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Lung cancer risk in relation to nicotinic acetylcholine receptor, CYP2A6 and CYP1A1 genotypes in the Bangladeshi population

Mohammad Safiqul Islam [a], Maizbha Uddin Ahmed [a], Muhammad Shahdaat Bin Sayeed [a], Abdullah Al Maruf [a], A.G.M. Mostofa [a], Syed Md Akram Hussain [b], Yearul Kabir [c], Ann K. Daly [d], Abul Hasnat [a,*]

[a] Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh
[b] Department of Oncology, Bangabandhu Sheikh Mujib Medical University, Dhaka-1000, Bangladesh
[c] Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka-1000, Bangladesh
[d] Institute of Cellular Medicine, Newcastle University, NE2 4HH, United Kingdom

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ABSTRACT

Background: *CYP1A1*, *CYP2A6* and *CHRNA5* are biologically plausible genes as risk factors for lung cancer but no studies have been reported in the Bangladeshi population.
Methods: We conducted this study to determine the prevalence and role of *CYP1A1*, *CYP2A6* and *CHRNA5* polymorphisms together with tobacco smoking in the development of lung cancer in Bangladesh. A case–control study was carried out on 106 lung cancer patients and 116 controls to investigate three allelic variants of the *CYP1A1* gene—rs4646903, rs1048943 and rs1799814; 2 variants of *CYP2A6* (*CYP2A6*1B1*, *CYP2A6*4*) and 1 variant of *CHRNA5* (rs16969968) using Polymerase Chain Reaction Restriction Fragment Length Polymorphism.
Results: Lung cancer risk was estimated as odds ratio (OR) and 95% confidence interval (CI) using unconditional logistic regression models adjusting for age, sex and smoking. A significantly elevated lung cancer risk was associated with heterozygous, mutant and combined heterozygous plus mutant variants of CYP1A1 rs4646903. A significant association was also found for heterozygous and heterozygous plus mutant variants of rs1048943 which was in linkage disequilibrium with rs4646903. The risk of lung cancer was decreased significantly in individuals carrying at least one CYP2A6 deletion (CYP2A6*4) allele. No association with lung cancer risk was found for *CHRNA5* rs16969968. When stratified by smoking, the effects of CYP1A1 and CYP2A6 polymorphisms on lung cancer susceptibility were found to be significant only in heavy smokers who had smoked 40 pack years or more (54% of all cases) but no associations were seen for lighter smokers. No association was also found with any polymorphism in the non-smokers in this study.
Conclusions: Our results indicate that the CYP1A1*2B allele (rs4646903 and rs1048943) is associated with an increased lung cancer risk and CYP2A6*4 is associated with a decreased lung cancer risk in the study population.

1. Introduction

Lung cancer is the most common form of cancer in the world [1]. Considering incidence and mortality in males, lung cancer is also the most prevalent cancer in Bangladesh (24.9% of incidence and 28.6% of mortality) [2]. In females, lung cancer (5.6%) is next to breast cancer (21.9%), cervical cancer (21.8%) and lip & oral cavity cancer (6.6%) with respect to incidence whereas in case of mortality lung cancer (7.6%) is next to cervical (19%) and breast cancer (15.4%) [2]. Total estimated lung cancer patients in Bangladesh were 196,000 among those aged ≥30 y [3,4]. According to the IARC 2010 report, new lung cancer cases in Bangladesh were 14951 in 2008 and the annual number of cases are estimated to rise to 43,048 by 2030 [2].

Tobacco smoking is the major risk factor for the development of lung cancer and about 80–90% of lung cancers are attributable to cigarette smoking [5]. Cigarette smoke contains about 4000 chemicals—at least 250 of them are known to be harmful and more than 60 are known to be carcinogenic [6]. These have been detected in mainstream cigarette smoke and most of the same carcinogens are also present in second-hand smoke [7]. Due to high production and consumption of tobacco, Bangladesh is in a high risk for the prevalence of lung cancer [8] and >57,000 people die each year in Bangladesh due to tobacco related diseases [4].

