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Original Article

Dentistry Section

Periodontal Initial Radiological Findings of Genetically Predisposed Finnish Adolescents

ANNA MARIA HEIKKINEN[1], ELMIRA PAKBAZNEJAD ESMAEILI[2], LEENA KOVANEN[3], HELLEVI RUOKONEN[4], KAISA KETTUNEN[5], JARI HAUKKA[6], TAINA TERVAHARTIALA[7], TIMO SORSA[8]

ABSTRACT

Introduction: Periodontitis is a multifactorial infectious disease of the supporting tissues of teeth in which bacterial, genetic and lifestyle factors such as smoking have an important role.

Aim: The aim was to examine if Bleeding On Probing (BOP ≥ 20%) and ≥ 4 mm deep pockets correlated with any suspicion of initial radiological findings of periodontitis and bone loss. We also investigated whether any pro-inflammatory-related candidate Single Nucleotide Polymorphisms (SNPs) were associated with any suspicion of radiological findings.

Materials and Methods: Altogether 47 generally healthy adolescent patients of one birth cohort had given their approval for their saliva samples to be used for DNA analysis. One participant was excluded after discrepant gender check. An oral radiologist analysed right and left bitewing radiographs of 47 patients. Clinical parameters such as BOP ≥ 20%, ≥ 4 mm pockets, Visible Plaque Index of all teeth (VPI%), as well as smoking habits were recorded. DNA was extracted and 71 SNPs from candidate genes for initial periodontitis were genotyped. The association between ≥ 4 mm pockets and BOP ≥ 20% with radiological findings and selected SNPs was modelled using logistic regression.

Results: Variants in Toll-Like Receptors 4 (TLR4) gene (rs498670) (OR=5.8, {CI95% 1.6–20.7}, p=0.02, FDR q-value=0.13) and TNFSF11 gene (rs2277438, OR=0.3 {CI95% 0.1–0.9}, p=0.002, FDR q-value=0.56) were associated with any suspicious radiological findings; however the significance vanished after False Discovery Rate analysis (FDR). The association between BOP ≥ 20% and any radiographic signs of periodontitis was found to be statistically significant, OR=1.6, CI 95% 1.0-2.4, p=0.04.

Conclusion: Only TLR4 (rs498670) and TNFSF11 (rs2277438) genes were found to have a positive correlation with radiological findings suggestive of initial periodontitis after adjustment for smoking and visible plaque.

Keywords: Genes, Periodontitis, X-rays

INTRODUCTION

Periodontitis is a multifactorial infectious disease of the supporting tissues of teeth in which bacteria, genetics and lifestyle factors such as smoking have an important role. It is thought to be a polygenic and inflammatory disease [1]. Periodontitis is initiated in a partly genetically determined host immune response triggered by pathogenic bacteria [2]. The host immune system plays an important role in the pathogenesis through an array of Pattern Recognition Receptors (PRRs) including CD14, TLRs and mannose-binding lectin [3,4]. It has previously been shown that a promoter variant of the CD14 gene leads to enhanced transcriptional activity and higher serum levels of CD14 [4]. TLR4 can specifically recognize Gram-negative bacteria and two variants, namely TLR4 +896A/G (rs4986790, also known as Asp299Gly) and1196C/T (rs4986791, also known as Thr339Ile), are associated with susceptibility to bacterial infections [3]. TLR SNPs can regulate progression of periodontitis [4]. The Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) is induced by Tumour Necrosis Factor Ligand Superfamily member 11 (TNFSF11), which in humans is encoded by the TNFSF11 gene and has been linked to periodontitis [5].

Periodontitis is more common among adults, the prevalence being about 60% in Finland [6], but the disease has also been reported to some extent in children and adolescents [7]. There are different forms of periodontitis in adolescents, some of them being rapidly destructive. Assessment of periodontal disease is primarily based on clinical examination, and radiographic examination is supplementary when justified clinically [8]. Intraoral radiographs such as bitewings and periapicals provide the best indication of the level of periodontal bony support and periodontitis-related changes [9,10]. Radiographs, however, may sometimes reveal periodontitis-related features and signs that are difficult to detect and confirm by clinical examination only [8]. Based on the radiographic evaluation of posterior bitewing radiographs, the prevalence of alveolar bone loss has been reported to be as high as 17.9% among 4–11-year-olds, 7% among 15–17-year-old and 10.4% among 15-year-old [11,12].

