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Original scientific article

MATRIX METALLOPROTEINASE 7 (*MMP-7*) AND TISSUE INHIBITOR OF METALLOPROTEINASES-2 (*TIMP-2*) DOWNREGULATION COMPLEMENTS *PLAG1* ONCOGENE OVEREXPRESSION IN PLEOMORPHIC ADENOMA PATHOGENESIS

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Anicic B., N. Nikolic, J. Carkic, D. Jelovac, Z. Jezdic, B. Dozic, V. Danilovic, J. Milasin (2021). *Matrix metalloproteinase 7 (MMP-7) and tissue inhibitor of metalloproteinases-2 (TIMP-2) downregulation complements plag1 oncogene overexpression in pleomorphic adenoma pathogenesis*.- Genetika, Vol 53, No.1, 339-348.

Pleomorphic adenoma (PA) is the most common neoplasm of salivary glands and consists of epithelial and mesenchymal components. Although a benign lesion, it harbors a potential for recurrence and malignant transformation. Also, due to its histological diversity and unpredictive behavior PA can represent both diagnostic and therapeutic challenge. Matrix metalloproteinases (MMPs) are well known modifiers of extracellular matrix (ECM) PA component and in conjunction with their endogenous tissue inhibitors (TIMPs) may influence PA tumor biology. PLAG1 oncogene also has an important role in PA; however, neither the exact mechanisms of its influence nor its interactions with other genes are completely elucidated. The aims of this study were to assess the expression of *PLAG1*, *MMP-2*,

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MMP-7, *MMP-9*, *TIMP-1* and *TIMP*-2 genes in PAs, and find a possible association of gene expression levels with clinical/epidemiological parameters of PA patients. Relative mRNA levels were assessed using Quantitative real-time PCR analyses in 15 PAs of the parotid gland and 5 normal salivary glands (NSGs). A statistically significant overexpression of *PLAG1* was observed in PA compared to NSG samples (P=0.010); PA had 5.48 times higher mRNA levels than NSG. Out of the three analyzed *MMP* genes, significantly lower levels of *MMP-7* were found in PA patients (P=0.026). *TIMP2* was also downregulated in PA samples, compared to NSGs (P=0.040). *MMP-7* and *TIMP-2 mRNA* levels were decreased 2.95 and 2.85 times respectively, in PA samples. No association was found between gene expression and clinical/epidemiological PA parameters. Our results suggest that *PLAG1* overexpression with concomitant *MMP7* and *TIMP2* downregulation may contribute to PA development.

Keywords: salivary gland, pleomorphic adenoma, *MMP-7, TIMP-2, PLAG1*, gene expression

INTRODUCTION

The field of salivary gland tumor biology is wide and challenging, given the numerous subtypes of both benign and malignant neoplasms that may develop in major and minor salivary glands. Salivary gland neoplasms are among those with the greatest cytomorphological and clinical heterogeneity creating, firstly, diagnostic difficulties, but also issues regarding treatment and disease outcome prediction (RITO *et al*., 2018). The 2017 World Health Organization Classification of Head and Neck Tumors defined at least 31 different benign and malignant epithelial tumors (EL-NAGGAR *et al*., 2017). Pleomorphic adenoma (PA) is the most common salivary gland neoplasm worldwide, accounting for 70–80% of abnormal SG growths (BOKHARI *et al*., 2020) and it mainly occurs in the superficial lobe of the parotid gland, ut can also affect the submandibular and minor salivary glands. It most often presents as a painless bulging mass of epithelial and myoepithelial tissues with minor stromal and mesenchymal components (SINGH *et al*., 2017).

An interesting feature of the genetic background of SG neoplasms is that they often arise from chromosomal translocations, which are usually considered a characteristic of hematological malignancies rather than solid tumors. Hence, for many years studies were mainly focused on chromosomal rearrangements, and indeed highly specific patterns of chromosome translocations have been described in SG neoplasms, such as t(11;19) (q14-21;p12-13) resulting in *CRTC1-MAML2* oncogenic fusion seen in many mucoepidermoid carcinomas (O'NEILL *et al*., 2009; ILIC-DIMITRIJEVIC *et al*., 2014), t(6:9)(q22-23;p23-24) translocations which predominantly result in *MYB-NFIB* gene fusions described in adenoid cystic carcinomas, etc. (BRILL *et al*., 2011).

