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Assessment of genotyping tools applied in genetic susceptibility studies of periodontal disease: a systematic review

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ABSTRACT

Objective: A systematic review to evaluate the various genotyping tools and study strategies employed to define genetic susceptibility to periodontitis.

Methods: The review was performed in accordance with Preferred Reporting Items for Systematic Review and Meta-Analysis guidelines. The search for publications referring to the genetic bases of periodontal disease was performed on the MEDLINE-PubMed and Cochrane Library databases, on trials registers, and on the web pages of regulatory agencies.

Results: We found 2439 potentially eligible articles, of which only 25 satisfied the established inclusion criteria and were processed for data extraction. The review revealed marked heterogeneity between studies, caused in part by the lack of a universally accepted definition for periodontitis phenotypes and by the variety of genotyping tools available. The most commonly used technique was genotyping candidate genes.

Conclusion: The few rigorous studies that have been published on genetic susceptibility to periodontitis are subject to severe methodological bias due to their design and the genotyping tools employed. Despite their limitations, candidate gene studies continue to be the predominant methodological approach, rather than genome-wide association studies. Further studies must be designed using a universally accepted, validated diagnostic criterion for periodontitis, analysing multiple genes and polymorphisms in combination with rare variants.

KEYWORDS: Periodontal disease, Genetics, Gene polymorphism

INTRODUCTION

The existence of a genetic component to periodontal disease (PD) has been confirmed in twin studies, which estimate that 38% to 82% of populational variability in the clinical parameters of periodontal disease is attributable to genetic factors (Michalowicz et al., 1991a, 19991b, 2000). It has been suggested that the genetic component of chronic periodontitis (CP) might have been overestimated (Torres de Heens, Loos, & van der Velden, 2010), but that it is more relevant in aggressive periodontitis (AgP), as has been demonstrated in familial aggregation studies (Benjamin & Baer, 1967; Marazita et al., 1994; Saxén & Nevanlinna, 1984).

As with diabetes, certain types of cancer, Alzheimer's disease, Crohn's disease, and schizophrenia, PD is considered to be genetically complex, resulting from the interaction of genes with the environment (Laine, Crielaard, & Loos, 2012; Stabholz, Soskolne, & Shapira, 2010). Two main hypotheses have been proposed regarding the genetic basis of complex diseases. The first is the common disease/common variant (CD/CV) hypothesis (Reich & Lander, 2001), in which it is assumed that genetic variants common in the general population but which individually have a weak effect are those that have the greatest influence on genetic susceptibility to complex disorders. These genetic variants are called polymorphisms when they are present with a frequency of at least 1% in the population (Hart, Marazita, & Wright, 2000). They include single nucleotide polymorphisms (SNPs), which are very common in the genome and consist of a change of one nucleotide base for another. The second hypothesis is the common disease/rare variant (CD/RV) hypothesis, in which the main contributors to susceptibility to complex diseases are rare variants (minor allele frequency <1%) present in the genome (Pritchard, 2001). The hypothesis that is deemed valid will thus determine the strategy applied to detect variants that favour disease. Most PD studies assume the CD/CV hypothesis; thus, the most common strategy consists of the search for polymorphisms that could affect the periodontal disease phenotype.

One of the first studies to identify genetic markers for PD was published in 1997 by Kornman et al (Kornman et al., 1997). Those authors analysed several polymorphisms of the interleukin-1 (*IL-1*) family (polymorphisms of *IL-1A* at position-889, of *IL-1B* at position -511 and +3953, and of *IL-1RN* intron 2) and found evidence of an association between *IL-1* and the severity of PD in nonsmokers, differentiating between individuals with mild and severe PD.

These encouraging results led to further genetic studies on PD that applied distinct methodological designs. One of those was the genome-wide linkage analysis, which has been widely used to study complex diseases such as schizophrenia (Stefansson et al., 2002) and type 2 diabetes (Nisticò et al., 1996); however, in contrast to its notable efficacy in the genetic mapping of Mendelian diseases, the technique has limited usefulness in the detection of alleles with a weak effect, which are common in complex diseases (Plomin, Haworth, & Davis, 2010).

Another design is the association study, which enables relationships to be established between a specific polymorphism and a disease or phenotypic trait. This approach determines differences in the frequency of a genetic marker in cases and controls to indicate a relationship between that marker and the disorder under investigation. The two principal methods in this context are the candidate gene association study (CGAS) and the genome-wide association study (GWAS). The CGAS is based on an analysis of genes that are linked to a certain disease through their function or their position in the genome. It is therefore essential for investigators to understand the pathophysiology of a disorder before performing this type of study. Based on the promising results from what was considered the first CGAS (Kornman et al., 1997), this type of design became the most widely used to analyse the genetics of PD. However, the very rapid development of genotyping technologies in recent years has led to substitution of CGASs with GWASs, because these latter studies theoretically enable us to identify new genetic markers. The first GWAS to study periodontitis was published in 2010 and established an association

between SNP rs1537415, present in gene *GLT6D1* (glycosyltransferase), and AgP (Schaefer et al., 2010a).

Given the complexity of PD, the role of genetics is significant in its onset, progression, and severity (Albandar & Tinoco, 2002). The identification of genetic risk factors associated with PD is therefore essential to improving prevention and treatment strategies for this disease (Song, Yao, He, & Xu, 2015).

The objective of this systematic review was to evaluate the genotyping tools and study strategies used in the literature to establish the genetic bases of PD and to determine their main limitations.

MATERIALS AND METHODS

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) review process was used to perform the present systematic review (Moher, Liberati, Tetzlaff, Altman, & PRISMA Group, 2009).The PRISMA checklist was followed in both the planning and the reporting of the review.

Focused Question

The research question formulated was based on PECO framework: If in the studies performed on patients with periodontitis reported in the literature (Population) in which the genetic basis of susceptibility to periodontal disease was determined (Exposition), we then analyse the limitations and potential biases of the genotyping tools used (Control), are the validity and reliability of the results confirmed (Outcome)?

Eligibility criteria

The following inclusion criteria were applied: studies published in English; case-control design; specification of the PD phenotype; a sample size equal to or greater than 100 individuals in either the case or control group, or in both groups. No limitations were placed on the year of publication or the age, population group, or country of origin of the participants. Publications with any of the following potential biases were excluded: a study group that included individuals with systemic diseases that could favour the onset or

augment the severity of PD; patients treated with drugs that provoke gingival hyperplasia; and pregnant women (pregnancy gingivitis). Reviews and meta-analyses were also excluded, even if they referred to genetic susceptibility to PD. We used the number of SNPs analysed as inclusion criteria for candidate gene studies. There have been many studies of this type that have analysed a reduced number of SNPs; they are generally lacking in quality control and have a lack of appropriate statistical corrections, Hardy-Weinberg equilibrium analysis or correspondence of cases and controls with a population background. Thus, due to the criteria that define the copy number variations (CNVs) (Lin, Naj, & Wang, 2013; Merikangas, Corvin, & Gallagher, 2009) and the limitations observed in those studies with a reduced number of SNPs, only the studies that analysed ≥10 SNPs were included.

Information sources

The search for articles was performed using the electronic databases of the National Library of Medicine, Washington DC, USA (MEDLINE, PubMed), and of the Cochrane Library, in addition to trial registers and the web pages of regulatory agencies. A manual search was performed of the most relevant journals in the field of periodontics of the last 10 years. In addition, "snowball" methods were also applied such as pursuing references of references from all selected full-text papers. The search was performed up to Jan 2018. Study selection was performed in accordance with the most recent recommendations of the Centre for Reviews and Dissemination of the University of York (Centre for Reviews and Dissemination, University of York, 2008).The following keywords were used: “periodontitis” and “periodontal disease”, in combination with “genetic polymorphism”, “single nucleotide polymorphism”, “mutation”, “genes”, “new genome sequencing”, “exome sequencing”, “whole genome sequencing”, “CNV”, and “genome wide association”. The “humans” filter was selected for all searches.

Search strategy

The search strategy used a combination of Medical Subject Headings terms and keywords for MEDLINE and Cochrane Library. The search strategy used is described in the supporting information (appendix S2).

Study selection

Study selection was performed using a two-stage screening process performed by two independent reviewers (P. D. and A. de C.). Disagreement on the inclusion or exclusion of a specific article was resolved by consensus. In the first phase, studies that did not satisfy the inclusion criteria were eliminated, as were those considered irrelevant based on their title or the content of the abstract. A full-text review of the remaining articles was then performed to confirm the inclusion criteria and to check quality. The most relevant data were then extracted. Concordance between the two reviewers was calculated using the Kappa coefficient for the first and second screenings.

Data collection process/data items

The eligibility criteria were applied after agreement had been achieved between the two reviewers for the studies to be included in the review. Data were gathered on the general characteristics of the study (year of publication, clinical parameters recorded, number of SNPs studied, genotyping method) and on their populational characteristics (population, age, number of participants, smoking, diabetes). The extracted data were presented in the same form in which they were described in the original studies, except for those studies with the same design but different forms of presentation of the results, in which case the results were processed to facilitate extraction of the data of interest.

Risk of bias in individual studies

We aimed to assess the risk of bias of the included studies through sensitivity analysis. The risk of bias/quality assessment for the individual studies was assessed according to the suggested scale (score of 0 to 20) adapted to genetic analysis of periodontal genetic association studies (Nibali, 2013).

Summary measures/synthesis of results

No meta-analysis was attempted for this systematic review; thus, no formal statistical analyses were performed.

RESULTS

Study selection

The search provided a total of 2439 potentially selectable articles (MEDLINE PubMed, n=2392; Cochrane, n=47; other sources, n=0) (Figure 1). Duplicate articles were eliminated by comparing the results obtained with each database, leaving 2412 articles potentially useful for the review. The titles and abstracts of these studies were then examined, and 87 articles were selected for full-text review; of these, 62 were excluded because they did not satisfy the inclusion criteria. This left a final count of only 25 studies for analysis in this review. The Kappa value for inter-reviewer concordance was 0.95 for the first screening (by title and abstract) and 0.89 for the second screening (full-text review).