This study is a part of a larger project and a part has been published previously [13-17]. Against this background, our aim was to determine whether BOP ≥ 20% and Deep Periodontal Pockets (PPD) in adolescents correlated with any suspicion of radiological findings or bone loss. We also investigated whether any pro-inflammatory, initial periodontitis- and caries-related candidate SNPs were associated with a suspicion of radiological findings suggestive of initial periodontitis.

MATERIALS AND METHODS

Study population

This cross-sectional study was carried out at the Kotka Health Centre in Eastern Finland in 2014–2015. This study was approved by the Ethical Committee of the Helsinki and Uusimaa Hospital District (Dnro 260/13/03/00/13) and was conducted according to the principles of the Declaration of Helsinki [18]. The participants gave their written informed consent. Altogether, 47 generally healthy participants of one birth cohort gave approval for saliva collection and DNA analysis. One participant was excluded after discrepant gender check.

The collection of all other clinical parameters such as BOP ≥ 20% [13-17], PPD; PPD1 (one or more ≥ 4 mm pocket), PPD2 (two or more ≥ 4 mm pockets), PPD3 (three or more ≥ 4 mm pockets), VPI% of all teeth [19], as well as smoking (no, yes) and gender, has been described earlier in our studies [15-17].

Radiological examinations

An oral radiologist analysed right and left bitewing radiographs of 47 patients and evaluated the condition of alveolar bone in the interproximal area of the premolars and molars. Special attention was given to the following conditions as described by Whaites E and Drage N (2013) [10]: the smoothness, continuousness, and cortication of the margin of the crestal bone, the sharpness of the angle between the crestal margin and cortical bone of the socket (lamina dura), the width of the periodontal ligament space, and a distance between crestal margin and cemento-enamel junction of more than 2 mm [20]. Perio 2 was set as: irregularity of margin of the crestal bone, irregularity of angle between crestal margin and lamina dura, widening of the periodontal ligament space, and horizontal Bone Loss (BL); distance between alveolar crest and cemento-enamel junction more than 2 mm [19] based on the criteria for periodontitis defined by Whaites E and Drage N 2013 [10].

Selection of genetic variants for genotyping

The process for selecting SNPs from pro-inflammatory- and initial periodontitis-related candidate genes, DNA extraction, genotyping, genotyping by Agilent Mass Array, and the genotyping quality control procedure has been described previously [16,17]. Genotyping success rates for the samples ranged from 93.5% to 100% / SNP. One subject was excluded because of a discrepancy in gender check results.

STATISTICAL ANALYSIS

The association between BOP ≥ 20% and radiological findings in bitewing X-rays (Perio 2 and BL) was modelled using logistic regression analysis [21]. The association between SNPs and radiological findings was modelled assuming an additive effect of SNPs with R package "SNP association" [16]. An unadjusted model and VPI-adjusted and smoking-adjusted models were calculated. The p-values were corrected for multiple testing using FDR correction [22,23]. False Discovery Rate (FDR) q-values less than 0.05 were considered significant.

	N*	%					
Female	17	37					
Male	29	63					
Smoking regularly	5	10.9					
Perio 2 positive†	33	71.7					
BL positive‡	17	37					
BOP > 20%§	6	13					
PPD1	28	60.9					
PPD2	21	45.7					
PPD3	16	34.8					
	Min.	1st Qu.	Median	Mean	SD	3rd Qu.	Max.
VPI	0	6.33	10.7	17.6	18.08	21.28	72.1
Age	15	15	15	15.78	0.92	17	17
BOP	0.6	4.2	8.8	12.17	13.15	14.08	68.5
PPD	0	0	1	4.24	6.92	4.75	32

[Table/Fig-1]: General characteristics, total N=46*.
*one participant was excluded after discrepant gender check
†)Perio 2 based on definitions given by Whaites E and Drage N 2013 [10]: irregularity of margin of the crestal bone, irregularity of angle between crestal margin and lamina dura, widening of the periodontal ligament space, and horizontal bone loss (BL); distance between alveolar crest and cemento-enamel junction more than 2 mm [19].
‡) BL = distance between alveolar crest and cemento-enamel junction more than 2 mm
§) [15-17].

