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Association of HLA class II alleles with suicidal behavior in a Transylvanian population

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ABSTRACT

Background: Suicide is a complex phenomenon determined by the interaction of various risk factors. The Major Histocompatibility Complex is the most polymorphic gene cluster of the entire human genome, being linked to both the regulation of the immune system and various psychiatric diseases. The aim of this study was to identify HLA-DQB1 and DRB1 alleles and genotypes susceptible to influence suicidal behavior.

Methods: We explored the association of HLA-DQB1 alleles with the suicidal behavior on a sample of 427 individuals (including 110 suicide attempters) from Transylvania, as well as the association of HLA-DRB1 alleles with the suicidal behavior on a sample of 271 individuals (including 50 suicide attempters), using the single specific primer-PCR (SSP-PCR) technique.

Results: We found that the HLA-DQB1*02, *03 and *06 alleles, the DQB1*02/*03, DQB1*02/*06, DRB1*12/*15 and DRB1*07/*13 genotypes, as well as the DQB1*06~DRB1*07 and DQB1*02~DRB1*13 haplotypes, were more frequent in suicide attempters. In contrast, the HLA-DQB1*04 and DQB1*13 alleles, the DQB1*02/*05 and DQB1*03/*05 genotypes and the DQB1*03~DRB1*13 haplotype were less frequent in the case group.

Conclusion: HLA-DQB1*02, *03 and *06 alleles and the DQB1*02/*03 and *02/*06 genotypes are susceptible to favor a suicide behavior, while the HLA-DQB1*04 and *13 alleles and the DQB1*02/*05 and *03/*05 genotypes were protective against such behavior. A similar analysis regarding the HLA-DRB1 alleles detected a possible risk for suicidal behavior among individuals possessing either the DRB1*12/*15 or the DRB1*07/*13 genotypes. DQB1*06~DRB1*07 and DQB1*02~DRB1*13 haplotypes were found susceptible to favor a suicidal behavior, while DQB1*03~DRB1*13 exhibited a protective influence.

Keywords: suicide attempts, genotype, HLA, haplotype, suicide behavior

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INTRODUCTION

Suicide is a serious health problem causing over 700,000 deaths yearly worldwide, while recording many more uncompleted attempts [1]. Men dominate complete suicide statistics, while women are more often involved in attempted suicides. For both sexes suicide rates increase with age [2]. Elders are most vulnerable because of inherent co-morbidities combined with various other risk factors. However, quite alarming remains the growing rate of suicides involving the young generation. Suicide is the fourth leading cause of death in 15-19 years old teenagers and young adults in their 20s [1].

Suicide is a complex phenomenon determined by the interaction between various genetic, psychosocial or environmental risk factors. Correlations between suicide and major depression, affective disorders, alcoholism and drug abuse, schizophrenia or personality disorders are well-documented [3-7]. Extensive evidence supports the idea that up to 90% of the suicide attempters present clinically diagnosable mental disorders and that more than 50% of the people who have committed suicide have had serious psychiatric conditions such as severe depression or bipolar disorder [6]. Suicidality, encompassing ideation and suicide attempts, is a heterogeneous behavior co-occurring with multiple psychiatric conditions [8]. Its heritability at genome-wide level is estimated at about 30-55% [5, 9]. A prior suicide attempt

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was found to be the single most important risk factor for suicide in the general population [1, 4, 8, 10].

Discovering objective signatures for suicidal behavior is a challenging task [11-13]. A common genetic material was found among several major psychiatric disorders [14, 15]. Various studies argued for the existence of a genetic vulnerability independent of the psychiatric condition [16]. As the suicidal behavior is determined by the complex interaction of several genes with stressful environmental factors, the identification of candidate genes suggested by genome-wide association studies (GWAS) was pursued from a polygenic perspective [17]. However, determining the specific contribution of certain genes to the suicide phenomenon remains a challenging ongoing process [5]. The Human Leukocyte Antigen (HLA) system, also known as the human major histocompatibility complex (MHC), contains the most polymorphic gene cluster of the entire human genome and was linked to a long list of medical conditions besides the autoimmune diseases [7, 18-21].

Several studies suggested a shared genetic background bridging autoimmune disorders to major psychosis. HLA has been seen for a long time as a susceptibility locus for psychoses [7, 22]. As most HLA genes have low recombination frequencies, allelic variations can prove to be good markers associated with either protection against or susceptibility to certain medical conditions.

The aim of this case-control observational study was to identify HLA-DQB1 and DRB1 alleles, genotypes and haplotypes susceptible to influence suicidal behavior. This study also contributes to the build-up of a more comprehensive picture of HLA alleles distribution in the Romanian general population.

