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GENETIC PREDISPOSITION TO THE RISK OF POTENTIALLY MALIGNANT AND MALIGNANT HEAD AND NECK DISEASES, TP53, TLR2, DEFB1 GENE POLYMORPHISM

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The study was devoted to the identifying associations between leukoplakia, head-and-neck carcinogenesis and single-nucleotide polymorphisms in three genes: the tumour-suppressor TP53 Arg72Pro (rs1042522), the cell-surface receptor TLR2 Arg753Gln (rs5743708) and the antimicrobial peptide gene DEFB1 G-20A (rs11362). The study involved 60 patients aged 25–55 years with leukoplakia (n=20), oncology (n=20) and control (n=20). Allelic variants of the genes were genotyped by allele-specific polymerase chain reaction. A genetic predisposition to diseases affecting the oral mucosa has been demonstrated for both the development of leukoplakia and the emergence of malignancies. A statistically significant association was observed between the rs1042522 TP53 Arg72Pro polymorphism, the rs11362 DEFB1 G-20A polymorphism and the risk of oral pathologies. Insight into these risk factors, coupled with early diagnostic screening, may enable individuals to adopt preventive measures that lower their probability of developing such conditions.

Key words: DNA methylation, oral cancer, premalignant lesions, biomarkers, early diagnosis.

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ГЕНЕТИЧНА СХИЛЬНІСТЬ ДО РИЗИКУ ВИНИКНЕННЯ ПОТЕНЦІЙНО ЗЛОЯКІСНИХ І ЗЛОЯКІСНИХ ЗАХВОРЮВАНЬ ГОЛОВИ ТА ШИЇ, ПОЛІМОРФІЗМ ГЕНІВ TP53, TLR2, DEFB1

Дослідження було присвячене виявленню асоціацій між лейкоплакією, канцерогенезом голови та шиї і однонуклеотидними поліморфізмами в трьох генах: супресора пухлин TP53 Arg72Pro (rs1042522), рецептора клітинної поверхні TLR2 Arg753Gln (rs5743708) та гена антимікробного пептиду DEFB1 G-20A (rs11362). У дослідженні взяли участь 60 пацієнтів віком 25–55 років з лейкозами (n=20), онкологією (n=20) та контрольна група (n=20). Алельні варіанти генів генотипували за допомогою алель-специфічної полімеразної ланцюгової реакції. Генетична схильність до захворювань, що вражають слизову оболонку порожнини рота, була продемонстрована як для розвитку лейкозу, так і для виникнення злоякісних новоутворень. Виявлено статистично значущий зв'язок між поліморфізмом rs1042522 TP53 Arg72Pro, поліморфізмом rs11362 DEFB1 G-20A та ризиком виникнення патологій ротової порожнини. Розуміння цих факторів ризику в поєднанні з раннім діагностичним скринінгом може дозволити людям вжити превентивних заходів, які знизять ймовірність розвитку таких станів.

Ключові слова: метилювання ДНК, рак порожнини рота, передракові захворювання, біомаркери, рання діагностика.

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Head and neck cancer is the sixth most common group of human malignancies, accounting for approximately 3 % of all cancers; 48 % of cases are localised in the oral cavity, and 90 % of those correspond to oral squamous cell carcinoma (OSCC). OSCC – which affects the lips, oral tongue, gingiva, floor of the mouth, palate and other intra-oral sites, including the buccal mucosa – is the most frequent malignant neoplasm of the oral cavity, with an annual incidence exceeding 300 000 new cases worldwide [4].

Most oral cancers are preceded by a potentially malignant stage [8], clinically defined by leukoplakia (a white patch), erythroplakia (a red patch) and oral submucous fibrosis (irreversible fibrosis of the submucosal tissue). The lesions occur most often on the alveolar mucosa, followed by the buccal mucosa, palate, tongue and floor of the mouth [2]. Multiple lesions in the same patient are not uncommon.

Leukoplakia is essentially idiopathic; nevertheless, tobacco smoking is a major risk factor, although such lesions may also arise in non-smokers. When smoking is implicated, cessation can lead to partial or complete regression of the lesion [5]. Reported progression rates of oral leukoplakia to invasive oral cancer vary widely, from 0 % to 36 % [13]. According to Chaturvedi et al. (2020), oral leukoplakia

confers a 40.8-fold increase in oral cancer risk and a five-year absolute risk of 3.3 % [6], findings consistent with a population-based Medicare study of adults aged ≥ 65 years in the United States [7].

