# Genome-wide Association Study of Periodontal Pocketing in Finnish Adults

**Paula Tegelberg**
University of Oulu
**Jussi Miikkael Leppilahti** ( jussi.leppilahti@oulu.fi )
University of Oulu
**Atte Ylöstalo**
University of Oulu
**Tellervo Tervonen**
University of Oulu
**Johannes Kettunen**
University of Oulu
**Anna Liisa Suominen**
University of Eastern Finland
**Pekka Ylöstalo**
University of Oulu

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# Abstract

**Background:** A genome-wide association study is an analytical approach that investigates whether genetic variants across the whole genome contribute to disease progression. The aim of this study was to investigate genome-wide associations of periodontal condition measured as deepened periodontal pockets (≥ 4 mm) in Finnish adults.

**Methods:** This study was based on the data of the national Health 2000 Survey (BRIF8901) in Finland and the Northern Finland Birth Cohort 1966 Study totalling 3,245 individuals. The genotype data were analyzed using the SNPTEST v.2.4.1. The number of teeth with deepened periodontal pockets (≥ 4 mm deep) was employed as a continuous response variable in additive regression analyses performed separately for the two studies and the results were combined in a meta-analysis applying a fixed effects model.

**Results:** Genome-wide significant associations with the number of teeth with ≥ 4 mm deep pockets were not found at the p-level of $< 5 \times 10^{-8}$, while in total 27 loci reached the p-level of $5 \times 10^{-6}$. Of the top hits, SNP rs4444613 in chromosome 20 showed the strongest association ($p = 1.35 \times 10^{-7}$).

**Conclusion:** No statistically significant genome-wide associations with deepened periodontal pockets were found in this study.


# Background

According to the current conception, susceptibility to the initiation and progression of periodontitis depends on the strength of the genetically determined immune response against bacteria [1, 2]. Modifying co-factors associated with periodontitis include age, gender, smoking, diabetes, obesity, and socioeconomic factors [3, 4].

Using a classic twin study design, Michalowicz and his group[5, 6] showed that approximately 50% of the variation in clinically determined adult periodontitis was attributed to genetic variance. A later twin study, which used self-reported data on periodontal condition, reported lower heritability estimates for periodontitis; 39% in women and 33% in men [5–7]. Overall, heritability seems to be higher for severe early-onset traits and among younger individuals [2].

Although the twin studies were crucial in determining the overall role of the genetic component behind periodontitis they could not be used for the identification of the number and location of the susceptibility genes. To achieve the latter goal, two widely used approaches have been used; a candidate gene association study and a genome-wide association study (GWAS). In a recent systematic review and meta-analysis, the authors concluded that up to one-third of periodontitis variance in the population was attributable to genetic factors [2]. In general, lower heritability was reported in studies using GWAS design in comparison to twin studies or other family studies [2].

GWAS enables screening of genetic variants across the whole genome linked to a disease under investigation. Unlike the candidate gene studies, in which the selection of target genes is based on their known role in the pathogenesis of the disease, GWAS offers an approach for the determination of novel polymorphisms that is not limited by prior knowledge. A number of studies have investigated genome-wide associations of different periodontitis phenotypes [2, 8]. In terms of the GWA approach in chronic periodontitis, several studies using various case definitions and populations of different ethnic origins have been published [9–21]. Only a few of these studies reported associations on the level of stringent genome-wide significance level ($p$-value $< 5 \times 10^{-8}$) [16, 18, 21, 22].

## Methods

In this study, the aim was to investigate genome-wide associations of the number of teeth with deepened periodontal pockets (PD $\geq$ 4 mm) in 30–65-year old Finnish adults. We used the data of two studies, the national Health 2000 Survey and the Northern Finland Birth Cohort 1966 Study (NFBC1966), collected using the following methods:

# The Health 2000 Survey

In the Health 2000 Survey, which consisted of a study of persons living in mainland Finland in 2000–2001, a clinical oral health examination was carried out on 6,335 individuals aged 30 years or over [23, 24] .

In the clinical oral health examination, dental plaque was measured from three predetermined teeth, on one surface each: from the buccal surface of the most posterior tooth in the upper right quadrant, from the lingual surface of the most posterior tooth in the lower left quadrant, and the labial surface of the lower canine in the lower left quadrant [25]. The presence of plaque was categorized into three categories: no visible plaque (value 1), visible plaque on gingival margins only (value 2), or visible plaque also elsewhere (value 3). The mean of these values was used as an individual plaque score in the statistical analyses.

