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The association of HLA-DQB1*0602 but not HLA-DRB1*15 with obstructive sleep apnea

Abstract

Purpose: Obstructive sleep apnea (OSA) is a sleep breathing disorder with unclear multifactorial pathogenesis. This study aimed to investigate the association between OSA and two human leukocyte antigens (HLA) alleles; DQB1*0602 and DRB1*15.

Methods: Forty patients with OSA and 40 control subjects were enrolled in the study. OSA diagnosis was made utilizing the Apnea-Hypopnea Index (AHI) ≥ 5 in overnight polysomnography (PSG). AHI was also used to determine OSA severity. Controls were randomly selected from healthy volunteers who had a low risk for OSA, utilizing the Berlin Questionnaire. Polymerase chain reaction (PCR) using Sequence Specific Primers (PCR-SSPs) was used to determine the association between HLA (HLA-DQB1*0602 and HLA-DRB1*15) and OSA, then statistical analyses of the results were performed.

Results: HLA-DQB1*0602 allele was found in 85% of all OSA patients and 50% of controls ($P < 0.001$). In patients with severe OSA, HLA-DQB1*0602 was present in the 92.9% compared with 66.7% in non-severe OSA ($P = 0.05$). HLA-DRB1*15 allele was found in 15% of OSA patients and 20% of controls, with no difference between the two groups ($P = 0.556$). No statistical difference was found in HLA-DRB1*15 between severe and non-severe OSA ($P = 0.499$). After adjusting for gender, HLA-DQB1*0602 allele was associated with increased odds of OSA (OR = 6.17, 95% CI 1.87-20.3, $p = 0.003$), but HLA-DRB1*15 allele was not associated with OSA (OR 0.45, 95% CI 0.12-1.73, $p = 0.242$).

Conclusions: The presence of HLA-DQB1*0602 allele, but not HLA-DRB1*15 allele, was significantly associated with OSA.

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Many medical conditions are increasingly recognized as having a genetic contribution to their susceptibility, pathogenesis, phenotypes, natural history and/or prognosis. Expanding knowledge of these genetic associations provides a better understanding of disorders, which may improve screening and diagnostic tools, impact preventive measures and therapeutic options [1,2].

A number of genetic factors partly control or influence several sleep traits, including circadian rhythm and sleep wake cycles, EEG patterns and response to sleep deprivation [2]. The impact of genes on many sleep disorders has also been identified [1,3-5]. Family clustering was observed in narcolepsy [6], which is a sleep disorder that is characterized by excessive daytime somnolence, sleep paralysis, hypnagogic hallucinations and cataplexy [7]. Narcolepsy has been reported to be associated with human leukocyte antigens (HLA) [5,8-11].

HLA are complex genetic regions on chromosome 6 that encode cell surface proteins. They are objective genetic markers that may be implicated directly in the pathogenesis of disease or associate disease susceptibility through genes within the major histocompatibility complex (MHC) [12]. Variations in the HLA regions are associated with variety of diseases and disorders that are related to the immune system [13].

Complex disorders that have polygenic or multifactorial pathogenesis are more difficult to study than disorders influenced by a single factor or gene [13]. Obstructive sleep apnea (OSA) is an example of a complex multifactorial disorder. It is a sleep breathing disorder that results from repetitive partial or complete cessation of airflow due to upper airway collapse during sleep, despite breathing effort. OSA is a major health problem, contributing to significant morbidity and mortality related to heightened risk of cardiovascular events and motor vehicle accidents [14-16].

The pathogenesis of OSA remains unclear and heritable components are among multiple diverse contributing mechanisms including immune system and inflammatory pathways [17-19]. Growing evidence suggests that genetic factors and their interaction with non-genetic factors influence the development of OSA [20,21]. The estimated genetic contribution to OSA pathogenesis is about 40%, while the rest is blamed on nongenetic factors [22]. Many interacting risk factors for OSA exist and are thought to be affected by genetic factors; including family history, gender, race, obesity and upper airway morphology [18,19,23,24,25]. Genetic factors were found to have a role in OSA development via pathways involved with ventilatory control, craniofacial anatomy, inflammation and immunity [26].

