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Preliminary data regarding the influence of the COL1a1 *rs2249492* polymorphism on the risk of malocclusion in the Romanian population

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Abstract. Malocclusion is a condition characterized by diverse phenotypic expression patterns, with a complex underlying genetic background. COL1a1 is one of the genes that has been previously associated with malocclusion, with one particular SNP, *rs2249492* (C>G, C>T), having been linked with an increased risk of skeletal class II malocclusion.

In this paper, making use of DNA sequencing and cephalometric measurements, we present preliminary data regarding the association between the *rs2249492* SNP and the risk of malocclusion in the Romanian population, illustrated as continuous, rather than categorical phenotypes. The results show a tendency towards a Class II pattern determined by mandibular retrognathism, rather than maxillary prognathism among the individuals possessing the mutant allele. Subsequent studies on larger sample sizes should include statistical analysis focused on associations between the *rs2249492* allele and

continuous phenotypic variation inside, but not restricted to Class II malocclusion, in order to acquire a more detailed picture of the interaction between the polymorphism and this complex condition.

Keywords: malocclusion, COL1a1, *rs2249492*, cephalometric measurements, SNP

Introduction

The term malocclusion encompasses various phenotypic expression patterns, caused by a complex genetic background and characterized by the unharmonious growth of the maxillary and mandibular structures, leading to a defective relationship between the dental arches (Laviana *et al*., 2021; Nishio and Huynh, 2016; Weaver *et al.,* 2017). According to Angle's classification, this complex condition can be divided into three groups – Class I, II and III. The last two types are the most frequent, impacting not only the patient's masticatory functions, but also their appearance and mental health, leading to a reduced quality of life in affected individuals (Graber *et al*., 2017; Li *et al*., 2010; Liu *et al*., 2009; Ma *et al.,* 2019).

Class II malocclusion encompasses phenotypes characterized by a convex facial pattern, as a consequence of either a protruded maxilla or a retruded mandible. Conversely, Class III phenotypes are characterized by concave facial profiles caused by either a retruded maxilla or a more protruded mandible, of which the most well-known is mandibular prognathism (Doraczynska-Kowalik *et al*., 2017; Hardy *et al*., 2012; Laviana *et al*., 2021; Li *et al*., 2010; Liu *et al.,* 2009).

A good, detailed understanding of the genetic factors underlying malocclusion is vital so that orthodontists are able to correctly diagnose and treat this condition (Weaver *et al.,* 2017; Zabrina *et al.,* 2021). This study is concerned with the COL1a1 gene, that encodes the pro-alpha chain of type I collagen and has been previously associated with malocclusion (Da Fontoura *et al*., 2015; Doraczynska-Kowalik *et al.,* 2017; Zabrina *et al.,* 2021). Mutations of this gene have been linked with various diseases, such as those related to facial anomalies and osteogenesis imperfecta. Out of these mutations, one particular SNP, *rs2249492* (C>G, C>T), has been linked with an increased risk of skeletal class II malocclusion (Da Fontoura *et al.,* 2015).

As such, this paper is aimed at presenting preliminary data regarding the influence of the *rs2249492* polymorphism on malocclusion risk in the Romanian population, with focus on continuous phenotypic variation represented by four different cephalometric measurements.

Materials and methods

2.1 Cephalometric measurements

In order to obtain preliminary data on the association between the *rs2249492* allele and malocclusion in the Romanian population, the sample set comprises a total of 12 individuals, of which 9 suffer from Class II malocclusion and 3 suffer from Class III, classified according to the clinical evaluation, facial photographs evaluation, dental cast study and cephalometric measurements.

With the purpose of getting a more detailed picture on the patients' complex phenotype, four cephalometric measurements performed in the OnyxCeph software and assessed with Steiner analysis and Witts appraisal were taken into account: the SNA, SNB, ANB and AoBo (the Witts appraisal). The SNA and SNB angles are measures of maxillary and mandibular position, respectively, while the ANB and AoBo are measures of jaw disparity (Ghergie *et al.,* 2013).

2.2 DNA extraction, PCR amplification and DNA sequencing

Genetic data were collected from the same sample set mentioned above.

Genomic DNA was extracted from buccal swabs, using the Animal and Fungi DNA Extraction Kit (Jena BioScience, Germany), according to the manufacturer's instructions.

