Received Date : 11-May-2016
Revised Date : 21-Jun-2016
Accepted Date : 15-Jul-2016
Article type : Systematic Review

Periodontal infectogenomics: systematic review of associations between host genetic variants and subgingival microbial detection

Running Title: Periodontal infectogenomics review

Keywords: genetic, bacteria, periodontitis, infectogenomics

Luigi Nibali [1], Anna Di Iorio [2], Olanrewaju Onabolu[3],Guo-Hao Lin [4]

[1] Clinical Oral Research Centre, Institute of Dentistry, Queen Mary University London (QMUL), London, United Kingdom

[2] Library Services, UCL Eastman Dental Institute, London, United Kingdom

[3] Private practice, London, United Kingdom

[4] Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry, Ann Arbor, MI, USA

Corresponding Author:
Luigi Nibali
Clinical Oral Research Centre
Institute of Dentistry

Queen Mary University London (QMUL)
London, United Kingdom
Tel.: 0044
Fax: 0044
E-mail: l.nibali@qmul.ac.uk

ABSTRACT

BACKGROUND: Recent research is increasingly showing that host genetic variants can affect the colonization by specific microbes. The aim of this study was to systematically investigate the associations between host genetic variants and subgingival microbial detection and counts.

MATERIALS AND METHODS: A systematic search of the literature was conducted in Ovid Medline, Embase, LILACS and Cochrane Library for studies reporting data on host genetic variants and detection of microbes subgingivally.

RESULTS: A total of 43 studies were included in the review, from an initial search of 3887 titles. Studies consisted mainly of candidate gene studies and of one genome-wide analysis. Some promising associations were detected between single nucleotide polymorphisms and microbial detection. The only feasible meta-analysis failed to show any association between Interleukin 1 *(IL1)* genetic variants and detection of periodontopathogenic bacteria subgingivally.

CONCLUSIONS: There is no evidence yet that neither*IL1* genetic polymorphisms nor other investigated genetic polymorphisms are associated with presence and counts of subgingival bacteria. Further studies on large populations with replication samples should clarify the possible effects of other genetic variants on the subgingival microbiota.

CLINICAL RELEVANCE:

Scientific rationale for the study: Genetic variants are thought to influence the composition of the subgingival biofilm (this has been named infectogenomics). However, potential associations have not been systematically investigated in periodontitis.

Principal findings: Despite some promising potential associations, no conclusions can be made yet on any gene variants associated with subgingival bacteria.

Practical implications: Although some indications exist that genetic variants can affect subgingival microbiota, the concept of infectogenomics needs further investigation in periodontitis.

FUNDING: No specific funding was obtained for the analysis reported in this paper.

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

BACKGROUND

Medical research in the last decades has brought an increased awareness of the magnitude and relevance of microbial colonization of the human body, to the extent that we now know that perhaps up to 90% of the cells and approximately 99% of the genomic material in the human body are microbial (Turnbaugh et al. 2007). Whilst most of these bacteria and microbial communities give essential benefits to their host, a handful of them predispose to human disease(McFall-Ngai et al. 2005). It is becoming increasingly clear that the microbial colonization depends on several factors including microbial virulence, lifestyle factors, environmental agents and also on the ability- largely genetically determined- of the host to respond to the microbial challenge(Cooke & Hill 2001, Blekhman et al. 2015). The term 'Infectogenomics' was introduced to define the effect of host genetic variants (most often single nucleotide polymorphisms, or SNPs) in i) influencing the response to microbial agents and the risk to develop pathological immune reactions and ii) influencing the actual colonization in a given ecological niche(Kellam & Weiss 2006). Recently, microbial agents are emerging as a possible cause not just of diseases traditionally considered as 'microbial' but also of other chronic diseases such as certain forms of cancer and rheumatoid arthritis. Therefore, the role of genetic variants in affecting microbial biofilm composition in many bodily ecosystems is gaining more interest (Nibali et al. 2014).

Data have emerged in the last 15 years of how host genetic variants may affect the presence and counts of specific bacteria in the subgingival niche(Socransky et al. 2000), in turn increasing the risk of developing periodontitis (Nibali et al. 2009). Although sparse studies have been published on the association of specific genetic variants with subgingival bacteria

such as ‘red complex’ bacteria or *Aggregatibacter actinomycetemcomitans*, these associations have still not been verified systematically. A better knowledge of the relationships between host genetic variantsandmicrobial colonization patterns in periodontitis could potentially help to better understand periodontal disease pathogenesis and could help with its management. The aim of this review was to systematically investigate the associations between host genetic variants and subgingival microbes.

MATERIALS AND METHODS

A systematic review protocol was written in the planning stages and the PRISMA checklist (Moher et al., 2009) was followed both in planning and reporting this review (checklist attached as supplemental material 1). A review protocol was prepared and registered with PROSPERO (reference CRD42015026928).

Focused question

- The question addressed was the following: is there anassociation between host genetic variants and detection and counts of specific microbes subgingivally?

PECO outline:

- Population: subjects with measures of periodontal disease or periodontal health
- Exposure: analysis of host genetic variants
- Comparisons: genotypes/allele frequency at different SNPs
- Outcomes: detection of specific microbes subgingivally

Eligibility criteria

Human studies reporting measures of associations between host genetic variants and detection of subgingival microbes were considered suitable for this review. Inclusion criteria were:

- Study designs:
  - case-control studies
  - cross-sectional studies
  - longitudinal studies or randomized controlled trails (RCTs) providing baseline genetic and microbial data
- Reporting measures of periodontal disease (periodontal diagnosis)
- Reporting analysis of host genetic variants (SNPs or other types of genetic variations)

- Reporting data on microbial detection or counts and/or proportions subgingivally (by host genetic variant)

Exclusion criteria were:

- Reviews
- Case reports
- Studies on animal models

Information sources

The literature search for the present systematic review was conducted at Ovid Medline (up to 10/09/2015), Embase (up to 13/09/2015), LILACS and Cochrane Library (both up to 14/09/2015). The reference lists of included articles and relevant reviews were manually searched. The search was complemented by a hand search of the journals most likely to publish studies on this topic in the last 20 years (*Journal of Clinical Periodontology, Journal of Dental Research, Journal of Periodontal Research* and *Journal of Periodontology*).

Search strategy

The search strategy used a combination of MeSH terms and key words described in supplemental material 2.

Study selection

Studies were selected by a two-stage screening approach carried out by two independent reviewers (authors A.D.I. and O.O.). Disagreements about inclusion or exclusion of a study were resolved by consulting an arbitrator (author L.N.).

The first-stage screening of titles and abstracts was carried out in order to eliminate irrelevant articles and those that did not meet the inclusion criteria established by this review. At the second-stage screening, following reading of the full-texts, the study eligibility was verified independently by both reviewers and the data extraction and quality assessment were performed for the included studies. The level of agreement between the two reviewers was calculated using Kappa statistics for first and second-stage screening.

Data collection process/ data items

Data were extracted based on the general study characteristics (authors and year of publication, country and study design) and population characteristics (number of participants,

age, gender, ethnicity, inclusion/exclusion criteria anddiagnosis of periodontal status). Specific data on genetic and microbial analysis, genetic variantsanalysed, microbes analysed, method used for genetic analysis and method used for microbial sampling and microbial detection/identificationwere extracted.

Risk of bias in individual studies

The risk of bias of the included case-control and cross-sectional studies was assessed through sensitivity analysis by using a recently-proposed score of 0 to 20 adapted to genetic analyses of periodontal studies (Nibali 2013). The 'Newcastle Ottawa tool to assess risk of bias' (Newcastle Ottawa scale http://www.ohri.ca/programs/clinical_epidemiology/oxford.htm) was used to assess risk of bias for longitudinal studies.

Summary measures/Synthesis of results/ Statistical methods

The study outcomes werethe risk ratio of detection of specific subgingival microbes (primary outcome) or the overall microbial counts or proportions (secondary outcome) in patients with different genotypes. Meta-analysis could be performed only for at least 3 papers investigating the same combination of SNPs and subgingival bacteria. The risk ratios of primary and secondary outcomeswere estimated using a computer program (Review Manager Version 5.0. Copenhagen; The Nordic Cochrane Centre, The Cochrane Collaboration, 2008).The contribution of the includedarticles wasweighted using inverse-variance method. Random effects meta-analyses of the selected studies were applied to avoid any bias being caused by methodological differences among studies. Forest plots were produced to graphically showthe difference in outcomes of groups with different genotypesusing number of SNPs with each genotypeas the analysis unit. A p value= 0.05 was used as the cut-off level for significance. Heterogeneity was assessed with chi-square tests and I^2 test, which ranges between 0% and 100% and where lower values represent less heterogeneity. In addition, funnel plots were used to assess the presence of the publication bias across studies.