MATERIALS AND METHODS

We analyzed associations of HLA class II alleles with the suicidal behavior on a case group of 110 subjects (85 men) from Cluj and surrounding Transylvanian counties, including patients of the Psychiatric Clinic of the Cluj County Emergency Hospital who fitted in the International Classification of Diseases ICD-10 diagnostic categories regarding suicide attempts (diagnostic groups X6x - Intentional self-poisoning, and X7x - Intentional self-harm) and completed suicides recorded at the Cluj-Napoca Institute of Legal Medicine. A number of 317 individuals (156 males) with no history of suicide attempts subjected to DNA paternity testing in the Molecular Biology Laboratory of the Cluj County Institute of Legal Medicine served as controls.

Venous blood samples (2 mL) were collected in EDTA-treated vacuettes from both patients and controls. Cranial blood samples were collected during autopsy from the deceased. DNA extraction and purification was performed with a Ready DNA Spin Kit (inno-train Diagnostik GmbH, Germany) following the manufacturer's protocol. HLA typing was performed by single specific primer-PCR (SSP-PCR) on a G-Storm thermocycler (LabTech International, UK) using two kits according to the manufacturer's instructions: a HLA-Ready Gene DQ kit (inno-train Diagnostik GmbH, Germany), followed by agarose gel electrophoresis with ethidium bromide, and a HLA-FluoGene DRDQ kit (inno-train Diagnostik GmbH, Germany), fluorescence detection being performed as an endpoint method in the FluoVista fluorescence reader (inno-train Diagnostik GmbH, Germany).

No kinship relations between the case-control groups or among each group were noted. All study protocols and procedures involving human subjects were approved by the Ethics Committee of Iuliu Haţieganu University of Medicine and Pharmacy of Cluj-Napoca (no. 270/3.07.2019). All enrolled subjects signed agreement statements regarding the publishing of the study results under confidentiality provisions, according to the World Medical Association Declaration of Helsinki, revised in 2000, Edinburgh.

The statistical analyses (frequencies, values of the T-test and Pearson's chi square test) were performed using an Epi Info™ software (version 7.2.4.0) developed by the Centers for Disease Control and Prevention (Atlanta, Georgia, USA). Tables were produced using the Microsoft Office Excel software (Microsoft Corp., Redmond, Washington, USA). The significance threshold was set at $p \leq .05$.

RESULTS

The results of the cross tabulation between the dependent variable (suicide attempt) and the independent variables (the occurrence of each of the HLA-DQB1 alleles) for the 110 cases and 317 controls brought out some significant findings. We found an increased frequency of the HLA-DQB1*02, *03 and *06 alleles in the case group, while DQB1*04 and *13 were less frequent, as presented in Table 1.

When analyzing the HLA-DQB1 genotypes, statistical significance was observed for two more frequent genotypes (Table 2), *02/*03 and *02/*06, and two less prevalent genotypes, *02/*05 and *03/*05.

A similar analysis regarding the HLA-DRB1 alleles was carried out for the 271 individuals (50 suicide attempters and 221 controls). No significant associations of the DRB1 alleles with suicide behavior were found (Table 3), but HLA-DRB1*14 and DRB1*15 were significantly more

Table 1. HLA-DQB1 Alleles Odds Ratio/Risk Ratio for suicidal behavior

HLA-DQB1	Case		Control		OR	CI 95% OR	RR	CI 95% RR	p
	n (220)	%	n (634)	%					
*01	9	4.1	46	7.3	.55	(.26; 1.13)	.62	(.34; 1.14)	.06
*02	42	19.1	37	5.8	3.81	(2.37; 6.11)	2.31	(1.81; 2.95)	**$4*10^{-7}$**
*03	57	25.9	119	18.8	1.51	(1.05; 2.17)	1.35	(1.05; 1.73)	**.02**
*04	9	4.1	55	8.7	.45	(.22; .92)	.53	(.28; .98)	**.02**
*05	10	4.6	48	7.6	.58	(.29; 1.17)	.65	(.37; 1.16)	.08
*06	18	8.2	25	3.9	2.17	(1.16; 4.06)	1.68	(1.16; 2.44)	**.01**
*07	14	6.3	44	6.9	.91	(.49; 1.70)	.93	(.58; 1.49)	.45
*08	1	.5	9	1.4	.32	(.04; 2.52)	.39	(.06; 2.48)	.23
*09	0	0	1	.2	0	Undefined	0	Undefined	.74
*10	0	0	8	1.3	0	Undefined	0	Undefined	.09
*11	23	1.5	89	14	.71	(.44; 1.16)	.77	(.53; 1.14)	.11
*12	3	1.4	4	.6	2.18	(.48; 9.81)	1.67	(.71; 3.97)	.26
*13	7	3.2	41	6.5	.48	(.21; 1.08)	.55	(.28; 1.10)	**.04**
*14	5	2.3	25	3.9	.57	(.21; 1.50)	.64	(.28; 1.43)	.17
*15	12	5.5	45	7.1	.76	(.39; 1.46)	.81	(.48; 1.35)	.25
*16	10	4.6	38	6	.75	(.37; 1.53)	.80	(.46; 1.40)	.27

frequent in the 10 suicide attempts conducted under the influence of illegal substances.

