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Effect of Genetic Polymorphisms of the Tumor Suppressor Gene (TP53) Pathway in Saudi Smokers and Non-Smokers

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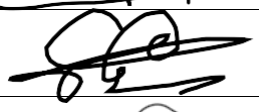
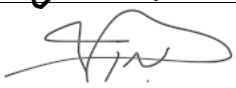
Prof. Dr. Saad Hussin Alkahtani	
Dr. Bader Obaid Almutairi	

22nd of April 2020

Dedication

This thesis is dedicated
To my Father
To my Mother God have mercy on her
To my Wife
To my children (Abdullah, Khaled and Rima)
For their endless love, support and encouragement

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First of all, my appreciation and thanks go to Allah the greatest for giving me the strength and ability to complete this master thesis and throughout my life. Then, I would like to express my sincere gratitude to my supervisor Dr. Mikhlid H Almutairi, for his continuous encouragement, guidance, patience, supervision and invaluable assistance throughout the project research and during writing of this thesis. He is not only a great supervisor, but also a great teacher in my life. Huge thanks also go to Dr. Bader O Almutairi for his guidance, advice, support, patience and encouragement during writing of this thesis.

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English Abstract

Background: Tumor suppresser gene (TP53) and its downstream genes (P21 and MDM2) play crucial roles in the regulation of DNA damage checkpoint (G1/S) of the cell cycle. Genetic polymorphisms in those genes have been implicated in developing various smoking-related diseases (SRDs). The aim of this study was to examine the potential association between cigarette smoking (CS) and the single nucleotide polymorphisms (SNPs) located in TP53, P21, and MDM2 genes, situated in the exons regions, among smokers and non-smoker from Saudi Arabian population.

Methods: TP53 rs1042522 (C/G), P21 rs1801270 (A/C), and MDM2 rs769412 (A/G) were investigated by genotyping 568 blood specimens, 283 were obtained from male and female smokers, and 285 were obtained from male and female non-smokers.

Results: Genetic and allelic alterations were detected between the rs1042522 variant tested here and the smoking patients in terms of patient age, patient gender, duration of CS, daily rate of CS, and types of smoking among Saudi smokers as compared to the control individuals. P21 rs1801270 polymorphism, however, was associated with allelic differences in smokers whose ages fall under 29 years, below 7 years of CS consumption, and among smokers who smoke shisha. In addition, our results showed that no genetic and allelic variations were detected between the MDM2 SNP rs769412 and the smoking subjects in all clinical parameters mentioned previously.

Conclusion: Our results demonstrate that rs1042522 polymorphism has a higher effector role in all clinical parameters of the smoking population, which increases the potential risk of developing SRDs. Therefore, this polymorphism could be used as a novel diagnostic biomarker for the early diagnosis of several diseases caused by CS in the Saudi population.

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List of Abbreviations

ASW	African ancestry in Southwest USA
ATM	Ataxia telangiectasia mutated kinase
CDKN1A	Cyclin-dependent kinase inhibitor 1A
CDKs	Cyclin-dependent kinases
CEU	Utah residents with Northern and Western European ancestry from the CEPH collection
CHD	Chinese in Metropolitan Denver, Colorado
CI	Confidence intervals
CO	Carbon monoxide
CRS	Riyadh region population in Saudi Arabia
CS	Cigarette smoking
CTD	C-terminal domain
Cyc	Cyclin
DBD	DNA binding domain
DNA	Deoxyribonucleic acid
DNA-PK	DNA-dependent protein kinase
EDTA	Ethylenediaminetetraacetic acid
FRET	Fluorescence resonance energy transfer
G1	Gap-1 phase
GIH	Gujarati Indians in Houston, Texas
HCB	Han Chinese in Beijing, China
JPT	Japanese in Tokyo, Japan
KD	Kilodalton
LWK	Luhya in Webuye, Kenya
MDM2	Murine Double Minute 2 gene
Mdm2	Murine double minute 2 protein
MEX	Mexican ancestry in Los Angeles, California

MGB	Minor Groove Binder
miRNA	Micro RNA
MKK	Maasai in Kinyawa, Kenya
ml	Milliliter
mRNA	Messenger RNA
NCBI	National Centre for Biotechnology Information
NES	Nuclear export signal
NFQ	Non-fluorescent quencher dye
ng	Nanogram
NLS	Nuclear localization signal
OD	Tetramerization domain
OR	Odds ratio
P21	P21 gene
p21	P21 protein
p53	Tumor suppressor protein
PCNA	proliferating cell nuclear antigen
PCR	Polymerase chain reaction
pRb	Retinoblastoma protein
PRR	Proline-rich region
RNA	Ribonucleic acid
rpm	Rotation per minute
S	Synthesis phase
SNP	Single nucleotide polymorphism
SRDs	Smoking-Related Diseases
TAD	Transactivation domain
TP53	Tumor suppressor gene
TSI	Toscans in Italy
UTRs	Untranslated regions
X^2	chi-squared

YRI	Yoruba in Ibadan, Nigeria
Zn	Zinc
µl	Microliter

Chapter 1: Introduction

1.1 Overview of Cigarette Smoking; Statistics and Facts

Cigarette smoking (CS) is a prime public health issue in both developed and developing countries (West, 2017). CS is considered as one of the major threats to world health at present and future (Edwards, 2004). It is toxic and contains many harmful constituents which effect on smokers and secondhand smokers (Pappas, 2011).

According to the International Agency for Research on Cancer, CS contains of 5000 chemical compounds, 81 chemical compounds of them are fully characterized “carcinogenic” (Smith et al., 2003) (Figure 1.1). There are three basic components exist in cigarettes, which are nicotine, carbon monoxide (CO) and tar, which are found to be implicated in numerous health problems (Calafat et al., 2004). For example, nicotine considers as a toxin that directly addictive or may enhance addiction (Lee et al., 2012), and may cause various disorders of central nervous system, gastrointestinal tract and immune system via its interaction with other risk factors, such as genetic susceptibility (Thomas et al., 2005). Also, CO considers as immunomodulatory toxic (Lee et al., 2012), and may increase the risk of smoking-induced cardiovascular diseases (Zevin et al., 2001). Moreover, cigarettes contain of other chemicals components, such as organic compounds,

inorganic compounds, aldehydes, aromatic amines, N-heterocyclic amines, N-nitrosamines polycyclic aromatic hydrocarbons, aza-arenes and metals (Figure 1.1), which may contribute to cardiopulmonary diseases and cancer (Hoffmann & I, 1997; Shihadeh et al., 2015).

Although CS contains different factor risks for the human body, there are approximately 1135 million smokers (928 million males and 207 million females) worldwide based on a study performed in 2012 (WHO, 2014). CS causes about five million deaths around the world due to Smoking-Related Diseases (SRDs) (Peto & Lopez, 2004). According to the World Health Organization, CS has been classified as the second leading risk factor for mortality worldwide (WHO, 2009).

In the Kingdom of Saudi Arabia (KSA), CS has existed for more than 50 years, despite it does not grow tobacco plant or manufacturing cigarettes or other tobacco products (Jarallah et al., 1999). There are numerous smokers among the Saudi population in both sexes (Al-Nozha et al., 2009); however, the prevalence of current CS is much higher in men than in women at various ages (Bassiony, 2009). According to the National Cancer Institute, at the age of more than 15 years, the number of Saudi smokers is approximately 37.6% in men

and 6% in women (Goffman, 2009). In fact, among the Saudi Arabian individuals, CS is identified as one of the serious risk factors for chronic obstructive lung disease (Al Ghobain et al., 2015) and coronary artery disease (Al-Nozha et al., 2009).

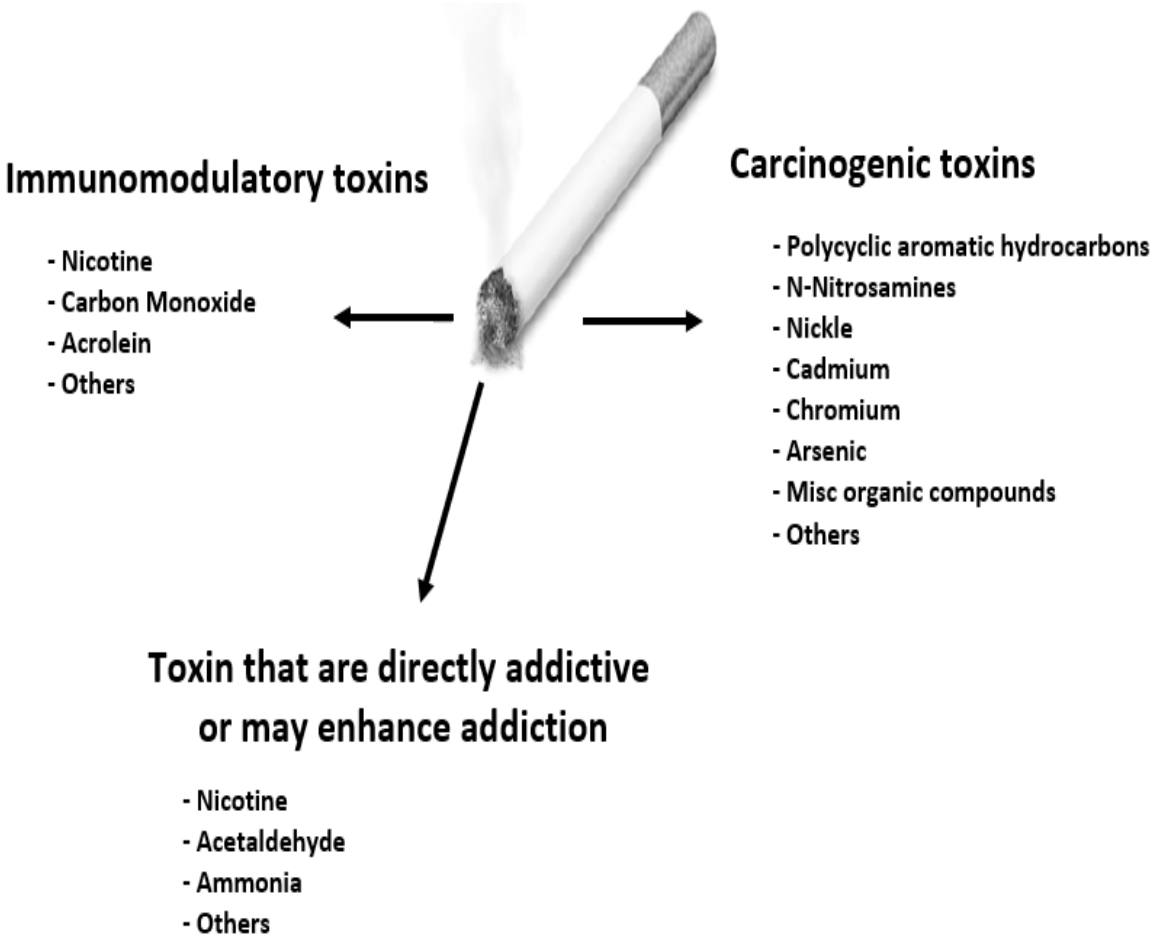

Figure 1.1: The components of cigarettes. Cigarettes contain carcinogenic toxins, immunomodulatory toxic and toxin that directly addictive or may enhance addiction (Adapted with modified from Lee et al., 2012).

1.2 The Relationship Between CS and Diseases

Human body consists of billions of small living units called cells that grow and divide normally as the body needed. Normally, these cells died and replaced by new cells when become old or damaged. When some cells of the body divide rapidly more than the body needed, they will become cancerous cells and may cause disease called cancer or tumor, and they have the ability to initiate anywhere on the human body (NCI, 2015).

In 1920s, the epidemiological index of the connection between CS and cancer began to arise. By 1950s, the role of CS in lung cancer was detected (Levin et al., 1950; Doll & Hill, 1964). Since then, evidence of the relationship between CS and cancer begin to accumulate. Moreover, CS may contribute to the development of various types of cancers, including lung cancer, oral cavity cancer, conductive airways cancer (Seifart & Plagens, 2007; Gibbons et al., 2014; Uppal et al., 2014; Kheradmand et al., 2017), breast cancer (Verde et al., 2016), mucinous ovarian cancer (Jordan et al., 2006), bladder, colorectal cancers (Kytola et al., 2017), and pancreatic cancer (Blackford et al., 2009). Also, the effect of CS has been related with the nose, stomach, liver, renal, colon, and myeloid leukemia cancers,

although the association to these tumours is weak (Doll, 1996, Chao et al., 2000).

Despite CS contributes to increase the risk of multiple diverse types of cancers, lung cancer is more closely associated with CS. Before the commercial production of cigarettes began, the incidence rate of lung cancer was so rare, but clinical observations and epidemiologic studies suggested a potential causative association between CS and an increase in lung cancer cases (White, 1990; Ochsner & DeBakey, 1999). Now, lung cancer become a major type of cancers that leading deaths in men and the second in women worldwide (Torre et al., 2015). Based on study of Robert N. Proctor (Proctor, 2001), it reported that CS is responsible for the majority of lung cancer cases of deaths. In 2012, it was estimated that 1,590,000 people around the world died due to lung cancer (Islami et al., 2015). There is irrefutable proof that CS is the main cause of bronchogenic carcinoma in approximately 85% - 90% of lung cancer victims. Also, there is evidence that the exposure of environmental tobacco smoke may lead to lung cancer in life-long non-smokers (Hackshaw et al., 1997; Blot & McLaughlin, 1998).

CS has been considered as the most extensively the behavioral risk factors. Furthermore, CS has been determined as the epidemic that causes many health effects and initiates various types of SRDs (Figure 1.2) (Kuper et al., 2002; Burns, 2003); for example, chronic pulmonary disease (Kopa & Pawliczak, 2018), cardiovascular disease, asthma (Kovacs et al., 2012), and periodontal disease (Kinane & Chestnutt, 2000). Numerous of studies have been suggested that CS may affect on another parts of body, including lower urinary tract such as the bladder and renal pelvis (Talaska et al., 1991; Airoldi et al., 2002). Also, CS may affect on digestive tract, including oral cavity, larynx, pharynx and esophagus (Akiba &. Hirayama, 1990; Vaughan et al., 1996), these parts may be converting into cancerous (Akiba &. Hirayama, 1990; Talaska et al., 1991; Vaughan et al., 1996; Airoldi et al., 2002). Moreover, examining the relationship between CS and diabetes mellitus have been detected, which may contribute to increase the potential of diabetes mellitus especially among heavy smokers (Rimm et al., 1993).

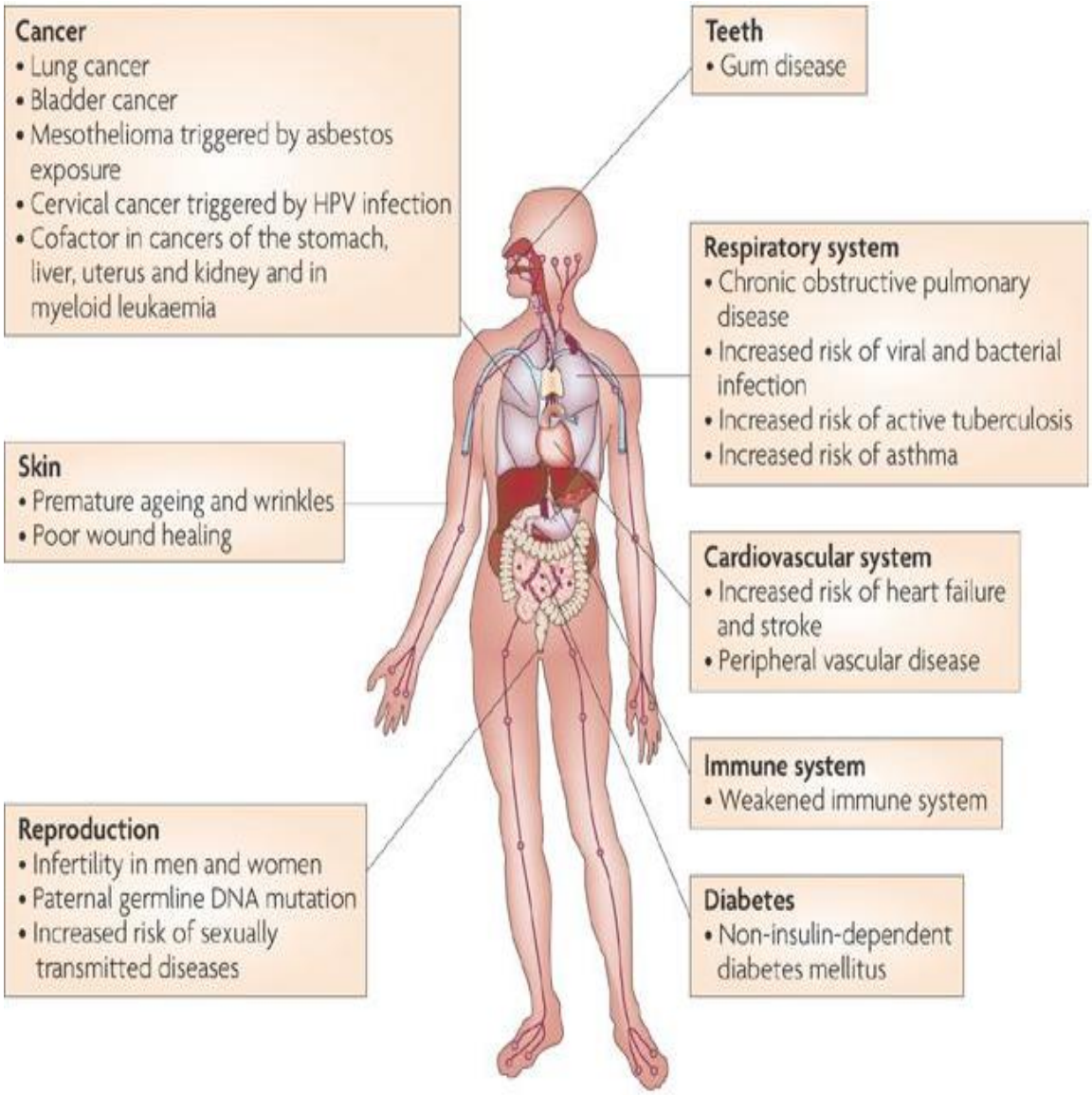

Figure 1.2: Diverse effects of SC. It has various effects on every aspect of human body health. CS causes most deaths that related with non-malignant cardiovascular, respiratory conditions and most types of cancer. CS associated with diverse health risks such as weakness of immune system, diabetes, poor wound healing, decreased fertility in males and females and an increased risk of sexually transmitted diseases. (Adapted from Stämpfli & Anderson, 2009).

