REVIEW ARTICLE

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Quality of methods and reporting in association studies of chronic periodontitis and *IL1A −889 and IL1B +3953/4* SNPs: A systematic review

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Objective: The aim of this systematic review was to evaluate the quality of reporting and methodology in genetic association studies between *IL1A −889* and *IL1B +3954* polymorphisms and chronic periodontitis.

Background: Evidence provided by periodontal research on genetic risk factors is of uttermost importance in clinical practice as a possible diagnostic and prognostic tool for periodontitis. