DNA repair, *NFKβ*, and *TP53* polymorphisms associated with potentially malignant disorders and oral squamous cell carcinoma in Argentine patients

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Objective. An important strategy in cancer prevention is to identify individual susceptibilities for cancer development through the genomic profile. Developing countries such as Argentina have no data on genetic composition. The aim of this study was to evaluate the single nucleotide polymorphisms of genes related to DNA repair (*XCCR3, XPD*), cell cycle arrest/apoptosis (*TP53*), and inflammation (*NFKβ*) of patients with precancer and oral cancer and to contribute to recognizing potential risk of developing these pathologies, and incorporate the risk patients into a clinical follow-up program in Córdoba, Argentina.

Study Design. A cross-sectional study was performed on 140 patients with oral squamous cell carcinoma (OSCC), oral potentially malignant disorders (OPMDs), and controls. Genotyping of single nucleotide polymorphisms was performed using allele-specific polymerase chain reaction or restriction fragment length polymorphism techniques. The variables were evaluated by bivariate and multivariate statistical methods, with $P < .05$ statistically significant.

Results. The multiple correspondence analyses showed that patients with OSCC are clustered with the T allele of *XRCC3 T241 M* and the C allele of *TP53 R72 P*, and patients with OPMDs are clustered with the T allele of *NFKβ-519*.

Conclusion. Our preliminary results showed that the C allele of the Pro72 variant of *TP53* was related to OSSC and OPMD, and the T allele of *NFKβ-519* is related to OPMDs in Argentine patients. (Oral Surg Oral Med Oral Pathol Oral Radiol 2021;131:339–346)

Oral potentially malignant disorders (OPMDs) and oral squamous cell carcinoma (OSCC) present a complex epidemiology because of their multifactorial nature. The identification of individual genetic predispositions for oral cancer development is an important strategy because it allows the development of new tools for the prevention and early detection of this disease[1,2] and thus prediction of the risk of becoming ill.[3] Developing countries such as Argentina have no data on genetic composition. Research on genetic predispositions is essential to afford knowledge that improves prevention strategies and to reduce the costs of cancer therapy.

Recently it was determined through next-generation sequencing that the *TP53* gene is one of the most mutated genes in head and neck squamous cell carcinomas (HNSCCs).[2,4-6] One of the single nucleotide polymorphisms (SNP) at position 72 of the *TP53* gene generates a protein derivative containing either arginine or proline. Cell culture studies related to the cellular functions of this polymorphism observed that the arginine variant (R72) has a suppressive action on malignant transformation and increases apoptotic activity.[2]

DNA repair mechanisms are essential for genome integrity and to prevent cancer development. Several genes are implicated in these processes, such as *XPD* and *XRCC3*.[7] The *XPD* gene is involved in nucleotide excision repair, and the *XRCC3* gene participates in repairing DNA double-strand breaks by homologous recombination.[1,7-10] An SNP of the *XPD* gene characterized by a variation of nucleotides at codon 751, which changes the amino acid Lys to Gln in the corresponding protein, is associated with different neoplasms.[7,9] One of most common polymorphisms of the *XRCC3* gene is a nonconservative substitution of Thr for Met at codon 241 (exon 7), associated with the radiation sensitivity of DNA damage.[11]

Chronic inflammatory processes are recognized as a hallmark of malignancy.[12,13] The *NFKβ-519-C* haplotype has been significantly associated with the risk of developing gastric cancer and head and neck cancer.[14]

Studying these polymorphisms could provide useful information about the early detection of OSCC. To our knowledge there are no genetic data on these SNPs in the city of Córdoba, Argentina, except for the SNP of the *TP53* codon 72.[2] Therefore, the aim of this work was to evaluate the SNPs of genes related to DNA repair (*XCCR3, XPD*), cell cycle arrest/apoptosis (*TP53*), and inflammatory processes (*NFKβ*) in patients with precancer and oral cancer in order to identify populations with potential risk of developing these pathologies in Córdoba, Argentina. This knowledge could be used to improve prevention and early diagnosis.

