RESEARCH ARTICLE

MDM2 (RS769412) G> a Polymorphism in Cigarette Smokers: A Clue for the Susceptibility to Smoking and Lung Cancer Risk

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Abstract

Cigarette smoke contains oxidants and free radicals which are carcinogen that can induce mutations in human. Single nucleotide polymorphisms (SNPs) are the most frequent mutation found in human genome. In present study, we have examined the association of Mdm2 (rs769412) A>G polymorphism in cigarette smokers to predict the smoking related risk of cancers. Our results show that smokers, 87% were found with AA genotype, 10% with heterozygous AG genotype, and 3% with GG genotype. The heterozygous AG genotype was observed in lower percentage of smokers (10%) as compared to non-smokers (18%), whereas, homozygous AA genotype was observed in lower percentage of non-smokers (81%) as compared to the smokers (87%). The results from present study support the association with an allele and AG genotype in the non-smokers. However, further studies are required to establish the role of Mdm2 (rs769412) C>T in cigarettes smokers and diseases.

Key words: Smokers - genome - genotype - SNPs - Mdm2 - rs769412 - cancer

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Introduction

Cigarette smoking, even light or intermittent active or prolonged passive types are biggest threat for many types of cancers mainly of lung cancer and cardiovascular disease (CVD) (Bjartveit and Tverdal 2005; Schane et al., 2010). According to the World Health Organization reports, approximately there are 1.3 billion smokers globally (World Health Organization., 2008) and, consequently, 5.4 million people die per annum (World Health Organization., 2011). As estimated, by 2030, around 8 million smokers will die every year due to health problems caused by smoking. Cigarette Smoking causes about 90% of all lung cancer deaths (Doll et al., 1994).

It is well documented that cancer is the biggest threat in the United Kingdom and smoking related patients are at high risk, particularly with lung cancers. (United Kingdom Lung Cancer Coalition, 2007). It has been reported by American Cancer Society (2012) that smoking may grow many other types of cancers. According to the World Health Organization (2011) smoking may cause of financial setback along with losses of lives worldwide including Europe and Asian countries. The prevalence of smoking is high amongst the male youth in lower income countries. As far as the Kingdome of Saudi Arabia is concerned, the prevalence of current smoking among adolescent ranges from 12-29.8%. Global Youth Tobacco Survey (GYTS) which was conducted by WHO (World Health Organization., 2011) reported that 34.5% senior secondary school students smoke sometime while 20% of them were regular smokers. Currenty the frequency rate of smoking is in between 2.4 and 37 % among young adults. Concurrently, that was 11.6% and 25% among adults and elderly population (Bassiony 2009). It may be a direct result of factors like urbanization, promotional advertising strategies of tobacco industries, modernization and misapprehensions associate smoking with maturity (World Health Organization, 2011).

Recently there was great interest shown among the scientists to study the relationship of cancer with single nucleotide polymorphisms (SNPs) such as p21, bcl2 or Mdm2 gene etc. Several studies have already been attempted in some of these genes. One of the candidate oncogenes, the murine double minute (Mdm2), that codes for Mdm2 gene, and negatively regulates tumor suppressor p^{53}. Mdm2 functions as E3 ubiquitin ligase identifies N-terminal trans-activation domain (TAD) of the p^{53} tumor suppressor and inhibits transcriptional activation of p^{53} (Oliner et al., 1992; Iwakuma and Lozano., 2003). Associations of different single nucleotide polymorphisms (SNPs) within genes with the predisposition of several diseases have been done and more researches are going in these directions. Mdm2 is one of the highly targeted and studied genes for their involvement in different types of cancers. The current work on Mdm2 have been observed and reported an association with the various types of cancers. For example, Mdm2 variants at 309 is associated with esophageal squamous cell carcinoma (Hong et al.,

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2005). In one of the studied, Mdm2 SNP 309 G allele observed an increased risk but the T-allele is integrated with earlier beginning of sporadic breast cancers in the Chinese population [Lum et al., 2008) in another study, variant at 285C may protect the effect of SNP309G allele (Knappskog and Lonning., 2011). The association of Mdm2 T309 G polymorphism with bladder cancer is also documented (Onat et al., 2006). A study on promoter SNPs of Mdm2 revealed the lack of association with lung cancer in a Chinese population (Hu et al., 2006).

