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ORIGINAL ARTICLE

Polymorphism in epigenetic regulating genes in relation to periodontitis, number of teeth, and levels of high-sensitivity C-reactive protein and glycated hemoglobin: The Tromsø Study 2015-2016

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Abstract

Background: The aim of this study was to investigate the association between periodontitis and four single nucleotide polymorphisms (SNPs) in genes involved in epigenetic regulation of DNA, and between these same SNPs and tooth loss, high-sensitivity C-reactive protein (hs-CRP), and glycated hemoglobin (HbA1c) levels.

Methods: We included participants with periodontal examination (n = 3633, aged: 40–93 years) from the Tromsø Study seventh survey (2015–2016), Norway. Periodontitis was defined according to the 2017 AAP/EFP classification system as no periodontitis, grades A, B, or C. Salivary DNA was extracted and genotyping was performed to investigate four SNPs (*rs2288349*, *rs35474715*, *rs34023346*, and *rs10010325*) in the sequence of the genes *DNMT1*, *IDH2*, *TET1*, and *TET2*. Association between SNPs and periodontitis was analyzed by logistic regression adjusted for age, sex, and smoking. Subgroup analyses on participants aged 40–49 years were performed.

Results: In participants aged 40-49 years, homozygous carriage of minor A-allele of *rs2288349* (*DNMT1*) was associated with decreased susceptibility to periodon-

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titis (grade A: odds ratio [OR] 0.55; $p = 0.014$: grade B/C OR 0.48; $p = 0.004$). The minor A-allele of *rs10010325* (*TET2*) was associated with increased susceptibility to periodontitis (grade A OR 1.69; $p = 0.035$: grade B/C OR 1.90; $p = 0.014$). In the entire sample, homozygous carriage of the G-allele of *rs35474715* (*IDH2*) was associated with having ≤24 teeth (OR 1.31; $p = 0.018$). Homozygous carriage of the A-allele of *TET2* was associated with hs-CRP≥3 mg/L (OR 1.37; $p = 0.025$) and HbA1c≥6.5% (OR 1.62; $p = 0.028$).

Conclusions: In this Norwegian population, there were associations between polymorphism in genes related to DNA methylation and periodontitis, tooth loss, low-grade inflammation, and hyperglycemia.

KEYWORDS
DNA methylation, epigenetics, periodontitis, single nucleotide polymorphism, SNPs

1 | INTRODUCTION

Periodontitis is characterized by irreversible destruction of the tooth-supporting tissues resulting in progressive attachment loss that can lead to tooth loss.[1] In Norway, 50%–72% of the population have periodontitis, of which 9%–20% have severe periodontitis.[2–4] Although a dysbiotic bacterial biofilm is the primary etiologic factor of periodontitis, its pathogenesis is multifactorial where genetic and epigenetic factors, diet, and smoking can also affect disease progression and prognosis.[5] Epigenetics refers to changes in the gene expression without altering the DNA sequence.[6] One of the major epigenetic mechanisms is DNA methylation, which involves the addition of methyl groups to the 5′ position at the base cytosine (5mC), which mainly occurs at CpG sites of the gene promoter. DNA methylation causes gene silencing, whereas DNA demethylation allows gene expression. DNA methyltransferases (DNMTs) are enzymes that catalyze methylation of DNA.[6] Interestingly, research has shown that 5mC can be further converted into 5-hydroxymethylcytosine (5hmC) through a reaction regulated by ten-eleven translocation (TET) proteins.[7] Although the conversion to 5hmC is catalyzed by TET enzymes, the reaction requires the co-factor alpha-ketoglutarate (α-KG), which is produced by isocitrate dehydrogenases (IDHs).[8] The generated 5hmC results in DNA demethylation.

Studies in the field of periodontology have found altered DNA methylation levels of inflammation-related genes,[9–11] while no differences in the gene expression level of DNMTs.[12,13] In contrast, a significantly larger proportion of TET2-positive cells was reported in individuals with periodontitis compared to individuals with gingivitis, but without corresponding differences at the transcriptional level.[13]

Recent research has shown a cross-talk between epigenetic and genetic variations through single nucleotide polymorphisms (SNPs).[14] SNPs are alterations in a single allele of a gene,[15] which can change the gene expression.[14,16,17] Tak & Farnham[16] found that SNPs in non-coding regions could disrupt CpG sites, and thereby alter the DNA methylation pattern of a larger gene area. In fact, SNPs that disrupt a CpG site could reduce the DNA methylation level as far as 10 kb.[18] In periodontitis, it has been suggested that SNPs in about 65 genes are associated with the disease[5]; however, results differ between populations.[17] Although SNPs in DNA methylation-related genes might be associated with periodontitis, this association has so far, to our knowledge, not been explored in population-based studies. Thus, exploring the role of epigenetics in the multifactorial pathogenesis of periodontitis may contribute to our understand of the disease.

Several studies have demonstrated that periodontitis is associated with elevated high-sensitivity C-reactive protein (hs-CRP)[19] and glycated hemoglobin (HbA1c) levels,[20,21] and with chronic systemic diseases. These associations may be partially explained by shared genetic risk factors; however, knowledge is still limited. Furthermore, little is known about the impact of genetic polymorphism on tooth loss; although, genetic risk seems to contribute to increased odds of losing more teeth according to previous studies.[22,23] Thus, the aims of the present study were to investigate the association between periodontitis and SNPs in the sequence of four genes involved in DNA methylation (*rs2288349* in *DNMT1*, *rs35474715* in *IDH2*, *rs34023346* in *TET1*, and *rs10010325* in *TET2*), as well as

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between the same SNPs and tooth loss, hs-CRP, and HbA1c levels.

