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

Massimo Mezzavilla[a,*,1], Luisa Zupin[a,1], Chiara Ottavia Navarra[b], Roberto Di Lenarda[b], Paolo Gasparini[a,b], Sergio Crovella[a,b], Antonietta Robino[a], Lorenzo Bevilacqua[b]

[a] Institute for Maternal and Child Health - IRCCS "Burlo Garofolo", 34137 Trieste, Italy
[b] Department of Surgical, Medical and Health Sciences, University of Trieste, 34149 Trieste, Italy

A B S T R A C T

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 ($p\text{-value} < 3.28 \times 10^{-06}$) 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; Styrkarsdottir et al., 2016a; Styrkarsdottir 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

* Corresponding author at: Institute for Maternal and Child Health - IRCCS "Burlo Garofolo", Via dell'Istria 65/1, 34137 Trieste, Italy.
E-mail address: massimo.mezzavilla@burlo.trieste.it (M. Mezzavilla).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.mgene.2020.100670

Table 1
Clinical characteristics of the study population.

Condition	Criteria	Age (mean ± standard deviation)	Sex (Males: Females)	% of Smokers
Healthy $n = 160$	No probing attachment loss, probing pocket depths ≤3 mm, bleeding on probing < 10%, no radiological bone loss	36.9 ± 14.0	36.3%: 63,7%	22%
Periodontitis $n = 442$	Interproximal clinical attachment loss of ≥3 mm at ≥2 non-adjacent teeth	54.6 ± 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 (n = 442)	Controls (n = 160)	p-value	OR (CI)
rs55872743 Chr3:29389808			3.28×10^{-06}	2.54 (1.72–3.84)
A/A	44.6% ($n = 197$)	68.0% ($n = 109$)		
A/C	45.0% ($n = 199$)	30.0% ($n = 48$)		
C/C	10.4% ($n = 46$)	2.0% ($n = 3$)		
rs7636555 Chr3:29397894			3.20×10^{-06}	2.56 (1.73–3.87)
T/T	45.0% (n = 199)	68.7% ($n = 110$)		
A/C	44.8% ($n = 198$)	29.3% ($n = 47$)		
C/C	10.2% ($n = 45$)	2.0% (n = 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 ≥ 10 were considered deleterious as indicated by Kircher et al. 2014). Then, a total of 3780 SNPs with CADD score ≥ 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 ≥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 α1(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.

Acknowledgements

We thank all the individuals who participated to the Friuli-Venezia Giulia Genetic Park project. A sincere thank you to Veronika Collovati for proofreading this manuscript. This study was partially supported by Fondo Trieste (2008), Regione FVG (L.26.2008) and the Italian Ministry of Health (RC 35/2017). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

AlJehani, Y.A., 2014. Risk factors of periodontal disease: review of the literature. Int. J. Dent. 2014, 182513. https://doi.org/10.1155/2014/182513.

Aulchenko, Y.S., Ripke, S., Isaacs, A., van Duijn, C.M., 2007. GenABEL: an R library for genome-wide association analysis. Bioinformatics 23, 1294–1296. https://doi.org/10.1093/bioinformatics/btm108.

Beck, J.D., Elter, J.R., Heiss, G., Couper, D., Mauriello, S.M., Offenbacher, S., 2001. Relationship of periodontal disease to carotid artery intima-media wall thickness: the atherosclerosis risk in communities (ARIC) study. Arterioscler. Thromb. Vasc. Biol. 21, 1816–1822.

Caton, J.G., Armitage, G., Berglundh, T., Chapple, I.L.C., Jepsen, S., Kornman, K.S., Mealey, B.L., Papapanou, P.N., Sanz, M., Tonetti, M.S., 2018. A new classification scheme for periodontal and peri-implant diseases and conditions - introduction and key changes from the 1999 classification. J. Periodontol. 89 (Suppl. 1), S1–S8. https://doi.org/10.1002/JPER.18-0157.

Cupples, L.A., Arruda, H.T., Benjamin, E.J., D'Agostino, R.B., Demissie, S., DeStefano, A.L., Dupuis, J., Falls, K.M., Fox, C.S., Gottlieb, D.J., Govindaraju, D.R., Guo, C.-Y., Heard-Costa, N.L., Hwang, S.-J., Kathiresan, S., Kiel, D.P., Laramie, J.M., Larson, M.G., Levy, D., Liu, C.-Y., Lunetta, K.L., Mailman, M.D., Manning, A.K., Meigs, J.B., Murabito, J.M., Newton-Cheh, C., O'Connor, G.T., O'Donnell, C.J., Pandey, M., Seshadri, S., Vasan, R.S., Wang, Z.Y., Wilk, J.B., Wolf, P.A., Yang, Q., Atwood, L.D., 2007. The Framingham Heart Study 100K SNP genome-wide association study resource: overview of 17 phenotype working group reports. BMC Med. Genet. 8 (Suppl. 1), S1. https://doi.org/10.1186/1471-2350-8-S1-S1.

