Original article:
Human Leukocyte Antigen Genetic Variations in Obstructive Sleep Apnea Patients that were positive for HLA-DQB1*0602.

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Abstract:
Background: Genetic variations in the HLA system may cause susceptibility to a large number of autoimmune and infectious diseases, and the complexity of HLA makes it hard to investigate HLA types associated with diseases. The association between HLA and Obstructive Sleep Apnea (OSA) is not well investigated due to the complexity of OSA pathogenesis; including genetic and non-genetic in different populations. Our previous study using PCR-SSPs technique showed that HLA-DQB1*0602 allele is associated with almost 6 times increase in risk in North Jordan OSA patients. Aim: The aim of this study was to see if there are any HLA genetic variations using DNA sequencing technique in the region in which HLA-DQB1*0602 is located that might interact with HLA-DQB1*0602 and affect OSA development in OSA patients who were positive for HLA-DQB1*0602 allele. Result: The DNA sequencing results showed 8 nucleotide substitution variations, which are p.G77E (c.230G>A), p.R80R (c.240C>G), p.Q85L (c.254A>T), p.R87P (c.260G>C), p.Y69D (c.205T>G), p.A70V (c.209C>T), p.A70A (c.210G>A), p.Y79Y (c.237C>T). Although only 5 variants resulted in amino acid change, all 8 variants were included in the statistical analysis, and none of these genetic variants was significant (p-value > 0.05). Additionally, eight haplotypes were detected. Some of these haplotypes might have a role in disease development through the interaction with HLA-DQB1*0602 and other genetic variants or could be as markers for OSA by the mechanism of linkage disequilibrium. Conclusion: Further studies are needed to explain the pathogenesis of OSA in terms of possible self or non-self-antigens involved.

Keywords: HLA (Human Leukocyte Antigen), OSA (Obstructive Sleep Apnea), SNPs (Single Nucleotide Polymorphisms), Polymerase Chain Reaction Sequence Specific Primers (PCR-SSPs)

Introduction
Obstructive Sleep Apnea (OSA) is a common sleep disorder that results from partial or complete collapse of the upper airway of the respiratory system during sleep1. The pathogenesis of OSA is not well understood and several factors involved in disease development include genetic and non-genetics components. Several genes in different pathways in human body can interfere with OSA development2. In the studies that investigated the genetic association with OSA, the Mendelian patterns of inheritance were not observed

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in most of OSA cases suggesting a multi-gene pathogenesis involved in the disease heritability. The impact of genes on the pathogenesis of OSA has been found in numerous studies which established that OSA aggregates within families. In children with OSA, over 43% of them in one study had at least one relative with OSA symptoms. The patients who have one affected relative are approximately 50% more likely to have OSA themselves. OSA and obesity share a substantial genetic basis; on the other hand, there is a potential importance of genetic factors unrelated to obesity that may determine OSA in those genes influencing susceptibility to OSA may therefore, can be related to OSA age, sex and total body fat adjusted variance, and make it hard to investigate HLA types related to OSA.

The impact of genes on the pathogenesis of OSA has been found in numerous studies which established that OSA aggregates within families. In children with OSA, over 43% of them in one study had at least one relative with OSA symptoms. The patients who have one affected relative are approximately 50% more likely to have OSA themselves. OSA and obesity share a substantial genetic basis; on the other hand, there is a potential importance of genetic factors unrelated to obesity that may determine OSA susceptibility. The heritability component the level of upper body fat relative to lower body fat ranges from approximately 30% to 50% of the phenotype’s age, sex and total body fat adjusted variance, therefore, can be related to OSA. OSA was reported in members of the same and different generations and in obese and non-obese family members suggesting an inherited syndrome.

Different studies found significant associations between different genes and OSA, including ADAM29, FLRT2, and SLC18A3. C-reactive protein and IL-6 genes with OSA, APOE epsilon 4 allele and OSA, tumor necrosis factor-α (−308) gene polymorphism which was significantly associated with OSA.

