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Familial tendency as a determinant of tooth loss during long-term periodontal therapy

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Abstract

Aim: Little evidence exists on how familial tendencies affect the long-term success of periodontal therapy. The aim of this study was to compare outcomes for two generations and their control patients treated in the same private practice.

Materials/methods: Parents and their children were observed for tooth loss between 1986 and 2017. Matching control groups were identified from the same practice, one for the parent and one for the children group. The control patients had *no* close family members with a history of periodontal diseases. Both the generations and control groups completed a similar course of periodontal therapy. The matching strategy aimed at making the groups as similar as possible with respect to well-known risk and prognostic factors. The data were analysed by multiple regression where the outcome was the number of teeth lost due to periodontal disease.

Results: A total of 435 patients were identified (148 parents, 154 children and 133 controls). 72 parents and 61 children (133) had more than 5 years follow-up (average 15.5 and 12.9 years, respectively). Balancing tests showed that the matching was successful. 65% of tooth loss was attributable to close family history. The regression showed that the parent generation lost 1.02 more teeth than the controls, while the children lost 0.61 more teeth.

Conclusion: Having close family members with a history of periodontal diseases is a strong prognostic factor affecting the long-term outcome of periodontal therapy.

KEYWORDS

dental scaling, environmental impacts, family characteristics, genetics, periodontal disease, prognostic factors, tooth loss, treatment outcome

1 | INTRODUCTION

Genetics has been shown to be a risk factor with a variance of up to one-third for developing periodontal diseases in populations (Laine, Crielaard, & Loos, 2012; Nibali, Iorio, Tu, & Vieira, 2017; Nibali et al., 2019). However, little evidence is available to assess the strength of a genetic pre-disposition as a prognostic factor for the long-term outcome of periodontal therapy.

Twin studies are the most powerful methods to assess the genetic aspects of periodontal diseases. Between 38% and 82% of the variance for these populations are due to genetic factors (Michalowicz et al., 1991, 2000; Kurushima, Bowyer, Ide, Hughes, & Steves, 2019).

The most convincing genetic evidence is reported from monozygotic and dizygotic twins suffering from early onset periodontitis (Corey, Nance, Hofstede, & Schenkein, 1993).

Patients with early onset periodontal diseases often carry specific combinations of risk alleles. Patients with later onset periodontal diseases also carry risk alleles; however, environmental factors, lifestyle factors and an ageing immune system play a larger part in these individuals (Schaefer, 2018).

Periodontal diseases elicit polygenic reactions where more than 20 genes modify the diseases (Hart, Marazita, & Wright, 2000). As the genes vary between patients, the dose-dependant response to specific bacteria will also vary resulting in different degrees of susceptibility (Laine et al., 2012). Variations in susceptibilities have also been reported for patients with different racial/ethnic backgrounds (Sanders et al., 2017; Schaefer, 2018).

Aggregations of periodontal diseases have been reported in certain families (Petit, Steenbergen, Timmerman, Graaff, & Velden, 1994; van der Velden et al., 1989). However, family studies are less powerful due to the problem of separating the genetic from the environmental factors. In addition, little is known about any possible parental influences on behaviour and habits such as for example oral hygiene, compliance and smoking. The problem of separating the genetic from the environmental- and lifestyle factors is not unique to periodontal diseases, a number of complex medical conditions such as type 2 diabetes and cardiovascular diseases share the same problem (Murea, Ma, & Freedman, 2012).

It has been shown that initial periodontal therapy followed by a periodontal maintenance programme is effective in saving the teeth for most patients (Hirschfeld & Wasserman, 1978; Fardal, Johannessen, & Linden, 2004). As little as 0.036 teeth are lost per year in patients undergoing long-term periodontal maintenance therapy (Fardal et al., 2004). However, it is not known whether this low tooth loss can also be extended to patients who have close family members with a history of periodontal diseases.

