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Deleterious variants in genes associated with bone mineral density are linked to susceptibility to periodontitis development

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ABSTRACT

Studies have investigated the relationship between periodontitis and the decrement of the bone mineral density (BMD). However, there is limited knowledge on the possible association between BMD-related genes and susceptibility to periodontitis development.

To fill this gap, a total of 157 genes, previously associated with BMD, were analyzed in 442 subjects affected by chronic periodontitis.

Candidate gene analyses resulted in two *RBMS3* deleterious variants, rs55872743 and rs7636555, significantly associated ($p\text{-value} < 3.28 \times 10^{-6}$) with periodontitis status.

These results will shed new light on the possible role of genes linked to BMD affecting periodontitis.

1. Introduction

Conditions correlated with decreased bone mineral density (BMD), such as osteoporosis and osteopenia, are included in the list of possible actors involved in the aetiology of periodontitis (Reddy and Morgan, 2013; AlJehani, 2014; Passos et al., 2013). Periodontitis is a chronic multifactorial inflammatory disease associated with dysbiosis of plaque biofilms and characterized by progressive destruction of the tooth-supporting apparatus (Papapanou et al., 2018a; Papapanou et al., 2018b). Many studies suggest a significant relationship between decreased skeletal BMD and reduction of teeth number or increased risk of periodontal disease development (Inagaki et al., 2005; Kim et al., 2014; Savić Pavičin et al., 2017); although, other authors reported lack of correlation (Elders et al., 1992; May et al., 1995).

Considering these controversial findings, we decided to focus our attention on genes related to bone remodelling and metabolism and to study their impact on periodontitis development. We selected a list of genes previously associated with BMD in European individuals (Lee et al., 2014; Pei et al., 2016a; Mullin et al., 2016; Stykarsdottir et al., 2016a; Stykarsdottir et al., 2016b; Pei et al., 2016b; Medina-Gomez et al., 2018; Moayyeri et al., 2014), and we studied the role of these

genes in the susceptibility towards periodontitis development in six isolated populations from North-East Italy (Esko et al., 2013). We decided to perform our association study in isolated populations since all individuals live in the same environment, and there is an overall genetic homogeneity. These aspects can be exploited for the identification of genes and rare variants associated with complex traits and diseases as previously demonstrated (Esko et al., 2013; Varilo and Peltonen, 2004).

2. Materials and methods

A total of 826 individuals (18–88 years) were enrolled in the “Friuli Venezia Giulia Genetic Park” project (Esko et al., 2013; Xue et al., 2017). The ethical committee of IRCCS Burlo Garofolo and the Ethics Committee of the University of Trieste approved the study (Prot. CE/V – 78, 06/08/2007), following the ethical standards of the 1975 Declaration of Helsinki (7th revision, 2013). A written informed consent was obtained from each participant.

The information regarding medical and individual data was recorded, and the periodontal disease was diagnosed as reported in our previous articles (Zupin et al., 2017a; Zupin et al., 2017b), following

Abbreviations: BMD, bone mineral density; CADD, Combined Annotation Dependent Depletion; CAL, Clinical Attachment Loss; IBS, identity by state (IBS); PPD, probing pocket depth; SNP, single nucleotide polymorphism

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Table 1
Clinical characteristics of the study population.

Condition	Criteria	Age (mean $\pm$ standard deviation)	Sex (Males: Females)	% of Smokers
Healthy <i>n</i> = 160	No probing attachment loss, probing pocket depths $\leq$ 3 mm, bleeding on probing < 10%, no radiological bone loss	36.9 $\pm$ 14.0	36.3%: 63.7%	22%
Periodontitis <i>n</i> = 442	Interproximal clinical attachment loss of $\geq$ 3 mm at $\geq$ 2 non-adjacent teeth	54.6 $\pm$ 12.4	48.4%: 51.6%	21%

Table 2
Genotype distribution of statistically significant SNPs in RBMS3 gene among periodontitis cases and controls.

SNP	Cases (<i>n</i> = 442)	Controls (<i>n</i> = 160)	p-value	OR (CI)
rs55872743 Chr3:29389808			3.28×10^{-06}	2.54 (1.72–3.84)
A/A	44.6% (<i>n</i> = 197)	68.0% (<i>n</i> = 109)		
A/C	45.0% (<i>n</i> = 199)	30.0% (<i>n</i> = 48)		
C/C	10.4% (<i>n</i> = 46)	2.0% (<i>n</i> = 3)		
rs7636555 Chr3:29397894			3.20×10^{-06}	2.56 (1.73–3.87)
T/T	45.0% (<i>n</i> = 199)	68.7% (<i>n</i> = 110)		
A/C	44.8% (<i>n</i> = 198)	29.3% (<i>n</i> = 47)		
C/C	10.2% (<i>n</i> = 45)	2.0% (<i>n</i> = 3)		

the criteria of the American Academy of Periodontology and the European Federation of Periodontology (Caton et al., 2018). Patients with gingivitis, edentulous and patients with incomplete data (total number = 224) were excluded from the study.