2 | MATERIALS AND METHODS

2.1 | Study population

This cross-sectional study was based on data of 3633 Norwegian adults (40-93 years old) from the seventh survey of the Tromsø Study (Tromsø7), conducted in 2015-2016.[24] Further description of the Tromsø Study and Tromsø7 is found in supplementary file 1. In addition to answering extensive questionnaires, the participants underwent a dental examination with pocket depth probing and a panoramic radiograph. The Committee for Medical and Health Research Ethics North, Norway, approved the study (ref.2014/940 and ref.406077 REC North). All participants provided written informed consent.

2.2 | Dental examination and case definition of periodontitis (phenotype)

Periodontal pocket depth (PD) was measured to the closest millimeter. Radiographic marginal bone level was measured on panoramic radiographs as described by Holde et al.[2] Further description of the periodontal examination and bone level measurement is provided in supplementary file 1.

Periodontitis was diagnosed primarily from bone loss according to the new AAP/EFP classification system of periodontal disease.[25,26] Periodontitis was graded based on an indirect estimation using bone loss as a function of age, that is, percentage of radiographic bone loss divided by the age of the participant (% bone loss/age). The most severe bone loss at any interproximal site in the mouth of participants who had interdental bone loss at ≥2 non-adjacent teeth was used for grading. It has been argued that the thresholds for defining different disease grades proposed by Tonetti et al. in 2018[26] (grade A: < 0.25, grade B: 0.25-1.0, grade C: > 1.0) are arbitrary.[27] When applying the definition of % bone loss/age in this study sample, very few periodontitis cases (2%) were classified as grade C, and the majority (65%) were classified as grade B. Therefore, we found it reasonable to use a modified threshold of 0.75 to define grades B and C (grade A: < 0.25, grade B: 0.25-0.75, grade C: > 0.75).

Four phenotype groups were created based on grading of periodontitis:

1) No bone loss (healthy, gingivitis)
2) Periodontitis grade A (representing a slow rate of bone loss per age)
3) Periodontitis grade B (representing a moderate rate of bone loss per age)
4) Periodontitis grade C (representing a rapid rate of bone loss per age)

2.3 | DNA extraction and genotyping

Saliva samples for genetic analysis were collected during the dental examination. Two ml of saliva was stored in Oragene DNA Self-Collection kits (DNA Gentotek Inc. Murrieta, CA, USA) until DNA extraction. The samples were diluted to 2 ng DNA/μL using TE buffer (10 mM Tris- HCl, 1 mM EDTA, pH 8.0). Genotyping was performed using TaqMan assays and polymerase chain reaction (PCR). Four gene polymorphisms involved in the epigenetic regulation were genotyped: *rs2288349*, *rs35474715*, *rs34023346*, and *rs10010325* (Table 1). These markers were selected based on the frequency of the less common allele predicted to be higher than 40%. Primers, probes, and universal master mix were provided by the Applied Biosystems (Applied Biosystems, Foster City, CA, USA). For the allelic discrimination assay, two TaqMan probes were used. Total quantitative polymerase chain reaction volume was 3 μL, containing 1.2 ng DNA/reaction, TaqMan PCR master mix + SNP assay. The thermal cycle was carried out by starting with a hold cycle at 95°C for 10 minutes, followed by 40 amplification cycles at 92°C for 15 seconds and at 60°C for 1 minute.

2.4 | Behavioral-, medical- and dental-related parameters

Participants who did not define their ethnicity as Norwegian, Sami, or of Finnish descent (Kven) and who had lived the greater part of their childhood outside of Norway were categorized as having foreign background. Smoking status was assessed by the question “Do you/did you smoke daily?” and categorized as never, former, and current smoker. Body mass index (BMI) was calculated based on weight and height measured at the clinical examination (weight in kilograms divided by the square of height in meters, kg/m^2) and categorized as: underweight/normal weight (<25.0 kg/m^2), overweight (25.0-29.9 kg/m^2), and obese (≥30.0 kg/m^2). Diabetes was defined by self-reported current diabetes and/or current use of diabetes medication and/or HbA1c ≥6.5%. HbA1c was analyzed by high-performance liquid chromatography (Tosoh G8:Tosoh Bioscience, San Francisco, USA). HbA1c≥6.5% is a risk factor for periodontitis progression[25,28] and is recommended as the cutoff for diagnosing diabetes and is also an established treatment metric for patients with diabetes.[29,30]

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TABLE 1 Characteristics of the SNPs.

Rs ID	Position, alleles	Nearest gene (SNP position)	Variation type	Function
rs2288349	chr19:10146569 (GRCh38.p13) G > A	*DNMT1* (intronic)	SNV (Single Nucleotide Variation)	An enzyme that regulates the DNA methylation process, which results in gene silencing.
rs35474715	chr15:90097681-90097682 (GRCh38.p13) delGGinsA/delG	*IDH2* (intronic)	Indel (Insertion and Deletion)	An enzyme that regulates the DNA hydroxymethylation process, which results in gene activation.
rs34023346	chr10:68621797 (GRCh38.p13) G > A/G > T	*TET1* (intronic)	SNV (Single Nucleotide Variation)	An enzyme that regulates the DNA hydroxymethylation process, which results in gene activation.
rs10010325	chr4:105185196 (GRCh38.p13) C > A/C > G/C > T	*TET2* (plus strand) *TET2-AS1* (negative strand) (intronic)	SNV (Single Nucleotide Variation)	An enzyme that regulates the DNA hydroxymethylation process, which results in gene activation.

