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## The influence of polymorphism of the MUC7 gene on the teeth and dental hygiene of students at a faculty of dentistry in Poland

Wpływ polimorfizmu genu MUC7 na stan uzębienia i higienę jamy ustnej u studentów Wydziału Stomatologii

### Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

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### Summary

#### Objective:

The aim of the study was to analyze polymorphism of the MUC7 gene and its correlation with the DMFT value and the Plaque Control Record by O'Leary.

#### Material/Methods:

The study was carried out on 158 students of a faculty of dentistry in Poland. Students were subjected to a clinical oral examination. The status of caries was determined using the decayed, missing and filled teeth (DMFT) value. The status of dental hygiene was examined by the Plaque Control Record (PCR Plaque Index by O'Leary T, Drake R, Naylor, 1972) index. Sherlock AX, a universal kit for DNA isolation from biological tracks (A&A BIOTECHNOLOGY), was used for DNA isolation. VNTR polymorphism in the MUC7 gene was examined by polymerase chain reaction (PCR).

#### Results:

The prevalence of the MUC7\*6/\*6 genotype was definitely higher than MUC7\*5/\*6. The distribution of prevalence of MUC7\*6/\*6 and MUC7\*5/\*6 in the control group was similar to another. The distribution of the value of the DMFT index in the group examined with MUC7\*6/\*6 was similar to the group with MUC7\*5/\*6. Statistical analysis did not show a significant correlation between genotypes of the MUC7 gene and DMFT and the Plaque Control Record index.

#### Conclusions:

This study does not show a correlation between the MUC7 genotypes and caries and oral hygiene of students.

#### Key words:

DMFT • Plaque Control Record index • polymorphism of MUC7 gene

### Streszczenie

#### Cel pracy:

Celem pracy była ocena zależności pomiędzy polimorfizmem genu MUC7 a wskaźnikami PUWZ i PCR.

#### Material/Metody:

Badanie przeprowadzono wśród 158 studentów Wydziału Stomatologii PUM. Badanie kliniczne jamy ustnej przeprowadzono za pomocą zgłębnika i lusterka w oświetleniu sztucznym w warunkach standardowego gabinetu dentystycznego. W ocenie stanu uzębienia posłużono się zapisem na diagramie z uwzględnieniem wskaźnika PUWZ, a stan higieny jamy ustnej oceniono wskaźnikiem PCR (Plaque Index by O'Leary T, Drake R, Naylor, 1972). Do izolacji DNA ze śladów



biologicznych użyto zestawu uniwersalnego Sherlock AX (A&A BIOTECHNOLOGY). VNTR polimorfizm genu MUC7 został zbadany przy użyciu Polymerase Chain Reaction (PCR).

**Wyniki:** Badania wykazały zdecydowanie częstsze występowanie genotypu MUC7\*6/\*6 w porównaniu do genotypu MUC7\*5/\*6. Rozkład częstości genotypu MUC7\*6/\*6 i genotypu MUC7\*5/\*6 w grupie kontrolnej był zbliżony do siebie. Rozkład wartości wskaźnika PUWZ w grupie badanej była zbliżona u osób zarówno z genotypem MUC7\*6/\*6 jak i MUC7\*5/\*6. Analiza statystyczna nie wykazała istotnej korelacji pomiędzy polimorfizmem genem MUC7, a wskaźnikami PUWZ i PCR.

**Wnioski:** Badania nie wykazały zależności pomiędzy genotypem MUC7 a chorobą próchnicową i higieną jamy ustnej badanych.

**Słowa kluczowe:** PUWZ • wskaźnik Plaque Control Record • polimorfizm genu MUC7

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## INTRODUCTION

Caries is the most widespread disease of the teeth. The following factors play an important role in the progression of caries: pathogenic bacteria, carbohydrates, the susceptibility of the surface of the enamel and time. Dental plaque is the most important factor which influences the development of tooth caries and gingivitis. The dental biofilm supports a micro-ecosystem of bacteria that exhibit a variety of physiological characteristics [19]. About 60 to 80% of dental plaque is composed of bacteria, which are immersed in a matrix. The organic matrix of the dental plaque is formed by glycoproteins of saliva and also consists of mucins, products of the bacterial metabolism (glucan and fructan) and cells of the epithelium, leucocytes, calcium and phosphorus. Mucins play an important role in the formation of dental pellicle which lubricates the dental surface. Dental plaque, which covers the surface of teeth, contributes to initiating the carious process. Dental plaque bacteria are responsible for enzymatic processes in which lactic acid is the final product. The examination of salivary proteins has shown that several salivary proteins (the proline-rich, parotid acid and double-band salivary proteins) exhibit genetic polymorphism, which may play an important role in the etiology of dental caries [1].