RESULTS

Study selection

Figure 1 shows the flowchart representing study selection and inclusion. The initial search resulted in 5072 papersat Ovid Medline, Embase, Cochrane Library and LILACS combined, which reduced to 3887 after removing conference abstracts, case reports and reviews. Followingfirst-stage screening of titles and abstracts, 71 articles qualified for full-text

screening (considered potentially suitable by at least one reviewer). After full text reading, 43 articles met the defined inclusion criteria and 28 were excluded (see Figure 1 for reasons for exclusion). Every effort was made to obtain any relevant missing data from the papers by contacting the authors by email. The kappa value for inter-reviewer agreement was 0.60 at title and abstract screening and 0.48 at full text reading.

Study characteristics

Table 1 reports the characteristics of the reviewed studies. Of the 43 included studies, most articles were written in English (n= 41), while 1 was written in Russian and 1 in Italian. The countries where the studies were conducted included Germany (n=10), Brazil (n=7), Czech Republic (n=5), UK (n=4), USA (n=3), Sweden (n=3), Japan (n=3), China (n=2), Belgium (n=1), Switzerland (n=1), Italy (n=1), India (n=1), Poland (n=1) and Russia (n=1). The patient sample ranged from 12 to 1020 patients. Study designs included case-control, cross-sectional and longitudinal treatment studies. The 43 papers reviewed were published in 2 decades, from 2000 to 2015.

Included cases ranged from chronic periodontitis (CP), aggressive periodontitis (AgP), chronic gingivitis (CG) and healthy periodontia or cases of patients treated with dental implants (Jansson et al. 2005). Some papers focused only on patients with specific medical history, such as HIV (Goncalves et al. 2009), renal transplant (Gong et al. 2013, Luo et al. 2013), coronary heart disease (Schulz et al. 2012), Crohn's disease (Stein et al. 2010), neutropenia (Ye et al. 2011) or pregnancy (Hirano et al. 2010, Wang et al. 2012). Two papers described large explorative genome analyses (85,947 SNPs) using the same patient cohort but with a different analytic approach (Divaris et al. 2012, Rhodin et al. 2014), while all other studies focused on a candidate gene with one or a few selected SNPs. Genetic analysis was generally performed by PCR after DNA extraction from blood samples (leukocytes) or buccal swabs, with some studies using a chair-side PST (Periodontal Susceptibility Test). Microbiological analyses generally were performed by PCR or checkerboard and occasionally culture (see Table 1 for details). Microbial outcomes included detection (presence/absence) or counts or proportions of bacteria. Target bacteria usually consisted of *Aggregatibacter acinomycetemcomitans, Poprhyromonas gingivalis, Tannerella forsythia, Treponema denticola, Prevotella intermedia*and *Fusobacterium nucleatum*. Up to 40 bacterial taxa were included for DNA checkerboard analysis (Socransky et al. 2000), one

paper included also viruses in the analysis (Tsarev & Nikolaeva 2010), while one study performed 16s rDNA pyrosequencing of the subgingival microbiota (Ye et al. 2011).

Synthesis of results

Some studies reported positive associations between genotypes and detection or counts/proportions of specific bacteria, while other papers reported lack of associations (see Table 1). Whole-genome explorative analyses failed to reveal genome-wide significant signals, but the results are suggestive that there is an association between 13 loci, and 'red' and 'orange' complex bacteria (Divaris et al. 2012). Using the gene-centric MAGENTA (meta-analysis gene set enrichment of variant associations) approach, 2 genes (KCNK1 and DAB2IP) showed a significant association with high periodontal pathogen colonization (red complex and*Porphyromonas gingivalis* respectively) (Rhodin et al. 2014). Among candidate gene studies, 13 investigated Interleukin 1 (*IL1*) SNPs, while other studies focused on a variety of SNPs. SNPs investigated in at least 3 published papers are detailed below:

Interleukin 1 genes

Some studies reported associations between *IL1* SNPs and detection of periodontopathogenic bacteria, while other studies focused on counts/proportions. While different *IL1* SNPs were investigated, most studies report on results based on '*IL1* composite genotype' (Kornman et al. 1997). Positivity for this composite genotype (*IL1*+) was defined as the presence of at least one copy of 'allele 2' for SNPs *IL1B* rs 1143634 (previously reported as *IL1B* +3953 or +3954) and *IL1 A* rs 1800587 (previously reported as *IL1A* -889).Among studies on 'bacterial counts', conflicting results were reported. Socransky and co-workersfound increased proportionsof several subgingival bacteria (*T. forsythia, T. denticola, F. nucleatum, Fusobacterium periodonticum, Campylobacter gracilis, Capnocytophaga sputigena, Streptococcus gordonii, Streptococcus constellatus. Streptococcus intermedius* and 3 *Capnocytophaga species)* in *IL1*+ subjects (Socransky et al. 2000). Increased *Campylobacter rectus*counts were found in a study on *IL1*+ chronic periodontitis patients compared with *IL1*- (Kowalski et al. 2006). On the other hand, among 151 chronic periodontitis patients in supportive periodontal care, IL1- subjects had an increased total bacterial load and increased levels of *A. actinomyecetemcomitans, P. gingivalis, Eubacterium nodatum* and *Streptococcus anginosus* compared with IL1+ subjects (Agerbaek et al. 2006), while another study reported no significant associations between *IL1* genotypes and microbial counts measured by PCR

(Papapanou et al. 2001). Owing to heterogeneity of the reported data, it was not possible to performmeta-analysis of the 'bacterial counts/proportions' outcome.

For the 'microbial detection' outcome, various*IL1* SNPs and different bacteria were investigated (see table 1 for details). Excluding populations with specific co-morbidities, a sufficient number of studies (at least 3) were conducted in Caucasians investigating '*IL1* composite genotype' and detection of *A. actinomycetemcomitans* and *P. gingivalis* in patients with periodontitis (including aggressive periodontitis, chronic periodontitis and mixed) (Checchi et al. 2004, Kratka et al. 2007, Nibali et al. 2008, Tsarev & Nikolaeva 2010, Schulz et al. 2011)(this was possible after obtaining additional individual data from the studies by Schulz et al. 2011 and Nibali et al. 2008). Meta-analysis of risk ratio of association between *IL1* composite genotype and detection of *A. actinomycetemcomitans* among selected studiesrevealed a non-statistically significant overall risk ratio of 0.79 (95% CI= 0.45 to 1.38, p= 0.40) (Figure 2). Moreover, the comparison presented a moderate to high degree of heterogeneity among selected studies (p value for chi-square test= 0.02, and I^2 test = 65%). The meta-analysis of the risk ratio for an association between *IL1* composite genotype and *P.gingivalis*presented an overall risk ratio of 1.24 (95% CI= 0.90 to 1.72), no statistical significance (p= 0.18) (Figure 3) and a high degree of heterogeneity (p value for chi-square test= 0.0002, and I^2 test = 82%).

Meta-analysis of 3 studies in patients with periodontitis(Kratka et al. 2007, Nibali et al. 2008, Schulz et al. 2011)reporting associations between *IL1* composite genotype and *T. forsythia* detection revealed an overall risk ratio of 1.01 (95% CI= 0.91 to 1.13), with no statistical significance (p= 0.80) (Figure 4) and a low degree of heterogeneity (p value for chi-square test= 0.82, and I^2 test = 0%).

Interleukin 6 gene

Three independent studies, all from the same research group, investigated associations between *IL6* SNPs and subgingival bacteria. Consistent results were reported regarding higher detection of *A. actinomycetemcomitans* in *IL6* -174 G (rs 1800795) homozygous subjects and in subjects with specific *IL6* genotypes and haplotypes (defined by IL6 -1363 rs2069827 and -1480 rs2069825)(Nibali et al. 2011, Nibali et al. 2012, Nibali et al. 2013). However, meta-analysis was not performedbecause of heterogeneity inethnicities of studied populations.