1.3 CS and DNA Damage

DNA damage is defined as any modification of the usual double helical structure of DNA that changes its coding properties or normal function in transcription or replication processes (Lindahl, 1993; Rao, 1993). DNA damage is a common occurrence in a cell's life (Sancar et al., 2004), and is considered as environmental agent that can leads to tumor initiation (Lowe et al., 2004). The structure of DNA can be damaged by exogenous agents that may cause mutations, cellular death, and cancers (Sancar et al., 2004). Among these exogenous agents, CS components may lead to development of mutations in nonmalignant tissues (Boran et al., 2017; Dylawerska et al., 2017).

1.4 CS and Mutations

A mutation is identified as a change in the nucleotide sequence of DNA molecule (Rosenberg & Rosenberg, 2012), and occurs with a frequency of less than 1% of normal population (Auer et al., 2012). There are many types of mutations, which can change the wild-type (the usual) DNA sequence (Figure 1.3). The common type of mutation is a substitution mutation, which occurs when a single base of one strand of DNA is replaced by another one. When one purine is substituted by another purine (for example, A to G) or one pyrimidine

is substituted by another pyrimidine (e.g., C to T) is called a transition mutation. However, when one purine is substituted by a pyrimidine (such as A to C) is called a transversion mutation (Rosenberg & Rosenberg, 2012).

Other types of mutations are rarer and more complex than substitutions, including a deletion mutation (occurs due to loss one or more nucleotide pairs in a DNA molecule), an insertion mutation (occurs due to addition one or more nucleotide pairs in a DNA molecule), an inversion mutation (occurs due to a 180° rotation of a segment of DNA without either loss or gain nucleotides in DNA sequence) (Rosenberg & Rosenberg, 2012).

In addition, there are other terms of mutations such as missense or non-synonymous mutation (occurs by replacement of one nucleotide in codon that changes one amino acid), silent mutations or synonymous mutation (occurs by replacement of one nucleotide in codon, but do not change amino acid), nonsense mutation (occurs by replacing one nucleotide that converts amino acid codon into stop codon, which leads to a shorter, or usually nonfunctional protein) and frameshift mutation (occurs by insertion or deletion one or two nucleotides that changes a gene's reading frame, which leads to

changing all subsequent amino acid remains in the growing polypeptide chain (Rosenberg & Rosenberg, 2012).

Most of these mutations are spontaneous, which occur during DNA replication, gene transcription or in the absence of any known cause, but sometimes they occur due to DNA damage by exposure to exogenous agents (Rosenberg & Rosenberg, 2012), such as CS (Yauk et al., 2007). These mutations are generally repaired by DNA repair pathways, including TP53 pathway in the human genome; however, if any mutation cases by CS among TP53 pathway genes and it does not repair, it may contribute to cancer initiation among diverse Human ethnicities (Gibbons et al., 2014; Liu et al., 2014; Wu et al., 2015; Kytola et al., 2017).

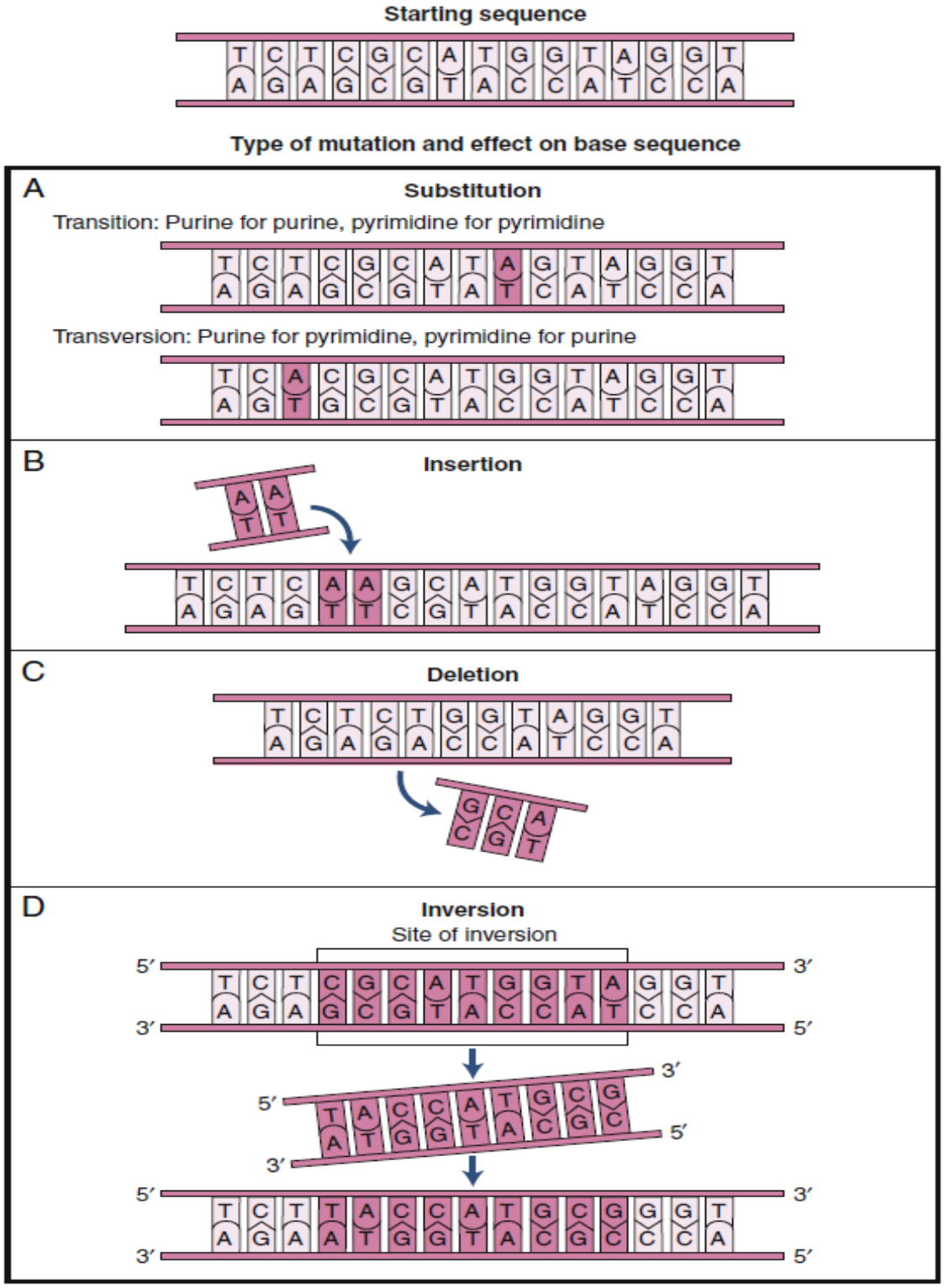

Figure 1.3: Types of mutations. Types of mutations occur in DNA molecule including substitution, insertion, deletion and inversion mutations (Adapted from Rosenberg & Rosenberg, 2012).

1.5 CS and Single Nucleotide Polymorphism

A single nucleotide polymorphism (SNP) is an alteration that occurs by substitution at a single base in a DNA sequence (Guo & Sun, 2002), and occurs with a frequency of 1% or greater of the population (Brookes, 1999). SNPs are the most common type of genetic variations in human genome, representing about 90% of sequence variations (Collins et al., 1998). It has been estimated that SNPs may frequent about one per 1000 bases (Taillon-Miller et al., 1998). Investigations have been shown that SNPs can occur in different regions of the genome, including coding sequences, introns, promoters and 5'- and 3'- untranslated regions (UTRs) (Deng et al., 2017). SNPs affect on the gene expression process by different ways depend on their location in the DNA genome. For example, SNPs affect on gene transcription and translation mechanisms if they occur in the exons which may alter the amino acid sequence of protein. However, if they occur within an intron or promoter, they might affect alternative splicing process of the mRNA or increased/decreased transcription, respectively (Abbas & Dutta, 2009; Deng et al., 2017). They also affect on mRNA stability and translation if they occur in the

UTRs regions (Aouacheria et al., 2007, Abbas & Dutta, 2009) (Figure 1.4).

SNPs have been widely detected in diverse diseases (Bonassi et al., 2005) and in various types of cancers, such as colon, head and neck, and breast cancers (Jelonek et al., 2010). SNPs in dominant oncogenes and tumor suppressor genes are associated with prevalence of new lung cancers (Eydian et al., 2016). Moreover, many of studies have shown that CS may implicate in the occurrence of the numerous of SNPs that initiate many types of cancers, such as lung, head and neck, laryngeal, gastric, colorectal and bladder cancers (Matullo et al., 2005; Stern et al., 2007; Werbrouck et al., 2008; Bau et al., 2009; Liu et al., 2010; Li et al., 2014). Therefore, examining genetic variations like SNPs and their implications of causing diseases could result in clinical progressions, via discovering precise genetic markers, which help to development of diagnosis, disease prevention as well as therapy (Saenko & Rogounovitch, 2018).

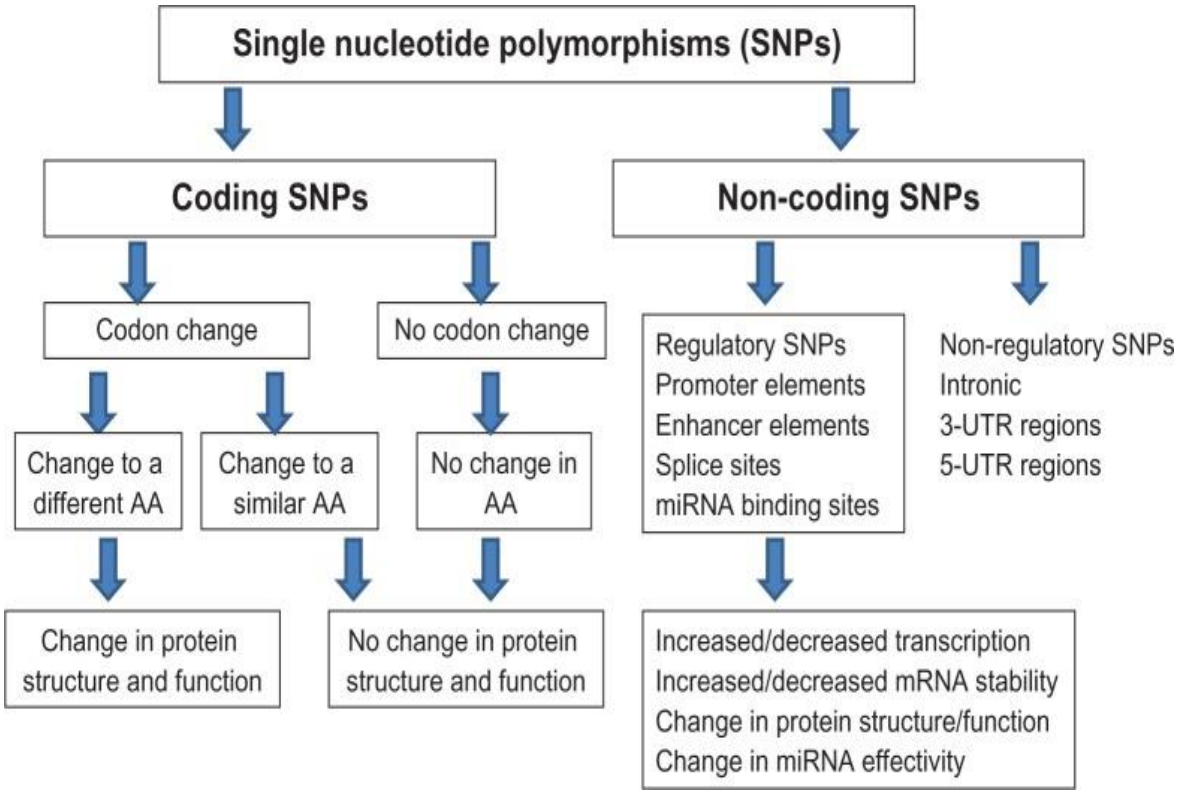

Figure 1.4: The biological consequences of SNPs types based on their location within genome. SNPs taking place in the coding sequences, exons, could result in codon change resulting in an amino acid (AA) or no change in an amino acid AA. Changes in AA may result in either change in protein structure or function. Noncoding SNPs may potentially result in changes in mRNA stability, transcription, protein structure or function, or change in miRNA binding site (Adapted from Abbas & Dutta, 2009).

1.6 Mechanism of TP53 and Its Downstream Genes in Repair of DNA Damage

TP53 and its downstream genes (P21 and MDM2) play crucial roles in the regulation of DNA damage (G1/S) checkpoint (Chen et al., 1994; Karimian et al., 2016). The whole process is divided into the following steps: (1) after DNA damage, the ataxia telangiectasia mutated kinase (ATM) (Maya et al., 2001) or DNA-dependent protein kinase (DNA-PK) (Shieh et al., 1997) rapidly phosphorylates p53, which enhance stability and activity of p53 due to releasing Mdm2 oncoprotein, which acts as negative regulator of p53 (Shieh et al., 1997; Maya et al., 2001), (2) once the p53 protein is activated, it induces P21 gene to encode p21 protein, (3) p21 protein can bind with Cyclin-dependent kinases (CDKs) and repress their activities, which leading to cell cycle arrest (El-Deiry et al., 1994; Jiang et al., 1994; Abbas and Dutta, 2009), (4) and dephosphorylation of retinoblastoma protein (pRb) (Broude et al., 2007), (5) which leads to increase an association of pRb and the E2F transcription factor that prevents the transition from the G1 phase to S phase (Zhang et al., 1999). This G1 arrest gives the cell time to repair the DNA damage (Satyanarayana et al., 2008; Neganova et al., 2011). Consequently, variation of SNPs of

any of cell cycle regulatory genes, such as TP53, P21 and MDM2 due to CS manner could cause a cell cycle deregulation, which may initiate a cancer (Wang et al., 2015; Ren et al., 2013).

1.7 The Relationship Between Mutation and Polymorphisms in TP53 Pathway Genes and Diseases

1.7.1 Effect of Mutation, and Polymorphisms in TP53 Gene

TP53 is described as a fundamental tumor suppressor gene or anti-oncogene (Kamada, 2016). In human, it mapped on the short arm of chromosome 17 (17pl3) (Catherwood et al., 2019). It encodes 53-KD (Kilodalton) nuclear phosphoprotein (p53) (Xu et al., 2017), which consists of 393 amino acid (Catherwood et al., 2019). The p53 protein comprises of several functional domains (Figure 1.5) which are a transactivation domain (TAD) (residues 1–61), a proline-rich region (PRR) (residues 61–94), a DNA binding domain (DBD) (residues 94–292), a linker region (residues 292–325), a tetramerization domain (OD) (residues 325–356), and C-terminal domain (CTD) (residues 356–393) (Vogelstein et al., 2000; Joerger & Fersht, 2010). TAD is responsible for binding with Mdm2 that controls the cellular activity of p53 (Weinberg et al., 2004; Toledo & Wahl, 2006), PRR is consists of five PXXP motifs (Hong et al., 2010) and plays a key role in the induction of apoptosis (Campbell et al., 2013), DBD is a core domain and plays an essential role in sequence-specific DNA binding (Joerger & Fersht, 2008; Chansaenroj et al.,

2013), OD is responsable for post-translational modifications and protein–protein interactions (Chene, 2001), and CTD is essential for binding to different partner proteins (Iida et al., 2016).