Two types of genetically different mucins can be distinguished in the oral cavity. There are two kinds of mucins: high molecular weight mucin MG1 (1000 kDa) and low molecular weight mucin MG2 (200–300 kDa) [14,17,20,24]. Salivary mucins are recognized as a major factor in the defense of teeth and oral mucosa against mechanical, chemical and microbial insults. These large glycosylated glycoproteins participate in the formation of the protective pellicle covering tooth enamel and soft oral mucosa, promote bacterial aggregation and clearance from the oral cavity and are responsible for the maintenance of viscoelastic, hydrophobic

and lubricative properties of saliva. They constitute a heterogeneous group of glycoproteins. MG1 mucin is encoded by the MUC5B gene, localized on chromosome 11 (11p15.5 position), but the gene responsible for the synthesis of mucin MG2 is found on the long shoulder of chromosome 4 (4q13-q21 position) [14,21]. Many MUC genes, including both MUC5B and MUC7, establish a high degree of the polymorphism, which is determined by the variable number of tandem repeats (VNTR), situated in the central exon of the gene [14]. The MUC7 gene encoding MG2 mucin can be divided into three distinct domains: unique 5'- and 3'-translated regions containing 4 and 1 potential N-glycosylation sites and a central tandem repeat domain which is composed of six tandem repeats of 69 nucleotides (23 amino acids) with a high number of Thr and Ser [4,12].

MG1 mucin is composed of 15% protein and 78% carbohydrate and appears to exist as oligomer and monomeric units. MG2 mucin is composed of 30% protein and 68% carbohydrate. It exists as a single polypeptide chain. MG1 and MG2 mucins contain O-linked and N-linked carbohydrate units.

MG1 mucin is synthesized in mucous cells of the submandibular, sublingual and some minor glands. MG2 mucin is produced only by mucous cells in the submandibular glands [8,21].

Salivary mucins are multifunctional molecules that have been implicated in the protection of oral surfaces. The high molecular weight mucin MG1 is responsible for the rheological properties of saliva: the lubrication of hard and soft tissues to minimize injury from mastication and other mechanical injury, the formation of the barrier to desiccation and to chemical and mechanical insults and the formation of a diffusion barrier between underlying tissues and the external environment. Both MG1 and MG2 create heterogeneous complexes with different proteins of saliva:

with amylase, proline-rich, serine and histidine. MG2 fuses with lactoferrin, statherin and SIgA (secretory immunoglobulins A) [8]. Both mucins MG1 and MG2 play an important role in the agglutination of bacterial cells and the colonization of the oral cavity. MG1 binds a few oral microorganisms, including *Haemophilus parainfluenzae* and *Helicobacter pylori*. MG2 mucin is considered an important component of the non-immune host defense system in the oral cavity. MG2 can bind to cariogenic strains including *Streptococcus mutans*. This binding requires a structural determinant in the N-terminal region.

The purpose of this study was to determine the influence of the polymorphism of the MUC7 gene on the severity of caries and dental hygiene.

## MATERIALS AND METHODS

### Subject selection

Protocol approval no. BN-001/64/06 was obtained from the Bioethics Committee of the Pomeranian Medical University in Szczecin (24 May 2006).

The research was carried out on 158 students of both sexes (117 females, 41 males) from the Faculty of Dentistry at the Pomeranian Medical University in Szczecin, Poland. The age of examined persons was 20–21 years. All subjects were randomly selected from the list of students. Students provided information and written consent and agreed to participate on a voluntary basis as part of a large prospective research project. None of the subjects (donors) was taking prescription medication or drugs at the time.