TNF-α gene

Four studies investigated associations between *TNFα* SNPs and subgingival bacteria. Two separate studies (Nibali et al. 2008, Trombone et al. 2009)found no associations between *TNFα*-308 A/G genotypes (rs 1800629) and detection of the studied bacteria, while a study on 175 Caucasian patients (with AgP, CP or healthy) reported an association between *TNFα* 308GG/238GG (rs361525) haplotype and higher*P. intermedia* detection (Schulz et al. 2008a). The same group found individuals with*TNFα* 308 AG orAA genotypes and with A-allele to be associated with higher *P. intermedia* detection in a separate study on CAD patients (Schulz et al. 2012). Meta-analysis for studies on*TNFα*was not possible owing to heterogeneity in ethnicity and medical history in the three reported studies.

Publication bias analysis

Table 2 reports results of risk of bias analysis of individual studies, showing a wide range of variability from a total score of 5 to a total score of 17 for case-control and cross-sectional studies. Table 3 shows results of risk of bias analysis based on Newcastle Ottawa scale for longitudinal studies, ranging from a total of 5 to 9. The items that were lacking in most studies were representativeness of cases, power calculation, universal case and control definition and methodological details on genetic analyses, including success rates of DNA extraction and of genotyping, good reproducibility and blind genotyping.

Funnel plots of the meta-analysis of the risk ratios of patients with the*IL1* composite genotype are shown in Supplemental Material 3 to 5 (for detection of *A. actinomycetemcomitans, P. gingivalis* and *T. forsythia* respectively). However, the symmetry of these plots could not be clearly assessed due to the small number of included studies.

DISCUSSION

This is the first systematic review, to our knowledge, to investigate associations between host genetic variants and detection and counts/proportions of periodontopathogenic bacteria subgingivally, based on the concept of periodontal infectogenomics. This was defined as the effect of host genetic variants in influencing the composition of the subgingival microbiota (Nibali et al. 2009). Such concept was borrowed from studies suggesting that SNPs or other genetic variants in the host can affect the response to the microbial challenge (Gage & Kosoy 2005, Kellam & Weiss 2006) and can affect the composition of microbial biofilms in the

human body (Frank et al. 2007, Craven et al. 2012,Blekhman et al. 2015,). More recently, the concept of genetic dysbiosis was introduced, to better describe the effect of genetic variants on determining subtle changes in the composition of biofilms, able to predispose to periodontitis and other chronic non-infectious human diseases (Nibali et al. 2014). Evidence exists of similar gut microbial composition in twins, suggesting an influence of genetic factors in establishing a 'core' gut microbiota (Turnbaugh et al. 2009). However, a recent study showed no differences in the total number of supra- and sub-gingival species shared by monozygotic and dizygotic twins, suggesting that genetic effect may not be evident in a mature, stable oral bacterial communities (Papapostolou et al. 2011).

Forty-three studies were included in the present review. The genetic and microbial analyses tipically involved the study of one or a selected panel of SNPs and one or a selected panel of bacteria supposed to have an effect on periodontal pathology. However, recent technology enabled researchers to expand this approach and to perform large genetic and microbiological analyses. These consisted of genome-wide SNP arrays including analysis of 85,947 SNPs (Divaris et al. 2012)and of microbial 16s rDNA pyrosequencing (Ye et al. 2011). The advantages and disadvantages of these approaches often both lie in their explorative nature which,while allowing concomitant analysis of a wide array of potentially relevant genes and bacteria, carries the risk of losing power and focus by multiple testing and by not taking into consideration a possible functional relevance to the periodontium.However, GWAS could also be interpreted with a more focused approach in the context of biological relevance. The GWAS included in this review (Divaris et al. 2012, Rhodin et al. 2014) performed periodontal infectogenomics analysis of 1020 White subjects participating in the Atherosclerosis Risk In Communities (ARIC) and focused on 8 periodontal pathogens analysed by checkerboard DNA-DNA hybridization. The authors detected no genome-wide significant signals, but suggestive evidence ($p<5 \times 10^{-6}$) of association for 13 genetic loci (including KCNK1, FBXO38, UHRF2, IL33, RUNX2, TRPS1, CAMTA1 and VAMP3) and "red" and "orange" complex microbiota. The same effect direction was detected in a second sample of 123 African-American participants(Divaris et al. 2012). Using a gene-centric analysis of the same population which takes into account multiple SNPs for each gene and adjusts statistical significance accordingly, 2 genes (KCNK1 and DAB2IP) showed association with high periodontal pathogen colonization (red complex and*Porphyromonas gingivalis* respectively). These two genes also exhibited suggestive genome-wide association with chronic periodontitis(Rhodin et al. 2014). These results may be promising but need

replication in independent cohorts. The first attempt to a 'wide' microbial periodontal infectogenomics approach was conducted on 14 Swedish patients with neutrophil defects, using 16sDNA pyrosequencing microbial analysis, but with clear limitation due to the small sample size (Ye et al. 2011).

Among the studies with a candidate-gene and candidate-bacteria approach included in this review, target SNPs were mainly within the*IL1, IL6, TNFα* genes, while target bacteria usually consisted of *A.acinomycetemcomitans, P.gingivalis, T.forsythia, T.denticola, P.intermedia*and*F.nucleatum*. Occasionally, other candidate SNPs and bacteria and viruses were included in the analysis. Meta-analysis of the so-called '*IL1* composite genotype' (Kornman et al. 1997) with microbial detection revealed conflicting results with no evidence of an association with detection of *A. actinomycetemcomitans, P. gingivalis* or *T. forsythia*. Data synthesis of associations between the*IL1* composite genotype and microbial counts/proportions subgingivally was equally inconclusive with reports of lack of associations (Papapanou et al. 2001), increased periodontopathogenic bacteria in *IL1+* subjects (Socransky et al. 2000) and increased periodontopathogenic bacteria in *IL1-* periodontitis patients in maintenance (Agerbaek et al. 2006). Meta-analysis for this outcome was not feasible due to the overwhelming heterogeneity of the studies.

Among other candidate genes implicated as genetic risk factors in periodontitis, subjects with thepro-inflammatory *IL6* genotypes (Fishman et al. 1998, Fife et al. 2005) show perhaps the most consistent associations with *A. actinomycetemcomitans* detection and counts in several independent studies and in different populations, although by the same research group. In particular, *IL6* genotypes (defined by genotypes in -174, -1363 and -1480 SNPs) were associated with increased chances of subgingival detection of *A. actinomycetemcomitans*in 267AgP and CP patients in the UK (Nibali et al. 2012) and with higher *A. actinomycetemcomitans*counts analysed by checkerboard DNA-DNA analysis in a rural population living in Andra Pradesh, India (Nibali et al. 2011). In 12 AgP patients selected based on their *IL6* genotypes ('pro-inflammatory *IL6* haplotype positive' vs. '*IL6* haplotype negative'), higher *A. actinomycetemcomitans* counts were detected subgingivally in *IL6* 'haplotype positive' subjects before treatment. Despite a reduction in *A. actinomycetemcomitans* counts after non-surgical and surgical treatment, these subjects showed again an increase in counts of *A. actinomycetemcomitans* 3 months after periodontal treatment (Nibali et al. 2013). Interestingly, two of the investigated *IL6*genotypes (rs1800795

and rs1800796) showed moderate association with high 'red complex' colonization in the GWAS reported above (Divaris et al. 2012), giving strength to this supposed effect. However, no other studies to reject or support these associations on *IL6* and subgingival bacteria have so far been published.