CYP1A1 expressed in the extra-hepatic tissues including lung, plays a potential role in the metabolism of polycyclic aromatic hydrocarbons (PAH) which are present in tobacco smoke. CYP1A1 expression is induced by PAHs and the extent of inducibility is associated with pulmonary PAH-related DNA adduction and lung cancer risk [9]. A number of variant alleles of CYP1A1 have been reported [10].

* Corresponding author. Tel.: +880 2 9667850x8164; fax: +880 2 8615583.
E-mail address: ahasnat99@yahoo.com (A. Hasnat).

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Two polymorphisms have been most frequently studied in relation to lung cancer risk [11]. The first variant rs4646903 (3798T>C; MspI or m1 polymorphism) is a point mutation in the MspI restriction site in the 3′-untranslated region of *CYP1A1* gene which is frequently found in Asian populations and is strongly associated with lung cancer risk [12]. The second polymorphism rs1048943 (2454A>G; m2 polymorphism) results in an amino acid change (Ile462Val) [13]. This variant is frequent in Asians and is associated with lung cancer risk even though rare in Caucasians [12,14,15]. The combination of rs4646903 and rs1048943 forms the *CYP1A1*2B* allele with individual alleles positive for the single SNPs only referred to as *CYP1A1*2A* and *CYP1A1*2C* [10]. Another CYP1A1 allele is *CYP1A1*rs1799814 (*CYP1A1*4*; 2452C>A; m4 polymorphism) which results in an amino acid change of Thr461Asn in the heme-binding domain [15,16]. The frequency in Caucasians is 3–6% [17]. *CYP1A1*4* is also detected in the Indian subcontinent but with a very low frequency [18,19]. There is evidence that levels of CYP1A1 are higher in those positive for *CYP1A1*2B* [20] though rs1048943 alone does not appear to result in altered enzyme activity in vitro [21].

The *CYP2A6* gene shows inter-individual variation of >100-fold in both CYP2A6 mRNA and protein levels [22]. It is a highly polymorphic gene and characterized by multiple gene conversions with *CYP2A7*, and several forms of gene deletions (*CYP2A6*4A-F*), duplications (*CYP2A6*1x2A*, *CYP2A6*1x2B*) and single nucleotide polymorphisms (SNP) in coding and regulatory regions (*CYP2A6**9A) [23,24]. Some alleles encode enzymes that are associated with absent (e.g. **4*) [25], reduced (e.g. **9*) [26], normal (e.g. **8*) [27] or increased (e.g. **1B*) [28] *CYP2A6* activity with markedly different frequencies among ethnic groups [29]. The frequency of the *CYP2A6*4 (*4A and *4D)* variant is high in Asian populations, ranging from 6.7 to 24.2% in Japanese, Koreans, and Chinese, but is much less prevalent in Caucasians and African-Americans [23,30].

Recent genome-wide association studies (GWAS) [31–33] have mapped a lung cancer susceptibility locus to chromosome 15q25.1 containing nicotinic acetylcholine receptor genes *CHRNA3*, *CHRNA5* and *CHRNB4* encoding the α3, α5, and β4 subunits of nAChRs respectively. This finding was recently confirmed by the International Lung Cancer Consortium [34] in white populations. Genetic variation in nicotinic acetylcholine receptors that affect the gene expression or protein function appears to be associated with smoking behavior and the risk of smoking-related lung cancer [35]. Although a series of studies has been conducted in Caucasians, Asians—mostly in Chinese, Japanese and Korean population, no study of *CYP1A1*, *CYP2A6* and *CHRNA5* as risk factors for lung cancer has been conducted on a Bangladeshi population. A few case–control studies of *CYP1A1* and *CYP2A6* have been conducted in Indian populations but no study has been reported yet on CHRNA5 except a study [36] on Gujarati Indians in Houston (GIH), Texas, USA. The ancestry in Indian subcontinent is unique [37] and the population demonstrates a high genetic differentiation and extensive sub-structuring due to racial admixture [37,38]. Bangladesh has a population possessing similar type of racial admixture and genetic diversity as populations in other part of Indian subcontinent. Therefore, we assume our population has some similarity with Indians but still having a unique genetic ancestral lineage.