The p53 plays a key role in the expression regulation and enhancing various activities of same effectors of cellular processes, such as cell-cycle arrest, proliferation, apoptosis, and DNA repair (Jaiswal et al., 2013). These P53-mediated responses are essential role not only in anti-cancer prevention in human, but also in responding to commonly used cancer treatments (Bourdon, 2007; Ferraiuolo et al., 2017; Cabezas et al., 2019).

Figure 1.5: Domain structure of p53 protein. The first domain of p53 which located in the N-terminal region is the transactivation domain (TAD), divided into two basic subdomains TAD1 and TAD2, the second domain is the proline rich region (PRR), flowed by the core region of p53 which is DNA-binding domain (DBD), then the linker region connect between the core domain and the tetramerization domain (OD), and the last domain of p53 that located in the C-terminal region is the C-terminal domain (Adapted with modified from (Joerger & Fersht, 2010)).

It is common for tumor suppressor genes to be inactivated by nonsense or frameshift mutations, while most TP53 mutations are missense, which causes the substitution of single amino acid at different sites (Petitjean et al., 2007, Kamada et al., 2016), and most of these mutations occur in DBD (Valverde et al., 2016). When such mutations occur in critical regions of the p53 gene, the result may be loss the mechanisms of normal growth control and can also promote cancers initiation (Hecht, 2018). Studies examining the role of p53 in tissue have revealed that p53 staining was more common and intense in the smoking group comparing to control group. This is because smoking could cause a mutation in the TP53 gene, which can lead to increase the mutated gene transcription (Toptaş et al., 2015). Mutations in TP53 gene is identified as the most common alteration where it is seen in 50% of human tumors (Cheok et al., 2011). The prevalence rate of TP53 mutations in various human tumors ranging from ~ 35% -50% in ovarian, colorectal, esophageal, head, neck, laryngeal, lung and skin tumors to ~ 6% in cervical tumor (Petitjean et al., 2007) (Figure 1.6). Furthermore, several studies mentioned that TP53 mutations are found in approximately 40% of human lung cancers and are widespread in smokers rather than non-smokers

(Greenblatt et al., 1994; Hernandez-Boussard & Hainaut, 1998). Also, TP53 mutations have been linked to CS in squamous cell carcinoma of head and neck cancers as well as on esophageal cancer and bladder cancer (Harris et al., 1993; Brennan et al., 1995). Moreover, it has been mentioned that treated squamous epithelial cells in the oral cavity with carcinogens in smoker can initiate neoplastic transformations. However, the exact mechanism of how CS results in neoplastic changes is still unclear (Pfeifer et al., 2002).

Single nucleotide substitution in the coding sequence of TP53 may be an indication of most types of cancer in human and lead to defect in the function of p53 (Whibley et al., 2009). Moreover, polymorphism in TP53 gene may contribute to alter p53 protein function (Murphy, 2006). Many functional SNPs at TP53 gene have been mentioned to be implicated with risk of initiating several types of human cancers (Yan et al., 2009; Francisco et al., 2011; Bellini et al., 2012; Karim, 2014) such as lung cancer (Eydian et al., 2016). Researchers have identified more than 200 SNPs in the TP53 gene (Whibley et al., 2009), including codon 72 polymorphism in exon 4, that occurs due to the replacement of C to G [substitution of proline (Pro) by arginine (Arg); rs1042522]. This type of p53 polymorphism

shows a remarkable alteration in the structure of the P53 protein (El Ghorayeb et al., 2016), and has been associated with cancer development in many ethnicity and CS manner (Wu et al., 2002; Lin et al., 2018). The P72R (rs1042522) SNP influence occurs in the PRR of p53 and affects on the apoptotic function of the p53 (Sakamuro et al., 1997; Pietsch et al., 2006; Khan et al., 2016). A previous study provided evidence that a possible positive correlation between smoking habits and rs1042522 polymorphism in TP53 in contrast with non-smokers (Francisco et al., 2011). Another study revealed that two SNPs (14181 (T>C) and 14201 (G>T)) in intron 7 of p53 gene are significantly correlated with the lung cancer risk (Phang et al., 2011). Also, the alteration in polymorphisms at p53 exon 4, introns 3 and 6 in smoker could be an important cause for lung cancer progression in the Caucasian population (Schabath et al., 2006). For this reason, in this thesis, we investigated the possible correlation between TP35 SNPs among smokers and non-smokers from Saudi Arabian population.

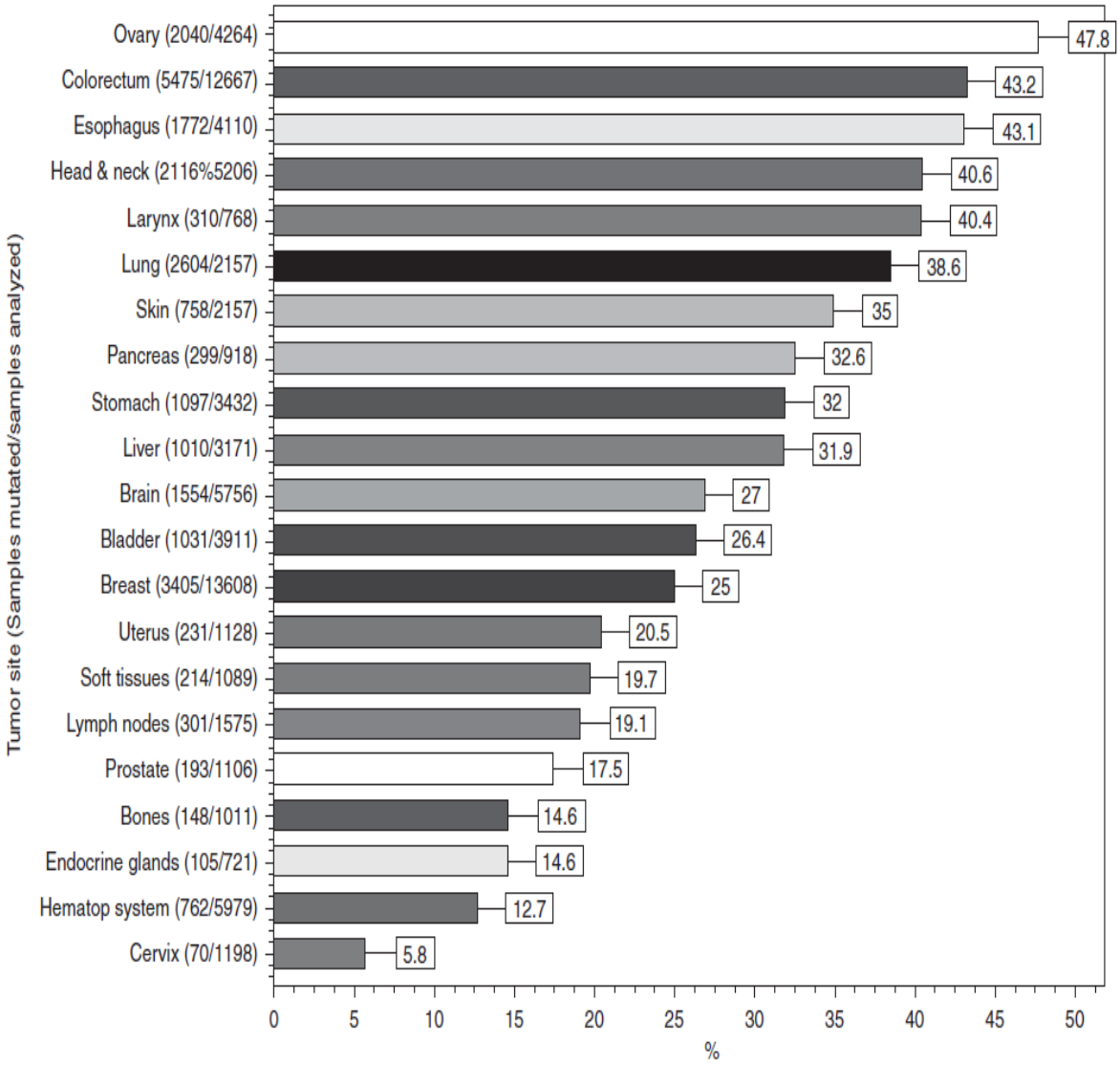

Figure 1.6: TP53 mutations prevalence in sporadic cancers. The proportion of tumors with somatic TP53 mutations is indicated. Data from IARC TP53 Database (R13, November 2008) (Adapted from (Petitjean et al., 2007)).

1.7.2 Effect of Mutation, and Polymorphisms in P21 Gene

P21 gene (also known as cyclin-dependent kinase inhibitor 1A, CDKN1A gene) is located on chromosome 6 short arm (6p21.2). It encodes a 21-kD protein called p21 which consists of 164 amino acids (El-Deiry et al., 1993; Coactivators & Schönthal, 2017). P21 protein has two cyclin-binding domains (Figure 1.7) which are cyc1(residues 17-24) and cyc2 (residues 153-159), a CDK domain (residues 53-58), and nuclear localization signal (NLS) domain (residues 140-153), as well as a proliferating cell nuclear antigen (PCNA) domain (residues 141-160) (Cazzalini et al., 2010). P21 binds with CDK subunit through CDK domain and with cyclin subunit through cyc1 or cyc2 domain to inhibiting the activity of cyclin-CDK complex (Chen et al., 1996; Al Bitar & Gali-Muhtasib, 2019). It can also bind and inhibit PCNA role which is a protein that play a key role in DNA replication via acting as cofactor of DNA polymerase (Cazzalini et al., 2010).

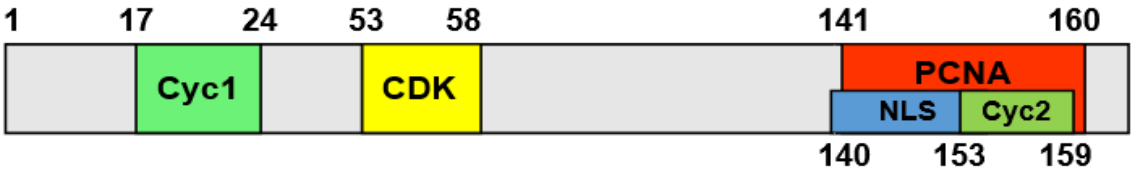

Figure 1.7: The major domains of p21 protein. P21 consists of cyc1 and cyc2 domains which are located in the N-terminal region and C-terminal region respectively. It contains of CDK domain. In the C-terminal region, there are also PCNA domain and NLS site (Adapted with modified from (Cazzalini et al., 2010)).

The p21 protein plays a crucial role in cell-cycle checkpoint regulation, where it binds to and inhibits the activity of CDK2 and contributes in inhibiting cell cycle progression in G1/S phase (Gartel et al., 1996; Harada & Ogden, 2000; Karimian et al., 2016).

Mutations of the P21 gene have been reported to be rare in human cancers (Galmarini et al., 2006). Although, several studies have found mutations of the P21 implicated in diverse types of cancers, including prostate (Gao et al., 1995), breast (Balbín et al., 1996), and bladder cancers (Lacombe et al., 1996), as well as oral squamous cell carcinomas (Ibrahim et al., 2002).

Polymorphisms of the P21 gene lead to changing transcripts and inhibiting apoptosis (Dumont et al., 2003). Several studies have identified, 42 SNPs of P21 gene, including 2 major p21 SNPs in codon 31 rs1801270 and in the 3′ untranslated regions (3′ UTRs) rs1059234. These SNPs may either independently or in combination, contribute to cancer initiation (Gravina et al., 2009; Ma et al., 2011). In the p21 rs1801270 polymorphism, a replacement of A to C in codon 31 causing a change in arginine (Arg) to serine (Ser) (Castro et al., 2009; Gravina et al., 2009).Thus, changing in the p21 protein structure may disrupt the regulation of cellular proliferation and

enhance carcinogenesis (Li et al., 2005). However, the p21 rs1801270 (R31S) polymorphism does not directly affect on the p21 functional domains (Staalesen et al., 2006).

A study presented that the p21 rs1801270 SNP was significantly correlated with increasing the cervical tumor risk in a Chinese population (Wang et al., 2012). Also, a Meta-analysis of another study suggested that, in the p21 rs1801270 SNP, Ser-allele and Ser/Ser genotype may be risk factors for gastrointestinal tract cancer in Asian populations (Dong et al., 2015). Many studies have investigated the effects of P21 SNPs on the lung tumor risk, but the results of these studies have been inconsistent (Själander et al. 1996; Shih et al. 2000; Su et al. 2003; Popanda et al. 2007). For this reason, in this thesis, we investigated the possible correlation between P21 SNP among smokers and non-smokers from Saudi Arabian population.

1.7.3 Effect of Mutation, and Polymorphisms in MDM2 Gene

The Murine Double Minute 2 (MDM2) gene is an important negative regulator of the p53 gene, which inhibits p53 expression (Moumen et al., 2007) and is mapped on the long arm of chromosome 12 (12q15). It encodes 90 KD nuclear protein (Mdm2) (Taş et al., 2017), which comprised of 491 amino acids (Wu, 2017). Mdm2 composes of several important domains (Figure 1.8) which are an N-terminal p53-binding domain (residues 17-125) that binds to TAD of p53 (Leng et al., 1995) and capable of interact with CTD of p53 (Poyurovsky et al., 2010), NLS, nuclear export signal (NES), a central domain which called acidic domain (residues 237-301) that capable of binding directly with DBD of p53 to inhibit the DNA binding function of p53 (Cross et al., 2011) and also capable of ubiquitinating p53 (Kawai et al., 2003), a zinc finger domain (residues 305-330) is responsible of interacting with ribosomal proteins, and a ring finger domain (residues 438-478) is a site of Mdm2 that consists of the residues ubiquitin E3 ligase that marks the p53 to initiate proteasome degradation activity (Kawai et al., 2003).

Mdm2 plays an essential role as E3 ubiquitin ligase in downregulation the p53 activity via binding to the transcriptional

activation domain of p53 and inhibiting its role in regulation of target genes, or through proteasome degradation (Zhang & Wang, 2000; Kawai et al., 2003; Wu, 2017). Moreover, increased levels of Mdm2 would inactivate the p53 functions in cell cycle arrest (Iwakuma, & Lozano, 2003). The overexpression of MDM2 has been seen to be associated with development and progression more than forty different types of cancers, including leukemias, solid and sarcomas tumors (Rayburn, 2005). These changes in stability and activity of MDM2 expression can result from gene amplification, increased transcription, enhanced translation, or mutations (Zhang & Wang, 2000).

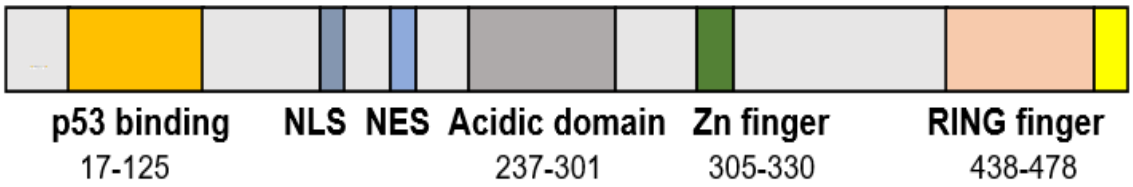

Figure 1.8: Domain structure of Mdm2 protein. The basic domains of Mdm2 are the following: the p53 binding domain is located in the N-terminal region, NLS, nuclear export signal (NES), the central domain is the acidic domain, the green region of the figure shows the zinc finger domain , and the pink region of the diagram indicates the ring finger domain which located in the C-terminal region of Mdm2 (Adapted with modified from (Wu, 2017)).

Mutations in MDM2 gene may affect the biological properties of Mdm2 protein which may lead to initiate cancers in human (SCHLOTT et al., 1997, Lindström, et al., 2007). Different studies have reported that mutations in MDM2 gene found in several types of human cancers including, osteosarcoma, hepatocarcinoma, leukemia (SCHLOTT et al., 1997), and liposarcoma (Tamborini et al., 2001).

Several studies have been carried out on the associations between SNPs within genes and with the predisposition of several diseases. For example, MDM2 SNP309 (rs2279744) is associated with cancer susceptibility in different types of cancers, including cervical, gastric, hepatocellular (Wo et al., 2011; Chen et al., 2014), prostate, ovarian (Chen et al., 2012; Ma et al., 2013) and bladder cancers (Onat et al., 2006). A pervious study showed that MDM2 SNP309 is associated with more advanced esophageal squamous cell carcinoma (Hong et al., 2005). A recent study proved that MDM2 SNP309 plays a role in endometrial cancer onset among postmenopausal women (Wujcicka et al., 2019). Different studied have been done on the association of MDM2 SNP309 with lung cancer and have yielded inconsistent results (Zhuo et al., 2012). For example, in the study by Zhang et al (2006), it provided significant correlation between MDM2

SNP309 and smoking for smokers who carry MDM2 GG genotypes, which could contribute to lung cancer development. In contrast, another pervious study suggested that no evidence for association between risk of lung cancer and MDM2 SNP309 in the Chinese population (Hu et al., 2006).