A strength of the studiescurrent systematic review is their range of conditions including periodontal health, AgP, CP, gingivitis as well as systemic health and other systemic conditions such as cardiovascular disease or Crohn's disease. A Limitation of the included studies is their heterogeneity, especially with regards to data reporting. For example, because of their complexity, only part of the data are often reported and it is difficult to calculate detection rates for each studied genotype. Furthermore, the small sample sizes of most studies is a clear limitation, as it may produce spurious results. In particular, risk of bias analysis revealed that only 15 of 43 included studies reported a priori sample size calculation for the main outcome. Another limitation of some studies is the case-control approach, with the use of care-seeking rather than population-based individuals, which has been shown to carry risk of bias (Wacholder et al. 1992, Grimes and Schulz 2005). A strength of this systematic review is the novelty of the studied subject and the inclusion of 43 papers. Based on this review, we conclude that the *IL1* composite genotype is not associated with specific subgingival microbial colonization patterns. We suggest that other gene variants showing promising associations with detection and counts of periodontopathogenic bacteria subgingivally need replication in large independent samples. Separate analyses should be reported for subjects affected by periodontal disease and periodontally-healthy individuals. Furthermore, studies should follow strict criteria such as STREGA for the conduct and reporting of periodontal genetic-microbial association studies (Little et al. 2009). Genome-wide approaches and comprehensive analyses of the microbial communities subgingivally, although presenting some analytical difficulties, represent the future for research in this field.A more detailed knowledge of the human oral microbiome could provide more information on its association with host genetic variants(Cross et al. 2016). Investigation of microbial colonization patterns in relation to transcriptome information in the affected gingival tissues may also offer valuable information on infectogenomics effects and insights into epigenetic changes.

ACKNOWLEDGMENTS

We gratefully acknowledge the help of Mrs Ieva Kacevice in the translation of one of the included articles and of the authors who have kindly sent raw data to allow meta-analysis.

REFERENCES

Agerbaek, M. R., Lang, N. P. & Persson, G. R. (2006) Microbiological composition associated with interleukin-1 gene polymorphism in subjects undergoing supportive periodontal therapy. *Journal of Periodontology***77**, 1397-1402.

Blekhman, R., Goodrich, J.K., Huang, K., Sun, Q., Bukowski, R., Spector, D., Keinan, A., Ley R.E., Gevers, D.& Clark, A.G. (2015) Host genetic variation impacts microbiome composition across human body sites. *Genome Biology* **15,** 16:191.

Borges, M. A., Figueiredo, L. C., Brito, R. B., Jr., Faveri, M. & Feres, M. (2009) Microbiological composition associated with vitamin D receptor gene polymorphism in chronic periodontitis. *Brazilian Oral Research***23**, 203-208.

Borilova Linhartova, P., Bartova, J., Poskerova, H., Machal, J., Vokurka, J., Fassmann, A. & Izakovicova Holla, L. (2015) Apolipoprotein E gene polymorphisms in relation to chronic periodontitis, periodontopathic bacteria, and lipid levels. *Archives of Oral Biology***60**, 456-462.

Borilova Linhartova, P., Vokurka, J., Poskerova, H., Fassmann, A. & Izakovicova Holla, L. (2013) Haplotype analysis of interleukin-8 gene polymorphisms in chronic and aggressive periodontitis. *Mediators of Inflammation***2013**, 342351.

Cavalla, F., Biguetti, C. C., Colavite, P. M., Silveira, E. V., Martins, W., Jr., Letra, A., Trombone, A. P., Silva, R. M. & Garlet, G. P. (2015) TBX21-1993T/C (rs4794067) polymorphism is associated with increased risk of chronic periodontitis and increased T-bet expression in periodontal lesions, but does not significantly impact the IFN-g transcriptional level or the pattern of periodontophatic bacterial infection. *Virulence***6**, 293-304.

Checchi, L., Gatto, M. R., Pattison, A. & Felice, P. (2004) [Genetic and microbiologic tests in periodontal disease]. *Minerva Stomatologica***53**, 345-353.

Cooke, G. S. & Hill, A. V. (2001) Genetics of susceptibility to human infectious disease. *Nature Reviews: Genetics***2**, 967-977.

Craven, M., Egan, C. E., Dowd, S. E., McDonough, S. P., Dogan, B., Denkers, E. Y., Bowman, D., Scherl, E. J. & Simpson, K. W. (2012) Inflammation drives dysbiosis and bacterial invasion in murine models of ileal Crohn's disease. *PLoS One***7**, e41594.

Cross, B, Faustoferri, R.C.& Quivey, R.G. Jr. (2016) What are we learning from the Human Oral Microbiome Project? *Current Oral Health Reports* **3**: 56-63.

Divaris, K., Monda, K. L., North, K. E., Olshan, A. F., Lange, E. M., Moss, K., Barros, S. P., Beck, J. D. & Offenbacher, S. (2012) Genome-wide association study of periodontal pathogen colonization. *Journal of Dental Research***91**, 21S-28S.

Ferreira, S. B., Jr., Trombone, A. P., Repeke, C. E., Cardoso, C. R., Martins, W., Jr., Santos, C. F., Trevilatto, P. C., Avila-Campos, M. J., Campanelli, A. P., Silva, J. S. & Garlet, G. P. (2008) An interleukin-1beta (IL-1beta) single-nucleotide polymorphism at position 3954 and red complex periodontopathogens independently and additively modulate the levels of IL-1beta in diseased periodontal tissues. *Infection and Immunity***76**, 3725-3734.

Fife, M. S., Ogilvie, E. M., Kelberman, D., Samuel, J., Gutierrez, A., Humphries, S. E. & Woo, P. (2005) Novel IL-6 haplotypes and disease association. *Genes and Immunity***6**, 367-370.

Finoti, L. S., Anovazzi, G., Pigossi, S. C., Corbi, S. C., Teixeira, S. R., Braido, G. V., Kim, Y. J., Orrico, S. R., Cirelli, J. A., Mayer, M. P. & Scarel-Caminaga, R. M. (2013a) Periodontopathogens levels and clinical response to periodontal therapy in individuals with the interleukin-4 haplotype associated with susceptibility to chronic periodontitis. *European Journal of Clinical Microbiology and Infectious Diseases***32**, 1501-1509.

Finoti, L. S., Corbi, S. C., Anovazzi, G., Teixeira, S. R., Steffens, J. P., Secolin, R., Kim, Y. J., Orrico, S. R., Cirelli, J. A., Mayer, M. P. & Scarel-Caminaga, R. M. (2013b) Association between IL8 haplotypes and pathogen levels in chronic periodontitis. *European Journal of Clinical Microbiology and Infectious Diseases***32**, 1333-1340.

Fishman, D., Faulds, G., Jeffery, R., Mohamed-Ali, V., Yudkin, J. S., Humphries, S. & Woo, P. (1998) The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6

transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *Journal of Clinical Investigation***102**, 1369-1376.

Frank, D. N., St Amand, A. L., Feldman, R. A., Boedeker, E. C., Harpaz, N. & Pace, N. R. (2007) Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proceedings of the National Academy of Sciences of the United States of America***104**, 13780-13785.

Gage, K. L. & Kosoy, M. Y. (2005) Natural history of plague: perspectives from more than a century of research. *Annual Review of Entomology***50**, 505-528.

Goncalves, L. S., Ferreira, S. M., Souza, C. O. & Colombo, A. P. (2009) Influence of IL-1 gene polymorphism on the periodontal microbiota of HIV-infected Brazilian individuals. *Brazilian Oral Research***23**, 452-459.

Gong, Y., Bi, W., Cao, L., Yang, Y., Chen, J. & Yu, Y. (2013) Association of CD14-260 polymorphisms, red-complex periodontopathogens and gingival crevicular fluid cytokine levels with cyclosporine A-induced gingival overgrowth in renal transplant patients. *Journal of Periodontal Research***48**, 203-212.

Grimes, D.A.& Schulz, K. F. (2005) Compared to what? Finding controls for case-control studies. *Lancet* **365**: 1429-1433.

Hirano, E., Sugita, N., Kikuchi, A., Shimada, Y., Sasahara, J., Iwanaga, R., Tanaka, K. & Yoshie, H. (2010) Peroxisome proliferator-activated receptor gamma polymorphism and periodontitis in pregnant Japanese women. *Journal of Periodontology***81**, 897-906.

Holla, L. I., Hrdlickova, B., Linhartova, P. & Fassmann, A. (2011) Interferon-gamma +874A/T polymorphism in relation to generalized chronic periodontitis and the presence of periodontopathic bacteria. *Archives of Oral Biology***56**, 153-158.

Holla, L. I., Hrdlickova, B., Vokurka, J. & Fassmann, A. (2012) Matrix metalloproteinase 8 (MMP8) gene polymorphisms in chronic periodontitis. *Archives of Oral Biology***57**, 188-196.

Jansson, H., Hamberg, K., De Bruyn, H. & Bratthall, G. (2005) Clinical consequences of IL-1 genotype on early implant failures in patients under periodontal maintenance. *Clinical Implant Dentistry and Related Research***7**, 51-59.

