The CHRNA5 polymorphism (rs16969968) and its association with waterpipe smoking addiction among Jordanians

Thaka’a K. Al-Omousha, Karem H. Alzoubib, Omar F. Khabour, Fawzi M. Alsheyaba, Ahmed Abu-siniyeh, Fadi A. Mayyas, Caroline O. Cobb, and Thomas Eissenberg

Department of Clinical Pharmacy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan; Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, Jordan University of Science and Technology, Irbid, Jordan; Department of Medical Laboratories, Faculty of Health Sciences, American University of Madaba, Madaba, Jordan; Department of Psychology, Virginia Commonwealth University, Richmond, VA, USA; Center for the Study of Tobacco Products, Virginia Commonwealth University, Richmond, VA, USA

ABSTRACT
Waterpipe smoking is a form of tobacco use that causes nicotine/tobacco dependence and has become a global health problem. In this study, the association of rs16969968 SNP in the CHRNA5 gene with waterpipe dependence was investigated. A total of 386 men and women who used a waterpipe to smoke tobacco were recruited and divided into less dependent and more dependent smokers based on their score on the Lebanon Waterpipe Dependence Scale (LWDS). Results showed a significant difference in the distribution of GG, GA, and AA genotypes by waterpipe dependence status (p < .001). The more dependent group showed a higher frequency of the AA genotype than the less dependent smokers’ group (38% vs. 23%, respectively). In addition, the more dependent smokers exhibited more A allele than less dependent smokers (53% vs. 37% respectively, p < .001). In conclusion, there is an association between the rs16969968 SNP and waterpipe dependence as assessed by the LWDS.

Introduction
Waterpipe smoking is a method of tobacco consumption that is prevalent in every world region (Logo et al., 2020; Mostafa, 2020; Nasser, Geng, & Al-Wesabi, 2020). In some countries, the rate of waterpipe use has surpassed that of cigarettes (Jawad et al., 2018). Waterpipe smoking is associated with several health problems (Reyes-Caballero et al., 2020; Sullman, Gras, Kagialis, Papa georgi, & Font-Mayolas, 2020), including cardiovascular diseases (Alomari, Khabour, Alzoubi, & Eissenberg, 2020; Nemmar et al., 2020), respiratory diseases (Kudhair, Alabid, Zayed, Lafta, & Taheri-Kaf rani, 2020; Nemmar, Al-Salam, Beegam, Yuvaraju, & Ali, 2019), and cancer (Kudhair et al., 2020; Sabi et al., 2020).

Similar to cigarettes, waterpipe tobacco smoke is rich in several types of toxic compounds derived from tobacco, charcoal, flavors, sugar, and other additives. Examples of such compounds are nicotine/cotinine that is associated with tobacco dependence, cyclic hydrocarbons that cause cancer, volatile aldehydes that induce lung diseases, and CO that predisposes to heart disease (Kaplan et al., 2019; Leavens et al., 2020). Studies have shown that waterpipe-induced exposure to nicotine has clear detrimental physiological effects in the body including dependence as assessed by the Lebanon Waterpipe Dependence Scale (LWDS) (Bahelah et al., 2019; El Hourani et al., 2019; Felu et al., 2020).

The CHRNA5/A3/B4 is a gene cluster on chromosome 5 that encodes the α5, α3, and β4 of the nicotinic acetylcholine receptor subunits (nAChR) (Duga et al., 2001; Eng, Kozak, Beaudet, & Zoghbi, 1991). Genome-wide association studies have shown strong associations between tobacco smoking dependence and genetic variations in the CHRNA5/A3/B4 gene cluster (Saccone et al., 2007; Wen, Yang, Cui, & Li, 2016). Similarly, candidate gene association studies have confirmed this strong association (Lee, Ahn, Seweryn, & Sadee, 2018; Liu et al., 2018). One single nucleotide polymorphisms (SNP) that showed strong relation to tobacco smoking behavior-dependence? is the rs16969968 SNP in exon 5 of CHRNA5 (Hubacek et al., 2019; Silva et al., 2019). This SNP replaces an aspartate into asparagine at nucleotide

CONTACT Karem Alzoubi khalzoubi@just.edu.jo Department of Clinical Pharmacy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid 22110, Jordan

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398 of the α5 subunit. It has been shown that α5nAChRs, which are located in the habenulo-interpeduncular nucleus, play a role in the nicotine self-administration rewarding mechanism in animal models. Interference with the α5nAChR pathway has been shown to prevent the aversive effects of nicotine on the mHb-IPN pathway (Antolin-Fontes, Ables, Görlich, & Ibáñez-Tallon, 2015; Besson et al., 2016).