A number of studies from the same periodontal practice setting have reported on the long-term outcomes, costs of periodontal and implant therapy as well as patients' behaviour, habits and inputs (Fardal, Johannessen, & Linden, 2001, 2002, 2003; Fardal et al., 2012; Fardal, Fardal, & Persson, 2013; Fardal, Grytten, Martin, Houlihan, & Heasman, 2016; Fardal & Linden, 2005, 2008, 2010; Fardal, 2006; Fardal & Hansen, 2007; Fardal & McCulloch, 2011; Fardal & Grytten, 2013, 2014; Fardal & Lygre, 2015). This setting, in a small rural community with a stable population from the same ethnic background where the principle investigator is the only specialist within one hour commuting distance, a good knowledge of local family relationships and a long-term follow-up of a sizable number of patients, provides a unique opportunity to study outcome, similarities and differences between parents, their offspring and control patients.

The aims of this study were to identify probands and control groups for parents and offspring being treated for periodontal diseases in the same practice and to compare their long-term outcomes. The hypothesis is that patients with close relative(s) being treated/undergoing maintenance therapy for periodontal diseases have a poorer long-term outcome than patients with no such family connections.

Clinical Relevance

Scientific rational for the study: Studies have shown the importance of genes in the development of periodontal diseases; however, little is known about the effects of a genetic pre-disposition on the outcome of periodontal treatment.

Principle findings: Having close family members being treated for periodontal diseases is an important prognostic outcome factor for the disease.

Practical implications: In the clinical situation, it is important to interview patients about close relatives with periodontal diseases. This, together with other risk and prognostic factors will help to create individual treatment profiles for patients.

2 | MATERIALS AND METHODS

2.1 | Study population—test and control groups

All the patients included in the present study were from a specialist practice in periodontics located in the south-western part of Norway. The specialist practice was established in 1986 and receives referrals from general dental practitioners, community dentists and physicians in Norwegian rural communities with a total population of 25–30,000. The area has approximately 25 dentists split evenly between private practice and the community dental service. The primary investigator is a specialist certified by the Norwegian Board of Health Supervision and is the only periodontal specialist in the area. The nearest specialist is located more than one hour away by car or public transport.

During the period 1986–2017, we identified-124 families (148 parents and 154 children) who had received periodontal therapy in the practice.

Patients who had a minimum of 5 years periodontal treatment including initial therapy and maintenance therapy before the end of the study in 2017 were included in the long-term observations. This reduced the original patient population to 72 parents and 61 children. Following standard conventions in the literature on field experiments, the two generations should be defined as the treatment groups (Gerber & Green, 2012). However, to avoid confusion as the control groups received the same type of treatment, the generation groups were referred to as the test groups. The patients in the test groups had been treated for periodontal disease, and they had close family members with a history a history of periodontal diseases or periodontal therapy.

TABLE 1 Descriptive statistics for the matching variables and the outcome variable

Variables	Parent generation		Children generation	
	Test group—Periodontal disease among close relatives (*n* = 72)	Control group—No periodontal disease among close relatives (*n* = 72)	Test group—Periodontal disease among close relatives (*n* = 61)	Control group—No periodontal disease among close relatives (*n* = 61)
Matching variables				
Initial examination				
Gender (Male = 1)	0.35 [0.23–0.46]	0.31 [0.20–0.41]	0.43 [0.30–0.55]	0.41 [0.28–0.54]
Age	67.9 [66.3–69.5]	66.4 [64.4–68.3]	52.2 [49.8–54.6]	52.3 [50.7–53.9]
Number of teeth present	24.1 [22.8–25.3]	24.2 [23.3–25.1]	27.4 [26.8–28.1]	26.2 [25.5–27.0]
Smoking				
Daily = 1	0.32 [0.21–0.43]	0.24 [0.14–0.34]	0.34 [0.22–0.47]	0.34 [0.22–0.47]
Number of cigarettes per day	5.0 [3.2–6.8]	3.4 [1.9–4.9]	5.7 [3.4–7.9]	5.1 [3.2–7.0]
Periodontal diagnosis at initial therapy				
Stage II = 1	0.07 [0.01–0.13]	0.10 [0.03–0.17]	0.08 [0.01–0.15]	0.15 [0.06–0.17]
Stage III = 1	0.57 [0.45–0.69]	0.68 [0.57–0.79]	0.66 [0.53–0.78]	0.64 [0.52–0.76]
Stage IV = 1	0.36 [0.25–0.47]	0.22 [0.12–0.32]	0.26 [0.15–0.37]	0.21 [0.11–0.32]
Maintenance phase				
Level of hygiene				
Good = 1	0.26 [0.16–0.37]	0.31 [0.20–0.41]	0.34 [0.22–0.47]	0.39 [0.27–0.52]
Moderate = 1	0.71 [0.60–0.81]	0.54 [0.42–0.66]	0.62 [0.50–0.75]	0.52 [0.40–0.65]
Poor = 1	0.03 [−0.01 to 0.07]	0.15 [0.07–0.24]	0.03 [−0.01 to 0.08]	0.08 [0.01–0.15]
Maintenance profile				
Compliant = 1	0.94 [0.89–1.00]	0.97 [0.93–1.00]	0.89 [0.80–0.97]	0.93 [0.87–1.00]
At the end of the study				
Number of observation years	15.5 [14.0–17.1]	14.8 [13.1–16.4]	12.9 [11.2–14.5]	11.6 [10.9–12.3]
Outcome variable				
Tooth loss—number of teeth	1.94 [1.10–2.78]	0.65 [0.39–0.92]	0.70 [0.30–1.11]	0.26 [0.09–0.43]