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MATERIALS AND METHODS

Patients

A cross-sectional study of patients of both sexes who spontaneously attended at the Oral Medicine Department, Facultad de Odontología, Universidad Nacional de Córdoba, Argentina, between 2014 and 2019 was designed (n = 140). The average number of new OSCC cases diagnosed per year is 6 and the average number of new OPMD cases per year is 10.[15]

Cases and controls were recruited according to the following criteria: Age ≥30 years old and alterations in the oral mucosa for more than 30 days. The controls and patients with OPMD/OSCC shared features such as age range, sex, geographic region, smoking and alcohol use, and the absence of neoplastic pathologies. The eligibility criteria included no corticoid therapy or chemotherapy treatment; no history of other cancers; no systemic diseases chronic alcoholism, drug use, and immunologic diseases; and no human papillomavirus infection.

The clinical, genetic, and environmental data were recorded in a single clinical history following our own protocols.[16,17] Patients were examined by previously calibrated dentists (kappa = 0.7) and were asked questions on lifestyle, age, sex, ethnicity, smoking and alcohol use, and other factors.

The study groups were as follows: (1) OSCC: Patients with a diagnosis of OSCC according to C00-C06 criteria (https://icd.who.int/browse10/2019/en#/C00); (2) OPMD: patients with a diagnosis of OPMD according to criteria described by Warnakulasuriya et al.[18] and World Health Organization criteria (https://icd.who.int/browse10/2019/en#/C00); and (3) controls: patients with oral lesions other than OSSC or OPMD. The controls enlisted in the same dental department from which the cases were recruited. They attended for reasons unrelated to cancer or precancer lesions.

All oral lesion diagnoses were encoded according to the *International Classification of Diseases*, 10th Revision (https://icd.who.int/browse10/2019/en#/C00). Classification of the site and morphology was described by the *International Classification of Diseases for Oncology*, third edition (https://www.who.int/classifications/icd/adaptations/oncology/en/). In addition, all lesions were confirmed by routine pathologic examination of biopsies stained with hematoxylin/eosin.[15]

DNA isolation and genotyping by restriction fragment length polymorphisms

Buccal cells for DNA extraction were collected from oral mucosa with normal clinical diagnosis (corroborated by Papanicolaou stain) from controls and patients with OPMD or OSCC using disposable sterile cytologic brushes as previously described by Zarate et al.[2] The cytobrushes were stored in sterile Eppendorf tubes at −30°C. DNA was isolated as previously described by Zarate et al.[2] At the beginning of this research, the choice of studied polymorphisms was made based on our previous meta-analysis, in which we found an association with OSCC in a worldwide population.[1]

