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Systematic Review

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

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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.

Key words: bacteria; genetic; infectogenomics; periodontitis

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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)

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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 variants and microbial 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.

Material 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 Appendix S1). A review protocol was prepared and registered with PROSPERO (reference CRD42015026928).

Focused question

- The question addressed was the following: is there an association 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 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 September 2015), Embase (up to 13 September 2015), LILACS and Cochrane Library (both up to 14 September 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 Appendix S2.

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 and diagnosis of periodontal status). Specific data on genetic and microbial analysis, genetic variants analysed, microbes analysed, method used for genetic analysis and method used for microbial sampling and microbial detection/identification were 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 0–20 adapted to genetic analyses of periodontal studies (Nibali 2013a). The "Newcastle Ottawa tool to assess risk of bias" (Newcastle Ottawa scale http://www.ohri.ca/)

was used to assess risk of bias for longitudinal studies.

Summary measures/synthesis of results/ statistical methods

The study outcomes were the 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 three papers investigating the same combination of SNPs and subgingival bacteria. The risk ratios of primary and secondary outcomes were estimated using a computer program (Review Manager Version 5.0.; The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark). The contribution of the included articles was weighted 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 show the difference in outcomes of groups with different genotypes using number of SNPs with each genotype as 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 papers at Ovid Medline, Embase, Cochrane Library and LILACS combined, which reduced to 3887 after removing conference abstracts, case reports and reviews. Following first-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 Fig. 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.

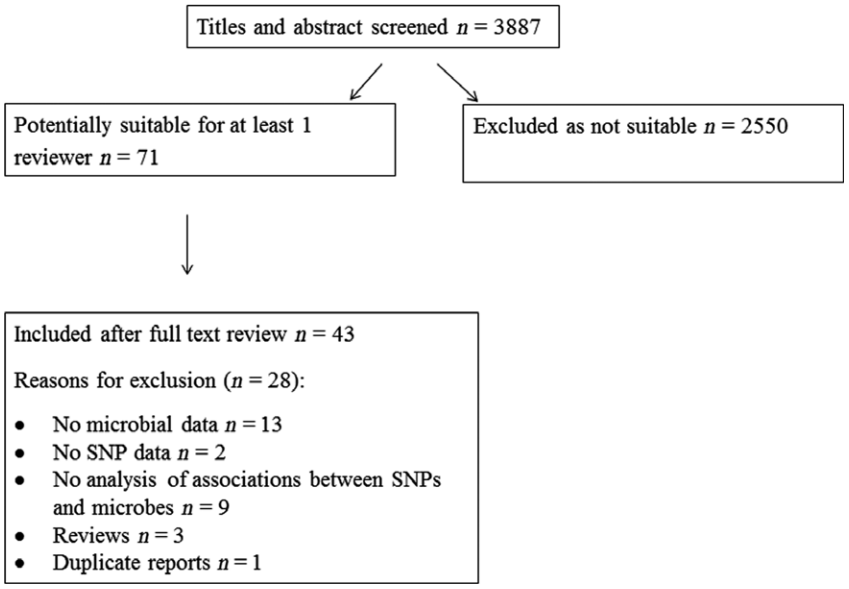

Fig. 1. Flowchart of study inclusion.

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 one was written in Russian and one 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 two 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), neutropaenia (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

Table 1. Summary of study characteristics and of genetic and microbiological methods and main findings for included studies

Authors	Study design	Ethnicity	No. patients	Clinical diagnosis	Genetic analysis		Microbiological analysis		Associations- main results
					Method	Analysed genes	Method	Analysed bacteria	
Agerbaek 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 Linhartova 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 Linhartova 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. (2013a)	CC	Caucasian	39	CP, H	PCR	IL4	qPCR	*Pg*, *Tf*, *Td*	IL4 TCI/CCI haplotype: higher levels of *Pg*, *Tf*, *Td*
Finoti et al. (2013b)	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*

Table 1. (continued)

Authors	Study design	Ethnicity	No. patients	Clinical diagnosis	Genetic analysis		Microbiological analysis		Associations- main results
					Method	Analysed genes	Method	Analysed bacteria	
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. (2013b)	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 al. (2008a)	CC	Caucasian	93	AgP, CP, H	PCR	IL10	PCR test	*Aa*, *Pg*, *Pi*, *Tf*, *Td*	IL10 ACC, ATA and ACC/ATA haplotypes: <*Pi*; IL10 GCC/GCC haplotypes: >*Pi*
Reichert et al. (2008b)	CC	Caucasian	198	AgP, CP, H	PCR	IFN, IL12	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

Table 1. (continued)

Authors	Study design	Ethnicity	No. patients	Clinical diagnosis	Genetic analysis		Microbiological analysis		Associations- main results
					Method	Analysed genes	Method	Analysed bacteria	
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-κB −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-Kuroki et al. (2009)	CC	Japanese	64	AgP, CP, H	PCR	IL1, Fc-γ, HLA	PCR	*Aa*, *Pg*, *Pi*, *Tf*, *Td*	Patients withHLADQB1 BamHI site: >*Tf* detection
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