Furthermore, MDM2 SNP354 (rs769412) A>G is a synonymous SNP (E354E), which does not directly affect directly on the functional domains of Mdm2 (Thrower et al., 2000; Boersma et al., 2006; Zhao et al., 2014). The SNP354 was associated with the risk of larynx carcinoma among alcohol drinkers (Wang et al., 2015). It was also significantly associated with breast cancer incidence (Boersma et al., 2006). In comparison, according to study on Caucasians and African-Americans in the United States, there was no relation between MDM2 SNP354 and lung cancer (Pine et al., 2006). For this reason, in this thesis, we investigated the possible correlation between MDM2 SNP among smokers and non-smokers from Saudi Arabian population.

1.8 Aims of This Study

So far, no study has been found related to the correlations between the polymorphisms of TP53, P21 and MDM2 genes and smoking among Saudi Arabian population. For this reason, the aim of the thesis is to investigate whether a relationship exists between the genetic variants of TP53 pathway and smoking to identify specific genetic markers for the prevention of the potential effects of CS on healthy participants and or the diagnosis of diseases related to CS. The specific aims are to:

1. Examine the global genotype and allele allocations of SNPs located in TP53 (rs1042522), P21 (rs1801270), and MDM2 (rs769412) genes, and their frequencies among smokers and non-smoker from Saudi Arabian population.
2. Evaluate the possible link of the genotypic distribution of TP53, P21 and MDM2 gene polymorphisms with clinical parameters of the two study groups.
3. Compare the allele variation of TP53, P21 and MDM2 polymorphisms between the Saudi Arabian population and other populations existing on the International HapMap project study groups.
4. Analyse the 3D structures resulting from TP53, P21 and MDM2 polymorphisms and their potential functional modification through the Phyre2 web portal.

Chapter 2: Materials and Methods

2.1 Materials

All chemicals, kits and equipment used during this study, and the names of the suppliers are presented in Table 2.1.

Table 2.1:Materials used in this study and their supplier

Materials	Supplier
BD Vacutainer® K2E (EDTA) (6ml)	BD-Plymouth, UK
Vortex Mixer	National labnet Co., Inc. Woodbridge, NJ, USA
Centrifuge	Eppendorf AG, Germany
DNeasy® Blood & Tissue Kit (250)	QIAGEN, Germany
NanoDrop 8000 Spectrophotometer	Thermo Fisher Scientific, USA
Mini Centrifuge	Woodbridge, KOREA
TaqMan® Genotyping Master Mix	Applied Biosystems by life biotechnologies™, USA
MicroMap® Fast Optical 96-Well Reaction Plate with Barcode (0.1 ml)	Applied Biosystems by life biotechnologies™, USA
QuantStudio 7 Flex Real-Tim PCR System	Applied Biosystems by life biotechnologies™, USA
Micropipettes	Eppendorf Reference 2, Germany
Pipette tips	Eppendorf Research, Germany
Eppendorf ThermoStat plus, 1.5 ml	Eppendorf AG, Germany
Ethanol absolute	Fisher Scientific, UK
Plate Centrifuge 5810 R	Eppendorf AG, Germany
MicroAmp™ Optical Adhesive Film	Applied Biosystems, USA
TaqMan™ SNP Genotyping Assay	Applied Biosystems by life biotechnologies™, USA

2.2 Methods

2.2.1 Ethical Approval Certificate

Written ethical assent for this study was reviewed and approved by the Research Ethics Committee of the College of Applied Medical Sciences, King Saud University (KSU), Riyadh, Saudi Arabia, with ethical approval reference number CAMS 13/3536. All participants smokers and non-smokers agreed to sign a written informed document of participation in this investigation, and they were provided a privacy statement describing their personal data protection. Clinical data were acquired through a self-completed questionnaire, including age, CS history, allergy symptoms or diseases, and number of cigarettes consumed per day.

2.2.2 Collection of Blood Samples

Our study population composed of 568 Saudi males and females, including 283 cigarette smokers and 285 non-smokers, which known as healthy controls. Three milliliter of blood was obtained from participants using ethylene-diamine tetra acetic acid (EDTA) vacutainer vials. These samples were collected between January and February 2019 from the Blood Donation Center at King Saud medical

city (Riyadh, Saudi Arabia). Participants who self-reported having inflammatory diseases and/or chronic respiratory failure were excluded from the study.

2.2.3 Genomic DNA Extraction

Genomic DNA was extracted from 200µl of peripheral blood, performed according to standard procedures using a DNeasy® Blood & Tissue Kit (QIAGEN, Germany). First, in a new clean 1.5 ml microcentrifuge tube, 20 µl of Proteinase K solution was pipetted into the bottom of the tube, 200µl of blood sample was transferred to the tube. Then, 200 µl of buffer BL was added and mixed by pulse vortexing for 15 sec, followed by incubation at 56ºC for 10 min using the Eppendorf ThermoStat plus. After that, the tubes were spun down briefly by using Mini Centrifuge. A volume of 200 µl of absolute ethanol was added to the samples, and mixed again by pulse vortexing for 15 sec.

The whole mixture was then transferred to the QIAGEN mini spin column (which fitted with a 2ml collection tube), and centrifuged at 8000 rpm for 1 min, then the collection tube was replaced with a new one. Then, 500 µl of buffer AW1 was added and centrifuged at 8000 rpm for 1 min and the collection tube was replaced again with a

new collection tube. After that, 500 µl of buffer AW2 was added and centrifuged again at 8000 rpm for 1 min, and replaced the collection tube with a new clean 1.5 mL microcentrifuge tube. Finally, 75 µl of AE was added to the tube and incubated for 1 min at room temperature, followed by centrifuging at full speed (14000 rpm) for 1 min.

2.2.4 Determination of DNA Concentration

The DNA concentrations were quantified using a NanoDrop 8000 Spectrophotometer. DNA sample purity was calculated by establishing the ratio of A260:A280nm and A260:A230 reads. The DNA was considered as "pure" when the ratio was around 2.0. Then, the purified DNA samples were stored at -20°C until further analysis.

2.2.5 SNPs Genotyping Test Using TaqMan® Assay

2.2.5.1 The Principle TaqMan® Assay

TaqMan® SNP Genotyping Assay was used to distinguish between two alleles of a specific SNP. This method involves by using forward and reverse primers to amplify a target sequence of target, which has two dye-labeled probes to detect a specific change in allele sequence at SNP site. The first probe is complementary to the wild-

type allele and is labeled at the 5′ site with reporter dye called VIC® dye, which detects the sequence of allele 1. However, the second probe is complementary to the variant allele, and is also labeled at the 5′ site with another reporter dye called FAM™ dye, which determines the sequence of allele 2 (Figure 2.1). At the 3′ site, the probes are labeled with a non-fluorescent quencher dye (NFQ). Also, the probes contain a Minor Groove Binder (MGB) at 3′ end, which binds with DNA to stabilize the probe/template complex.

This assay used the fluorescence resonance energy transfer (FRET) technology, which has a 5′ reporter dye and a 3′ quencher dye that are covalently attached to the wild type and variant allele probes. Based on FRET technology, the quencher dye absorbs the energy of the reporter dyes. A new strand of DNA is synthesized by Taq DNA polymerase. This polymerase encounters the annealed probe and the 5`reporter dye of the appropriate probe which is be separated as a result of the 5`→ 3` Taq exonuclease activity. At this point, the fluorescence signal is no longer quenched and can now be detected with laser excitation. SNP can be detected as homozygotes for either Allele 1 or Allele 2, or heterozygotes that carry both alleles (Schleinitz et al., 2011).

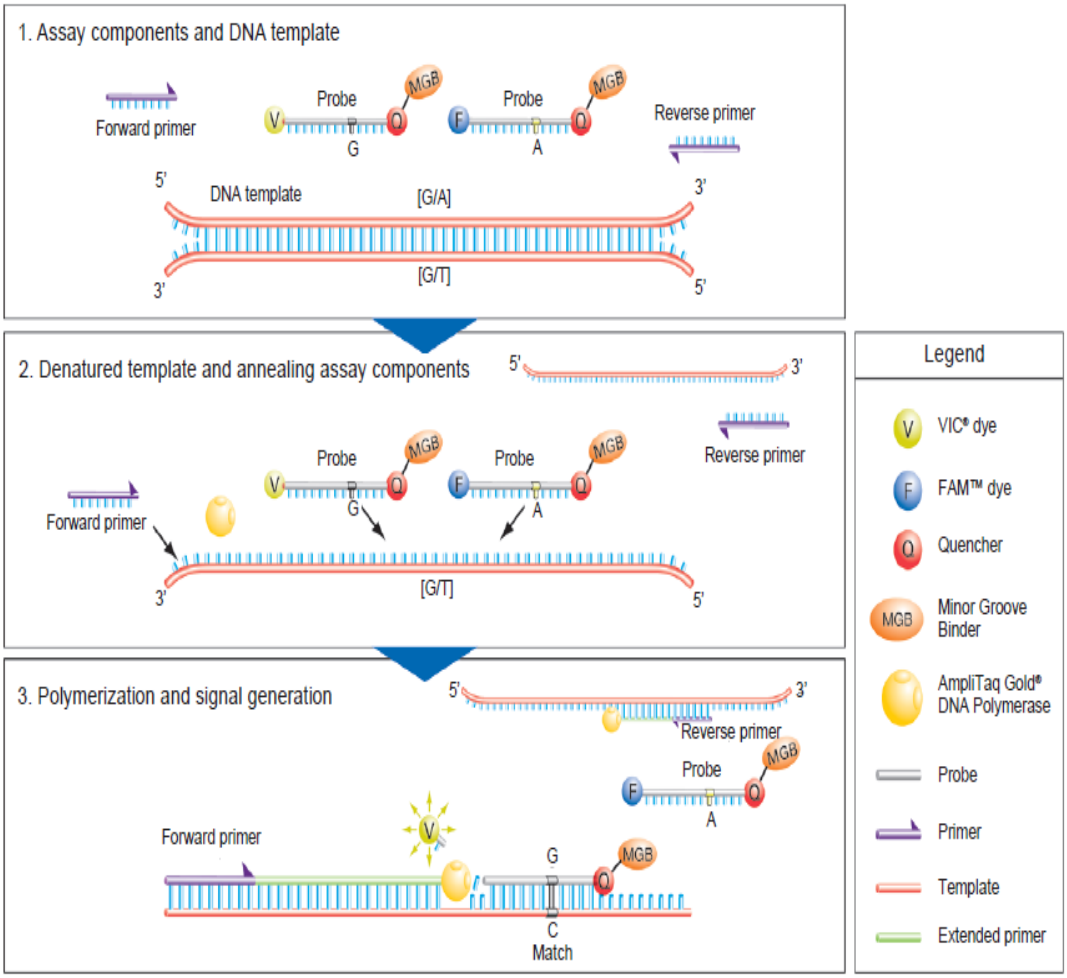

Figure 2.1: **Principle of allelic discrimination using TaqMan® SNP Genotyping Assays.** Panel 1: figure indicates the assay components and DNA template: Forward and reverse primers used to amplify the desired polymorphic sequence, two dye-labeled probes at 5`site used to detect specific allele. A 3` NFQ used to quench the fluorescence. The MGB at the 3`site used to stabilizes the probe/template complex. Panel 2: figure refers to the denatured template and the components of annealing assay. Panel 3: figure indicates the polymerization process and signal generation: Taq polymerase enzyme begins synthesizing a new strand and encounters the probe. From the appropriate probe, the 5`- bound fluorescence dye is separated due to 5`→ 3` exonuclease activity of the Taq enzyme. Now, laser excitation can detect the fluorescence signal because the dye is no longer quenched. (Adapted from Applied Biosystems, 2010).

2.2.5.2 Preparation of TaqMan® SNP Genotyping Assay

Genomic DNA blood samples of 10 ng were prepared before genotyping. The selected three SNPs in the current study are presented in the Table 2.2.

Table 2.2: Lists of SNPs used in this study

SNP ID	Gene	Location	Polymorphism
rs1042522	*TP53*	Exon	C > G
rs1801270	*P21*	Exon	A > C
rs769412	*MDM2*	Exon	A > G

In a 96 well plate, each sample was genotyped in a volume of 10µl per reaction. Each well contained 8µl of reactions, which listed in Table 2.3 and 2µl of diluted DNA to reach a final volume of 10µl. The plate was sealed by using adhesive film and then briefly centrifuged to collect the contents and remove air bubbles.

Table 2.3: Reagents Used for Preparation of TaqMan Genotyping Assay

Reagent	Amount/Reaction (µL)
2 × TaqMan® Genotyping Master Mix	5 µl
40 × TaqMan® Genotyping SNP Assay	0.25 µl
DNase-free water	2.75 µl
Total	8µl

Allelic discrimination of the SNPs was performed using a QuantStudio™ 7 Flex Real-time PCR System instrument with the Sequence Detection Software (Almutairi et al., 2019). The real-time

PCR amplification processes were prepared under the reaction conditions as shown in Table 2.4

Table 2.4: The real-time PCR program

Step Name	Temperature	Time	Cycles
Pre-denaturation	95 °C	7 min	1
Denaturation	95 °C	30 sec	40
Annealing	60 °C	1 min	
Extension	72 °C	30 sec	
Final extension	72°C	5 min	1

2.2.6 Statistical Analysis

Both group differences were examined as described in a previous work (Almutairi et al., 2019; Almutairi & Semlali, 2019) by comparing the allelic and genotypic frequencies using the chi-squared (X^2) test. The Statistical Package for the Social Sciences (SPSS) version 16.0 statistical software was utilized to examine statistical significance, where $P<0.05$ represents statistical significance. The Hardy–Weinberg equilibrium test was performed using Fisher's exact test (two-tailed). Lastly, the odds ratio (OR) was measured with 95% confidence intervals (CI) to test the connection strength.

2.2.7 3D Structure Analysis

The 3D structures analysis was performed to predict the potential structural modification and damaging effects of p53, p21and Mdm2 SNPs. After getting the proteins sequences from NCBI, the analysis was built up using the Phyre2 web which considered as one of the most widely programs for protein structure prediction and modeling (Kelley et al. 2007). Then, the 3D structures of p53, p21 and Mdm2 proteins were visualized by using the PyMOL software. Finally, we added the SNPs to the proteins sequences to analyze the potential structural modification of proteins by using the PyMOL software.

Chapter 3: Results

3.1 Clinical and Demographic Data of the Study Participants

Basic clinical data characteristics of smoking and non-smoking participants are represented in Table 3.1. The total numbers of populations used in the current study were 568 Saudi individuals, including 283 male and female smokers, and 285 male and female non-smokers. In fact, there were no significant differences between the two classes in age, gender and smoking characteristics. The median ages of smoking participants and non-smoking control group were almost equal (29.76±7.09 and 29.13±8.83, respectively). Based on the gender, male smokers were (91.5%), while female smokers were (8.5%). According to the smoking duration, we divided smokers into two groups, the first group was those who had smoked cigarettes for 7 years or less (61.0%), and the second group was those who had smoked cigarettes for a period of more than 7 years (39.0%). Also, based on the daily CS rate, we separated smokers into two classes as following, those who had consumed 12 CS or less a day (51.4%), and those who had consumed more than 12 CS a day (48.6%). In addition, we divided smoking participants depending on the smoking types into two categories, smokers who had smoked cigarettes (75.5%), and smokers who had smoked shisha (24.5%).

Table 3.1: Clinical and demographic data of the study participants

Variable	Smokers	Non-smokers
Number	283	285
Age (years), median ± average	29.76±7.09	29.13±8.83
Age (years)		
≤ 29 years	152 (53.7%)	174 (61.3%)
> 29 years	131 (46.3%)	110 (38.7%)
Gender		
Males	259 (91.5%)	201 (70.5%)
Females	24 (8.5%)	84 (29.5%)
Years of smoking		
≤ 7 years	172 (61.0%)	----------
> 7 years	110 (39.0%)	----------
Quantity of cigarette smoking per day		
≤ 12 times	145 (51.4%)	----------
> 12 times	137 (48.6%)	----------
Type of smoking		
Cigarette	213 (75.5%)	----------
Shisha	69 (24.5%)	----------

3.2 Genotypic Distribution of TP53, P21, and MDM2 Gene Polymorphisms Among Smokers and Non-Smokers

Three SNPs were genotyped in 568 Saudi participants, 283 smokers and 285 non-smokers by using TaqMan genotyping assay. These SNPs are rs1042522 C>G (P72R) in TP53 gene, rs1801270 A>C (R31S) in P21 gene, and rs769412 A>G (E354E) in MDM2 gene. All characteristics of selected SNPs are described in Table 3.2.