An increased prevalence of the DRB1*12/*15 and DRB1*07/*13 genotypes was observed among suicide attempters. Odds Ratio could not be calculated as neither of the two genotypes were identified in the controls. However, Risk Ratios were significant: 5.60 for DRB1 *12/*15 (found in two patients, p=.03) and 5.7021 for DRB1 *07/*13 (identified in three patients, p =.01) (Table 4).

Regarding the 1084 HLA-DQB1~DRB1 haplotypes of the 271 subjects significant results were found for two relatively frequent combinations in the case group: DRB1*07~DQB1*06 (p=.01, found in 5 patients and 3 controls) and DRB1*13~DQB1*02 (p<.05, found in 3 patients and 2 controls). In contrast, the DRB1*13~DQB1*03 haplotype (p=.04) was found in 1 patient and 24 controls (Table 5).

DISCUSSION

For half a century HLA has been seen as a susceptibility locus for psychiatric disorders, a condition strongly associated with the suicide behavior. The HLA system also plays a prominent role in the regulation of viral infections, of particular importance in the context of the COVID-19 pandemic [23]. HLA polymorphisms bear a significant impact on numerous diseases. While no di-

Table 2. HLA-DQB1 Genotypes Odds Ratio/Risk Ratio for suicidal behavior

HLA-DQB1 Genotype	Case		Control		OR	CI 95% OR	RR	CI 95% RR	p
	n (110)	%	n (317)	%					
*01/*05	0	0	1	.3	0	Undefined	0	Undefined	.74
*02/*02	7	6.4	12	3.8	1.73	(.66; 4.50)	1.46	(.79; 2.69)	.19
*02/*03	26	23.6	39	12.3	2.21	(1.27; 3.84)	1.72	(1.21; 2.45)	**<.01**
*02/*04	0	0	1	.3	0	Undefined	0	Undefined	.74
*02/*05	5	4.6	36	11.4	.37	(.14; .97)	.45	(.19; 1.04)	**.02**
*02/*06	14	12.7	12	3.8	3.71	(1.66; 8.29)	2.25	(1.51; 3.34)	**<.01**
*02/*07	0	0	1	.3	0	Undefined	0	Undefined	.74
*03/*03	22	2	41	12.9	1.68	(.95; 2.98)	1.44	(.98; 2.12)	.05
*03/*04	0	0	7	2.2	0	Undefined	0	Undefined	.12
*03/*05	9	8.2	66	2.8	.34	(.16; .71)	.42	(.22; .79)	**<.01**
*03/*06	10	9.1	37	11.7	.76	(.36; 1.58)	.81	(.45; 1.44)	.29
*03/*11	0	0	1	.3	0	Undefined	0	Undefined	.74
*04/*05	1	.9	4	1.3	.72	(.08; 6.49)	.77	(.13; 4.50)	.62
*04/*06	0	0	2	.6	0	Undefined	0	Undefined	.55
*05/*05	6	5.5	22	6.9	.77	(.31; 1.96	.82	(.40; 1.70)	.39
*05/*06	9	8.2	27	8.5	.96	(.44; 2.10)	.97	(.54; 1.75)	.55
*06/*06	1	.9	8	2.5	.35	(.04; 2.87)	.43	(.07; 2.72)	.28

Table 3. HLA-DRB1 Alleles Odds Ratio/Risk Ratio for suicidal behavior

HLA-DRB1	Case n (100)	%	Control n (442)	%	OR	CI 95% OR	RR	CI 95% RR	p
*01	9	9	45	1.2	.87	(.41;1.85)	.89	(.48;1.67)	.44
*03	7	7	46	1.4	.65	(.28; 1.48)	.69	(.34; 1.42)	.20
*04	9	9	49	11.1	.79	(.38; 1.67)	.83	(.44; 1.55)	.34
*07	14	14	43	9.7	1.51	(.79; 2.88)	1.39	(.85; 2.27)	.14
*08	1	1	9	2	.49	(.06; 3.88)	.54	(.08; 3.48)	.42
*09	0	0	1	.2	0	Undefined	0	Undefined	.82
*10	0	0	8	1.8	0	Undefined	0	Undefined	.19
*11	23	23	88	19.9	1.20	(.71; 2.02)	1.16	(.76; 1.76)	.29
*12	3	3	4	.9	3.39	(.75; 15.38)	2.36	(.99; 5.67)	.12
*13	7	7	41	9.3	.74	(.32; 1.69)	.77	(.38; 1.57)	.31
*14	5	5	25	5.6	.88	(.33; 2.35)	.90	(.40; 2.04)	.51
*15	12	12	45	1.2	1.20	(.61; 2.37)	1.16	(.68; 1.99)	.35
*16	10	10	38	8.6	1.18	(.57; 2.46)	1.14	(.64; 2.05)	.39

