PERIODONTAL INFECTOGENOMICS: THE EFFECT OF HOST GENETIC VARIANTS ON SUBGINGIVAL MICROBIAL COLONIZATION. A SYSTEMATIC REVIEW
Investigators:

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1. BACKGROUND

Medical research in the last decades has brought an increased awareness of the magnitude and importance of microbial colonization of the human body, to the extent that we now know that 90% of the cells in the human body are microbial. Whilst most of these bacteria and microbial communities give essential benefits to their host, a handful of them predispose to human disease (McFall et al. 2005). It is clear now that the effects of microbial colonization depend on several factors including microbial virulence, environmental agents and also on the ability of the host to respond to the microbial challenge. This is, at least in part, genetically determined (Cooke & Hill 2001). The term ‘Infectogenomics’ was introduced to define the effect of host genetic variants (namely single nucleotide polymorphisms, or SNPs) in influencing the response to infective agents and therefore the risk to develop disease (Kellam & Weiss 2005). Recently, microbial agents are emerging as a possible cause not just of disease traditionally considered as ‘infective’ but also of other chronic diseases such as cancer and rheumatoid arthritis (Nibali et al. 2014).

Periodontal diseases are characterized by an inflammatory reaction against members of the oral microbiota (Socransky & Haffajee 2012). Periodontitis (PD), a destructive form of periodontal disease, affects the supporting apparatus of the teeth, leading to apical migration of the epithelial attachment and resorption of connective tissue and alveolar bone, often resulting in early tooth loss. Evidence has emerged in the last 15 years of how host genetic variants may predispose to the presence of specific bacteria subgingivally (Socransky & Haffajee 2000, Nibali et al. 2007). This may in turn increase the risk of developing PD (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 (Nibali et al. 2007, Nibali et al. 2008, Divaris et al. 2012, Cavalla et al. 2015), these associations have still not been verified systematically. A better knowledge of which host genetic variant predispose to microbial colonization below the gingival margin and to the development of progression of periodontal disease could potentially help the understanding of periodontal disease pathogenesis and help with its management. For example, subjects with a genetically-determined tendency to increased proliferation of periodontopathogenic bacteria may benefit more from adjunctive antibiotic therapy.

AIM

The aim of this systematic review is to appraise the existing literature on periodontal infectogenomics measured as detection of subgingival microbes based on host genetic variants.
2. REVIEW QUESTION

Broad question:
- What is the association between host genetic variants and detection 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

3. MATERIALS AND METHODS

4.1 IDENTIFYING RESEARCH EVIDENCE

The search will be conducted through the electronic databases MEDLINE, EMBASE, LILACS and The Cochrane Database (including the Central Register of Controlled Trials (CENTRAL)) and will be complemented by a search through the reference lists of included studies. No language restriction will be included in the initial search. Among published literature, peer-reviewed studies, reports, book chapters and conference abstracts will be screened. Narrative or systematic reviews on the topic will be searched in order to identify suitable papers. The search strategy is described below:

MEDLINE:

1. exp Genetic Predisposition to Disease/
2. exp Genotype/
3. Genome, Human/
4. exp Genomics/
5. exp Host-Pathogen Interactions/
6. exp Genetic Variation/
7. exp Genetic Association Studies/
8. Alleles/
9. (single nucleotide polymorphism* or SNP* or allel* or ((gene* or geno*) adj5 (varia* or diver* or differ*))).mp.
10. or/1-9
11. exp Bacterial Infections/
12. exp Infection/
13. exp Bacteria/
14. exp Microbiota/
15. Dysbiosis/
16. (bacter* or microb* or pathogen* or biofilm* or microorganism* or dysbio* or (infect* adj5 agent*)).mp.
17. or/11-16
18. exp Periodontal Diseases/
19. exp Periodontics/
20. exp Mouth/
21. (subgingiva* or gingiv* or periodont* or periopathogen* or periodontopath* or ((dental or tooth or teeth or oral*) adj5 plaque)).mp.
22. or/18-21
23. 10 and 17 and 22