High-sensitivity C-reactive protein (hs-CRP) was measured in blood samples by a particle-enhanced immunoturbidimetric assay (Cobas 8000, Roche diagnostics, Mannheim, Germany). The hs-CRP groups were categorized as <3 mg/L and ≥3 mg/L. A low-grade inflammation, that is, hs-CRP≥3 mg/L has been reported in individuals with periodontitis.[31] History of cardiovascular diseases (CVD, including myocardial infarction or stroke), cancer, and rheumatoid arthritis was based on questionnaires. General health was categorized as excellent/good, neither good nor bad (moderate), and very bad/bad. Toothbrushing frequency was categorized into twice/day or more, or once/day or less often. The number of teeth was determined based on panoramic radiographs and was categorized into ≥25 teeth and ≤24 teeth because loss of four or more teeth due to periodontitis is one of the complexity factors in the 2017 classification scheme.[26] The number of teeth with PD ≥5 mm and ≥6 mm was dichotomized as 0 or ≥1.

2.5 | Statistical analysis

Characteristics of participants in relation to periodontitis are expressed as numbers and percentages for categorical variables and as means ± standard deviations (SD) for continuous variables. The statistical significance of differences between groups were assessed by analysis of variance (ANOVA) and Pearson's χ^2 test. In the analyses of the whole study population, non-periodontitis and grade A were merged into one group (reference group) because of few participants without periodontitis, and grade B and grade C were analyzed separately and against the reference group. In the subgroup analyses (aged 40-49 years), non-periodontitis was used as reference group and periodontitis grade B and C were combined. The subgroup of 40-49 years old was chosen because we wanted to compare participants with no periodontitis with participants with different grades of periodontitis, and the largest proportion of healthy individuals (61.5%) was found in this age group.

All statistical analyses were performed in STATA software (STATA, StataCorp, College Station, TX, USA, version 16.0). To investigate whether gene polymorphisms were associated with periodontitis, we used the «genass» package for number of statistical tests on genotype data analysis and «genhw» for the Hardy-Weinberg equilibrium (HWE) tests. The *p*-values of allelic, genotypic, and Cochran-Armitage Linear Trend Test are reported. «Genass» statistical tests are further described in supplementary file 1.

In the entire sample, we used logistic regression models adjusted for age, sex, and smoking to explore the associations between periodontitis, number of teeth, hs-CRP, HbA1c, and SNP markers. In the 40-49 years subgroup, the associations between periodontitis and SNP markers were analyzed using logistic regression models adjusted for sex and smoking. The most general codominant model was applied, which compares heterozygous and homozygous for the minor allele genotypes to the homozygous for the most frequent allele. Statistical significance for the multiplicative interaction between SNP markers, sex, and smoking (never-smokers and ever-smokers) for all models were tested using overall Wald test. Results from multiple

binomial logistic regression models are presented as adjusted odds ratios (ORs) and 95% confidence intervals (CIs). Sensitivity analysis, when participants with foreign background were excluded, was conducted for the age group of 40-49 years, as this group included more participants with a foreign background than other age groups. We report uncorrected p-values throughout the manuscript. All tests were two-tailed, and p-values < 0.05 were statistically significant.

3 | RESULTS

3.1 | Characteristics of the study population

Characteristics of the study population are presented in Table 2 and characteristics of the subgroup 40-49 years are presented in (See Supplementary Table 1 in online Journal of Periodontology). The proportion of respondents with no periodontitis was higher in the 40-49-year age-group (61.5%) compared with the older age groups, and smoking was more common among participants with grade B and C periodontitis. In the 40- to 49-year subgroup, smoking was more common among those with periodontitis compared to those with no periodontitis.

3.2 | Hardy-Weinberg equilibrium (HWE)

Results on the HWE test are presented in Table 3 for the whole study cohort. Only one SNP (G > A) *rs2288349* (*DNMT1*) was significantly outside HWE (p = 0.015) in the no periodontitis/grade A group. For other SNPs, no departure from HWE was detected. When we split the no periodontitis/grade A group, the no periodontitis group was not outside HWE (416 (0.56)/324 (0.44), p = 0.517); however, the periodontitis grade A group was outside HWE (1275 (0.58)/911 (0.42), p = 0.001). In subgroup analysis of participants aged 40-49 years, no departure from HWE was detected (Table 4).

3.3 | Interactions between SNPs, sex and smoking

There were no statistically significant interactions between any of the SNPs and sex or smoking, neither in the entire sample nor in the 40- to 49-year subgroup (data not shown) for any tested logistic regression models.

3.4 | Associations between SNPs and periodontitis in the whole study cohort and the 40- to 49-year subgroup

3.4.1 | (G > A) *rs2288349* (*DNMT1*)

In the entire sample, the genotype distributions of the polymorphisms of *rs2288349* (*DNMT1*) in the periodontitis grade B group differed significantly from the no periodontitis/periodontitis grade A (p = 0.014) (Table 3). The heterozygous genotype AG versus GG *rs2288349* (*DNMT1*) was inversely associated with periodontitis grade B (OR 0.79; 95%CI: 0.67-0.93; p = 0.005) when compared with the no periodontitis/grade A group. In the 40-49 years subgroup, homozygous genotype of the minor A-allele AA versus GG of *rs2288349* (*DNMT1*) was associated with decreased susceptibility to periodontitis grade A (OR 0.55; 95%CI: 0.34-0.89; p = 0.014) and grade B/C (OR 0.48; 95%CI: 0.30-0.79; p = 0.004) when compared with the no periodontitis group (Table 4).

3.4.2 | (X > G) *rs35474715* (*IDH2*) and (G > A) *rs34023346* (*TET1*)

No clear statistically significant associations were found between periodontitis and SNP *rs35474715* (*IDH2*) or SNP *rs34023346* (*TET1*) neither for the entire sample nor for the 40-49 years subgroup (Tables 3 and 4).

