4	250
17	10	5	0	2	425
18	10	16	13	3	490
19	12	13	11	4	503
20	10	16	11	3	1170
21	8	14	8	3	501
22	10	14	13	3	503
23	11	12	10	3	308
24	7	13	12	3	285
25	12	16	16	4	386
26	7	9	9	3	191
27	9	16	13	4	461
28	11	8	11	3	800
29	10	12	10	3	705
30	12	17	18	4	351
31	9	14	12	3	381
32	12	20	20	5	638
33	8	18	17	5	449
34	12	13	8	2	465
35	12	12	11	2	134
36	10	13	11	3	418
37	11	8	9	3	790
38	5	17	13	4	340
39	5	13	12	3	343
40	12	16	15	4	154
41	6	9	9	3	40

Statistical analysis

First, univariate analysis is applied to the results and MHC alleles, whose p values are smaller than 0.20, are found (Table 2). Then, backward model selection method multivariate logistic regression analysis is performed to these alleles and protective/risk factor alleles are determined (Table 2). When power of the study is 80 and alpha value is 0.05, detectable odds ratio is calculated as 2.5 for 20% exposure. According to the results of multivariate logistic regression analysis, alleles whose values lower than 1 are evaluated as protective

(reducing risk) and alleles whose values higher than 1 are evaluated as risk factors (increasing risk).Correct classification rate (The percentage of Cases Correctly Classified) is found to be 79.8% according to the results of multivariate logistic regression analysis.

Table 2. Results of univariate analysis

Allel Name	Allel Number	Control	Patient	P Value
A1	0	69	36	0.143
	1	16	5	
	2	3	0	
A3	0	73	29	0.116
	1	14	11	
	2	1	1	
A26	0	83	35	0.102
	1	5	6	
	2	0	0	
B14	0	87	37	0.035
	1	1	4	
	2	-	-	
B15	0	84	35	0.073
	1	4	6	
	2	-	-	
B18	0	69	39	0.020
	1	19	2	
	2	-	-	
B27	0	82	41	0.176
	1	6	0	
	2	0	0	
B35	0	59	34	0.081
	1	25	6	
	2	4	1	
B37	0	88	39	0.099
	1	0	2	
	2	0	0	
B51	0	71	25	0.021
	1	16	15	
	2	1	1	
C1	0	86	36	0.076
	1	2	4	
	2	-	-	
C4	0	55	33	0.071
	1	31	7	
	2	2	1	
C17	0	86	37	0.053
	1	1	4	
	2	1	-	
DQ3	0	30	20	0.108
	1	41	16	
	2	17	5	
DQ5	0	56	19	0.026
	1	31	19	
	2	1	3	
DRB4	0	58	35	0.021
	1	29	6	
	2	1	-	
DRB8	0	87	36	0.012
	1	1	5	
	2	-	-	
DRB14	0	76	29	0.050
	1	12	12	
	2	-	-	
DRB3.3	0	83	41	0.142

	1	4	0	
	2	1	0	
DRB5.2	0	84	36	
	1	4	5	0.142
	2	0	0	

Table 3. Results of Multivariate logistic regression analysis

Allel Name	Allel Count	Odds Ratio (confidence interval)	p value
A1	0	reference	
	1	0.141 (0.031 – 0.651)	0.012
	2	-	
B18	0	reference	
	1	0.069 (0.010 – 0.470)	0.006
	2	-	
B35	0	reference	
	1	0.263 (0.088 – 0.786)	0.017
	2	0.043 (0.002 – 1.079)	0.056
DQ5	0	reference	
	1	1.675 (0.035 – 4.419)	0.297
	2	50.554 (2.577 – 991.7)	0.010
DRB8	0	reference	
	1	46.07 (3.217 – 660.4)	0.005
	2	-	
DRB5.2	0	reference	
	1	8.098 (0.089 – 73.33)	0.063
	2	-	

RESULTS

A1, B18, B35 alleles are determined as protective and DQ5, DRB8, DRB5.2 alleles are determined as increasing the risk of disease. Single copy of A1, B18 and B35 alleles decreased the risk of disease 7.092, 14.493 and 3.802 times respectively. Double copy of B35 allele decreased the risk of disease 23.256 times. DRB8 and DRB5.2 alleles increase the risk of disease 46.070 and 8.098 times respectively while the double copy of DQ5 allele increases the risk of disease 50.554 times.