Table 4. HLA-DRB1 Genotypes Odds Ratio/Risk Ratio for suicidal behavior

HLA-DRB1 Genotype	Case n (50)	%	Control n (221)	%	OR	CI 95% OR	RR	CI 95% RR	p
*01/*03	1	2	6	2.7	.73	(.09; 6.21)	.77	(.12; 4.81)	.62
*01/*04	0	0	6	2.7	0	Undefined	0	Undefined	.29
*01/*07	1	2	4	1.8	1.11	(.12; 1.12)	1.09	(.18; 6.38)	.64
*01/*08	1	2	1	.5	4.49	(.28; 73.03)	2.74	(.67; 11.23)	.34
*01/*10	0	0	2	.9	0	Undefined	0	Undefined	.66
*01/*11	2	4	12	5.4	.73	(.16; 3.35)	.76	(.21; 2.83)	.50
*01/*13	1	2	6	2.7	.73	(.09; 6.21)	.77	(.12; 4.81)	.62
*01/*14	0	0	2	.9	0	Undefined	0	Undefined	.66
*01/*15	0	0	3	1.4	0	Undefined	0	Undefined	.54
*01/*16	3	6	3	1.4	4.64	(.91; 23.70)	2.82	(1.22; 6.54)	.08
*03/*03	0	0	1	.5	0	Undefined	0	Undefined	.82
*03/*04	2	4	6	2.7	1.49	(.29; 7.63)	1.37	(.40; 4.67)	.45
*03/*07	0	0	4	1.8	0	Undefined	0	Undefined	.44
*03/*10	0	0	1	.5	0	Undefined	0	Undefined	.82
*03/*11	2	4	10	4.5	.88	(.19; 4.14)	.90	(.25; 3.27)	.61
*03/*12	0	0	1	.5	0	Undefined	0	Undefined	.82
*03/*13	0	0	2	.9	0	Undefined	0	Undefined	.66
*03/*14	1	2	3	1.4	1.48	(.15; 14.56)	1.36	(.24; 7.58)	.56
*03/*15	1	2	4	1.8	1.11	(.12; 1.12)	1.09	(.18; 6.38)	.64
*03/*16	0	0	6	2.7	0	Undefined	0	Undefined	.29
*04/*04	0	0	2	.9	0	Undefined	0	Undefined	.66
*04/*07	3	6	7	3.2	1.95	(.49; 7.83)	1.67	(.62; 4.45)	.27
*04/*11	2	4	7	3.2	1.27	(.26; 6.32)	1.21	(.35; 4.23)	.52
*04/*13	0	0	7	3.2	0	Undefined	0	Undefined	.24
*04/*15	1	2	6	2.7	.73	(.09; 6.21)	.77	(.12; 4.81)	.62
*04/*16	1	2	4	1.8	1.11	(.12; 1.12)	1.09	(.18; 6.38)	.64
*07/*07	1	2	5	2.3	.88	(.10; 7.72)	.90	(.15; 5.49)	.69
*07/*10	0	0	1	.5	0	Undefined	0	Undefined	.82
*07/*11	3	6	6	2.7	2.29	(.55; 9.48)	1.86	(.71; 4.85)	.22
*07/*13	3	6	0	0	Undefined	Undefined	5.70	(4.40; 7.39)	**.01**
*07/*14	0	0	3	1.4	0	Undefined	0	Undefined	.55
*07/*15	2	4	4	1.8	2.26	(.40; 12.70)	1.84	(.58; 5.87)	.31
*07/*16	0	0	4	1.8	0	Undefined	0	Undefined	.44
*08/*03	0	0	1	.5	0	Undefined	0	Undefined	.82
*08/*04	0	0	1	.5	0	Undefined	0	Undefined	.82
*08/*11	0	0	3	1.4	0	Undefined	0	Undefined	.54

(continued on page 19)

(Table 4 continued from page 18)