Table 3.2: Description of the selected SNPs

Gene	SNP ID	SNP location	Variation type	Amino acid/nucleotide change	Alleles change
TP53	rs1042522	NC_000017.11:g.7676154	Exon	C/G (P72R)	C/G
P21	rs1801270	NC_000006.12:g.36684194	Exon	A/C (R31S)	A/C
MDM2	rs769412	NC_000012.12:g.68839435	Exon	A/G (E354E)	A/G

Table 3.3 shows the genotype and allele frequencies of TP53, P21, and MDM2 polymorphisms in smoking participants and controls. Among these SNPs, a statistically significant association was observed in rs1042522 in the TP53 gene with smoking behavior. In controls, the genotypic allocations of CC, CG and GG genotypes were 27%, 49% and 24%, respectively; whereas in smoker participants were found to be 8%, 52% and 40%, respectively. The heterozygous CG, homozygous GG and CG+GG genotypes and the G allele showed a significant difference when compared with the CC, and C reference

alleles (OR= 3.80, P<0.00001; OR= 5.80, P<0.00001; OR= 4.47, P<0.00001 and OR= 2.075, P<0.00001, respectively; Table 3.3). However, there was no significant correlations are found between smokers in contrast with non-smokers in genetic variants of P21 rs1801270, and MDM2 rs769412 except the C allele of rs1801270. The genotype distributions of rs1801270 SNP of AA, AC and CC genotypes among controls were 7%, 33% and 60%, respectively; while in smoker participants were 5%, 27% and 68%, respectively. Interestingly, there was an association between the C allele of rs1801270 variant in smokers than those with the A reference allele (OR= 1.33, P=0.049). In contrast, MDM2 rs769412 SNP was distributed as 85% AA, 15% AG and 0% GG for controls and 84%, 16% and 0%, respectively for smoking participants.

Table 3.3: Genotype distributions of TP53, P21, and MDM2 gene SNPs among smokers and non-smokers

Gene	SNP	Alleles	Controls		Smokers		OR	95% CI	X^2	P value
			N	Percent	N	Percent				
TP53	rs1042522	**Total**	**285**	**100%**	**283**	**100%**				
		CC	78	27%	22	8%	Ref			
		CG	138	49%	148	52%	3.80	2.24-6.44	26.61	**<0.00001***
		GG	69	24%	113	40%	5.80	3.31-10.16	41.56	**<0.00001***
		CG+GG	207	73%	261	92%	4.47	2.69-7.42	37.58	**<0.00001***
		C	294	52%	192	34%	Ref			
		G	276	48%	374	66%	2.075	163-2.63	36.17	**<0.00001***
P21	rs1801270	**Total**	**274**	**100%**	**278**	**100%**				
		AA	19	7%	14	5%	Ref			
		AC	91	33%	76	27%	1.133	0.533-2.410	0.11	0.74481
		CC	164	60%	188	68%	1.556	0.756-3.201	1.46	0.22696
		AC+CC	255	93%	264	95%	1.405	0.690-2.862	0.88	0.34692
		A	129	24%	104	19%	Ref			
		C	419	76%	452	81%	1.33	1.00-1.78	3.87	**0.049***
MDM2	rs769412	**Total**	**282**	**100%**	**282**	**100%**				
		AA	239	85%	236	84%	Ref			
		AG	42	15%	45	16%	1.085	0.687-1.714	0.12	0.72645
		GG	1	0%	1	0%	1.013	0.063-16.285	0.00	0.99289
		AG+GG	43	15%	46	16%	1.083	0.689-1.704	0.12	0.72896
		A	520	92%	517	92%	Ref			
		G	44	8%	47	8%	1.074	0.700-1.650	0.11	0.74292

*P < 0.05, Ref = Reference allele

3.3 Association Between TP53, P21, and MDM2 SNPs and Smokers' Ages

To determine any relationship between the ages of smokers and TP53, P21, and MDM2 polymorphisms, we categorized all smokers and non-smokers into two groups, whom aged 29 years or below (≤ 29 years) and whom aged above age 29 years (> 29 years). The numbers of smokers and non-smokers were 152 and 174, respectively in individuals more than 29 years old, while they were 131, and 110, respectively in individuals less than 29 years old (Table 3.1). The results analysis showed an association between TP53 SNP rs1042522 and all allelic frequencies and genotypic distributions among smokers 29 years or less than those in controls (Table 3.4). The CG, GG and CG+GG genotypes and the G allele showed a significant correlation with CS effects among younger smoking participants, but not with non-smoking participants (OR= 4.095, P= 0.00007 for CG; OR= 4.783, P= 0.00003 for GG; OR= 4.338, P= 0.00001 for CG+GG; and OR= 1.774, P= 0.00035 for G allele) (Table 3.4). In addition, the same SNP also showed at CG, GG and CG+GG genotypes and the G allele a significant association with an increasing effects of CS among older smokers (> 29 years) as compared to controls (OR= 3.616, P=

0.00077 for CG; OR= 7.794, P<0.00003 for GG; OR= 4.880, P<0.00007 for CG+GG; and OR= 2.590, P<0.00003 for G allele) (Table 3.5).

Among younger smokers (≤ 29 years), the genotype allocations of AA, AC and CC genotypes of P21 SNP rs1801270 were found to be 7%, 31% and 62%, respectively in control, while were 3%, 24% and 73%, respectively in smokers. The heterozygous AC and homozygous CC frequency in younger smokers did not show any significant difference when compared with younger non-smokers, whereas the C allele frequency showed in younger smokers a significant relationship when compared with the A reference allele (OR= 1.556, P= 0.03280) (Table 3.4). However, among older smoker (> 29 years), they did not show any significant differences when compared with older non-smokers. The genotype distributions of AA, AC and CC genotypes of the rs1801270 SNP were found to be 7%, 37% and 56%, respectively in older non-smokers and 7%, 31% and 62%, respectively in older smokers. Also, the C allele frequency in older smokers did not show significant difference when compared with the A reference allele (Table 3.5).

As for MDM2 SNP rs769412, the results did show any significant association between CS and age neither in younger nor in older smokers as shown in the Table 3.4 and Table 3.5, respectively. In younger smokers (≤ 29 years), the genotype frequencies for this SNP were 85% AA, 14% AG and 1% GG in non-smoking controls and 87% AA, 13% AG and 0% GG in smoking participants. The phenotypes were 92% A and 8% G in controls and 93% A and 7% G in smoking participants (Table 3.4). While in older smokers, the genotype frequencies for this SNP were 84% AA, 16% AG and 0% GG for non-smokers and 80% AA, 19% and 1% GG for smokers. In addition, the phenotypes were 92% A and 8% G in non-smokers and 90% A and 10% G in smokers (Table 3.5).

Table 3.4: Comparison of genotypic allocations of TP53, P21, and MDM2 gene polymorphisms in smokers with entire controls in age ≤ 29 years

Gene	SNP	Allele	Controls		≤ 29 years		OR	95% CI	X^2	P value
			N	Percent	N	Percent				
TP53	rs1042522	**Total**	**174**	**100%**	**152**	**100%**				
		CC	44	25%	11	7%	Ref			
		CG	84	48%	86	57%	4.095	1.982-8.463	15.85	**0.00007***
		GG	46	27%	55	36%	4.783	2.219-10.309	17.32	**0.00003***
		CG+GG	130	75%	141	93%	4.338	2.149-8.757	18.85	**0.00001***
		C	172	49%	108	36%	Ref			
		G	176	51%	196	64%	1.774	1.294-2.431	12.79	**0.00035***
P21	rs1801270	**Total**	**165**	**100%**	**149**	**100%**				
		AA	11	7%	5	3%	Ref			
		AC	51	31%	36	24%	1.553	0.497-4.856	0.58	0.44682
		CC	103	62%	108	73%	2.307	0.775-6.868	2.36	0.12415
		AC+CC	154	93%	144	97%	2.057	0.698-6.065	1.77	0.18278
		A	73	22%	46	15%	Ref			
		C	257	78%	252	85%	1.556	1.035-2.340	4.56	**0.03280***
MDM2	rs769412	**Total**	**173**	**100%**	**151**	**100%**				
		AA	147	85%	131	87%	Ref			
		AG	25	14%	20	13%	0.898	0.476-1.691	0.11	0.73837
		GG	1	1%	0	0%	0.374	0.015-9.258	0.89	0.34593
		AG+GG	26	15%	20	13%	0.863	0.460-1.619	0.21	0.64629
		A	319	92%	282	93%	Ref			
		G	27	8%	20	7%	0.838	0.460-1.527	0.33	0.56313

*P < 0.05, Ref = Reference allele

Table 3.5: Comparison of genotypic allocations of TP53, P21, and MDM2 gene polymorphisms in smokers with entire controls in age > 29 years

Gene	SNP	Allele	Controls		> 29 years		OR	95% CI	X^2	P value
			N	Percent	N	Percent				
		Total	**110**	**100%**	**131**	**100%**				
		CC	34	31%	11	8%	Ref			
		CG	53	48%	62	48%	3.616	1.670-7.828	11.32	**0.00077***
TP53	rs1042522	GG	23	21%	58	44%	7.794	3.385-17.946	25.97	**<0.00003***
		CG+GG	76	69%	120	92%	4.880	2.333-10.209	19.95	**<0.00007***
		C	121	55%	84	32%	Ref			
		G	99	45%	178	68%	2.590	1.787-3.754	25.74	**<0.00003***
		Total	**108**	**100%**	**129**	**100%**				
		AA	8	7%	9	7%	Ref			
		AC	40	37%	40	31%	0.889	0.312-2.536	0.05	0.82566
P21	rs1801270	CC	60	56%	80	62%	1.185	0.432-3.252	0.11	0.74129
		AC+CC	100	93%	120	93%	1.067	0.397-2.867	0.02	0.89818
		A	56	26%	58	22%	Ref			
		C	160	74%	200	78%	1.207	0.791-1.840	0.76	0.38208
		Total	**108**	**100%**	**131**	**100%**				
		AA	91	84%	105	80%	Ref			
		AG	17	16%	25	19%	1.275	0.648-2.508	0.49	0.48196
MDM2	rs769412	GG	0	0%	1	1%	2.602	0.105-64.655	0.86	0.35294
		AG+GG	17	16%	26	20%	1.325	0.676-2.597	0.68	0.41076
		A	199	92%	235	90%	Ref			
		G	17	8%	27	10%	1.345	0.712-2.539	0.84	0.35942

*P < 0.05, Ref = Reference allele

3.4 The Relationship Between TP53, P21, and MDM2 SNPs and Gender of Smokers

We also investigated a correlation between TP53, P21, and MDM2 gene polymorphisms and gender of smokers among 201 controls and 259 smokers of males, and 84 controls and 24 smokers of females. The results supported an association between polymorphism rs1042522 in TP53 gene and smokers versus non-smokers in both types of genders. In males, as presented in Table 3.6, the CG, GG and CG+GG genotypes and G allele presented a significant increased correlation with CS among the male smokers compared with the male controls (OR= 3.510, P<0.00005; OR= 5.213, P<0.00002; OR= 4.058, P<0.00007 and OR= 1.982, P= <0.00003, respectively). The same significant results were observed in females as shown in Table 3.7. although the number of female smokers were small compared to the health females, the CG, GG and CG+GG genotypes and G allele also presented a higher increased association with CS among female smokers compared with controls (OR= 11.304, P= 0.02626; OR= 32.378, P= 0.00042; OR= 18.724, P= 0.00386 and OR= 4.438, P= 0.00009, respectively).

However, the results of this study did not show any significant relationship between polymorphism rs1801270 in P21 gene and CS in either gender. In males, the percentage of genotype distributions AA, AC, CC were 7%, 31% and 62% in non-smokers, and 5%, 27% and 68% in smokers, respectively (Table 3.6). While in females, the percentage of genotype distributions AA, AC, CC of the same SNP were 7%, 38% and 55% in healthy controls, and 0%, 39% and 61% in smoking participants, respectively (Table 3.7).

Finally, in either gender, the MDM2 SNP rs769412 results also did not display any correlation between the genotypic frequencies and CS. The genotype distributions of this variant were 83% and 89% for the AA reference allele, 17% and 10% for homozygous AG, and 0% and 1% for a double-mutant allele GG in healthy males and healthy females, respectively. However, In male and female smokers, these values were 84% and 83% for the AA reference allele, 16% and 17% for homozygous AG, and 0% for both double-mutant GG, respectively (Tables 3.6 and 3.7).

Table 3.6: Genotype and allele frequencies of SNPs in TP53, P21, and MDM2 gene in male smokers with overall controls

Gene	SNP	Allele	Controls		Male		OR	95% CI	X^2	P value
			N	Percent	N	Percent				
		Total	**201**	**100%**	**259**	**100%**				
		CC	55	27%	22	8%	Ref			
		CG	99	49%	139	54%	3.510	2.010-6.130	20.72	**<0.00005***
TP53	rs1042522	GG	47	24%	98	38%	5.213	2.848-9.541	30.82	**<0.00002***
		CG+GG	146	73%	237	92%	4.058	2.375-6.934	28.91	**<0.00007***
		C	209	52%	183	35%	Ref			
		G	193	48%	335	65%	1.982	1.519-2.586	25.70	**<0.00003***
		Total	**194**	**100%**	**255**	**100%**				
		AA	13	7%	14	5%	Ref			
		AC	61	31%	67	27%	1.020	0.444-2.341	0.00	0.96291
P21	rs1801270	CC	120	62%	174	68%	1.346	0.611-2.966	0.55	0.45921
		AC+CC	181	93%	241	95%	1.236	0.567-2.695	0.29	0.59292
		A	87	22%	95	19%	Ref			
		C	301	78%	415	81%	1.263	0.911-1.750	1.96	0.16107
		Total	**198**	**100%**	**258**	**100%**				
		AA	164	83%	216	84%	Ref			
		AG	34	17%	41	16%	0.916	0.557-1.506	0.12	0.72838
MDM2	rs769412	GG	0	0%	1	0%	2.279	0.092-56.315	0.76	0.38403
		AG+GG	34	17%	42	16%	0.938	0.571-1.540	0.06	0.79987
		A	362	91%	473	92%	Ref			
		G	34	9%	43	8%	0.968	0.605-1.549	0.02	0.89186

*P < 0.05, Ref = Reference allele

Table 3.7: Genotype and allele frequencies of SNPs in TP53, P21, and MDM2 gene in female smokers with overall controls

Gene	SNP	Allele	Controls		Female		OR	95% CI	X^2	P value
			N	Percent	N	Percent				
		Total	**84**	**100%**	**24**	**100%**				
		CC	23	27%	0	0%	Ref			
		CG	39	47%	9	37%	11.304	0.629-203.248	4.94	**0.02626***
TP53	rs1042522	GG	22	26%	15	63%	32.378	1.827-573.843	12.43	**0.00042***
		CG+GG	61	73%	24	100%	18.724	1.094-320.471	8.35	**0.00386***
		C	85	51%	9	19%	Ref			
		G	83	49%	39	81%	4.438	2.023-9.733	15.40	**0.00009***
		Total	**80**	**100%**	**23**	**100%**				
		AA	6	7%	0	0%	Ref			
		AC	30	38%	9	39%	4.049	0.208-78.705	1.73	0.18831
P21	rs1801270	CC	44	55%	14	61%	4.236	0.225-79.876	1.85	0.17334
		AC+CC	74	93%	23	100%	4.101	0.223-75.545	1.83	0.17593
		A	42	26%	9	20%	Ref			
		C	118	74%	37	80%	1.463	0.652-3.286	0.86	0.35456
		Total	**84**	**100%**	**24**	**100%**				
		AA	75	89%	20	83%	Ref			
		AG	8	10%	4	17%	1.875	0.512-6.864	0.92	0.33655
MDM2	rs769412	GG	1	1%	0	0%	1.228	0.048-31.272	0.27	0.60608
		AG+GG	9	11%	4	17%	1.667	0.465-5.976	0.62	0.42933
		A	158	94%	44	92%	Ref			
		G	10	6%	4	8%	1.436	0.430-4.801	0.35	0.52496

*P < 0.05, Ref = Reference allele

3.5 Association Between SNPs in TP53, P21, and MDM2 Genes and Daily CS Rate

According to the quantity of CS per day, we therefore classified the smoking study subjects into two classes, moderate smokers whom had smoked ≤12 times per day, which include 145 subjects and heavy smokers whom had smoked >12 times per day, which include 137 subjects. This classification was in order to examine the association between the daily rate of CS consuming and genetic differences of polymorphism allelic of TP53, P21, and MDM2 Genes. The analyses of this investigation showed a significant association of TP53 SNP rs1042522 with moderate and heavy smokers. In moderate smokers (≤ 12 times/day), the CG, GG, CG+GG genotypes and G allele of TP53 SNP were significantly associated with CS as compared to non-smokers (OR= 3.068, P= 0.00035; OR= 4.441, P<0.00005; OR= 3.526, P= 0.00002 and OR= 1.905 , P= 0.00001, respectively; Table 3.8). In heavy smokers (>12 times/day), they were also significantly correlated with CS when compared to controls (CG: OR= 5.087, P= 0.00001; GG: OR= 8.054, P<0.00002; CG+GG: OR= 6.076, P= <0.00002 and G: OR= 2.251, P= <0.00001; Table 3.9).