Note: Mean and proportions. 95% confidence interval in brackets

Matched control groups were identified from the same practice, one for the parent generation and one for the children generation. The control patients had been treated for periodontal disease, but they had *no* close family members (parents, children or siblings) with a history of periodontal diseases or periodontal therapy. This was confirmed by both patients' interviews and a search in the practice database. The matching process was done on a case control basis. Adding the two generations and the two control groups, a total of 435 patients were examined, and 266 patients were followed long-term (≥5 years).

For parents in both the test and control groups, the study ended in 2017. All patients were then alive, and the majority of the patients were in the active compliant maintenance phase (Table 1). Thus, it is not possible that treatment for control parents was carried out much more recently than for test parents.

For both the test and control groups, adopted and stepchildren were excluded as well as patients with a non-North European ethnic background.

The study design is illustrated in Figure 1. Our design allowed us to compare differences in outcome between test and control groups separately for an older (parents) and a younger (children) age group. As age is a prognostic factor for long-term tooth loss, we expected the largest effect on outcome for the parent generation (Fardal et al., 2004). Note that with our study design, it is difficult to study inter-generational transmission of periodontal therapy outcomes. The parent and children generations were not directly comparable due to possible socioeconomic, oral hygiene, dental awareness/motivational, dental supply and dental insurance differences at the equivalent ages. In addition, periodontal therapy has progressed and changed over the lifespan of the generations. However, simple non-parametric intra-familial comparisons were carried out as described in Section 2.5.

2.2 | Matching variables

The aim of our matching strategy was to make the test and control groups as similar as possible with respect to a number of factors including well-known risk and prognostic factors, which influence the outcome of periodontal therapy. Our matching variables were measured at three time points:

At the initial examination/initial therapy: Gender, age, number of teeth present, smoking (daily or not, and the number of cigarettes smoked per day) and periodontal diagnosis (stages I–IV) (Tonetti, Greenwell, & Kornman, 2018).

During the maintenance phase: Level of hygiene (good, moderate or poor) as outlined by Fardal and Linden (2005) and maintenance profile (compliant or not).

At the end of the study: The number of observation years.

Systemic disorders that have a major impact on the loss of periodontal tissue by influencing periodontal inflammation: All patients were assessed for systemic disorders as described by Albandar, Susin, and Hughes (2018).

2.3 | Types of periodontal therapy provided

Patients in both the test and control groups completed a similar course of periodontal therapy including non-surgical therapy and when appropriate, surgical intervention. Initial therapy included oral hygiene instruction, scaling and root planing using standard curettes (Gracey and Colombia patterns). In the initial phase, scaling and root planing procedures were completed without the use of local anaesthesia. The whole mouth was treated over a series of visits at 2–4 weeks interval. Oral hygiene was reinforced repeatedly and based on individual needs. The patients received a thorough explanation of the periodontal anatomy and the disease process involved in periodontitis. Special emphasis was placed on the importance of periodontal maintenance therapy following the initial definitive therapy.

Periodontal surgery was prescribed for patients who had sites with bleeding on probing or persistent deep pocketing at re-assessment 6 weeks after the completion of initial therapy. Following the completion of the initial definitive periodontal therapy, a diagnosis using the stages from I-IV was recorded for each patient (Tonetti et al., 2018). The diagnosis was based on the periodontal support of remaining teeth, healing after periodontal therapy, assessed level of plaque control, smoking habits and systemic periodontal risk factors.