OSCC constitutes the most common (~ 90 %) malignant tumour among squamous cell cancers of the head and neck. Arising from the oral mucosa, it is frequently linked to excessive tobacco and alcohol intake, which induces mutations in key tumour-suppressor genes, most notably p53. It is widely accepted that most sporadic tumours result from a multistep accumulation of genetic alterations that change epithelial-cell behaviour through loss of chromosomal heterozygosity, ultimately culminating in fully invasive squamous cell carcinoma [10].

Hereditary factors, such as genetic polymorphisms, are increasingly studied as potential prognostic determinants in diverse malignancies. Genes regulate normal cell growth, DNA repair, cell-cycle control, apoptosis, cell differentiation and oxidative stress. Particular attention has focused on the codon 72 Arg/Pro polymorphism of the TP53 gene (TP53 Arg72Pro). TP53, located on chromosome 17p13, is one of the most frequently mutated genes in human cancers and is a pivotal driver of carcinogenesis. Numerous studies show that Toll-like receptors (TLRs) can modulate innate-immune activation and thereby foster cancer development under inflammatory conditions [3].

Epithelial cells express a wide array of antimicrobial peptides that constitute a first line of defence and maintain microbial homeostasis. Beyond their antimicrobial functions, these peptides possess immunomodulatory properties, bridging innate and adaptive immunity. β-Defensins, for example, act as chemokines and signalling molecules for antigen-presenting cells and thus serve as initiators of the adaptive immune response.

The purpose of the study was to identify associations between leukoplakia, head-and-neck carcinogenesis and single-nucleotide polymorphisms in three genes: the tumour-suppressor TP53 Arg72Pro (rs1042522), the cell-surface receptor TLR2 Arg753Gln (rs5743708) and the antimicrobial peptide gene DEFB1 G-20A (rs11362).

Materials and methods. The study involved 60 patients aged 25–55 years with leukoplakia (n=20), oncology (n=20) and control (n=20).

Dental examination was conducted in the dental office at the Department of Epidemiology and Prevention of Major Dental Diseases, Pediatric Dentistry and Orthodontics of the SE “The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine” (SE “ISMFS NAMS”).

DNA isolation from buccal epithelial cells was performed according to a modified method using Chelex [12]. DNA concentration and purity were determined spectrophotometrically (Nanophotometr, Implen, Germany) by taking a 2.5 μl aliquot directly from the tube with the DNA solution.

Allelic variants of the TP53 Arg72Pro (rs1042522), TLR2 Arg753Gln (rs5743708) and DEFB1 G-20A (rs11362) genes were genotyped by allele-specific polymerase chain reaction (AS-PCR). For each locus, two parallel reactions – specific for the wild-type and mutant allele – were assembled in 0.2 mL Eppendorf tubes containing 20 μL of reaction buffer, 100 nM of each allele-specific oligonucleotide primer and 100–150 ng of genomic DNA. A negative control was prepared by adding 2.5 μL of nuclease-free water in place of template DNA to both reaction types.

PCR amplification was carried out on a LabCycler thermal cycler (SensQuest, Germany) under the following conditions: initial denaturation at 94 °C for 15 min, followed by 35 cycles of 94 °C for 15 s, 64 °C for 15 s and 72 °C for 20 s. All primers were synthesised by Metabion GmbH (Germany).

Amplicons were separated by horizontal electrophoresis in 2 % agarose prepared in 1×tris-borate–EDTA (TBE) buffer at 100 V for 45 min. A GeneRuler 100 bp DNA Ladder served as the molecular-weight marker. Gels were stained with ethidium bromide and visualised under ultraviolet transillumination.

Statistical processing of the obtained results, including the test for deviation from the Hardy-Weinberg equilibrium (HWE) and the assessment of the association of genotypes and alleles between groups by the Pearson $\chi 2$ method, was carried out using the DeFinetti genetic statistics program on the website of the Institute of Genetics (Munich, Germany). The degree of association of genotype and alleles of patients with leukoplakia, neoplasia and controls was calculated by the value of the odds ratio (OR) with 95 % confidence interval and Pearson's $\chi 2$ test. The difference was considered to be statistically significant at $p<0.05$ [1].

Results of the study and their discussion. The distribution and comparison of frequencies of genotypes and alleles of rs1042522 TP53 Arg72Pro and rs5743708 TLR2 Arg753Gln polymorphisms in patient groups presented in Table 1.