Kellam, P. & Weiss, R. A. (2006) Infectogenomics: insights from the host genome into infectious diseases. *Cell***124**, 695-697.

Kornman, K. S., Crane, A., Wang, H. Y., di Giovine, F. S., Newman, M. G., Pirk, F. W., Wilson, T. G., Jr., Higginbottom, F. L. & Duff, G. W. (1997) The interleukin-1 genotype as a severity factor in adult periodontal disease. *Journal of Clinical Periodontology***24**, 72-77.

Kowalski, J., Gorska, R., Dragan, M. & Kozak, I. (2006) Clinical state of the patients with periodontitis, IL-1 polymorphism and pathogens in periodontal pocket--is there a link? (An introductory report). *Advances in Medical Sciences***51 Suppl 1**, 9-12.

Kratka, Z., Bartova, J., Krejsa, O., Otcenaskova, M., Janatova, T. & Duskova, J. (2007) Interleukin- 1 gene polymorphisms as assessed in a 10-year study of patients with early-onset periodontitis. *Folia Microbiologica***52**, 183-188.

Little, J., Higgins, J. P., Ioannidis, J. P., Moher, D., Gagnon, F., von Elm, E., Khoury, M. J., Cohen, B., Davey-Smith, G., Grimshaw, J., Scheet, P., Gwinn, M., Williamson, R. E., Zou, G. Y., Hutchings, K., Johnson, C. Y., Tait, V., Wiens, M., Golding, J., van Duijn, C., McLaughlin, J., Paterson, A., Wells, G., Fortier, I., Freedman, M., Zecevic, M., King, R., Infante-Rivard, C., Stewart, A. & Birkett, N. (2009) Strengthening the reporting of genetic association studies (STREGA): an extension of the STROBE Statement. *Human Genetics***125**, 131-151.

Luo, Y., Gong, Y. & Yu, Y. (2013) Interleukin-10 gene promoter polymorphisms are associated with cyclosporin A-induced gingival overgrowth in renal transplant patients. *Archives of Oral Biology***58**, 1199-1207.

McFall-Ngai, M. J., Henderson, B. P. & Ruby, E. G. (2005) *The influence of cooperative bacteria on animal host biology*. Cambridge: Cambridge University Press.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G. & Group, P. (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Annals of Internal Medicine***151**, 264-269, W264.

Nibali, L. (2013) Suggested guidelines for systematic reviews of periodontal genetic association studies. *Journal of Clinical Periodontology***40**, 753-756.

Nibali, L., D'Aiuto, F., Ready, D., Parkar, M., Yahaya, R. & Donos, N. (2012) No association between A actinomycetemcomitans or P gingivalis and chronic or aggressive periodontitis diagnosis. *Quintessence International***43**, 247-254.

Nibali, L., Donos, N. & Henderson, B. (2009) Periodontal infectogenomics. *Journal of Medical Microbiology***58**, 1269-1274.

Nibali, L., Henderson, B., Sadiq, S. T. & Donos, N. (2014) Genetic dysbiosis: the role of microbial insults in chronic inflammatory diseases. *Journal of Oral Microbiology***6**. doi:10.3402/jom.v6.22962.

Nibali, L., Madden, I., Franch Chillida, F., Heitz-Mayfield, L., Brett, P. & Donos, N. (2011) IL6 -174 genotype associated with Aggregatibacter actinomycetemcomitans in Indians. *Oral Diseases***17**, 232-237.

Nibali, L., Pelekos, G., D'Aiuto, F., Chaudhary, N., Habeeb, R., Ready, D., Parkar, M. & Donos, N. (2013) Influence of IL-6 haplotypes on clinical and inflammatory response in aggressive periodontitis. *Clinical Oral Investigations***17**, 1235-1242.

Nibali, L., Ready, D. R., Parkar, M., Brett, P. M., Wilson, M., Tonetti, M. S. & Griffiths, G. S. (2007) Gene polymorphisms and the prevalence of key periodontal pathogens. *Journal of Dental Research***86**, 416-420.

Nibali, L., Tonetti, M. S., Ready, D., Parkar, M., Brett, P. M., Donos, N. & D'Aiuto, F. (2008) Interleukin-6 polymorphisms are associated with pathogenic bacteria in subjects with periodontitis. *Journal of Periodontology***79**, 677-683.

Papapanou, P. N., Neiderud, A. M., Sandros, J. & Dahlen, G. (2001) Interleukin-1 gene polymorphism and periodontal status. A case-control study. *Journal of Clinical Periodontology***28**, 389-396.

Papapostolou, A., Kroffke, B., Tatakis, D. N., Nagaraja, H. N. & Kumar, P. S. (2011) Contribution of host genotype to the composition of health-associated supragingival and subgingival microbiomes. *Journal of Clinical Periodontology***38**, 517-524.

Reichert, S., Machulla, H. K., Klapproth, J., Zimmermann, U., Reichert, Y., Glaser, C., Schaller, H. G. & Schulz, S. (2008a) Interferon-gamma and interleukin-12 gene polymorphisms and their relation to aggressive and chronic periodontitis and key periodontal pathogens. *Journal of Periodontology***79**, 1434-1443.

Reichert, S., Machulla, H. K., Klapproth, J., Zimmermann, U., Reichert, Y., Glaser, C., Schaller, H. G. & Schulz, S. (2009) Interleukin-2 -330 and 166 gene polymorphisms in relation to aggressive or chronic periodontitis and the presence of periodontopathic bacteria. *Journal of Periodontal Research***44**, 628-635.

Reichert, S., Machulla, H. K., Klapproth, J., Zimmermann, U., Reichert, Y., Glaser, C. H., Schaller, H. G., Stein, J. & Schulz, S. (2008b) The interleukin-10 promoter haplotype ATA is a putative risk factor for aggressive periodontitis. *Journal of Periodontal Research***43**, 40-47.

Reichert, S., Stein, J. M., Klapproth, J., Zimmermann, U., Reichert, Y., Glaser, C., Schaller, H. G. & Schulz, S. (2011) The genetic impact of the Q551R interleukin-4 receptor alpha polymorphism for aggressive or chronic periodontitis and the occurrence of periodontopathic bacteria. *Archives of Oral Biology***56**, 1485-1493.

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**, 882-890.

Schulz, S., Hierse, L., Altermann, W., Klapproth, J., Zimmermann, U., Reichert, Y., Glaser, C., Kluttig, A., Stein, J. M., Schaller, H. G. & Reichert, S. (2010) The del/del genotype of the nuclear factor-kappaB -94ATTG polymorphism and its relation to aggressive periodontitis. *Journal of Periodontal Research***45**, 396-403.

Schulz, S., Machulla, H. K., Altermann, W., Klapproth, J., Zimmermann, U., Glaser, C., Kluttig, A., Stein, J., Schaller, H. G. & Reichert, S. (2008a) Genetic markers of

tumour necrosis factor alpha in aggressive and chronic periodontitis. *Journal of Clinical Periodontology***35**, 493-500.

Schulz, S., Schlitt, A., Lutze, A., Lischewski, S., Seifert, T., Dudakliewa, T., Gawe, R., Werdan, K., Hofmann, B., Glaser, C., Schaller, H. G. & Reichert, S. (2012) The importance of genetic variants in TNFalpha for periodontal disease in a cohort of coronary patients. *Journal of Clinical Periodontology***39**, 699-706.

Schulz, S., Stein, J. M., Altermann, W., Klapproth, J., Zimmermann, U., Reichert, Y., Glaser, C., Schaller, H. G. & Reichert, S. (2011) Single nucleotide polymorphisms in interleukin-1gene cluster and subgingival colonization with Aggregatibacter actinomycetemcomitans in patients with aggressive periodontitis. *Human Immunology***72**, 940-946.

Schulz, S., Zissler, N., Altermann, W., Klapproth, J., Zimmermann, U., Glaser, C., Schaller, H. G. & Reichert, S. (2008b) Impact of genetic variants of CD14 and TLR4 on subgingival periodontopathogens. *International Journal of Immunogenetics***35**, 457-464.

Shimomura-Kuroki, J., Yamashita, K. & Shimooka, S. (2009) Tannerella forsythia and the HLA-DQB1 allele are associated with susceptibility to periodontal disease in Japanese adolescents. *Odontology***97**, 32-37.