Activation of the *PLAG1* gene on chromosome 8q12 is the most frequent gain-of-function mutation found in pleomorphic adenomas of the salivary glands and represents the result of different recurrent chromosomal translocations involving, for instance the β-catenin gene on 3p21 in t (3;8 (p21;q12), the leukemia inhibitory factor receptor gene on 5p13 found in t (5;8)(p13;q12) and others (VOZ *et al*., 2000). *PLAG1* role in PA tumorigenesis is undeniable;

however, its interactions with other genes involved in PA development are less well characterized.

PAs are known to comprise both epithelial and myoepithelial cells embedded in a mesenchyma-like stroma (ITO *et al.*, 2009), and the extracellular matrix (ECM) component represents a crucial regulator of tumor growth and progression (PEREIRA *et al.*,2005). ECM molecules are modified by matrix metalloproteinases (MMPs), a family of enzymes that influences the behavior of cells by creating space for their migration, by releasing ECM-bound growth factors and by activating signaling molecules (PAGE-MCCAW *et al.*, 2007). Another important element affecting salivary gland tumor biology is the subtle balance between matrix MMPs and their endogenous tissue inhibitors (TIMPs), which functions as a regulator of ECM turnover (ZHANG *et al.*, 2009). Thus, the aims of the present study were (a) to assess the expression of the following genes: *PLAG1*, *MMP-2*, *MMP-7*, *MMP-9*, *TIMP-1* and *TIMP-2* in pleomorphic adenomas of the salivary glands and (b) to find a possible relationship between these molecular markers and clinical/epidemiological parameters of PA patients.

MATERIALS AND METHODS

A total of 20 samples of salivary gland tissue were included in the present study. The study group consisted of fifteen pleomorphic adenomas of the parotid gland and the control group of five normal salivary glands (NSGs) i.e. histopathologically tumor free glands that were obtained from patients with oral cancer undergoing radical neck dissection (Figure 1). The patients were recruited from July to October 2020 at the Clinic for Maxillofacial Surgery, School of Dental Medicine, University of Belgrade. This study was performed according to the ethical principles of the Helsinki Declaration, and the patients signed a written informed consent.

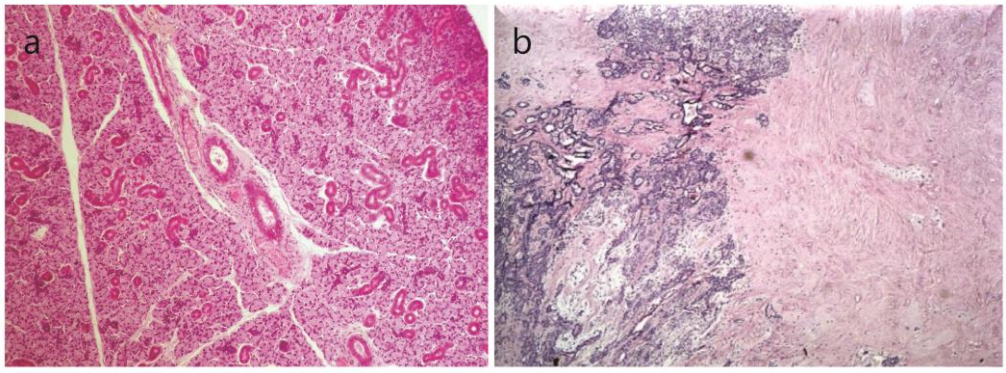

Figure 1. Specimens of normal salivary gland **(a)** and pleomorphic adenoma exhibiting typical histological features, i.e. the presence of epithelial component with ductal structures (left) and a mesenchymal myxoid component (right) **(b)** (magnification 20X).

Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA), and complementary DNA (cDNA) was synthesized through a reverse transcription reaction using

Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA).