In contrast, the rs1801270 SNP of P21 gene analyses did not present any relationship between this variant and smoking in either moderate or heavy smokers when compared to non-smokers. In controls of both classes, the genotype frequencies were distributed into 7% AA, 33% AC and 60% CC. However, they were allocated as 5% for AA, 28% for AC and 67% for CC in moderate smokers and 6% for AA, 26% for AC and 68% for CC in heavy smokers, (Tables 3.8 and 3.9, respectively). Furthermore, the allelic distributions of this SNP were 24% for A allele and 67% for C allele in controls, while they were distributed as 18% for A allele and 82% for C allele in moderate smokers; however, in heavy smokers, they were distributed as 19% and 81%, respectively; Table 3.8 and 3.9.

Regarding to MDM2 SNP rs769412, the results of this polymorphism also did not show any correlation with CS rate in moderate and heavy smoking subjects when compared with non-smoking subjects. The genotype distributions of AA, AG and GG were 85%, 15% and 0%, respectively among controls in both categories. However, they were allocated as 86%, 14% and 0%, respectively among moderate smokers (Table 3.8), and were allocated as 81%, 18%, and 1%, respectively among heavy smokers (Table 3.9).

In addition, the allelic variations of this SNP were 92% A and 8% G for non-smokers in both categories, but moderate and heavy smokers were distributed as 93% and 90% for allele A and 7% and 10% for allele G, respectively; Tables 3.8 and 3.9.

Table 3.8: Comparison of genotype frequencies of TP53, P21, and MDM2 gene polymorphisms in smokers whom smoking ≤12 times/day

Gene	SNP	Allele	Controls		≤ 12 times/day		OR	95% CI	X^2	P value
			N	Percent	N	Percent				
		Total	**285**	**100%**	**145**	**100%**				
		CC	78	27%	14	10%	Ref			
		CG	138	49%	76	52%	3.068	1.628-5.785	12.77	**0.00035***
TP53	rs1042522	GG	69	24%	55	38%	4.441	2.272-8.680	20.63	**<0.00005***
		CG+GG	207	73%	131	90%	3.526	1.917-6.486	17.93	**0.00002***
		C	294	52%	104	36%	Ref			
		G	276	48%	186	64%	1.905	1.424-2.548	19.10	**0.00001***
		Total	**274**	**100%**	**142**	**100%**				
		AA	19	7%	6	5%	Ref			
		AC	91	33%	39	28%	1.357	0.504-3.658	0.37	0.54499
P21	rs1801270	CC	164	60%	95	67%	1.834	0.708-4.753	1.60	0.20597
		AC+CC	255	93%	134	95%	1.664	0.649-4.266	1.15	0.28448
		A	129	24%	51	18%	Ref			
		C	419	76%	229	82%	1.382	0.963-1.985	3.09	0.07879
		Total	**282**	**100%**	**143**	**100%**				
		AA	239	85%	123	86%	Ref			
		AG	42	15%	20	14%	0.925	0.521-1.645	0.07	0.79127
MDM2	rs769412	GG	1	0%	0	0%	0.646	0.026-15.985	0.51	0.47345
		AG+GG	43	15%	20	14%	0.904	0.509-1.603	0.12	0.72933
		A	520	92%	266	93%	Ref			
		G	44	8%	20	7%	0.889	0.513-1.538	0.18	0.67299

*P < 0.05, Ref = Reference allele

Table 3.9: Comparison of genotype frequencies of TP53, P21, and MDM2 gene polymorphisms in smokers whom smoking >12 times/day

Gene	SNP	Allele	Controls		> 12 times/day		OR	95% CI	X^2	P value
			N	Percent	N	Percent				
		Total	**285**	**100%**	**137**	**100%**				
		CC	78	27%	8	6%	Ref			
		CG	138	49%	72	52%	5.087	2.329-11.113	19.31	**0.00001***
TP53	rs1042522	GG	69	24%	57	42%	8.054	3.591-18.065	31.05	**<0.00002***
		CG+GG	207	73%	129	94%	6.076	2.841-12.994	26.43	**<0.00002***
		C	294	52%	88	32%	Ref			
		G	276	48%	186	68%	2.251	1.664-3.046	28.29	**<0.00001***
		Total	**274**	**100%**	**137**	**100%**				
		AA	19	7%	8	6%	Ref			
		AC	91	33%	36	26%	0.940	0.378-2.338	0.02	0.89338
P21	rs1801270	CC	164	60%	93	68%	1.347	0.567-3.197	0.46	0.49836
		AC+CC	255	93%	129	94%	1.201	0.512-2.819	0.18	0.67276
		A	129	24%	52	19%	Ref			
		C	419	76%	222	81%	1.314	0.916-1.885	2.21	0.13676
		Total	**282**	**100%**	**138**	**100%**				
		AA	239	85%	112	81%	Ref			
		AG	42	15%	25	18%	1.270	0.738-2.188	0.75	0.38779
MDM2	rs769412	GG	1	0%	1	1%	2.134	0.132-34.427	0.30	0.58447
		AG+GG	43	15%	26	19%	1.290	0.755-2.206	0.87	0.35070
		A	520	92%	249	90%	Ref			
		G	44	8%	27	10%	1.281	0.775-2.118	0.94	0.33227

*P < 0.05, Ref = Reference allele

3.6 Frequencies of TP53, P21, and MDM2 SNPs According to Smoking Duration

Based on the years of smoking, we separated the smoking populations into 172 short-term smokers (≤ 7 Years) and 110 long-term smokers (> 7 years) to assess the correlation of TP53, P21, and MDM2 gene SNPs with duration of CS. As shown in Table 3.10 and Table 3.11, the genotype differences and statistical analyses of those SNPs in short-term and long-term smokers are described, respectively compared to the non-smoking populations.

The frequencies of CG, GG and CG+GG genotype and G allele of TP53 SNP rs1042522 showed a significant association with an increasing in both short-term and long-term smokers when compared with non-smokers. In short-term smokers, the OR ratio and *P* value were (OR= 2.959, P= 0.00021; OR= 4.389, P<0.00001; OR= 3.436, P<0.00008, and OR= 1.914, P<0.00003, respectively; Table 3.10). However, in long-term smokers, the OR ratio and *P* value were (OR= 6.557, P= 0.00002; OR= 10.626, P<0.00004; OR= 7.913, P<0.00006, and OR= 2.381, P<0.00001, respectively, Table 3.11).

The genotype frequencies of P21 SNP rs1801270 did not support any significant correlation with CS in short- or long-term

smokers when compared to non-smokers (Table 3.10 and Table 3.11,). The genotype distributions of controls were 7% AA, 33% AC and 60% CC, while in short-term and long-term smokers, they were distributed as 4% and 7% AA, 25% and 31% AC and 71% and 62% CC, respectively. However, the frequency of C allele showed a significant in short-term smokers when compared with controls (OR= 1.596, P= 0.00882, Table 3.10), but did not display that in long-term smokers (OR= 1.059, P= 0.76203, Table 3.11).

In MDM2 rs769412 SNP, no significant associations were observed in both groups of years of smoking. The percentage of genotype distributions of AA, AG and GG in controls were 85%, 15% and 0%, respectively. Among short-term smokers, these ratios were 86%, 14% and 0%, respectively (Table 3.10), whereas they were 80%, 19% and 1%, respectively among long-term smokers (Table 3.11). The wild-type A allele was distributed at 92% among controls and at 93% and 89% in the short- and long-term smokers, respectively compared to the G mutant allele distribution of 8% among non-smoking populations, 7% in the short-term smokers and 11% in the long-term smokers (Tables 3.10 and 3.11).

Table 3.10: Genotype frequencies of TP53, P21, and MDM2 gene SNPs in smokers with entire controls in participants whom smoking for ≤ 7 years

Gene	SNP	Allele	Controls		≤ 7 Years		OR	95% CI	X^2	P value
			N	Percent	N	Percent				
		Total	**285**	**100%**	**172**	**100%**				
		CC	78	27%	17	10%	Ref			
		CG	138	49%	89	52%	2.959	1.643-5.330	13.78	**0.00021***
TP53	rs1042522	GG	69	24%	66	38%	4.389	2.352-8.189	23.22	**<0.00001***
		CG+GG	207	73%	155	90%	3.436	1.954-6.041	19.91	**<0.00008***
		C	294	52%	123	36%	Ref			
		G	276	48%	221	64%	1.914	1.454-2.520	21.65	**<0.00003***
		Total	**274**	**100%**	**167**	**100%**				
		AA	19	7%	6	4%	Ref			
		AC	91	33%	42	25%	1.462	0.544-3.925	0.57	0.44965
P21	rs1801270	CC	164	60%	119	71%	2.298	0.891-5.928	3.10	0.07813
		AC+CC	255	93%	161	96%	1.999	0.782-5.112	2.17	0.14105
		A	129	24%	54	16%	Ref			
		C	419	76%	280	84%	1.596	1.123-2.270	6.86	**0.00882***
		Total	**282**	**100%**	**168**	**100%**				
		AA	239	85%	145	86%	Ref			
		AG	42	15%	23	14%	0.903	0.521-1.562	0.13	0.71432
MDM2	rs769412	GG	1	0%	0	0%	0.549	0.022-13.558	0.61	0.43640
		AG+GG	43	15%	23	14%	0.882	0.510-1.523	0.20	0.65141
		A	520	92%	313	93%	Ref			
		G	44	8%	23	7%	0.868	0.515-1.466	0.28	0.59710

*P < 0.05, Ref = Reference allele

Table 3.11: Genotype frequencies of TP53, P21, and MDM2 gene SNPs in smokers with entire controls in participants whom smoking for > 7 years

Gene	SNP	Allele	Controls		> 7 years		OR	95% CI	X^2	P value
			N	Percent	N	Percent				
TP53	rs1042522	**Total**	**285**	**100%**	**110**	**100%**				
		CC	78	27%	5	4%	Ref			
		CG	138	49%	58	53%	6.557	2.524-17.035	18.53	**0.00002***
		GG	69	24%	47	43%	10.626	3.999-28.234	29.82	**<0.00004***
		CG+GG	207	73%	105	96%	7.913	3.109-20.140	24.91	**<0.00006***
		C	294	52%	68	31%	Ref			
		G	276	48%	152	69%	2.381	1.712-3.311	27.32	**<0.00001***
P21	rs1801270	**Total**	**274**	**100%**	**111**	**100%**				
		AA	19	7%	8	7%	Ref			
		AC	91	33%	34	31%	0.887	0.355-2.216	0.07	0.79793
		CC	164	60%	69	62%	0.999	0.418-2.391	0.00	0.99863
		AC+CC	255	93%	103	93%	0.959	0.407-2.261	0.01	0.92433
		A	129	24%	50	23%	Ref			
		C	419	76%	172	77%	1.059	0.730-1.536	0.09	0.76203
MDM2	rs769412	**Total**	**282**	**100%**	**113**	**100%**				
		AA	239	85%	90	80%	Ref			
		AG	42	15%	22	19%	1.391	0.787-2.460	1.30	0.25503
		GG	1	0%	1	1%	2.656	0.164-42.907	0.51	0.47455
		AG+GG	43	15%	23	20%	1.420	0.810-2.490	1.51	0.21897
		A	520	92%	202	89%	Ref			
		G	44	8%	24	11%	1.404	0.832-2.369	1.63	0.20187

*P < 0.05, Ref = Reference allele

3.7 A Comparison Between the Effect of Shisha Smoking and CS on TP53, P21, and MDM2 Polymorphisms

To evaluate the risk associated with CS based on type of smoking, all smokers were grouped into two categories as smokers whom had smoked cigarettes, which include 213 individuals and those whom had smoked Shisha, which include 69 individuals. For TP53 SNP rs1042522, the genotype distributions and allele frequencies among smokers showed a significant allocation when compared with controls in both categories. In cigarette smokers (Table 3.12), the heterozygous CG showed approximately 5-fold increase of correlation with CS (OR= 4.783; CI= 2.526-9.055; P<0.00003), while the homozygous GG genotypes showed nearly 8-fold stronger association with CS (OR= 7.826; CI= 4.022-15.228; P<0.00006). The two variants together CG+GG were 5.797-fold more correlated with CS (OR= 5.797; CI= 3.123-10.760; P<0.00001). In addition, the G phenotype showed more than 2-fold increase correlation with CS as compared to C phenotype (OR= 2.271; CI= 1.748-2.951; P<0.00005, Table 3.12)

In shisha smokers for the same SNP, the CG genotype appeared to have more than two-fold increased correlation with shisha smoking

(OR= 2.324; CI= 1.066-5.067; P= 0.03050), while the GG genotype was approximately three-fold more associated with shisha smoking compared to the reference CC (OR= 2.889; CI= 1.252-6.665; P= 0.01054). Furthermore, a combination of the two alleles CG+GG showed 2.512-fold increase association with shisha smoking (OR= 2.512; CI= 1.190-5.304; P= 0.01314). The G allele phenotype showed slightly lower association with shisha smoking (OR= 1.608; CI= 1.101-2.347; P= 0.01345, Table 3.13).

This study also showed that the genotype frequencies results of P21 SNP rs1801270 did not show any significant correlation with smoking in both types of smoking when compared with controls. The genotype distributions of AA, AC and CC of non-smokers were 7%, 33% and 60%, respectively; whereas in cigarette and shisha smokers were 6% and 3% AA, 28% and 25% AC, and 66% and 72% CC, respectively (Table 3.12 and Table 3.13). Also, the frequency of C allele did not show any significant difference with smoking in cigarette smokers' category (OR= 1.262, P= 0.14373, Table 3.12), but showed a significant correlation in shisha smokers' category compared to controls (OR= 1.608, P= 0.04104, Table 3.13).

Lastly, the MDM2 rs769412 results did not appear any significant association in either category of smoking types when compared with non-smokers. The genotype allocations of AA, AG and GG of controls were found to be 85%, 15% and 0%, respectively. However, among cigarette smokers, the genotype distributions were 84%, 16% and 0%, respectively (Table 3.12), while they were 82%, 18% and 0%, respectively among shisha smokers (Table 3.13). The frequency of G allele also did not show any significant association in both categories when compared with controls as shown in Table 3.12 and Table 3.13.

