Research article

CHRNA5 rs16969968 and CHRNA3 rs578776 polymorphisms are associated with multiple nicotine dependence phenotypes in Bangladeshi smokers

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ABSTRACT

Background: A recent study has identified the role of *CHRNA5-A3-B4* gene cluster variants rs16969968 and rs578776 of nicotinic acetylcholine receptors (nAChRs) on smoking status in Bengali ethnicity. The aim of the current study was to investigate whether these rs16969968-rs578776-rs11072768 single nucleotide polymorphisms (SNPs) of *CHRNA5-A3-B4* gene cluster were associated with nicotine dependence (ND) and related phenotypes.
Methods: The Fagerstrom Test for Nicotine Dependence (FTND) and Cigarette Dependence Scale (CDS-12) were used to assess the degree of ND, and genotyping was done using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method on a cohort of 129 male smokers participating in a structured questionnaire-based survey.
Results: Smokers with AA genotype of *CHRNA5* rs16969968 SNP were at significantly increased risk of developing ND compared to its wild type variant with odds ratio (ORs) of 1.20 (FTND: 95% CI 0.25–5.37, p = 0.253) and 2.48 (CDS-12: 95% CI 0.46–13.26, p = 0.081), respectively. Conversely, smokers with AA genotype of *CHRNA3* rs578776 variant had a strong protective effect against ND development (ORs = 0.27, 95% CI 0.09–0.80, p = 0.076). There was no such link reported in *CHRNB4* rs11072768 variant carriers. Similarly, G-A/G-A diplotype of rs16969968_rs578776 variants was discovered to be a protective factor against ND. Moreover, demographic features such as age, occupation and dwelling status were found to be significantly associated with ND.
Conclusion: Taken together, *CHRNA5-A3-B4* gene cluster variants rs16969968 and rs578776 as well as specific demographic characteristics regulate ND and related smoking phenotypes in Bangladeshi male smokers. Further studies with large sample sizes are required to substantially validate the significance.

1. Introduction

Smoking is one of the leading causes of premature death worldwide. Study conducted on more than 200,000 people has reported that about 67 percent of smokers perished from smoking-related illness and the relative risk for adverse health effects increases with intensity of smoking [1]. In 2015, global estimated death due to smoking was 6.4 million and it has been predicted that by 2030 it will be approximately 6.8 million which is appalling [2, 3]. Besides, in Bangladesh, the death rate due to smoking has been reported to be 19% by World Health Organization (WHO) [4]. Global Adult Tobacco Survey (GATS) conducted by WHO in Bangladesh has reported that approximately 35.3% adults are currently smoking among which 46% are men and 25.2% are women and among the non-smokers 39% adults are exposed to smoking [5]. Even though

different smoking cessation treatments are available, many smokers failed to act upon their intentions to quit smoking and diversity in quitting occurs due to nicotine dependence (ND). The majority of dependents are not able to stop smoking, even with professional assistance [6] and in spite of the known health benefits. Various restorative factors [7, 8, 9] are contributing to relapse after cessation among which genetic factors play an important role and can be attributable to a 50% risk for failed attempts to quit smoking [4, 5]. Recently, Genome-wide association studies (GWAS) have highlighted some new genetic variants such as *CHRNA5-A3-B4* gene cluster associated with nicotine dependence (ND), which can also be candidates for the prediction of the success of the treatment of this dependence [10].

ND is a complex behavioral condition that has a slow onset and develops due to prolonged smoking which can be characterized by

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tolerance, cravings, withdrawal symptoms during periods of abstinence, and loss of control over the amount despite the harmful consequences. Nicotine is the main chemical constituent and psychoactive ingredient of cigarettes responsible for ND development which gives a temporary pleasing effect in the brain after inhalation and absorption, and dissipation of its transient effect impulsivity to smoke arises again. It exerts its psychopharmacological activity by binding with functionally diverse neuronal nicotinic acetylcholine receptors (nAChRs), which exists as homo or hetero pentameric and ligand gated ion channel [11, 12, 13, 14]. Among its 12 different subtypes, α5, α3, β4 receptor subunits are often co-expressed and co-regulated on chromosome 15q25, and encoded by *CHRNA5-A3-B4* gene cluster [10]. Polymorphism in this gene cluster alters the receptor composition as well as binding of nicotine to this receptor which leads to changes in functional and behavioral outcomes. Additionally, variation in this gene cluster has been found associated with ND and various smoking related phenotypes [12, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32] as well as lung cancer [33, 34, 35] which makes this a plausible candidate to develop better assessment methods and produce more personalized therapy for cessation. The most well-known hereditary influence related to ND is a missense mutation at rs16969968 (G>A) on the nAChRs *CHRNA5*, resulting in an amino acid alteration from aspartate to asparagine at the 398th position [36, 37, 38, 39]. *CHRNA3* rs578776 (G>A) is a 3'-UTR variant and the minor allele genotype (AA) was found to be a protective factor against heaviness of smoking as well as ND [15, 27, 36, 38, 39, 40, 41, 42] and *CHRNB4* rs11072768 (T>G) is an intron variant significantly associated with cigarette per day (CPD) in Chinese males and lung cancer indirectly through smoking behavior [12, 43, 44, 45].