Elders, P.J., Habets, L.L., Netelenbos, J.C., van der Linden, L.W., van der Stelt, P.F., 1992. The relation between periodontitis and systemic bone mass in women between 46 and 55 years of age. J. Clin. Periodontol. 19, 492–496.

Elter, J.R., Champagne, C.M.E., Offenbacher, S., Beck, J.D., 2004. Relationship of periodontal disease and tooth loss to prevalence of coronary heart disease. J. Periodontol. 75, 782–790. https://doi.org/10.1902/jop.2004.75.6.782.

Esko, T., Mezzavilla, M., Nelis, M., Borel, C., Debniak, T., Jakkula, E., Julia, A., Karachanak, S., Khrunin, A., Kisfali, P., Krulisova, V., Kučinskienė, Z.A., Rehnström, K., Traglia, M., Nikitina-Zake, L., Zimprich, F., Antonarakis, S.E., Estivill, X., Glavač, D., Gut, I., Klovins, J., Krawczak, M., Kučinskas, V., Lathrop, M., Macek, M., Marsal, S., Meitinger, T., Melegh, B., Limborska, S., Lubinski, J., Paolotie, A., Schreiber, S., Toncheva, D., Toniolo, D., Wichmann, H.-E., Zimprich, A., Metspalu, M., Gasparini, P., Metspalu, A., D'Adamo, P., 2013. Genetic characterization of northeastern Italian population isolates in the context of broader European genetic diversity. Eur. J. Hum. Genet. 21, 659–665. https://doi.org/10.1038/ejhg.2012.229.

Fritz, D., Stefanovic, B., 2007. RNA-binding protein RBMS3 is expressed in activated hepatic stellate cells and liver fibrosis and increases expression of transcription factor Prx1. J. Mol. Biol. 371, 585–595. https://doi.org/10.1016/j.jmb.2007.06.006.

Inagaki, K., Kurosu, Y., Yoshinari, N., Noguchi, T., Krall, E.A., Garcia, R.I., 2005. Efficacy of periodontal disease and tooth loss to screen for low bone mineral density in Japanese women. Calcif. Tissue Int. 77, 9–14. https://doi.org/10.1007/s00223-004-0275-x.

Jiang, F., Stefanovic, B., 2008. Homeobox gene Prx1 is expressed in activated hepatic stellate cells and transactivates collagen alpha1(I) promoter. Exp. Biol. Med. (Maywood) 233, 286–296. https://doi.org/10.3181/0707-RM-177.

Kiel, D.P., Demissie, S., Dupuis, J., Lunetta, K.L., Murabito, J.M., Karasik, D., 2007. Genome-wide association with bone mass and geometry in the Framingham Heart Study. BMC Med. Genet. 8 (Suppl. 1), S14. https://doi.org/10.1186/1471-2350-8-S1-S14.

Kim, J.W., Kong, K.A., Kim, H.Y., Lee, H.S., Kim, S.J., Lee, S.H., Sim, K.W., Kim, M.R., Lee, J.H., 2014. The association between bone mineral density and periodontitis in Korean adults (KNHANES 2008-2010). Oral Dis. 20, 609–615. https://doi.org/10.1111/odi.12179.

Kircher, M., Witten, D.M., Jain, P., O'Roak, B.J., Cooper, G.M., Shendure, J., 2014. A general framework for estimating the relative pathogenicity of human genetic variants. Nat. Genet. 46, 310–315. https://doi.org/10.1038/ng.2892.

Lee, S.H., Kang, M.I., Ahn, S.H., Lim, K.-H., Lee, G.E., Shin, E.-S., Lee, J.-E., Kim, B.-J., Cho, E.-H., Kim, S.-W., Kim, T.-H., Kim, H.-J., Yoon, K.-H., Lee, W.C., Kim, G.S., Koh, J.-M., Kim, S.-Y., 2014. Common and rare variants in the exons and regulatory regions of osteoporosis-related genes improve osteoporotic fracture risk prediction. J. Clin. Endocrinol. Metab. 99, E2400–E2411. https://doi.org/10.1210/jc.2014-1584.