Studies examining genetic factors that contribute to waterpipe tobacco smoking dependence are lacking and are needed to inform waterpipe tobacco smoking cessation interventions. Therefore, the aim of the present investigation was to examine the association between rs16969968 SNP of the CHRNA5 gene and waterpipe tobacco smoking dependence.

Method

Participants and procedure
A total of 386 waterpipe smokers recruited from waterpipe cafés, public gardens, and parks, and via social media completed this study. The objectives of the study were explained to the participants, and their consent to participate was obtained before data collection. Participants (>18 years old) who smoke waterpipe for at least the past 12 months were eligible to participate in the study. Waterpipe smokers were first screened by asking them about type of other tobacco products that have used. Individuals who reported using other types of tobacco (dual smoker, about 47.3% of the invited subjects: 347 out of 733) were excluded from the study. Participants’ demographics, tobacco use behavior, and dependence characteristics were collected using a structured questionnaire. Blood samples for genomic DNA analysis were obtained using EDTA tubes in specialized medical laboratories. The study procedures were permitted by the Research Ethics Committee of King Abdullah University Hospital.

Waterpipe dependence score

The waterpipe dependence score was obtained using the Lebanon Waterpipe Dependence Scale-10J (LWDS-10J) as previously described (Alzoubi et al., 2013; Primack et al., 2014; Khabour, Abu-Eitah, Alzoubi, Abu-Siniyeh, & Eissenberg, 2020). Smokers were divided into less dependent and more dependent smokers based on their LWDS score (≥15 and <15, respectively).

Isolation of genomic DNA

DNA was isolated using DNA extraction kits (Promega, Madison, WI, USA) as previously described (Alzoubi et al., 2013; Khabour et al., 2020; Sabi et al., 2020). After extraction, the quality and quantity of the isolated DNA were assayed using Bio-Rad SmartSpect_3000 device (Hertfordshire, UK). DNA samples were stored at 20°C until used for genotyping analysis.

Genotyping of rs16969968 SNP

The rs16969968 SNP was genotyped using PCR and Restriction Fragment Length Polymorphism technique. The DNA fragment containing the SNP was amplified by PCR using F: 5'-CGC CTT TGG TCC GCA AGA TA-3' and R: 5'-TGC TGA TGG GGG AAG TGG AG-3' primers (Stevens et al., 2008). The reaction volume was 25 μl containing 2X PCR master mix (Promega, Madison, WI, USA), 1 μl of each primer, 5 ng of genomic DNA, and nuclease-free water. The reaction mix was subjected to 5 min of 94°C, then 35 cycles of heating at 94°C for 35 s, annealing at 58°C for 35 s and extension at 72°C for 1 min, and a final extension at 72°C for 7 min using thermal cycler. Successful amplification was indicated by the presence of 435-bp fragment on 2.5% agarose gel. Digestion with Taq1 restriction enzyme (from Fermentas, Germany) was done by incubating 10 μl of PCR product with 2 units of Taq1 enzyme at 60°C for 4 h and then visualized as indicated above.

Statistical analysis

The SPSS software was used for statistical analysis. Parameters were presented as mean ± SD or frequencies as appropriate. Data were examined among the overall sample and by gender using the chi-squared test or Student’s t-test as appropriate. The sample calculation was performed via G. Power software (Fritz Faul, Kiel, Germany). P < .05 was considered statistically significant.

