TP53 Polymorphisms in Smokers’ and Nonsmokers’ Pericoronal Follicles of Asymptomatic Impacted Third Molars

E Burhanoglu, MÇ Aksoy¹, PA Kosar²

Background and Purpose: The investigators designed and implemented a prospective cohort study composed of smoking and nonsmoking patients with asymptomatic fully impacted mandibular third molars. The objective of the paper was to evaluate 21 single-nucleotide polymorphisms (SNP) on the TP53 gene in smokers’ (S) and nonsmokers’ (NS) pericoronal follicles of asymptomatic impacted third molars. Materials and Methods: Matrix-assisted desorption/ionization time of flight mass spectrometry was used for SNP analysis of 21 regions in the TP53 gene. Descriptive statistics and Chi-square tests were computed with a P value of 0.05. Results: Ten of the 21 SNPs related to oral pathologies according to NCBI dbSNP, were detected; in these, the genotypic frequencies showed no differences between the S and NS groups (P > 0.05). The results showed a high ratio of SNPs without correlation between smoking and TP53 gene status. Conclusion: Further studies should examine the entire TP53 gene to elucidate how smoking affects it in larger study populations.

Keywords: Dental follicle, matrix-assisted desorption/ionization time of flight mass spectrometry, single-nucleotide polymorphism, smoking, TP53

Original Article

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The extraction of impacted teeth is the most common surgical procedure performed in oral surgery, but the extraction of asymptomatic impacted teeth remains controversial. Studies on asymptomatic fully impacted teeth have focused on the histopathologic and immunohistochemical findings in their follicles. Dentigerous cysts, odontogenic keratocystic tumors, odontomas, and ameloblastomas are some of the pathologies that may arise from the follicles of asymptomatic impacted teeth. Although rare, malignancies have been reported as arising from these follicles.[1]

Cigarette smoke is comprised of approximately 5000 chemicals, of which 81 are classified “carcinogenic” by the International Agency for Research on Cancer,[2] free radicals and the chemicals in cigarette smoke form DNA adducts and cause DNA damage.[3,4]

Human cells have various protective mechanisms that can prevent damaged DNA from replicating itself,[5] and p53 is one of the most well-known tumor suppression proteins.[6] In healthy tissues, p53 protein is found in low levels, but because of its short half-life (6–20 min), it cannot be detected in routine immunohistochemical procedures.[7] In cases of cellular stress or DNA damage, its transcription increases and it arrests cells in the G1 phase of the cell cycle to provide time for DNA repair.[8] If the damage is irreparable, then it directs the cell to apoptosis.[9]

Studies investigating p53 in tissue have found that p53 staining was more common and intense in the smoking group than the control group, which suggested that one effect of smoking could be some kind of mutation in the TP53 gene that leads to the increased transcription of the mutated and more stable p53 protein, leading to its accumulation in tissues.[1]

Access this article online

Quick Response Code:

Website: www.njcponline.com
DOI: 10.4103/njcp.njcp_434_17

How to cite this article: Burhanoğlu E, Aksoy MÇ, Kosar PA. TP53 polymorphisms in smokers' and nonsmokers' pericoronal follicles of asymptomatic impacted third molars. Niger J Clin Pract 2018;21:967-73.

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TP53 mutation is the most common genetic mutation encountered in human cancers; there is an accompanying TP53 mutation in 50% of squamous cell carcinomas. Smoking is a major etiologic factor for squamous cell carcinoma of the head-and-neck regions, and it has been suggested that the repetitive exposure of squamous epithelial cells in the oral cavity to carcinogens in cigarette smoke can cause neoplastic transformations. However, the exact mechanism of how cigarette smoke causes neoplastic changes still requires elucidation.[3]

The aim of this article is to investigate the TP53 status in the dental follicles of asymptomatic impacted lower third molar teeth in smoking and nonsmoking patients and to assess whether a relationship exists between TP53 status and smoking.