AgP, aggressive periodontitis; CAD, coronary artery disease; CB, checkerboard DNA-DNA hybridization, IL1+, "IL-1 composite genotype" positive; CC, case-control studies where periodontitis cases where compared with healthy controls; CD, Crohn's disease; CG, chronic gingivitis; CMV, cytomegalovirus; CP, chronic periodontitis; CS, cross-sectional studies of periodontitis cases or general population, without presence of pre-selected controls; EBV, Epstein–Barr virus; GO, gingival overgrowth; H, healthy; HSV, herpes virus; IL1−, "IL1 composite genotype" negative; L, longitudinal studies; NS, no significant associations detected; PD, periodontitis; SNP, single nucleotide polymorphism; SPT, supportive periodontal therapy; *Aa*, *Aggregatibacter acinomycetemcomitans*; *Cg*, *Campylobacter gracilis*; *Co*, *Capnocytophaga ochracea*; *Cr*, *Campylobacter rectus*; *Cs*, *Campylobacter showae*; *Cs*, *Capnocytophaga sputigena*; *Ec*, *Eikenella corrodens*; *En*, *Eubacterium nodatum*; *Fn*, *Fusobacterium nucleatum*; *Fp*, *Fusobacterium periodonticum*; *Pg*, *Porphyromonas gingivalis*; *Pi*, *Prevotella intermedia*; *Pm*, *Peptostreptococcus micros*; *Sa*, *Streptococcus anginosus*; *Sc*, *Streptococcus constellatus*; *Sg*, *Streptococcus gordonii*; *Si*, *Streptococcus intermedius*; *Td*, *Treponema denticola*; *Tf*, *Tannerella forsythia*.

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, two genes (KCNK1 and DAB2IP) showed a significant association with high periodontal pathogen colonization (red complex and *P. 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 three 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* rs1143634 (previously reported as *IL1B* +3953 or +3954) and *IL1A* rs1800587 (previously reported as *IL1A* −889). Among studies on "bacterial counts," conflicting results were reported. Socransky et al. (2000) found increased proportions of several subgingival bacteria (*T. forsythia*, *T. denticola*, *F. nucleatum*, *Fusobacterium periodonticum*, *Campylobacter gracilis*, *Capnocytophaga sputigena*, *Streptococcus gordonii*, *Streptococcus constellatus*. *Streptococcus intermedius* and three *Capnocytophaga* species) in *IL1*+ subjects. Increased *Campylobacter rectus* counts were found in a study on *IL1*+ CP patients compared with *IL1*− (Kowalski et al. 2006). On the other hand, among 151 CP patients in supportive periodontal care, IL1− subjects had an increased total bacterial load and increased levels of *A. actinomycetemcomitans*, *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 perform meta-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 three) were conducted in Caucasians investigating "*IL1* composite genotype" and detection of *A. actinomycetemcomitans* and *P. gingivalis* in patients with periodontitis (including aggressive periodontitis, CP 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 studies revealed a non-statistically significant overall risk ratio of 0.79 (95% CI = 0.45–1.38, $p = 0.40$) (Fig. 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 = 0.90–1.72), no statistical significance ($p = 0.18$) (Fig. 3) and a high degree of heterogeneity (p-value for chi-square test = 0.0002, and I^2 test = 82%).

Meta-analysis of three 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 = 0.91–1.13), with no statistical significance ($p = 0.80$) (Fig. 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 (rs1800795) homozygous subjects and in subjects with specific *IL6* genotypes and haplotypes (defined by IL6 −1363 rs2069827 and −1480 rs2069825)

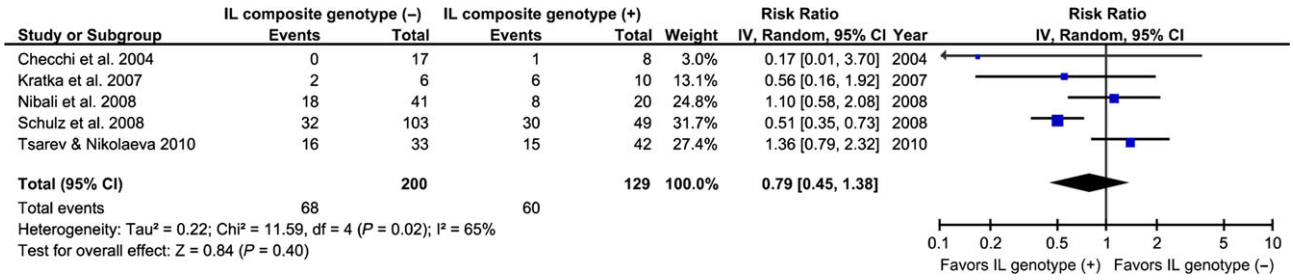

Fig. 2. Forest plot presenting risk ratio of *Aggregatibacter actinomycetemcomitans* subgingival detection in patients with periodontitis by *IL1* composite genotype (overall risk ratio = 0.79, 95% CI = 0.45–1.38, $p = 0.40$, moderate to high degree of heterogeneity).