Table 1

Distribution and comparison of frequencies of genotypes and alleles of rs1042522 TP53 Arg72Pro and rs5743708 TLR2 Arg753Gln polymorphisms in patient groups

Polymorphism	rs1042522 TP53 Arg72Pro				
Genotype, allele	ArgArg	ArgPro	ProPro	Alele Arg	Alele Pro
Comparison of frequencies	ArgPro<> ArgArg	ArgPro+ProPro <> ArgArg DM	ProPro<>ArgArg+ArgPro RM	Arg<>Pro	–
OR (95 % CI) leukoplakia-control	2.667 (1.163–6.113)	2.667 (1.417–5.020)	2.250 (0.994–5.092)	2.429 (1.485–3.972)	–
χ2, p-value	4.707	8.595	3.176	12.057	–
OR (95 % CI) oncology-control	4.000 (1.815–8.814)	2.667 (1.417–5.020)	1.000 (0.397–2.519)	1.889 (1.142–3.124)	–
χ2, p-value	11.604	8.595	0.056	5.641	–
Polymorphism	rs5743708 TLR2 Arg753Gln				
Genotype, allele	ArgArg	ArgGln	GlnGln	Alele Arg	Alele Gln
Comparison of frequencies	ArgGln<> ArgArg	ArgGln+GlnGln <>ArgArg DM	GlnGln<>ArgArg+ArgGln RM	Arg<>Gln	–
OR (95 % CI) leukoplakia-control	1.000 (0.054–18.575)	1.000 (0.054–18.575)	–	1.000 (0.058–17.182)	–
χ2, p-value	0.056	0.051	0.505	0.053	–
OR (95 % CI) oncology-control	2.250 (0.170–29.769)	2.250 (0.170–29.769)	–	2.111 (0.176–25.350)	–
χ2, p-value	3.176	3.008	0.505	2.919	–

Note. CI – confidence interval; DM – dominant model; RM – recessive model; HWE – Hardy-Weinberg equilibrium. Significant values of the odds ratio (95 % CI) and values of p<0.05 are highlighted in bold.

The TP53 gene plays a pivotal role in regulating the cell cycle and apoptosis, making it a central target in cancer-prevention strategies. Although the impact of its polymorphisms on therapeutic outcomes is still under investigation, elucidating their effects may facilitate the development of personalized treatment approaches.

A significant association was identified between the homozygous GG genotype of the codon-72 Arg/Pro polymorphism in TP53 and disease risk: odds ratio (OR)=2.667, 95 % confidence interval (CI) 1.163–6.113; Pearson's χ^2, p=0.031 for oral leukoplakia, and OR=4.000, 95 % CI 1.815–8.814; χ^2, p<0.001 for malignancy.

When leukoplakia is detected at an early stage, the prognosis is favorable; comprehensive therapy can halt progression and prevent complications. By contrast, advanced lesions tend to evolve into cancer, markedly reducing both life expectancy and quality of life. The heterozygous GC genotype and the G allele of the same polymorphism were likewise linked to an elevated risk of both leukoplakia and cancer (p=0.004).

Toll-like receptor 2 (TLR2) signaling has been implicated in various autoimmune conditions, chronic inflammation and inflammation-driven cancers. Such settings create a microenvironment rich in growth and survival factors that foster oncogenesis. TLRs are typically expressed on immune cells, including B lymphocytes and monocytes. In the present study, however, differences in genotype and allele frequencies of the TLR2 Arg753Gln polymorphism (rs5743708) between the study groups were not statistically significant, indicating that replacement of arginine with glutamine at residue 753 does not influence head-and-neck cancer risk. A larger, more representative cohort may be necessary to detect effects of the minor genotypes.

Future investigations focusing on genetic markers and activation mechanisms of TLR2 could clarify its role in head-and-neck cancer susceptibility and lead to more effective treatments – for example, by employing TLR2-targeted agents as adjuvants or antagonists in oncologic therapy.

The distribution and comparison of frequencies of genotypes and alleles of rs11362 DEFB1 G-20A polymorphism in patient groups presented in Table 2.