Several non-HLA genetic associations were identified in the pathogenesis of OSA [21, 22,26] and numerous candidate genes have been reported in different populations [20,22,27-30].

The association of HLA with increased susceptibility to diverse respiratory disorders has been described [31-38]. There is strong evidence suggesting that (HLA-DQB1*0602 allele) is related to narcolepsy [5, 8-11]. Hypocretin is a neurotransmitter that plays a role in the regulation of wakefulness and sleep [39]. It has been suggested that the autoimmune attack of DQ0602 (DQB1*0602 and DQA1*0102) on hypocretin-secreting neurons is responsible for narcolepsy [40]. Narcolepsy and OSA share the major symptom of excessive sleepiness and may, therefore, share genetic causative factors.

Rheumatoid arthritis is an inflammatory disorder that has been found to have high association with sleep disturbances and sleep apneas [41-43]. Some HLA types that are associated with rheumatoid arthritis (HLA-DRB1 alleles / HLA-DRB1*15 allele) may also play a role in OSA development [44].

The relation between HLA system and OSA is still a field for research. The current study aimed to investigate the association of two alleles with OSA in Northern Jordan: HLA-DQB1*0602, which is associated with narcolepsy; and HLA-DRB1*15, which is associated with rheumatoid arthritis.

Methods

The study was performed at Princess Haya Biotechnology Center at Jordan University of Science and Technology and the Pulmonary and Sleep Medicine Clinic at King Abdullah University Hospital, Jordan.

The study was approved by the Institutional Review Board at the Jordan University of Science and Technology. Written informed consent was obtained from the patients and healthy control subjects after counselling about the study.

Study subjects

This study included 40 patients with OSA (27.5% females and 72.5% males) and 40 control subjects (70.0% females and 30.0% males) in Northern Jordan. Study subjects were selected from OSA patients who agreed to participate in the study. Patients' ages ranged from 24–75 years. All patients presented initially to the Pulmonary and Sleep Medicine Clinic at King Abdullah University Hospital from 2014 -2015 with snoring, excessive daytime somnolence, witnessed apneas or other sleep apnea-related symptoms, and were diagnosed with OSA

utilizing overnight polysomnography (PSG), which remains the gold standard diagnostic test for OSA [45,46].

For the diagnosis and severity of OSA, PSG relies on a number of respiratory events (apneas and/or hypopneas) during sleep, and this is expressed as average number of events per hour of sleep or the Apnea-Hypopnea Index (AHI) [14,47,48]. These respiratory events have the same health consequences [15, 16]. In adults, an AHI of less than five events per hour is considered normal. Mild OSA is defined as AHI between five and 15 events per hour, moderate OSA is defined as AHI between 15 and 30 events per hour and severe OSA is defined as AHI greater than 30 events per hour [47,48].

Patients with central sleep apnea or others sleep disorders and patients with immune disorders, rheumatoid arthritis or similar chronic inflammatory diseases were excluded from this study. There were no patients with cardinal symptoms of narcolepsy who merited further investigation utilizing the diagnostic test for this disease; i.e., the multiple sleep latency test [7].

Controls were randomly selected from healthy volunteer subjects presenting for routine checkup. Controls, excluding the ones who are at moderate or high risk for OSA, underwent initial screening by the well-validated Berlin Questionnaire. Control subjects who had any known medical disorder, or had any symptom of OSA or other sleep related symptoms (including snoring, excessive daytime somnolence or fatigue and witnessed apneas), were also excluded. Neither patients nor controls received blood transfusion or bone marrow transplantation, to avoid false positive results that may occur from receiving blood from donors with positive target HLA alleles.