**EMBASE:**

1. exp genetic predisposition/
2. exp genotype/
3. human genome/
4. exp genomics/
5. host pathogen interaction/
6. genetic variability/
7. genetic association/
8. allele/
9. exp genetic heterogeneity/
10. (single nucleotide polymorphism* or SNP* or allel* or ((gene* or geno*) adj5 (varia* or diver* or differ*)).mp.
11. or/1-10
12. exp bacterial infection/
13. exp infection/
14. exp bacteria/
15. exp microflora/
16. dysbiosis/
17. (bacter* or microb* or pathogen* or biofilm* or microorganism* or dysbio* or (infect* adj5 agent*)).mp.
18. or/12-17
19. exp periodontal disease/
20. exp periodontics/
21. exp mouth/
22. exp dentition/
23. (subgingiva* or gingiv* or periodont* or periopathogen* or periodontopath* or ((dental or tooth or teeth or oral*) adj5 plaque)).mp.
24. or/19-23
25. 11 and 18 and 24

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**Cochrane Library:**

#1. MeSH descriptor: [Genetic Predisposition to Disease] explode all trees
#2. MeSH descriptor: [Genotype] explode all trees
#3. MeSH descriptor: [Genome, Human] this term only
#4. MeSH descriptor: [Genomics] explode all trees
#5. MeSH descriptor: [Host-Pathogen Interactions] explode all trees
#6. MeSH descriptor: [Genetic Variation] explode all trees
#7. MeSH descriptor: [Genetic Association Studies] explode all trees
#8. MeSH descriptor: [Alleles] this term only
#9. (single next nucleotide next polymorphism*) or SNP* or allel* or ((gene* or geno*) near/5 (varia* or diver* or differ*))
#10. [or #1-#9]
#11. MeSH descriptor: [Bacterial Infections] explode all trees
#12. MeSH descriptor: [Infection] explode all trees
#13. MeSH descriptor: [Bacteria] explode all trees
#14. MeSH descriptor: [Microbiota] explode all trees
#15. MeSH descriptor: [Dysbiosis] this term only
#16. bacter* or microb* or pathogen* or biofilm* or microorganism* or dysbio* or (infect* near/5 agent*)
#17. [or #11-#16]
#18. MeSH descriptor: [Periodontal Diseases] explode all trees
#19. MeSH descriptor: [Periodontics] explode all trees
#20. MeSH descriptor: [Mouth] explode all trees
#21. subgingiva* or gingiv* or periodont* or periopathogen* or periodontopath* or ((dental or tooth or teeth or oral*) near/5 plaque)
4.2 STUDY SELECTION

The inclusion criteria for studies in the systematic review are:

- Study designs:
  - case-control studies
  - cross-sectional studies
  - longitudinal studies or RCTs providing baseline genetic and microbial data
- Reporting measures of periodontal disease reported (periodontal diagnosis)
- Reporting analysis of host genetic variants (SNPs)
- Reporting data on microbial detection subgingivally (by host genetic variant)

Exclusion criteria are:

- Reviews
- Case reports
- Studies on animal models

Study selection will be conducted by two independent reviewers (AD, OO) in the following stages:

1. Initial screening of potentially-suitable titles and abstracts against the inclusion criteria to identify potentially relevant papers

2. Screening of the full papers identified as possibly relevant in the initial screening

Studies will be excluded if not meeting the inclusion criteria (such as for instance animal studies, conference abstracts or reviews). Following the screening of titles and abstracts (steps 1 and 2), the studies included by both reviewers will be compared and a complete database for step 3 will be formed joining all studies selected by at least one reviewer. Following step 3, in case of a disagreement between reviewers, the decision about study eligibility will be made trying to reach a consensus between the two reviewers. In case of continued disagreement, a third reviewer or
arbitrator (LN) will judge study inclusion. The agreement value between reviewers will be calculated after step 2 and after step 3.

1.3. DATA EXTRACTION

We aim to assess precision, directness, applicability and risk of bias of the included studies. A standardized data extraction form to be used in the study is attached, where data from eligible studies will be recorded. In particular, the following data will be recorded:

- Study design
- Number of patients included
- Patients’ demographics (age, ethnicity, gender, smoking, socio-economic factors)
- Definition and diagnosis of periodontal disease/health
- SNPs analysed
- Microbes analysed
- Method used for genetic analysis
- Method used for microbial sampling technique and microbial detection

4.4 QUALITY ASSESSMENT

The risk of bias will be defined as a systematic error or deviation from the truth, in results or inferences (Verhagen et al. 1998).