Probing pocket depths were measured using a ball-pointed WHO periodontal probe with markings at 3.5 and 5.5 mm. Measurements were taken at four sites per tooth (distal angle on the buccal side, midbuccal, midlingual and mesial angle on the lingual side). Only the deepest pocket on each tooth was recorded as follows: periodontal pocket 4–5 mm deep and $\geq$ 6 mm deep. A variable, the number of teeth with $\geq$ 4 mm deep periodontal pockets, was then created for statistical analyses. Third molars and radices were not included in the periodontal examination.

# The NFBC 1966 Study

The NFBC 1966 Study is a life-span cohort study of individuals born in 1966 in the two northernmost provinces in Finland [26]. In 2012–2013, when the subjects were 46 years old, a comprehensive oral health examination was included in the study for the first time. It was carried out on 1,964 persons living in Oulu or within a 100 km radius from Oulu, the largest city in northern Finland.

In the periodontal examination, plaque was registered from the buccal sites of the teeth as follows: no visible plaque (value 0), plaque when lightly touching the tooth surface by the tip of a periodontal probe (LM 8-520B, Lääkintämuovi, Finland) or visible plaque (value 1). For the statistical analyses, the figures were converted into a percentual plaque score, i.e. percentage of sites with value 1, to better correspond to the scale used in the Health 2000 Survey (1–3, continuous variable). Probing pocket depths were measured at four sites per tooth (mesiobuccal, midbuccal, distobuccal and midoral). The probing force was calibrated for every subject using a letter scale (corresponding to 25g). The number of teeth with ≥ 4 mm deep periodontal pockets was calculated for the statistical analyses. Wisdom teeth and radices were excluded from the periodontal examination.

Subjects with diabetes mellitus (T1DM or T2DM) and rheumatoid arthritis were excluded from both surveys based on a health interview (Health 2000) and questionnaire data (NFBC 1966) on general health. In addition, we excluded subjects over the age of 65 from the Health 2000 Survey. In both surveys, data on smoking were collected using data of the health interview and the questionnaire and categorized as non-smoker or smoker (including both former and current smokers).

The basic characteristics of altogether 3,906 participants in the two surveys are presented in Table 1.

# Genotyping

The Health 2000 DNA samples were genotyped using Illumina Humanhap 610k array and the NFBC 1966 using Illumina Humanhap 310k array. The genotypes were called with Illuminus software and imputed to 1000g reference v1 using IMPUTE2 software (http://mathgen.stats.ox.ac.uk/impute/impute_v2.html).

# Statistical analysis

The SNP-TEST v 2.4.1, which is a test for the analysis of SNP association in GWAS (https://mathgen.stats.ox.ac.uk/genetics_software/snptest/snptest_v2.4.1.html), was used. Variants were filtered prior to the meta-analysis by minor allele frequency (MAF; < 0.01) and additive model information measure (< 0.7).

Genome-wide association studies were performed separately for the two studies using additive regression models adjusted for population stratification, age, gender, smoking, and plaque. The number of teeth with deepened periodontal pockets (≥ 4 mm) was used as a continuous outcome variable. The results were combined in a meta-analysis applying a fixed effects model and using GWAMA, a software for performing genome-wide association meta-analysis (https://www.geenivaramu.ee/en/tools/gwama)

(Table 2). Manhattan plots were created using the qqman package for R software (https://cran.r-project.org/web/packages/qqman/index.html) (Fig. 1).

While a total of 3,245 subjects were included in the final age- and gender-adjusted analyses the fully adjusted analyses were based on the data of 2,650 subjects (Table 2). The threshold for statistical significance on the genome-wide level was defined as p-value < $5 \times 10^{-8}$. Another threshold was set at p-level of $5 \times 10^{-6}$ for reporting so called “suggestive” associations (Additional file 1).

# Results

In the national Health 2000 Survey and the NFBC 1966 Study, 51.5% and 46.5% of the participants were males, and 70.4% and 68.9% non-smokers, respectively (Table 1). The mean number of teeth with ≥ 4 mm deep periodontal pockets was 4.1 (median 2.0) in the Health 2000 Survey and 2.1 (median 0.0) in the NFBC 1966 Study.