Socransky, S. S., Haffajee, A. D., Smith, C. & Duff, G. W. (2000) Microbiological parameters associated with IL-1 gene polymorphisms in periodontitis patients. *Journal of Clinical Periodontology***27**, 810-818.

Stein, J. M., Lammert, F., Zimmer, V., Granzow, M., Reichert, S., Schulz, S., Ocklenburg, C. & Conrads, G. (2010) Clinical periodontal and microbiologic parameters in patients with Crohn's disease with consideration of the CARD15 genotype. *Journal of Periodontology***81**, 535-545.

Trombone, A. P., Cardoso, C. R., Repeke, C. E., Ferreira, S. B., Jr., Martins, W., Jr., Campanelli, A. P., Avila-Campos, M. J., Trevilatto, P. C., Silva, J. S. & Garlet, G. P. (2009) Tumor necrosis factor-alpha -308G/A single nucleotide polymorphism and red-complex periodontopathogens are independently associated with increased levels

of tumor necrosis factor-alpha in diseased periodontal tissues. *Journal of Periodontal Research***44**, 598-608.

Tsarev, V. N. & Nikolaeva, E. N. (2010) [Polymorphism of IL1alpha and IL1beta genes and bacterial invasion in patients with chronic generalized periodontitis]. *Stomatologiia (Mosk)***89**, 19-23.

Turnbaugh, P.J., Ley, R.E., Hamady, M., Fraser-Liggett, C.M., Knight, R.&Gordon, J.I. (2007) The human microbiome project. *Nature***449**:804–10.

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., Sogin, M. L., Jones, W. J., Roe, B. A., Affourtit, J. P., Egholm, M., Henrissat, B., Heath, A. C., Knight, R. & Gordon, J. I. (2009) A core gut microbiome in obese and lean twins. *Nature***457**, 480-484.

Wacholder, S., Silverman, D.T., McLaughlin, J.K. & Mandel, J.S. (1992) Selection of controls in cse-control studies. II. Types of controls. *American Journal of Epidemiology* **135**: 1029-1041.

Wang, Y., Sugita, N., Kikuchi, A., Iwanaga, R., Hirano, E., Shimada, Y., Sasahara, J., Tanaka, K. & Yoshie, H. (2012) FcgammaRIIB-nt645+25A/G gene polymorphism and periodontitis in Japanese women with preeclampsia. *International Journal of Immunogenetics***39**, 492-500.

Wolf, D. L., Neiderud, A. M., Hinckley, K., Dahlen, G., van de Winkel, J. G. & Papapanou, P. N. (2006) Fcgamma receptor polymorphisms and periodontal status: a prospective follow-up study. *Journal of Clinical Periodontology***33**, 691-698.

Ye, Y., Carlsson, G., Wondimu, B., Fahlen, A., Karlsson-Sjoberg, J., Andersson, M., Engstrand, L., Yucel-Lindberg, T., Modeer, T. & Putsep, K. (2011) Mutations in the ELANE gene are associated with development of periodontitis in patients with severe congenital neutropenia. *Journal of Clinical Immunology***31**, 936-945.

TABLE LEGENDS:

Table 1: Summary of study characteristics andof genetic and microbiological methods and main findings for included studies. CC= case-control studies where periodontitis cases where compared with healthy controls; CS= cross-sectional studies of periodontitis cases or general population, without presence of pre-selected controls; L= longitudinal studies; CP= Chronic Periodontitis; AgP= Aggressive Periodontitis; CG= Chronic Gingivitis; H= Healthy; PD= Periodontitis; CD= Crohn's disease; CAD= Coronary Artery Disease; GO= Gingival Overgrowth; SPT= Supportive Periodontal Therapy; SNP= Single Nucleotide Polymorphism; CB= checkerboard DNA-DNA hybridization, IL1+= 'IL-1 composite genotype' positive; IL1- = 'IL1 composite genotype' negative; NS= no significant associations detected; *Aa= Aggregatibacter acinomycetemcomitans, Cg= Campylobacter gracilis, Cr= Campylobacter rectus, Cs= Campylobacter showae,Co= Capnocytophaga ochracea, Cs= Capnocytophaga sputigena, Ec= Eikenella corrodens, En=Eubacterium nodatum, Fn= Fusobacterium nucleatum, Fp= Fusobacterium periodonticum, Pm= Peptostreptococcus micros, Pg= Porphyromonas gingivalis, Pi= Prevotella intermedia, Sa= Streptococcus anginosus, Sc=Streptococcus constellatus. Si= Streptococcus intermedius, Sg= Streptococcus gordonii,Td= Treponema denticola, Tf= Tannerella forsythia,* HSV= Herpes Virus, CMV= Cytomegalovirus, EBV= Epstein-Barr Virus.

Table 2: Quality assessment of included case-control studies with the scoring system previously proposed (Nibali (2013).

Table 3: Quality assessment of included longitudinal studies with the Newcastle Ottawa scale.

FIGURE LEGENDS:

Figure 1: Flowchart of study inclusion

Figure 2: Forest plot presenting risk ratio of *A. actinomycetemcomitans* subgingival detection in patients with periodontitis by *IL1* composite genotype (overall risk ratio=0.79, 95% CI= 0.45 to 1.38,p= 0.40, moderate to high degree of heterogeneity).

Figure 3: Forest plot presenting risk ratio of *P. gingivalis* subgingival detection in patients with periodontitis by *IL1* composite genotype (overall risk ratio=1.24, 95% CI= 0.90 to 1.72,p= 0.18, high degree of heterogeneity).

Figure 4: Forest plot presenting risk ratio of *T. forsythia* subgingival detection in patients with periodontitis by *IL1* composite genotype (overall risk ratio=1.01, 95% CI= 0.91 to 1.13,p= 0.80, low degree of heterogeneity).

SUPPLEMENTAL MATERIAL LEGENDS:

Supplemental Material 1: PRISMA checklist
Supplemental Material 2: Summary of search strategy.
Supplemental Material 3: Funnel plot of meta-analysis of risk ratio of*A. actinomycetemcomitans* subgingival detection in patients with periodontitis by *IL1* composite genotype.
Supplemental Material 4: Funnel plot of meta-analysis of risk ratio of*P. gingivalis* subgingival detection in patients with periodontitis by *IL1* composite genotype
Supplemental Material 5: Funnel plot of meta-analysis of risk ratio of*T. forsythia* subgingival detection in patients with periodontitis by *IL1* composite genotype.

Authors	Study design	Ethnicity	No. patients	Clinical diagnosis	Genetic analysis		Microbiological analysis		Associations- main results
					Method	Analysed genes	Method	Analysed bacteria	
Agerbaeck et al. 2006	CS	Caucasian	151	CP in SPT	PCR	IL1	CB	40 taxa	IL-1 -: > total bacterial load and >levels of Aa, En, Pg, Sa
Borges et al. 2009	CC	Caucasian	60	CP, H	PCR	VDR	CB	38 taxa	NS
Borilova et al. 2013	CC	Caucasian	492	AgP, CP, H	RT PCR	IL8	DNA microarray	Aa, Pg, Pi,Tf,Td,Pm, Fn	Healthy subjects with IL8 +396 T allele: < Fn detection; AgP patients with IL8 −251T allele: > Aa detection; CP patients with IL8 +781 CC genotype: < Tf detection
Borilova et al. 2015	CC	Caucasian	469	CP, H	RT PCR	ApoE	DNA microarray	Aa, Pg, Pi,Tf,Td,Pm, Fn	NS
Cavalla et al. 2015	CC	Mixed	608	CP, CG, H	RT PCR	TBX21	PCR	Pg, Tf, Td	NS
Checchi et al. 2004	CS	Caucasian	25	CP	Commercial kit	IL1	PCR?	Pg, Pi	NS
Divaris et al. 2012	CS	Caucasian + Blacks	1020 + 123	Range H-PD	Genome-wide SNP array	85,947 SNPs	CB	Aa, Pg, Pi,Cr,Fn,Pn, Tf,Td	No genome-wide significant signals but 16 loci providing suggestive evidence of association