Quantitative real-time PCR analyses were performed to assess the relative gene expression levels of PLAG1 oncogene, matrix metalloproteinase (*MMP*) -2, -7 and -9, the tissue inhibitor of metalloproteinases (*TIMP*) -1 and -2, using the SYBR-green based fluorescence quantification system (SensiFAST™ SYBR® HiROX Kit, Bioline, London, UK) and specific primers (Table 1). Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as an endogenous control for the normalization and relative gene expression levels in all samples were defined as the ratio of each gene of interest to *GAPDH* expression, using the comparative Ct ($2^{-\Delta\Delta Ct}$) method.

Descriptive statistics was presented as mean ± standard deviation values. Statistical analyses were conducted using SPSS 22.0 (SPSS, Chicago, IL, USA) or the GraphPad Prism 6.0 software. Kolmogorov–Smirnov normality test was performed for the outcome values and depending on the distribution, Student's t-test or Mann-Whitney U-test were performed. The Spearman's rank correlation coefficient (ρ) was used to assess the relationship between relative expression levels of the investigated genes. All p values less than 0.05 were considered significant.

Table 1. Sequences of primers used in the study and the corresponding amplicons' lengths

Gene	Sense and antisense	bp
PLAG1	5'-ATCACCTCCATACACACGACC-3' 5'-AGCTTGGTATTGTAGTTCTTGCC-3'	76
MMP-2	5'-CTCCTGAATGCCCTTGATGT-3' 5'-ATGACAGCTGCACCACTGAG-3'	425
MMP-7	5'-CCAATGAATGAATGAATGGATG-3' 5'-GTATGGGACATTCCTCTGATCC-3'	180
MMP-9	5'-GAACAAATACAGCTGGTTCC-3' 5'-TACCCTATGTACCGCTTCAC-3'	345
TIMP-1	5'-AGTCAACCAGACCACCTTATACCA-3' 5'-TTTCATAGCCTTGGAGGAGCTGGTC-3'	386
TIMP-2	5'-CTCTGTGACCCAGTCCATCC-3' 5'-ATGCACATCACCCTCTGTGA-3'	177
GADPH	5'-TCATGACCACAGTCCATGCCATCA-3' 5'-CCCTGTTGCTGTAGCCAAATTCGT-3'	450

bp – base pair

RESULTS

The relative gene expression of *PLAG1* oncogene, matrix metalloproteinases -2, -7 and -9 and tissue inhibitors of metalloproteinases -1 and -2 genes was assessed in fifteen pleomorphic adenoma (PA) cases and five normal salivary glands (NSGs) (Table 2, Figure 2).

A highly significant overexpression of *PLAG1* was observed in PA compared to NSG samples (p=0.010) (Figure 2a). Levels of *PLAG1* mRNA were 5.48 times higher in PA than in controls.

Table 2. The mean ± standard deviation values for relative gene expression levels in pleomorphic adenomas (PA) and normal salivary glands (NSGs)

	Group	Mean±SD	p value*
PLAG1	PA (N=15)	0.074537±0.098197	**0.010**
	NSG (N=5)	0.013582±0.003639	
MMP-2	PA (N=15)	0.032152±0.104362	0.206
	NSG (N=5)	0.026929±0.036044	
MMP-7	PA (N=15)	0.033852±0.049544	**0.026**
	NSG (N=5)	0.099983±0.068824	
MMP-9	PA (N=15)	0.000175±0.000290	0.359
	NSG (N=5)	0.000049±0.000049	
TIMP-1	PA (N=15)	0.627082±0.69193	0.896
	NSG (N=5)	0.410688±0.26028	
TIMP-2	PA (N=15)	0.046952±0.026906	**0.040**
	NSG (N=5)	0.133800±0.094328	