HLA-DRB1 Genotype	Case n (50)	%	Control n (221)	%	OR	CI 95% OR	RR	CI 95% RR	p
*09/*16	0	0	1	.5	0	Undefined	0	Undefined	.82
*10/*11	0	0	1	.5	0	Undefined	0	Undefined	.82
*10/*13	0	0	2	.9	0	Undefined	0	Undefined	.66
*11/*11	4	8	10	4.5	1.83	(.55; 6.11)	1.60	(.67; 3.81)	.25
*11/*13	1	2	9	4.1	.48	(.06; 3.88)	.53	(.08; 3.48)	.25
*11/*14	0	0	4	1.8	0	Undefined	0	Undefined	.44
*11/*15	3	6	8	3.6	1.70	(.43; 6.65)	1.51	(.56; 4.10)	.33
*11/*16	2	4	8	3.6	1.11	(.23; 5.39)	1.09	(.31; 3.86)	.58
*12/*13	0	0	1	.5	0	Undefined	0	Undefined	.82
*12/*14	0	0	1	.5	0	Undefined	0	Undefined	.82
*12/*15	2	4	0	0	Undefined	Undefined	5.60	(4.34; 7.24)	**.03**
*12/*16	1	2	1	.5	4.49	(.28; 73.03)	2.74	(.67; 11.23)	.34
*13/*08	0	0	1	.5	0	Undefined	0	Undefined	.82
*13/*13	1	2	1	.5	4.49	(.28; 73.03)	2.74	(.67; 11.23)	.34
*13/*14	0	0	1	.5	0	Undefined	0	Undefined	.82
*13/*15	0	0	6	2.7	0	Undefined	0	Undefined	.29
*13/*16	0	0	4	1.8	0	Undefined	0	Undefined	.44
*14/*04	0	0	1	.5	0	Undefined	0	Undefined	.82
*14/*10	0	0	1	.5	0	Undefined	0	Undefined	.82
*14/*14	0	0	1	.5	0	Undefined	0	Undefined	.82
*14/*15	3	6	4	1.8	3.46	(.75; 15.99)	2.41	(.98; 5.88)	.12
*14/*16	1	2	3	1.4	1.48	(.15; 14.56)	1.36	(.24; 7.58)	.56
*15/*08	0	0	1	.5	3.46	(.75; 15.99)	2.41	(.98; 5.88)	.12
*15/*15	0	0	3	1.4	0	Undefined	0	Undefined	.82
*15/*16	0	0	3	1.4	0	Undefined	0	Undefined	.54
*16/*08	0	0	1	.5	0	Undefined	0	Undefined	.82
*16/*16	1	2	0	0	Undefined	Undefined	5.51	(4.28; 7.10)	.18

Table 5. HLA DRB1~DQB1 Haplotypes Odds Ratio/Risk Ratio - suicidal behavior

HLA-DRB1~DQB1 Haplotype	Case n (200)	%	Control n (884)	%	OR	CI 95% OR	RR	CI 95% RR	p
DRB1*01~DQB1*02	2	1	10	1.1	.88	(.19; 4.06)	.90	(.25; 3.22)	.61
DRB1*01~DQB1*03	2	1	20	2.3	.44	(.10; 1.88)	.49	(.13; 1.84)	.34
DRB1*01~DQB1*04	1	.5	1	.1	4.44	(.28; 71.25)	2.72	(.68; 1.93)	.18
DRB1*01~DQB1*05	12	6	52	5.9	1.02	(.53; 1.95)	1.02	(.60; 1.72)	.53
DRB1*01~DQB1*06	1	.5	7	.8	.63	(.08; 5.15)	.68	(.11; 4.25)	.55
DRB1*03~DQB1*02	7	3.5	50	5.7	.61	(.27; 1.35)	.65	(.32; 1.32)	.14
DRB1*03~DQB1*03	4	2	19	2.2	.93	(.31; 2.76)	.94	(.38; 2.31)	.58
DRB1*03~DQB1*04	0	0	1	.1	0	Undefined	0	Undefined	.82
DRB1*03~DQB1*05	2	1	17	1.9	.52	(.12; 2.25)	.57	(.15; 2.11)	.29
DRB1*03~DQB1*06	1	.5	5	.6	.88	(.10; 7.60)	.90	(.15; 5.43)	.69
DRB1*04~DQB1*02	4	2	14	1.6	1.27	(.41; 3.89)	1.21	(.50; 2.89)	.43
DRB1*04~DQB1*03	12	6	59	6.6	.89	(.47; 1.69)	.91	(.54; 1.55)	.44
DRB1*04~DQB1*04	0	0	2	.2	0	Undefined	0	Undefined	.66
DRB1*04~DQB1*05	1	.5	12	1.4	.37	(.05; 2.82)	.41	(.06; 2.73)	.28
DRB1*04~DQB1*06	1	.5	11	1.2	.40	(.05; 3.11)	.45	(.07; 2.94)	.32
DRB1*07~DQB1*02	14	7	39	4.4	1.63	(.87; 3.07)	1.46	(.92; 2.34)	.09
DRB1*07~DQB1*03	8	4	31	3.5	1.15	(.52; 2.53)	1.12	(.59; 2.10)	.43
DRB1*07~DQB1*05	1	.5	13	1.5	.34	(.04; 2.59)	.38	(.06; 2.55)	.24
DRB1*07~DQB1*06	5	2.5	3	.3	7.53	(1.75; 31.77)	3.45	(1.99; 5.99)	**.01**
DRB1*08~DQB1*02	0	0	1	.1	0	Undefined	0	Undefined	.82
DRB1*08~DQB1*03	0	0	4	.5	0	Undefined	0	Undefined	.44
DRB1*08~DQB1*04	1	.5	7	.8	.63	(.08; 5.15)	.68	(.11; 4.25)	.55