Genomic DNA was used as a template for allele specific polymerase chain reaction (PCR) and restriction fragment length polymorphisms. *TP53 R72 P, XPD Lys751 Gln, XRCC3 T241 M*, and *NFKB1*-519 C>T polymorphisms were analyzed after PCR amplification in 3% agarose gels. *TP53 R72 P* (rs 1042522; G>C) polymorphisms were analyzed using primers 5′-GCCAGAGGCTGCTCCCCC-3′ and 5′-CGTGCAAGTCACAGACTT-3′ for proline (177 bp) or 5′-TCCCCCTTGCCGTCCCAA-3′ and 5′-CTGGTGCAGGGGCCACGC-3′ for arginine (141 bp). For *XPD Lys751 Gln* rs13181 polymorphisms, *XPD* was amplified by PCR using primers 5′-TCAAACATCCTGTCCCTACT-3′ and 5′-CTGCGATTAAAGGCTGTGGA-3′. The *XPD* PCR product (344 bp) was digested with PstI at 37°C for 3 h, and the polymorphisms were determined as AA (234 and 110 bp), AC (234, 171, 110, and 63 bp), or CC (171, 110, and 63 bp). For *XRCC3 T241 M* rs861539 polymorphisms, *XRCC3* was amplified by PCR using primers 5′-GGTCGAGTGACAGTCCAAAC-3′ and 5′-TGCAACGGCTGAGGGTCTT-3′. The *XRCC3* PCR product (455 bp) was digested with NlaIII at 37°C for 3 h, and the polymorphisms were determined as CC (315 and 140 bp), CT (315, 210, 140, and 105 bp), or TT (210, 140, and 105 bp). For *NFKB1*-519 rs 2233408 C/T polymorphisms, *NFKB1* was amplified by PCR using primers 5′-GCTTTCACAACTTCTACCTG-3′ and 5′-AGAGTGGAAATGATGGCTG-3′. The *NFKB1* PCR product (188 bp) was digested with MnlI at 37°C for 6 h, and the polymorphisms were determined as CC (188 bp) or CT+TT (121 and 67 bp). It was not possible to determine all polymorphisms in all patients. One representative gel is shown in Figure 1. Reagents were obtained from Biodynamics (Buenos Aires, Argentina; Taq polymerase and reagents for mix PCR) and Thermo-Fisher Scientific (Buenos Aires, Argentina; restriction enzymes).

PCR products were obtained in a 50 μL final volume. PCR amplification was carried out on a BioRad, Brazil, iCycler thermal cycler using the following protocol: 5 min at 95°C, 30 s at 95°C, 30 s at 60°C to 55°C depending on the SNP, and 45 s at 72°C for 35 cycles, with an additional 5 min at 72°C after the last cycle. The PCR products were separated on a 3% TBE (tris/borate/EDTA) agarose gel and stained with ethidium bromide. A DNA ladder marker (GeneRuler 100 bp, Thermo Scientific, Buenos Aires) was used to determine the size of the DNA fragment. The reaction with all reagents excluding the template DNA was used as a negative control.

After amplification of the SNP by PCR, the product obtained (50 μL) was transferred to 1.5 mL Eppendorf

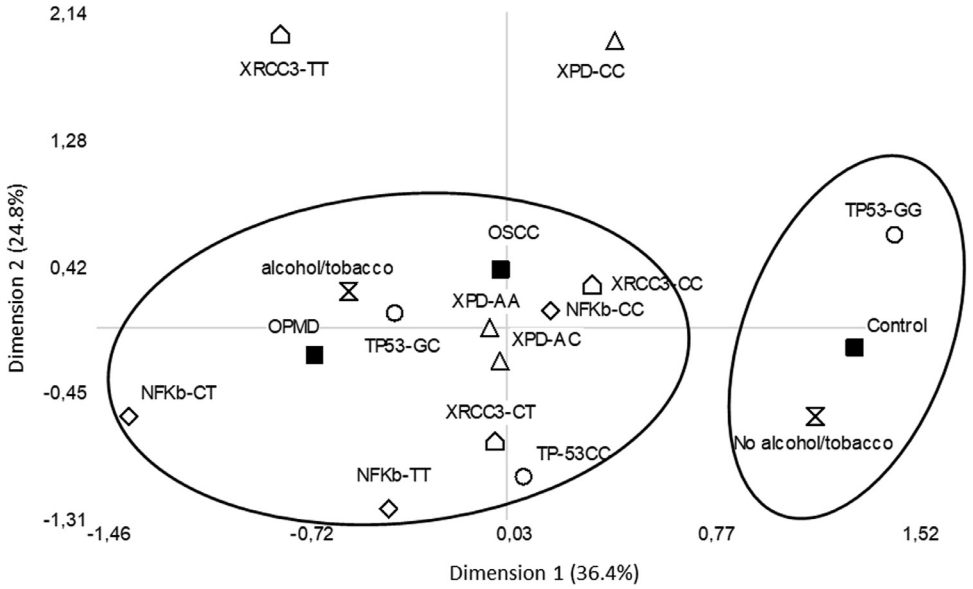

Fig. 1. Multiple correspondence analysis (projection on the first 2 dimensions). Accumulated inertia was 36.4%. Dimension 1, 24.8%. Dimension 2. OPMD, oral potentially malignant disorders; OSCC, oral squamous cell cancer. Genotypes of *XPDLys751 Gln*: AA-CC-AC; genotypes of *NFKβ-519*: CC-CT-TT; genotypes of *XRCC3 T241 M*: CC-CT-TT.