In one of the study emphasized by Zhuo et al. (2012), that Mdm2 polymorphism might be an important factor of lung cancer threat, even for the population who never smokers.

Genetic propensity to lung cancer is probably due to both polygenic and heterogeneous, conferred by low penetrance and variable arrangements of comparatively common variants. (Shields and Harris., 2000; Zhou et al., 2005). Moreover, it is important to mention that the smoking-gene interactions are the key of lung cancer predisposition (Sasco et a., 2004). Hence, this study was undertaken to examine the association of Mdm2 variant rs769412 (A>G) with the cigarette smoking behavior. The polymorphism of interest was chosen because that had been reported to be associated with the cancer of breast. Hence we thought of to test the hypothesis, if the same polymorphism may be associated with the smoking behavior and possible in turn with the smoking related diseases. The outcome of this study will allow us understand the relationship between smoking behavior and possible occurrences of smoking related cancers in Saudi population.

Materials and Methods

In this study, 300 smokers of age range 18-50 years (33+3.5 years) and 300 age matched nonsmokers were recruited. Samples were collected from local clinics. The approval of this deigned study was taken by the Ethical Committee, Riyadh Saudi Arabia. The volunteers were asked to submit their consent for the following study. All the groups were drawn from the central region of the Kingdom of Saudi Arabia.

Sample collection

The volunteer participated in this study were selected on the basis of number of cigarette the smoke daily. Those who smoked more than five cigarettes were regarded as smokers and those don not smoke any kind of tobacco product were regarded as non-smokers. The volunteers were recruited at the community not at hospitals. There was no female subject in this study because of the some local contrivances. About 5mL of venous blood was drawn using sterile heparinized syringe from smokers and nonsmoker individuals for genotyping.

DNAExtraction:

Genomic DNA was extracted by using QIAamp blood DNA extraction kit as instructed by the manufacturing company.

Genotyping by TaqMan assay

Genotyping were carried using predesigned TaqMan® assays in accordance with the manufacturer's instructions on ABI Prism 7500 fast (Applied Biosystems, Foster City CA) in 96-well plates. Briefly, after pre PCR cycling for 2 minutes at 50°C, 10 minutes at 95°C, 20 μl PCR reactions were run for 40 cycles of amplification at 92°C denaturation for 15 seconds, 62°C annealing/extension for 60 seconds. Failed genotypes were re run.

Statistical methods

Genotype frequency deviations were analyzed by χ^2 test. Ethnicity specific effect and genotype specific risk were assessed by odds ratio (OR). Risk associated with each SNP was estimated by combining allelic, dominant and recessive OR and 95% confidence intervals (CI). To evaluate the strength of the association between smoking behavior and polymorphism, ORs with 95%CIs were calculated. We further explored the relationship of co-dominant, dominant, recessive model and allele versus allele model, respectively. Analysis of data was performed using SPSS software. The statistical method was used on two sided probability. Differences were considered significant whenever the value was ($P \leq 0.05$).

Results

We analyzed the polymorphic status of Mdm2(rs769412) (G>A) by Taman based Real Time PCR analysis. Based on the previous work, this is the rest study in the Saudi Arabian population, which examined the association of the Mdm2 gene polymorphism with cigarette smokers.