Study Design

Using the polymerase chain reaction-sequence-specific primers (PCR-SSPs) technique, we investigated the relationship between those two HLA alleles and OSA in North Jordanian

patients. Both alleles were studied in both OSA patients and controls. Blood samples were collected from both groups and DNA samples were studied. The workflow included sample collection, genomic DNA extraction, primer design and optimization, polymerase chain reaction PCR using Sequence Specific Primers (PCR-SSPs), gel electrophoresis and statistical analysis.

Venous blood samples (3 ml) were collected into EDTA tubes from 40 patients and from 40 control individuals. Genomic DNA was isolated from peripheral blood lymphocytes. Genomic DNA was extracted from blood samples by using a commercially available kit (Puregene Blood Kit; Qiagen, Germany).

SSP that bind HLA-DQB1*0602 were used according to the primer sequence [49]. Websites and online Primer 3 software were used to design the primers for HLA-DRB1*15 and for the first exon of HLA-DRB1 gene as an internal positive control for both alleles. The annealing temperature for PCR primers was determined (for the first exon of HLA-DRB1 gene as internal control, for HLA-DRB1*15 and HLA-DQB1*0602) by performing gradient PCR reaction using DNA samples.

PCR-SSPs technique was done using 2x PCR Master Mix Solution (i-MAX™ II) kit (iNtRON Biotechnology, Korea). PCR reactions were carried out using two pairs of primers to amplify two different regions in two different HLA loci (HLA-DRB1*15 and HLA-DQB1*0602), and one pair to amplify the internal positive control. The amplification protocol included initial denaturation at 94°C for 5 min and 30 cycles of 94°C for 30 s, 58°C for 45 s, and 72°C for 45 s and a final extension at 72°C for 5 min, using Veriti 96 well Thermal Cycler (Applied Biosystem, USA).

The PCR product was loaded into wells of 2.3% agarose gel (Bio Basic, Canada), separate ladder (100 bp) (Fermentas, Thermo Fisher Scientific, USA) was used to determine the PCR product size. Product size for PCR primers (annealing

TABLE 1. Product size for PCR primers

HLA gene/ allele	Forward Primer	Reverse Primer	Product size
DQB1*0602 allele	5'-CGTGCGTCTTGTGACCAGAT-3'	5'-GCTGTTCCAGTACTCGGCAT-3'	121 bp
First exon of HLA-DRB1 gene as internal control	5'-AGCATCTCTGACCAGCAACT-3'	5'-AGGCCCCCTTACACAAGTCTC-3'	501 bp
HLA-DRB1*15 allele	5'-TTTCCTGTGGCAGCCTAAGA-3'	5'-CTCTCCACAACCCCGTAGTT-3'	246 bp

HLA, human leukocyte antigen; bp, base pair.

Annealing temperature of 58°C and 30 cycles.

FIGURE 1. Separation of PCR products of (HLA-DQB1*0602) allele by 2.3% agarose gel. Lane M: 100 base pair ladder; lane -ve: negative control.

FIGURE 2. Separation of PCR products of (HLA-DRB1*15) allele by 2.3% agarose gel. Lane M: 100 base pair ladder; lane -ve: negative control.

temperature of 58 °C and 30 cycles) is shown in Table 1. Ethidium bromide (Bio Basic, Canada) was added (1.5 µl) to the buffer to stain the DNA for visualization under ultraviolet transilluminator provided with a gel documentation system (BioRad, USA). Separation of PCR products of the HLA-DQB1*0602 and HLA-DRB1*15 alleles by 2.3% agarose gel are shown in Figures 1 and 2.

Lanes 2 and 3 in Figure 1 indicated the presence of the DQB1*0602 allele with a 121 bp amplified product, shown by

a horizontal red arrow, and lanes 1, 4 and 5 indicated the absence of the DQB1*0602 allele. Lanes 2, 15, 16 and 17 in Figure 2 indicated the presence of DRB1*15 allele with 246 bp amplified product, shown by a horizontal red arrow, and lanes 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 indicated the absence of the DRB1*15 allele.