tubes. Then 6 μL of potassium acetate (3 M) was added, and 2 to 4 vol of ethanol 100% solution potassium acetate. The mixture was stirred and left for 10 min at −20˚C. Then it was centrifuged at 13,000 rpm for 15 min at 4˚C. The supernatant was removed and the precipitate was washed with 100 μL of 70% ethanol at −20˚C, followed by centrifugation at 13,000 rpm for 5 min at 4˚C and removal of the supernatant. The solution was centrifuged once more at 13,000 rpm for 30 s at 4˚C and the supernatant was removed. The pellet was dried for approximately 30 min to 1 h at room temperature and then resuspended in 50 μL of sterile Milli-Q water and an 8-μL aliquot was taken for restriction analysis. Finally, digestion with the appropriate restriction enzyme according to the protocol described in the literature for each SNP was performed.[2,7,10,14]

Statistical analysis

The critical level for establishing statistical significance was set at $P < .05$. The categorical data are reported as absolute and relative percentage frequencies, and quantitative variables are reported as medians and ranges. The kappa test was performed to evaluate the concordance among dentists, with a value of ≥0.6 indicating good agreement. Fisher's test was used to evaluate bivariate association of variables and odds ratios (ORs) and their associated 95% confidence intervals (CIs) are reported. To visualize similar groups among variables we performed exploratory multiple correspondence analysis (MCA). This exploratory method uses plots to determine whether the groups of interest could be differentiated by studied variables. The plot interpretation was based on the variables located approximately in the same region of space by parameters such as total inertia[19] and a logistics model. Data were analyzed using Infostat software professional version 2019 (Universidad Nacional de Cordoba) and R (www.r-project.org) software version 3.6.1.

Ethics approval

The Research and Ethics Committee of the Ministry of Health of the province of Cordoba (No. 1379) approved this study. All patients signed an informed consent form to participate and the study was conducted in accordance with the Declaration of Helsinki.

RESULTS

Women comprised 53.6% of the study population (controls: 23, OSCC: 14, OPMD: 38) and men comprised 46.4% (controls: 15, OSCC: 27, OPMD: 23). A significantly higher percentage of men was observed among patients diagnosed with OSCC (65.9%; Table I). Women comprised a nonsignificant majority of patients diagnosed with OPMD (62.3%; Table II).

The tongue was the most frequent anatomic area of malignant lesions in patients with OSCC (63.2%; Table I), whereas the buccal mucosa was the most frequent location in patients with OPMD (47.5%; Table II). Patients who consumed alcohol had an increased risk of developing OSCC (OR = 3.33; 95% CI, 1.04, 10.64) or OPMD (OR = 4.62; 95% CI, 1.52, 14.1; Tables I and II), and smokers had an increased risk of developing OSCC (OR = 5.09; 95% CI, 1.46,