**Materials and Methods**

The study was conducted in accordance with the Declaration of Helsinki and the study design was approved by the Local Ethical Committee (Süleyman Demirel University Faculty of Medicine Ethical Committee, Decision No. 125). The study was supported by the Süleyman Demirel University Scientific Research Projects Coordination Department (Project No. 3608-D2-13). The inclusion criteria were patients aged 18 or above who were systemically healthy, had not used antibiotics or analgesic drugs for a minimum of 1-month before the surgical procedure, and had a clinically asymptomatic fully impacted lower third molar teeth with pericoronal radiolucency narrower than 2.5 mm on a panoramic radiograph.[10] Patients who were younger than 18 years, had accompanying systemic disease, had used antibiotics or analgesic drugs in the previous month, and/or had clinically symptomatic impacted lower third molar teeth with a pericoronal radiolucency wider than 2.5 mm on a panoramic radiograph were excluded from the study.

The patients’ sociodemographic characteristics, smoking habits for the smoker group (smoking duration, daily consumption, and packs/year), the status of bone retention in the teeth, and the position of the teeth according to Winter’s classification were recorded. The widest region of the pericoronal radiolucencies was measured with the Planmeca Romexis (Planmeca Oy, Helsinki, Finland) software.

Routine surgical extraction procedures were performed under local anesthetic by experienced surgeons. The follicles were separated from the teeth with the help of a lancet and kept in saline solution at 4°C. Specimens were sent to a private laboratory with a cold chain for single-nucleotide polymorphism (SNP) analysis. Before the polymerase chain reaction (PCR), the quantification of genomic DNA was confirmed with ultraviolet spectrophotometer measurements of 260–280-nm wavelength results at 1.8–2.0. After quantitation, most of the DNA was diluted to a stock concentration of 50 ng/μl. For the analysis, a matrix-assisted desorption/ionization time of flight mass spectrometry (MALDI-TOF MS; Sequenom, San Diego, USA) procedure was carried out on 21 regions of the TP53 gene (selected from the NCBI dbSNP database) (TP53 dbSNP 2015). Nucleic acid changes were detected by amplifying target regions using the genomic software of MassARRAY® TYPER 4.0 (Agena Bioscience Inc., San Diego, USA) and specially designed primer pairs. Unconnected deoxynucleotide triphosphates in the amplification products were neutralized with shrimp alkaline phosphatase. These products were then subjected to elongation reaction with iPLEX (Agena Bioscience Inc., San Diego, USA). The iPLEX reaction products were treated with a cationic exchange resin to remove ions such as Na+, K+, Mg+, and to minimize the background contamination, and then centrifuged. SNPs that are classified as either pathologic or likely pathologic in the database and associated with oral pathologies were included in the study. Furthermore, some SNPs were eliminated and replaced with SNPs in the kit design [Figure 1].

Chi-square test for independence was used for the statistical analysis of the relationship between SNP occurrence and smoking, and a Z-test was used to compare the two ratio’s critical values.

**Results**

One hundred follicles were examined in the paper and the main reason for extraction being prophylactic followed by orthodontic treatment. The study group consisted of the follicles from 50 smoking participants and the control group consisted of those from the 50 nonsmoking participants. Out of the 100 follicles, 58 were from female and 42 were from male patients, and ages were in the range 18–50 (22.9 ± 5.9). Patients’ gender and smoking status, bone retention status, and angulation according to the Winter’s classification of the impacted teeth were as given in Table 1.

The shortest duration of smoking habit was 7 months, while the longest was 33 years. The mean duration of smoking habit was 7.7 ± 6.4 years. The number of cigarettes consumed varied between two cigarettes a month to 30 cigarettes a day. The mean cigarette consumption was 12.5 ± 7.5 cigarettes each day. In terms of packs per year, the mean cigarette consumption was 4.9 ± 5.1 (varying between 0.1 and 25).

MALDI-TOF MS analysis detected 10 SNPs on the TP53 gene [Table 2]: rs2287497, rs2287498, rs2287499, rs2909430, rs8079544, rs9895829, rs12947788,
rs12951053, rs17551157, and rs17880560 [Figure 2a-c]. Four of these SNPs (rs2287497, rs2287498, rs2287499, and rs17551157) were on the WRAP53 gene, which is a 5’ flanking gene and a negative regulator of TP53.

According to the Chi-square analysis results, the Chi-square test statistic calculated between the base sequence of the rs2287497 gene region and smoking status was found to be 3.8, which was not statistically significant. Furthermore, as a result of the Z-test, the difference between the C base rate (38/50-0.8) and the C base rate (45/50-0.9) among the nonsmokers (NS) in the rs2287497 gene was not statistically significant.