Table 3.12: Distribution of genotype frequencies of selected SNPs in TP53, P21, and MDM2 gene in smokers whom smoke cigarettes

Gene	SNP	Allele	Controls		Cigarette		OR	95% CI	X^2	P value
			N	Percent	N	Percent				
		Total	**285**	**100%**	**213**	**100%**				
		CC	78	27%	13	6%	Ref			
		CG	138	49%	110	52%	4.783	2.526-9.055	26.04	**<0.00003***
TP53	rs1042522	GG	69	24%	90	42%	7.826	4.022-15.228	42.78	**<0.00006***
		CG+GG	207	73%	200	94%	5.797	3.123-10.760	36.91	**<0.00001***
		C	294	52%	136	32%	Ref			
		G	276	48%	290	68%	2.271	1.748-2.951	38.39	**<0.00005***
		Total	**274**	**100%**	**209**	**100%**				
		AA	19	7%	12	6%	Ref			
		AC	91	33%	58	28%	1.009	0.456-2.233	0.00	0.98205
P21	rs1801270	CC	164	60%	139	66%	1.342	0.629-2.862	0.58	0.44520
		AC+CC	255	93%	197	94%	1.223	0.580-2.580	0.28	0.59618
		A	129	24%	82	20%	Ref			
		C	419	76%	336	80%	1.262	0.924-1.723	2.14	0.14373
		Total	**282**	**100%**	**213**	**100%**				
		AA	239	85%	179	84%	Ref			
		AG	42	15%	33	16%	1.049	0.639-1.722	0.04	0.84963
MDM2	rs769412	GG	1	0%	1	0%	1.335	0.083-21.491	0.04	0.83788
		AG+GG	43	15%	34	16%	1.056	0.647-1.723	0.05	0.82815
		A	520	92%	391	92%	Ref			
		G	44	8%	35	8%	1.058	0.666-1.681	0.06	0.81163

*P < 0.05, Ref = Reference allele

Table 3.13: Distribution of genotype frequencies of selected SNPs in TP53, P21, and MDM2 gene in smokers whom smoke shisha

Gene	SNP	Allele	Controls		Shisha		OR	95% CI	X^2	P value
			N	Percent	N	Percent				
		Total	**285**	**100%**	**69**	**100%**				
		CC	78	27%	9	13%	Ref			
		CG	138	49%	37	54%	2.324	1.066-5.067	4.68	**0.03050***
TP53	rs1042522	GG	69	24%	23	33%	2.889	1.252-6.665	6.54	**0.01054***
		CG+GG	207	73%	60	87%	2.512	1.190-5.304	6.15	**0.01314***
		C	294	52%	55	40%	Ref			
		G	276	48%	83	60%	1.608	1.101-2.347	6.11	**0.01345***
		Total	**274**	**100%**	**68**	**100%**				
		AA	19	7%	2	3%	Ref			
		AC	91	33%	17	25%	1.775	0.378-8.331	0.54	0.46200
P21	rs1801270	CC	164	60%	49	72%	2.838	0.639-12.614	2.04	0.15340
		AC+CC	255	93%	66	97%	2.459	0.559-10.823	1.51	0.21956
		A	129	24%	21	15%	Ref			
		C	419	76%	115	85%	1.686	1.017-2.794	4.17	**0.04104***
		Total	**282**	**100%**	**68**	**100%**				
		AA	239	85%	56	82%	Ref			
		AG	42	15%	12	18%	1.219	0.603-2.467	0.31	0.58059
MDM2	rs769412	GG	1	0%	0	0%	1.413	0.057-35.142	0.23	0.62848
		AG+GG	43	15%	12	18%	1.191	0.590-2.406	0.24	0.62563
		A	520	92%	124	91%	Ref			
		G	44	8%	12	9%	1.144	0.587-2.230	0.16	0.69330

*P < 0.05, Ref = Reference allele

3.8 Comparison of Saudi Arabian Population Results with Other Results Reported for Different Populations

Genotyping results of non-smokers for the selected SNPs were used to compare Riyadh region population in Saudi Arabia (CRS), from which we collected our samples, with other previously studied populations. We studied the rs1042522 SNP genotypes in 285 samples, the rs1801270 SNP genotypes in 274 samples and the rs769412 SNP genotypes in 282 samples from CRS. The results for the selected SNPs are presented in Tables 3.14-3.16. The allelic and genotypic frequencies for rs1042522 SNP was significantly different among European (CEU; $P<0.00001$) and Nigerian (YRI; $P=0.00682$) populations when compared to Saudi population (Table 3.14). For P21 rs1801270 SNP, the frequency of the various alleles differ significantly between CEU, Chinese (HCB), Japanese (JPT), Chinese in Metropolitan (CHD), Luhya Kenyan (LWK), and Italian (TSI) populations in contrast with Saudi population from Riyadh Region with P values of less than 0.005 (Table 3.15). For MDM2 rs769412, genotyped samples of CRS population showed significantly different results compared to JPT ($P=0.001857$) and LWK ($P=0.001266$)

populations, but they did not show any difference when compared with the other populations (Table 3.16).

Table 3.14: Comparison of frequency of TP53 SNP in different populations

SNP ID	Populations	Samples (N)	Genotype frequency N (%)		X^2	P value
			C	G		
rs1042522	**CEU**	120	28 (0.23)	92 (0.77)	27.54	**<0.00001**
	HCB	90	43 (0.48)	47 (0.52)	0.354	0.55145
	JPT	88	39 (0.44)	49 (0.56)	3.650	0.05604
	YRI	118	78 (0.66)	40 (0.34)	7.318	**0.00682**
	CRS	285	147 (0.52)	138 (0.48)	Refs	

CEU: Utah residents with Northern and Western European ancestry from the CEPH collection. **HCB:** Han Chinese in Beijing, China. **JPT:** Japanese in Tokyo, Japan. **YRI:** Yoruba in Ibadan, Nigeria. **CRS:** Saudi population residing in the Riyadh region of central Saudi Arabia.

Table 3.15: Comparison of frequency of P21 variant in other populations

SNP ID	Populations	Samples (N)	Genotype frequency N (%)		X^2	P value
			A	C		
rs1801270	**CEU**	224	9 (0.04)	215 (0.96)	37.17	**<0.00001**
	HCB	86	40 (0.47)	46 (0.53)	16.62	**<0.00001**
	JPT	170	66 (0.39)	104 (0.61)	12.26	**0.000462**
	YRI	224	69 (0.31)	155 (0.69)	2.697	0.100554
	ASW	98	26 (0.27)	72 (0.73)	0.179	0.672645
	CHD	168	86 (0.51)	82 (0.49)	35.86	**<0.00001**
	GIH	176	32 (0.18)	144 (0.82)	1.811	0.178423
	LWK	180	58 (0.32)	122 (0.68)	4.218	**0.039999**
	MEX	98	25 (0.26)	73 (0.74)	0.139	0.709696
	MKK	284	74 (0.26)	210 (0.74)	0.024	0.876376
	TSI	176	18 (0.10)	158 (0.90)	15.03	**0.000106**
	CRS	274	66 (0.24)	208 (0.76)	Refs	

CEU: Utah residents with Northern and Western European ancestry from the CEPH collection. **HCB:** Han Chinese in Beijing, China. **JPT:** Japanese in Tokyo, Japan. **YRI:** Yoruba in Ibadan, Nigeria. **ASW:** African ancestry in Southwest USA. **CHD:** Chinese in Metropolitan Denver, Colorado. **GIH:** Gujarati Indians in Houston, Texas. **LWK**: Luhya in Webuye, Kenya. **MEX:** Mexican ancestry in Los Angeles, California. **MKK:** Maasai in Kinyawa, Kenya. **TSI:** Toscans in Italy. **CRS:** Saudi population residing in the Riyadh region of central Saudi Arabia.

Table 3.16: Genotype frequencies of MDM polymorphism in Saudi population compared to other populations

SNP ID	Populations	Samples (N)	Genotype frequency N (%)		X^2	P value
			A	G		
rs769412	**CEU**	226	210 (0.93)	16 (0.07)	0.708	0.400258
	HCB	86	83 (0.97)	3 (0.03)	2.075	0.149684
	JPT	172	170 (0.99)	2 (0.01)	9.685	**0.001857**
	YRI	226	201 (0.89)	25 (0.11)	1.465	0.226213
	ASW	98	84 (0.86)	13 (0.13)	2.477	0.115505
	CHD	170	163 (0.96)	7 (0.04)	2.568	0.109046
	GIH	174	160 (0.92)	14 (0.08)	0.035	0.851414
	LWK	180	148 (0.82)	32 (0.18)	10.391	**0.001266**
	MEX	100	92 (0.92)	8 (0.08)	0.000	1.000000
	MKK	286	252 (0.88)	34 (0.12)	2.526	0.111949
	TSI	176	164 (0.93)	12 (0.07)	0.187	0.665383
	CRS	282	259 (0.92)	23 (0.08)	Refs	

CEU: Utah residents with Northern and Western European ancestry from the CEPH collection. **HCB:** Han Chinese in Beijing, China. **JPT:** Japanese in Tokyo, Japan. **YRI:** Yoruba in Ibadan, Nigeria. **ASW:** African ancestry in Southwest USA. **CHD:** Chinese in Metropolitan Denver, Colorado. **GIH:** Gujarati Indians in Houston, Texas. **LWK**: Luhya in Webuye, Kenya. **MEX:** Mexican ancestry in Los Angeles, California. **MKK:** Maasai in Kinyawa, Kenya. **TSI:** Toscans in Italy. **CRS:** Saudi population residing in the Riyadh region of central Saudi Arabia.

3.9 Structural and Functional Analysis of TP53, P21, and MDM2 Polymorphism

The selected SNPs of TP53, P21, and MDM2 gene were located in the exon regions. So, we examined their potential effects on p53, p21 and Mdm2 protein by Phyre2 server and PyMOL software. The result indicated that the wild type structure of p53 has a proline amino acid in the location number 72 at PRR (residues 61–94) of p53 protein (Figure 3.1), while the P72R (rs1042522) SNP affected on the p53 structure and function especially in the PRR by substitution the proline by an arginine in the location number 72 of p53 protein (Figure 3.2). So, the P72R polymorphism was damaging and appeared to be correlated with CS in the Saudi society.

Also, the results showed that the wild type structure of P21 has an arginine amino acid which located at the location number 31 of p21 protein (Figure 3.3). The R31S (rs1801270) replaced this arginine residue by a serine amino acid in the location number 31 of p21 protein (Figure 3.4).So, the R31S was a non- synonymous SNP that changed the amino acid sequence of p21, but it has not affected on the functional domains of p21 and has not correlated with CS in the Saudi society.

Based on the Phyre2 and PyMOL results, the structure of Mdm2 protein has not changed with E354E (rs769412) SNP, because it was a synonymous SNP and did not changed the Mdm2 sequence and gave the same amino acid (glutamic acid) in the location number 354 of the Mdm2 (Figure 3.5). So, the E354E was not associate with CS and was not recognized in Saudi smokers.

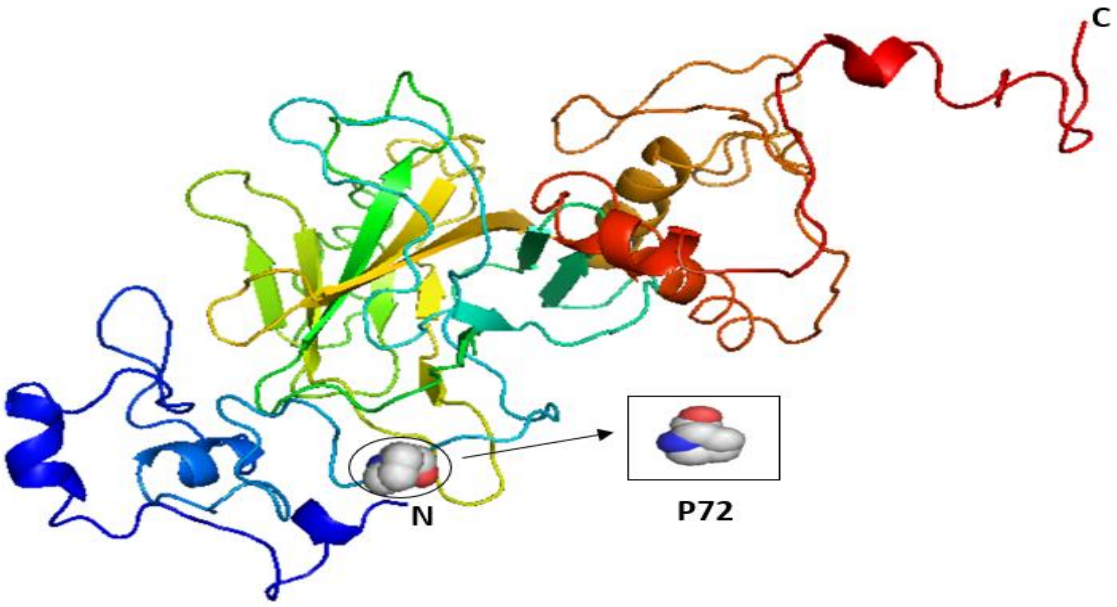

Figure 3.1: Wild type structure of p53 (P72). The figure shows the wild type structure of p53 protein. P72 refers to the Proline amino acid which located at the location number 72 of p53 protein. Letter N refers to N-terminus (free amino group (NH2-)), is the beginning of amino acid chain. C letter refers to C-terminal (a free carboxyl group (-COOH)), is the end of amino acid chain.

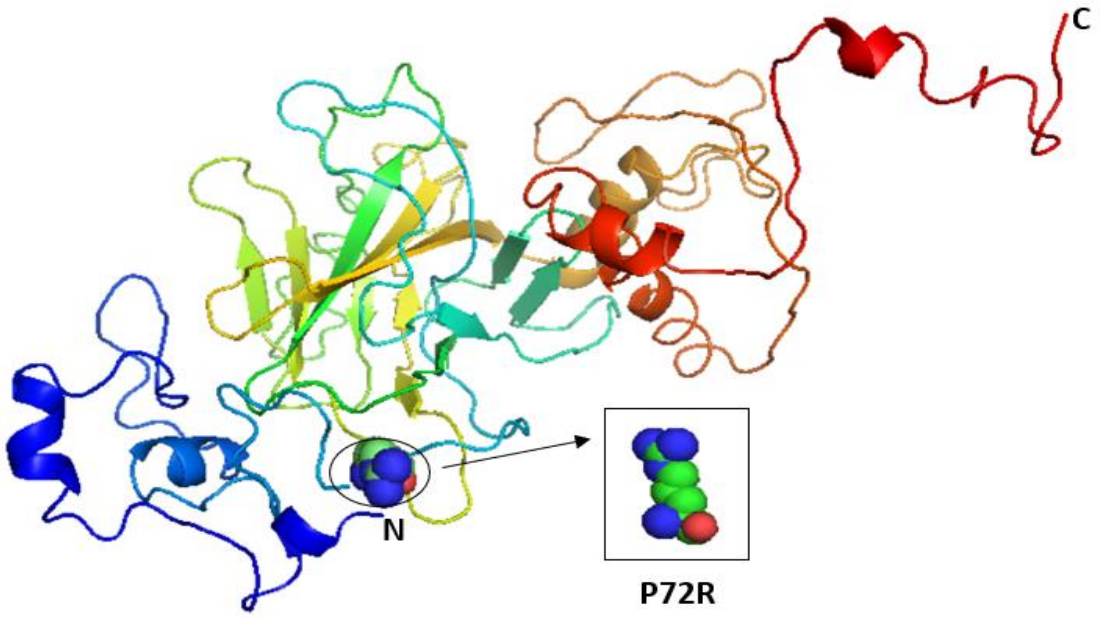

Figure 3.2: Structure of p53 with SNP (P72R). The diagram shows the changing of p53 protein after SNP (P72R) that leads to substitute Proline by Arginine in the location number 72 of p53 as identified in the p53 protein of Saudi smokers.

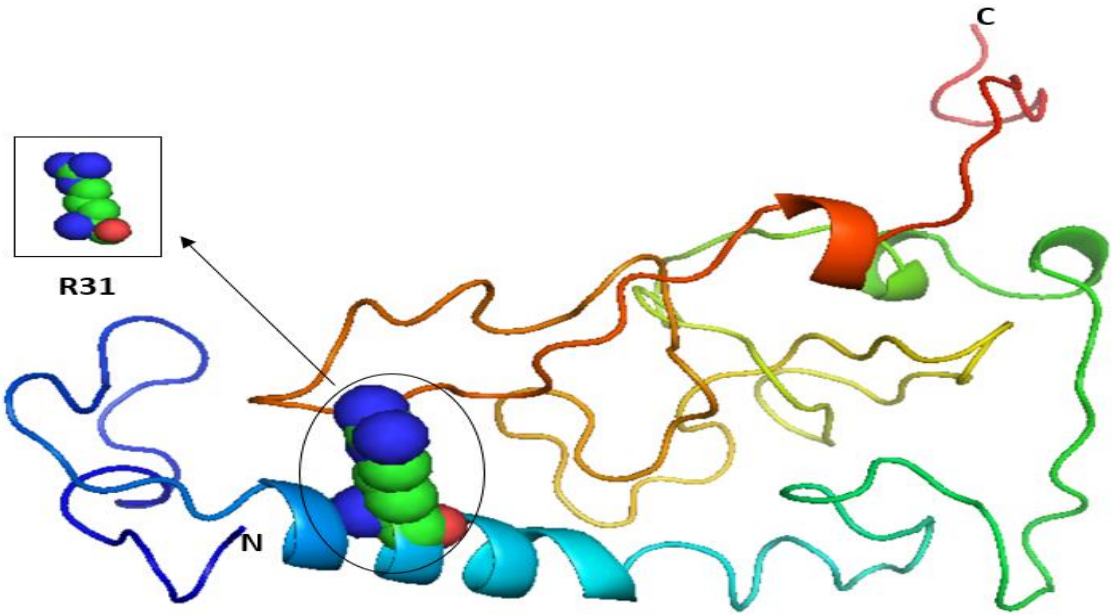

Figure 3.3: 3D structure of normal p21 protein (R31). The diagram displays the wild type structure of p21 protein. P31 indicates the Arginine amino acid which located at the location number 31 of p21 protein.