DISCUSSION

Onset and exacerbation of OCD is associated with GABHS infections and autoimmune hypothesis for OCD is claimed by some studies[16-19]. After the observation of relationship between Sydenham's chorea and OCD, the characterization of PANDAS is made[20,21]. Monoclonal antibodies that carry B-lymphocyte antigens (D8/17) are claimed as sensitivity markers for the early onset of rheumatic fever, OCD and PANDAS[22].

Antigen presentation and T cell activation appear to be key to triggering an autoimmune response prompting investigation of many genes within this pathway for association with auto-immune diseases including several within the MHC[23]. The gene encoding myelin oligodendrocyte glycoprotein is found to have an important role in autoimmune diseases such as OCD and multiple sclerosis due to being located on the distal end (6p21.3) of HLA gene[24].

In this study, 3 alleles (A1, B18, B35) are determined as protective and 3 alleles (DQ5, DRB8, DRB5.2) are determined as increasing the risk of disease. Important function of loci such as HLA-DR/-DQ, which belongs to HLA class II, on presentation of exogenic antigens to T-helper cells are determined in different autoimmune diseases[25]. Similarity of these findings with PANDAS etiology and risk increasing alleles found in our study being HLA class II is remarkable. Albeit there is a generalization that exogenic antigen presentation is done by HLA class II and endogenic antigen presentation is done by HLA class I, sometimes opposite is also true and immune response can change according to this[26,27]. Furthermore, determination of the strong relationship between DQ and DRB loci and consideration of this relationship as a characteristic of autoimmune diseases in previous studies is also worth noting[28-30].

This study determined that the single copy of DRB8 and DRB5.2 alleles increase the risk of disease 46.070 and 8.098 times respectively while the double copy of DQ5 allele increases the risk of disease 50.554 times (Table 2). These alleles all belong to HLA class-II. Although non-mutual mechanisms are proposed to explain the DR/DQ relationship, determination of the association between these loci in our study is meaningful[28,29,31].

In addition, single copy of A1, B18 and B35 alleles decreased the risk of disease 7.092, 14.493 and 3.802 times respectively. Also, double copy of B35 allele decreased the risk of disease 23.256 times. According to these results, B35 allele is noticeable. All protective alleles found in this study belong to HLA class-I and these findings support the previous results that show the role of viruses and bacteria, which triggers CD8+ T-cells, on autoimmune diseases[10].

Determination of related genes in PANDAS is an important step to understand the biological risk factors. This study will also help the identification of non-genetic elements causing the disease. On one hand, determination of the markers for PANDAS will help researchers to examine the related genes and the effects of gene products on development of the disease. On the other hand, elimination of the effects of genetic elements will help the better understanding of the role of environmental factors. In addition, determination of these genetic markers will help the researchers to understand the collective effect of genetic and non-genetic element on disease phenotype.

The major limitation of this study is the lack of patient samples. When associating HLA haplotypes with autoimmunity, obtaining a big pool of patients from the same population is the major difficulty that also affects results. This study would have better outcomes with a bigger pool of patients. However, we tried to overcome this problem by increasing the number of controls and managed to decrease confidence intervals. Still, these results will be useful for future meta-analysis studies.

In conclusion, this study determined that some HLA alleles increase the OCD risk significantly while some alleles are protective considerably. However, more studies are needed on this topic to be conclusive. The relationship between SNP polymorphisms on HLA loci and autoimmune diseases together with gene panels related to immune response should be researched.

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