We detected several publications in which samples derived from the same study group were analysed. In 2010, Chai et al. analysed samples gathered between May 2005 and August 2007 from a primary care clinic in the Prince Philip Dental Hospital of the Faculty of Dentistry of the University of Hong Kong; they analysed the SNPs present in *C5* (Chai, Song, Zee, & Leung, 2010a) and *FCGR* (Chai, Song, Zee, & Leung, 2010b). In 2010, Schaefer et al. conducted the first GWAS to study periodontitis (Schaefer et al., 2010a). They analysed the German population with AgP and replicated the association in a panel of Dutch patients with AgP. Subsequently, these cohorts were examined in a large candidate-gene association study by Schaefer et al. in 2014 and 2015 (Schaefer et al., 2014; Schaefer et al., 2015). Also in 2010, Schaefer et al. analysed 12 SNPs in the gene *DEFB1* in individuals from Germany and the Netherlands with CP and AgP (Schaefer et al., 2010), and in 2011 performed an analysis of 51 SNPs in genes *CDKN2A* and *CDKN2B* for individuals with AgP and of 37 SNPs in the same genes but in individuals with CP (Schaefer et al., 2011). Also in 2011, Scapoli et al. studied 14 candidate genes,

including some belonging to the *IL-1* cluster that had already been studied a year earlier in the same population sample (with the exception of 25 new participants from the University of Turin) (Scapoli et al., 2010, 2011) and with the same results. In 2014, de Jong et al. (de Jong et al., 2014) analysed cases and controls described and studied previously by Schaefer et al. in 2010 (Schaefer et al., 2010a). The difference between the two studies was that de Jong et al. only investigated SNPs in genes *SLC23A1* and *SLC23A2* instead of the whole genome, as the earlier study had done. One year later, in 2015, Schaefer et al. (Schaefer et al., 2015), investigating the genetic risk factors shared between coronary artery disease and periodontitis, analysed the same German/Austrian, Dutch, and Turkish cohorts as Schaefer et al. in 2010 and 2013 (Schaefer et al., 2010a, 2013). In addition, patients from the Netherlands and from Turkey were studied at a whole genome level by Munz et al. in 2017 (Munz et al. 2017). Finally, Offenbacher et al. (Offenbacher et al., 2016) and Munz et al. (Munz et al., 2017) analysed the same German population whose cases were previously described by Schaefer 2015 and whose controls were recruited from the Competence Network FOCUS (Food Chain Plus) (Müller et al., 2015), the Dortmunder Gesundheits studie (Berger, 2012), and the Heinz Nixdorf Recall Studies 1-3 (Schmermund et al., 2002).

Study characteristics

The principal characteristics of the 25 studies selected for this review are summarised in Table 1 and in the following sections: *Diagnosis of periodontal disease*, *Demographics*, *Quality assessment*, *Clinical parameters*, *Study methodology*, *Genotyping method*, and *Genetic results.*

Diagnosis of periodontal disease

Most of the studies (20 of 25) were published in 2010 or later; nevertheless, the diagnostic criteria for the PD phenotype in ten of these studies were those proposed by Armitage in 1999 (Armitage, 1999), and another three used other sources from that same year (Flemmig, 1999; Lang et al., 1999). In the remaining 12 studies, the diagnostic criteria

used to determine the phenotype were not specified, although the values of the clinical parameters used to select the participants were given.

Demographics

The population samples in nine articles included German individuals and in six included Dutch individuals. The populations in the remaining studies were from Japan (4), China (4), Italy (3), Brazil (2), United States (2), England (1), Macedonia (1), and Turkey (1). The sample size of the studies reviewed varied between 208 (Schaefer, 2010b) and 8295 participants (Munz et al., 2017) (Table 1). The mean age — obtained by weighting the means across studies — of the individuals with AgP was 37 years and of those with CP was 50 years. Data on the participants' smoking habits were only recorded in 17 of the 25 studies included in the review (Chai et al, 2010a, Chai et al, 2010b; de Jong et al., 2014; Feng et al., 2014; Fiebig et al., 2008; Kobayashi et al., 2009a, 2009b; Munz et al., 2017; Offenbacher et al., 2016; Schaefer et al., 2010a, 2010b, 2011, 2013, 2014, 2015; Shang et al., 2016; Zupin et al., 2017). In one study, smoking was considered an exclusion criterion (Zhang et al., 2014), and seven provided no specific data on smoking (Table 1). The number of individuals with diabetes was only reported in seven studies (de Jong et al., 2014; Feng et al., 2014; Offenbacher et al., 2016; Schaefer et al., 2010a, 2010b, 2011; Zhang et al., 2014), and a history of diabetes was considered an exclusion criterion in six studies (Chai et al., 2010a, 2010b; Fiebig et al., 2008; Kobayashi et al., 2009b; Schaefer et al., 2015; Shang et al., 2016).The remaining 12 articles made no reference to diabetes (Table 1).

Quality assessment

Random sample selection from the general population was performed in eight studies (de Jong et al., 2014; Schaefer et al., 2010a, 2010b, 2011, 2013, 2014; Zhang et al., 2014; Zupin et al., 2017), whereas in 16 studies the participants were recruited from hospitals, dental clinics, research centres, and universities. Randomisation of participant selection was not specified in one study, though the origin of the controls was a blood transfusion

centre _considered as the general population_ and the possibility that individuals with periodontitis had been included was noted (Brett et al., 2005).

Twenty of the publications examined did not confirm the presence of at least one examiner or specify his or her level of training (Atanasovska-Stojanovska, Popovska, Trajkov, & Spiroski, 2013; Brett et al., 2005; Chai et al., 2010a, 2010b; de Jong et al., 2014; Feng et al., 2014; Gunji et al., 2007; Kobayashi et al., 2009a, 2009b; Munz et al., 2017; Offenbacher et al., 2016; Scapoli et al., 2010, 2011; Schaefer et al., 2010a, 2013, 2014, 2015; Suzuki et al., 2004; Zhang et al., 2014; Zupin et al., 2017). In two studies, the clinical parameters were gathered by a single examiner with experience (Schaefer et al., 2010b, 2011), and in two (Letra et al., 2012; Shang et al., 2016) of the remaining three studies, the degree of concordance between the examiners was defined using Cohen's kappa coefficient (Garlet et al., 2012).

Clinical parameters

The oral health status variables recorded in the studies and included in the review are summarised in Table 3. The following parameters were evaluated most frequently: probing pocket depth, also defined as probing depth (14 studies) (Atanasovska-Stojanovska et al., 2013; Chai et al., 2010a, 2010b; Gunji et al., 2007; Kobayashi et al., 2009a, 2009b; Letra et al., 2012; Offenbacher et al., 2016; Schaefer et al., 2010b, 2011; Shang et al., 2016; Suzuki et al., 2004; Zhang et al., 2014; Zupin et al., 2017); clinical attachment loss, also defined as clinical attachment level or probing attachment level (13 studies) (Atanasovska-Stojanovska et al., 2013; Chai et al., 2010a, 2010b; Feng et al., 2014; Kobayashi et al., 2009a, 2009b; Letra et al., 2012; Offenbacher et al., 2016; Schaefer et al., 2010b, 2011; Shang et al., 2016; Zhang et al., 2014; Zupin et al., 2017); bleeding on probing (11 studies) (Atanasovska-Stojanovska et al., 2013; Chai et al., 2010a, 2010b; Kobayashi et al., 2009a, 2009b; Offenbacher et al., 2016;Schaefer et al., 2010b, 2011; Suzuki et al., 2004; Zhang et al., 2014; Zupin et al., 2017); and alveolar bone loss (12 studies) (Chai et al., 2010a; de Jong et al., 2014; Fiebig et al., 2008; Kobayashi et al., 2009b; Schaefer et al., 2010a, 2010b, 2011, 2013,2014 2015; Suzuki et

al., 2004; Zhang et al., 2014). Some of the selected studies also reported other variables, including the number of natural teeth (eight studies) (Chai et al., 2010b; Fiebig et al., 2008; Gunji et al., 2007; Kobayashi et al., 2009b; Schaefer et al., 2010b, 2011, 2014; Zupin et al., 2017); variables related to the accumulation of bacterial plaque (four studies) (Kobayashi et al., 2009a, 2009b; Offenbacher et al., 2016; Zupin et al., 2017); furcation exposure (3 studies) (Schaefer et al., 2010b, 2011, 2014); tooth mobility (two studies) (Suzuki et al., 2004; Zhang et al., 2014); loss of interproximal insertion (two studies) (Scapoli et al., 2010, 2011); the gingival index (two studies) (Atanasovska-Stojanovska et al., 2013; Offenbacher et al., 2016); and degree of gingival recession (two studies) (Zhang et al., 2014; Zupin et al., 2017).

Study methodology

Twenty-one (84%) of the 25 selected publications used the CGAS design (Atanasovska-Stojanovska et al., 2013; Brett et al., 2005; Chai et al., 2010a, 2010b; de Jong et al., 2014; Fiebig et al., 2008; Gunji et al., 2007; Kobayashi et al., 2009a, 2009b; Letra et al., 2012; Scapoli et al., 2010, 2011; Schaefer et al., 2010b, 2011, 2013, 2014, 2015; Shang et al., 2016; Suzuki et al., 2004; Zhang et al., 2014; Zupin et al., 2017). Five of those studies analysed a single candidate gene: formyl peptide receptor 1 (*FPR-1*), β-defensin 1 (*DEFB1*), complement component 5 (*C5*), Fc γ receptor (*FCGR*), and interleukin-8 (*IL-8*) (Chai et al., 2010a, 2010b; Gunji et al., 2007; Schaefer et al., 2010b; Zhang et al., 2014). Of the 13 articles that used GWAS methodology, nine were excluded from the review because they did not satisfy the selection criteria (Divaris et al., 2012, 2013; Freitag-Wolf et al., 2014; Hong, Shin, Ahn, Lee, & Kim, 2015; Rhodin et al., 2014; Sanders et al., 2017; Shaffer et al., 2014; Shimizu et al., 2015; Teumer et al., 2013) (Table 2); only four studies were included (16% of the total of articles included in the review) (Feng et al., 2014; Munz et al., 2017; Offenbacher et al., 2016; Schaefer et al., 2010a). Only the data related to the analysis of the independent German sample of the series by Offenbacher et al. (Offenbacher et al., 2016) were included in the review. The data corresponding to the

GWAS initially performed were not examined because the controls of the studied cohort included patients with periodontitis.

Genotyping method

In seven of the selected studies, the genotyping of cases and of controls was performed using TaqMan probes (Fiebig et al., 2008; Kobayashi et al., 2009b; Letra et al., 2012; Munz et al., 2017; Schaefer et al., 2010b, 2011; Suzuki et al., 2004). The Sequenom platform was used in seven of the review articles (Chai et al., 2010a, 2010b; Munz et al., 2017; Scapoli et al., 2010, 2011; Shang et al., 2016; Zhang et al., 2014) and the Illumina platform in another seven publications (Munz et al., 2017; Offenbacher et al., 2016; Schaefer et al., 2013, 2014, 2015; Zhang et al., 2014; Zupin et al., 2017). Polymerase chain reaction (PCR) was used as the genotyping method in two studies (Brett et al., 2005; Scapoli et al., 2011), and a variant of PCR in a further two (Atanasovska-Stojanovska et al., 2013; Gunji et al., 2007) (restriction fragment length polymorphism [RFLP]PCR and single specific primer [SSP]PCR, respectively). The following genotyping platforms were employed in the remaining articles: Affymetrix (de Jong et al., 2014; Schaefer et al., 2010a, 2015), nano-Invader DNA chip system (Kobayashi et al., 2009a, 2009b), and SNPlex (Schaefer et al., 2010b, 2011) (Table 4).