The Chi-square test statistic calculated between the base sequence of the rs2287498 gene region and smoking status was found to be 1.5, which was not statistically significant. In the rs2287498 gene, the difference between the prevalence of the G base rate (29/50-0.6) in NS and the prevalence of G in smokers (33/50-0.7) was not statistically significant.

The Chi-square test statistic calculated between the base sequence of the rs2287499 gene region and smoking status was found to be 3.3, which was not considered statistically significant. In the rs2287499 gene area, the difference between the prevalence of the G base incidence in NS (43/50-0.9) and in smokers (48/50-1) was not statistically significant.

The Chi-square test statistic that is calculated between the base sequence of the rs2909430 gene region and smoking status was found to be 1.7, that between the base sequence of the rs8079544 gene region and smoking status was found to be 1.9, that between the base sequence of the rs9895829 gene region and smoking status was found to be 1.9, that between the base sequence of the rs12947788 gene region and smoking status was found to be 1, and that between the base sequence of the rs12951053 gene region and smoking status was found to be 1, none of which are statistically significant.

The Chi-square test statistic calculated between the base sequence of the rs17551157 gene region and smoking status was found to be 2.2 and was not statistically significant.
Table 2: Base substitutions of observed single nucleotide polymorphisms (SNPs) and reference SNP Number

<table>
<thead>
<tr>
<th>Reference SNP number (SNP500 cancer ID)</th>
<th>Base substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2287497 (WRAP53-07)</td>
<td>C&gt;T</td>
</tr>
<tr>
<td>rs2287498 (WRAP53-08)</td>
<td>Ex1+19C&gt;T</td>
</tr>
<tr>
<td>rs2287499 (WRAP53-11)</td>
<td>Ex1-230C&gt;G</td>
</tr>
<tr>
<td>rs2909430 (TP53-66)</td>
<td>IVS4-91G&gt;A</td>
</tr>
<tr>
<td>rs8079544 (TP53-09)</td>
<td>IVS1-112C&gt;T</td>
</tr>
<tr>
<td>rs9895829 (TP53-65)</td>
<td>IVS4-125A&gt;G</td>
</tr>
<tr>
<td>rs12947788 (TP53-10)</td>
<td>IVS7+72G&gt;A</td>
</tr>
<tr>
<td>rs12951053 (TP53-11)</td>
<td>IVS7+92T&gt;G</td>
</tr>
<tr>
<td>rs17551157</td>
<td>C=DEL</td>
</tr>
<tr>
<td>rs17880560</td>
<td>delCAGGTC</td>
</tr>
</tbody>
</table>

SNP=Single-nucleotide polymorphism

Table 3: Allele frequencies of different rs regions in smoking and nonsmoking patients

<table>
<thead>
<tr>
<th>Smoking</th>
<th>Genotype</th>
<th>x²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsmoking (-)</td>
<td>AA</td>
<td>0.900</td>
</tr>
<tr>
<td></td>
<td>Aa</td>
<td>0.760</td>
</tr>
<tr>
<td>Smoker (+)</td>
<td>AA</td>
<td>0.980</td>
</tr>
<tr>
<td></td>
<td>Aa</td>
<td>0.920</td>
</tr>
</tbody>
</table>

AA=Homozygote major allele; Aa=Heterozygote allele; aa=Homozygote minor allele; += Positive means Smoking; -= Negative means non smoking

sequences of the rs2287497, rs2287498, rs2287499, rs2909430, rs8079544, rs9895829, rs12947788, rs12951053, rs17551157, and rs17880560 gene regions are independent from cigarette smoking. The allele frequencies are given in Table 3.

Statistical analysis showed no correlation between smoking and SNP occurrence. The Chi-square values for each SNP and smoking habit were in the range 1.0–3.8.