Mdm2 Polymorphism

As shown in Table1, the genotypic distributions in smokers were determined as 87% for the AA, 10% for the heterozygous AG status, and 3% for the GG, respectively. The homozygous GG genotype was seen only two percent lower in non-smokers (1%) in comparison of smokers (3%).Heterozygous AG genotype was seen in higher percentage of non-smokers (18%) when compared to smokers (10%). But homozygous dominant genotype AA was found in high percentage (87%) in smokers then that of non-smokers (81%). There was significant difference

Table 1. Distribution of Mdm2 (rs769412). Genotypes and Allelic Frequencies of the Study Population

	Genotypes					
	AA (n)	%	AG (n)	%	GG (n)	%
Non-Smokers n (%)	243	81	54	18	3	1
Smokers n (%)	261	87	30	10	9	3

*χ^2 (2 d. f.)=10.500; p=0.005 for genotypes

Table 2. Distribution of Mdm2 (rs769412) Allele Type and Allilic Frequencies

	Allelotypes			
	A (n)	a.f.	G (n)	a.f.
Non-Smokers (n)	540	0.9	60	0.1
Smokers (n)	552	0.92	48	0.08

*χ^2 (1 d. f.) = 1.231; p=0.267 for allelic frequency; a.f.=allelic frequency

Table 3. Odds Ratio with 95%CI of Mdm2 (rs769412) Gene in Smokers

Genotypes		Odds Ratio	95%CI	p-value
	AA versus AG	0.517	0.320-0.835	0.006
	AA versus GG	2.793	0.747-10.438	0.112
	AG versus GG	5.4	1.357-21.481	0.013
Dominant Model	AA versus AG+GG	0.637	0.409-0.992	0.045
Recessive Model	GG versus AG+GG	0.228	0.058-0.896	0.024
Allelotypes	A/G	0.783	0.526-1.165	0.226

among AA, AG and GG genotypes of smokers and non-smokers (χ^2=10.5, p<0.005). Also, A or G allele was not found to be a significant factor associated with smokers (χ^2=1.23, p=0.26) (Table-2). Hence, these groups were combined before further statistical analysis (Table 3). Data showed that AA versus AG, AG versus GG and dominant model AA versus AG+GG as well as recessive model GG versus AG+GG genotypes exhibited significant difference between smokers and non-smokers (OR, 0.515 (95%CI 0.319-0.735), p=0.006; OR 5.4 (95%CI 1.357-21.481), p=0.01; OR 0.637 (95%CI 0.410-0.972), p=0.04; OR 0.218 (95%CI 0.048-0.886), p=0.02 respectively). Other model AA versus GG did not showed any significant differences (p= 0.11) (Table-3).

Frequency of A allele was 0.9 and 0.92 among non-smokers and smokers respectively whereas, for G allele it was 0.1 and 0.08 among and non-smokers and smokers respectively. Neither the A allele nor the G alleles were significantly associated with smokers. However a higher percentage of AA in comparison of lower percentage of AG genotypes in smokers suggests that transition mutation of A allele to G favor the smoking behavior. In other words this case a reverse mutation G>A have a protective role against cigarette smoking and that seems to be a factor for A to G transition mutation and heterozygous condition at rs769412 in Mdm2 gene.

Discussion

Cigarette smoking is an ongoing a major health threat, which contributes considerably to cancer of nasal cavities, nasopharynx, liver, kidney (renal cell carcinoma), stomach and uterine cervix, lung, oral cavity, larynx, urinary bladder, esophagus, pharynx, pancreas, renal pelvis for adenocarcinoma of the oesophagus, and myeloid leukemia (Sasco et al., 2004).Widespread cigarette smoke comprises of 8% tar, 92% gaseous components, which contain more than 1015 active free radicals/puff (Pryor and Stone 1993). In cigarette smoke there are 55 carcinogenic compounds have been reported, polycyclic aromatic hydrocarbons (PAH), Nitrosamines, Heterocyclic amines, Aza-arenes and aromatic amines etc. that have been evaluated by the International Agency for Research on Cancer (IARC) (Hecht., 2012). Many active free radicals and other oxidants present in all kinds of cigarette smoke are capable of oxidizing the biomolecules including DNA and may result in consequential disorders such as altered gene expression and cancer (Pryor and Stone., 1993).