In accordance with those previous studies and as association differs in different races and ethnic groups, our previous work established a strong association between this gene cluster variants and smoking status (SS) [21]. Therefore, the current study was aimed to further explore the hypothesis that occurrences of these three SNPs (rs16969968-rs578776-rs11072768) in *CHRNA5-A3-B4* gene cluster control several ND traits in Bangladeshi male smokers.

2. Materials and methods

2.1. Study design and population

A total of 129 smokers defined by WHO [46] were enrolled from our previously published study [21] but was limited only to male population due to unavailability and privacy concerns of female smokers. A prior power analysis (β = 0.2) determined that the power of the analysis of variance (ANOVA) was ≥0.8 at a 95% confidence interval (CI), indicating that the sample size (n = 129) of the study was sufficient. Each participant signed a written consent form before being enrolled and completed self-reported measures for his smoking history and behavior. Study protocol was approved by the ethical committees and the study was conducted according to the declaration of Helsinki and its further amendments [47].

2.2. Phenotype analysis

Primary data was collected by a questionnaire-based survey method where Fagerstrom Test for Nicotine Dependence (FTND) and Cigarette Dependence Scale (CDS-12), two scales were used for the measurement of degree of ND. The FTND scale measures the degree of ND from 2 (low) to 8 (high), relates with the withdrawal symptoms, ability to stop smoking, physiological dependence and tolerance, heavily weighted for cigarettes per day (CPD) and time from waking until the first cigarette (TFC) but fails to differentiate very low dependent smokers [48, 49, 50]. Whereas, the 12-items CDS scale is brief, self-administered, slightly better predictor of withdrawal symptoms and self-efficacy after cessation, has test-retest ability and suited for both high and low dependent smokers ranging from 12 (lowest) to 60 (highest) but has no 'gold standard' for measuring ND. Since CDS-12 scale is briefer and more sensitive than FTND scale but has no 'gold standard' for ND both the scales were used in order to measure the ND more precisely. Here, we assessed ND with some smoking-related phenotypes-smoking initiation (SI), CPD, TFC in association with these two scores i.e., FTND, CDS-12.

SI was assessed by comparing smokers of ≤16 years to smokers of >16 years of age as previous study reported no observable genetic effect

Table 1. Correlation between demographic characteristics of smokers and different smoking phenotypes.

Characteristics	SI age			CPD			TFC			FTND			CDS		
	≤16 years	>16 years	p-value	≤20	>20	p-value	≤30	>30	p-value	<4	≥4	p-value	<40	≥40	p-value
Age (years)															
<25	4	27	0.024*	31	0	0.022*	6	25	0.0005*	22	9	0.029*	22	9	0.027*
25–50	15	38		56	14		37	32		31	39		33	37	
>50	8	8		15	7		16	7		9	13		8	14	
Weight (kg)															
<60	6	24	0.516	34	11	0.256	24	21	0.603	27	18	0.327	18	27	0.098
60–80	18	44		61	9		32	38		44	26		39	31	
>80	3	5		7	1		3	5		7	1		6	2	
Occupation															
Student	11	42	0.377	53	1	0.0001*	16	38	0.0004*	34	20	0.026*	34	20	0.038*
Service	3	9		16	3		8	11		11	8		11	8	
Business	5	10		15	5		13	8		7	13		6	14	
Others	8	12		18	12		22	7		10	20		12	18	
Dwelling status															
Urban	15	46	0.497	57	3	0.0005*	22	38	0.014*	40	20	0.0004*	39	21	0.003*
Rural	12	27		45	18		37	26		22	41		24	39	
PTE															
Yes	15	37	0.665	53	9	0.447	30	32	0.925	32	30	0.787	31	31	0.785
No	12	36		49	12		29	32		30	31		32	29	

PTE, Parental Tobacco Exposure; SI, Smoking initiation; CPD, Cigarette per day; TFC, Time from waking until the First Cigarette; FTND, Fagerstrom Test for Nicotine Dependence; CDS-12, Cigarette Dependence Scale -12 (*) marks the values which are statistically significant where p < 0.05 indicates statistical significance.