Results

The total sample (N = 386) was divided into two groups (less dependent smokers, 51.0% vs. more dependent smokers, 49.0%) according to their LWDS dependence score (Table 1). The mean age in the less dependent smokers’ group was 34.1 years, whereas it was 38.5 in the more dependent smokers’ group (P > .05). The percentage of males in the less dependent smokers’ group was 61.9% versus 69.8% in the more dependent smokers’ group. The mean LWDS was significantly different between the two groups (Table 1).

| Variable               | Less dependent smokers (n = 197) | More dependent smokers (n = 189) |
|------------------------|---------------------------------|-------------------------------|
| Age (mean ± SD)        | 34.1 ± 11.6                     | 38.5 ± 9.5                    |
| Age range              | 19–68                           | 19–70                         |
| Gender (females)       | 61.9                            | 69.8                          |
| Average dependence score | 10.3 ± 3.2                     | 21.6 ± 3.4                    |

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groups (10.3 in less dependent vs. 21.5 in the more dependent, \( p < .001 \)).

Genotyping of rs16969968 in the CHRNA5 gene is shown in Table 2. There was a statistically significant difference in the distribution of GG, GA, and AA genotypes among waterpipe smoking groups (\( p < .001 \)). The frequency of AA genotype was higher in the more dependent smokers group compared to the less dependent smokers’ group (38% vs. 23%, respectively). In addition, the A allele was more enriched among the more dependent smokers’ group than the less dependent smokers’ group (53% vs. 37%, respectively, \( p < .001 \)).

When the gender of the participants was considered, the rs16969968 A allele was associated with more dependent smokers in both males and females (Table 3, \( p < .01 \)). However, the association of the AA genotype was only significantly associated with males. Thus, rs16969968 SNP seems to be associated with more dependence on waterpipe smoking.

Table 2. The rs16969968 genotype and allele frequencies among waterpipe smokers.

| Genotype/allele | Less dependent smokers (\( N = 197 \)) | More dependent smokers (\( N = 189 \)) | Odds ratio (95% CI) | \( p \) value |
|----------------|----------------------------------------|---------------------------------------|---------------------|----------------|
| Genotype       |                                        |                                       |                     |                |
| GG            | 98(50)                                 | 60(32)                                | 1.00                | <.001          |
| GA            | 54(27)                                 | 57(30)                                | 0.58 (0.35–0.95)    |                |
| AA            | 45(23)                                 | 72(38)                                | 0.38 (0.23–0.63)    |                |
| Allele        |                                        |                                       |                     |                |
| Allele G      | 250(63.0)                              | 177(47.0)                             |                     |                |
| Allele A      | 144(37.0)                              | 201(53.0)                             |                     |                |

Table 3. rs16969968 genotype and allele frequencies among waterpipe smokers according to gender.

| Genotype/allele | Less dependent smokers (\( N = 197 \)) | More dependent smokers (\( N = 189 \)) | Odds ratio (95% CI) | \( p \) value |
|----------------|----------------------------------------|---------------------------------------|---------------------|----------------|
| Males          |                                        |                                       |                     |                |
| GG            | 60(49)                                 | 41(31)                                | 1.00                | <.01          |
| GA            | 34(28)                                 | 40(30)                                | 0.58 (0.32–1.06)    |                |
| AA            | 28(23)                                 | 51(39)                                | 0.38 (0.20–0.69)    |                |
| Allele G      | 154(63.0)                              | 122(46)                               |                     | <.001          |
| Allele A      | 90(37.0)                               | 142(54)                               |                     |                |
| Females       |                                        |                                       |                     |                |
| GG            | 38(51)                                 | 19(33)                                | 1.00                | .097         |
| GA            | 20(27)                                 | 17(30)                                | 0.59 (0.25–1.38)    |                |
| AA            | 17(23)                                 | 21(37)                                | 0.40 (0.17–0.94)    |                |
| Allele G      | 96 (64)                                | 55(48)                                |                     | .021         |
| Allele A      | 54(36)                                 | 59(52)                                |                     |                |

Discussion

Nicotine dependence maintains tobacco-smoking behavior that causes increased morbidity and mortality among cigarette smokers. Different genetic variations have been found to impact nicotine dependence among human populations (Saccone et al., 2007). In recent years, waterpipe use has dramatically increased at the global level (Jawad et al., 2018; Qasim et al., 2019). The present findings showed a strong association between the rs16969968 SNP of the CHRNA5 gene with waterpipe tobacco dependence.