It is well certitude fact that lung cancer is instigate by the smoking, which is the major cause of mortality in European country where as in America the survival rate of lung cancer patients are greater as 43%. Tobacco smoking is the main factor attributed to lung cancer, but its likeliness is probably enhanced by common genetic variations. The p^{53} triggers cell cycle arrest, senescence, DNA repair and apoptosis when cells are exposed to numerous forms of cellular stress, including DNA damage. However despite smoking being a major contributor to lung cancer, not all smokers develop it (Liu et al., 2005). Giving rise to the possibility that certain individuals might be more vulnerable to cigarette smoke. Studies demonstrating familial aggregation by Schwartz and Ruckdeschel, 2006 have given evidence of a genetic component responsible for lung cancer risk. All in all, inclination to lung cancer might be a limited extend to the inter individual genetic variation in the form common allele variants or single nucleotide polymorphisms (SNP).

Problematic inactivation of the TP^{53} tumor suppressor gene in lung carcinogenesis is a recurring, early event. Consistent with the p^{53} tumor suppressor functions, including growth arrest, triggers cell cycle arrest, senescence, DNA repair and apoptosis when cells are exposed to various forms of cellular stress, including DNA damage. About >90% of small cell lung cancers and >50% of non–small cell lung cancers have p^{53} mutations (Mechanic et al., 2007). Mdm2 is interrelated with p^{53}, which play an important role for blocking its transcriptional activity. (Freedman and Levine., 1999).

Recently there has been great interest in studying the association of lung cancer with SNPs, which are the main sources of human genetic variation and may increase individual's vulnerability to cancer (Boersma et al., 2006). Mdm2 SNP354 (rs769412) causes an A>G base change at codon 354 which generates Sp1 binding site, though it is not leading to amino acid change. However, Rajaraman et al have found associated of Mdm2 SNP354 (rs769412) with breast cancer (Rajaraman et al., 2007). In an African-American population study of Mdm2 SNP354 (rs769412) showed the lack of association between occurrence of lung cancer in smokers (Pine et al, 2006). However, in one study, Rajaraman (2007) reported the protective role of Ex12+162A>G (rs769412) in case of galioma cancer.

There have been a few studies on rs769412 of Mdm2gene for their association of lung cancer due to smoking behavior. The present study analyzes for the first time the Mdm2 variant (rs769412) A>G in Saudi Arabian smokers. Results indicated that Mdm2 (rs769412) A>G single nucleotide polymorphism was found significantly associated with non-smokers (p=0.005) (Table1). Our findings are in consistence with the previous findings (Boersma 2006 and Rajaraman 2007), but not supported byPine et al., 2006).

However, the lack of the available literatures and the extensive studies on the variation of Mdm2(rs769412)

A>G and their interrelation with the lung cancer and smoking behavior jeopardizes the supports to our finding. Hence, there is a great need of study the variation in different populations for different disease. Nevertheless, this study may be useful for the further researches on Mdm2 SNPs in smokers and their associated diseases.

References

American Cancer Society (2012). Cancer treatment and survivorship facts & figures 2012-2013. Atlanta.

Bassiony MM (2009). Smoking in Saudi Arabia. *Saudi Medical J*, **30**, 876-81.

Bjartveit K, Tverdal A (2005). Health consequences of smoking 1-4 cigarettes per day. *Tob Control*, **14**, 315-20.

Boersma BJ, Howe TM, Goodman JE (2006). Association of breast cancer outcome with status of p^{53} and MDM2 SNP309. *J Natl Cancer Inst*, **98**, 911-9.

Doll R, Peto R, Wheatley K, et al (1994). Mortality in relation to smoking: 40 years' observations on male British doctors. *BMJ*, **309**, 901-11.

Freedman DA, Levine AJ (1999). Regulation of the p^{53} protein by the MDM2 oncoprotein: Thirty-Eighth GHA Clowes Memorial Award Lecture. *Cancer Res*, **59**, 1-7.