*Mann-Whitney U Test, significant at $p<0.05$

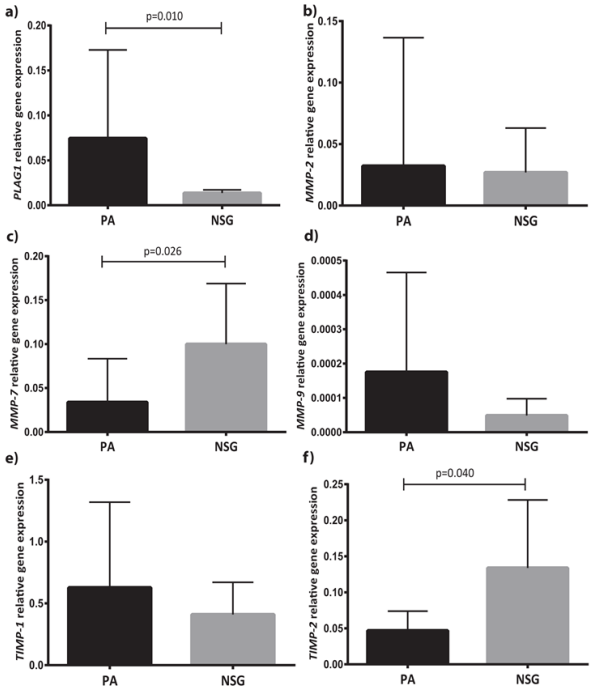

Figure 2. Relative gene expression levels of *PLAG1* **(a)**, *MMP-2* **(b)**, *MMP-7* **(c)**, *MMP-9* **(d)**, *TIMP-1* **(e)** and *TIMP-2* **(f)** in pleomorphic adenoma (PA) and normal salivary gland (NSG) specimens. Significant differences ($p < 0.05$) are indicated on the graph.

Out of the three analyzed *MMPs*, only *MMP-7* levels were altered in PA compared to controls. Namely, *MMP-7* was significantly down-regulated in PA samples (p=0.026) (Figure 2c). *TIMP-2* was also downregulated in PA, compared to NSGs (p=0.040) (Figure 2f). *MMP-7* and *TIMP-2* mRNA levels were decreased 2.95 and 2.85 times respectively, in PA compared to normal tissue. Moreover, a highly significant positive correlation was observed between *MMP-7* and *TIMP-2* relative gene expression levels in the PA group (ρ=0.768, p=0.001).

No association was observed between relative gene expression levels of either of the analyzed genes and the tumor size, nor epidemiological parameters such as gender, age and smoking status (data not presented).

DISCUSSION

Genetics and epigenetics play a fundamental role in the ethiopathogenesis of pleomorphic adenomas, the most frequent type of salivary gland tumors. Not only PA are the most frequent type of SG tumors, but this benign neoplasm has also the capacity to relapse and undergo malignant transformation (NIKOLIC *et al*., 2015). Although the golden standard for the diagnosis of SG tumors are their morphological and histological characteristics, there is an obvious need of improving the currently existing system of their classification with new molecular markers.

Pleomorphic adenoma gene 1 (*PLAG1*) has been shown to function as an oncogene in several human tumors, i.e. its overexpression has been documented in lipoblastomas, gastrointestinal stromal tumors, angiomyofibroblastomas, synovial sarcomas, etc. (MATSUYAMA *et al*., 2012). In the present study, a 5.5 fold increase of PLAG1 expression has been found in PA compared to control tissue, confirming some previous findings on the crucial role of this oncogene activation in PA pathogenesis (DE BRITO *et al*., 2016a; DE BRITO *et al*., 2016b; BRODETSKYI *et al*., 2020; LEE *et al*., 2019). In our study, no correlation could be established between *PLAG1* expression and clinical/epidemiological parameters. There are only sporadic reports dealing with *PLAG1* expression as a prognostic marker and only in pituitary tumors a correlation between *PLAG1* down-regulation and tumor aggressiveness has been established (YU *et al*., 2016). However, it is estimated that *PLAG1* may help in differential diagnosis of salivary gland benign and malignant tumors (ROTELLINI *et al*., 2014). Moreover, it also appears that *PLAG1* expression can discriminate pleomorphic carcinomas originating from pleomorphic adenomas from carcinomas arising *de novo* (BAHRAMI *et al*., 2012).