Table 2. Association of *CHRNA5-A3-B4* gene cluster SNPs with different smoking phenotypes.

Characteristics	SI age				CPD				TFC				FTND				CDS			
	≤16 years	>16 years	Adjusted OR (95%CI)	p-value	≤20	>20	Adjusted OR (95%CI)	p-value	≤30	>30	Adjusted OR (95%CI)	p-value	<4	≥4	Adjusted OR (95% CI)	p-value	<40	≥40	Adjusted OR (95% CI)	p-value
rs16969968 G>A																				
GG	21	50	Ref		73	16	Ref		45	44	ref		43	46	Ref		46	43	ref	
GA	5	18	0.65 (0.21–1.99)	0.153	22	5	1.03 (0.33–3.08)	0.30	11	16	0.59 (0.27–1.53)	0.121	16	11	0.62 (0.27–1.42)	0.104	15	12	0.81 (0.35–1.95)	0.237
AA	1	5	0.45 (0.05–4.30)	0.168	7	0	0.29 (0.02–5.13)	0.132	3	4	0.69 (0.16–3.34)	0.228	3	4	1.20 (0.25–5.37)	0.253	2	5	2.48 (0.46–13.26)	0.081
rs578776 G>A																				
GG	6	29	Ref		37	5	Ref		20	22	ref		17	25	Ref		19	23	ref	
GA	12	32	1.76 (0.57–5.13)	0.096	43	13	2.16 (0.74–6.64)	0.05	30	25	1.25 (0.54–2.77)	0.159	27	29	0.70 (0.33–1.58)	0.147	28	28	0.74 (0.34–1.76)	0.201
AA	8	11	3.49 (0.96–10.54)	0.016*	18	3	1.18 (0.26–5.44)	0.23	9	13	0.75 (0.27–1.96)	0.198	15	6	0.27 (0.09–0.80)	0.076*	13	8	0.48 (0.17–1.40)	0.08
rs11072768 T>G																				
TT	11	29	Ref		42	11	Ref		26	28	ref		29	24	Ref		28	25	ref	
TG	13	28	1.20 (0.47–3.10)	0.223	41	6	0.57 (0.18–1.69)	0.092	23	23	1.09 (0.48–2.40)	0.280	24	23	1.19 (0.50–2.41)	0.221	25	22	0.94 (0.44–2.20)	0.301
GG	2	14	0.38 (0.07–1.91)	0.081	16	4	0.92 (0.27–3.31)	0.32	9	11	0.85 (0.32–2.43)	0.275	8	12	1.78 (0.63–4.96)	0.09	9	11	1.33 (0.45–3.9)	0.192

PTE, Parental Tobacco Exposure; SI, Smoking initiation; CPD, Cigarette per day; TFC, Time from waking until the First Cigarette; FTND, Fagerstrom Test for Nicotine; Dependence; CDS-12, Cigarette Dependence Scale -12 (*) marks the values which are statistically significant after correcting for multiple testing.

in subjects who began daily nicotine use after the age of 16 [51]. CPD was used as a measurement of tobacco consumption where heavy smokers who smoked 20 or more cigarettes per day were compared with light smokers who smoked less than 20 cigarettes per day [52]. TFC was applied for measuring the craving phenotype and the ability to quit as well. ND was evaluated using FTND and CDS-12 scores. Participants were divided into two groups based on their scores: low dependence (<4 scores for FTND, ≤40 scores for CDS-12) and high dependence (≥4 scores for FTND, >40 scores for CDS-12).

2.3. Genotype analysis

Genomic DNA was extracted from all 129 smokers' blood samples and genotyped for *CHRNA5* rs16969968, *CHRNA3* rs578776, *CHRNB4* rs11072768 variants using Polymerase Chain Reaction -Restriction Fragment Length Polymorphism (PCR-RFLP) method [53]. Details of the genotype analysis can be found in our previously published study [21].