PCR amplification and Sanger sequencing (Macrogen Europe, The Netherlands) were carried out on a fragment of the COL1a1 gene containing the *rs2249492* SNP. The primers amplified a region of 500 bp length and had the following sequence (Da Fontoura *et al*., 2015; Doraczynska-Kowalik *et al.,* 2017):

COL1a1_F GTAAGGTTGAATGCACTTTTGGTTT
COL1a1_R GTGAGTGCCCAGAATCCCCA

The PCR reaction setup that led to the obtained results was as follows: 25µL total reaction volume, 1×PCR Buffer, 2.5 mM MgCl2, 0.2 mM dNTPs (Bioline, Meridian Bioscience, USA), 0.5 pM of forward and reverse Primers and 1.25 units/reaction MangoTaq Polymerase (Bioline, Meridian Bioscience, USA). The PCR parameters were: 95°C for 5 min (initial denaturation), followed by 35 cycles of 95°C for 30 s (denaturing), 66°C for 30 s (annealing), 72°C for 30 s (extension) and a final extension for 5 min at 72°C.

Results and discussion

The obtained results indicate a high tendency towards the mutant T allele in the investigated cohort, with 7 out of 9 Class II patients and all 3 Class III patients having this SNP, and only 2 Class II individuals having the reference C allele. Complete cephalometric measurements were obtained for 9 out of 12 individuals and the results are presented below, in Table 1.

When looking at the Class II group, the results are indicative of the previously confirmed association between the *rs2249492* T allele and an increased risk of developing Class II malocclusion (Da Fontoura et al., 2015). What can also be noted is the fact that all 3 members of the Class III group have the same mutant T allele. However, further inquires on larger sample sizes are necessary in order to confirm an association between the *rs2249492* SNP and Class III malocclusion.

Next, when analyzing the results of the cephalometric measurements, complex phenotypes can be observed, suggesting that the individuals' Class II and III skeletal pattern may not be simply described as either mandibular or maxillary prognathism.

The SNA angle, which indicates the position of the maxilla, has a mean value of 82º, with higher values indicating maxillary prognathism and lower values representing maxillary retrognathism (Doraczynska-Kowalik *et al*., 2017; Ghergie *et al.,* 2013). In the case of the Class II group, while 2 patients display SNA values lower than the mean, 3 have values around the mean and 1 has a value higher than the mean. For the Class III individuals, two highly different measurements were obtained, with one having an above-the-mean value and the other, lower than the mean.

The SNB angle, which indicates the position of the mandible, has a mean value of 80º, with mandibular prognathism being reflected by higher values and mandibular retrognathism by lower values (Doraczynska-Kowalik *et al*., 2017; Ghergie *et al*., 2013). In this case, again, the Class III individuals have opposing values, while the Class II group is characterized by values indicative of mandibular retrognathism.

Lastly, the ANB (mean of 0-2º) and AoBo (mean of 0-2 mm) angles, that are used to assess the level of jaw disparity (Doraczynska-Kowalik *et al.,* 2017; Ghergie *et al.,* 2013), reinforce the idea of complex malocclusion phenotypes. While all Class II individuals can be classified as such on the basis of the ANB, only 4 present a Class II skeletal pattern on the basis of the Witts' appraisal. As far as the Class III individuals are concerned, the ANB values suggest opposing skeletal patterns, while the AoBo values are indicative of Class III skeletal patterns.

Considering how essential it is to understand the complex genetic background of this condition in order to establish better preventive measures as well as efficient, personalized therapies (Dehesa-Santos *et al.,* 2021; Moreno Uribe and Miller, 2016; Xue *et al.,* 2010), attention needs to be paid to the correlations between genotype and continuous phenotypic variation (Da Fontoura *et al*., 2015). Of course, given the small sample size of this preliminary study, no statistically significant associations could be found, however a tendency towards a Class II pattern driven by mandibular retrognathism, rather than maxillary prognathism among the individuals possessing the mutant allele can be observed. Further studies on larger sample sizes should include statistical analysis focused on associations between the *rs2249492* allele and continuous phenotypic variation inside, but not limited to, Class II malocclusion, in order to obtain a more detailed picture on the interaction between the SNP and this complex condition (Da Fontoura *et al*., 2015).

Table 1. *rs2249492* alleles and cephalometric measurements.
Grey areas signify a lack of data.

Nr. crt.	Class	Allele	SNA º	SNB º	ANB º	AoBo (mm)
1	II	C	78.3	70.2	8.1	3
2	II	T	81.9	74.3	7.7	3
3	II	T	82.2	76.9	5.4	-2
4	II	C				
5	II	T				
6	II	T	80	74	6	1.96
7	II	T	83.3	77.5	5.8	3
8	II	T	82.1	77.8	4.3	3
9	II	T	79.7	76.2	3.5	1
10	III	T	83.4	81.7	1.7	-6
11	III	T				
12	III	T	74.4	70.4	4	-3

Conclusions

This paper offers promising preliminary data regarding the influence of the COL1a1 gene on the risk of developing malocclusion in the Romanian population, as well as a useful experimental setup to be used in further studies of this kind.

While there have been previous discussions regarding the different prevalence of malocclusion across distinct geographic areas (Da Fontoura *et al.,* 2015; Hardy *et al*., 2012), studies on the region of Romania have yet to be

included (Ghergie *et al.,* 2013). As such, our study presents initial data that can be utilized as starting hypotheses for more extensive studies focused on the population of Romania.