Appendix S3 reports the genotyping technology used in the various studies in relation to the level of evidence of association between genes and PD. The greatest level of evidence in the present review (moderate) was achieved by the *SLC23A1* and *SLC23A2*genes. De Jong et al. found a significant association between these genes and AgP in whites (de Jong et al., 2014). Cases and controls were genotyped using the Affymetrix Human Mapping 500K Array set.

Genetic results

In 21 articles, an association was established between some of the SNPs analysed and the presence of CP, AgP, or PD in general (Table 4). In the four remaining studies, no association of any type was found between the polymorphisms studied and PD (Fiebig et al., 2008; Kobayashi et al., 2009a; Offenbacher et al., 2016; Scapoli et al., 2010). In the

present systematic review, a significant association between the presence of PD and a total of 54 SNPs was found. Of the 54 SNPs, 29 were associated with AgP, 21 with CP, two with both PD phenotypes, and two with PD in general.

The most common associations in the studies reviewed were between the interleukin-6 gene (*IL6*) and CP and AgP (Brett et al., 2005; Scapoli et al., 2011), between the vitamin D receptor gene (*VDR*) and CP (Brett et al., 2005; Kobayashi et al., 2009b), and between the interleukin-1B gene (*IL1B*) and AgP and CP (Atanasovska-Stojanovska et al., 2013; Brett et al., 2005) (Table 4). However, the level of evidence of these associations was very weak (appendix S3). Another gene related to *IL6*, the interleukin-6 signal transducer (*IL6ST*), was found to be associated with AgP in the study conducted by Suzuki et al. (Suzuki et al., 2004); the odds ratios (ORs) for the SNPs present in these genes associated with PD were between 2.1 and 3.6.

An OR>4 for four of the SNPs was significantly associated with PD. The polymorphisms were present in the genes *PTGDS* (OR,5.1), *KRT23* (OR,6.3), *EGF* (OR,4.8), and *C5* (OR,6.1) (Chai et al., 2010a; Suzuki et al., 2004).

Moderate evidence of an association was detected for only two genes, *SLC23A1 and SLC23A2* (de Jong et al., 2014). In different studies and ethnic populations, several genes showed a weaker evidence association level with periodontal disease (appendix S3).

Publication bias analysis

Appendix S3 reports the results of the risk of bias analysis of individual studies included in the present review. The quality evaluation scale of the periodontal genetic association studies proposed by Nibali (Nibali, 2013) was used for the quality evaluation. CGAS and GWAS study quality scores ranged from a total of nine to a total of 14 (out of a maximum total of 20). In addition to the suggested scoring system, a threshold was used to determinate the “sturdiness” of the included publications, defining as “robust GWAS” the genome-wide association study with independent replication and a quality score >10 and

as “robust CGAS” the candidate gene association study including at least 1000 participants and with a quality score >10 (Nibali, Di Iorio, Tu, & Vieira, 2017).

DISCUSSION

This is the first systematic review, to our knowledge, to assess genotyping tools applied in genetic susceptibility studies on periodontal disease. The study of genetic associations with periodontitis can be carried out through various approaches, such as linkage analysis, family studies and association studies. However, due to its advantages the last approach is more widely used today. There are two major types of association studies: candidate gene and genome wide association studies. In the association studies carried out to date, the genotyping tools used to study the genetic basis of periodontal disease are diverse. TaqMan, Sequenom and Illumina are among the most commonly used genotyping platforms in the studies included in this review. The various approaches and tools used present certain limitations, which can lead to weak studies and erroneous associations.

The present study is not exempt from several methodological limitations that should be considered when extrapolating our results. The influence of participant selection criteria on the results of studies on genetic susceptibility to PD remains unknown. The most controversial include variability in the definition and phenotype of PD and in the clinical parameters evaluated (American Academy of Periodontology Task Force Report on the Update to the 1999 Classification of Periodontal Diseases and Conditions, 2015; Armitage, 1999; Leroy, Eaton, & Savage, 2010; Tonetti, Claffey, & European Workshop in Periodontology group C, 2005) and in the inclusion of individuals with risk factors for PD, such as smoking (Kinane & Chestnutt, 2000) or diabetes (Salvi, Carollo-Bittel, & Lang, 2008). The limitation of the literature search to English-language publications can also introduce a potential bias in the selection of articles (Moher, Pham, Lawson, & Klassen, 2003; Pham, Klassen, Lawson, & Moher, 2005). We included only case-control studies with at least 100 participants because publications with small study cohorts (<100

individuals) greatly contribute to the risk of false-positive or false-negative results (Burgner, Jamieson, & Blackwell, 2006; Newton-Cheh & Hirschhorn, 2005). Common or rare Copy Number Variations (CNVs) have been associated with genetic susceptibility for many diseases (Merikangas et al., 2009), and as such remain one of many viable categories of genomic variation to be explored for possible genetic contributions to disease (Lin et al., 2013). Threshold parameters for calling a CNV recommend analysing CNVs spanning at least 10 SNPs (Lin et al., 2013). However, the exclusion of articles with fewer than 10 SNPs could also constitute a methodological limitation of the present study.

The predominant methodological design in the research evaluated was the candidate gene approach. In these association studies, a hypothesis is established a priori not only on the effect of certain genes on the risk of developing the disease but also on the presence of functional variants of these genes (Wilkening, Chen, Bermejo, & Canzian, 2009). The genes targeted in this analysis were selected based on a previous understanding of the mechanisms involved in the molecular biology of the disease. This study design thus presents limitations, given that there could be genes that have an influence on the development of the disease but whose function or involvement in key pathways of the disease process remain undefined. The number of variants analysed in studies that use this design is therefore limited.

GWASs also have a series of limitations. As occurs with other diseases, some of the genes associated with PD do not express a known biological function that could explain this association. In GWASs, it is essential to guarantee the power of the study, because the analysis of thousands of SNPs in a small sample will lead to a high risk of finding false associations, mainly because of random fluctuations in allele frequency between cases and controls (Schaefer, Jepsen, & Loos, 2011). In addition, GWASs need to be automated procedures for determining genotypes, because the assigned genotypes are not manually curated; thus, erroneous genotypes could go unnoticed thus introducing false positive associations.

To date, 13 studies using GWAS methodology have been published in the databases selected, although only four satisfied the inclusion criteria for the present review (Schaefer et al., 2010a; Feng et al., 2014; Munz et al., 2017; Offenbacher et al., 2016). ~~The first GWAS was performed by Schaefer et al. in 2010 (Schaefer et al., 2010a). Those authors analysed the genomes of 283 white individuals of German nationality with AgP and found a strong association between polymorphism rs1537415, located in gene *GLT6D1*, and AgP; this association was confirmed on repeating the study in a group of Dutch patients. Four years later, Feng et al. (Feng et al., 2014) used the GWAS technique to study the DNA of a group of 866 individuals from the dental register of the University of Pittsburgh. They analysed more than 500,000 SNPs and compared the 20 most relevant SNPs with a bank of samples from Brazilian patients, confirming that locus 16q22.3 contributed to the onset of CP.~~ The authors of the two most recent GWASs agree in stating potential biases, such as the origin of the participant population, but they draw attention to the importance of GWAS in the design of future studies on the genetic bases of PD using multiple candidate genes. ~~In 2016, Offenbacher et al. (Offenbacher et al., 2016) examined the association of more than 2.1 million markers with CP in a larger Dental Atherosclerosis Risk in Communities cohort (n=4766 Northern European). *BEGAIN* and *UBE3D* showed associations with severe CP and with moderate CP, respectively. These data were not included in the review because the control group was formed of individuals with mild periodontitis. They also analysed an independent German sample including 717 AgP cases, but no association was found (Offenbacher et al., 2016). The data corresponding to this cohort was included in the present review. One year later, Munz et al. conducted a GWAS using German and Dutch AgP samples (896 cases and 7104 controls), validated the association in a German CP sample (993 cases and 1416 controls), and replicated the positive findings in a Turkish AgP sample (223 cases and 564 controls). They found an association of *SIGLEC5* and *DEFA1A3* with periodontitis at a genome-wide level in the pooled samples (Munz et al., 2017).~~

Of the four articles that used GWAS methodology and were included in the review, two were considered robust GWASs (Munz et al., 2017; Schaefer et al., 2010a), but the association of genes found with the PD was considered weak, because the results were not replicated in an independent GWAS or in a robust CGAS. Furthermore, association studies performed at a genome-wide level do not tag rare variants (Cirulli & Goldstein, 2010). As stated in the CD/RV hypothesis, rare variants can contribute substantially to the risk of developing a disease such as PD. Rare variants have modest-to-small effect sizes on phenotypic variation; thus, their analysis has the potential to reveal novel variants implicated in complex diseases (Auer & Lettre, 2015). GWASs performed to date in PD have been based on the CD/CV hypothesis and have not taken rare variants into account in the design of the arrays, due to their high cost. The rapid improvement in the technology for measuring genomic variation has allowed the appearance of DNA sequencing-based approaches, such as next generation sequencing (NGS) and custom arrays. These tools help ascribe the contribution of rare variants to complex traits and diseases, thus eliminating this recognised limitation of GWASs (Cirulli & Goldstein, 2010). The studies included in the present review show considerable heterogeneity with regard to the genotyping techniques employed, reflecting ever-advancing technological developments. Chronologically, PCR was the first genotyping method to be described and was followed by variants of the same process (PCR-SSP and PCR-RFLP). PCR-SSP uses specific probes to amplify the DNA. Two PCRs are necessary to type a single SNP using this method, one with the specific primer and another with the common primer; additionally, this PCR variant requires a larger amount of DNA than other methods (Bugert et al., 2003). PCR-RFLP is based on the selective amplification of certain restriction fragments of a DNA sample, which gives robust and unequivocal results as long as a suitable endonuclease is used. The problem with this technique is the need for post-PCR DNA processing, which increases the risk of contamination (Dorak, 2006). Other studies have used the TaqMan genotyping system (Applied Biosystems, Foster City, CA, USA). This technology consists of a fluorophore covalently bonded to the 5´ terminus of a probe,