3.4.3 | (C > A) *rs10010325* (*TET2*)

In the entire sample, there were no statistically significant associations for SNP *rs10010325* (*TET2*) (Table 3). In the 40-49 years subgroup, heterozygous carriage of the minor A-allele of *rs10010325* (*TET2*) was associated with increased susceptibility to periodontitis grade B/C (OR 1.83; 95%CI: 1.22-2.72; p = 0.003) when compared with the no periodontitis group. Homozygous carriage of the minor A-allele of *rs10010325* (*TET2*) was associated with increased susceptibility to periodontitis grade A (OR 1.69; 95%CI: 1.04-2.74; p = 0.035), and grade B/C (OR 1.90; 95%CI: 1.14-3.15; p = 0.014) when compared with the no periodontitis group (Table 4).

3.5 | Association between SNP (X > G) *rs35474715* (*IDH2*) polymorphism and number of teeth in the entire sample

In the entire sample, homozygous carriage of the minor G-allele of *rs35474715* (*IDH2*) was associated with having

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TABLE 2 Characteristics of the study sample (participants aged 40-93 years [$n = 3633$]).

	No periodontitis $n = 390$ (10.7%)	Periodontitis grade A $n = 1123$ (30.9%)	Periodontitis grade B $n = 1892$ (52.1%)	Periodontitis grade C $n = 228$ (6.3%)
Demographic and behavioral characteristics				
Sex, n (%)				
Women	218 (55.9)	618 (55.0)	932 (49.3)	105 (46.1)
Men	172 (44.1)	505 (45.0)	960 (50.7)	123 (53.9)
Age (years) (mean (SD), min-max)	49.9 (9.8), 40-87	55.4 (10.8), 41-93	60.4 (10.7), 40-91	57.6 (8.9), 40-78
Age group (years), n (%)				
40-49	240 (61.5)	396 (35.3)	359 (19.0)	46 (20.2)
50-59	98 (25.1)	374 (33.3)	502 (26.5)	73 (32.0)
60-69	28 (7.2)	239 (21.3)	617 (32.6)	85 (37.3)
70-93	24 (6.2)	114 (10.1)	414 (21.9)	24 (10.5)
No. of participants with foreign background[a], n (%)	21 (5.4)	33 (2.9)	53 (2.8)	9 (4.0)
Smoking status, n (%)				
Never	250 (64.4)	593 (53.8)	648 (35.0)	48 (21.6)
Former	116 (29.9)	407 (36.9)	927 (50.0)	105 (47.3)
Current	22 (5.7)	102 (9.3)	279 (15.0)	69 (31.1)
Medical parameters				
BMI (kg/m^2), (mean [SD])	27.1 (4.5)	27.1 (4.3)	27.4 (4.5)	28.3 (4.8)
BMI groups, n (%)				
≤24.9	131 (33.7)	376 (33.5)	579 (30.7)	65 (28.6)
25.0-29.9	167 (42.9)	484 (43.2)	854 (45.3)	82 (36.1)
≥30	91 (23.4)	261 (23.3)	454 (24.1)	80 (35.2)
hs-CRP≥3 mg/L, n (%)	43 (11.1)	145 (13.0)	289 (15.3)	56 (24.7)
HbA1c≥6.5 %, n (%)	9 (2.3)	52 (4.7)	118 (6.3)	18 (8.1)
Diabetes, n (%)	17 (4.4)	68 (6.1)	148 (7.8)	23 (10.1)
Previous myocardial infarction or stroke, n (%)	12 (3.1)	52 (4.6)	154 (8.1)	17 (7.5)
Previous or current cancer, n (%)	20 (5.2)	78 (7.1)	178 (9.8)	19 (8.6)
Previous or current rheumatoid arthritis, n (%)	12 (3.2)	50 (4.6)	95 (5.3)	14 (6.4)
General health, n (%)				
Excellent/good	282 (72.3)	793 (71.1)	1292 (68.9)	137 (60.4)
Neither good nor bad	91 (23.3)	268 (24.0)	477 (25.4)	75 (33.0)
Bad/very bad	17 (4.4)	54 (4.8)	106 (5.7)	15 (6.6)
Dental related parameters				
No of teeth, (mean [SD])	25.4 (5.6)	25.4 (4.4)	23.3 (5.4)	21.3 (6.4)
≥25 teeth, n (%)	307 (78.7)	867 (77.2)	1020 (53.9)	89 (39.0)
≤24 teeth, n (%)	83 (21.3)	256 (22.8)	872 (46.1)	139 (61.0)
Toothbrushing frequency, n (%)				
1 time/day or less often	72 (18.6)	178 (16.2)	413 (22.3)	53 (24.0)
No. of participants with one or more teeth with PPD ≥ 5 mm, n (%)	56 (14.4)	286 (25.5)	870 (46.0)	185 (81.1)
No. of participants with one or more of teeth with PPD ≥ 6 mm, n (%)	9 (2.3)	51 (4.5)	321 (17.0)	121 (53.1)

Values are numbers (percentages) for categorical variables and mean (SD) for continuous variables.

Note: Periodontitis was diagnosed primarily from RBL according to the new AAP/EFP classification system of periodontal disease (Caton et al 2018; Tonetti et al 2018). The modified threshold of 0.75 RBL/age to define grade B and C (grade A: < 0.25, grade B: 0.25–0.75, grade C: > 0.75) was used.

Abbreviations: BMI, body mass index; HbA1c, glycated hemoglobin A1c AAP/EFP, American Academy of Periodontology/European Federation of Periodontology; hs-CRP, high-sensitivity C-reactive protein; RBL, radiographic bone loss; SD, standard deviation.