Table I. Population features: controls vs patients with OSCC

Population features	*Variable*	*Cutoff*	*Control (n = 38) AF (RF%)*	*OSCC (n = 41) AF (RF%)*	*P value*	*OR*	*95% CI*	
							LB	*UB*
Demographics	Sex	Male	15 (39.5)	27 (65.9)	Reference category			
		Female	23 (60.5)	14 (34.1)	**.026**	**0.33**	**0.12**	**0.87**
	Age	<45 years	11 (28.9)	5 (12.2)				
		≥ 45 years	27 (71.1)	36 (87.8)	.0722	2.85	0.92	8.84
Risk factors	Tobacco use*	Never/quit	30 (78.9)	18 (44.4)	Reference category			
		Current	8 (21.1)	23 (57.6)	**.0107**	**5.09**	**1.46**	**17.77**
	Alcohol consumption†	Never/quit	26 (68.4)	16 (39.4)	Reference category			
		Current	12 (31.6)	25 (60.6)	**.0438**	**3.33**	**1.04**	**10.64**
Clinical outcomes	Anatomic site of lesion	Tongue		26 (63.2)	**.0004**	No estimate		
		Jugal mucosa		10 (23.7)				
		Other site		5 (13.2)				

$P < .05$ indicates statistical significance (bold values).
OSCC, oral squamous cell cancer; *AF*, absolute frequency; *RF%*, % relative frequency; *OR*, odds ratio; *CI*, confidence interval; *LB*, lower bound; *UB*, upper bound.
*Current consumption of at least one cigarette/day over a 1-year minimal period.
†Current consumption of 2 drinks/week over a 1-year minimal period.

Table II. Population features: controls vs patients with OPMD

Population features	*Variable*	*Cutoff*	*Control (n = 38) AF (RF%)*	*OPMD (n = 61) AF (RF%)*	*P value*	*OR*	*95% CI*	
							LB	*UB*
Demographics	Sex	Male	15 (40.6)	23 (37.7)	Reference category			
		Female	23 (59.4)	38 (62.3)	.8218	1.1	0.46	2.67
	Age	<45 years	11 (28.9)	7 (11.5)				
		≥ 45 years	27 (71.1)	54 (88.5)	**.0303**	**3.43**	**1.12**	**10.5**
Risk factors	Tobacco use*	Never/quit	30 (78.9)	16 (26.2)	Reference category			
		Current	8 (21.1)	45 (73.8)	**.0001**	**45**	**10.5**	**186.7**
	Alcohol consumption†	Never/quit	26 (68.4)	19 (31.1)	Reference category			
		Current	12 (31.6)	42 (68.9)	**.0066**	**4.62**	**1.52**	**14.1**
Clinical outcomes	Anatomic site of lesion	Tongue		17 (27.9)	.0679	No estimate		
		Jugal mucosa		29 (47.5)				
		Other site		15 (24.6)				

$P < .05$ indicates statistical significance (bold values).
OPMD, oral potentially malignant disorder; *AF*, absolute frequency; *RF%*, % relative frequency; *OR*, odds ratio; *CI*, confidence interval; *LB*, lower bound; *UB*, upper bound.
*Current consumption of at least one cigarette/day over a 1-year minimal period.
†Current consumption of 2 drinks/week over a 1-year minimal period.

17.77) or OPMD (OR = 45; 95% CI, 10.5, 186.7; Tables I and II).

The frequency distribution of all genotypes was in Hardy-Weinberg equilibrium ($P > .05$) for controls, but the *NFKβ-519* polymorphism displayed a T-allele imbalance, although this may have been attributable to sample size.

All three genotypes were detected in all of the SNPs studied (*TP53 Arg72 Pro, XPDLys751 Gln, XRCC3 Thr241 Met*, and *NFKβ-519*.

It was observed that the C allele of *TP53 R72 P* correlates with the presence of OPMD and OSCC ($P = .0061$; Table III). The frequency of genotypes GC +CC in controls was 60% and a considerable increase was observed in patients with OPMD (81.8%) and OSCC (85.5%; Table III). Patients carrying the C allele were 4 and 3 times more likely to develop premalignant (OR = 4.67; 95% CI, 1.65-13.22) or malignant (OR = 3.14; 95% CI, 1.09-9.07) lesions, respectively (Table IV).