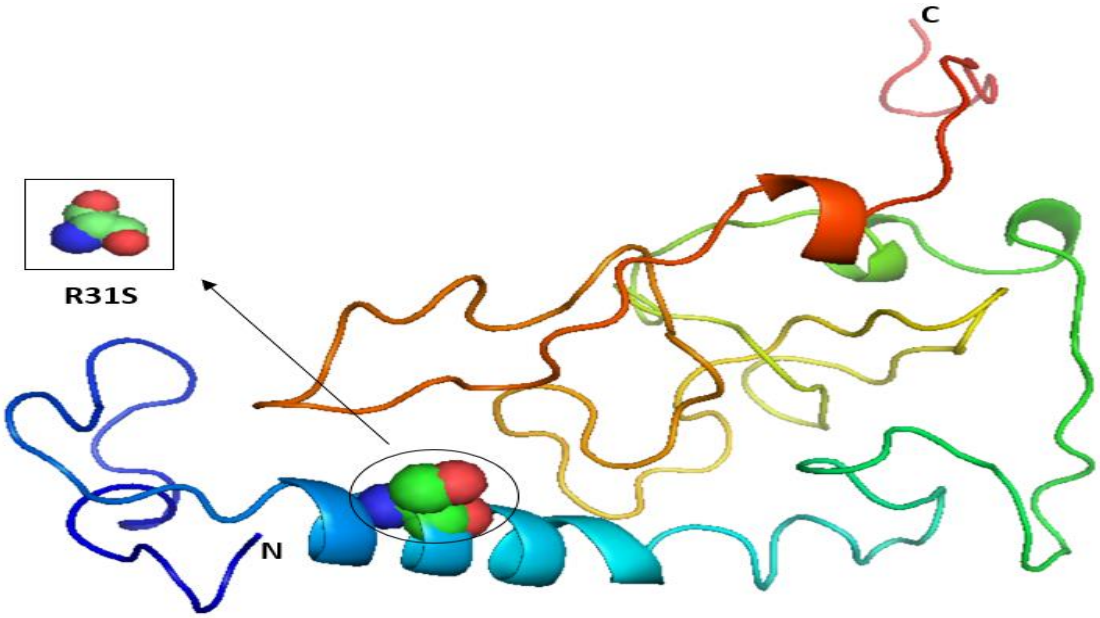

Figure 3.4: 3D structure of p21 with SNP (R31S). The diagram presents the SNP (R31S) in the p21 protein that leads to replaced Arginine by Serine in the location number 31 of p21 as recognized in the p21 protein of Saudi smokers, but it did not directly influence on functional domains of p21 protien.

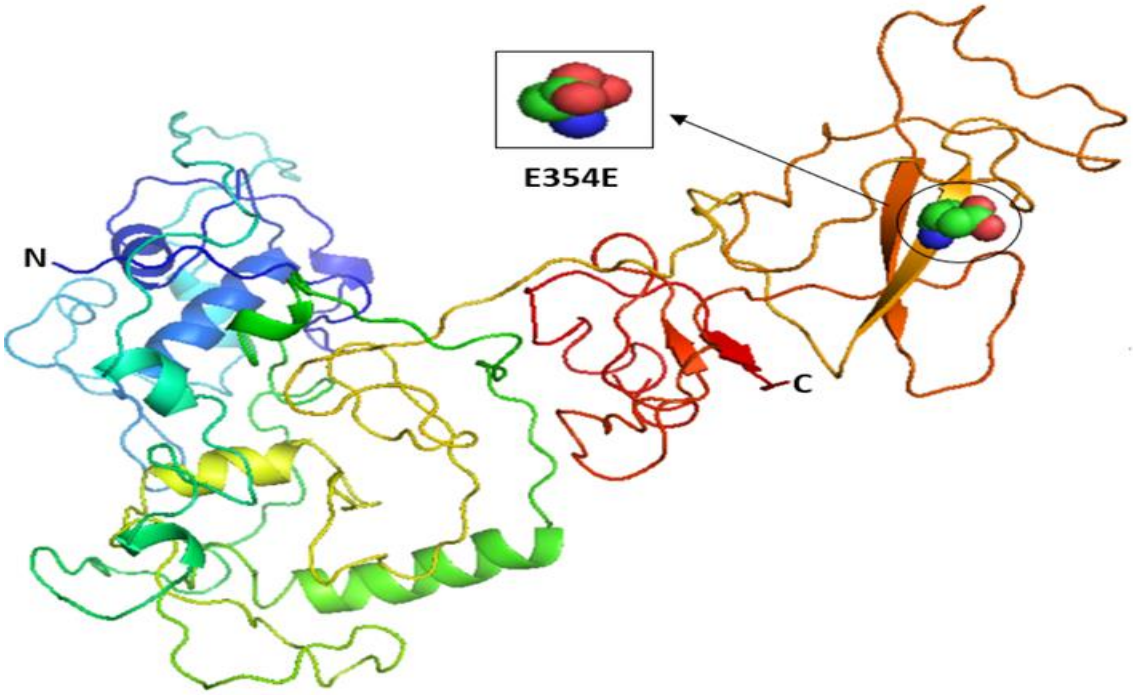

Figure 3.5: **Normal and mutated structure of Mdm2 (E354E).** The diagram displays the structure of Mdm2 protein. E354E indicates the synonymous SNP that does not change the glutamic acid in the location number 354 of Mdm2 protein which did not recognized among Saudi smokers.

Chapter 4: Discussion

It has been revealed that CS can cause the progression of multiple autoimmune diseases – chronic pulmonary, vascular, allergies, and cancer (Qiu et al., 2017)., including 80% of lung cancer (Shereef et al., 2015). Among Saudi Arabian adolescents, CS has become a major public health issue (Algorinees et al., 2016), and consumption of smokeless tobacco appears to contribute to oral cancer risk (Alharbi & Quadri, 2018). The damaging impacts of CS are attributed to multiple chemical and free radical components that can lead to the formation of DNA adducts, which then result in DNA damage (Pfeifer et al., 2002; Yoshitomi et al., 2014).

Furthermore, CS is a major etiologic factor for head and neck squamous cell carcinoma, and it has been reported that repetitive CS exposure of squamous epithelial cells in the oral cavity can cause neoplastic transformations. However, the real mechanism through which CS causes malignancies still requires clarification (Pfeifer et al., 2002). Cells in humans have several protective pathways that can inhibit DNA damage from duplicating itself (Pagès & Fuchs, 2002), and the genes in the TP53 pathway are considered to be one of the most recognized tumor suppression proteins (Brázda et al., 2014). In cases of DNA damage initiated by tobacco's chemical carcinogens,

the transcription of TP53 increases in order to arrest the cell cycle of the damaged cells at the G1/S phase to provide time for DNA repair genes to repair the damaged DNA (Jan, 2001; Bourdon, 2007). If the damage is irreparable, then cells go directly to the apoptosis pathway to prevent the damaged DNA's proliferation (Ferraiuolo et al., 2017). Therefore, the TP53 pathway has a very essential protective function and is normally called the guardian of the genome (Nguyen et al., 2017).

An example of genetic variations that can increase the risk for several cancers in human are SNPs, which contribute in the modification of DNA repair efficiency by altering protein function (de, 2002; Xi et al., 2004). All previous data support the hypothesis that TP53 SNPs and CS may contribute to the development of SRDs. A previous literature review showed no prior work evaluating the association between genetic variation of the TP53 pathway genes and CS effects. Thus, the main goal of this thesis is to investigate the potential role of correlations between the CS and the exonic genetic polymorphisms rs1042522 in TP53 gene, rs1801270 in P21 gene, and rs769412 in MDM2 gene using 283 samples from Saudi smokers and 285 samples from Saudi non-smokers to identify genetic markers that

could be beneficial in decreasing disease risk caused by CS among healthy individuals. The present study focuses on investigating the allocations of TP53 pathway gene polymorphisms in genomic DNA isolated from the peripheral blood cells of CS subjects compared to non-CS subjects.

In the TP53 pathway, TP53 rs1042522, P21 rs1801270, and MDM2 rs769412 polymorphisms play a crucial role in genomic instability and DNA damage. They have also been reported to be associated with cancer risk and pathologic characteristics (Chen et al., 2015). General significant relations between TP53 and P21 gene polymorphisms and smoking behaviour were observed in the study population. However, no global genetic and allelic differences were detected between the MDM2 SNP and the smoking individuals. In addition, genetic and allelic alterations were detected between the TP53 variant tested here and the smoking patients in terms of patient age, patient gender, duration of CS, daily rate of CS, and types of smoking among Saudi smokers as compared to the control individuals. P21 polymorphism, however, was associated with allelic differences in smokers whose ages fall under 29 years, below 7 years of CS consumption, and among smokers who smoke shisha. Conversely, our

results showed that no genetic and allelic variations were detected between the MDM2 SNP and the smoking subjects in all clinical parameters mentioned previously.

The p53 human tumor suppressor protein plays an essential role in cell cycle arrest regulation (Ren et al., 2013). Multiple polymorphisms in TP53 gene have been found to be connected to cancer susceptibility (Hrstka et al., 2009). Among them, the rs1042522 variant is the most widely examined polymorphism in TP53 gene. The rs1042522 polymorphism is located at exon 4 in codon 72 and is the missense substitution of C>G, which causes an amino acid transversion of proline (Pro) by arginine (Arg). Such amino acid transversion alters the P53 protein function (Grochola et al., 2010).

Given the fundamental role of TP53 rs1042522 polymorphism in tumorigenesis, many researchers attempted to explain their roles in certain cancer type risk. For example, previous studies found that rs1042522 conferred higher susceptibility to thyroid cancer among the Indian population (Khan et al., 2015), and is associated with increased lung adenocarcinoma risk in Chinese females (Ren et al., 2013). Interestingly, our results in the present study showed that genotypes

and allele frequencies of TP53 rs1042522 were higher among smoking individuals and may lead to the risk of SRDs development. The higher increased risk was found in all clinical parameters tested here, which include smokers' age, years of smoking, daily smoking, gender, and smoking types. Similar TP53 rs1042522 results were found, but in different populations and different malignancies (Li et al., 2013; Lin et al., 2013; Ren et al., 2013; Khan et al., 2015; Kumari et al., 2016).

The P21 rs1801270 genetic variant is also known as P21 Arg31Ser, which takes place in codon 31 and leads to amino acid change from arginine (Arg) to serine (Ser). This SNP is located in an exon region, which could alter the p21 protein's function (Chedid et al., 1994). Several studies have assessed the relationship between rs1801270 polymorphism and the risk of several types of human malignancies, including gastric cancer, breast cancer, and cervical cancer. However, the results remain inconclusive (Qiu et al., 2010; Li et al., 2011; Wang et al., 2012; Dong et al., 2015; Martinez-Nava et al., 2016).

In that sense, a previous study has revealed a correlation between the p21 rs1801270 A allele and the decrease of cervical

cancer susceptibility in Chinese women population (Wang et al., 2012). However, Taghavi et al. proposed that rs1801270 is not a genetic biomarker for esophageal carcinoma in northeastern Iran (Taghavi et al., 2010). A possible reason for this difference in results may be that the carcinogenesis mechanism may vary between diverse types of cancer sites and the rs1801270 polymorphism may exert several effects on multiple cancers (Xiao et al., 2017).

Our analysis showed that the rs1801270 C allele of P21 gene leads to the risk of SRDs development. The increase in risk was more evident among younger smokers, smokers who have consumed CS for less than seven years, and shisha users. Our results with the rs1801270 polymorphism are consistent with some previous reports (Powell et al., 2002; Wu et al., 2003; Li et al., 2005), but different from other studies (Shih et al., 2000; Polakova et al., 2009), which identify a deleterious effect of the rs1801270 C allele in smokers. This discrepancy is probably because of ethnic differences or sample size (Birgander et al., 1996). The study shows that the age of smokers significantly ($p < 0.05$) influences the association between cigarette consumption and the P21 gene for rs1801270 C allele. In smokers over 29 years old, the distribution of the C allele has no effects,

whereas it has harmful effects in smokers under 29 years old. A previous study reported that younger smokers are at greater risk of developing breast cancer (Verde et al., 2016) and that CS is more harmful at younger ages (Verde et al., 2016; Pérez-Rubio et al., 2017). Therefore, our results suggest that prevention of CS in young adults should be taken seriously.

The MDM2 gene is a negative regulator of p53 (Reza et al., 2020) and is also involved in tumor growth and metastasis (Qiu et al., 2008). MDM2 rs769412 SNP generates an A>G base change at codon 354, which causes Sp1 binding site, but this variant does not contribute to an amino acid substitution (Boersma et al., 2006). The rs769412 SNP showed the lack of relationship between lung cancer occurrence in smokers among African-American people (Pine et al., 2006). Nevertheless, in one study, Rajaraman et al. (2007) reported the protective status of rs769412 in a case of glioma cancer (Rajaraman et al., 2007). The present study investigates for the first time the MDM2 variant rs769412 in Saudi Arabian smokers compared to non-smokers. In our study, results indicated that the Mdm2 polymorphism rs769412 was not found to have a more positive, significant association with smoking individuals than with non-

smoking individuals. Therefore, our findings are in consensus with the previous findings of studies in different population (Boersma et al., 2006; Pine et al., 2006; Jiao et al., 2016; Reza et al., 2002), but not supported by Rajaraman et al. (2007) study (Rajaraman et al., 2007). Therefore, there is a great need to study the variation of MDM2 SNP rs769412 for diverse diseases in diverse populations. Nevertheless, this study may be useful for further research on smokers regarding MDM2 SNPs and their association with different diseases.

In conclusion, our results demonstrate that rs1042522 polymorphism has a higher effector role in all clinical parameters of the smoking population, which increases the potential risk of developing SRDs. However, the rs1801270 polymorphism was only associated with increasing risk among younger smokers, smokers who have consumed CS for less than seven years, and shisha users. Therefore, a novel diagnostic biomarker may exist for the early diagnosis of several diseases caused by CS in this sub-group of the Saudi population. Furthermore, the number of female samples was insufficient to identify any statistically significant relationship which may found in the P21 and MDM2 polymorphisms.

Recommendation

1- Further studies with sufficiently larger samples, functional analysis, and using various other populations are suggested to confirm our findings and to investigate the relation between the genetic variations of TP53, P21, and MDM2 and the effects of smoking.

2- Examine the potential association between CS and SNPs located in TP53, P21, and MDM2 genes in TP53 (rs1042522), P21 (rs1801270), and MDM2 (rs769412) genes in other exons regions, among smokers and non-smoker from Saudi Arabian population.

3- Study the possible association between TP53, P21 and MDM2 gene polymorphisms and SRDs such as asthma, chronic lung diseases and periodontal diseases among Saudi Arabian population.

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Arabic Abstract

خلفية الدراسة: الجين المثبط للأورام (TP53) والجينات المرتبطة بنفس المسار (P21 و MDM2)، تلعب دوراً حاسماً في تنظيم مرحلة (G1/S) من دورة حياة الخلية والمعنية بالكشف عن ضرر الحمض النووي منقوص الأكسجين. التغيرات الوراثية في تلك الجينات لها علاقة بظهور أمراض مختلفة مرتبطة بالتدخين. الهدف من هذه الدراسة هو فحص الارتباط المحتمل بين تدخين السجائر وتعدد الأشكال الوراثية المفردة (SNPs) الموجودة في مناطق الخرجونات في جين TP53 وP21 و MDM2 للمدخنين وغير المدخنين السعوديين.

منهجية الدراسة: تم فحص تعدد الأشكال الوراثية المفردة TP53 rs1042522 (C/G) وP21 rs1801270 (A/C) و MDM2 rs769412 (A/G) عن طريق التنميط الجيني لـ 568 عينة دم، 283 عينة منها تم الحصول عليها من ذكور وإناث مدخنين ، 285 عينة من ذكور وإناث غير مدخنين.

النتائج: تم الكشف عن التغيرات الوراثية والأليلية بين المتغير rs1042522 الذي تم اختباره والمرضى المدخنين من حيث عمر المريض، وجنس المريض، ومدة التدخين، والمعدل اليومي لـ التدخين، وأنواع التدخين بين المدخنين السعوديين مقارنة بالأشخاص الغير مدخنين كعينة ضابطة. ومع ذلك، ارتبط تعدد الأشكال الوراثية P21 rs1801270 بالاختلافات الأليلية لدى المدخنين الذين تقل أعمارهم عن 29 عامًا، وأقل من 7 سنوات يدخنون السجائر، وبين المدخنين الذين يدخنون الشيشة. بالإضافة إلى ذلك، أظهرت نتائجنا أنه لم يتم الكشف عن أي اختلافات جينية وأليلية بين تعدد الأشكال الوراثية MDM2 rs769412 والأشخاص المدخنين في جميع المعلمات السريرية المذكورة سابقًا.

الخاتمة: توضح نتائجنا أن تعدد الأشكال الوراثية المفردة rs1042522 لها دور مؤثر أعلى في جميع المعلمات السريرية لدى الأشخاص المدخنين، مما يزيد من المخاطر المحتملة

لتطور الأمراض المرتبطة بالتدخين. لذلك، يمكن استخدام تعدد الأشكال لوراثية المفردة هذه كمؤشر حيوي تشخيصي جديد للتشخيص المبكر للعديد من الأمراض التي يسببها تدخين السجائر للأشخاص السعوديين.