As already stated, MMPs and TIMPs, and more specifically their balanced expression, are very important factors in the maintenance/remodeling of the extracellular matrix. Disruptions in this balance may lead to different pathological conditions, including tumors. Out of the three *MMP* and two *TIMP* genes analyzed in the present study, *MMP-7* and *TIMP-2* displayed approximately three times lower mRNA levels in PA compared to controls. Importantly, there was a positive correlation of their down-regulation pointing to their joint involvement in PA development. To the best of our knowledge, this is the first report on *MMP-7* expression in PA. It must be emphasized however that in several types of salivary gland carcinomas (acinic cell carcinoma, adenoid cell carcinoma and mucoepidermoid carcinoma) lower levels of MMP-7 were associated with increased aggressiveness and poorer survival (LUUKKAA *et al*., 2010; HÄMETOJA *et al*., 2021). Data regarding the expression of TIMP-2 in SG tumors are also rather scarce. There are only few comparative studies dealing with the distinction between benign and

malignant tumors as well as between different types of SG carcinomas based on TIMPs expression and their ratio to MMPs. Usually, this MMP/TIMP ratio was found to be increased in carcinomas compared to adenomas (NAGEL *et al*., 2004). Contrary to our findings, several authors have found high levels of immunohistochemical expression of matrix metalloproteinase tissue inhibitors, including TIMP-2 in pleomorphic adenomas (SOUZA FREITAS *et al*., 2018). It must be stressed that some of the inconsistencies in the literature are probably due to different techniques applied in expression analyses, very heterogeneous tumor types included in the studies and, sometimes, somewhat small sample sizes.

In conclusion, our findings support the view that *PLAG1* gene is a key player in PA development. Additionaly, the present study uncovered the involvement of *MMP-7* coupled with *TIMP-2* in the pathogenic process. However, a larger cohort of patients is necessary in order to provide a consistent hypothesis about the cross-talk of the analyzed molecular markers in this salivary gland neoplasm.

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REFERENCES

ABBASI, H.A. and M., KAZEMI (2011): Detection of polymorphism at the insulin like growth factor-I gene in Mazandaran native chicken using polymerase chain reaction-restriction fragment length polymorphism method. Am. J. Anim. Vet. Sci., *6*: 80–83.

ABDALHAG, M.A., T., LI, L., DUAN, T., ZHANG, G., ZHANG, J., WANG, Y., WANG (2016): Association analysis of *IGF-I* gene expression with growth and reproductive traits in Jinghai yellow chickens. Genet. Mol. Res., *15*: (4).

ADEOLU, A.I. AND V.U., OLEFORUH – OKOLEH (2014): Biotechnological approach in genetic diversity conservation of Nigerian indigenous chickens for future breed development: problems and prospects. J. Life Sci. Res. Dis.,*1*: 40-46.

AL-HASSANI, A.S., D.H., AL-HASSANI, I.A., ABDUL-HASSAN (2015): Association of Insulin-Like Growth Factor-1 Gene Polymorphism at 279 Position of the 5'UTR Region with Body Weight Traits in Broiler Chicken. Asian J. Poult. Sci., *9*(4): 213 - 222.

AMILLS, M., N., JIMENEZ, D., VILLALBA, M., TOR, E., MILINA, D., CUBILO, C., MARCOS, A., FRANCESCH, A., SANCHEZ, J., ESTANY (2003): Identification of three single nucleotide polymorphisms in the chicken insulin-like growth factor 1 and 2 genes and their associations with growth and feeding traits. Poult. Sci., *82*: 1485–1493.

BABAYI, M.S., S., HAMIDREZA, G., ABOLFAZL, Z., NOSRATOLLAH (2014): IGF-I Gene Polymorphism and its Associations with Some Growth traits in West-Azerbaijan Native chicken using PCR-RFLP Techniques. Bull. Env. Pharmacol. Life Sci., *3*(6): 29-32.

BHATTACHARYA, T.K., R.N., CHATTERJEE, K., DUSHYANTH, C., PASWAN, R., SHUKLA, M., SHANMUGAM (2015): Polymorphism and expression of insulin-like growth factor 1 (IGF1) gene and its association with growth traits in chicken. Bri. Poult. Sci., *56*(4): 398 – 407.

BIAN, L.H., S.Z., WANG, Q.G. WANG, S., ZHANG (2008): Variation at the insulin-like growth factor 1 gene and its association with body weight traits in the chicken. J. Anim. Breed. Genet., *125*: 265-270.

FAO (2007): The State of the World's animal genetic resources for Food and Agriculture Organization. Rome, Italy.