After the completion of cause related or corrective therapy, all patients were seen between one and three times per year in the specialist practice for maintenance care. The maintenance visits with the specialist practitioner alternated with visits to the general dental practitioner such that all patients were seen in total between 2 and 4 times per year. Written instructions were given both to the referring dentist and the patient outlining the plans for maintenance therapy. During each maintenance visit, scaling, root planing and polishing of the teeth were routinely performed according to the specific needs of each patient. The interval between recall visits was shortened or lengthened as appropriate according to the stability of the periodontal condition.

During the maintenance period, sites with increasing probing depth were treated with repeated scaling and root planing. Subsequently, if there were clinical signs of residual sub-gingival calculus or persistent inflammation, surgical intervention was performed. In addition, systemic or topical antibiotic therapy was used in acute exacerbations of periodontal disease. The re-treatment was carried out according to the descriptions of Fardal and Linden (2005).

2.4 | Analyses

We carried out two types of analyses. First, we did a balancing test of all the matching variables. For each of the test and control groups, we calculated descriptive statistics for each of the matching variables. A successful matching implied that the mean values should be similar across the groups. Second, we tested for differences between the test and control groups by estimating the following regression model:

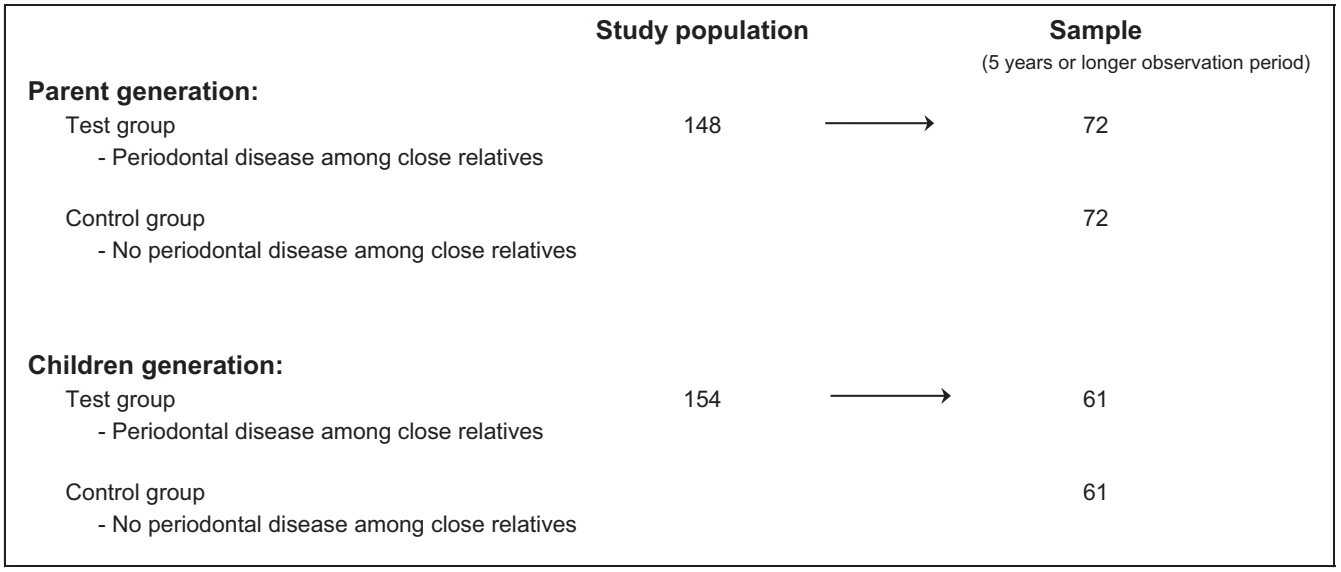

FIGURE 1 An illustration of the study design

$$\text{Tooth loss} = \alpha_0 + \beta_1 \text{Test} + \Sigma\gamma \text{ Matching variables} \quad (1)$$

Tooth loss is the number of teeth lost due to periodontal disease, measured at the end of the observation period. Test is a dummy variable that equals 1 for patients who had a history of periodontal disease among their close relatives. A positive and significant regression coefficient β_1 indicates that having a history of periodontal disease among close relatives leads to more tooth loss.