(continued on page 20)

(Table 5 continued from page 19)

HLA-DRB1~DQB1 Haplotype	Case n (200) %		Control n (884) %		OR	CI 95% OR	RR	CI 95% RR	p
DRB1*08~DQB1*05	1	.5	4	.5	1.11	(.12; 9.94)	1.08	(.19; 6.29)	.64
DRB1*08~DQB1*06	0	0	2	.2	0	Undefined	0	Undefined	.66
DRB1*09~DQB1*03	0	0	1	.1	0	Undefined	0	Undefined	.82
DRB1*09~DQB1*05	0	0	1	.1	0	Undefined	0	Undefined	.82
DRB1*10~DQB1*02	0	0	2	.2	0	Undefined	0	Undefined	.66
DRB1*10~DQB1*03	0	0	1	.1	0	Undefined	0	Undefined	.82
DRB1*10~DQB1*05	0	0	11	1.2	0	Undefined	0	Undefined	.10
DRB1*10~DQB1*06	0	0	2	.2	0	Undefined	0	Undefined	.66
DRB1*11~DQB1*02	4	2	12	1.4	1.48	(.47; 4.65)	1.36	(.58; 3.21)	.34
DRB1*11~DQB1*03	34	17	117	13.2	1.34	(.88; 2.04)	1.27	(.91; 1.75)	.10
DRB1*11~DQB1*04	0	0	2	.2	0	Undefined	0	Undefined	.66
DRB1*11~DQB1*05	5	2.5	29	3.3	.76	(.29; 1.98)	.79	(.35; 1.80)	.38
DRB1*11~DQB1*06	3	1.5	16	1.8	.83	(024; 2.86)	.85	(.30; 2.43)	.52
DRB1*12~DQB1*02	0	0	1	.1	0	Undefined	0	Undefined	.82
DRB1*12~DQB1*03	3	1.5	4	.5	3.35	(.74; 15.09)	2.34	(.99; 5.56)	.12
DRB1*12~DQB1*05	1	.5	2	.2	2.22	(.20; 24.56)	1.81	(.36; 9.02)	.46
DRB1*12~DQB1*06	2	1	1	.1	8.92	(.80; 98.85)	3.64	(1.62; 8.18)	.09
DRB1*13~DQB1*02	3	1.5	2	.2	6.72	(1.11; 4.46)	3.29	(1.59; 6.80)	**<.05**
DRB1*13~DQB1*03	1	.5	24	2.7	.18	(.02; 1.34)	.21	(.03; 1.46)	**.04**
DRB1*13~DQB1*04	0	0	1	.1	0	Undefined	0	Undefined	.82
DRB1*13~DQB1*05	1	.5	15	1.7	.29	(.04; 2.22)	.34	(.05; 2.25)	.17
DRB1*13~DQB1*06	9	4.5	40	4.5	.99	(.47; 2.08)	10	(.54; 1.82)	.57
DRB1*14~DQB1*02	1	.5	5	.6	.88	(.10; 7.60)	.90	(.15; 5.43)	.69
DRB1*14~DQB1*03	0	0	7	.8	0	Undefined	0	Undefined	.24
DRB1*14~DQB1*05	7	3.5	36	4.1	.85	(.37; 1.95)	.88	(.44; 1.75)	.45
DRB1*14~DQB1*06	2	1	2	.2	4.45	(.62; 31.82)	2.73	(1.02; 7.33)	.16
DRB1*15~DQB1*02	3	1.5	6	.7	2.23	(.55; 8.99)	1.82	(.72; 4.62)	.22
DRB1*15~DQB1*03	6	3	15	1.7	1.79	(.69; 4.68)	1.57	(.79; 3.12)	.17
DRB1*15~DQB1*04	0	0	2	.2	0	Undefined	0	Undefined	.66
DRB1*15~DQB1*05	5	2.5	24	2.7	.92	(.35; 2.44)	.93	(.42; 2.09)	.55
DRB1*15~DQB1*06	10	5	43	4.9	1.03	(.51; 2.09)	1.02	(.58; 1.82)	.53
DRB1*16~DQB1*02	0	0	10	1.1	0	Undefined	0	Undefined	.13
DRB1*16~DQB1*03	4	2	16	1.8	1.11	(.37; 3.35)	1.09	(.45; 2.63)	.52
DRB1*16~DQB1*05	16	8	44	5	1.66	(.92; 3.01)	1.48	(.96; 2.30)	.07
DRB1*16~DQB1*06	0	0	6	.7	0	Undefined	0	Undefined	.29