2.4. In-silico gene expression analysis

To explore the tissue specific expression of *CHRNA5-A3-B4* gene cluster and its associated SNPs of interest, an in-silico gene expression analysis was performed using Genotype-Tissue Expression (GTEx) portal [54]. Furthermore, to correlate the expression of these three genes with two major histological subtypes of lung cancer i.e., lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) as well as with smoking status, UALCAN, an interactive web tool based on TCGA dataset [55], was used.

2.5. Statistical analysis

Chi-square test ($\chi 2$) was applied to measure the association of smokers' demographic features with several smoking phenotypes. To perform multiple regression analyses for assessing odds ratios (ORs) and 95% confidence interval (CI), SPSS software package, version 17.0 (SPSS, Inc., Chicago, IL) was used and were adjusted to age, occupation and dwelling status. In all the analyses, p value less than 0.05 was considered as statistically significant. In addition, data, if required, were corrected for multiple comparisons using Bonferroni method in order to prevent false positive results.

3. Results

3.1. Association between demographic characteristics of smokers and smoking phenotypes

Age of the smokers was found to be a statistically significant factor in influencing many smoking-related phenotypes, such as age of SI, CPD, TFC, FTND and CDS-12, whereas occupation and dwelling status were important contributing factors primarily for phenotypes related to the intensity of nicotine dependence. Individual body weight and parental tobacco exposure didn't seem to impact any ND traits. Detailed results of the correlation analysis between demographic characteristics and smoking phenotypes are shown in Table 1.

3.2. Correlation of CHRNA5-A3-B4 gene cluster with smoking phenotypes

To examine the influence of these investigated SNPs on the age of daily smoking initiation, we reported a significant association of rs578776 AA genotype with late onset of smoking (OR = 3.49, 95% CI = 0.96–10.54, p = 0.016). However, no such link was discovered for the other two SNPs (Table 2).

Furthermore, the CPD value was used to assess the intensity of nicotine consumption, and our study found no significant connection between the three SNPs studied and the amount of tobacco consumed (Table 2).
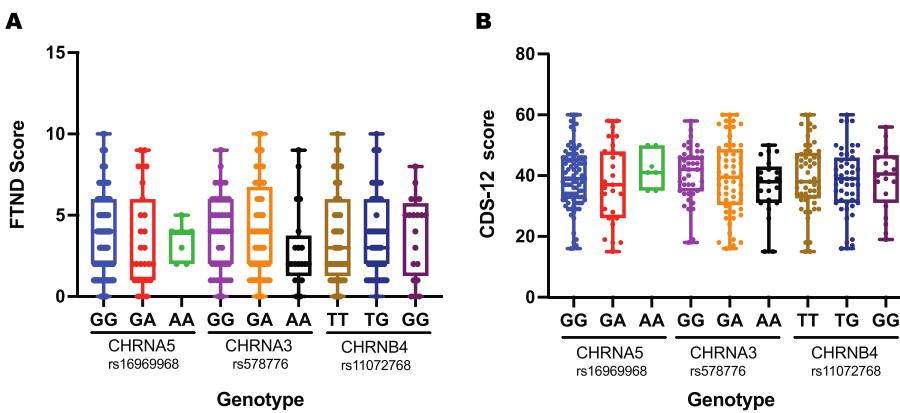
Figure 1. Distribution of nicotine dependence test scores among smokers of different *CHRNA5-A3-B4* genotypes A) FTND score, B) CDS-12 score.

FTND, CDS scores, and TFC phenotype were recorded afterwards to study the influence of these selected polymorphisms on the degree of ND in smokers. According to the FTND and CDS-12 scores, rs16969968 AA genotype contributed to the development of ND. Conversely, given its high linkage disequilibrium (D' = 1.0) and low correlation (r2 = 0.2478) with rs16969968 in Bengali ethnicity (Supplementary Figure 1), the rs578776 AA genotype showed a protective function against ND. In addition, the TFC phenotype was found to be unrelated to the SNPs studied (Table 2). Figure 1 shows the FTND and CDS-12 scores distribution among smokers of different CHRNA5-A3-B4 genotypes.

To further dig into the collective role of rs16969968 and rs578776 SNPs, different diplotypes, matched pair of haplotypes on homologous chromosomes [56], of these two polymorphisms having at least <5.0% frequency were correlated with their FTND scores. The diplotype analysis revealed that individuals having two low-risk G alleles for rs16969968 along with two low-risk A alleles for rs578776 were associated with significantly reduced risk of ND (OR = 0.2167, 95% CI = 0.05–0.95, p = 0.043) (Figure 2).