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Ethic statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the University of Medicine and Pharmacy "Iuliu Haţieganu" Cluj-Napoca (97/08.03.2017). Informed consent was obtained from all subjects involved in the study.

References

Da Fontoura, C. S. G., Miller, S. F., Wehby, G. L., Amendt, B. A., Holton, N. E., Southard, T. E., Allareddy, V., & Moreno Uribe, L. M. (2015). Candidate gene analyses of skeletal variation in malocclusion. *Journal of Dental Research*, 94(7), 913–920. https://doi.org/10.1177/0022034515581643

Dehesa-Santos, A., Iber-Diaz, P., & Iglesias-Linares, A. (2021). Genetic factors contributing to skeletal class III malocclusion: a systematic review and meta-analysis. *Clinical Oral Investigations*, 25(4), 1587–1612. https://doi.org/10.1007/s00784-020-03731-5

Doraczynska-Kowalik, A., Nelke, K. H., Pawlak, W., Sasiadek, M. M., & Gerber, H. (2017). Genetic factors involved in mandibular prognathism. *Journal of Craniofacial Surgery,* 28(5), e422–e431. https://doi.org/10.1097/SCS.0000000000003627

Ghergie, M., Festila, D., Kelemen, B., & Lupan, I. (2013). Myo1H Gene Single Nucleotide Polymorphism rs10850110 and the Risk of Malocclusion in the Romanian Population. *Acta Medica Transilvanica*, 2(42), 281–284.

Graber LW, RL, Jr, V., KWL, V., & GJ., H. (2017). Orthodontics: Current Principles and Techniques. (6th Ed. El).

Hardy, D. K., Cubas, Y. P., & Orellana, M. F. (2012). Prevalence of angle class III malocclusion: A systematic review and meta-analysis. *Open Journal of Epidemiology,* 02(04), 75–82. https://doi.org/10.4236/ojepi.2012.24012

Laviana, A., Thahar, B., Melani, A., Mardiati, E., Putri, L., & Zakyah, A. D. (2021). Role of matrilin-1 (MATN1) polymorphism in class III skeletal malocclusion with mandibular prognathism in Deutero-Malay race: a case-control study. *Egyptian Journal of Medical Human Genetics*, 22(1). https://doi.org/10.1186/s43042-021-00131-6

Li, Q., Zhang, F., Li, X., & Chen, F. (2010). Genome scan for locus involved in mandibular prognathism in pedigrees from China. *PLoS ONE*, 5(9), 1–4. https://doi.org/10.1371/journal.pone.0012678

Liu, Z., McGrath, C., & Hagg, U. (2009). The impact of malocclusion/orthodontic treatment need on the quality of life a systematic review. *Angle Orthodontist*, 79(3), 585–591. https://doi.org/10.2319/042108-224.1

Ma, J., Huang, J., & Jiang, J. hui. (2019). Morphological analysis of the alveolar bone of the anterior teeth in severe high-angle skeletal Class II and Class III malocclusions assessed with cone-beam computed tomography. *PLoS ONE,* 14(3), 1–13. https://doi.org/10.1371/journal.pone.0210461

Moreno Uribe, L. M., & Miller, S. F. (2016). Genetics of the dentofacial variation in human malocclusion. *Physiology & Behavior*, 176(1), 100–106. https://doi.org/10.1111/ocr.12083.Genetics

Nishio, C., & Huynh, N. (2016). Skeletal Malocclusion and Genetic Expression: An Evidence-Based Review. *Journal of Dental Sleep Medicine*, 03(02), 57–63. https://doi.org/10.15331/jdsm.5720

Weaver, C. A., Miller, S. F., da Fontoura, C. S. G., Wehby, G. L., Amendt, B. A., Holton, N. E., Allareddy, V., Southard, T. E., & Moreno Uribe, L. M. (2017). Candidate gene analyses of 3-dimensional dentoalveolar phenotypes in subjects with malocclusion. *American Journal of Orthodontics and Dentofacial Orthopedics,* 151(3), 539–558. https://doi.org/10.1016/j.ajodo.2016.08.027

Xue, F., Wong, R. W. K., & Rabie, A. B. M. (2010). Genes, genetics, and Class III malocclusion. *Orthodontics and Craniofacial Research*, 13(2), 69–74. https://doi.org/10.1111/j.1601-6343.2010.01485.x

Zabrina, S., Ramadhanti, Jazaldi, F., Gultom, F. P., & Auerkari, E. I. (2021). Genetic and epigenetic aspects of class III malocclusion with mandibular prognathism phenotypes. *AIP Conference Proceedings*, 2344(March), 10–16. https://doi.org/10.1063/5.0047282