FAYEYE, T.R., K. L., AYORINDE, V., OJO, D.M., ADESINA (2006): Frequency and influence of, show major genes on body weight and body size parameters of Nigeria local chickens. Live. Res. Rur. Dev., *18*(37): 1-9.

HALL, T.A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser., *41*: 95-98.

HAVENSTEIN, G.B., P.R. FERKET, M.A. QURESHI (2003): Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci., *82*: 1500-1508.

HORST, P. (1989): Native fowl as reservoir for genomes and major genes with direct and indirect effects on adaptability and their potential for tropical oriented breeding plans. Arch. Anim. Breed., *53*: 93-101.

IBE, S.N. (2001): Problems and proposal of developing Nigeria poultry breeds. J. Science Agric. Food Tech. Envir.,*1*: 1-3.

ILORI, B.M., M., WHETO, S.O., DUROSARO, K., AKANO, A.O., ADEBAMBO, O.A., ADEBAMBO (2016): Polymorphism of *IGF-1* Promoter and the UTR Regions of Nigerian Locally Adapted Chickens. J. Biol. Agric. Healthc., *6*: No.10.

KADLEC, J., B., HOSNEDLOVÁ, V., REHOUT, J., CÍTEK, L., VECEREK, L., HANUSOVA (2012): Insulin-like growth factor-I gene polymorphism and its association with growth and slaughter characteristics in broiler chickens. J. Agrobiol., *28*(2): 157–163.

KUHNLEIN, U., L., NI, S., WEIGEND, J.S., GAVORA, W., FAIRFULL, D., ZADWORNY (1997): DNA polymorphisms in the chicken growth hormone gene: response to selection for disease resistance and association with egg production. Anim. Genet., *28*: 116123.

LARON, Z. (2001): Insulin-growth factor 1 (IGF 1): A growth hormone. J. Clin. Pathol. Mol. Pathol., *54*:311–316.

LEI, M., Q.H., NIE, X., PENG, D.X., ZHANG, Q., ZHANG (2005): Single nucleotide polymorphisms of the chicken insulin-like factor binding protein 2 gene associated with chicken growth and carcass traits. Poult. Sci., *84*: 1191–1198.

LIU, X. (2009): Association of Single Nucleotide Polymorphisms with Phenotypic Production Traits in Broiler Chickens. *Thesis submitted to the Faculty of the Graduate School of the University of Maryland, College Park, in partial fulfilment of the requirements for the degree of Master of Science*. Pp 81.

LI, H., W., ZHU, K., CHEN, X., WU, Q., TANG, Y., GAO (2008): Associations between GHR and IGF-1 Gene Polymorphisms, and Reproductive Traits in Wenchang Chickens. Turk. J. Vet. Anim. Sci., *32*(4): 281-285.

MITTANCK, D.W., S.W., KIM, P., ROTWEIN (1997): Essential promoter elements are within the 5' untranslated region of human insulin-like growth factor 1 exon 1. Mol. Cell.Endocrinol., *2*: 153 -163.

NAGARAJA, S.C., S.E., AGGREY, J., YAO, D., ZADWORNY, R.W., FAIRFULL, U., KUHNLEIN (2000): Trait association of a genetic marker near the IGF-I gene in Egg-laying chickens. J. Hered., *91*:150–156.

ODUBOTE, I.K. (2015): Genetic Diversity of Nigerian Local Chicken. Research Gate: 10.13140/RG.2.1.2837. 9044 https://www.researchgate.net/publication/277014164

PANDEY, N.K., R.P., SINGH, V.K., SAXENA, N., SHIT, R., SINGH, R.K., SHARMA, K.V., SASTRY (2013): Effect of IGF1 gene polymorphism and expression levels on growth factors in Indian colored broilers. Livest. Sci.,*155*: 157-164.

PETERS, S.O., C.O.N., IKEOBI, M.O., OZOJE, O.,A., FAMAKINNWA, Y.S., OSHODI, O.A., OLUFUMILAYO (2007): Egg quality of the Nigeria local chicken as influenced by some major genes. Nig. J. Anim. Prod., *34* (1): 25-31.

PROMWATEE, N., B., LAOPAIBOON, T., VONGPRALUB, Y., PHASUK, S., KUNHAREANG, W., BOONKUM M., DUANGJINDA (2013): Insulin-like growth factor I gene polymorphism associated with growth and carcass traits in Thai synthetic chickens. Genet. Mol. Res., *12*(4): 4332-4341.