3.3. Correlation between CHRNA5-A3-B4 gene expression and lung cancer risk

Our in-silico mRNA expression analysis of these three genes in different regions of brain and lungs revealed that the median expression of both *CHRNA5* and *CHRNA3* genes is highest in the cerebellum region, whereas basal ganglia express the highest level of *CHRNB4* gene. Compared to the cerebellum, *CHRNA5* and *CHRNB4* genes are relatively lowly expressed in lung tissues, approximately by 3-fold and 4-fold, respectively and the expression of *CHRNA3* was found negligible (only 1.2% relative to cerebellum) (Supplementary Figure 2 and Supplementary Table 1).

To correlate these expression data with lung cancer risk, expression between normal and lung cancer was obtained from the UALCAN database. *CHRNA5* gene was found to be significantly upregulated in lung cancer patients irrespective of their cancer subtypes, ethnicities and smoking habits (Figure 3), suggesting a link between high *CHRNA5* expression and lung cancer risk. In contrast to *CHRNA5* overexpression in lung primary tumors, expression of *CHRNA3* gene showed no significant difference between normal and lung cancer patients except for Caucasian ethnicity and LUSC subtypes (Figure 4), which could possibly be justified by the low expression level of this gene in lungs compared to major brain regions. In case of *CHRNB4* gene expression, a trend similar to *CHRNA5* expression was observed. However, it is important to highlight the fact that *CHRNB4* expression exhibits insignificant results for Asian lung cancer patients (Figure 5). More importantly, despite the existence of some sort of relationship between normal and lung cancer patients' gene expression for *CHRNA5-A3-B4* gene cluster in terms of their smoking habits, none of these genes showed any significant difference when a

comparison was made between smoker and non-smoker lung cancer patients.

Our analysis of *CHRNA5* rs16969968 polymorphism also reported a significantly low expression for AA genotype compared to GG genotype (p = 5.00 × 10-09) in lung tissue (Supplementary Figure 3). No lung tissue specific data was found for *CHRNA3* rs578776 and *CHRNB4* rs11072768 polymorphisms in the GTEx portal.

4. Discussion

Numerous lines of evidence including ours have indicated a compelling link between *CHRNA5-A3-B4* gene cluster variants (rs16969968-rs578776-rs11072768) and SS in Bangladeshi male smokers. In the present study, which primarily focused on the transition from regular smoking to addiction, we performed analyses to explore their relationship with ND by utilizing two independent ND measurement scales (FTND and CDS-12) covering a broad spectrum of smoking related phenotypes including smoking initiation age, smoking quantity and craving as well as dependence.

Individuals carrying AA genotype of *CHRNA5* rs16969968 variant had 1.20 (FTND) and 2.48 (CDS-12) higher odds of developing ND, if compared with noncarriers, replicating several previously reported studies [18, 26, 26]. However, our findings failed to reach sufficient statistical significance considerably owing to the small study population or probably a true finding in reference to few previous similar reports [57, 58, 59]. Association of rs16969968 AA genotype with ND was well documented by in vitro functional studies [18, 60]. A change from aspartic acid (D398) to asparagine (N398) in rs16969968 variant initiates a series of event including the reduction of receptor's function, concomitant prolongation of receptor desensitization and reduction of
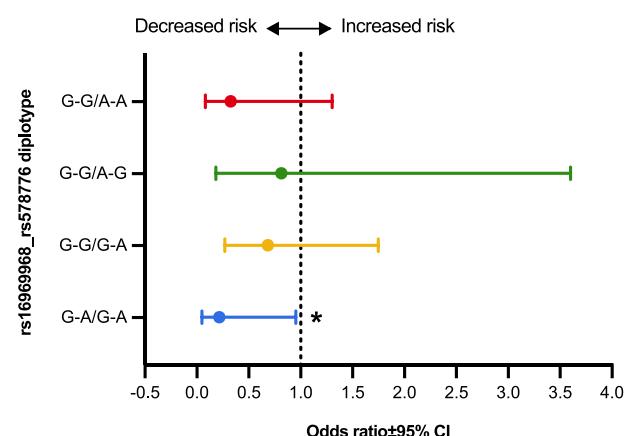