2. Materials and methods

2.1. Study design

The study was a case–control study conducted on 106 lung cancer patients and 116 healthy volunteers matched by age, sex and smoking status. Lung cancer patients were recruited from Ahsania Mission Cancer and General Hospital, Dhaka Medical College Hospital and Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. Patients were histologically diagnosed with lung cancer according to the International Association of Lung Cancer [39] between the period of January 2009 and December, 2011. After physical examination controls were selected by matching age, sex and smoking status to lung cancer patients. Smoking information, demographic characteristics, and lifestyle factors were collected through interviews by trained nurses in the presence of expert physicians. No lung cancer case had a history or evidence of any other severe diseases like cardiovascular disease, kidney disease, previous cancer, and metastasized cancer and if preset they were excluded from the study. Controls were not relatives to the patients and no subject had a history or evidence of hepatic, renal, gastrointestinal or hematologic deviations or any acute or chronic diseases based on medical history, clinical examination and laboratory investigation (hematology, blood biochemistry and urine analysis).

Current smokers had been smoking regularly and nonsmokers had never smoked during his/her lifetime. Those smokers who quit for >1 y before the recruitment were considered as former smokers. Current and ex-smokers were considered as ever smokers. In ever smokers, subjects with a pack-years value ≥40 were considered

Table 1
Primers, PCR conditions, restriction enzymes and expected DNA fragments on digestion to genotype the selected polymorphisms.

Polymorphism	Primers	PCR condition	No. of cycles	RE	Digestion condition	DNA fragments
CYP1A1 rs4646903 (m1 or MspI)	FP 5′-CAGTGAAGAGGTGTAGCCGCT-3′ RP 5′-TAGGAGTCTTGTCTCATGCCT-3′	94 °C 30 s 62 °C 30 s 72 °C 30 s	36	*Msp*I	37 °C (incubated over night)	AF 340 NH 340 HE 340, 200, 140 MH 200, 140
CYP1A1 rs1048943 (Ile462Val)	FP: 5′-GAACTGCCACTTCAGCTGTCT-3′ RP: 5′-CCAGGAAGAGAAAGACCTCCCAGCGGGCCA-3′	94 °C 1 min 68 °C 1 min 70 °C 1 min	35	*Nco*I	37 °C (incubated over night)	AF 195 NH 163, 32 HE 195, 163, 32 MH 195
CYP1A1 rs1799814 (CYP1A1*4)	FP 5′-CCACTCACTTGACACTTCTG-3′ RP 5′-TAGACAGAGTCTAGGCCTCA-3′	93 °C 1 min 55 °C 1 min 30 s 70 °C 2 min	35	*Bsa*I	37 °C (incubated over night)	AF 381 NH 201, 180 HE 381, 201, 180 MH 381
*CYP2A6*1A* *CYP2A6*1B1*, *CYP2A6*4*	FP: 5′-CACCGAAGTGTACCCTATGCTG-3′ RP: 5′-AAAATGGGCATGAACGCCC-3′	94 °C 1 min 56 °C 1 min 72 °C 2 min	30	*Bsu*36I *BstU*I	Initially incubated over night with *Bsu36 I* at 37 °C and then incubated with *BstU* I at 60 °C for 12 h	AF 1338 *1A 800, 434,104 *1B1 800, 285, 149, 104 *4 759, 285, 149, 104, 41
CHRNA5 (rs16969968)	FP: 5′-CGCCTTTGGTCCGCAAGATA-3′ RP: 5′-TGCTGATGGGGGAAGTGGAG-3′	94 °C 1 min 57 °C 30 s 74 °C 1 min	35	*Taq*I	65 °C (incubated over night)	AF 435 NH 290, 145 HE 435, 290, 145 MH 435

AF, amplified fragment; NH, normal homozygote; HE, heterozygote; MH, mutant homozygote; and RE, restriction endonuclease.