TABLE 2. Distribution of HLA-DQB1*0602 allele and HLA-DRB1*15 allele among patients with OSA and healthy controls

	Group				<i>P</i> -value
	Control subjects		Obstructive Sleep Apnea Syndrome		
Gender	n	%	n	%	<0.001
Female	28	70.0	11	27.5	
Male	12	30.0	29	72.5	
HLA-DQB1*0602 allele	20	50.0	34	85.0	<0.001
HLA-DRB1*15 allele	8	20.0	6	15.0	0.556

HLA, human leukocyte antigen

TABLE 3: HLA-DQB1*0602 and HLA-DRB1*15 alleles in relation to OSA severity

OSA Severity	# Patients	Presence of DQB1*0602 (# patients)	Presence of DRB1*15 (# patients)
Non-severe OSA (mild-moderate)	12	8/12 66.7%	3/12 25%
Severe OSA	28	26/28 92.9%	3/28 10.7%
Total	40	34/40 85%	6/40 15%
		$P = 0.050$	$P = 0.499$

HLA, human leukocyte antigen; OSA, obstructive sleep apnea

TABLE 4. Multivariate analysis of the association between both HLA-DQB1*0602 and HLA-DRB1*15 alleles and OSA

Variable	Odds ratio	95% confidence interval		P-value
		Lower limit	Upper limit	
Gender (male vs. females)	5.94	2.08	16.96	0.001
HLA-DRB1*15 allele (yes vs. no)	0.45	0.12	1.73	0.242
HLA-DQB1*0602 allele (yes vs. no)	6.17	1.87	20.30	0.003

HLA, human leukocyte antigen

Statistical analysis

Statistical analyses were performed using the IBM SPSS software (version 21). Chi-squared test was used to determine the association of HLA-DQB1*0602 and HLA-DRB1*15 alleles with OSA patients (and control subjects). Multivariate analysis was used to determine the association between OSA and HLA-DQB1*0602 and HLA-DRB1*15 alleles to adjust for the confounding factors. Odds ratio and their 95%

confidence intervals were reported. A P -value of < 0.05 was considered statistically significant.

Results

PCR results from the 40 OSA patients showed that the HLA-DQB1*0602 allele was found in 85% of patients and 50% of controls ($P < 0.001$), while the HLA-DRB1*15 allele was found in 15% of patients and 20% of controls. No

difference was seen between the two groups ($P=0.556$) (Table 2).

Four patients (10%) were positive for both alleles and six patients (15%) had either of the alleles. Six control subjects (15%) were positive for both alleles and 18 subjects (45%) had neither of the alleles. All patients who were negative for the HLA-DQB1*0602 allele were also negative for HLA-DRB1*15 allele.

Severe OSA was present in 70% of the patients, while mild to moderate OSA was present in 30% of the patients. HLA-DQB1*0602 was present in the 92.9% of patients with severe OSA compared with 66.7% in non-severe OSA ($P=0.05$). In contrast, the presence of HLA-DRB1*15 was not different between patients with severe and non-severe OSA ($P=0.499$) (Table 3).

Table 4 shows the multivariate analysis of the association between both HLA-DQB1*0602 and HLA-DRB1*15 alleles and OSA after adjusting for gender. The presence of HLA-DQB1*0602 allele was associated with increased odds of OSA (OR = 6.17, CI 1.87-20.3), $P=0.003$, but HLA-DRB1*15 allele was not associated with OSA (OR 0.45, CI 0.12-1.73), $P=0.242$.

Discussion

In this study we investigated the association of two HLA alleles with the presence of OSA. The major findings are as follows: 1) most of OSA patients had HLA-DQB1*0602 allele; 2) a stronger association was noticed in severe OSA cases, suggesting a possible contribution to its severity; and 3) there was no association between HLA-DRB1*15 and OSA.