The regression model was estimated separately for the parent and children generations. This was done both with and without matching variables included. If the matching had been successful, the coefficient should be similar in size with and without the matching variables included. We did not report the estimates for the matching variables as they had the same signs and were of similar sizes as previously reported (Fardal et al., 2004).

2.5 | Intra-familial comparisons

In spite of the generations not being directly comparable, some data were used for exploring possible intra-familial similarities and differences: Gender distributions, medical histories, smoking histories, common teeth missing and stratification of tooth loss.

2.6 | Ethics approval

The present quality assurance and evaluation project was by definition exempted from approval by the Norwegian Regional Committees for Medical and Health Research Ethics (REC) by their following general statement: "Quality assurance and evaluation that is part of the health service, even if the projects are carried out using scientific methodology and with the purpose of generating knowledge that is intended to be published provided that clinical studies are of the same type as the ordinary diagnosis and treatment for the disease in question are exempted from approval by REC" (Regional Committees for Medical & Health Research Ethics, 2012).

The manuscript is in compliance with the STROBE checklist.

3 | RESULTS

3.1 | Descriptive statistics—matching variables

The test and control groups were well balanced with respect to the well-known risk and prognostic factors that influence the outcome of periodontal therapy (Table 1). For all the matching variables, the point estimates were closely similar, and the 95% confidence intervals overlapped across groups.

3.2 | Descriptive statistics—numbers of teeth lost

For the parent generation, the number of teeth lost for those who had a history of periodontal disease among their close relatives was 1.94 (Table 1). The figure for those who had no history of periodontal disease among their close relatives was 0.70. The 95% confidence intervals for these point estimates did not overlap indicating that having a history of periodontal disease is an important prognostic predictor for periodontal treatment outcome.

For the children generation, the number of teeth lost was 0.65 for those who had a history of periodontal disease among their close relatives (Table 1). The corresponding figure for those who did not have a close relative with a history of periodontal disease was 0.26. The 95% confidence interval for these point estimates overlapped. Most likely, this is due to low statistical power due to the low number of observations (n = 61).

Variable	Parent generation		Children generation	
	I	II	III	IV
Test group				
Periodontal disease among close relatives = 1	1.29*	1.02*	0.44*	0.61*
Standard errors	(0.44)	(0.44)	(0.22)	(0.20)
95% confidence interval	[0.41–2.16]	[0.15–1.88]	[0.01–0.87]	[0.20–1.01]
Matching variables included	No	Yes	No	Yes
Number of observations	144	144	122	122

Note: Regression coefficients with standard errors and 95% confidence intervals.
*$p < .05$.

TABLE 2 The effect of having a history of periodontal disease among close relatives on the number of teeth lost due to periodontal disease

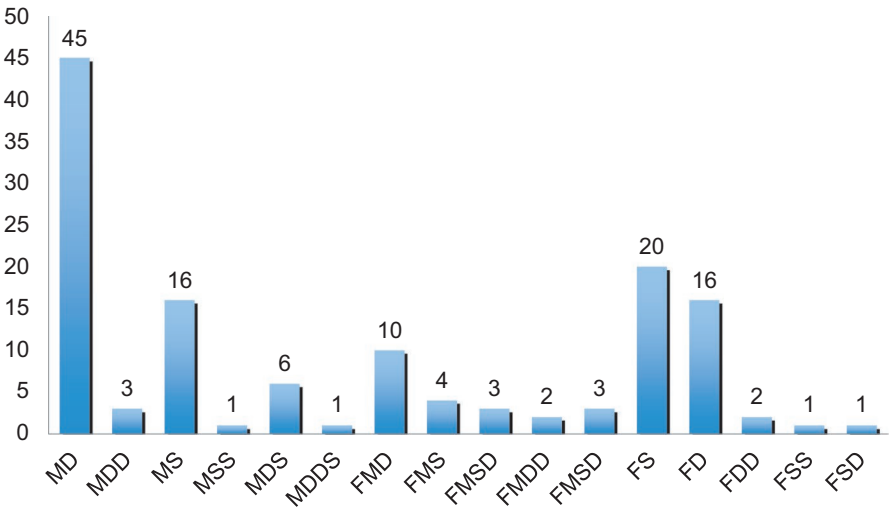

FIGURE 2 Frequencies of family relationships referred for periodontal treatment. D, daughter; F, father; M, mother; S, son