The *XPD* and *XRCC3* SNPs were not associated with OPMD or OSCC (Table III), but the T allele of *NFKβ-519* was significantly associated with OPMD ($P = .0193$; Table II). Patients carrying the T allele of *NFKβ-519* were 9 times more likely to develop OPMD (OR = 9.26; 95% CI, 1.13, 75.68; Table IV).

We performed MCA to analyze the distribution of SNPs according to diagnosis. MCA showed that the adjusted inertia for the first 2 dimensions was 61.2%. The first dimension explained 36.4% of data variability, and the categories were mainly organized along the first axis (Figure 1). Patients with OSCC and OPMD

Table III. Genotypes of studied SNPs by group

Gene SNPs	*Genotype*	*Control N (%)*	*OPMD N (%)*	*OSSC N (%)*	*P value*
TP53 Arg72 Pro	GG*	14 (40)	10 (18.2)	8 (19.5)	**.0364**
rs1042522	GC	16 (45.7)	38 (69.1)	26 (63.4)	
	CC	5 (14.3)	7 (12.7)	7 (17.1)	
	GC+CC	21 (60.0)	45 (81.8)	35 (85.5)	**.0061**
XPDLys751 Gln	AA*	14 (41.2)	23 (40.3)	12 (31.6)	.7952
rs13181	AC	17 (50.0)	31 (54.4)	22 (57.9)	
	CC	3 (8.8)	3 (5.3)	4 (10.5)	
	AC +CC	20 (58.8)	34 (59.6)	26 (68.4)	.6236
NFKB-519 C/T	CC*	32 (96.9)	38 (77.6)	31 (91.2)	**.0187**
rs2233408	CT	0 (0.0)	5 (10.2)	0 (0.0)	
	TT	1 (3.0)	6 (12.2)	3 (8.8)	
	CT+TT	1 (3.0)	11 (22.4)	3 (8.8)	**.0193**
XRCC3-Thr241 Met	CC*	16 (50.0)	20 (47.6)	12 (41.4)	.4003
rs861539	CT	15 (46.9)	19 (45.2)	12 (41.4)	
	TT	1 (3.1)	3 (7.1)	5 (17.2)	
	CT+TT	16 (50.0)	21 (50.0)	17 (58.6)	.7851

$P < .05$ indicates statistical significance (bold values).
SNP, single nucleotide polymorphism; *OPMD*, oral potentially malignant disorder; *OSCC*, oral squamous cell carcinoma.
*Healthy allele.

Table IV. Regression logistic models for each SNP

SNP	*Diagnosis*	*OR*	*95% CI*		*P value*
			LB	*UB*	
TP53 Arg72 Pro	Control	1			
rs1042522	OPMD	4.67	1.65	13.22	**.0037**
	OSCC	3.14	1.09	9.07	**.0341**
XPDLys751 Gln	Control	1			
rs13181	OPMD	1.03	0.44	2.46	.9382
	OSCC	1.52	0.58	3.99	.3984
XRCC3 Thr241 Met	Control	1			
rs861539	OPMD	1.1	0.44	2.76	.8391
	OSCC	1.42	0.51	3.9	.5004
NFKβ-519 C/T	Control	1			
rs 2233408	OPMD	9.26	1.13	75.68	**.0378**
	OSCC	3.1	0.31	31.4	.3389

Model: $\text{Logit}_{(\text{variant allele presence})} = \beta_0 + \beta_1$ diagnosis. Adjusted by lifestyle risks (alcohol/tobacco use). Control is a reference category. Bold indicates statistical significance.
SNP, single nucleotide polymorphism; *OR*, odds ratio; *CI*, confidence interval; *LB*, lower bound; *UB*, upper bound; *OPMD*, oral potentially malignant disorder; *OSCC*, oral squamous cell carcinoma.

were associated with the GC/CC genotypes of *TP53 R72 P*, CT/TT genotypes of *NFKβ*-519,[14] heterozygous genotype of *XRCC3 T241 M* and *XPD Lys751 Gln*, and lifestyle risks including alcohol and tobacco use. The controls were related to the GG genotype of *TP53 R72 P* and no lifestyle risks (Figure 1).