ROY, R.N., A., CECUTTI, A.H., CERULATH, W.M., STEINBERG, B.R., BHAVANI (1997): Endimetrial transcripts of human insulin-like growth factors arise by differential promoter usage. Mol. Cell. Endocrinol., *13*:11-19.

SHAH, T., S., DESHPANDE, U., DASGUPTA, K.M., SINGHAND, C.G., JOSHI (2012): Genotypic and Allelic Frequencies of IGF1 and IGF2 Genes in Broilers Analysed by Using PCR-RFLP Techniques. Iranian J. Appl. Anim. Sci., *2*(4): 357-360.

TAMURA, K., G., STECHER, D., PETERSON, A., FILIPSKI, S., KUMAR (2013): MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol. Biol Evol., *30*:2725-2729.

YAKUBU, A., A.E., SALAKO, M., DE DONATO, M.I., TAKEET, S.O., PETERS, M., OKPEKU, M., WHETO, I.G., IMUMORIN (2017): Nucleotide sequence variability analysis of major histocompatibility complex Class II DQA1 gene in Nigerian goats. Genetika (Belgrade), *49*(3): 865-874.

ZHOU, H., A.D., MITCHELL, J.P., MCMURTRY, C.M., ASHWELL, S.J., LAMONT (2005): Insulin-like growth factor-I gene polymorphism associations with growth, body composition, skeleton integrity and metabolic traits in chickens. Poult. Sci., *84*: 212-219.

SNIŽENA EKSPRESIJA MATRIKSNE METALOPROTEINAZE 7 (*MMP-7*) I TKIVNOG INHIBITORA METALOPROTEINAZE 2 (*TIMP-2*) U SADEJSTVU SA POVIŠENOM EKSPRESIJOM *PLAG1* ONKOGENA UČESTVUJE U PATOGENEZI PLEOMORFNOG ADENOMA

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Izvod

Pleomorfni adenom (PA) je najčešća neoplazma pljuvačnih žlezda i sastoji se od epitelnih i mezenhimskih komponenti. Iako benigna, ova lezija poseduje potencijal recidiviranja i maligne transformacije. Takođe, zbog histološke raznolikosti i ponašanja koje nije uvek moguće predvideti, PA može da predstavlja ozbiljan dijagnostički i terapijski izazov. Matriksne metaloproteinaze (MMP) su dobro poznati modifikatori vanćelijskog matriksa i zajedno sa njihovim endogenim tkivnim inhibitorima (TIMP) mogu uticati na biologiju PA. Onkogen *PLAG1* takođe ima važnu ulogu u PA; međutim, ni tačni mehanizmi njegovog delovanja niti interakcije sa drugim genima nisu u potpunosti razjašnjeni. Ciljevi ove studije bili su da se odredi nivo ekspresije *PLAG1, MMP-2, MMP-7. MMP-9, TIMP-1 i TIMP-2* gena u PA i pronađe eventualna veza između njihove ekspresije i kliničkih/epidemioloških parametara u grupi pacijenata sa PA. Relativni nivo genske ekspresije utvrđen je primenom kvantitativne PCR analize u realnom vremenu u 15 PA parotidne žlezde i 5 normalnih pljuvačnih žlezda (NSG). Ekspresija *PLAG*1 bila je 5,48 puta viša u PA u poređenju sa uzorcima NSG (P = 0,010). Od tri analizirana *MMP*-a, samo je *MMP-7* gen pokazao kod pacijenata sa PA značajno niži nivo nego u kontrolama (P = 0,026); ekspresija *TIMP2* je takođe bila smanjena u uzorcima PA, u poređenju sa NSG (P = 0,040), 2,95 puta za *MMP-7*, odnosno 2,85 puta za TIMP-2. Nije utvrđena povezanost između ekspresije gena i kliničkih/demografskih parametara PA. Naši rezultati sugerišu da prekomerna ekspresija *PLAG1* sa istovremenim smanjenjem ekspresije *MMP7* i *TIMP2* može doprineti razvoju PA.

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