Hecht SS (2012). Lung carcinogenesis by tobacco smoke, *Int J Cancer*, **131,** 2724-32**.**

Hong Y, Miao X, Zhang X (2005). The role of P^{53} and MDM2 polymorphisms in the risk of esophageal squamous cell carcinoma. *Cancer Res*, **15**, 9582-587.

Hu Z, Ma H, Lu D (2006). Genetic variants in the MDM2 promoter and lung cancer risk in a Chinese population. *Int J Cancer*, **1**, 1275-278.

Iwakuma T, Lozano G (2003). MDM2, an introduction. *Mol Cancer Res*, **1**, 993-1000

Knappskog S, Lonning PE (2011). Effects of the MDM2 promoter SNP285 and SNP309 on Sp1 transcription factor binding and cancer risk. *Transcription*, **11**, 207-10.

Liu G, Zhou W, Christiani DC (2005). Molecular epidemiology of non-small cell lung cancer. *Semin Respir Crit Care Med*, **26**, 265-72.

Lum SS, Chua HW, Li H et al (2008). MDM2 SNP309 G allele increases risk but the T allele is associated with earlier onset age of sporadic breast cancers in the Chinese population. *Carcinogenesis*, **29**, 754-61.

Mechanic LE, Bowman ED, Welsh JA et al (2007). Common genetic variation in TP^{53} is associated with lung cancer risk and prognosis in African Americans and somatic mutations in lung tumors. *Cancer Epidemiol Biomarkers Prev*, **16**, 214-22.

Oliner JD, Kinzler KW, Meltzer PS et al (1992). Amplification of a gene encoding a p^{53}-associated protein in human sarcomas. *Nature*, **358**, 80-83.

Onat OE, Tez M, Ozçelik T (2006). MDM2 T309G polymorphism is associated with bladder cancer. *Anticancer Res*, **26**, 3473-475.

Pine SR, Mechanic LE, Bowman ED, et al (2006). MDM2 SNP309 and SNP354 are not associated with lung cancer risk. *Cancer Epidemiol Biomarkers Prev*, **15**, 1559-561.

Pryor WA, Stone, K (1993). Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. *Ann N Y Acad Sci*, **686**, 12-27.

Rajaraman P, Wang SS, Rothman N et al (2007). Polymorphisms in apoptosis and cell cycle control genes and risk of brain tumors in adults. *Cancer Epidemiol Biomarkers Prev*, **16**, 1655-61.

Sasco AJ, Secretan MB, Straif K (2004). Tobacco smoking and cancer: a brief review of recent epidemiological evidence. *Lung Cancer*, **2**, 3-9.

Schane RE, Ling PM, Glantz SA (2010). Review health effects of light and intermittent smoking. A review. *Circulation*. **121**, 1518-22.

Schwartz AG, Ruckdeschel JC (2006). Familial lung cancer: genetic susceptibility and relationship to chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*, **173**, 16-22.

Shields P, Harris C (2000). Cancer risk and low-penetrance susceptibility genes in gene- environment interactions. *J Clin Oncol*, **18**, 2309-15.

United Kingdom Lung Cancer Coalition, Lung Cancer Plan (2007). Improving lung cancer survival in the UK, UK Lung Cancer Coalition November.

World Health Organization (2008). Report on the global tobacco epidemic, 2008-The MPOWER package, Geneva.

World Health Organization (2011). Report on the global tobacco epidemic (2011). Warning about the dangers of tobacco. Geneva: Switzerland.

Zhou W, Liu G, Park S (2005). Gene-smoking interaction associations for the ERRC1 polymorphisms in the risk of lung cancer. *Cancer Epidemiol Biomarkers Prev*, **14**, 491-6.

Zhuo W, Zhang L, Zhu B (2012). Association of MDM2 SNP309 variation with lung cancer risk: evidence from 7196 cases and 8456 controls. *PLoS ONE*. **7**, 41546.