The Human Leukocyte Antigen (HLA) forms a 7.6 Mb region on chromosome 6p21, and it is considered the most gene dense and complex region within the human genome encoding 252 expressed loci. There are two primary classes of HLA molecules which are class I and class II, with differences in their structure and in their function. Some HLA alleles can make resistance to some diseases, but on the other hand some HLA alleles especially HLA class II alleles can make susceptibility to several diseases.

There is strong evidence suggesting that a specific HLA allele is related to a sleep disorder called Narcolepsy that shared with OSA the major symptoms of excessive day time sleepiness, this HLA allele is associated with OSA. The HLA-DQB1*0602 allele was also found to be associated with OSA. Other HLA alleles were also found to be associated with OSA such as HLA-A11 and HLA-DRB1*09 alleles, and HLA-A2. The combination of non-genetic and genetic factors that interfere with disease development can be present and make it hard to investigate HLA types related to diseases.

The studies that have been done on diseases like OSA focused on specific genes or alleles; however, variants in those genes influencing susceptibility to OSA may also exist. Association of single gene polymorphisms need to be supported by other studies focusing on the gene expression that is influenced by those SNPs to identify their functional consequences.

The haplotype is defined by a series of single nucleotide polymorphisms that are found at a linked loci on the same chromosome which there is a high linkage disequilibrium and can vary in there frequencies from one population to another. The analysis of haplotypes in genetics studies of human diseases became more important since many associations have been detected between many haplotypes and diseases, but to investigate the association between HLA system and OSA it is reasonable that studying those diseases that are known to be associated with HLA and either share major manifestation of OSA or have increased association with OSA.

The aim of this study is to see if there are any HLA variations using DNA sequencing technique in the region where HLA-DQB1*0602 allele is located that might interfere with HLA-DQB1*0602 as gene-gene interaction and affect OSA development in OSA patients who were positive for HLA-DQB1*0602 allele that was detected in our previous study using polymerase chain reaction sequence specific primers (PCR-SSPs) technique which showed that HLA-DQB1*0602 allele is associated with OSA.

Materials and Methods

Ethical clearance:

This study has been approved by the Institutional Research Board (IRB) at Jordan University of Science and Technology and was performed at Princess Haya Biotechnology Center and the Sleep Medicine Clinic at King Abdullah University Hospital, Jordan. Both patients and healthy control subjects signed a written informed consent after counseling about the study. Forty North Jordanian patients diagnosed with OSA by overnight polysomnograms (29 males and 11 females); age range (24–75 years). According to their AHI, 70% of patients had severe OSA, 15% had moderate, and 5% had mild OSA. Forty North Jordanian healthy subjects (28 females and 12 males) were randomly selected as controls after screening for OSA risk utilizing Berlin questionnaire and excluding those at moderate or high risk.

Peripheral blood samples from both OSA patients and controls were collected into 3ml-EDTA tubes (a total of 80 samples, a 3 ml each). Genomic DNA was isolated from venous blood lymphocytes by using a commercially available kit (Puregene Blood Kit; Qiagen, Germany), the polymerase chain reaction was carried out using 2x PCR Master mix Solution.
was considered statistically significant and logistic regression for odd ratio and confident interval.

Results
A total of 8 variations were identified in OSA patients and controls that were positive for HLA-DQB1*0602. All these variations were found in the third exon of DQB1 gene in which HLA-DQB1*0602 allele is located. All these variations are nucleotide substitution presented as homozygotes and some are heterozygotes, and three of these variations are synonymous that do not change the amino acid (Table 1).

All the variations were not significantly different between case and controls. We did the statistical analysis in 2 ways (all 8 variants, and only the 5 variants resulting in amino acid change) and both are included. All variants might be significant because of linkage disequilibrium with other genetic variants (Table 2). Some of these variations that were detected in this study forms haplotypes, eight haplotypes were detected (Table 3).

Discussion
This study is the first genetic analysis study that investigate and detect the variations using Sanger sequencing technique by direct sequencing the region in which HLA-DQB1*0602 allele is located with North Jordanian OSA patients who are positive for HLA-DQB1*0602 allele.