Gene	Marker	Allele	OR	lower	upper	p-value	FDR q-value
TLR4	rs498670	A	5.78	1.62	20.69	0.002	0.13
TNFSF11	rs2277438	G	0.28	0.09	0.87	0.02	0.56
IL1RN	rs3087266	T	3.19	0.82	12.4	0.06	0.8
SYN1	rs6520279	C	2.3	0.9	5.86	0.06	0.8
TNFSF11	rs9533156	T	0.43	0.16	1.16	0.08	0.81

[Table/Fig-2]: Associations between Perio 2†) and SNPs, results adjusted for smoking and VPI.
†)Perio 2 based on definitions given by Whaites E and Drage N 2013 [10]: irregularity of margin of the crestal bone, irregularity of angle between crestal margin and lamina dura, widening of the periodontal ligament space, and horizontal BL; distance between alveolar crest and cemento-enamel junction more than 2 mm [19].

Gene	Marker	Allele	OR	lower	upper	p-value	FDR q-value
IL1B	rs1143633	A	0.3	0.08	1.1	0.06	0.9
TLR4	rs498670	A	2.4	0.85	6.79	0.09	0.9
IL1RN	rs4251985	T	2.55	0.83	7.9	0.09	0.9
VDR	rs1544410	A	0.41	0.14	1.25	0.1	0.9
MPO	rs11575868	A	0.21	0.02	1.92	0.11	0.9

[Table/Fig-3]: Associations between bone loss (BL)‡) and SNPs, results adjusted for smoking and VPI.
‡)BL = distance between alveolar crest and cemento-enamel junction more than 2 mm

Gene	Marker	Allele	OR	lower	upper	p-value	FDR q-value
TLR4	rs498670	A	5.02	1.47	17.19	0.004	0.22
TNFSF11	rs2277438	G	0.3	0.1	0.9	0.03	0.67
VDR	rs7975232	C	0.41	0.14	1.2	0.09	0.67
IL10	rs1800871	T	0.66	0.24	1.83	0.09	0.67
IL10	rs1800872	A	0.66	0.24	1.83	0.09	0.67

[Table/Fig-4]: Associations between Perio 2†) and SNPs, results unadjusted.
†)Perio 2 based on definitions given by Whites E and Drage N 2013 [10]: irregularity of margin of the crestal bone, irregularity of angle between crestal margin and lamina dura, widening of the periodontal ligament space, and horizontal bone loss (BL); distance between alveolar crest and cemento-enamel junction more than 2 mm [19].

Gene	Marker	Allele	OR	lower	upper	p-value	FDR q-value
MPO	rs11575868	A	0.16	0.02	1.45	0.05	0.98
IL1B	rs1143633	A	0.36	0.11	1.13	0.07	0.98
TLR4	rs498670	A	2.33	0.88	6.15	0.07	0.98
IL1RN	rs4251985	T	2.43	0.87	6.77	0.08	0.98
VDR	rs1544410	A	0.47	0.17	1.34	0.14	0.98

[Table/Fig-5]: Associations between bone loss (BL)‡) and SNPs, results unadjusted.
‡)BL = distance between alveolar crest and cemento-enamel junction more than 2 mm

RESULTS

Of all participants, 72% (n=33) had a suspicion of radiographic signs of periodontitis (Perio 2) and 37% (n=17) had BL (BL= distance between alveolar crest and cemento-enamel junction of more than 2 mm), see [Table/Fig-1].

Initially TLR4 gene (rs498670) (OR=5.8, {CI95% 1.6–20.7}, p=0.02, FDR q-value=0.13) and TNFSF11 gene (rs2277438, OR=0.3 {CI95% 0.1–0.9}, p=0.002, FDR q-value=0.56) adjusted for VPI and smoking, showed an association with any suspicious radiological findings (Perio 2) suggestive of initial periodontitis in adolescents; however, this significance disappeared after false discovery rate analysis both for adjusted and unadjusted values, see [Table/Fig-

2,3]. No associations between BL and SNPs were found both for adjusted and unadjusted values [Table/Fig-4,5].