which enables genotyping of the samples to be performed. By using allele-specific probes in the PCR, the amplification and detection steps are combined so that, in contrast to other methods, no additional post-PCR processing is required. The estimated error rate with this technique is less than 1 in every 2000 genotyping assays (Ranade et al., 2001). When the aim is to analyse a larger number of SNPs, these basic methods are costly and time-consuming if used alone, making them uneconomical in studies demanding a high yield. A system used for massive SNP analysis is SNPlex (Tobler et al., 2005), a method based on capillary electrophoresis. This method is a good alternative to previous genotyping methods because it only requires a small amount of DNA and uses easily accessible electrophoresis instruments (Tobler et al., 2005). Another genotyping tool is the novel nano-Invader DNA chip system. This approach is a miniaturised genotyping technique, in which the maximum sample volume is of 45 nanolitres. The sample is deposited on a polycarbonate slide with a capacity to perform 5040 tests per chip; in contrast to the other genotyping tools, this system does not require the PCR purification and hybridisation steps. PCR amplification and other time-intensive processing phases that require special apparatus to hold the amplified products are tedious but necessary when employing other methods of DNA typing, such as Sequenom (Sequenom, San Diego, CA, USA), Affymetrix (Affymetrix, High Wycombe, UK) and Illumina (Illumina, San Diego, CA, USA). Sequenom's MassARRAY technology incorporates a genotyping method based on the mass of the nucleotide added to the end of the primer. The mass is measured in a mass spectrometer using the MALDI-TOF technique (Jurinke, van den Boom, Cantor, & Köster, 2002). Given that this technique is based on an intrinsic property of the molecules — their absolute mass (Brookes, 1999)— it avoids the introduction of modifications at a molecular level, such as the fluorescent marking characteristic of other methods (Meyer & Ueland, 2011). Sequenom's MassARRAY has certain drawbacks; it involves an initial purification stage that makes automation difficult and requires elaborate equipment, increasing its cost (Dorak, 2006). The Affymetrix technology employs probes synthesised directly on a quartz wafer. Two types of array were described in the selected

publications: the Affymetrix Human Mapping 500K array set (including 500,000 SNPs) (Affymetrix & Inc, 2006) and the Affymetrix Genome-Wide Human SNP array 5.0 (including 500,568 SNPs) (Affymetrix, 2007). In addition, Affymetrix makes it possible to perform a genome-wide analysis in a single experiment (Kennedy et al., 2003; Thermo Fisher Scientific Inc., 2017). The Illumina Human OmniBeadChip, Illumina Human 610-Quav1_B BeadChip, and Illumina Inmunochip arrays are based on the Illumina Bead Array technology, which uses a fibreoptic or silica substrate onto which small silica beads (3 microns) are applied (Shen et al., 2005). Each bead is covered by hundreds of thousands of copies of a specific oligonucleotide, so that the fragments of DNA analysed will bind to the oligonucleotides if they possess the complementary region (Illumina, 2015). The Illumina Immunochip, the most recent of the three arrays, is a custom-made Illumina Infinium genotyping array that includes 196,524 SNPs, designed to perform deep replication and fine mapping of established GWAS loci from major inflammatory and autoimmune diseases (Cortes & Brown, 2011). However, Immunochip has the following weaknesses: The chip depends on the power of the initial GWAS for its marker selection and does not cover the whole genome. Second, Immunochip is designed for use in white European populations. Third, many rare variants are not represented on the chip because they have yet to be identified. Another weakness is that a proportion of very rare variants will probably not be accurately genotyped by the chip, although based on early indications, the chip performs this genotyping well (Cortes & Brown, 2011). Another Illumina array used in the included studies was the Illumina Exome 12v1 (in which12 denotes the number of samples that can be run on a single exome chip and v1 denotes version 1), hereafter referred to as an exome chip. Markers on exome chips are primarily exome SNPs (over 92% of the SNPs are in the exome) (Exome Chip Consortia, 2011). The exonic content consists of more than 240,000 markers representing diverse populations (European, African, Chinese, and Hispanic). The exome chip includes 196,524 SNPs and is designed to concentrate on rare variants rather than on common ones. The Affymetrix and Illumina arrays increase the genomic cover for genome-wide

analysis and for the detection of copy number variations (CNVs) compared with other methods (Illumina, 2015; Thermo Fisher Scientific Inc., 2017). In addition, both platforms have genotype calling algorithms.

The selection of one or another tool is determined by the number of SNPs and samples that they can analyse, and by their yield. The genotyping platforms most frequently employed in the studies selected for this review were TaqMan, Sequenom, and Illumina. TaqMan was one of the most common techniques employed, although it has a moderate yield and is limited in terms of the number of SNPs and samples that can be analysed (Ranade et al., 2001). Another tool used was Sequenom, which enables a moderate number of SNPs and samples to be studied, with a high yield (thousands of genotypes per day) (Jurinke et al., 2002). Illumina SNP arrays have a high capacity for the analysis of markers (including rare variants) and samples, and with a high yield (one million genotypes per day) (Shen et al., 2005). PCR and its variants, PCR-RFLP and PCR-SSP, were the second-most frequently used tools, despite their great limitations (Dorak, 2006). These were followed by Affymetrix SNP arrays, enabled to analyse a high number of SNPs and samples, and with a high yield (one million genotypes per day) (Kennedy et al., 2003). Like Illumina, Affymetrix allows creation of customized arrays incorporating desire markers, including rare variants. Another tool used was the nano-Invader, a miniaturised genotyping technique that, despite achieving good precision, sensitivity, speed, and yield in genotyping without PCR amplification, presents pipetting and evaporation issues due to the small sample volume used (Nomura, Kondo, Nagano, Matsui, & Egashira, 2007). Only two reports included in this review describe the use of this tool; both studies were written by the same group (Kobayashi et al., 2009a, 2009b). Later studies have used other genotyping tools, suggesting that because of the rapid advances in genotyping techniques, the nano-Invader method is less effective than its successors. Last in the list was SNPlex, with a high yield (thousands of genotypes per day) that allows the analysis of a moderate number of markers and samples (Tobler et al., 2005).

As described above, genotyping rare variants is a difficult process but an interesting approach to study risk of developing a disease such as PD. Today, the best approach to screen for novel variations and evaluate the contribution of rare variants to complex diseases is sequencing by NGS and custom arrays. However, conducting whole-genome sequencing in large cohorts using this sequencing technology remains economically prohibitive. Given that 98% of the human genome is noncoding, and therefore more difficult to interpret, whole-exome sequencing methods were developed to sequence only the coding regions of the genome (exome). The Affymetrix and Illumina platforms introduced the Affymetrix Axiom exome array and the exome chip, respectively, as an affordable alternative to exome sequencing. The Axiom exome array and the exome chip only enable researchers to test a predetermined set of variants. In the study performed by Schaefer et al. in 2014 (Schaefer et al., 2014), included in the present review, a significant association was found between the rare variant rs62481981 at the *IRF5* gene after covariate adjustment (p=.0012; 801 AgP, 1476 controls). However, the association lost significance after correction for multiple testing in the replication. Schaefer et al. genotyped all candidate genes with the Illumina Immunochip. The authors noted that the lack of statistical power was not sufficient to provide evidence of the association of less frequent variants.

Therefore, due to inappropriate sample recruitment (lack of a universally accepted diagnostic criterion for periodontitis, the small sample size studied, and not taking into account ethnicity differences and environmental effects) and the poor study designs used to investigate genetic susceptibility (CGAS approach with low sample size, not adjusting for covariates, no study of rare variants, and no replication of the results in an independent sample), few true genetic associations for PD have been identified.

Conclusions

There are a wide variety of definitions and variables analysed to establish a diagnosis of PD. In addition, studies on the genetic susceptibility of PD use different criteria for

selecting participants. All these factors make the selection of a single methodological strategy for the analysis of genetic susceptibility to PD a challenging task.

Despite their inability to identify new or unexpected loci associated with PD in the genome and their other limitations, candidate gene studies continue to be the most widely employed to analyse genetic susceptibility to PD. However, with advances in scientific knowledge and genotyping technologies, CGSs are gradually being replaced by GWASs. No optimal genotyping tool yet exists for the genetic study of PD. The genotyping technology to be used must be considered; but also, depending on the specific human population that will be studied, the SNPs selected by each platform must also be taken into consideration. Therefore, despite the intrinsic limitations of SNP arrays, those that enable evaluation of specific rare population variants could be the most suitable. However, no studies on PD with a GWAS design that take into account rare variants have been published to date.

Future studies should therefore apply strict, validated diagnostic criteria for PD, using large study cohorts, adjusting the results according to the relevant risk factors, and including an analysis of multiple genes and polymorphisms in combination with rare variants.

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REFERENCES

Affymetrix, I. (2007). Data Sheet, Genome-Wide Human SNP Array 5.0. Retrieved from http://tools.thermofisher.com/content/sfs/brochures/genomewide_snp5_datasheet.pdf

Affymetrix, & Inc. (2006). Data Sheet, GeneChip® Human Mapping 500K Array Set. Retrieved from https://tools.thermofisher.com/content/sfs/brochures/500k_datasheet.pdf

Albandar, J. M., & Tinoco, E. M. B. (2002). Global epidemiology of periodontal diseases in children and young persons. *Periodontology 2000*, *29*, 153–76. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12102707

American Academy of Periodontology Task Force Report on the Update to the 1999 Classification of Periodontal Diseases and Conditions. (2015). *Journal of Periodontology*, *86*(7), 835–838. https://doi.org/10.1902/jop.2015.157001

Armitage, G. C. (1999). Development of a classification system for periodontal diseases and conditions. *Northwest Dentistry*, *79*(6), 31–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11413609

Atanasovska-Stojanovska, A., Popovska, M., Trajkov, D., & Spiroski, M. (2013). IL1 cluster gene polymorphisms in Macedonian patients with chronic periodontitis. *Bratislavske Lekarske Listy*, *114*(7), 380–385. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23822621

Auer, P. L., & Lettre, G. (2015). Rare variant association studies: considerations, challenges and opportunities. *Genome Medicine*, *7*(1), 16. https://doi.org/10.1186/s13073-015-0138-2

Benjamin, S. D., & Baer, P. N. (1967). Familial patterns of advanced alveolar bone loss in adolescence (periodontosis). *Periodontics*, *5*(2), 82–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/5228312

Berger, K. (2012). [DHS: The Dortmund health study]. *Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz*, *55*(6–7), 816–21. https://doi.org/10.1007/s00103-012-1492-5

Brett, P. M., Zygogianni, P., Griffiths, G. S., Tomaz, M., Parkar, M., D'Aiuto, F., & Tonetti, M. (2005). Functional Gene Polymorphisms in Aggressive and Chronic Periodontitis. *Journal of Dental Research*, *84*(12), 1149–1153. https://doi.org/10.1177/154405910508401211

Brookes, A. J. (1999). The essence of SNPs. *Gene*, *234*(2), 177–86. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10395891

Bugert, P., Lese, A., Meckies, J., Zieger, W., Eichler, H., & Klüter, H. (2003). Optimized sensitivity of allele-specific PCR for prenatal typing of human platelet alloantigen single nucleotide polymorphisms. *BioTechniques*, *35*(1), 170–4. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12866418

Burgner, D., Jamieson, S. E., & Blackwell, J. M. (2006). Genetic susceptibility to infectious diseases: big is beautiful, but will bigger be even better? *The Lancet. Infectious Diseases*, *6*(10), 653–63. https://doi.org/10.1016/S1473-3099(06)70601-6

Centre for Reviews and Dissemination, University of York. (2008). Retrieved November 16, 2017, from https://bmchealthservres.biomedcentral.com/articles/10.1186/s12913-017-2703-4

Chai, L., Song, Y.-Q., Zee, K.-Y., & Leung, W. K. (2010a). Single nucleotide polymorphisms of complement component 5 and periodontitis. *Journal of Periodontal Research*, *45*(3), 301–308. https://doi.org/10.1111/j.1600-0765.2009.01234.x

