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Review

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

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ABSTRACT

Objective: A systematic review to evaluate the various genotyping tools and study strategies employed to define genetic susceptibility to periodontitis.
Methods: The review was performed in accordance with Preferred Reporting Items for Systematic Review and Meta-Analysis guidelines. The search for publications referring to the genetic bases of periodontal disease was performed on the MEDLINE-PubMed and Cochrane Library databases, on trials registers, and on the web pages of regulatory agencies.
Results: We found 2439 potentially eligible articles, of which only 25 satisfied the established inclusion criteria and were processed for data extraction. The review revealed marked heterogeneity between studies, caused in part by the lack of a universally accepted definition for periodontitis phenotypes and by the variety of genotyping tools available. The most commonly used technique was genotyping candidate genes.
Conclusion: The few rigorous studies that have been published on genetic susceptibility to periodontitis are subject to severe methodological bias due to their design and the genotyping tools employed. Despite their limitations, candidate gene studies continue to be the predominant methodological approach, rather than genome-wide association studies. Further studies must be designed using a universally accepted, validated diagnostic criterion for periodontitis, analysing multiple genes and polymorphisms in combination with rare variants.

1. Introduction

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

As with diabetes, certain types of cancer, Alzheimer's disease, Crohn's disease, and schizophrenia, PD is considered to be genetically complex, resulting from the interaction of genes with the environment (Laine, Crielaard, & Loos, 2012; Stabholz, Soskolne, & Shapira, 2010). Two main hypotheses have been proposed regarding the genetic basis of complex diseases. The first is the common disease/common variant (CD/CV) hypothesis (Reich & Lander, 2001), in which it is assumed that genetic variants common in the general population but which individually have a weak effect are those that have the greatest influence on genetic susceptibility to complex disorders. These genetic variants

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are called polymorphisms when they are present with a frequency of at least 1% in the population (Hart, Marazita, & Wright, 2000). They include single nucleotide polymorphisms (SNPs), which are very common in the genome and consist of a change of one nucleotide base for another. The second hypothesis is the common disease/rare variant (CD/RV) hypothesis, in which the main contributors to susceptibility to complex diseases are rare variants (minor allele frequency < 1%) present in the genome (Pritchard, 2001). The hypothesis that is deemed valid will thus determine the strategy applied to detect variants that favour disease. Most PD studies assume the CD/CV hypothesis; thus, the most common strategy consists of the search for polymorphisms that could affect the periodontal disease phenotype.

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

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

Another design is the association study, which enables relationships to be established between a specific polymorphism and a disease or phenotypic trait. This approach determines differences in the frequency of a genetic marker in cases and controls to indicate a relationship between that marker and the disorder under investigation. The two principal methods in this context are the candidate gene association study (CGAS) and the genome-wide association study (GWAS). The CGAS is based on an analysis of genes that are linked to a certain disease through their function or their position in the genome. It is therefore essential for investigators to understand the pathophysiology of a disorder before performing this type of study. Based on the promising results from what was considered the first CGAS (Kornman et al., 1997), this type of design became the most widely used to analyse the genetics of PD. However, the very rapid development of genotyping technologies in recent years has led to substitution of CGASs with GWASs, because these latter studies theoretically enable us to identify new genetic markers. The first GWAS to study periodontitis was published in 2010 and established an association between SNP rs1537415, present in gene *GLT6D1* (glycosyltransferase), and AgP (Schaefer, Richter, Nothnagel, Manke et al., 2010).

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

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

2. Materials and methods

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

2.1. Focused question

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

2.2. Eligibility criteria

The following inclusion criteria were applied: studies published in English; case-control design; specification of the PD phenotype; a sample size equal to or greater than 100 individuals in either the case or control group, or in both groups. No limitations were placed on the year of publication or the age, population group, or country of origin of the participants. Publications with any of the following potential biases were excluded: a study group that included individuals with systemic diseases that could favour the onset or augment the severity of PD; patients treated with drugs that provoke gingival hyperplasia; and pregnant women (pregnancy gingivitis). Reviews and meta-analyses were also excluded, even if they referred to genetic susceptibility to PD. We used the number of SNPs analysed as inclusion criteria for candidate gene studies. There have been many studies of this type that have analysed a reduced number of SNPs; they are generally lacking in quality control and have a lack of appropriate statistical corrections, Hardy-Weinberg equilibrium analysis or correspondence of cases and controls with a population background. Thus, due to the criteria that define the copy number variations (CNVs) (Lin, Naj, & Wang, 2013; Merikangas, Corvin, & Gallagher, 2009) and the limitations observed in those studies with a reduced number of SNPs, only the studies that analysed ≥10 SNPs were included.

2.3. Information sources

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

2.4. Search strategy

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

2.5. Study selection

Study selection was performed using a two-stage screening process performed by two independent reviewers (P. D. and A. de C.). Disagreement on the inclusion or exclusion of a specific article was resolved by consensus. In the first phase, studies that did not satisfy the inclusion criteria were eliminated, as were those considered irrelevant