DISCUSSION

Complex human diseases such as oral cancer and their various biological responses have been linked to multiple polymorphisms.[19,20] To our knowledge, there is no scientific literature on population characterization in Córdoba, Argentina, of the genetic polymorphisms evaluated in this study or in relation to the variations that can be observed in a population of patients with oral cavity cancers.

The current Argentine population has a heterogeneous ethnic origin, conferred mainly by waves of European immigrants and their integration with the native population.[21] It is estimated that around 90% of the population descends from Europeans, mainly Italians and Spaniards, with some indigenous legacy inherited by more than 50% of the population. Genetic components of African origin have also been established in at least 5% of the population.[21]

The Cordoba population showed high frequencies of the G allele of *TP53 R72 P*, corroborating our own previous[2] and other studies.[22,23] The patients studied were mainly from the city of Córdoba and some were from the interior of the province. It is known that there is a clear trend in the geographic distribution of the alleles of codon 72, with a correlation between the distance from the Equator and the G allele; Argentina is in the southern part of South America between the 21st and 55th parallels.[22] Populations such as African populations living near the Equator tend to have a greater proportion of the C allele, compared to northern Europeans, in whom the G allele predominates.[23]

The *XPD Lys751 Gln* SNP presented significantly different proportions in each study group. The allele frequency of Gln (genetic variant) varied from 10% in patients with OSCC to 9% in controls and 5% in patients with OPMD. The Gln allele is the most common of the 5 polymorphisms described for this gene, having an allele frequency of 22%.[24] However, no association was observed between the genotypes of the

XPD Lys751 Gln and cases or controls. Studies of colorectal cancer showed no association between the 751 Gln variant and this cancer in Polish patients.[25] However, studies conducted in digestive tract and lung cancers in Chinese populations showed an association between the Gln/Gln phenotype and cancer.[26]

The T allele of the *XRCC3 T241 M* polymorphism showed a significantly low (3%) proportion in the control groups and in patients with OPMD. A similar proportion has been observed in China, India, and Thailand.[27] On the other hand, the most prevalent genotypes in both OSCC and OPMD were CC and CT. This agrees with studies conducted in populations with European descent, Americans, and Italians in Brazil.[28,29,27,31]

The polymorphism *XRCC3 T241 M* involved in double-strand break repair has also been studied in patients with HNSCC with contradictory results.[29] In relation to the presence of the SNP polymorphism *XRCC3 T241 M* exon 7 and oral precancer/oral cancer, we did not observe a significant association between these pathologies and this polymorphism. Other authors found this association in bladder cancer,[30] lung and colorectal cancers,[30] and epithelial cell carcinoma.[32] In a study of patients with HNSCC, *XRCC3 T241 M* was observed to correlate significantly with age of onset. This SNP was related to patients under the age of 45 with the T allele.[33] In our study, most of the patients (80% on average) were over 45 years old.

In relation to the SNP *NFKB-519*, significantly different proportions of the genotypes identified in patients with OPMD were observed; in particular, the heterozygous genotype was identified in these patients, unlike controls and patients with OSCC, in whom this genotype was not detected. In studies of patients with hepatocellular carcinoma, a significant association was observed between the presence of this polymorphism and the pathology[34]; nevertheless, in another study, no significant relationship was observed between the presence of this polymorphism and HNSCC.[1]

The MCA plot showed that the presence of the heterozygous and homozygous mutated allele of *TP53 R72 P* and *NFKβ*-519 and heterozygous DNA repair genes *XRCC3* and *XPD* was related to the occurrence of OSCC or OPMD.