Chai, L., Song, Y.-Q., Zee, K.-Y., & Leung, W. K. (2010b). SNPs of Fc-gamma Receptor Genes and Chronic Periodontitis. *Journal of Dental Research*, *89*(7), 705–710. https://doi.org/10.1177/0022034510365444

Cirulli, E. T., & Goldstein, D. B. (2010). Uncovering the roles of rare variants in common disease through whole-genome sequencing. *Nature Reviews. Genetics*, *11*(6), 415–25. https://doi.org/10.1038/nrg2779

Cortes, A., & Brown, M. A. (2011). Promise and pitfalls of the Immunochip. *Arthritis Research & Therapy*, *13*(1), 101. https://doi.org/10.1186/ar3204

de Jong, T. M. H., Jochens, A., Jockel-Schneider, Y., Harks, I., Dommisch, H., Graetz, C., … Schaefer, A. S. (2014). SLC23A1 polymorphism rs6596473 in the vitamin C transporter SVCT1 is associated with aggressive periodontitis. *Journal of Clinical*

Periodontology, *41*(6), 531–540. https://doi.org/10.1111/jcpe.12253

Divaris, K., Monda, K. L., North, K. E., Olshan, A. F., Lange, E. M., Moss, K., … Offenbacher, S. (2012). Genome-wide Association Study of Periodontal Pathogen Colonization. *Journal of Dental Research*, *91*(7_suppl), S21–S28. https://doi.org/10.1177/0022034512447951

Divaris, K., Monda, K. L., North, K. E., Olshan, A. F., Reynolds, L. M., Hsueh, W.-C., … Offenbacher, S. (2013). Exploring the genetic basis of chronic periodontitis: a genome-wide association study. *Human Molecular Genetics*, *22*(11), 2312–2324. https://doi.org/10.1093/hmg/ddt065

Dorak, T. M. (2006). *Real-time PCR*. (M. Tevfik Dorak, Ed.), *Real-time PCR* (ilustrada,). Oxford: Taylor & Francis. https://doi.org/10.4016/17251.01

Exome Chip Consortia. (2011). Exome Chip Design Wiki Site. Retrieved January 25, 2018, from https://genome.sph.umich.edu/wiki/Exome_Chip_Design

Feng, P., Wang, X., Casado, P. L., Küchler, E. C., Deeley, K., Noel, J., … Vieira, A. R. (2014). Genome wide association scan for chronic periodontitis implicates novel locus. *BMC Oral Health*, *14*(1), 84. https://doi.org/10.1186/1472-6831-14-84

Fiebig, A., Jepsen, S., Loos, B. G., Scholz, C., Schäfer, C., Rühling, A., … Grössner-Schreiber, B. (2008). Polymorphisms in the interleukin-1 (IL1) gene cluster are not associated with aggressive periodontitis in a large Caucasian population. *Genomics*, *92*(5), 309–315. https://doi.org/10.1016/j.ygeno.2008.07.004

Flemmig, T. F. (1999). Periodontitis. *Annals of Periodontology*, *4*(1), 32–37. https://doi.org/10.1902/annals.1999.4.1.32

Freitag-Wolf, S., Dommisch, H., Graetz, C., Jockel-Schneider, Y., Harks, I., Staufenbiel, I., … Schaefer, A. S. (2014). Genome-wide exploration identifies sex-specific genetic effects of alleles upstream NPY to increase the risk of severe periodontitis in men. *Journal of Clinical Periodontology*, *41*(12), 1115–1121. https://doi.org/10.1111/jcpe.12317

Garlet, G. P., Horwat, R., Ray, H. L., Garlet, T. P., Silveira, E. M., Campanelli, A. P., … Silva, R. M. (2012). Expression Analysis of Wound Healing Genes in Human Periapical Granulomas of Progressive and Stable Nature. *Journal of Endodontics*, *38*(2), 185–190. https://doi.org/10.1016/j.joen.2011.09.011

Gunji, T., Onouchi, Y., Nagasawa, T., Katagiri, S., Watanabe, H., Kobayashi, H., … Ishikawa, I. (2007). Functional polymorphisms of the FPR1 gene and aggressive periodontitis in Japanese. *Biochemical and Biophysical Research Communications*, *364*(1), 7–13. https://doi.org/10.1016/j.bbrc.2007.09.105

Hart, T. C., Marazita, M. L., & Wright, J. T. (2000). The impact of molecular genetics on oral health paradigms. *Critical Reviews in Oral Biology and Medicine: An Official Publication of the American Association of Oral Biologists*, *11*(1), 26–56. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10682900

Hong, K.-W., Shin, M.-S., Ahn, Y.-B., Lee, H.-J., & Kim, H.-D. (2015). Genomewide association study on chronic periodontitis in Korean population: results from the Yangpyeong health cohort. *Journal of Clinical Periodontology*, *42*(8), 703–710. https://doi.org/10.1111/jcpe.12437

Illumina. (2015). BeadArray Microarray Technology. Retrieved November 16, 2017, from https://www.illumina.com/science/technology/beadarray-technology.html

Jurinke, C., van den Boom, D., Cantor, C. R., & Köster, H. (2002). The use of MassARRAY technology for high throughput genotyping. *Advances in Biochemical Engineering/biotechnology*, *77*, 57–74. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12227737

Kennedy, G. C., Matsuzaki, H., Dong, S., Liu, W., Huang, J., Liu, G., … Jones, K. W. (2003). Large-scale genotyping of complex DNA. *Nature Biotechnology*, *21*(10), 1233–1237. https://doi.org/10.1038/nbt869

Kinane, D. F., & Chestnutt, I. G. (2000). Smoking and periodontal disease. *Critical Reviews in Oral Biology and Medicine: An Official Publication of the American Association of Oral Biologists*, *11*(3), 356–65. Retrieved from

http://www.ncbi.nlm.nih.gov/pubmed/11021635
Kobayashi, T., Murasawa, A., Ito, S., Yamamoto, K., Komatsu, Y., Abe, A., … Yoshie, H. (2009a). Cytokine Gene Polymorphisms Associated With Rheumatoid Arthritis and Periodontitis in Japanese Adults. *Journal of Periodontology*, *80*(5), 792–799. https://doi.org/10.1902/jop.2009.080573
Kobayashi, T., Nagata, T., Murakami, S., Takashiba, S., Kurihara, H., Izumi, Y., … Yoshie, H. (2009b). Genetic risk factors for periodontitis in a Japanese population. *Journal of Dental Research*, *88*(12), 1137–1141. https://doi.org/10.1177/0022034509350037
Kornman, K. S., Crane, A., Wang, H. Y., di Giovine, F. S., Newman, M. G., Pirk, F. W., … Duff, G. W. (1997). The interleukin-1 genotype as a severity factor in adult periodontal disease. *Journal of Clinical Periodontology*, *24*(1), 72–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9049801
Laine, M. L., Crielaard, W., & Loos, B. G. (2012). Genetic susceptibility to periodontitis. *Periodontology 2000*, *58*(1), 37–68. https://doi.org/10.1111/j.1600-0757.2011.00415.x
Lang, N., Bartold, P., Cullinan, M., Jeffcoat, M., Mombelli, A., & Murakami, S. (1999). International classification workshop. Consensus report: Aggressive periodontitis. *Ann Periodontol*, *4*, 53. Retrieved from https://www.scienceopen.com/document?vid=f2290b73-7a95-4540-94eb-609d8fe3ad06
Leroy, R., Eaton, K. A., & Savage, A. (2010). Methodological issues in epidemiological studies of periodontitis--how can it be improved? *BMC Oral Health*, *10*(1), 8. https://doi.org/10.1186/1472-6831-10-8
Letra, A., Silva, R. M., Rylands, R. J., Silveira, E. M., de Souza, A. P., Wendell, S. K., … Vieira, A. R. (2012). MMP3 and TIMP1 variants contribute to chronic periodontitis and may be implicated in disease progression. *Journal of Clinical Periodontology*, *39*(8), 707–716. https://doi.org/10.1111/j.1600-051X.2012.01902.x
Lin, C.-F., Naj, A. C., & Wang, L.-S. (2013). Analyzing copy number variation using SNP array data: protocols for calling CNV and association tests. *Current Protocols in Human Genetics*, *79*, Unit 1.27. https://doi.org/10.1002/0471142905.hg0127s79
Marazita, M. L., Burmeister, J. A., Gunsolley, J. C., Koertge, T. E., Lake, K., & Schenkein, H. A. (1994). Evidence for autosomal dominant inheritance and race-specific heterogeneity in early-onset periodontitis. *Journal of Periodontology*, *65*(6), 623–630. https://doi.org/10.1902/jop.1994.65.6.623
Merikangas, A. K., Corvin, A. P., & Gallagher, L. (2009). Copy-number variants in neurodevelopmental disorders: promises and challenges. *Trends in Genetics : TIG*, *25*(12), 536–544. https://doi.org/10.1016/j.tig.2009.10.006
Meyer, K., & Ueland, P. M. (2011). Use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for multiplex genotyping. *Advances in Clinical Chemistry*, *53*, 1–29. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21404912
Michalowicz, B. S., Aeppli, D. P., Kuba, R. K., Bereuter, J. E., Conry, J. P., Segal, N. L., … Pihlstrom, B. L. (1991). A twin study of genetic variation in proportional radiographic alveolar bone height. *Journal of Dental Research*, *70*(11), 1431–5. https://doi.org/10.1177/00220345910700110701
Michalowicz, B. S., Aeppli, D., Virag, J. G., Klump, D. G., Hinrichs, E., Segal, N. L., … Pihlstrom, B. L. (1991). Periodontal Findings in Adult Twins. *Journal of Periodontology*, *62*(5), 293–299. https://doi.org/10.1902/jop.1991.62.5.293
Michalowicz, B. S., Diehl, S. R., Gunsolley, J. C., Sparks, B. S., Brooks, C. N., Koertge, T. E., … Schenkein, H. A. (2000). Evidence of a Substantial Genetic Basis for Risk of Adult Periodontitis. *Journal of Periodontology*, *71*(11), 1699–1707. https://doi.org/10.1902/jop.2000.71.11.1699
Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., & PRISMA Group. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Annals of Internal Medicine*, *151*(4), 264–9, W64. Retrieved from