Most of OSA patients in this study (85%) had the HLA-DQB1*0602 allele. The probability of having OSA in the presence of HLA-DQB1*0602 is almost six times higher than in the absence of HLA-DQB1*0602. OSA was severe in 70 % of patients and HLA-DQB1*0602 was present in 92.9 % of these patients, which indicates stronger association of HLA-DQB1*0602 with OSA and predisposition to greater severity. Having some OSA patients who were negative for HLA-DQB1*0602 (15%), even with severe disease in some of these patients, indicated that factors other than HLA-DQB1*0602 (genetic and non-genetic) were also involved in OSA development. PCR results in this study showed that 50% of the controls had the HLA-DQB1*0602 allele. In the Jordanian population, the frequency of DQB1*0602 was found to be 0.1069 (11%) according to Sánchez-Velasco et al. [50], who used 100 blood samples collected from adults of both sexes in Amman and an additional 46 samples collected in the Jordan Valley (Dead

Sea) to detect the frequency of HLA alleles. This frequency is lower than what we found in our study. This difference may be attributed to multiple factors, including the difference in the geographical area and date of the study as well as the sample size and the technique used in the study. Manzotte et al. [51] found that HLA-DQB1*0602 allele was associated with electroencephalographic differences in individuals with OSAS (higher theta power during sleep Stage 1, and lower delta power during sleep Stages 1 and 2) suggesting that HLA-DQB1*0602 is a potential genetic factor influencing sleep physiology in individuals diagnosed with OSA.

There was no statistical difference in the frequency of HLA-DRB1*15 in patients (15%) and controls (20%) ($P=0.556$), suggesting both its normal distribution among the north Jordanian population and the lack of an association between this HLA allele and OSA. Other studies have shown no association between HLA-DRB1*15 and OSA patients. Brunetti et al. [52] investigated the influence of HLA antigens and OSAS in childhood; there was no significant difference in frequency of HLA-DRB1*15 between OSA patients and the controls, but HLA-B65 expression was found to be significantly higher in children with sleep disordered breathing as compared with controls (10.5% vs. 3.61; post-Bonferroni correction (Pypc) < 0.04). Similarly, Lee et al. [53] reported an analysis of HLA in Korean patients with OSA that revealed no association between HLA-DRB1*15 and OSA, however, an association was shown between OSA and the HLA-A11 and DRB1*09 alleles and between OSA severity and HLA-DRB1*08 allele. Other diverse alleles in the HLA system have been linked to OSA. Yoshizawa et al. [54] studied HLA-A, B, C and DR antigens in Japanese patients with OSAS. They reported a markedly increased frequency of HLA-A2 antigen in the patients with OSAS compared with normal controls (81.3% vs 40.6%). They also reported that HLA-B39 was found more frequently in patients with OSAS but not in the normal controls. No significant deviation was observed in the frequencies of HLA-C and DR antigens between the OSAS patients and the controls. This study, however, did not use the standard definitions of apneas, hypopnea and AHI. No significant deviation was observed in the frequencies of HLA-C and DR antigens between the OSAS patients and the controls. This study, however, did not use the standard definitions of apneas, hypopnea and AHI.

HLA-A33, HLA-DRB1*03, DQB1*02 were implicated with OSA in a study that implicated also HLA-B7, B65, B63, B73 with primary snoring [55].

Our study was limited by the absence of polysomnographic data for healthy controls, which could not

be done due to limited resources and the difficulty in convincing low-risk patients to undergo polysomnography without a clear indication if needed. Another limitation was the small sample size, although statistical significance was achieved for the study population.

In view of our results, future research is needed to look into excessive sleepiness and find out whether this is a trait that HLA-DQB1*0602 modifies in patients with OSA and other sleep disorders associated with excessive sleepiness, and to investigate it as an early step in the pathogenesis of those disorders, among other underlying mechanisms, rather than merely as a resulting symptom.

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