While no significant genome-wide associations ($p$-value < $5 \times 10^{-8}$) with the number of teeth with ≥ 4 mm deep pockets were found in this study 27 loci, in total, reached the $p$-level of $5 \times 10^{-6}$ in the analyses (Fig. 1, Additional file 1). We used three different sets of co-variates in the analyses (Table 2.) and discovered that the SNP with the lowest p-value, rs4444613 in chromosome 20 ($p = 1.35 \times 10^{-7}$), was found when a full set of co-variates including smoking and plaque was used (Table 2.). We also observed that different top hits emerged when different co-variates were used. From beyond the top hits, only one SNP, rs7452509, emerged twice; in the fully adjusted analysis ($1.22 \times 10^{-6}$) and when adjusting for age, gender, smoking and population stratification ($2.23 \times 10^{-6}$) (Additional file 1).

Table 1
Basic characteristics of the study populations

| | Health 2000 Survey | NFBC 1966 Study |
|---|---|---|
| *N* | 2,068 | 1,838 |
| Age, years | 30–65 | 46 |
| Gender | | |
| Female | 48.5 | 53.5 |
| Male | 51.5 | 46.5 |
| Smoking status | | |
| Non-smoker | 70.4 | 68.9 |
| Current smoker | 29.4 | 21.4 |
| Missing data | 0.0 | 9.7 |
| [1]Plaque | 1.8[1] | 1.5[2] |
| Number of teeth with PD ≥ 4 mm, mean (median) | 4.1 (2.0) | 2.1 (0.0) |
| [1]The mean of maximum plaque values (1, 2 or 3); [2] scale 1–3. | | |

Table 2
Association results of the top SNPs in the meta-analysis

| | *n* | Chromosome | Position | SNP | *p*-value |
|---|---|---|---|---|---|
| (i) | 3245 | 1 | 79069409 | rs187209330 | $1.81 \times 10^{-6}$ |
| (ii) | 2925 | 14 | 91608044 | rs147203970 | $8.06 \times 10^{-7}$ |
| (iii) | 2650 | 20 | 13340138 | rs4444613 | $1.35 \times 10^{-7}$ |
| | | 15 | 76021782 | rs11630851 | $9.39 \times 10^{-7}$ |
| | | 20 | 13341944 | rs6042048 | $9.41 \times 10^{-7}$ |
| Adjusted for:<br>(i) Age, gender, and population stratification.<br>(ii) Age, gender, smoking status, and population stratification.<br>(iii) Age, gender, smoking status, plaque, and population stratification. | | | | | |

# Discussion

In this study, which was the first GWAS on periodontal condition in the Finnish adult population, no statistically significant associations were found. However, in total 27 SNPs showed associations at the p-level < 5 x $10^{-6}$; of these rs4444613 was the stongest one ($p = 1.35 \times 10^{-7}$). In line with a number of earlier studies, which required statistical evidence of association at a *p*-value level of < 5 x $10^{-8}$, we conclude that there were no associated genetic loci with the presence of deepened periodontal pockets in this population. It is likely that a larger sample size would be required to uncover genetic variation underlying the periodontal condition in this population. Our results verify that neither a single nor a few genes exist that lead to the risk of periodontal pocketing; instead, the genetic contribution is polygenic in nature with each locus having a small contribution to the cumulative heritable risk of periodontal pockets.

In terms of earlier GWAS on chronic periodontitis, only a few loci have reached the genome-wide significance. Sanders et al (2017) found an association with TSNAXDISC1 noncoding mRNA (lead signal: rs149133391) in a large population of Hispanics and Latinos and the variant was replicated independently in an African-American but not in a European-American sample. In a genetically isolated population in Italy, four SNPs in the EFCAB4B gene (rs242016, rs242014, rs10491972, and rs242002) were found to be significantly associated with localized periodontitis but the findings have not been replicated so far [18].