http://www.ncbi.nlm.nih.gov/pubmed/19622511
Moher, D., Pham, B., Lawson, M. L., & Klassen, T. P. (2003). The inclusion of reports of randomised trials published in languages other than English in systematic reviews. *Health Technology Assessment (Winchester, England)*, *7*(41), 1–90. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14670218
Müller, N., Schulte, D. M., Türk, K., Freitag-Wolf, S., Hampe, J., Zeuner, R., … Laudes, M. (2015). IL-6 blockade by monoclonal antibodies inhibits apolipoprotein (a) expression and lipoprotein (a) synthesis in humans. *Journal of Lipid Research*, *56*(5), 1034–42. https://doi.org/10.1194/jlr.P052209
Munz, M., Willenborg, C., Richter, G. M., Jockel-Schneider, Y., Graetz, C., Staufenbiel, I., … Schaefer, A. S. (2017). A genome-wide association study identifies nucleotide variants at SIGLEC5 and DEFA1A3 as risk loci for periodontitis. *Human Molecular Genetics*, *26*(13), 2577–2588. https://doi.org/10.1093/hmg/ddx151
Newton-Cheh, C., & Hirschhorn, J. N. (2005). Genetic association studies of complex traits: design and analysis issues. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *573*(1–2), 54–69. https://doi.org/10.1016/j.mrfmmm.2005.01.006
Nibali, L. (2013). Suggested guidelines for systematic reviews of periodontal genetic association studies. *Journal of Clinical Periodontology*, *40*(8), 753–6. https://doi.org/10.1111/jcpe.12128
Nibali, L., Di Iorio, A., Tu, Y.-K., & Vieira, A. R. (2017). Host genetics role in the pathogenesis of periodontal disease and caries. *Journal of Clinical Periodontology*, *44*, S52–S78. https://doi.org/10.1111/jcpe.12639
Nisticò, L., Buzzetti, R., Pritchard, L. E., Van der Auwera, B., Giovannini, C., Bosi, E., … Todd, J. A. (1996). The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. Belgian Diabetes Registry. *Human Molecular Genetics*, *5*(7), 1075–80. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8817351
Nomura, S., Kondo, M., Nagano, M., Matsui, K., & Egashira, T. (2007). Development of a novel nano-Invader DNA chip system. *Journal of Biochemical and Biophysical Methods*, *70*(5), 787–95. https://doi.org/10.1016/j.jbbm.2007.05.012
Offenbacher, S., Divaris, K., Barros, S. P., Moss, K. L., Marchesan, J. T., Morelli, T., … North, K. E. (2016). Genome-wide association study of biologically informed periodontal complex traits offers novel insights into the genetic basis of periodontal disease. *Human Molecular Genetics*, *25*(10), 2113–2129. https://doi.org/10.1093/hmg/ddw069
Pham, B., Klassen, T. P., Lawson, M. L., & Moher, D. (2005). Language of publication restrictions in systematic reviews gave different results depending on whether the intervention was conventional or complementary. *Journal of Clinical Epidemiology*, *58*(8), 769–76. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16086467
Plomin, R., Haworth, C. M. A., & Davis, O. S. P. (2010). Common disorders are quantitative traits. *Nature Reviews Genetics*, *10*(12), 872. https://doi.org/10.1038/nrg2670
Pritchard, J. K. (2001). Are Rare Variants Responsible for Susceptibility to Complex Diseases? *The American Journal of Human Genetics*, *69*(1), 124–137. https://doi.org/10.1086/321272
Ranade, K., Chang, M. S., Ting, C. T., Pei, D., Hsiao, C. F., Olivier, M., … Botstein, D. (2001). High-throughput genotyping with single nucleotide polymorphisms. *Genome Research*, *11*(7), 1262–8. https://doi.org/10.1101/gr.157801
Reich, D. E., & Lander, E. S. (2001). On the allelic spectrum of human disease. *Trends in Genetics: TIG*, *17*(9), 502–10. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11525833
Rhodin, K., Divaris, K., North, K. E., Barros, S. P., Moss, K., Beck, J. D., & Offenbacher, S. (2014). Chronic Periodontitis Genome-wide Association Studies. Gene-centric and gene set enrichment analyses. *Journal of Dental Research*, *93*(9), 882–890.

https://doi.org/10.1177/0022034514544506
Salvi, G. E., Carollo-Bittel, B., & Lang, N. P. (2008). Effects of diabetes mellitus on periodontal and peri-implant conditions: update on associations and risks. *Journal of Clinical Periodontology*, *35*(8 Suppl), 398–409. https://doi.org/10.1111/j.1600-051X.2008.01282.x
Sanders, A. E., Sofer, T., Wong, Q., Kerr, K. F., Agler, C., Shaffer, J. R., … Divaris, K. (2017). Chronic Periodontitis Genome-wide Association Study in the Hispanic Community Health Study / Study of Latinos. *Journal of Dental Research*, *96*(1), 64–72. https://doi.org/10.1177/0022034516664509
Saxén, L., & Nevanlinna, H. R. (1984). Autosomal recessive inheritance of juvenile periodontitis: test of a hypothesis. *Clinical Genetics*, *25*(4), 332–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6713708
Scapoli, C., Borzani, I., Guarnelli, M. E., Mamolini, E., Annunziata, M., Guida, L., & Trombelli, L. (2010). IL-1 Gene Cluster is Not Linked to Aggressive Periodontitis. *Journal of Dental Research*, *89*(5), 457–461. https://doi.org/10.1177/0022034510363232
Scapoli, C., Mamolini, E., Carrieri, A., Guarnelli, M. E., Annunziata, M., Guida, L., … Trombelli, L. (2011). Gene--gene interaction among cytokine polymorphisms influence susceptibility to aggressive periodontitis. *Genes and Immunity*, *12*(6), 473–480. https://doi.org/10.1038/gene.2011.28
Schaefer, A. S., Bochenek, G., Jochens, A., Ellinghaus, D., Dommisch, H., Güzeldemir-Akçakanat, E., … Schreiber, S. (2015). Genetic Evidence for *PLASMINOGEN* as a Shared Genetic Risk Factor of Coronary Artery Disease and PeriodontitisCLINICAL PERSPECTIVE. *Circulation: Cardiovascular Genetics*, *8*(1), 159–167. https://doi.org/10.1161/CIRCGENETICS.114.000554
Schaefer, A. S., Bochenek, G., Manke, T., Nothnagel, M., Graetz, C., Thien, A., … Schreiber, S. (2013). Validation of reported genetic risk factors for periodontitis in a large-scale replication study. *Journal of Clinical Periodontology*, *40*(6), 563–572. https://doi.org/10.1111/jcpe.12092
Schaefer, A. S., Jepsen, S., & Loos, B. G. (2011). Periodontal genetics: a decade of genetic association studies mandates better study designs. *Journal of Clinical Periodontology*, *38*(2), 103–7. https://doi.org/10.1111/j.1600-051X.2010.01653.x
Schaefer, A. S., Jochens, A., Dommisch, H., Graetz, C., Jockel-Schneider, Y., Harks, I., … Loos, B. G. (2014). A large candidate-gene association study suggests genetic variants at IRF5 and PRDM1 to be associated with aggressive periodontitis. *Journal of Clinical Periodontology*, *41*(12), 1122–31. https://doi.org/10.1111/jcpe.12314
Schaefer, A. S., Richter, G. M., Dommisch, H., Reinartz, M., Nothnagel, M., Noack, B., … Schreiber, S. (2011). CDKN2BAS is associated with periodontitis in different European populations and is activated by bacterial infection. *Journal of Medical Genetics*, *48*(1), 38–47. https://doi.org/10.1136/jmg.2010.078998
Schaefer, A. S., Richter, G. M., Nothnagel, M., Laine, M. L., Rühling, A., Schäfer, C., … Schreiber, S. (2010b). A 3’ UTR transition within DEFB1 is associated with chronic and aggressive periodontitis. *Genes and Immunity*, *11*(1), 45–54. https://doi.org/10.1038/gene.2009.75
Schaefer, A. S., Richter, G. M., Nothnagel, M., Manke, T., Dommisch, H., Jacobs, G., … Schreiber, S. (2010a). A genome-wide association study identifies GLT6D1 as a susceptibility locus for periodontitis. *Human Molecular Genetics*, *19*(3), 553–562. https://doi.org/10.1093/hmg/ddp508
Schmermund, A., Möhlenkamp, S., Stang, A., Grönemeyer, D., Seibel, R., Hirche, H., … Erbel, R. (2002). Assessment of clinically silent atherosclerotic disease and established and novel risk factors for predicting myocardial infarction and cardiac death in healthy middle-aged subjects: rationale and design of the Heinz Nixdorf RECALL Study. Risk Factors, Evaluation of Coronary Calcium and Lifestyle. *American Heart Journal*, *144*(2), 212–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12177636

Shaffer, J. R., Polk, D. E., Wang, X., Feingold, E., Weeks, D. E., Lee, M.-K., … Marazita, M. L. (2014). Genome-Wide Association Study of Periodontal Health Measured by Probing Depth in Adults Ages 18−49 years. *G3: genes - genomes - genetics*, *4*(2), 307–314. https://doi.org/10.1534/g3.113.008755

Shang, D., Dong, L., Zeng, L., Yang, R., Xu, J., Wu, Y., … Zhang, N. (2016). Two-stage comprehensive evaluation of genetic susceptibility of common variants in FBXO38, AP3B2 and WHAMM to severe chronic periodontitis. *Scientific Reports*, *5*(1), 17882. https://doi.org/10.1038/srep17882

Shen, R., Fan, J.-B., Campbell, D., Chang, W., Chen, J., Doucet, D., … Oliphant, A. (2005). High-throughput SNP genotyping on universal bead arrays. *Mutation Research*, *573*(1–2), 70–82. https://doi.org/10.1016/j.mrfmmm.2004.07.022

Shimizu, S., Momozawa, Y., Takahashi, A., Nagasawa, T., Ashikawa, K., Terada, Y., … Furuichi, Y. (2015). A Genome-wide Association Study of Periodontitis in a Japanese Population, *94*(4), 555–561. https://doi.org/10.1177/0022034515570315

Song, L., Yao, J., He, Z., & Xu, B. (2015). Genes related to inflammation and bone loss process in periodontitis suggested by bioinformatics methods. *BMC Oral Health*, *15*(1), 105. https://doi.org/10.1186/s12903-015-0086-7

Stabholz, A., Soskolne, W. A., & Shapira, L. (2010). Genetic and environmental risk factors for chronic periodontitis and aggressive periodontitis. *Periodontology 2000*, *53*(1), 138–153. https://doi.org/10.1111/j.1600-0757.2010.00340.x

Stefansson, H., Petursson, H., Sigurdsson, E., Steinthorsdottir, V., Bjornsdottir, S., Sigmundsson, T., … Stefansson, K. (2002). Neuregulin 1 and Susceptibility to Schizophrenia. *The American Journal of Human Genetics*, *71*(4), 877–892. https://doi.org/10.1086/342734

Suzuki, A., Ji, G., Numabe, Y., Muramatsu, M., Gomi, K., Kanazashi, M., … Kamoi, K. (2004). Single nucleotide polymorphisms associated with aggressive periodontitis and severe chronic periodontitis in Japanese. *Biochemical and Biophysical Research Communications*, *317*(3), 887–892. https://doi.org/10.1016/j.bbrc.2004.03.126

Teumer, A., Holtfreter, B., Völker, U., Petersmann, A., Nauck, M., Biffar, R., … Kocher, T. (2013). Genome-wide association study of chronic periodontitis in a general German population. *Journal of Clinical Periodontology*, *40*(11), 977–985. https://doi.org/10.1111/jcpe.12154