DISCUSSION

We found SNP rs498670 in the TLR4 gene to be associated with radiological findings suggestive of initial periodontitis; however, after FDR analysis the significance disappeared. In their meta-analysis, Jin SH et al., (2016) reported an association between TLR4C>G (rs7873784) SNP and chronic periodontitis in Asians [24]. TLR4 has been shown to play an important part in innate immunity by identifying lipid-based structures of bacteria, and thus being involved in intracellular signalling [2]. Genes related to host immunity and inflammatory response including cytokines, enzymes and cell surface receptors have been investigated, although often with conflicting results, possibly due to population-specific differences in allele frequencies [25].

Chronic periodontitis is common and the main cause of tooth loss in adults, while aggressive periodontitis has its onset primarily during early adolescence, and is rare in Finland [26]. In a Finnish birth cohort study, we previously reported that 10% of 15- to 16-year-old adolescents suffer from initial periodontitis [13,14] when at least 4 mm deep pockets and ≥ 2 mm bone loss are taken into account. Heikkinen AM et al., (2016a, 2016b, 2016c) have reported that 15- to 17-year-old Finnish adolescents had initial periodontitis with three or more pockets at least 4 mm deep, and that these genetically predisposed cases of initial periodontitis could conveniently be identified with aMMP-8 chair-side test (PerioSafe®) [15-17].

Early diagnosis of initial periodontitis is important for targeted prevention and improves prognosis of the disease in the long run. For this reason, systematic examination of periodontal tissues during routine dental examination of children and adolescents has been strongly recommended by the American Academy of Periodontology's Research, Science and Therapy Committee [7] as well as bitewing X-rays if necessary to confirm the diagnosis of initial periodontitis. It is important to detect susceptible patients as early as possible [15-17].

Perschbacher's statement in White SC and Pharoah MJ 2014 should be borne is mind: "Features that are not well delineated by diagnostic images are most apparent clinically, and features that the radiograph best demonstrates are difficult to identify and evaluate clinically" [8]. An incompatibility between clinical findings and radiological findings with respect to initial signs of periodontitis has also been reported [27]. An overview of the radiographic evaluation of early periodontal BL in adolescents has demonstrated the impact of subjective criteria on assessment leading to different interpretations of periodontal condition [28]. It should be borne in mind that in the present study, horizontal BL was recorded when the distance between alveolar crest and CEJ was 2 mm or more. There is some controversy in the literature, as some investigators permit a distance of 1.5 mm between CEJ and alveolar crest [8] and others a distance of 2 mm–3 mm in the absence of BL [10].

LIMITATION

One limitation of the present study was the relatively small number of adolescents due to the unwillingness of adolescent patients and their parents to give permission for genetic study.

CONCLUSION

As a conclusion, TLR4 gene (rs498670) and TNFSF11 gene (rs2277438) were associated with a suspicion of radiological findings suggestive of initial periodontitis in adolescents; however, this significance disappeared after false discovery rate analysis. In the future, a broader genome-wide SNP analysis is needed in a larger sample of adolescents.

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PARTICULARS OF CONTRIBUTORS:
1. Department of Oral and Maxillofacial Diseases, Head and Neck Center, University of Helsinki, Clinicum, Helsinki, Uusimaa, Finland.
2. Department of Oral and Maxillofacial Diseases, Head and Neck Center, University of Helsinki, Clinicum, Helsinki, Uusimaa, Finland.
3. Department of Oral and Maxillofacial Diseases, Head and Neck Center, University of Helsinki, Clinicum, Helsinki, Uusimaa, Finland.
4. Department of Oral and Maxillofacial Diseases, Head and Neck Center, University of Helsinki, Clinicum, Helsinki, Uusimaa, Finland.
5. FIMM, Institute for Molecular Medicine Finland, Helsinki, Uusimaa, Finland.
6. Docent, Department of Public Health, Clinicum, University of Helsinki, Uusimaa, Finland.
7. Docent, Department of Oral and Maxillofacial Diseases, Head and Neck Center, University of Helsinki, Clinicum, Helsinki, Uusimaa, Finland.
8. Professor, Department of Oral and Maxillofacial Diseases, Head and Neck Center, University of Helsinki, Clinicum, Helsinki, Uusimaa, Finland.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Anna Maria Heikkinen,
Department of Oral and Maxillofacial Diseases, Head and Neck Center,
University of Helsinki and Helsinki, Clinicum, Helsinki, Uusimaa, Finland,
Haartmaninkatu 8, FI-00290 Helsinki, Finland.
E-mail: anna.m.heikkinen@helsinki.fi

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