Shungin et al (2019) found a single-risk locus of SIGLEC5 (rs12461706, $p = 3.9 \times 10^{-9}$) by combining a questionnaire-based proxy phenotype of “loose teeth” with clinically verified periodontitis data. Different variants of SIGLEC5 were also found as shared risk loci in studies combining AgP and severe CP cases in German, Dutch and Turkish populations [17], and in a meta-analysis of German and Dutch AgP, and European-American and German CP cases [22]. In addition, significant variants for DEF1A3 (rs2978951, rs2738058), MTND1P5 (rs16870060), and LOC107984137 near the SHISA9 gene (rs729876) were found in these studies combining CP and AgP cases [17, 22]

Overall, the evidence for the genetic basis of CP based on the GWAS approach has so far been only modest. Moreover, the studies where SNPs exceeded the threshold of a significant association reported no SNPs in common, so there has been hardly any overlap in the genetic variants among the numerous suggestive associations reported to date [9, 11, 12]. In similar fashion, the 27 suggestive SNPs ($p < 5 \times 10^{-6}$) found in this study were not found in the GWAS catalog (https://www.ebi.ac.uk/gwas/), which lists in total l54 suggestive SNPs associated with periodontitis (published before 10th of March 2021). Moreover, different top SNPs have emerged for different case definitions of periodontitis even in the same population [19]. It has also appeared that the significant gene variants linked with the susceptibility to periodontitis using the candidate gene approach[1] turned out to be non-significant in the GWAS approaches [11, 12, 19].

Although the search for genome-wide significant variants has not produced consistent results, it does not mean that such variants do not exist. In fact, Laine and colleagues (2012) suggested that there might be

at least 10 to 20 modifying genes associated with periodontitis and that these genes may vary in different disease forms and in different ethnic populations. According to a recent estimation, variants in at least 65 genes could be associated with periodontitis [27]. Apart from the variation in the DNA sequence, there may also be changes in gene expression. Growing evidence exists that epigenetic changes, which are not captured by GWASs, play a role in the inflammatory pathways of periodontitis through activation or inactivation of genes [28]. This may, in part, hamper demonstration of the genetic component in periodontitis using the GWAS approach.

The fact that several definitions for periodontitis were used partly explains the heterogeneity between the results of earlier genetic studies. Commonly used measures for periodontal tissue destruction include periodontal attachment level, probing pocket depth, the latter two combined, and radiographic bone loss. We used the number of teeth with periodontal probing depth ≥ 4 mm, a surrogate measure of existing inflammatory periodontal condition. This does not take into account possible gingival retraction, which is common in older individuals, and therefore may underestimate the severity of periodontitis in the population. The new periodontitis classification system, based on stages of clinical attachment loss and treatment complexity, and grades of disease progression rate[29] has not been used so far in GWAS of periodontitis but will eventually help in standardizing the periodontitis phenotype in future studies.

We made some restrictions to the study population. First, we excluded individuals with diabetes mellitus and rheumatoid arthritis due to their links with periodontitis [30, 31]. In addition, to reduce the effect of tooth loss in the older part of the population, the study population was restricted to subjects under 66 years of age in the Health 2000 Survey. In light of earlier studies, which have shown that, compared to older age groups, heritability has a stronger effect on periodontal condition in subjects aged < 65 years[7] or ≤ 60 years[19] the latter restriction is relevant. Finally, the most common risk factors for periodontitis, smoking and dental plaque, were controlled for using them as covariates in the analyses.

To summarize, while the results of the meta-analysis based on the data of two Finnish cohorts showed no significant genome-wide associations with deepened periodontal pockets, 27 SNPs were found that can be tested in future studies using larger sample sizes.

# Abbreviations

Chronic periodontitis (CP), Aggressive periodontitis (AgP), Genome-wide association (GWA), genome-wide association study (GWAS), Northern Finland Birth Cohort 1966 (NFBC 1966), single nucleotide polymorphism (SNP), Translin-associated factor X Disrupted in schizophrenia 1 (TSNAXDISC1), EF-hand calcium binding domain 4B (EFCAB4B), Sialic Acid Binding Ig Like Lectin 5 (SIGLEC5), defensin alpha 1-3 DEF1A3, MT-ND1 pseudogene 5 (MTND1P5), shisa family member 9 (SHISA9), glycosyltransferase 6 domain containing 1 (GLT6D1).

# Declarations

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# Ethics approval and consent to participate

The study was conducted in accordance with the principles of the Helsinki declaration. Participation in the Health 2000 Survey and NFBC 1966 Study was voluntary. The participants provided informed written consent for the studies. The Ethical Committee of Northern Ostrobothnia Hospital District (74/2011) and the Ethical Committee for Research in Epidemiology and Public Health at the Hospital District of Helsinki and Uusimaa (5/2000) approved the studies.

# Consent for publication

Not applicable.