rect HLA association with an increased risk for depression was found in a GWAS on individuals of European ancestry, 14 HLA alleles were found to be associated with several autoimmune diseases with evidence for a bidirectional relationship with major depression, the main risk factor for suicidal behavior [24]. Several GWAS studies linked the MHC region with schizophrenia (often associated with suicide), highlighting differential functions (protective vs. risk factors) attributable to HLA molecules [22]. A recent GWAS demonstrated that HLA-DRB1 and HLA-DQB1 genetic diversity modulates responses to lithium in bipolar affective disorders, suggesting that an HLA-mediated low inflammatory background may contribute to an efficient response in patients with bipolar affective disorders [25]. Future studies may determine the role of HLA in reducing suicide prevalence through an effective diagnosis and treatment of psychiatric disorders that pose suicidal risks. GWAS studies followed by HLA imputation-based methods are likely to provide valuable clues towards understanding the HLA genetic contribution to psychiatric disorders [23].

A recent study also found HLA-DR polymorphisms to be involved in SARS-CoV-2 infection [26]. Another study [27] suggested COVID-19 susceptibility roles for HLA-A*03, HLA-B*39 and HLA-C*16 and a protective role for HLA-A*32. The impact of the COVID-19 on suicide rates has been reflected in various studies [28-30].

Our findings add to the data seeking to explain shady facets of this serious health problem. We found that the HLA-DQB1*02 allele belonging to the so-called 8.1 ancestral haplotype (3.8 times more likely to be present in patients exhibiting suicidal behavior, $p=4x10^{-7}$) increases the risk of a suicide attempt 2.3 times, as do the HLA-DQB1*03 (p=.02) and HLA-DQB1*06 (p=.01) alleles.

In contrast, HLA-DQB1*04 (p=.02) and HLA-DQB1*13 (p=.04) were found to act as protective alleles. Additional testing might confirm the protective influence of DQB1*05 (p=.08), as reported in a previous study [31]. Based on our risk ratio values, other alleles presumably susceptible to alleviate the risk of suicidal behavior are HLA-DQB1*08, *01 or *14 (RR of .6-.7), in contrast to HLA-DQB1*12 (RR 1.67). In accordance with these data, a Tunisian study found the DQB1*02 allele to be the most susceptible and DQB1*05 the most protective in schizophrenia patients [32]. On the other hand, an American study on a huge cohort of mainly Caucasian deceased organ donors [33] which associated intentional violent death with 21 HLA-DR and 10 HLA-DQ alleles found both DQB1*02 and DQB1*05 to be significantly associated with increased risks for violent death.

Regarding the HLA-DQB1 genotypes, the *02/*03 (OR=2.21, RR=1.72, p<.01) and *02/*06 (OR=3.71, RR=2.25, p<.01) specimens were found susceptible to elicit a suicidal behavior, while *03/*05 and *02/*05 were identified as protective genotypes. When associating suicide attempts documented in the 427 subjects' medical records with the occurrence of certain genotypes for which we found significant results, the Odds Ratio and Risk Ratio estimates were OR=.34, RR=.42, p<.01 for DQB1*03/*05 and OR=.37, RR=.45, p=.02 for DQB1*02/*05, suggesting that both of these exert a protective role against developing a suicide behavior. Based on these numbers, the later (involving the otherwise very harmful DQB1*02) may argue for the protective influence of the DQB1*05 allele. The Pearson's Chi-square test also produced significant results in these cases.

In a GWAS of the MHC region, DRB1*03:01 was found to play a protective role against schizophrenia [34]. In contrast, HLA-DRB1*03:01 (along with HLA-DQB1*02:01 and HLA-B*08:01) was found to be associated with depression after correcting for multiple testing [24]. Other studies concluded that the HLA-DRB1*03~DQB1*02 sub-haplotype of the 8.1 ancient haplotype is more frequent in bipolar disorder patients with a history of suicidal behavior [35], while the HLA-DRB1*11~DQB1*07 haplotype is more prevalent in autism spectrum disorders [36]. The HLA-DQA1~DRB1 region was also associated with prolonged longevity [20]. A UK study associated HLA-DRB1*13:02 with a high risk of case definition symptomatic COVID-19, a lower impact being attributed to HLA-DRB1*15 [26].