Ferreira et al. 2008	CC	Mixed	292	CP, H	PCR	IL1	PCR	Aa, Pg, Tf, Td	NS
Finoti et al. 2013	CC	Caucasian	39	CP, H	PCR	IL4	qPCR	Pg, Tf, Td	IL4 TCI/CCI haplotype: higher levels of Pg, Tf, Td
Finoti et al. 2013	CS	Mixed	65	CP, H	PCR	IL8	qPCR	Pg, Tf, Td	NS
Goncalves et al. 2009	CC	Mixed	105	CP, H (2 arms with HIV)	PCR	IL1	CB	33 bacterial species	NS
Gong et al. 2013	CS	Ns	204	Renal transplant with and without GO	PCR	CD14	PCR	Aa, Pg, Pi, Td, Tf	GO patients with CD14 -260 CT + TT genotype: > detection of Pg, Td, and Tf and red complex bacteria
Hirano et al. 2010	CS	Japanese	130	CP or H (all pregnant)	PCR	PPAR	PCR	Aa, Pg, Pi, Tf	NS
Holla et al. 2011	CC	Caucasian	498	CP, H	PCR	IFN	DNA microarray	Aa, Pg, Pi,Tf,Td,Pm, Fn	NS
Holla et al. 2012	CC	Caucasian	619	CP, H	PCR	MMP8	Commercial kit	Aa, Pg Pi,Tf,Td,Pm, Fn	NS
Jansson et al. 2005	L	Ns	22	Patients with dental implants	PCR	IL1	PCR	Aa, Pg, Pn	NS

Kowalski et al. 2006	CS	Ns	16	CP	Commercial kit	IL1	Commercial kit	Aa, Pg, Pi,Ec,Cr,Fn, Tf,Pm,Td	IL1 + subjects: higher Cr
Kratka et al. 2007	L	Ns	20	AgP	Commercial kit	IL1	Commercial kit	Aa, Pg, Pi,Tf,Td	NS
Luo et al. 2013	CS	Chinese	202	Renal transplant with and without GO	PCR	IL10	PCR	Aa, Pg, Pi,Td,Tf	GO patients with ATA haplotype: higher detection and counts of Pg and Td
Nibali et al. 2007	CS	Mixed	45	AgP	RT PCR	Fc-γ, FPR,TLR	Culture + PCR	Aa, Pg, Tf	IL6 -174 GG genotype and Fc-γ haplotypes: more detection of Aa
Nibali et al. 2008	CS	Mixed	107	AgP, CP	RT PCR	IL1, IL6, TLR,TNF	PCR	Aa, Pg, Tf	IL6 -6106 AA and IL6 haplotypes: > detection of Aa
Nibali et al. 2011	CS	Indians	251	Range H-PD	RT PCR	IL6	CB	40 taxa	IL6 - 174 GG genotype: > counts of Aa and detection and counts of Cs
Nibali et al. 2012	CS	Mixed	267	AgP, CP	RT PCR	IL6	PCR	Aa, Pg	IL-6 -1480 CC and -174 GG genotypes: > detection of Aa and Pg
Nibali et al. 2013	L	Caucasian	12	AgP	RT PCR	IL6	PCR	Aa	IL6 haplotypes: > counts of Aa before and after treatment
Papapanou et al. 2001	CC	Caucasian	205	CP, H	PCR	IL1	CB	19 bacterial strains	NS
Reichert et	CC	Caucasian	93	AgP, CP,	PCR	IL10	PCR test	Aa, Pg, Pi,	IL10 ACC, ATA and ACC/ATA

al. 2008a				H				Tf, Td	haplotypes: < Pi; IL10 GCC/GCC haplotypes: > Pi
Reichert et al. 2008b	CC	Caucasian	198	AgP, CP, H	PCR	IFN,IL 12	PCR	Aa, Pg, Pi, Tf, Td	IFN-γ 874 AA: < detection of Aa; IFN-γ 874 TA: > detection of Pi
Reichert et al. 2009	CC	Caucasian	200	AgP, CP, H	PCR	IL2	PCR test	Aa, Pg, Pi, Tf, Td	IL-2 -330, 166 TT-TT haplotype: > detection of Pg and red complex
Reichert et al. 2011	CC	Caucasian	243	AgP, CP, H	PCR	IL4	PCR test	Aa, Pg, Pi, Tf, Td	NS
Rhodin et al. 2014	CS	Caucasian	1020	Range H-PD	Genome-wide SNP array	85,947 SNPs	CB	Aa, Pg, Pi,Cr,Fn,Pn, Tf,Td	KCNK1 gene: >red complex becteria detection; DAB2IP gene: > Pg detection
Schulz et al. 2008a	CC	Caucasian	175	AgP, CP, H	PCR	TNF	PCR test	Aa, Pg, Pi, Tf, Td	TNFα 308GG / 238GG haplotype: > Pi detection
Schulz et al. 2008b	CC	Caucasian	213	AgP, CP, H	PCR	CD14, TLR4, ThR	PCR test	Aa, Pg, Pi, Tf, Td	PD patients with CD14 TT genotype: < Pi detection
Schulz et al. 2010	CC	Caucasian	222	AgP, CP, H	PCR	TLR2, NFkB	PCR test	Aa, Pg, Pi, Tf, Td	NF-kappaB -94 del/del: > Aa detection
Schulz et al. 2011	CC	Caucasian	248	AgP, CP, H	PCR	IL1	PCR test	Aa, Pg, Pi, Tf, Td	rs1800587, rs1143634 and IL1+: > Aa detection in the AgP group
Schulz et al. 2012	CS	Caucasian	942	CP, H (all with CAD)	PCR	TNF	PCR test	Aa, Pg, Pi, Pm, Tf, Td, Fn,Cr,En,Ec Cs,Cg,Co	TNF-α 308 AG+AA genotype and A-allele: > Pi detection
Shimomura et al. 2009	CC	Japanese	64	AgP, CP, H	PCR	IL1, Fc-γ,	PCR	Aa, Pg, Pi, Tf, Td	Patients withHLADQB1 BamHI site: > Tf detection

						HLA			
Socransky et al. 2000	CS	Ns	108	CP	PCR	IL1	CB	40 taxa	IL1 +: > counts of Tf, Td, Fn, Fp, Cg, Cs, Sc, Si, Sg and 3 Capnocytophaga species
Stein et al. 2010	CS	Caucasian	147	CP, H (all with CD)	PCR	CARD 15	Dot-blot hybridization	Aa, Pg, Tf, Pi, Cr	CARD15 mutations: < Pi detection
Trombone et al. 2009	CC	Mixed	304	CP, H	PCR	TNF	PCR	Aa, Pg, Tf, Td	NS
Tsarev & Nikolaeva 2010	CC	Caucasian	95	AgP, CP, H	Commercial kit	IL1	Commercial kit	Aa, Pg, Pi, Tf, Td, HSV1, HSV2, CMV, EBV	IL1 +: > detection of Pi, Tf, Td, Pg, HSV2 and EBV
Wang et al. 2012	CC	Japanese	119	CP, H (all post-birth)	PCR	Fc-γ	PCR	Aa, Pg, Pi	NS
Wolf et al. 2006	CC	Caucasian	205	CP, H	PCR	Fc-γ	CB	19 bacterial strains	NS
Ye et al. 2011	CS	Caucasian	14	CP, H (all with neutropenia)	Ns	ELANE	16s rDNA pyrosequencing	No specific target	No statistical analysis presented