The multifactorial diseases such as OSA are the biggest challenge facing medical molecular genetics due to the diverse mechanisms that may involve in disease etiology, so it is very important to know the genetic determinants that predispose to the disease as well as the non-genetic factors to explore the possibility of prevention or early intervention. Involvement of genetic abnormalities in the pathogenesis of OSA was demonstrated in many non-HLA genetic associations, however, the relation between HLA system and OSA was not adequately studied. The severity of the disease can vary between patients even if the cases were sporadic; this indicates that the disease risk depends on a complex interaction between several genetic variations and non-genetic factors.

Eight genetic variations were identified by sequencing technique, the variations are p.G77E (c.230G>A), p.R80R (c.240C>G), p.Q85L (c.254A>T), p.R87P (c.260G>C), p.Y69D (c.205T>G), p.A70V (c.209C>T), p.A70A (c.210G>A), p.Y79Y (c.237C>T), all of them are reported in ensemble database (http://www.ensembl.org), and none of them was significant (p-value > 0.05). As the explanation for odds ratio showed that none of the variations has...
Table 1: DNA variations in OSA patients and controls that were positive for HLA-DQB1*0602 allele.

<table>
<thead>
<tr>
<th>Type of variation</th>
<th>Amino acid change</th>
<th>Variant Nomenclature</th>
<th>Codon</th>
<th>Position</th>
<th>HLA-DQB1*0602</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missense variant</td>
<td>G/E p.G77E</td>
<td>c.230G&gt;A</td>
<td>gGg/gAg</td>
<td>6:32664947</td>
<td>Variation number 1 (rs1049083)</td>
</tr>
<tr>
<td>Synonymous variant</td>
<td>R/R p.R80R</td>
<td>c.240C&gt;G</td>
<td>cgC/cgG</td>
<td>6:32664937</td>
<td>Variation number 2 (rs3210148)</td>
</tr>
<tr>
<td>Missense variant</td>
<td>Q/L p.Q85L</td>
<td>c.254A&gt;T</td>
<td>cAg/cTg</td>
<td>6:32664923</td>
<td>Variation number 3 (rs1140313)</td>
</tr>
<tr>
<td>Missense variant</td>
<td>R/P p.R87P</td>
<td>c.260G&gt;C</td>
<td>cGg/cGg</td>
<td>6:32664917</td>
<td>Variation number 4 (rs1130380)</td>
</tr>
<tr>
<td>Missense variant</td>
<td>Y/D p.Y69D</td>
<td>c.205T&gt;G</td>
<td>Tac/Gac</td>
<td>6:32664972</td>
<td>Variation number 5 (rs1130370)</td>
</tr>
<tr>
<td>Missense variant</td>
<td>A/V p.A70V</td>
<td>c.209C&gt;T</td>
<td>gCg/gTg</td>
<td>6:32664968</td>
<td>Variation number 6 (rs1063318)</td>
</tr>
<tr>
<td>Synonymous variant</td>
<td>A/A p.A70A</td>
<td>c.210G&gt;A</td>
<td>gcG/gcA</td>
<td>6:32664967</td>
<td>Variation number 7 (rs1049082)</td>
</tr>
<tr>
<td>Synonymous variant</td>
<td>Y/Y p.Y79Y</td>
<td>c.237C&gt;T</td>
<td>taC/taT</td>
<td>6:32664940</td>
<td>Variation number 8 (rs1049073)</td>
</tr>
</tbody>
</table>

Table 2: Differences in the variations between cases and controls.