# Availability of data and materials

NFBC 1966 data is available from the University of Oulu, Infrastructure for Population Studies. Permission to use the data can be applied for research purposes via electronic material request portal. In the use of data, we follow the EU general data protection regulation (679/2016) and Finnish Data Protection Act. The use of personal data is based on cohort participant's written informed consent at his/her latest follow-up study, which may cause limitations to its use. Please, contact NFBC project center (NFBCprojectcenter@oulu.fi) and visit the cohort website (www.oulu.fi/nfbc) for more information.

Health 2000 data that support the findings of this study are available from the Finnish Institute for Health and Welfare. However, restrictions apply to the availability of these data, which were used under license for the current study, and are therefore not publicly available. Nevertheless, data are available from the authors upon reasonable request and with permission from the NFBC and the Finnish Institute for Health and Welfare.

# Competing interests

The authors declare that they have no competing interests.

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# Authors' contributions

PY, AY, TT, PT, JK and ALS conceptualised and designed the study. AY, JK, and ALS designed and performed the statistical analysis. PT, JL, PY, ALS and TT reviewed literature, and prepared and edited the manuscript. All the authors have read and approved the final manuscript for publication.

# Corresponding author

Correspondence to Jussi Leppilahti

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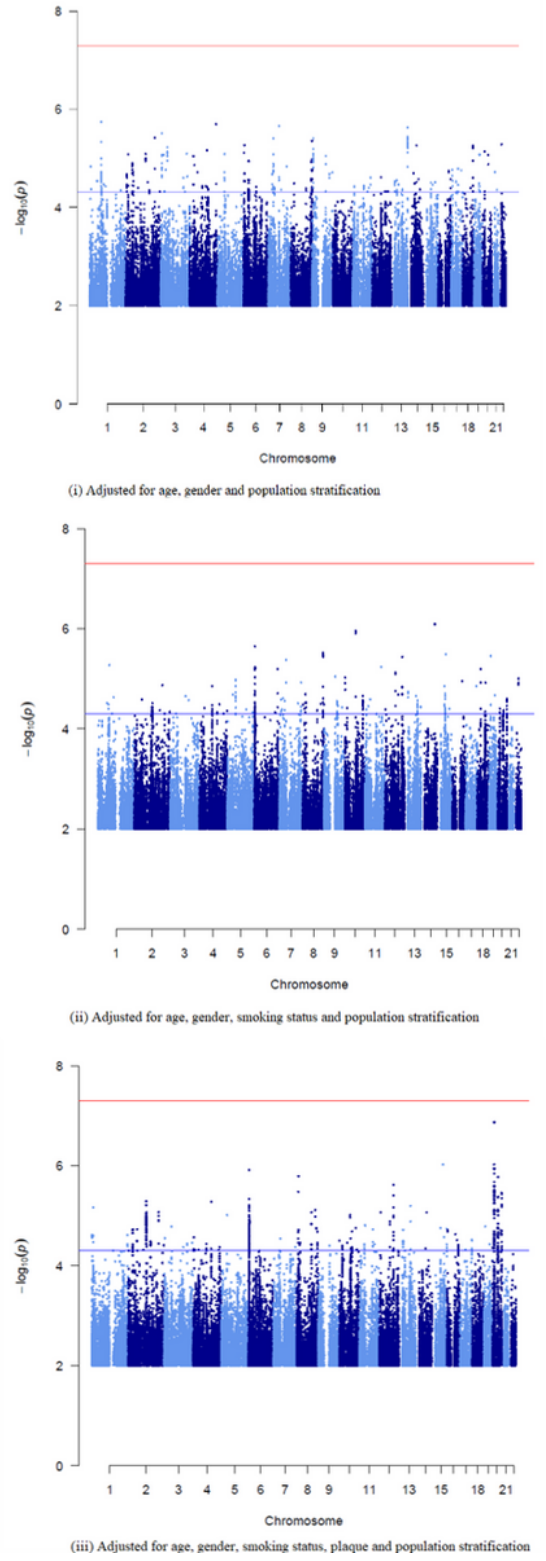
# Figures




## Figure 1

Manhattan plots for genome-wide association study of the number of teeth with ≥ 4 mm deep periodontal pockets based on different adjustments (i–iii); the position of the variants on each chromosome (on the x-axis) in relation to their – log10 (p-values) in the GWAS (on the y-axis). The red line demarks the limit of a statistically significant p-value.

# Supplementary Files

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