Figure 2. Association of different rs16969968_rs578776 diplotypes with FTND scores. *p < 0.05.
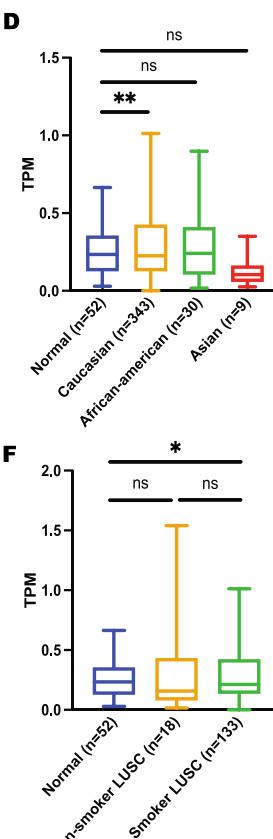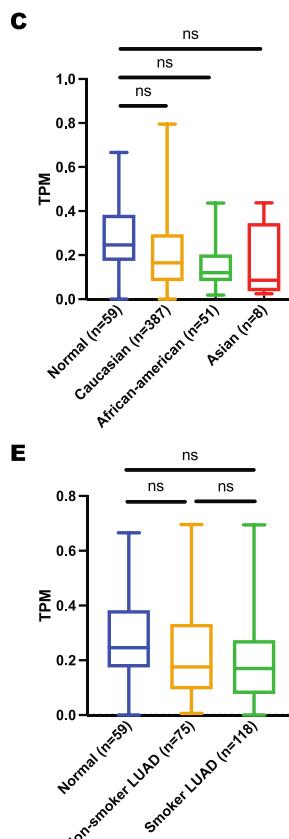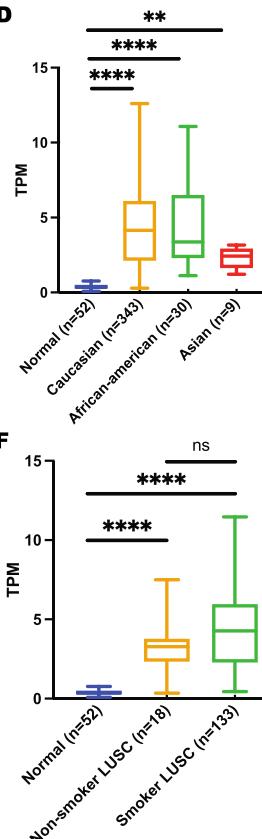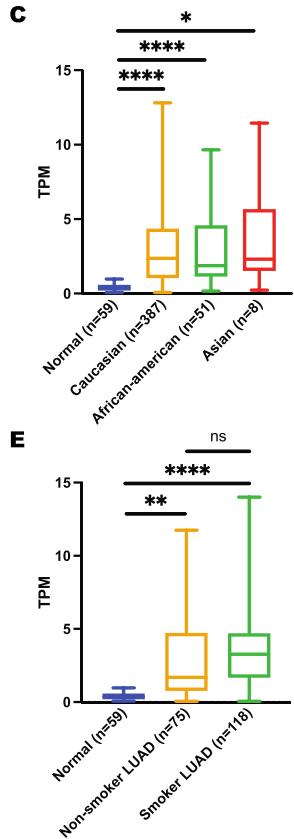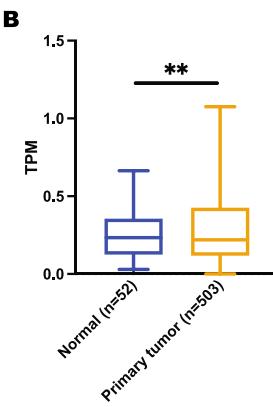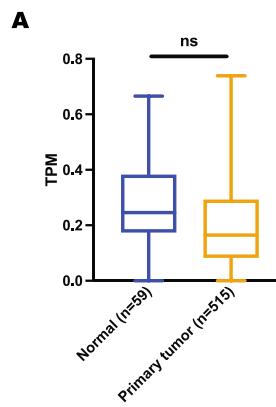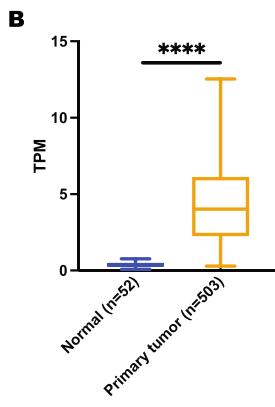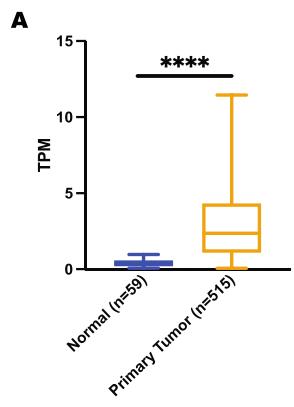
Figure 3. Expression analysis of *CHRNA5* gene using UALCAN A) expression in LUAD, B) expression in LUSC, C) expression in LUAD based on patient's ethnicity, D) expression in LUSC based on patient's ethnicity, E) expression in LUAD based on patient's smoking habit, F) expression in LUSC based on patient's smoking habit. *p < 0.05, **p < 0.01, ****p < 0.0001, ns p > 0.05.