The presence of a T allele in *XRCC3 241 M*, which results in an amino acid change from threonine to methionine, has a biological effect on the function of the enzyme and/or interaction with other proteins involved in DNA damage and repair.[35] The *XRCC3* gene is one of the genes that participate in the pathway of base excision repair (BER) of DNA, which is a first-line mechanism that prevents genome instability, because it contributes to cell defense against the majority of endogenous and exogenous sources of genotoxic lesions. BER in base lesions is initiated by a damage-specific DNA glycosylase, which eliminates the aberrant base.[36] In addition, the presence of the C allele (genetic variant) of *TP53 R72 P* is related to the occurrence of OSCC. It is known that the heterozygous genotype may be associated with a high risk of developing both OSCC and OPMD.[2] This may be because the C allele of *TP53 R72 P* is less efficient in suppressing cell transformation and takes longer to induce apoptosis. However, numerous studies linking *TP53 R72 P* with cancer have reported controversial results. In a study conducted in Taiwan, it was reported that the Arg/Arg phenotype increases the risk of developing OSCC by 2.7 times.[37] In a study conducted in northern Iran, no significant association between genotypes *TP53 R72 P* and oral cancers was found.[38] Similar observations were made by other authors in studies conducted in a non-Hispanic white population and in an indigenous population with head and neck carcinomas.[39] On the other hand, Twu et al. reported that the heterozygous G/C genotype is associated with increased risk of hypopharyngeal squamous cell carcinoma in a population in Taiwan. They suggested that ethnicity is a critical factor in determining the effects of different *TP53 R72 P* alleles on cancer predisposition.[39]

Several studies have linked p53 with DNA repair mechanisms. These studies showed that DNA not repaired by single-strand breaks triggers a p53/Sp1-dependent negative regulation of APE1. The latter is the endonuclease responsible for the DNA incision during BER. It is important to note that impaired function of p53 leads to a failure of BER coordination.[36,39,40]

The presence of the heterozygous genotype of *NFKβ SNP* was associated with OPMD diagnosis (Figure 1). Pathologies such as oral lichen have been associated with chronic inflammatory processes.[40,41] Of the total number of patients with OPMD included in this study, 64% had oral lichens. Moreover, it is known that the transcription factor NF-$\kappa\beta$ regulates multiple aspects of innate and adaptive immune functions and serves as a fundamental mediator of inflammatory responses.[41,42]

OPMD involved oral lichen planus and oral leukoplakia, but identifying whether there is a common genetic basis for malignant transformation would facilitate prevention and follow-up. Although they are 2 separate clinical entities with unknown etiology, both share a premalignant potential.[43]

Our results indicate that the roles played by polymorphisms in cancer or precancerous development are different in different populations; it is thus possible that the variations are due to geographic and ethnic differences as well as factors such as alcohol and tobacco use and other lifestyle factors. The data obtained in the many studies on polymorphisms in DNA repair genes do not always coincide. This could be because the specific balance between apoptotic signals and the effects

of repair is different in different tissues. Less efficient variants may result in protective signals in some tissues, whereas in others they may activate risk factors.

A limitation of this study may be the rather small number of patients and controls included, although the results of this study match the allele frequencies of genes *TP53 R72 P* or *XRCC3 T241 M* observed in larger population samples in Argentine patients.[44-46]

CONCLUSION

Our findings that the C allele of Pro72 variant of *TP53* was related to the occurrence of OSSC and OPMD, and the T allele of *NFKβ-519* is related to the occurrence of OPMD in Argentine patients have not previously been reported in the literature. These studies have potential benefits to enable the identification of predictive biomarkers and groups at higher risk of oral cancer and the implementation of a variety of health care strategies. The recognition of individual susceptibility that enhances the risk of oral premalignant or malignant lesion development through genetic biomarkers such as *TP53* and *NFKβ* polymorphisms could provide a promising tool for oral precancer or cancer risk assessment. An important aspect of oral carcinogenesis is individual genetic susceptibility; that is, the presence of different types of polymorphisms that may or may not favor the development of cancer. However, the results of this study should be confirmed in further research including a larger number of patients and in different regions of Argentina.

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