Thermo Fisher Scientific Inc. (2017). Affymetrix. Microarray Analysis. Retrieved November 16, 2017, from https://www.thermofisher.com/es/es/home/life-science/microarray-analysis/affymetrix.html?category=34003&categoryIdClicked=34003&rootCategoryId=34003&navMode=34003&aId=aboutNav

Tobler, A. R., Short, S., Andersen, M. R., Paner, T. M., Briggs, J. C., Lambert, S. M., … Wenz, H. M. (2005). The SNPlex genotyping system: a flexible and scalable platform for SNP genotyping. *Journal of Biomolecular Techniques: JBT*, *16*(4), 398–406. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16522862

Tonetti, M. S., Claffey, N., & European Workshop in Periodontology group C. (2005). Advances in the progression of periodontitis and proposal of definitions of a periodontitis case and disease progression for use in risk factor research. Group C consensus report of the 5th European Workshop in Periodontology. *Journal of Clinical Periodontology*, *32 Suppl 6*(s6), 210–3. https://doi.org/10.1111/j.1600-051X.2005.00822.x

Torres de Heens, G. L., Loos, B. G., & van der Velden, U. (2010). Monozygotic twins are discordant for chronic periodontitis: clinical and bacteriological findings. *Journal of Clinical Periodontology*, *37*(2), 120–128. https://doi.org/10.1111/j.1600-051X.2009.01511.x

Wilkening, S., Chen, B., Bermejo, J. L., & Canzian, F. (2009). Is there still a need for candidate gene approaches in the era of genome-wide association studies? *Genomics*, *93*(5), 415–9. https://doi.org/10.1016/j.ygeno.2008.12.011

Zhang, N., Xu, Y., Zhang, B. B., Zhang, T., Yang, H., Zhang, B. B., … Zhong, D. (2014). Analysis of interleukin-8 gene variants reveals their relative importance as genetic

susceptibility factors for chronic periodontitis in the Han population. *PloS One*, *9*(8), e104436. https://doi.org/10.1371/journal.pone.0104436

Zupin, L., Navarra, C. O., Robino, A., Bevilacqua, L., Di Lenarda, R., Gasparini, P., & Crovella, S. (2017). NLRC5 polymorphism is associated with susceptibility to chronic periodontitis. *Immunobiology*, *222*(5), 704–708. https://doi.org/10.1016/j.imbio.2017.01.001

FIGURE LEGEND

Figure1. Flow diagram of the complete study selection process and the results obtained at each stage.

Table(s)

TABLES

Table 1. Demographic characteristics of the study populations analysed in the articles included in the review.

Reference	Cases				Controls			Ethnic Group (Country)	Smoking	Diabetes
	Diagnosis	n	Origin	Age (years)	n	Origin	Age (years)			
(Suzuki et al., 2004)	AgP	134	U and Col	40 ± 11	125	U and Col	40 ± 15	Asian (Japan)	NS	NS
	CP	117	U and Col	54 ± 9						
(Brett et al., 2005)	AgP*	51	H	NS	100	Pop	NS	Caucasian (United Kingdom)	NS	NS
	CP*	57	H	NS						
(Fiebig et al., 2008)	AgP	415	U	30 ± 2	874	Pop	48 ± 4	Caucasian (Germany and The Netherlands)	S	S*
(Gunji et al., 2007)	AgP*	25	U	33 ± 6	349	U	NS	Asian (Japan)	NS	NS
(Kobayashi et al., 2009a)	PD*	117	DC, H and U	52 ± 1	108	NS	51 ± 1	Asian (Japan)	S	NS
(Kobayashiet al., 2009b)	AgP*	172	H	32 ± 6	303	H	38 ± 13	Asian (Japan)	S	S*
	CP*	147	H	53 ± 7						
(Schaefer et al., 2010a)	AgP	283	Pop	30 ± 5	972	H	51 ± NS	Caucasian (Germany)	S	S
(Schaefer et al., 2010b)	AgP*	532	Pop	33 ± NS	1472	Pop	42 ± NS	Caucasian (Germany and The Netherlands)	S	S
	CP*	805	Pop	54 ± NS	319	Pop	43 ± NS			
(Scapoli et al., 2010)	AgP†	95	U and RC	43 ± 8	121	Col	30 ± 5	Caucasian (Italy)	NS	NS
(Chai et al., 2010a)	PD*	229	DC, H and U	44 ± 7	207	DC, H and U	42 ± 8	Asian (China)	S	S*
(Chai et al., 2010b)	CP‡	190	DC, H and U	46 ± 5	169	DC, H and U	45 ± 6	Asian (China)	S	S*
(Scapoli et al., 2011)	AgP†	122	U and RC	43 ± 7	246	U	30 ± 8	Caucasian (Italy)	NS	NS
(Schaefer et al., 2011)	AgP*	159	Pop	34 ± 5	421	Pop	32 ± 9	Caucasian (The Netherlands)	S	S
	CP*	154	Pop	45 ± 10		Pop				
(Letra et al., 2012)	CP	169	U and Col	50 ± NS	506	U and Col	46 ± NS	Caucasian (Brazil and USA)	NS	NS
(Atanasovska-Stojanovska et al., 2013)	CP*	114	U	39 ± 10	301	U	35 ± 10	NS (Macedonia)	NS	NS
(Schaefer et al., 2013)	AgP	600	Pop	NS	1448	Pop	NS	Caucasian (Germany)	S	NS

(de Jong et al., 2014)	AgP	283	Pop	30 ± 5	979	Pop	51 ± 13	Caucasian (Germany)	S	S
(Feng et al., 2014)	CP	712	U	57 ± NS	1973	U	55 ± NS	Caucasian, African and other (Brazil and USA)	S	S
(Schaefer et al., 2014)	AgP	828	Pop	NS	2119	Pop and U	NS	Caucasian (Germany and The Netherlands)	S	NS
(Zhang et al., 2014)	CP*	400	Pop	50 ± 9	750	Pop	50 ± 8	Asian (China)	S*	S
(Schaefer et al., 2015)	AgP	1042	U	NS	3094	Pop	NS	Caucasian (Germany and The Netherlands)	S	S*
(Shang et al., 2016)	CP	471	H	NS	1312	Pop	NS	Asian (China)	S	S*
(Offenbacher et al., 2016)	AgP	717	U	NS	4210	Pop	NS	Caucasian (Germany)	S	S
(Zupin et al., 2017)	CP*	399	Pop	NS	148	Pop	NS	Caucasian (Italy)	S	NS
(Munz et al., 2017)	AgP	1116	H and U	NS	7179	Pop	NS	Caucasian (Germany, The Netherlands and Turkey)	S	NS

Abbreviations: PD, periodontal disease; AgP, aggressive periodontitis; CP, chronic periodontitis; S, specified; NS, not specified. S*, specified as exclusion criterion; U, university; H, hospital; DC, dental clinic; RC, research centre; Col, College of Dentists; Pop, general population.
*Armitage, 1999 (Armitage, 1999).
†Lang et al., 1999 (Lang et al., 1999).
‡ Fleming, 1999 (Flemmig, 1999).
Age is expressed as mean ± SD.

Table 2. Clinical parameters recorded in publications included in the review.

Reference	Clinical parameters				
	PPD	**CAL**	**BOP**	**Alveolar bone loss**	**Others**
(Suzuki et al., 2004)	R	NR	R	R: On x-rays	Degree of tooth mobility
(Brett et al., 2005)	NR	NR	NR	NR	X-rays and 6-point periodontal probing
(Fiebig et al., 2008)	NR	NR	NR	R: On x-rays	Number of teeth present
(Gunji et al., 2007)	R	NR	NR	NR	Number of natural teeth present
(Kobayashi et al., 2009a)	R	R	R	NR	Number of missing teeth, accumulation of supragingival plaque, mean distance from the free gingival margin to bottom of pocket and mean distance from the cement-enamel junction to bottom of pocket
(Kobayashiet al., 2009b)	R	R	R	R: On x-rays (2 sites per tooth)	Number of natural teeth, presence/absence of supragingival plaque (4 sites per tooth)
(Schaefer et al., 2010a)	NR	NR	NR	R: On x-rays	NR
(Schaefer et al., 2010b)	R	R	R	R: On x-rays and orthopantomography	Number of natural teeth and of exposed furcations
(Scapoli et al., 2010)	NR	NR	NR	NR	Loss of interproximal insertion > 2 mm on probing of any tooth
(Chai et al., 2010a)	R	R	R	R: On orthopantomography	NR
(Chai et al., 2010b)	R	R	R	NR	Number of natural teeth
(Scapoli et al., 2011)	NR	NR	NR	NR	Interproximal insertion loss > 2 mm on probing of any tooth
(Schaefer et al., 2011)	R	R	R	R: On x-rays and orthopantomography	Number of natural teeth and of exposed furcations
(Letra et al., 2012)	R	R	NR	NR	NR
(Atanasovska-Stojanovska et al., 2013)	R	R	R	NR	Löe and Silness gingival index
(Schaefer et al., 2013)	NR	NR	NR	R: On x-rays	NR
(de Jong et al., 2014)	NR	NR	NR	R	NR
(Feng et al., 2014)	NR	R	NR	NR	NR
(Schaefer et al., 2014)	NR	NR	NR	R: On x-rays	Number of natural teeth and of exposed furcations
(Zhang et al., 2014)	R	R	R	R: Vertical or horizontal loss of alveolar bone	Degree of gingival recession, tooth mobility and chronic gingival bleeding
(Schaefer et al., 2015)	NR	NR	NR	R: On x-rays and orthopantomography	NR
(Shang et al., 2016)	R	R	NR	NR	NR
(Offenbacher et al., 2016)	R	R	R	NR	Number of missing teeth, gingival index and plaque index
(Zupin et al., 2017)	R	R	R	NR	X-rays, number of teeth present, O´Leary plaque index and gingival recession
(Munz et al., 2017)	NR	NR	NR	NR	NR

Abbreviations: R, recorded; NR, not recorded; PPD, probing pocket depth; CAL, clinical attachment loss; BOP, bleeding on probing.

Table 3. Genome-wide association studies (GWASs) not included in the review and reasons for their exclusion.

Reference	Reasons for Exclusion
(Divaris et al., 2012)	Investigation of the susceptibility loci for colonisation with periodontal microbiota.
(Divaris et al., 2013)	The control group was formed of individuals with mild periodontitis.
(Teumer et al., 2013)	The control group was formed of individuals with mild periodontitis.
(Rhodin et al., 2014)	The control group was formed of individuals with mild periodontitis.
(Shaffer et al., 2014)	Not a case-control study. Analysis of two distinct phenotypes related to CP.
(Freitag-Wolf et al., 2014)	Selected only sex-specific SNPs.
(Hong et al., 2015)	The control group was formed of individuals with a healthy periodontium or mild periodontitis.
(Shimizu et al., 2015)	The control group was formed of individuals with systemic diseases (cerebral aneurysm, oesophageal cancer, endometrial cancer, chronic obstructive pulmonary disease or glaucoma).
(Sanders et al., 2017)	Not a case-control study.