The DNA was extracted from peripheral blood with the EZ1 DNA investigator kit (Qiagen, Milan, Italy) and then analyzed with the Illumina 370 k high-density SNP array (Illumina, Inc., San Diego, CA, USA) on the Illumina HiScan system at CBM (Cluster in Biomedicine, Trieste, Italy). Genotype calling and SNPs imputation were performed (for details see Zupin et al. 2017 (Zupin et al., 2017a)).

Based on the literature review, we investigated a set of genes, which are known to be involved in bone mineral density, (see Supplementary Table 1). Among them we included 122 genes identified by Lee et al. (Lee et al., 2014) (of which 3 of them, *FAM9B*, *KAL1*, *C11ORF98*, were not covered in our array), and other 38 genes (discovered more recently) (Pei et al., 2016a; Mullin et al., 2016; Styrkarsdottir et al., 2016a; Styrkarsdottir et al., 2016b; Pei et al., 2016b; Medina-Gomez et al., 2018; Moayyeri et al., 2014), leading to a total of 157 genes.

For all variants within these genes, we obtained the Combined Annotation Dependent Depletion (CADD) score to assess the deleteriousness of each polymorphism, using the CADD application version 1.3 (Kircher et al., 2014). This score predicts the possible impact of genetic variant and strongly correlates with both molecular functionality and pathogenicity, considering protein-altering and regulatory variation (Kircher et al., 2014).

Only variants with CADD score $\geq$ 10 were considered deleterious as indicated by Kircher et al. 2014). Then, a total of 3780 SNPs with CADD score $\geq$ 10 covering 157 genes were used for the association analysis, as described in Supplementary Table 1.

A mixed additive model linear regression was conducted using MixABEL and the GRAMMAR+ method (Aulchenko et al., 2007): the case status was the dependent variant while the SNP dosages were the tested independent variable. Sex, age and smoking status (yes/no) were included in the model as covariates. The genomic kinship matrix was estimated using the *ibs* function from the GenABEL R package (Aulchenko et al., 2007), as random effect. All statistical tests were performed with R software 3.5.0 version (R core Team, 2018), and the statistical significance was applied at *p-value* < .00005, following Bonferroni correction (0.05/3780 SNPs selected).

3. Results

We enrolled 442 patients affected by periodontitis (cases) and 160 individuals without any signs of periodontitis (controls). The patients' characteristics are reported in Table 1. From association analysis, considering all SNPs with CADD score $\geq$ 10, emerged that only two SNPs within *RBMS3* gene resulted significantly associated with periodontitis: rs55872743 (*p-value* = 3.28×10^{-06} , OR = 2.54) (CADD = 16.53, MAF = 0.2057) and rs7636555 (*p-value* = 3.20×10^{-06} , OR = 2.56) (CADD = 17.72, MAF = 0.2067). Table 2 reports genotype distribution among cases and controls and shows that for both SNPs, the C/C genotype was the one associated with a major risk of developing the disease.

4. Discussion

In this study, we observed the association between *RBMS3*-rs55872743 and rs7636555 polymorphisms and periodontitis condition, being the C derived allele for both SNPs more frequent among cases respect to controls. The two associated variants are in strong linkage disequilibrium in the European population ($r^2 > 0.9$) and are considered deleterious using the CADD score (CADD score > 15). Furthermore, C allele is relatively frequent in the European populations (frequency = 31%). The two variants identified were not formerly associated with any oral disease. However, one study observed a statistically significant correlation between *RBMS3* variation and periodontitis (Beck et al., 2001; Elter et al., 2004), and other *RBSM3* genetic variants were associated with BMD in different ethnic groups (Lee et al., 2014; Yang et al., 2013; Nicoletti et al., 2012; Cupples et al., 2007; Kiel et al., 2007). To note, in hepatic stellate cells *RBMS3* can promote gene expression of Prx1 (Fritz and Stefanovic, 2007) that in turn increments mRNA level of collagen $\alpha 1(I)$ (Jiang and Stefanovic, 2008), the most abundant component of bone tissue (Yang et al., 2013).

Periodontitis is a pathology involving an alveolar bone loss where a low bone mineral density may be associated (Oztürk Tonguç et al., 2012). Molecules related to BMD, such as *RBMS3*, could potentially have an impact on the development of the periodontitis. The novelty of our approach consisted in the combination of a gene-focused analysis followed by in silico functional assessment of deleteriousness (using CADD score).

In conclusion, our data suggest that two common deleterious

variants in *RBMS3* are potentially linked to the risk of periodontitis development, encouraging further functional studies in order to disclose the role of these genetic variants and genes linked to bone mineral density in susceptibility to periodontal disease.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mgene.2020.100670>.

Declarations of Competing Interest

None.

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