May, H., Reader, R., Murphy, S., Khaw, K.T., 1995. Self-reported tooth loss and bone mineral density in older men and women. Age Ageing 24, 217–221.

Medina-Gomez, C., Kemp, J.P., Trajanoska, K., Luan, J., Chesi, A., Ahluwalia, T.S., Mook-Kanamori, D.O., Ham, A., Hartwig, F.P., Evans, D.S., Joro, R., Nedeljkovic, I., Zheng, H.-F., Zhu, K., Atalay, M., Liu, C.-T., Nethander, M., Broer, L., Porleifsson, G., Mullin, B.H., Handelman, S.K., Nalls, M.A., Jessen, L.E., Heppe, D.H.M., Richards, J.B., Wang, C., Chawes, B., Schraut, K.E., Amin, N., Wareham, N., Karasik, D., Van der Velde, N., Ikram, M.A., Zemel, B.S., Zhou, Y., Carlsson, C.J., Liu, Y., McGuigan, F.E., Boer, C.G., Bønnelykke, K., Ralston, S.H., Robbins, J.A., Walsh, J.P., Zillikens, M.C., Langenberg, C., Li-Gao, R., Williams, F.M.K., Harris, T.B., Akesson, K., Jackson, R.D., Sigurdsson, G., den Heijer, M., van der Eerden, B.C.J., van de Peppel, J., Spector, T.D., Pennell, C., Horta, B.L., Felix, J.F., Zhao, J.H., Wilson, S.G., de Mutsert, R., Bisgaard, H., Styrkársdóttir, U., Jaddoe, V.W., Orwoll, E., Lakka, T.A., Scott, R., Grant, S.F.A., Lorentzon, M., van Duijn, C.M., Wilson, J.F., Stefansson, K., Psaty, B.M., Kiel, D.P., Ohlsson, C., Ntzani, E., van Wijnen, A.J., Forgetta, V., Ghanbari, M., Logan, J.G., Williams, G.R., Bassett, J.H.D., Croucher, P.I., Evangelou, E., Uitterlinden, A.G., Ackert-Bicknell, C.L., Tobias, J.H., Evans, D.M., Rivadeneira, F., 2018. Life-course genome-wide association study meta-analysis of total body BMD and assessment of age-specific effects. Am. J. Hum. Genet. 102, 88–102. https://doi.org/10.1016/j.ajhg.2017.12.005.

Moayyeri, A., Hsu, Y.-H., Karasik, D., Estrada, K., Xiao, S.-M., Nielson, C., Srikanth, P., Giroux, S., Wilson, S.G., Zheng, H.-F., Smith, A.V., Pye, S.R., Leo, P.J., Teumer, A., Hwang, J.-Y., Ohlsson, C., McGuigan, F., Minster, R.L., Hayward, C., Olmos, J.M., Lyytikäinen, L.-P., Lewis, J.R., Swart, K.M.A., Masi, L., Oldmeadow, C., Holliday, E.G., Cheng, S., van Schoor, N.M., Harvey, N.C., Kruk, M., del Greco M, F., Igl, W., Trummer, O., Grigoriou, E., Luben, R., Liu, C.-T., Zhou, Y., Oei, L., Medina-Gomez, C., Zmuda, J., Tranah, G., Brown, S.J., Williams, F.M., Soranzo, N., Jakobsdottir, J., Siggeirsdottir, K., Holliday, K.L., Hannemann, A., Go, M.J., Garcia, M., Polasek, O., Laaksonen, M., Zhu, K., Enneman, A.W., McEvoy, M., Peel, R., Sham, P.C., Jaworski, M., Johansson, Å., Hicks, A.A., Pludowski, P., Scott, R., Dhonukshe-Rutten, R.A.M., van der Velde, N., Kähönen, M., Viikari, J.S., Sievänen, H., Raitakari, O.T., González-Macías, J., Hernández, J.L., Mellström, D., Ljunggren, Ö., Cho, Y.S., Völker, U., Nauck, M., Homuth, G., Völzke, H., Haring, R., Brown, M.A., McCloskey, E., Nicholson, G.C., Eastell, R., Eisman, J.A., Jones, G., Reid, I.R., Dennison, E.M., Wark, J., Boonen, S., Vanderschueren, D., Wu, F.C.W., Aspelund, T., Richards, J.B., Bauer, D., Hofman, A., Khaw, K.-T., Dedoussis, G., Obermayer-Pietsch, B., Gyllensten, U., Pramstaller, P.P., Lorenc, R.S., Cooper, C., Kung, A.W.C., Lips, P., Alen, M., Attia, J., Brandi, M.L., de Groot, L.C.P.G.M., Lehtimäki, T., Riancho, J.A., Campbell, H., Liu, Y., Harris, T.B., Akesson, K., Karlsson, M., Lee, J.-Y., Wallaschofski, H., Duncan, E.L., O'Neill, T.W., Gudnason, V., Spector, T.D., Rousseau, F., Orwoll, E., Cummings, S.R., Wareham, N.J., Rivadeneira, F., Uitterlinden, A.G., Prince, R.L., Kiel, D.P., Reeve, J., Kaptoge, S.K., 2014. Genetic determinants of heel bone properties: genome-wide association meta-analysis and replication in the GEFOS/GENOMOS consortium. Hum. Mol. Genet. 23, 3054–3068. https://doi.org/10.1093/hmg/ddt675.