The quality of the included studies will be assessed through sensitivity analysis as it could impact on the overall results and conclusions (‘Systematic reviews, CRD’s guidance for undertaking reviews in health care’, University of York, 2008). The ‘Newcastle Ottawa tool to assess risk of bias’ (Newcastle Ottawa scale [http://www.ohri.ca/programs/clinical_epidemiology/oxford.htm]) will be used to assess risk of bias for longitudinal studies. The ‘Cochrane Collaboration’s tool for assessing risk of bias in randomized trials’ (Higgins et al. 2011) will be used to assess risk of bias for RCTs.

- **Case-control studies** (a study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories and a maximum of two stars for Comparability)
  - **Selection**
    1) Is the case definition (periodontitis/ gingivitis/ metabolic syndrome) adequate?
      a) yes, with independent validation
      b) yes, eg record linkage or based on self reports
      c) no description
2) Representativeness of the cases
   a) consecutive or obviously representative series of cases
   b) potential for selection biases or not stated
3) Selection of Controls
   a) community controls
   b) hospital controls
   c) no description
4) Definition of Controls
   a) no history of disease (endpoint)
   b) no description of source

- Comparability
1) Comparability of cases and controls on the basis of the design or analysis
   a) study controls for periodontitis/gingivitis/metabolic syndrome
   b) study controls for any additional factor

- Exposure
1) Ascertainment of exposure
   a) secure record
   b) structured interview where blind to case/control status
   c) interview not blinded to case/control status
   d) written self-report or medical record only
   e) no description
2) Same method of ascertainment for cases and controls
   a) yes
   b) no
3) Non-Response rate
   a) same rate for both groups
   b) non respondents described
   c) rate different and no designation

- Longitudinal cohort studies (A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability):

Selection
1) Representativeness of the exposed cohort
   a) truly representative of the average cases in the community
   b) somewhat representative of the average cases in the community
   c) selected group of users eg nurses, volunteers
   d) no description of the derivation of the cohort
2) Selection of the non exposed cohort
   a) drawn from the same community as the exposed cohort
   b) drawn from a different source
   c) no description of the derivation of the non-exposed cohort
3) Ascertainment of exposure*
   a) secure record (eg surgical records)
   b) structured interview
   c) written self-report
Nibali et al.- Infectogenomics review 1.0- August 2015- confidential

d) no description

* Specifically for this review, ‘secure record’ is defined by clinical diagnosis of furcation involvement (measured with Naber’s probe or equivalent), while ‘unsecure diagnosis’ is based on radiographic or other assessments

4) Demonstration that outcome of interest was not present at start of study
   a) yes
   b) no

Comparability
1) Comparability of cohorts on the basis of the design or analysis
   a) study controls for treatment provided
   b) study controls for any additional factor

Outcome
1) Assessment of outcome
   a) independent blind assessment
   b) record linkage
   c) self report
   d) no description

2) Was follow-up long enough for outcomes to occur
   a) yes (select an adequate follow up period for outcome of interest)
   b) no

3) Adequacy of follow-up of cohorts
   a) complete follow up - all subjects accounted for
   b) subjects lost to follow up unlikely to introduce bias (small number lost or description provided of those lost)
   d) high follow up rate and no description of those lost
   e) no statement

- **Randomized controlled studies (RCTs)**
The following items related to risk of bias in RCTs will be reviewed (Higgins et al. 2011):
  - Selection bias (random sequence generation, allocation concealment)
  - Performance bias (blinding of participants and personnel)
  - Detection bias (blinding of outcome assessment)
  - Attrition bias (incomplete outcome data)
  - Reporting bias (selective reporting)

In addition to this quality assessment, we will further assess study quality based on the following:
  - Subject or site/tooth used as unit of analysis (score 0 for site/tooth, 1 for subject, 2 for unclear)