3.3 | Regression results

For the parent generation, the regression coefficient was 1.29 ($p < .05$) with no matching variables included (Table 2). The coefficient was only slightly lower when the matching variables were included. This indicated that the matching had been successful. For both estimates, the 95% confidence interval overlapped. In clinical terms, the result implied that those patients who had relatives with a history of periodontal disease lost slightly more than one tooth during the observation period compared to those patients who did not have such relatives.

For the children generation, the regression coefficient was 0.44 ($p < .05$) without the matching variables included (Table 2). The coefficient increased to 0.61 ($p < .05$) when the matching variables were included. For both estimates, the 95% confidence interval overlapped. As expected, the numbers of teeth lost were less in the children generation than in the parent generation.

For both the parent and children populations, the regression estimates reported in Table 2 were similar to the differences in the number of teeth lost between the test and control groups (Table 1). This also supported that the matching had been successful.

3.3.1 | Systemic disorders

One patient each had obesity and diabetes (1.6%) in the children generation. 10% of the parent generation used blood sugar lowering medication. They were all well controlled and within the national level of 11% for the age group (Strøm et al., 2014).

3.4 | Intra-familial comparisons

3.4.1 | Gender distribution

The distribution between parent and offspring referred for periodontal therapy showed a much higher mother and daughter proportion than any other combination (Figure 2).

3.4.2 | Medical histories

There were no matches in medical histories or medications between parents and offspring. In terms of medication, there was a tendency

of prescribing cholesterol-lowering medication to children of parents with histories of cardiovascular diseases.

3.4.3 | Smoking histories

About 52.2% of children from smoking parents also smoked, while only 35.1% of children smoked who had non-smoking parents.

3.4.4 | Teeth missing at the initial examination

About 42 out of the 124 families (33.9%) had at least one common tooth missing at the initial examination.

3.4.5 | Stratification

Stratification of tooth loss showed that 92.1% of children from well-maintained parents were also well-maintained. The extreme downhill parents all had well-maintained children. One extreme downhill child had parents with well-maintained conditions. The definition of well-maintained, downhill and extreme downhill were according to Hirschfeld and Wasserman (1978).

3.4.6 | Outcome for children with both parents receiving treatment

About 29 children had both parents receiving treatment; only one patient lost 4 teeth (downhill) while the others were well-maintained (loosing ≤ 3 teeth).

4 | DISCUSSION

This is the first study to isolate and quantify the effects of having close relatives with periodontal disease on the long-term treatment outcomes. This was possible by controlling for age, gender, smoking, initial numbers of teeth, initial diagnosis, oral hygiene levels and compliance. In addition, the patients were from the same ethnic background, treated by the same clinician, no over-representation of obesity or diabetes or other systemic disorders-and the test and control groups had the same number of patients. Approximately, 65% of tooth loss was attributable to close family history. The test group of the parent generation had three times more tooth loss than their control group. Similarly, the test group of children had 2.7 times more tooth loss than their control group (Table 1).

The parents had 2.6 times more tooth loss than the children generation. This was mainly due to the lower age of the children generation and the fact that the children started their treatment at an earlier age. It is important to diagnose and start treatment early as it has been shown that signs of periodontal disease may be evident in the late twenties for aggressive types of the disease (Thorbert-Mros, Cassel, & Berglund, 2017).

About 33% of children and parents were missing the equivalent tooth/teeth. A twin study from Denmark suggested that genetic factors explained 36% of the total variation of missing teeth from tooth loss (Kurushima et al., 2017). Although it is tempting to compare the studies, there are substantial differences in the genetic material. In addition, the twin study included 23% edentulous patients and 47% with less than 20 teeth, while the present study had no edentulous patients and both generations had on averages more than 23 teeth.