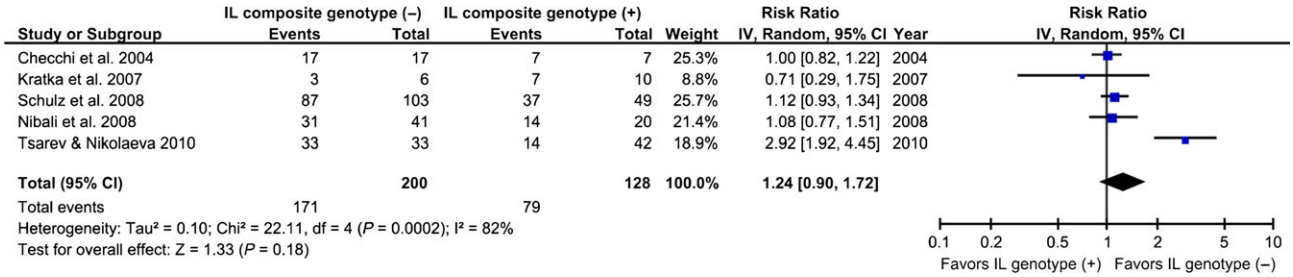

Fig. 3. Forest plot presenting risk ratio of *Poprhyromonas gingivalis* subgingival detection in patients with periodontitis by *IL1* composite genotype (overall risk ratio = 1.24, 95% CI = 0.90–1.72, $p = 0.18$, high degree of heterogeneity).

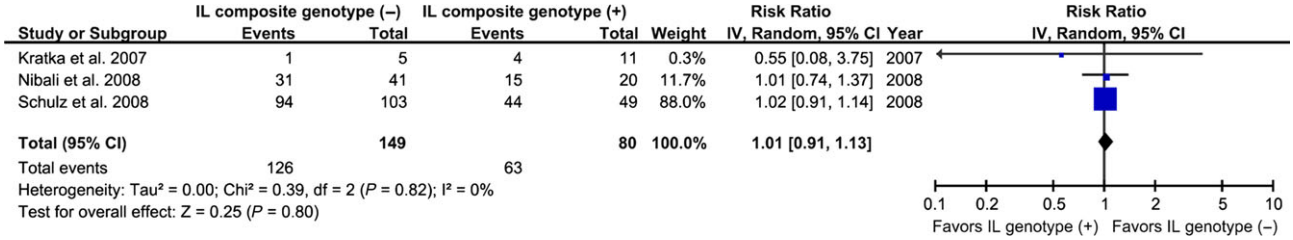

Fig. 4. Forest plot presenting risk ratio of *Tannerella forsythia* subgingival detection in patients with periodontitis by *IL1* composite genotype (overall risk ratio = 1.01, 95% CI = 0.91–1.13, $p = 0.80$, low degree of heterogeneity).

(Nibali et al. 2011, 2012, 2013b). However, meta-analysis was not performed because of heterogeneity in ethnicities 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 or AA genotypes and with A-allele to be associated with higher *P. intermedia* detection in a separate study on coronary artery disease 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 Appendix S3–S5 (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 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

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

Authors	Selection (4 items)	Comparability (1 item)	Exposure (3 items)	Study design (4 items)	Genetic analysis (8 items)
Agerbaek et al. (2006)			★	★	★★★★★
Borges et al. (2009)	★★★		★★★	★★	★★★★★
Borilova Linhartova et al. (2013)	★★★	★	★	★★★	★★★★★★
Borilova Linhartova et al. (2015)	★★★	★	★	★★★	★★★★★★
Cavalla et al. (2015)	★★★	★	★★★	★★★	★★★★★★★
Checchi et al. (2004)	★★		★	★	★★★
Divaris et al. (2012)	★★★	★	★★★	★★★	★★★★★★
Ferreira et al. (2008)	★★★	★	★★★	★	★★★★★
Finoti et al. (2013a)	★	★	★★★	★★	★★★★
Finoti et al. (2013b)	★	★	★★★	★★★	★★★★
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-Kuroki 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 3. Quality assessment of included longitudinal studies with the Newcastle Ottawa scale

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

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, Genome-Wide Association Studies (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 and focused on eight 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, two genes (KCNK1 and DAB2IP) showed association with high periodontal pathogen colonization (red complex and *P. gingivalis* respectively). These two genes also exhibited suggestive genome-wide association with CP (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. actinomycetemcomitans*, *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 the pro-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 267 AgP 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" *versus* "*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. 2013b). 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 studies included in the current 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 size 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 & 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.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. PRISMA checklist.
Appendix S2. Summary of search strategy.
Appendix S3. Funnel plot of meta-analysis of risk ratio of *Aggregatibacter actinomycetemcomitans* subgingival detection in patients with periodontitis by *IL1* composite genotype.
Appendix S4. Funnel plot of meta-analysis of risk ratio of *Poprhyromonas gingivalis* subgingival detection in patients with periodontitis by *IL1* composite genotype.
Appendix S5. Funnel plot of meta-analysis of risk ratio of *Tannerella forsythia* subgingival detection in patients with periodontitis by *IL1* composite genotype.

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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.