Mullin, B.H., Walsh, J.P., Zheng, H.-F., Brown, S.J., Surdulescu, G.L., Curtis, C., Breen, G., Dudbridge, F., Richards, J.B., Spector, T.D., Wilson, S.G., 2016. Genome-wide association study using family-based cohorts identifies the WLS and CCDC170/ESR1 loci as associated with bone mineral density. BMC Genomics 17, 136. https://doi.org/10.1186/s12864-016-2481-0.

Nicoletti, P., Cartsos, V.M., Palaska, P.K., Shen, Y., Floratos, A., Zavras, A.I., 2012. Genomewide pharmacogenetics of bisphosphonate-induced osteonecrosis of the jaw: the role of RBMS3. Oncologist 17, 279–287. https://doi.org/10.1634/theoncologist.2011-0202.

Oztürk Tonguç, M., Büyükkaplan, U.S., Fentoglu, O., Gümüs, B.A., Çerçi, S.S., Kirzioglu, F.Y., 2012. Comparison of bone mineral density in the jaws of patients with and without chronic periodontitis. Dentomaxillofac Radiol. 41, 509–514. https://doi.org/10.1259/dmfr/21900076.

Papapanou, P.N., Sanz, M., Buduneli, N., Dietrich, T., Feres, M., Fine, D.H., Flemmig, T.F., Garcia, R., Giannobile, W.V., Graziani, F., Greenwell, H., Herrera, D., Kao, R.T., Kebschull, M., Kinane, D.F., Kirkwood, K.L., Kocher, T., Kornman, K.S., Kumar, P.S., Loos, B.G., Machtei, E., Meng, H., Mombelli, A., Needleman, I., Offenbacher, S., Seymour, G.J., Teles, R., Tonetti, M.S., 2018a. Periodontitis: consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J. Periodontol. 89 (Suppl. 1), S173–S182. https://doi.org/10.1002/JPER.17-0721.

Papapanou, P.N., Sanz, M., Buduneli, N., Dietrich, T., Feres, M., Fine, D.H., Flemmig, T.F., Garcia, R., Giannobile, W.V., Graziani, F., Greenwell, H., Herrera, D., Kao, R.T., Kebschull, M., Kinane, D.F., Kirkwood, K.L., Kocher, T., Kornman, K.S., Kumar, P.S., Loos, B.G., Machtei, E., Meng, H., Mombelli, A., Needleman, I., Offenbacher, S., Seymour, G.J., Teles, R., Tonetti, M.S., 2018b. Periodontitis: consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J. Clin. Periodontol. 45 (Suppl. 20), S162–S170. https://doi.org/10.1111/jcpe.12946.

Passos, J.S., Vianna, M.I.P., Gomes-Filho, I.S., Cruz, S.S., Barreto, M.L., Adan, L., Rösing, C.K., Cerqueira, E.M.M., Trindade, S.C., Coelho, J.M.F., 2013. Osteoporosis/osteopenia as an independent factor associated with periodontitis in postmenopausal women: a case–control study. Osteoporos. Int. 24, 1275–1283. https://doi.org/10.1007/s00198-012-2130-7.

Pei, Y.-F., Hu, W.-Z., Hai, R., Wang, X.-Y., Ran, S., Lin, Y., Shen, H., Tian, Q., GEFOS-seq Consortium, AOGC Consortium, UK10K Consortium, Lei, S.-F., Zhang, Y.-H., Papasian, C.J., Deng, H.-W., Zhang, L., 2016a. Genome-wide association meta-analyses identified 1q43 and 2q32.2 for hip Ward's triangle areal bone mineral density. Bone 91, 1–10. https://doi.org/10.1016/j.bone.2016.07.004.