**DISCUSSION**

The extraction of impacted teeth is the most common surgical procedure in oral surgery, but the prophylactic extraction of asymptomatic fully impacted teeth remains controversial. Studies on these teeth have focused on histopathological changes and immunohistochemical stainings as seen on the dental follicles of such teeth. The term “asymptomatic” refers to impacted teeth that show no signs of illness, including radiographic findings, and studies involving these teeth should have pericoronal radiolucencies no wider than 2.5 mm; this limit can be extended to 3 mm if analog radiographs are used to account for magnification errors.[11]

In immunohistochemistry, p53 is one of the most commonly investigated proteins, which is a tumor suppressor protein found at very low levels in healthy tissue.[8] In cases, in which cellular stress causes DNA damage in cells, p53 expression increases and arrests the cell cycle in the G1 phase to allow time for DNA repair.[8] If the damage is irreparable, then p53 directs the cell to apoptosis and prevents the proliferation of damaged DNA.[9] This very important protective function has afforded p53, the honor of being called the “guardian of the genome.”[12]

**TP53** is the most frequently mutated gene in human cancers,[3,13] and there is a **TP53** mutation in more than 50% of cancers in the head-and-neck regions.[14] While smoking is a major etiological factor in cancer development, it also causes specific mutations in the **TP53** gene by forming DNA adducts in hotspot regions.[15]

In the presented paper, the researchers investigated 21 SNPs in the **TP53** gene using MALDI-TOF MS, of which 10 were detected (rs2287497, rs2287498, rs2287499, rs2909430, rs8079544, rs9895829, rs12947788, rs12951053, rs17551157, and rs17880560). The allele frequencies of the SNPs in this paper population were in concordance with previous studies except for rs17880560 and rs2287499.

Rs2287497 is an intronic change that takes place in the 5’ flanking gene of **TP53**; **WRAP53** is a negative regulator of **TP53** and partly overlaps with it.[16] In this SNP, a
rs2287498 is an exonic change in the second exon of WRAP53. In this SNP, a silent mutation takes place with a guanine (G) to adenine (A) nucleotide exchange that does not affect the coding of the phenylalanine amino acid. Although this is a silent mutation, it has been reported that the SNP in rs2287498 is related to ovarian and breast cancer. The allele frequencies in the presented study population were 0.8 (A/A), 0.4 (A/G), and 0.6 (G/G), and these results are in concordance with the paper of Međrek et al. Garcia-Closas et al. reported that a healthy homozygote genotype in healthy Polish and Norwegian populations was found to be 0.8, whereas in individuals with breast cancer, this ratio was found to be 0.9. On comparing subgroups of breast cancer, there was an increased risk of estrogen-receptor-negative breast cancer in the presence of SNP in the rs2287498 region.

rs2287499 is in the first exon of the WRAP53 gene. A G nucleotide is substituted for a C nucleotide, which results in glycine amino acid coding. Garcia-Closas et al. reported that SNPs in rs2287499 may be associated with estrogen-receptor-negative breast cancer. The study population’s allele frequencies were found to be 0 (C/C), 0.1 (C/G), and 0.9 (G/G), which are in accordance with previous studies that reported high frequencies of the C/C genotype. Bonab et al. reported a healthy homozygote genotype ratio of 0.7, a heterozygote genotype ratio of 0.3, and a homozygous polymorphic genotype ratio of 0.1 in the control group of Iran’s Azeri population; meanwhile, these ratios were respectively reported as 0.7, 0.3, and 0 in the patient group. Thus, they suggested that SNPs in the rs2287499 region were not associated with breast cancer. Garcia-Closas et al. reported that the rate of the homozygous healthy genotype incidence was 0.8 in the healthy population and 0.5 in the patient population; the heterozygote genotype incidence was 0.3 in the healthy group, 0.2 in the patient group; the homozygote polymorphic genotype was found to be 0 in both the healthy and patient groups.

rs2909430 is an intronic change in TP53’s fourth intron. This SNP, in which an A nucleotide is substituted for a G nucleotide, is associated with a decreased risk of invasive breast cancer in women younger than 50. Mechanic et al. reported an increased risk of lung cancer with a worse prognosis in the African–American population with this SNP and accompanying SNPs in rs1042522, rs9895829, rs1625895, and rs12951053. However, this was not the case for the Caucasian population. The allele frequencies in the study population were 0.7 (A/A), 0.3 (A/G), and 0 (G/G), which are in concordance with previous studies. Pineda et al. reported that SNPs commonly found in the TP53 gene were not associated with bladder cancer.