In general, it is difficult to identify and quantify parental influences on behaviour and habits. The present study showed a high proportion of mothers and daughters. A high percentage of women being treated for periodontal diseases has previously been reported both in the present setting (Fardal, Johannessen, & Linden, 2003; Fardal et al., 2004; Fardal & McCulloch, 2011; Fardal & Grytten, 2013, 2014) and in a review of external studies (Chambrone, Chambrone, Lima, & Chambrone, 2010). It is not clear whether women are more susceptible to periodontal diseases or whether they are more agreeable to referral for specialist treatment. A close and dominant mother and daughter relationship may also contribute to the referral pattern. This type of a close relationship has been the focus of attention for the medical/psychiatric and psychological research for many years (Shrier, Shrier, & Tompsett, 2004).

Another possible parental influence was the fact that 52.2% of children of parents who smoked were also smokers, while only 35.1% of children from non-smoking parents were smokers.

There are a number of limitations in the present study:

1. Systemic diseases were not statistically matched due to the complexities and possible inaccuracies involved: A. The relative risk for the various systemic diseases is not known making it impossible to find matching controls. B. Some patients may develop disorders/diseases during the observation period. C. It is not fully understood if a disease, which is being controlled by treatment, can still have an effect on periodontal disease (for example, diabetes) D. It is uncertain what effects the treatment itself has on the disease. E. A risk factor for developing periodontal diseases may not also be a prognostic outcome factor.

   In the present study, obesity and diabetes were not over-represented. In addition, there were no other serious systemic disorders present.

2. The patients' family history data may not be accurate. However, a number of unique features of this practice setting and the study design suggest a high degree of accuracy. It has previously been shown that close relatives were the most important source of information for new patients referred to this practice (Fardal & Hansen, 2007). All patients were interviewed to identify any close relatives with a history of periodontal disease. If patients were unsure about this at their initial interview, they

were requested to ask their relatives and to report back at their next appointment. No attempts were made to go beyond parents, children and siblings. A similar methodology has been described previously and has been shown to be successful in general medical practice for identifying common systemic diseases within families (Acheson, Wiesner, Zyzanski, Goodwin, & Stange, 2000; Guttmacher, Collins, & Carmona, 2004; Rich et al., 2004; Walter & Emery, 2006). Patient interviews have been shown to be reliable and of great value in determining whether a patient, other family members or future generations are at increased risk of developing a specific disease. Commonly, in these interviews, health information about a patient and his or her relatives are obtained across two to three generations (Doktoronline, 2009; United States National Institute of Health, 2019).

To a large extent, we were able to check the reliability of the information given about the relatives and their history of periodontal disease. This is because the catchment area of the practice encompassed most families. During a period of more than 30 years, this practice has been the only specialist practice in periodontics in the area. Virtually, all patients in need of periodontal therapy were registered in the practice records, and nearly all of them have completed their periodontal treatment here (Fardal & Hansen, 2007). This is mainly because in Norway, all patients have their travelling expenses for specialist care covered by the state (Helsenorge.no., 2019). This only applies if the patient attends the nearest specialist clinic. In our study, the group of control patients had been treated for periodontal disease, but they had no close family members (parents, children or siblings) with a history of periodontal disease or periodontal therapy. The principle investigator (ØF) checked whether there were any close relatives with history of periodontal disease in the practice records; none were identified.

3. Lack of genetic testing: A recent review concluded that genetic testing is not accurate enough to enable the stratification of patients for diagnosis and periodontal treatment (Schaefer, 2018).
4. Although the follow-up period was adequate to analyse tooth loss, the period was still too short to conclude on the lifetime outcome for these patients.
5. It strengthens the study to have all patients in the same practice setting and being treated by the same clinician; however, there are possible bias associated with a retrospective methodology from such a setting.
6. The use of tooth loss may not provide a complete picture of periodontal outcomes. Loss of periodontal support, increasing tooth mobility, pocket depths and patients' perceptions of their periodontal health are also important outcome measurements but are more difficult to diagnose accurately and to use for predicting future progress. We believe that tooth loss is a reasonable outcome measure. It is easy to measure, and it is an ultimate end point to evaluate the success or failure of periodontal treatment.

In conclusion, within the limits of this study, having close relatives with periodontal disease is a strong prognostic treatment factor. The successful matching strategy introduced in the present study is a useful model for future research in terms of identifying and quantifying the strengths of individual prognostic and risk factors. The approach is an important alternative to and an improvement over the basic and traditional association studies.

CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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