### Clinical examination

The research consisted of the clinical and genetic examination. Students were subjected to a clinical oral examination. The status of the caries was determined using the decay, missing, or filled teeth (DMFT) index by a single examiner calibrated according to World Health Organization (WHO) and diagnostic criteria [23]. Polymorphism of the MUC7 gene was analyzed in all examined students and separately in subjects with a low DMFT index ( $\leq 7$ ) and a high DMFT index ( $\geq 15$ ).

The status of dental hygiene was determined using the Plaque Control Record (Plaque Index by O'Leary T, Drake R, Naylor, 1972) index, which was obtained by the examination of four dental surfaces of all teeth [6]. The Plaque Control Record index includes the percentage of dental surfaces with dental plaque.

It was accepted that up to 20% of dental surfaces with dental plaque was good hygiene (PCR 1 according to O'Leary), but more than 20% was poor hygiene (PCR 2 according to O'Leary).

### DNA isolation

Genetic research was performed to obtain isolated DNA. DNA was essential for the identification of polymorphism of the MUC7 gene. Material for DNA isolation of the examined group (E) was obtained from the epithelial

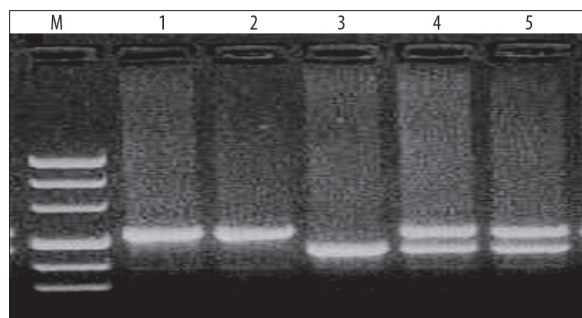


Fig. 1. Ethidium bromide-stained agarose gel used for the genotyping of VNTR polymorphism MUC7; Lanes: M – DNA molecular weight marker pUC Mix 8 (MBI Fermentas); 1, 2 – homozygote MUC7\*6/\*6, 3 – homozygote MUC7\*5/\*5, 4, 5 – heterozygote MUC7\*5/\*6

layer of the buccal mucosa. A swab test was carried out by rubbing “back and forth” the buccal mucosa of both sides ten times. Material for the genetic procedure was obtained from students who were not allowed to eat, drink or use chewing gum 0.5–1 h before the examination. The swabs were then left for 24 hours at room temperature (ca. 20°C) in order to dry. After this sample probes could be stored for around three months. Sherlock AX, a universal kit for DNA isolation from biological tracks (A&A BIOTECHNOLOGY), was used to isolate the DNA. This kit consists of using electrovalent-exchangeable membranes which detect DNA. The authors carried out research in a control group (n=158) to compare occurrence of polymorphism of the MUC7 gene. Material for examinations in the control group (C) determined the DNA genome, isolated from leucocytes of the cord blood of newborn babies with QIAapm® Mini Kit (Qiagen).

### Identification of VNTR polymorphism in exon 3 of the MUC7 gene

VNTR polymorphism in the MUC7 gene was examined by PCR with a pair of primers (sense primer: 5'-GTAGCTACATTAGCACCAGTG-3' and antisense primer: 5'-TTCAGAAGTGTGTCAGGTGCAAG-3'), previously described by Kirkbride et al. [11]. Two common alleles (MUC7\*5 and MUC7\*6) containing five and six tandem repeats in the VNTR region MUC7 should yield a PCR product of 521 and 590 bp, respectively.

The PCR reaction was carried out in a total volume of 10  $\mu$ L containing: 20 ng of template DNA, 4 pM of each primer and 1x PCR Master Mix (Fermentas) with Taq DNA polymerase. The amplification was performed with initial denaturation at 94°C for 5 min, and then 36 cycles: denaturation at 94°C for 20 s, annealing at 64°C for 40 s, and extension at 72°C for 40 s. The final 72°C incubation was extended by 8 minutes. The fragments were separated by electrophoresis on 2% agarose gel, and stained with ethidium bromide. The results were recorded with photographs of gels in UV light (Figure 1).