Pei, Y.-F., Xie, Z.-G., Wang, X.-Y., Hu, W.-Z., Li, L.-B., Ran, S., Lin, Y., Hai, R., Shen, H., Tian, Q., Zhang, Y.-H., Lei, S.-F., Papasian, C.J., Deng, H.-W., Zhang, L., 2016b. Association of 3q13.32 variants with hip trochanter and intertrochanter bone mineral density identified by a genome-wide association study. Osteoporosis Int. 27, 3343–3354. https://doi.org/10.1007/s00198-016-3663-y.

R core Team, 2018. R: A Language and Environment for Statistical Computing. http://www.R-project.org.

Reddy, M.S., Morgan, S.L., 2013. Decreased bone mineral density and periodontal management. Periodontology 2000 (61), 195–218. https://doi.org/10.1111/j.1600-0757.2011.00400.x.

Savić Pavičin, I., Dumančić, J., Jukić, T., Badel, T., 2017. The relationship between periodontal disease, tooth loss and decreased skeletal bone mineral density in ageing women. Gerodontology 34, 441–445. https://doi.org/10.1111/ger.12290.

Styrkarsdottir, U., Thorleifsson, G., Eiriksdottir, B., Gudjonsson, S.A., Ingvarsson, T., Center, J.R., Nguyen, T.V., Eisman, J.A., Christiansen, C., Thorsteinsdottir, U., Sigurdsson, G., Stefansson, K., 2016a. Two rare mutations in the *COL1A2* GENE associate with low bone mineral density and fractures in Iceland: *COL1A2* GENE MUTATIONS AND LOW BMD. J. Bone Miner. Res. 31, 173–179. https://doi.org/10.1002/jbmr.2604.

Styrkarsdottir, U., Thorleifsson, G., Gudjonsson, S.A., Sigurdsson, A., Center, J.R., Lee, S.H., Nguyen, T.V., Kwok, T.C.Y., Lee, J.S.W., Ho, S.C., Woo, J., Leung, P.-C., Kim, B.-J., Rafnar, T., Kiemeney, L.A., Ingvarsson, T., Koh, J.-M., Tang, N.L.S., Eisman, J.A., Christiansen, C., Sigurdsson, G., Thorsteinsdottir, U., Stefansson, K., 2016b. Sequence variants in the PTCH1 gene associate with spine bone mineral density and osteoporotic fractures. Nat. Commun. 7, 10129. https://doi.org/10.1038/ncomms10129.

Varilo, T., Peltonen, L., 2004. Isolates and their potential use in complex gene mapping efforts. Curr. Opin. Genet. Dev. 14, 316–323. https://doi.org/10.1016/j.gde.2004.04.008.

Xue, Y., Mezzavilla, M., Haber, M., McCarthy, S., Chen, Y., Narasimhan, V., Gilly, A., Ayub, Q., Colonna, V., Southam, L., Finan, C., Massaia, A., Chheda, H., Palta, P., Ritchie, G., Asimit, J., Dedoussis, G., Gasparini, P., Palotie, A., Ripatti, S., Soranzo, N., Toniolo, D., Wilson, J.F., Durbin, R., Tyler-Smith, C., Zeggini, E., 2017. Enrichment of low-frequency functional variants revealed by whole-genome sequencing of multiple isolated European populations. Nat. Commun. 8, 15927. https://doi.org/10.1038/ncomms15927.

Yang, T.-L., Guo, Y., Li, J., Zhang, L., Shen, H., Li, S.M., Li, S.K., Tian, Q., Liu, Y.-J., Papasian, C.J., Deng, H.-W., 2013. Gene-gene interaction between RBMS3 and ZNF516 influences bone mineral density. J. Bone Miner. Res. 28, 828–837. https://doi.org/10.1002/jbmr.1788.

Zupin, L., Robino, A., Navarra, C., Pirastu, N., Di Lenarda, R., Gasparini, P., Crovella, S., Bevilacqua, L., 2017a. *LTF* and *DEFB1* polymorphisms are associated with susceptibility toward chronic periodontitis development. Oral Dis. 23, 1001–1008. https://doi.org/10.1111/odi.12689.

Zupin, L., Navarra, C.O., Robino, A., Bevilacqua, L., Di Lenarda, R., Gasparini, P., Crovella, S., 2017b. NLRC5 polymorphism is associated with susceptibility to chronic periodontitis. Immunobiology 222, 704–708. https://doi.org/10.1016/j.imbio.2017.01.001.