Previous studies suggested that the CHRNA5-A3-B4 gene cluster has a strong association with various smoking-related phenotypes including nicotine dependence and cigarette smoking heaviness (Lassi et al., 2016). The rs16969968 in CHRNA5 was correlated with nicotine dependence among cigarette smokers (Ware, van den Bree, & Munafò, 2011). A case-control study showed that genetic variations in CHRNA5 may boost the development of nicotine dependence in Caucasian and African-American populations (Sherva et al., 2008). Further, a genome-wide association study showed several haplotypes in CHRNA5 and CHRNA3 genes that were correlated with nicotine dependence among European Americans (Li, Bao, Xu, Bao, & Zhang, 2012). The association of SNPs (rs16969968), in heterozygous and homozygous patterns, in CHRNA5 with nicotine dependence and risk to develop lung malignancies was revealed in the Indian population (Pandey et al., 2017). Finally, an association was found between rs16969968 SNP and the smoking phenotype in the European population but not in African populations. Thus, genetic variants in the CHRNA5-A3-B4 gene cluster may be linked to the smoking phenotype in some but not all individuals.

In the present investigation, the AA genotype of rs16969968 SNP was enriched among more dependent waterpipe smokers’ group but not the less dependent smokers’ group. When gender was considered, the AA genotype was associated with more waterpipe tobacco dependence among men but not women. In cigarette smoking, a case-control study that was focused on women who smoke tobacco cigarettes, with heavy- and light-smoking phenotypes, showed that the AA genotype of CHRNA5 rs16969968 was associated with heavy smoking (Conlon & Bewick, 2011). Thus, gender might influence genetic contribution to tobacco use behaviors.
It is worth to mention that the frequency of the G allele of the rs16969968 in the Jordanian population is higher than that of A allele. Similar distribution of rs16969968 alleles in the Palestinian and Algerian Arab populations was reported (Ayesh, Al-Masri, & Abed, 2018; Mimouni et al., 2020). The observed trend in the distribution of rs16969968 alleles is similar to that reported in other world populations (Aroche et al., 2020; Hubacek et al., 2019; Silva et al., 2019).

Different factors may influence the tobacco-smoking phenotype including genetic, environmental factors, and ethnicity. For example, some CHRNA5-A3-B4 gene cluster variants may increase the progression of nicotine dependence among European populations but are infrequent in African, American, and Asian populations. Thus, tobacco smoking behavior and its association with genetic variants should be examined in different populations to enhance smoking cessation intervention efforts.

Among the limitations of this study is that pure waterpipe smokers are not abundant. Therefore, it was very hard to recruit a large sample size. Future studies that include larger samples are needed to confirm the present findings. In addition, waterpipe dependence was measured using LWDS. This scale items were reported by to be correlated adequately with measurements of nicotine metabolites, exhaled carbon monoxide levels, and the frequency of waterpipe smoking (Primack et al., 2014; Salameh, Waked, & Aoun, 2008). In a recent study, a new scale (named the Syrian Center for Tobacco Studies [SCTS]-13), for nicotine dependence associated with waterpipe smoking was published (Alam et al., 2020). Confirming the present findings using the SCTS-13 scale is strongly recommended.

Conclusion
The current data confirmed that rs16969968 SNP located in the nAChR gene cluster CHRNA5-A3-B4 is strongly associated with waterpipe smoking dependence among exclusive waterpipe tobacco smokers.

Disclosure statement
Dr. Eissenberg is a paid consultant in litigation against the tobacco industry and also the electronic cigarette industry and is named on a patent for a device that measures the puffing behavior of electronic cigarette users. Other authors report no conflict of interest.

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ORCID
Karen H. Alzoubi http://orcid.org/0000-0002-2808-5099
Omar F. Khabour http://orcid.org/0000-0002-3006-3104
Ahmed Abu-sinjeyh http://orcid.org/0000-0002-1175-5017
Nour A. Al-Sawalha http://orcid.org/0000-0002-7401-8065
Fadia A. Mayyas http://orcid.org/0000-0002-2086-7205
Caroline O. Cobb http://orcid.org/0000-0003-3258-252X
Thomas Eissenberg http://orcid.org/0000-0002-8277-5437

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