[a]Participants who did not define their ethnicity as Norwegian, Sami or of Finnish descent (Kven) and who had lived the greater part of their childhood outside of Norway.

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TABLE 3 Association between four SNP markers and periodontitis in participants aged 40-93 ($n = 3633$).

SNP	No periodontitis/ periodontitis grade A $n = 1513$ (41.7%)	Periodontitis grade B $n = 1892$ (52.1%)	Periodontitis grade C $n = 228$ (6.3%)
(G > A) *rs2288349 (DNMT1)*			
Undetermined, n (%)	50 (3.3)	71 (3.8)	13 (5.7)
Alleles G/A, n (%)	1691 (0.58)/1235 (0.42)	2152 (0.59)/1490 (0.41)	251 (0.58)/179 (0.42)
HWE, *p*-value	0.015	0.168	0.835
Allelic test, *p*-value[a]		0.289	0.820
Genotype, n (%)			
GG	466 (31.8)	650 (35.7)	74 (34.4)
AG	759 (51.9)	852 (46.8)	103 (47.9)
AA	238 (16.3)	319 (17.5)	38 (17.7)
Genotypic test, *p*-value[a]		0.014	0.553
Trend test GG vs. AG vs. AA, *p*-value[a]		0.287	0.815
Codominant model, Adjusted OR (95% CI), *p*-value[a,b]			
AG vs. GG		0.79 (0.67, 0.93), $p = 0.005$	0.87 (0.61, 1.22), $p = 0.408$
AA vs. GG		0.94 (0.76, 1.18), $p = 0.605$	1.04 (0.66, 1.63), $p = 0.871$
(X > G) *rs35474715 (IDH2)*			
Undetermined, n (%)	33 (2.2)	43 (2.3)	5 (2.2)
Alleles X/G, n (%)	1621 (0.55)/1339 (0.45)	2097 (0.57)/1601 (0.43)	219 (0.49)/227 (0.51)
HWE, *p*-value	0.988	0.365	0.636
Allelic test, *p*-value[a]		0.113	0.025
Genotype, n (%)			
XX	444 (30.0)	585 (31.6)	52 (23.3)
GX	733 (49.5)	927 (50.1)	115 (51.6)
GG	303 (20.5)	337 (18.2)	56 (25.1)
Genotypic test, *p*-value[a]		0.231	0.078
Trend test XX vs. GX vs. GG, *p*-value[a]		0.111	0.025
Codominant model, Adjusted OR (95% CI), *p*-value[a,b]			
GX vs. XX		0.90 (0.76, 1.06), $p = 0.213$	1.16 (0.80, 1.68), $p = 0.431$
GG vs. XX		0.83 (0.67, 1.03), $p = 0.093$	1.50 (0.98, 2.32), $p = 0.064$
(G > A) *rs34023346 (TET1)*			
Undetermined, n (%)	74 (4.9)	78 (4.1)	5 (2.2)
Alleles G/A, n (%)	1477 (0.51)/1401 (0.49)	1771 (0.49)/1857 (0.51)	225 (0.50)/221 (0.50)
HWE, *p*-value	0.342	0.622	0.638
Allelic test, *p*-value[a]		0.045	0.732
Genotype			
GG	388 (27.0)	427 (23.5)	55 (24.7)
GA	701 (48.7)	917 (50.6)	115 (51.6)
AA	350 (24.3)	470 (25.9)	53 (23.8)
Genotypic test, *p*-value[a]		0.078	0.695
Trend test GG vs. GA vs. AA, *p*-value[a]		0.045	0.734
Codominant model, Adjusted OR (95% CI), *p*-value[a,b]			
GA vs. GG		1.18 (0.98, 1.42), $p = 0.072$	1.22 (0.85, 1.76), $p = 0.285$
AA vs. GG		1.24 (1.01, 1.53), $p = 0.044$	1.05 (0.68, 1.63), $p = 0.817$

(Continues)

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TABLE 3 (Continued)

SNP	No periodontitis/ periodontitis grade A $n = 1513$ (41.7%)	Periodontitis grade B $n = 1892$ (52.1%)	Periodontitis grade C $n = 228$ (6.3%)
(C > A) *rs10010325 (TET2)*			
Undetermined, n (%)	140 (9.3)	163 (8.6)	8 (3.5)
Alleles C/A, n (%)	1515 (0.55)/1231 (0.45)	1903 (0.55)/1555 (0.45)	240 (0.55)/200 (0.45)
HWE, *p*-value	0.507	0.536	0.138
Allelic test, *p*-value[a]		0.913	0.806
Genotype, n (%)			
CC	424 (30.9)	530 (30.7)	60 (27.3)
AC	667 (48.6)	843 (48.8)	120 (54.5)
AA	282 (20.5)	356 (20.5)	40 (18.2)
Genotypic test, *p*-value[a]		0.991	0.259
Trend test CC vs. AC vs. AA, *p*-value[a]		0.913	0.807
Codominant model, Adjusted OR (95% CI), *p*-value[a,b]			
AC vs. CC		1.02 (0.86, 1.22), $p = 0.792$	1.38 (0.97, 1.98), $p = 0.076$
AA vs. CC		1.03 (0.83, 1.28), $p = 0.764$	1.01 (0.64, 1.60), $p = 0.969$

Note: Trend test is Cochran-Armitage Linear Trend Test (tests for a linear trend in the proportions).
Abbreviations: OR, odds ratio; CI, confidence interval; SNP, single nucleotide polymorphism, MAF, minor allele frequency, HWE, Hardy-Weinberg equilibrium.
[a]In comparison to the group no periodontitis/grade A.
[b]Performed by multiple logistic regression analysis; adjusted for age, sex, and smoking.