rs8079544 is found in the first intron of TP53, where a T nucleotide is substituted for a C nucleotide. Few studies have focused on this SNP, and no association has yet been established with any cancer type. The allele frequencies were 1 (C/C) and 0.1 (C/T), and to T/T homozygosity was detectable in the study group. These results are in agreement with the NCBI dbSNP CAUC1 population. Pineda et al. reported that a healthy homozygote genotype ratio of 0.9, a heterozygote genotype ratio of 0.1, and a homozygote polymorphic genotype ratio of 0 in a healthy population among Spanish races. These rates were no different from those seen in patients who had been diagnosed with bladder cancer.

rs9895829 is an intronic polymorphism in the TP53 gene’s fourth intron where a G nucleotide has been substituted for an A nucleotide. In patients with bladder cancer, the G allele was found in higher frequencies than in the healthy group. In the present paper, allele frequencies were reported as 1 (A/A) and 0.1 (A/G), but no G/G homozygosity was detected; these results concur with the NCBI dbSNP CAUC1 population.

rs12947788 is an A-to-G nucleotide exchange in intron 7. Studies have associated this SNP with malign pleural mesothelioma and lung cancer. In this paper, the respective frequencies of A/A, A/G, and G/G genotypes were 0, 0.1, and 0.9, which were again in concordance with the NCBI db SNP CAUC1 population. Sprague et al. reported that TP53 polymorphisms were associated with an increased risk of breast cancer. In another study comparing colorectal cancer cases with a healthy control group, it was reported that the T allele, which is a minor allele, was seen less often in patients with colorectal cancer, but the difference was not statistically significant.

rs12951053 is an A-to-C nucleotide exchange in intron 7. In the literature, studies have related this SNP with invasive breast cancer, lung cancer, malign pleural mesothelioma, and cervical cancer. However,
instead of an A-to-C exchange, these studies investigated a T-to-G exchange. The genotype frequencies of the study population were 0.9 (A/A), 0.1 (A/C), and 0 (C/C). SNP in rs17880560 is the addition/deletion of 6 base pairs (bps) in the sequence of the 3'-untranslated region of the TP53 gene. Very few studies have investigated this SNP, although Sagne et al. investigated the addition of 6 bps to the sequence and found that, if seen together with another germline mutation in the TP53 gene, this SNP was associated with later cancer onset. The study population’s allele frequencies were 0.1 (CACGGC/CACGGG), 0.3 (CACGGC/delCACGGGC), and 0.6 (delCACGGC/delCACGGGC).

rs17551157 is an intronic change in the WRAP53 region of the TP53 gene. Little is known about this SNP and no association with any disease has been established for it. The allele frequencies of the study group were 0 (C/C), 0.1 (C/delC), and 0.9 (delC/delC).

There are very few studies on the possible roles of these SNPs in various cancer types and although some SNPs have been associated, the results were inconclusive. The reasons for this uncertainty are population-based genotypic frequency discrepancies, the distinct effects of specific SNPs in different populations, and difficulties determining the possible interactions between SNPs (phenotypic affect).

In this paper, the researchers standardized study specimens by collecting dental follicles from patients who had asymptomatic fully impacted lower third molars and had not been administered analgesics or antibiotics for a month before surgery. The adverse effects of smoking were assessed by comparing the study group of smoker patients and the control group of NS patients.

The results of this paper show a high ratio of SNPs without any correlation between smoking and TP53 gene status. Furthermore, there were no correlations between gender, tooth angulation, tooth developmental stage, and TP53 status, and so the researchers can assume that these SNPs were not a result of smoking but rather other environmental causes or perhaps ethnic characteristics of the study population. It is common knowledge that the rates of genotypes in different populations may vary. A major limitation of this paper was that only one part of the TP53 gene was examined. Further studies should be carried out on larger populations and total gene sequence analysis should be carried out to help assess the possible effects of smoking on the TP53 gene.

Financial support and sponsorship
This work was financially supported by a research grant from the Süleyman Demirel University Scientific Research Projects Coordination Department, Project Number: 3608-D2-13.

Conflicts of interest
There are no conflicts of interest.

REFERENCES