Table 2

Distribution and comparison of frequencies of genotypes and alleles of rs11362 DEFB1 G-20A polymorphism in patient groups

Polymorphism	rs11362 DEFB1 G-20A				
Genotype, allele	GG	GA	AA	Alele G	Alele A
Comparison of frequencies	GA<>GG	GA+AA<>GG DM	AA<>GG+GA RM	G<>A	–
OR (95 % CI) leukoplakia-control	0.750 (0.414–1.358)	0.976 (0.555–1.714)	11.111 (1.395–88.521)	1.333 (0.832–2.137)	–
χ2, p-value	0.641	0.021	6.240	1.161	–
OR (95 % CI) oncology-control	0.500 (0.262–0.953)	0.976 (0.555–1.714)	25.000 (3.284–190,315)	1.714 (1.082–2.715)	–
χ2, p-value	3.850	0.021	17.430	4.813	–

Note. CI – confidence interval; DM – dominant model; RM – recessive model; HWE – Hardy-Weinberg equilibrium. Significant values of the odds ratio (95 % CI) and values of $p<0.05$ are highlighted in bold.

Analysis of the G-20A polymorphism of the DEFB1 gene (rs11362) showed that the functionally intact GG genotype – both in the homozygous (GG) and heterozygous (GA) state – appears protective against the development of oral leukoplakia and neoplasia, although the differences did not reach statistical significance. By contrast, the mutant AA genotype was significantly associated with an elevated risk of both oral leukoplakia (odds ratio [OR] = 11.111; 95 % confidence interval [CI] 1.395–88.521; Pearson's χ^2, $p = 0.013$) and head-and-neck neoplasia (OR = 25.000; 95 % CI 3.284–190.315; χ^2, $p < 0.001$).

Our data therefore indicate a statistically significant over-representation of the rare A allele and the AA genotype of DEFB1 (−20G/A) among patients with leukoplakia and with head-and-neck carcinoma compared with controls. The AA genotype increases the likelihood of leukoplakia roughly 11-fold ($p = 0.013$) and carcinoma 25-fold ($p < 0.001$). In the control group, the predominant genotype and allele were GG and G, respectively.

Several studies have shown that the TP53 Arg72Pro mutation, which yields either arginine (G) or proline (C), produces three distinct genotypes – GG, GC and CC – that differ in their capacity to induce growth arrest and apoptosis. The GG variant displays enhanced mitochondrial localisation in tumour cell lines, whereas the CC variant more strongly trans-activates p21 and triggers growth arrest. An association of the CC TP53 Arg72Pro genotype with oral leukoplakia has been observed in women over 45 years of age who neither smoke nor consume alcohol, supporting the view that oral leukoplakia is a mucocutaneous condition marked by non-specific inflammation leading to severe destruction of the epithelial basal layer [14]. Expression of TLR2, TLR3, TLR4, TLR5, TLR7 and TLR9 has been demonstrated in cells of oral squamous-cell carcinoma. These findings suggest that TLR activation may have prognostic value in tumourigenesis, even though the precise biological role of each TLR in oral carcinogenesis remains to be elucidated [9]. Antimicrobial peptides (AMPs) are attracting attention because of their differential regulation in cancers such as oral squamous-cell carcinoma, indicating their potential as novel anticancer agents. These small cationic peptides are key effectors of innate immunity, particularly in the oral cavity, where they are produced by the salivary glands and epithelium to combat microbial invasion. AMPs exhibit both antimicrobial and anticancer activities, disrupting microbial membranes and inducing cytotoxicity in cancer cells by binding to exposed phosphatidylserine residues. Some AMPs also promote release of tumour antigens and damage-associated molecular patterns. Given the growing resistance to conventional chemotherapy, AMPs represent a promising avenue for developing effective oncologic therapeutics. Besides their direct cytotoxic effects, AMPs can activate adaptive immunity and function as tumour-suppressor genes [11]. Genetic variation in DEFB1 has been linked to Crohn's disease, severe acute pancreatitis and chronic gastritis. Notably, the −20 G > A SNP (rs11362) influences DEFB1 expression [15]. Such polymorphisms can modulate human β-defensin-1 production and, consequently, alter innate immune responses and oral health. Defensins may thus serve as prospective biomarkers for diagnostic or prognostic purposes and/or as components of novel therapeutic modalities.

Conclusions

1. A genetic predisposition to diseases affecting the oral mucosa has been demonstrated for both the development of leukoplakia and the emergence of malignancies.

2. A statistically significant association was observed between the rs1042522 TP53 Arg72Pro polymorphism, the rs11362 DEFB1 G-20A polymorphism and the risk of oral pathologies.

3. Insight into these risk factors, coupled with early diagnostic screening, may enable individuals to adopt preventive measures that lower their probability of developing such conditions.

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