≤24 teeth (OR 1.31; 95%CI: 1.05-1.65; $p = 0.018$) (Table 5). The other SNPs were not significantly associated with the number of teeth (data not shown).

3.6 | Associations between SNP (C > A) *rs10010325* (*TET2*) and levels of hs-CRP≥3 mg/L and HbA1c≥6.5% in the entire sample

In the entire sample, homozygous carriage of the minor A-allele of *rs10010325* (*TET2*) was associated with levels of hs-CRP≥3 mg/L (OR 1.37; 95%CI: 1.04-1.80; $p = 0.025$) and HbA1c≥6.5% (OR 1.62; 95%CI: 1.05-2.51; $p = 0.028$) (Table 6). The other SNPs were not associated with hs-CRP and HbA1c levels (data not shown).

4 | DISCUSSION

Few studies have evaluated the expression of markers related to DNA methylation in individuals with periodontitis.[12,13,32] In the present study, we have investigated the presence of SNPs in genes related to the DNA methylation process and their association with periodontitis, tooth loss and biomarkers of low-grade inflammation and hyperglycemia in data from a population-based study.

The main findings of the present study are: (a) the *DNMT1* AA and AG genotypes were associated with lower susceptibility for periodontitis, (b) the *TET2* AA and AC genotypes were associated with higher susceptibility for periodontitis in the age group 40-49 years, (c) the *TET2* AA genotype was associated with elevated levels of hs-CRP and HbA1c, and (d) the *IDH2* GG genotype was associated with reduced number of teeth.

In the present study, we investigated the distribution of genetic variance G > A for the *DNMT1* SNP *rs2288349*, located in an intron on the DNA minus strand. We found that individuals with the *DNMT1* AG and AA genotypes had a significantly lower risk of having periodontitis. This indicates that the A allele is "protective" for periodontitis, although it is not known how the genotype affects the function of DNMT1. DNMT1 has previously been found to be essential in maintenance of hematopoietic stem cells,[33] and could thereby affect the immune response. Previous studies on expression level of DNMT1 in periodontitis are few and contradicting.[12,13,34] However, in vitro and in vivo experimental models have shown that *Porphyromonas gingivalis* influences DNMT1 expression and that DNMT1 may have protective functions against periodontitis.[35,36] Nevertheless, further studies are needed both to clarify the role of DNMT1 in periodontitis, and how it is influenced by the *rs2288349* genotype.

We have previously found a significantly larger proportion of TET2-positive cells in periodontitis than in

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TABLE 4 Association between four SNP markers and periodontitis in participants aged 40-49 ($n = 1041$).

SNP	No periodontitis $n = 240$ (23.1%)	Periodontitis grade A $n = 396$ (38.0%)	Periodontitis grade B/C $n = 405$ (38.9%)
(G > A) *rs2288349 (DNMT1)*			
Undetermined, n (%)	10 (4.2)	10 (2.5)	9 (2.2)
Alleles G/A, n (%)	242 (0.53)/218 (0.47)	455 (0.59)/317 (0.41)	480 (0.61)/312 (0.39)
HWE, *p*-value	0.535	0.136	0.456
Allelic test, *p*-value[a]		0.030	0.006
Genotype			
GG	66 (28.7)	127 (32.9)	149 (37.6)
AG	110 (47.8)	201 (52.1)	182 (46.0)
AA	54 (23.4)	58 (15.0)	65 (16.4)
Genotypic test, *p*-value[a]		0.030	0.026
Trend test GG vs. AG vs. AA, *p*-value[a]		0.028	0.007
Codominant model, adjusted OR (95% CI), *p*-value[a,b]			
AG vs. GG		0.93 (0.64, 1.37), $p = 0.717$	0.70 (0.47, 1.04), $p = 0.078$
AA vs. GG		0.55 (0.34, 0.89), $p = 0.014$	0.48 (0.30, 0.79), $p = 0.004$
Codominant model, adjusted OR (95% CI), *p*-value[a,b,c]			
AG vs. GG		0.97 (0.65, 1.44), $p = 0.871$	0.69 (0.46, 1.04), $p = 0.075$
AA vs. GG		0.56 (0.34, 0.92), $p = 0.022$	0.51 (0.31, 0.84), $p = 0.008$
(X > G) *rs35474715 (IDH2)*			
Undetermined, n (%)	4 (1.7)	8 (2.0)	7 (1.7)
Alleles X/G, n (%)	256 (0.54)/216 (0.46)	410 (0.53)/366 (0.47)	459 (0.58)/337 (0.42)
HWE, *p*-value	0.679	0.889	0.171
Allelic test, *p*-value[a]		0.630	0.234
Genotype, n (%)			
XX	71 (30.0)	109 (28.1)	139 (34.9)
GX	114 (48.3)	192 (49.5)	181 (45.5)
GG	51 (21.6)	87 (22.4)	78 (19.6)
Genotypic test, *p*-value[a]		0.866	0.450
Trend test, XX vs. GX vs. GG, *p*-value[a]		0.633	0.247
Codominant model, Adjusted OR (95% CI), *p*-value[a,b]			
GX vs. XX		1.16 (0.79, 1.72), $p = 0.451$	0.90 (0.61, 1.33), $p = 0.602$
GG vs. XX		1.06 (0.66, 1.71), $p = 0.803$	0.81 (0.50, 1.31), $p = 0.389$
Codominant model, Adjusted OR (95% CI), *p*-value[a,b,c]			
GX vs. XX		1.14 (0.77, 1.69), $p = 0.501$	0.83 (0.56, 1.21), $p = 0.330$
GG vs. XX		1.04 (0.65, 1.66), $p = 0.878$	0.68 (0.42, 1.09), $p = 0.107$
(G > A) *rs34023346 (TET1)*			
Undetermined, n (%)	7 (2.9)	19 (4.8)	7 (1.7)
Alleles G/A, n (%)	255 (0.55)/211 (0.45)	385 (0.51)/369 (0.49)	396 (0.50)/400 (0.50)
HWE, *p*-value	0.263	0.332	0.270
Allelic test, *p*-value[a]	0.214	0.214	0.088
Genotype			
GG	74 (31.8)	103 (27.3)	93 (23.4)
GA	107 (45.9)	179 (47.5)	210 (52.8)
AA	52 (22.3)	95 (25.2)	95 (23.9)