Figure 4. Expression analysis of *CHRNA3* gene using UALCAN A) expression in LUAD, B) expression in LUSC, C) expression in LUAD based on patient's ethnicity, D) expression in LUSC based on patient's ethnicity, E) expression in LUAD based on patient's smoking habit, F) expression in LUSC based on patient's smoking habit. *p < 0.05, **p < 0.01, ****p < 0.0001, ns p > 0.05.

calcium ions permeability [60, 61], which ultimately generates an increased demand for nicotine in particular brain areas leading to development of ND [27, 36, 62]. In addition, conservation of D398 amino acid across divergent species [18] and a drastic reduction of sensitivity to nicotine induced behaviors and seizures in *CHRNA5* mutant mouse model [63] might also reason the high ND in *CHRNA5* rs16969968 AA genotype carriers. The *CHRNA3* rs578776 variant AA genotype was found to be protective against ND and late-onset smoking which corroborates several previous studies [18, 64]. A study conducted on the brain reward system reported that rs578776 protective A-allele carriers show normal intrinsic reward sensitivity to intrinsically pleasant activity in smokers whereas G-allele carriers demonstrate increased sensitivity to nicotine induced stimulation [65]. Moreover, Hong and colleagues identified rs578776 at-risk G-allele mediated overactivation of a specific

brain circuit in dorsal anterior cingulate cortex and left anterior thalamus regions using fMRI technology [66], which might be sensitive to nicotine exposure suggesting a plausible link between rs578776 at-risk G-allele and ND. Being an intron variant, *CHRNB4* rs11072768 polymorphism has been found to be associated with smoking cessation, CPD [12, 43] as well as lung cancer [45]. Molecular study performed on B4 subunit null mice reported an increased tolerance development after chronically treated with nicotine [67], which might lead to neuroadaptation and subsequently to ND. Surprisingly, our analysis could not identify any association of rs11072768 with any of the investigated smoking-related phenotypes.