<table>
<thead>
<tr>
<th>Variation Number</th>
<th>Control N=20</th>
<th>Patient N=34</th>
<th>OR</th>
<th>CI (95%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variation number 1 (rs1049083)</td>
<td>7 35</td>
<td>7 20.6</td>
<td>0.481</td>
<td>0.139-1.662</td>
<td>0.243</td>
</tr>
<tr>
<td>Variation number 2 (rs3210148)</td>
<td>8 40</td>
<td>11 32.4</td>
<td>0.717</td>
<td>0.228-2.260</td>
<td>0.57</td>
</tr>
<tr>
<td>Variation number 3 (rs1140313)</td>
<td>8 40</td>
<td>8 23.5</td>
<td>0.462</td>
<td>0.140-1.525</td>
<td>0.201</td>
</tr>
<tr>
<td>Variation number 4 (rs1130380)</td>
<td>7 35</td>
<td>8 23.5</td>
<td>0.389</td>
<td>0.115-1.319</td>
<td>0.124</td>
</tr>
<tr>
<td>Variation number 5 (rs1130370)</td>
<td>3 15</td>
<td>4 11.8</td>
<td>0.756</td>
<td>0.151-3.783</td>
<td>0.733</td>
</tr>
<tr>
<td>Variation number 6 (rs1063318)</td>
<td>3 15</td>
<td>3 8.8</td>
<td>0.548</td>
<td>0.100-3.020</td>
<td>0.486</td>
</tr>
<tr>
<td>Variation number 7 (rs1049082)</td>
<td>7 35</td>
<td>7 20.6</td>
<td>0.481</td>
<td>0.139-1.662</td>
<td>0.243</td>
</tr>
<tr>
<td>Variation number 8 (rs1049073)</td>
<td>2 10</td>
<td>2 5.9</td>
<td>0.563</td>
<td>0.073-4.340</td>
<td>0.577</td>
</tr>
</tbody>
</table>
Table 3: Haplotypes in OSA patients and controls that were positive for HLA-DQB1*0602.

<table>
<thead>
<tr>
<th>Haplotype frequency in patients and in controls</th>
<th>Variations in the haplotype</th>
<th>Haplotype number</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 controls 6 patients</td>
<td>c.230G&gt;A, c.240C&gt;G, c.254A&gt;T, c.260G&gt;C</td>
<td>1)</td>
</tr>
<tr>
<td>7 controls 6 patients</td>
<td>c.240C&gt;G, c.254A&gt;T, c.260G&gt;C</td>
<td>2)</td>
</tr>
<tr>
<td>1 patient</td>
<td>c.240C&gt;G, c.205T&gt;G, c.209C&gt;T</td>
<td>5)</td>
</tr>
<tr>
<td>1 patient</td>
<td>c.240C&gt;G, c.210G&gt;A, c.237C&gt;T</td>
<td>6)</td>
</tr>
<tr>
<td>1 patient</td>
<td>c.240C&gt;G, c.205T&gt;G, c.210G&gt;A</td>
<td>7)</td>
</tr>
<tr>
<td>1 patient</td>
<td>c.205T&gt;G, c.209C&gt;T, c.254A&gt;T, c.260G&gt;C</td>
<td>8)</td>
</tr>
</tbody>
</table>

Some of these haplotypes might have a role in disease development and progression through the interaction with HLA-DQB1*0602. Since HLA-DQB1*0602 was found to be associated with OSA [19, 20], and the attack of HLA-DQB1*0602 and HLA-DQA1*0102 on hypocretin-secreting neurons in narcolepsy [30]; this suggesting that the five variations that change the amino acids might interaction with HLA-DQB1*0602 and HLA-DQA1*0102 to attack on hypocretin-secreting neurons. Still this needs to be more investigated because there could be other variants in different genes including HLA genes combine with the 8 variants in this study by the mechanism of linkage disequilibrium and form more specific haplotypes that might interfere with OSA development, or work as markers for OSA.

**Conclusion**

Eight HLA genetic variants were detected in this study by Sanger sequencing in the region in which HLA-DQB1*0602 is located, and some of these variations might have a role in OSA development. Financial Support.

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**Conflict of interest** None

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Data gathering: All authors.

Writing and submitting manuscript: All authors.

**Editing and approval of final draft:** Thamer A. Al-Qatarneh, Ammar K. Daoud
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