Table 4. Characteristics of the genetic analysis in the articles included in the review.

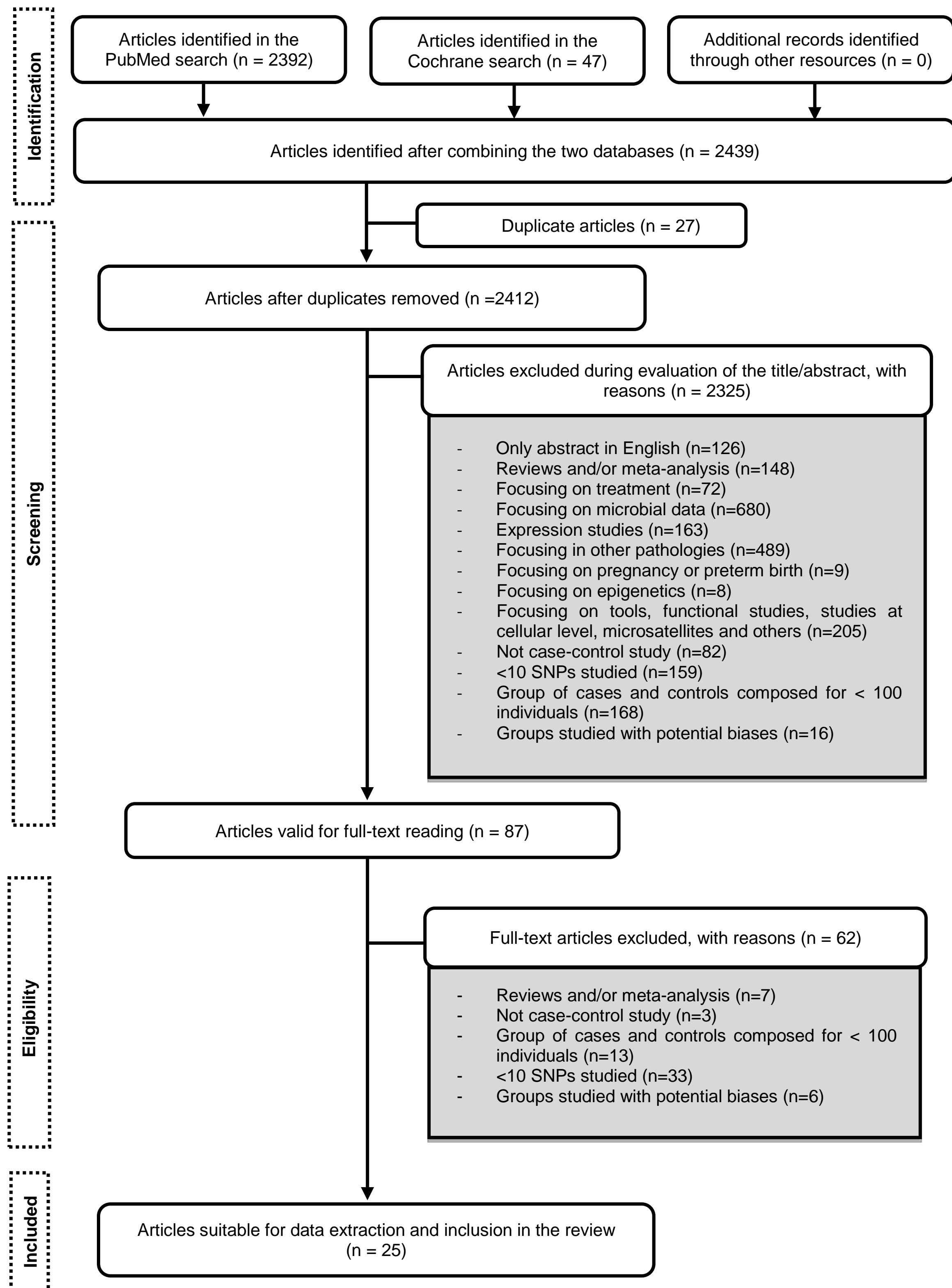
Reference	SNPs Studied (n)	Genotyping Method	Results			
			Associated gene (polymorphism)	**OR**	**95%CI**	**Association**
(Suzuki et al., 2004)	310	TaqMan	*COL1A1* (NA)	2.5	1.4-4.6	AgP
			COL4A1 (NA)	2.1	1.3-3.5	AgP
			PTGDS (NA)	5.1	1.5-18.2	AgP
			KRT23 (NA)	6.3	1.4-29.2	AgP
			IL6ST (NA)	NA	NA	AgP
			CTSD (NA)	3.2	1.6-6.5	CP
			EGF (NA)	4.8	1.5-14.7	CP
			CTSD (NA)	NA	NA	CP
			HSPG2(NA)	NA	NA	CP
			COL17A1(NA)	NA	NA	CP
(Brett et al., 2005)	10	PCR	*IL1B*(NA)	3.6	1.4-9.7	AgP
			IL6(NA)	3.1	1.5-6.3	AgP and CP
			VDR (NA)	2.7	1.4-5.4	CP
			TLR4(NA)	3.0	1.2-7.6	PD
(Fiebig et al., 2008)	10	TaqMan	ND	ND	ND	ND
(Gunji et al., 2007)	30	PCR-RFLP	*FPR1* (rs11666254)	2.0	NA	AgP
			FPR1 (rs12460836)	2.0	NA	AgP
(Kobayashi et al., 2009a)	16	Novel nano-Invader DNA chip system	ND	ND	ND	ND
(Kobayashi et al., 2009b)	35	Novel nano-Invader DNA chip system, (PCR)-Invader and TaqMan	*VDR* (NA)	2.5	1.4-4.3	CP
(Schaeferet al., 2010a)	500.568	Affymetrix Human Mapping 500k Array set and Affymetrix Genome-Wide Human SNP Array 5.0(controls of GWAS 2)	*GLT6D1* (rs1537415)	1.67 (GWAS 1) 1.65 (GWAS 2)	1.27-2.18 1.26-2.17	AgP
(Schaefer et al., 2010b)	12	SNPlex and TaqMan	*DEFB1* (rs1047031)	1.3	1.1-1.6	AgP and CP
(Scapoli et al., 2010)	70	Sequenom MassARRAY	ND	ND	ND	ND
(Chai et al., 2010a)	11	iPLEX Sequenom MassARRAY	*C5* (rs17611)	6.1	1.3-28.2	PD
(Chai et al., 2010b)	102	iPLEX Sequenom MassARRAY	*FCGR3A* (rs445509)	1.0	0.5-0.8	CP
(Scapoli et al., 2011)	28	Sequenom MassARRAY and PCR	*IL6*(NA)	2.5	1.5-4.4	AgP
(Schaefer et al., 2011)	51/37*	SNPlex and TaqMan	*CDKN2B* (rs3217992)	2.5	1.3-5.1	AgP
			CDKN2B-AS1 (rs518394)	2.3	1.1-4.9	AgP

(Munz et al., 2017)	502.332/15^	iPLEX Sequenom MassARRAY, Illumina Human	*CIDEFA1A3S1* ((rs297851))	2.6	1.2-5.4	AgP
			CDKN2B-AS1 (rs11790231)	NA	NA	AgP
			CDKN2B-AS1 (rs1360590)	2.1	1.1-3.9	CP
			CDKN2B-AS1 (rs10965224	NA	NA	CP
(Letra et al., 2012)	34	TaqMan	*TIMP1* (rs5906435)	NA	NA	CP
			MMP3 (rs679620)	NA	NA	CP
			MMP3 (rs650108)	NA	NA	CP
(Atanasovska-Stojanovska et al., 2013)	22	PCR-SSP	*IL1B* (NA)	2.1	1.4-3.3	CP
(Schaefer et al., 2013)	268	Illumina Inmunochip	Upstream of *IL10* (rs61815643)	0.8	0.7-0.9	AgP
			Upstream of *IL10* (rs6667202)	0.8	0.7-0.9	AgP
			Upstream of *IL10* (rs4072226)	0.8	0.7-1.0	AgP
(de Jong et al., 2014)	27	Affymetrix Human Mapping 500K Array set	*SLC23A1* (rs6596473)	1.3	1.0-1.5	AgP
			SLC23A1 (rs10063949)	1.4	1.0-1.9	AgP
			SLC23A2 (rs6990309)	1.5	1.1-2.1	AgP
			SLC23A2 (rs1519860)	1.6	1.1-2.3	AgP
			SLC23A2 (rs1715395)	1.4	1.0-2.1	AgP
(Feng et al., 2014)	620.901/20[†]	Illumina Human610-Quadv1_B BeadChip	Intergenic (rs1477403)	NA	NA	CP
			Intergenic (rs2833017)	NA	NA	CP
			Intergenic (rs9305434)	NA	NA	CP
			Intergenic (rs2048126)	NA	NA	CP
(Schaefer et al., 2014)	~3.000	Illumina Inmunochip	*IRF5* (rs62481981)	3.1	1.6-6.1	AgP
			PRDM1 (rs6923419)	0.7	0.6-0.9	AgP
(Zhang et al., 2014)	23	iPLEX Sequenom MassARRAY	*IL8* (rs4073)	1.2	1.0-1.4	CP
(Schaefer et al., 2015)	46	Illumina Inmunochip and Affymetrix Human Mapping 500K Array set	*PLG* (rs4252120)	1.3	1.3-1.4	AgP
(Shang et al., 2016)	65	Sequenom MassARRAY	*FBXO38* (rs10043775)	1.2	NA	CP
(Offenbacher et al., 2016)	~2.1 million/37'	Illumina Human BeadChips	ND	ND	ND	ND
(Zupin et al., 2017)	203	Illumina Exome 12v1	*NLRC5* (rs289723)	2.0	1.3-3.1	CP

		OmniBeadChip and TaqMan				
			DEFA1A3 (rs2738058)	1.3	1.1-1.4	AgP
			SIGLEC5 (rs4284742)	1.3	1.2-1.5	AgP
			SLC1A3 (rs1122900)	1.3	1.1-1.4	AgP

Abbreviations: PD, periodontal disease; AgP, aggressive periodontitis; CP, chronic periodontitis; OR, odds ratio; CI, confidence interval; ND, no data; NA, data not available.
* In the association study for AgP, 51 SNPs were analysed. In the association study for CP, 37 SNPs were analysed.
† In the association study for CP, 620 901 SNPs were analysed. An independent test for CP was performed in two Caucasians populations (Porto Alegro and Río de Janeiro), analysing 20 SNPs.
' In the association study for CP, 2.135.235SNPs were analysed. An independent test for CP was performed in German sample, analysing 47 SNPs.
^ In the discovery stage 502.332 SNPs were analysed. A replication was performed in a sample of Turkish descent, analysing 15 SNPs.

Figure(s)



Supplementary Material
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