Analysis of different diplotypes of rs16969968_rs578776 also detected that smoker with common allele homozygous for rs16969968 and
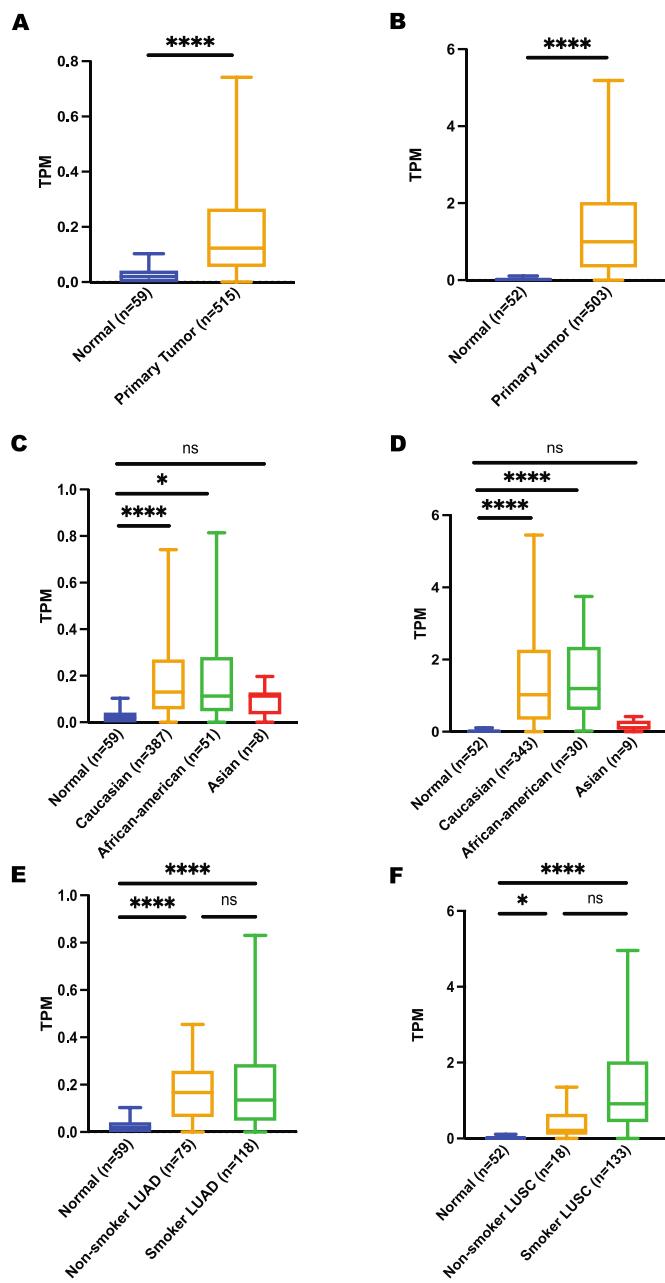
Figure 5. Expression analysis of *CHRNB4* gene using UALCAN A) expression in LUAD, B) expression in LUSC, C) expression in LUAD based on patient's ethnicity, D) expression in LUSC based on patient's ethnicity, E) expression in LUAD based on patient's smoking habit, F) expression in LUSC based on patient's smoking habit. *p < 0.05, **p < 0.01, ****p < 0.0001, ns p > 0.05.

minor allele homozygous for rs578776 demonstrated an overall protection against ND, showing an independent role of each SNP. This finding was also potentially backed by the low correlation ($r^2 = 0.2478$) value reported in BEB ethnicity (current study) and differences between rs578776 related dorsal anterior cingulate cortex (dACC)-thalamus circuit and rs16969968 related dACC-ventral striatum circuit [66].

ND features may also be modified by many social environment factors, according to social cognitive theory [68]. Age, occupation, and residence status were all found to be strongly correlated with ND-related traits in this study. On the other hand, parental smoking exposure had no effect on future ND development, which is in direct opposition to the social cognitive paradigm [68]. This discrepancy could possibly be due to other confounding factors which might be ruled out by large scale population study.

Our study also investigated the mRNA expression pattern of *CHRNA5-A3-B4* gene cluster in normal and lung cancer patients using an in-silico approach. We reported overexpression of *CHRNA5* and *CHRNB4* in lung cancer patients compared to normal volunteers which is consistent with several previously published results [33, 34, 45, 69]. However, *CHRNB4* expression was found to be nonsignificant in case of Asian ethnicity which in a way corroborates our findings regarding rs11072768 and ND. No such association was found for *CHRNA3* gene. In case of smoker lung cancer patients, expression of all three genes was significantly higher than that of normal individuals. Interestingly, there was no significant difference in expression of *CHRNA5-A3-B4* gene region revealed between non-smokers (n = 18) and smokers (n = 133) lung cancer patients, hence, requiring a large-scale investigation. SNP specific expression analysis using GTEx portal showed low expression of rs16969968 AA genotype in normal lung tissues and no data was found for other two SNPs. Altogether, based on the findings of our preliminary in-silico analysis, a comprehensive study comprising a substantial number of lung cancer patients with varying smoking status is required to establish any link among *CHRNA5-A3-B4* gene cluster, ND and lung cancer risk.

In conclusion, our findings showed that rs16969968 and rs578776 SNPs of *CHRNA5-A3-B4* gene cluster on chromosome 15q25 and certain demographic features influence ND in Bangladeshi male smokers, though our study was limited by small sample size and lack of any biochemical biomarker e.g. serum/urine cotinine (COT), trans-3'-hydroxy cotinine (3HC) levels measurement. This study replicating the gene to phenotype association with those self-reported phenotypes and genetic measurements allowed a further step towards understanding the relationship between the variants and ND in the Bangladeshi population. Further study with a large sample size should be performed to substantially validate these findings.

Declarations

Author contribution statement

Nusrat Islam Chaity: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Mohd Nazmul Hasan Apu: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no competing interests.

Additional information

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