While no significant associations with suicide attempt were found in our research regarding the HLA-DRB1 alleles for the 271 tested individuals, a possible increased risk for suicidal behavior was detected in individuals possessing either the DRB1*12/*15 or the DRB1*07/*13 genotypes. Risk Ratios were significant: 5.60 for DRB1 *12/*15 (found in two patients, p=.03) and 5.70 for DRB1 *07/*13 (found in three patients, p=.01). However, Odds Ratio could not be calculated as neither combination was identified in the controls.

Regarding the Transylvanian population, a study on 2,719 controls [37] analyzing the relative frequencies of HLA-DRB1 alleles found DRB1*11 (19%, n = 1033) to be the most frequent HLA-DRB1 allele, as was in Hungary, Greece, Serbia, Croatia, Slovakia, and Italy. Second most frequent were DRB1*03 and DRB1*13 (both 11.4%, n = 620), followed by DRB1*07 (10.6%, n = 576) and DRB1*16 (10.3%, n = 560). In our case group of 50 patients with suicidal behavior the most frequent HLA-DRB1 alleles were *11 (23%, n = 23), *07 (14%, n = 14), *15 (12%, n = 12) and *16 (10%, n = 10).

Of the HLA-DQB1~DRB1 haplotypes, susceptible of eliciting a suicidal behavior were found to be DRB1*07~DQB1*06 (p=.01) and DRB1*13~DQB1*02 (p<.05), while DRB1*13~DQB1*03 (p=.04) was identified as a protective haplotype. Additional analyses might certify the harmful influence of DRB1*16~DQB1*05, DRB1*12~DQB1*06 or DRB1*07~DQB1*02.

The main limitation of our study consisted in the analysis of a Caucasian population of little ethnic variability from six Transylvanian counties and was also negatively impacted by the absence of a comprehensive HLA allele frequency database regarding Romanian patients with suicidal behavior, covering various ethnic subgroups from all regions of the country. To provide valid conclusions, the association of the suicide behavior with certain HLA alleles needs to consider underlying psychiatric antecedents, along with other demographic, socio-economic and environmental factors. More studies are needed to outline a clearer picture on a national scale.

Last but not least, research regarding the suicide phenomenon is negatively influenced by the COVID-19 pandemic of recent years, which impacts our living standards and degrades the mental health of the general population as debatable restrictions are imposed, lifted, reinstated. While a directly quantifiable impact may not be apparent yet, a growing number of studies predict that the confluence of factors such as distress caused by health problems and economic concerns, depressive moods when coping with the premature death of loved ones, social isolation or diminished psychiatric services will ultimately convey higher suicide rates [29]. Economic uncertainty in particular was seen as a highly stressful factor associated with suicide [28]. In this context, the need for suicide prevention initiatives is imperative. Several suicide risk modeling algorithms have been designed lately [10, 38-39]. Any new findings on this topic contribute to more efficient prediction measures.

CONCLUSIONS

We explored the association of HLA-DQB1 alleles with the suicidal behavior on a sample of 427 individuals from Transylvania including 110 suicide attempters and found that the HLA-DQB1*02, *03 and *06 alleles, as well as HLA-DQB1*02/*03 and *02/*06 genotypes, were susceptible to elicit a suicidal behavior, while the HLA-DQB1*04 and *13 alleles, along with the DQB1*03/*05 and *02/*05 genotypes, were identified as protective against the development of a suicidal behavior.

We failed to identify DRB1 alleles significantly associated with suicide attempts on a sample of 271 individuals including 50 suicide attempters, but we identified HLA-DRB1*12/*15 and DRB1*07/*13 as two possibly harmful genotypes.

We found that DRB1*07~DQB1*06 and DRB1*13~DQB1*02 haplotypes are susceptible of eliciting a suicidal behavior, while DRB1*13~DQB1*03 was identified as a protective haplotype.

More studies are needed to produce a comprehensive HLA allele frequency database for the Romanian population, covering various ethnic subgroups from all regions of the country, and to establish associations susceptible to influence the risk of suicide behavior. Suicide prevention initiatives are needed to prevent increased suicide rates in the context of the COVID-19 pandemic and any new findings on this topic help to implement such programs.

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AUTHORS' CONTRIBUTIONS

MEV: Conceptualization, methodology, investigation, resources, writing original draft preparation. MLV: Conceptualized and designed, validation, methodology. SB: Conceptualization, writing, review and editing. GZN: writing, review, formal analysis, data curation. SIR: Formal analysis, data curation, methodology. CVS: Review, validation, resources. HGC: Data curation, resources, validation. HVM: Validation, supervision, data curation, conceptualization.

All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

No conflicts of interest exist for any of the study authors.

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