### Statistical analysis

The STATISTICA program was used to verify whether there was any influence among genotype distribution, the DMFT index and the Plaque Control Record. For describing nominal, categorical variables a number and percentage were

Table 1. The distribution of frequency of appearance of MUC7\*5/\*6 and MUC7\*6/\*6 genotypes in the examined and the control group

Group	MUC7*5/*6		MUC7*6/*6	
	n	[%]	n	[%]
E	37	23	121	77
C	39	25	119	75
Total	76		240	

E – examined group, C – control group.

Table 2. The distribution DMFT index in the group of persons with MUC7\*5/\*6 and MUC7\*6/\*6 genotypes

Genotype	DMFT					
	Low			High		
	n	Mean ±SD	p-value	n	Mean ±SD	p-value
MUC7*5/*6 (n=37)	6	5.3±1		5	15.8±0.8	
MUC7*6/*6 (n=121)	16	5.1±2.4	0.54	26	16.3±1.4	0.59

Table 3. The distribution the PCR index (Plaque Control Record) in the group of persons with MUC7\*5/\*6 and MUC7\*6/\*6 genotypes

Genotype	PCR1		PCR2		n	p-value
	n	%	n	%		
MUC7*5/*6	6	16	31	84	37	0.09
MUC7*6/*6	7	6	114	94	121	
Total	13		145		158	

applied. For examining the differences in prevalence between the MUC7 genotype and DMFT the Mann-Whitney U-test was used. The prevalence of the MUC7 genotype and Plaque Control Record was compared with Chi-square test with Yates correction. The acceptable level for type I error was set to 0.05.

## RESULTS

The results of this study are shown in Tables 1, 2 and 3. The prevalence of the MUC7\*6/\*6 genotype was definitely higher than MUC7\*5/\*6 (Table 1).

In the examined group 77% of persons (n=121) had the genotype MUC7\*6/\*6 and 23% MUC7\*5/\*6 (n=37). The distribution of prevalence of MUC7\*6/\*6 and MUC7\*5/\*6 in the control group was similar to the examined group. The mean DMFT in the whole examined population was 11.39. There were no caries-free persons in the examined group of students. For the evaluation of the dependence of the DMFT value on the genotype a statistical analysis was conducted in two groups: with low DMFT ≤7 (n=22) and high DMFT ≥15 (n=31) (Table 2).

The number of examined persons with a low and high DMFT index was similar in the group of persons with MUC7\*5/\*6 and MUC7\*6/\*6 and did not differ statistically. Also we did not find essential statistical differences

between mean of DMFT in low and high DMFT groups with MUC7\*5/\*6 and MUC7\*6/\*6 genotypes.

The statistical analysis did not show a significant correlation between the value of the PCR index and the genotypes of the MUC7 gene (Table 3).

## DISCUSSION

The average value of DMFT index in the investigated group of patients was 11.39. Due to the lack of DMFT index data concerning the group of age 20-21-year-olds in epidemiological research, the authors decided to compare DMFT index results given in epidemiological research for 18-year-olds. The average value of the DMFT index obtained in our investigation was significantly higher in comparison with the DMFT index from epidemiological research carried out in Poland among 18-year-olds in 2008. The research which was carried out by Jodkowska in years 1995, 2004 and 2008 considering the assessment of the status of oral health among 18-year-old young people showed that the average value of the DMFT index among these investigated groups was respectively 9.2, 8.0 and 7.65 [10]. The average value of the DMFT index in 1999 among 18-year-old people from Szczecin was 7.70 [5]. Differences in the intensity of caries and the high average value of the DMFT index obtained in the author's research may be result of higher age

of examined patients. The value of average DMF index increases with age. No caries-free persons (DMFT=0) were found in the examined group of students; therefore persons with DMFT index  $\leq 7$  (n=21) determined the group with a low risk of caries. A similar division of DMFT index was conducted by Banderas-Tarabay et al. where persons with DMFT  $\leq 4$  determined the group with a low risk of caries [2]. Within the oral cavity two types of mucins [3,5,6,17] were distinguished: high-molecular-weight mucin MG1 encoded by the MUC5B gene [7] and low-molecular-weight mucin MG2 encoded by the MUC7 gene [4,9,13,16,23].