(Continues)

TABLE 4 (Continued)

SNP	No periodontitis $n = 240$ (23.1%)	Periodontitis grade A $n = 396$ (38.0%)	Periodontitis grade B/C $n = 405$ (38.9%)
Genotypic test, *p*-value[a]		0.461	0.066
Trend test, GG vs. GA vs. AA, *p*-value[a]		0.227	0.087
Codominant model, Adjusted OR (95% CI), *p*-value[a,b]			
GA vs. GG		1.25 (0.83, 1.86), $p = 0.283$	1.45 (0.96, 2.17), $p = 0.076$
AA vs. GG		1.23 (0.77, 1.97), $p = 0.395$	1.42 (0.88, 2.30), $p = 0.153$
Codominant model, Adjusted OR (95% CI), *p*-value[a,b,c]			
GA vs. GG		1.34 (0.89, 2.00), $p = 0.157$	1.42 (0.93, 2.15), $p = 0.103$
AA vs. GG		1.29 (0.81, 2.07), $p = 0.287$	1.35 (0.82, 2.20), $p = 0.234$
(C > A) *rs10010325 (TET2)*			
Undetermined, n (%)	23 (9.6)	26 (6.6)	15 (3.7)
Alleles C/A, n (%)	263 (0.61)/171 (0.39)	395 (0.53)/345 (0.47)	409 (0.52)/371 (0.48)
HWE, *p*-value	0.346	0.169	0.206
Allelic test, *p*-value[a]		0.016	0.006
Genotype			
CC	83 (38.2)	112 (30.3)	101 (25.9)
AC	97 (44.7)	171 (46.2)	207 (53.1)
AA	37 (17.1)	87 (23.5)	82 (21.0)
Genotypic test, *p*-value[a]		0.067	0.006
Trend test, CC vs. AC vs. AA, *p*-value[a]		0.020	0.006
Codominant model, Adjusted OR (95% CI), *p*-value[a,b]			
AC vs. CC		1.34 (0.91, 1.97), $p = 0.132$	1.83 (1.22, 2.72), $p = 0.003$
AA vs. CC		1.69 (1.04, 2.74), $p = 0.035$	1.90 (1.14, 3.15), $p = 0.014$
Codominant model, Adjusted OR (95% CI), *p*-value[a,b,c]			
AC vs. CC		1.41 (0.95, 2.09), $p = 0.085$	1.81 (1.20, 2.74), $p = 0.005$
AA vs. CC		1.82 (1.20, 3.00), $p = 0.020$	2.06 (1.22, 3.48), $p = 0.007$

Note: Trend test is Cochran-Armitage Linear Trend Test (tests for a linear trend in the proportions).
Abbreviations: OR, odds ratio; CI, confidence interval; SNP, single nucleotide polymorphism, MAF, minor allele frequency, HWE, Hardy-Weinberg equilibrium.
[a]In comparison to the group no periodontitis.
[b]Performed by multiple logistic regression analysis; adjusted for sex and smoking.
[c]Very few participants had other than Caucasian ethnicity, thus we did not include ethnicity as a variable in the analyses.

TABLE 5 Association between *rs35474715* (*IDH2*) SNP and number of teeth in participants aged 40-93 years ($n = 3633$).

Codominant model (X > G) *rs35474715 (IDH2)*	No. of teeth ≥ 25 vs. no. of teeth ≤ 24	
	OR (95% CI)	*p*-value[a]
GX vs. XX	1.15 (0.96, 1.37)	0.140
GG vs. XX	1.31 (1.05, 1.65)	0.018

Abbreviation: SNP, single nucleotide polymorphism.
[a]Performed by multiple logistic regression analysis; adjusted for age, sex, and smoking.

gingivitis lesions using immunohistochemistry, but without corresponding changes at the gene-expression level.[13] Thus, in the present study, we wanted to investigate the role of *TET2* genetic variants and their potential association with periodontal disease. We found that *TET2* AA and AC genotypes were associated with periodontitis. The AA genotype was also associated with elevated levels of HbA1c and hs-CRP. Of note, a previous study on the Tromsø7 cohort found that periodontitis grade B/C was associated with a higher concentration of CRP.[31] It was previously suggested that TET2 is involved in both

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TABLE 6 Association between *rs10010325* (*TET2*) SNP and levels of hs-CRP ≥3 mg/L and HbA1C ≥ 6.5 % in participants aged 40-93 years (n = 3633).

Codominant model (C > A) *rs10010325 (TET2)*	OR (95% CI)	*p*-value[a]
hs-CRP < 3 mgL vs hs-CRP ≥ 3 mgL		
AC vs. CC	1.10 (0.87, 1.39)	0.404
AA vs. CC	1.37 (1.04, 1.80)	0.025
HbA1c < 6.5% vs. HbA1c ≥ 6.5%		
AC vs. CC	1.17 (0.80, 1.71)	0.416
AA vs. CC	1.62 (1.05, 2.51)	0.028

Abbreviations: CI, confidence interval; HbA1c, glycated hemoglobin A1c; hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio; SNP, single nucleotide polymorphism.