Furthermore, specific elements of study design with relation to genetic analyses will be evaluated as proposed recently with a score of 0 to 20 (Nibali et al. 2013):
<table>
<thead>
<tr>
<th>Category</th>
<th>Item</th>
<th>Positive answer (score 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SELECTION</strong></td>
<td>Adequate case definition</td>
<td>Full mouth probing and accepted independently-defined diagnosis adopted (e.g. EFP diagnoses) (or validated diagnosis by scoring bone loss in full mouth radiographs)</td>
</tr>
<tr>
<td></td>
<td>Representativeness of cases</td>
<td>Consecutive or obviously representative cases selected (no evidence of selection bias)</td>
</tr>
<tr>
<td></td>
<td>Selection of controls</td>
<td>Community/hospital controls geographically and ethnically matched to the cases</td>
</tr>
<tr>
<td></td>
<td>Definition of controls</td>
<td>Absence of disease assessed (full mouth probing done and accepted independently-defined diagnosis of health adopted)</td>
</tr>
<tr>
<td><strong>COMPARABILITY</strong></td>
<td>Confounders</td>
<td>Cases and controls balanced for smoking, socio-economic status, BMI (or confounders adjusted for)</td>
</tr>
<tr>
<td><strong>EXPOSURE</strong></td>
<td>Ascertainment of exposure</td>
<td>Secure record</td>
</tr>
<tr>
<td></td>
<td>Same method of ascertainment for cases and controls</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Non-response rate</td>
<td>Same for cases and controls</td>
</tr>
<tr>
<td><strong>STUDY</strong></td>
<td>Power calculation</td>
<td>Performed a priori</td>
</tr>
<tr>
<td><strong>METHODOLOGY/DESIGN</strong></td>
<td>Statistics</td>
<td>Well-described tests of significance for primary outcome</td>
</tr>
<tr>
<td></td>
<td>Corrected statistics</td>
<td>correction for false-positive (type I) error</td>
</tr>
<tr>
<td></td>
<td>Odds ratios and confidence intervals</td>
<td>Provided</td>
</tr>
<tr>
<td><strong>GENETIC ANALYSES</strong></td>
<td>Success rate of DNA extraction</td>
<td>Reported good rates</td>
</tr>
<tr>
<td></td>
<td>Success rate of genetic assessment</td>
<td>Reported good rates</td>
</tr>
<tr>
<td></td>
<td>Genotype counts</td>
<td>Provided in table or text</td>
</tr>
<tr>
<td></td>
<td>Hardy-Weinberg equilibrium</td>
<td>Satisfied</td>
</tr>
<tr>
<td></td>
<td>Primer sequence</td>
<td>Provided or referenced</td>
</tr>
<tr>
<td></td>
<td>Reproducibility</td>
<td>Described genotyping method to allow replication, validated genotyping accuracy</td>
</tr>
<tr>
<td></td>
<td>Genotyping blind to case-control status</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Proposed inheritance model</td>
<td>Inheritance model-free approach (not exclusively dominant or recessive pattern proposed)</td>
</tr>
</tbody>
</table>
4.5 DATA SYNTHESIS

Studies will be summarised narratively initially by chief characteristics to determine similarity. We aim to stratify results separately according to periodontal diagnosis (periodontitis, gingivitis, health) if possible.

Differences in the main outcome (microbial detection) will be assessed directly where possible (when compared within the same study) or indirectly where feasible according to study criteria (comparison between results of different studies). A meta-analysis will be considered appropriate and will be performed in the presence of a significant number of similar studies addressing the same question (and analyzing the same gene variants and subgingival microbes) and judged of acceptable quality. However, we anticipate that, owing to likely heterogeneity, meta-analysis may not be feasible. If possible, pairwise comparisons using traditional meta-analysis method or network meta-analysis will be conducted.

4.6 PROTOCOL AMENDMENTS DURING THE REVIEW

If during the search or data synthesis process, a change of direction is considered necessary or an additional review question is posed, the review may need to undergo a protocol modification. In that case, the impact of the change on the literature search and data extraction forms will be assessed, as these may require modification. Protocol amendments will be documented in a protocol addendum.

5. ADMINISTRATIVE PROCEDURES

Data Collection and Case Report Forms (CRFs)

It is the responsibility of the Investigators to maintain copies of case-report forms to be used for analysis. The Investigators have the responsibility for ensuring that all documents are completed and maintained according to the study protocol.

Monitoring

Prior to the commencement of the study, an initiation meeting will be held with the appropriate study personnel to review the objectives and procedures of the clinical trial.
6. STUDY TIMELINE
The literature search will start in October 2015, data extraction will be completed by February 2016, and data entry and analysis will be completed by April 2016. Manuscript preparation will take up to August 2016.

7. REFERENCES
• Cavalla F, Biguetti CC, Colavite PM, Silveira EV, Martins W Jr, Letra A, et al. 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 periodontopathic bacterial infection. *Virulence* 2015; 6(3):293-304.