In examinations carried out by Biesbrock et al. the polymorphism of the MUC7 gene was determined with a changeable number of tandem individuals (VNTR) in the central exon [3]. The MUC7\*6 and MUC7\*5 alleles are the most often appearing alleles of the MUC7 gene. It was confirmed by Kirkbride et al. and by authors of this work [11]. The prevalence of the MUC7\*6/\*6 genotype in the examined population in this work was definitely higher than of the MUC7\*5/\*6 genotype.

The research which was carried out by Van Niew Amerogen et al. shows that both types of mucin have an influence on the progression of the caries process and they wield an enormous influence on the susceptibility of the patient to caries [22]. Slomiany and Piotrowski examined the population of persons and analyzed the presence and the amount of salivary mucins divided between examined persons who were caries-resistant and caries-susceptible [17]. He judged the percentage contents of mucins MG1 and MG2 in saliva, and got scores which confirmed the link between the amount of mucins MG1 and MG2 and susceptibility to caries. Persons who were caries-resistant definitely had a larger percentage content of mucin MG2, which was smaller than MG1. In the case of persons susceptible to caries, presence of the opposite relation was found. Banderas-Tarabay et al. carried out a study amongst dentistry students (aged 17–42 years). The purpose of this study was to examine the presence of the polymorphism of glycoproteins of saliva, including mucin MG2 and the MUC7 gene, and its possible influence on caries [2]. The research findings are comparable to scores achieved by the authors of this study. In their examinations, Banderas-Tarabay et al. proved that the presence of the polymorphism of all glycoproteins of saliva, including mucin MG2, is correlated with the health of the oral cavity and the value of the DMFT index [2]. Moreover, persons with a high DMFT index had smaller contents of mucin MG1 and MG2. The results of this research showed that the distribution of patients, which was made by Slomiany and Piotrowski [18], in resistance to caries and susceptibility to caries is correct. It is now a well-known fact that both types of mucins are involved in the formation of dental plaque (firebrands mucin MG1) and in the adhesion process of bacterial cells. In the present study no associations between salivary mucins genotypes and dental caries were observed. The study population with MUC7\*5/\*6 was relatively small and this probably

accounts for the differences in genotype distributions from those expected for the general population. The results presented by Anderson suggested that salivary protein polymorphisms are genetic markers for human caries resistance [1]. In this study we did not observe a connection between MUC7 gene polymorphism and dental caries. It is possible to suppose that the presence of the MUC7\*6/\*6 genotype is a factor which contributes to the stagnation of dental plaque and indirectly influences the presence and the advancement of the carious process. This statement confirms the results obtained in this study, in which higher dental plaque stagnation was observed in persons with the MUC7\*6/\*6 genotype. The mechanism behind the action of this process remains unknown. Perhaps the polymorphism of the MUC7 gene in some way sails on the property of mucin which it encodes, increases its viscosity and in the process leads to the increased accretion of dental plaque. Mehrota et al. discovered the appearance of two isoforms of mucin MG2, MG2a and MG2b. Examinations were carried out on their origins in the submandibular and sublingual saliva [12]. Bobek et al. demonstrated that the amino acid composition of both forms of mucin MG2 were identical, but they differed in their sialic acid and fucose contents [4]. The examinations carried out show that mucin as a result of bacterial exoglycosidases can change their properties and become the substrate essential for the metabolism of microorganisms. It suggests that MG1 and MG2 together with different glycoproteins of saliva can have an essential influence on the microflora of the oral cavity and the accretion of dental plaque and increase the risk of caries. Unfortunately, this aspect among others requires further study. Our results point to the need to tie the genetic testing together with the biochemical analysis of saliva and increase the number of examined persons. The examination of salivary protein polymorphisms in oral disease is of interest, but also in terms of defining the relative contributions of environmental and genetic factors in the etiology of dental caries. The knowledge of the genotype of the MUC7 gene of the patient can contribute to the identification of persons with a high risk of caries and create individual preventive programs which will be effective in preventing this disease [7,15].

## CONCLUSIONS

This study concludes that the genetic polymorphism of the MUC7 gene is present in the population studied. Genotypes of the MUC7 gene are not good predictors of caries in Polish students.

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The authors have no potential conflicts of interest to declare.