[a]Performed by multiple logistic regression analysis; adjusted for age, sex, and smoking.

initiation and resolution of inflammation.[37] TET2 is a vital regulator of 5mC demethylation, and it can oxidate 5mC in RNA in innate immune cells.[38] In addition, TET2 has been linked to inflammation by other epigenetic mechanisms, such as histone modifications and by influencing inflammasome signaling.[37] In addition to inflammation, mutations in *TET2* were shown to play a role in the development of myeloid cancer and cardiovascular disease.[39] To the best of our knowledge, there are no published studies on the relation between HbA1c and mutations in *TET2*, although there are reports suggesting a connection between HbA1c and TET2 expression.[40,41] In a study on diabetic foot ulcers, an increase in TET2 and α-KG was found in the wound fluid, indicating a local epigenetic change in the wound. Interestingly, α-KG and HbA1c were positively correlated.[40] α -KG has a vital role in the TET2-directed process of converting 5hmC back to cytosine, thereby demethylating the DNA.[42] Diabetics with an HbA1c around 10% had decreased 5hmC compared to healthy controls, which was attributed to destabilized TET2 due to hyperglycemia.[41]

Certain biological mechanisms can be involved in the association between *TET2* and CRP seen in the present study. *TET2* and *DNMT3a* are among the genes commonly mutated in individuals with a state known as clonal hematopoiesis of indeterminate potential (CHIP).[43–45] About 10% of individuals aged 70–80 develop this non-heritable form of mutations in hematopoietic cells.[46] This condition occurs without evidence of hematologic malignancy, dysplasia, or cytopenia, and does not induce any symptoms. However, pro-inflammatory state, elevated hs-CRP-levels, atherosclerosis, and a two-fold increase in cardiovascular disease risk, independent of traditional risk factors, were reported in CHIP carriers.[43] Moreover, it has been shown using in vitro models that *CRP* gene expression is regulated by DNA methylation, that is, TET2 overexpression was found to enhance the expression of the *CRP* gene and promote inflammation, while overexpression of DNMT3a had the opposite effect and reduced the *CRP* gene expression.[47] Thus, further studies on *TET2* gene polymorphisms and their biological functions in relation to shared genetic risk factors between periodontitis and systemic inflammatory diseases would be of interest.

Interestingly, *IDH2* polymorphism was related to reduced number of teeth in the present study. Functional studies are needed to elaborate on the protective role of IDH2 and its role in tooth loss, which according to our results may not be related to periodontitis but could be associated with other causes of tooth loss, such as dental caries.

In the present study, we had separate analysis of the 40-49 years subgroup because it contained the largest proportion of healthy individuals, but still had a substantial proportion of individuals with periodontitis grade B and C. Based on previous research,[5] we hypothesized that those who have developed moderate or severe periodontitis at such an early age may have a particular genetic disposition for the diseases. Moreover, in the 40-49 years subgroup, clinical characteristics were more homogeneous across periodontitis groups, possibly reducing the risk of confounders.

The SNPs investigated in the current study are all within introns. Previously, it was thought that these SNPs are irrelevant to gene expression and/or disease. However, it is now proven that SNPs within introns can influence both functional elements such as intron splice enhancers and silencers, as well as contribute to disease susceptibility.[48] Further studies are needed to analyze if and how the *DNMT1* and *TET2* SNPs investigated in the present study may contribute to periodontitis susceptibility. In-depth understanding of the association between these SNPs and periodontitis might also explain why the DNA methylation levels in patients with periodontitis receiving conventional periodontal therapy are not restored to the DNA methylation levels of healthy individuals, as reported by Asa'ad et al.[49]

There are some limitations of the present study. The data are cross-sectional, meaning that we cannot determine causal relationships. Even if we adjusted for known confounding factors there might be other unknown factors that account for the observed associations. We have also studied only a few SNPs. It is therefore not possible to determine their specific role. Further studies are needed to investigate if these SNPs are at transcription factor binding sites or if they are in a regulatory site or connected to a trans-regulatory element.

The major strength of this study is the large, homogenous, and population-based sample where data were

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collected with trained personnel using standardized protocols and validated methods and instruments. We also used grading to define periodontitis phenotypes because it represents the percentage of bone loss and reflects historical disease experience at a given age and includes risk factors that may have affected bone loss over the individual's life course.[27] Bone loss is also found to be the best predictor of future disease progression in the absence of treatment.[50] Even though periodontal pocket depth is not considered in grading, most participants with grade B and C periodontitis had deep periodontal pockets, indicating that they had severe periodontitis.

5 | CONCLUSIONS

In this Norwegian population, there were associations between polymorphism in certain genes related to DNA methylation and periodontitis, especially in age-group 40-49 years. *DNMT1* was associated with less susceptibility to periodontitis while *TET2* was associated with increased susceptibility, as well as with low-grade inflammation and hyperglycemia. *IDH2* was associated with tooth loss. Since DNMT1 increases DNA methylation, which leads to gene silencing, while TET2 results in demethylation and gene activation, further studies on their encoding genes and their combined influence on periodontitis should be a subject to further studies on the mechanisms behind the pathogenesis/progression of periodontitis.

AUTHOR CONTRIBUTIONS

All authors contributed to conception and design of the study. B.J. and N.O. planned the oral health part in the Tromsø study and were involved in data collection. A.R.V. supervised the DNA extraction and genotyping. N.P. performed the statistical analysis with statistical advice from statistician M.P. B.J., F.A., L.L., and N.P. drafted the manuscript. All authors have been involved in data interpretation and revising it critically and have given final approval of the version to be published.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

This research used data from the seventh survey of the Tromsø Study. The dataset generated and analyzed during the current study is not publicly available. Data are available upon application to the Tromsø Study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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