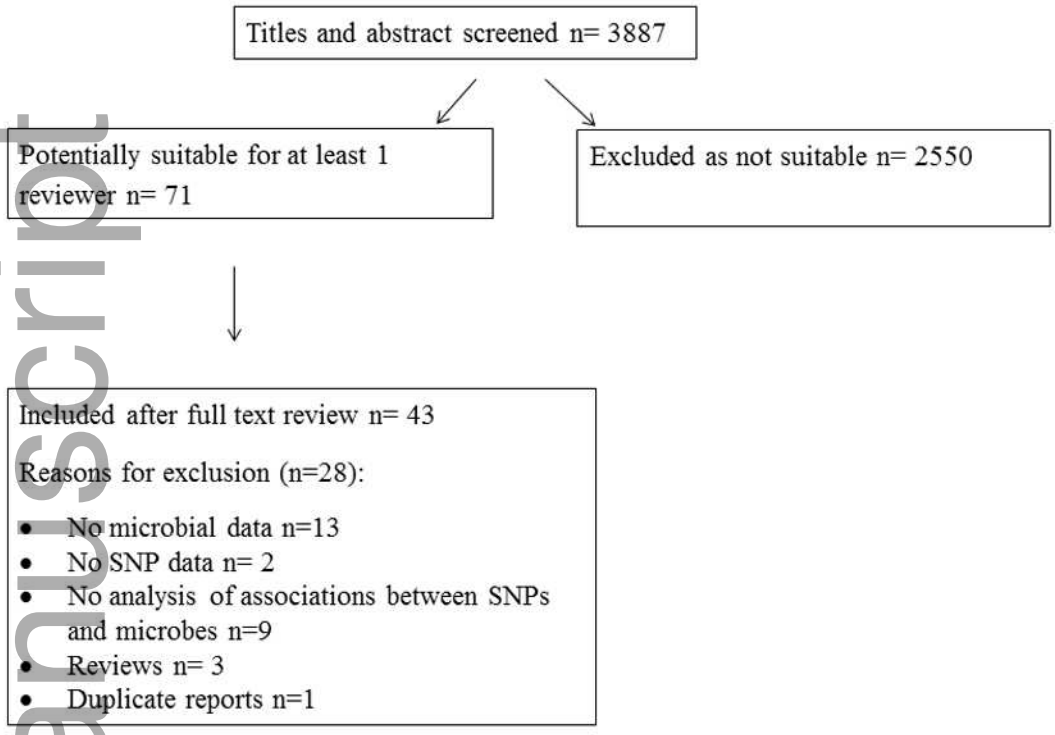
Authors	Selection (4 items)	Comparability (1 item)	Exposure (3 items)	Study design (4 items)	Genetic analysis (8 items)
Agerbaeck et al. 2006			★	★	★★★★★
Borges et al. 2009	★★★		★★★	★★	★★★★★
Borilova et al. 2013	★★★	★	★	★★★	★★★★★★
Borilova et al. 2015	★★★	★	★	★★★	★★★★★★
Cavalla et al. 2015	★★★	★	★★★	★★★	★★★★★★★
Checchi et al. 2004	★★		★	★	★★★
Divaris et al. 2012	★★★	★	★★★	★★★	★★★★★★
Ferreira et al. 2008	★★★	★	★★★	★	★★★★★
Finoti et al. 2013	★	★	★★★	★★	★★★★
Finoti et al. 2013	★	★	★★★	★★★	★★★★
Goncalves et al. 2009	★	★	★★★	★	★★★
Gong et al. 2013	★★★		★★★	★	★★★★
Hirano et al. 2010	★★	★	★★★	★	★★★★★
Holla et al. 2011	★★★	★	★★★	★★★	★★★★
Holla et al. 2012	★★★	★	★★★	★★★	★★★★★
Kowalski et al. 2006	★		★		★★★
Luo et al. 2013	★★★		★★★	★★★	★★★★★★
Nibali et al. 2007	★		★	★★	★★★★★
Nibali et al. 2008	★★		★	★★	★★★★★★
Nibali et al. 2011	★★★★	★	★★★	★★	★★★★★★
Nibali et al. 2012	★★		★	★★	★★★★★
Papapanou et al. 2001	★★	★	★★★	★	★★★★★
Reichert et al. 2008a	★★★	★	★★★	★★★	★★★
Reichert et al. 2008b	★★★	★	★★★	★★★	★★★★
Reichert et al. 2009	★★★	★	★★★	★★	★★★
Reichert et al. 2011	★★★	★	★★★	★★★★	★★★★
Rhodin et al. 2014	★★★	★	★★★	★★★	★★★★★★
Schulz et al. 2008a	★★	★	★★★	★★★★	★★★★★
Schulz et al. 2008b	★★	★	★★★	★★★	★★★★★

Schulz et al. 2010	★★★★	★	★★★	★★★	★★★★
Schulz et al. 2011	★★★★	★	★★★	★★★★	★★★★
Schulz et al. 2012	★★★★		★★★	★★★	★★★★
Shimomura et al. 2009	★		★★	★★	★★★★
Socransky et al. 2000			★	★	★★★★★
Stein et al. 2010	★		★★	★★★	★★★★★★
Trombone et al. 2009	★★★	★	★★★	★★	★★★★★
Tsarev & Nikolaeva 2010	★★		★★★	★★	★★★★
Wang et al. 2012	★★	★	★★	★★★	★★★★★
Wolf et al. 2006	★★	★	★★★	★★	★★★★
Ye et al. 2011	★★★		★★★		★★

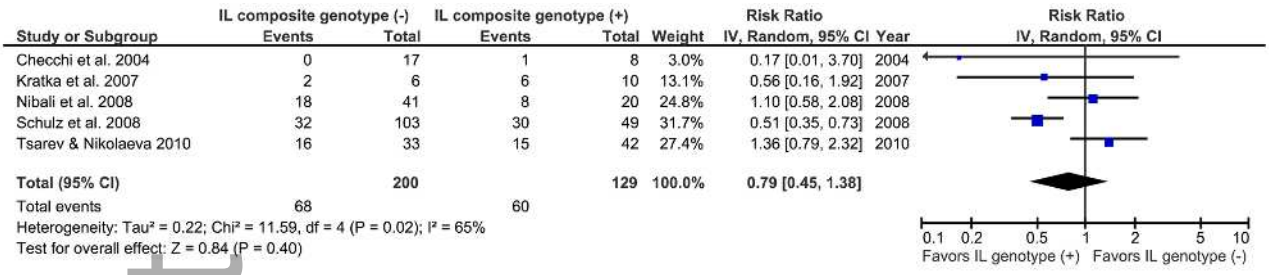
Table 2.

	Selection	Comparability	Outcome
Jansson et al. 2005	★★★		★★
Kratka et al. 2007	★★★★	★	★★
Nibali et al. 2013	★★★★	★★	★★★

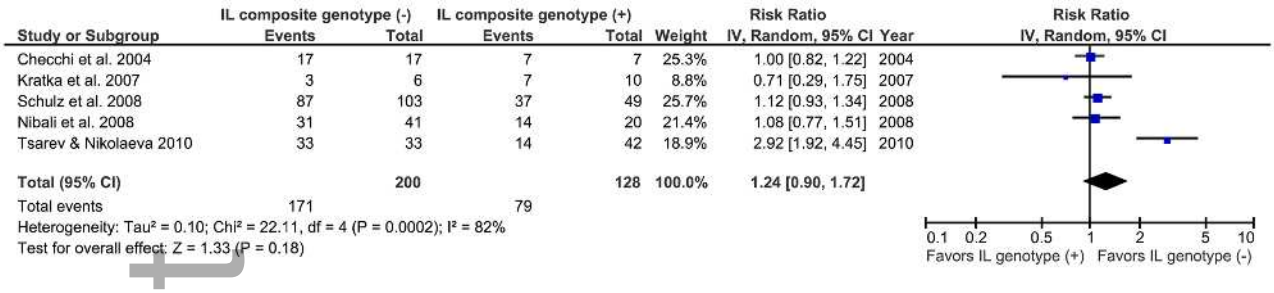
Table 3.



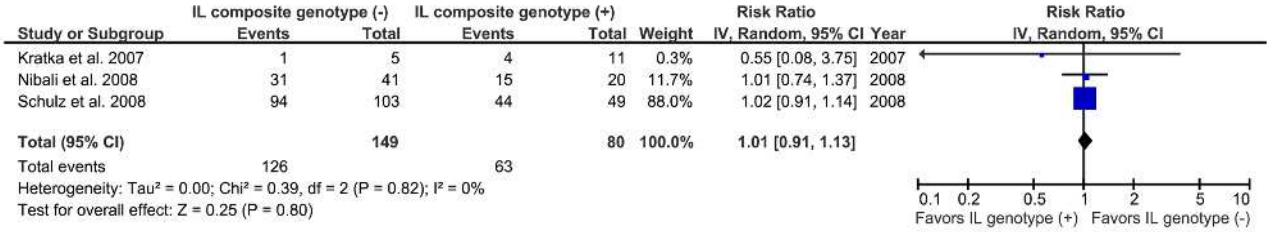
jcpe_12600_f1.tif



jcpe_12600_f2.tif



jcpe_12600_f3.tif



jcpe_12600_f4.tif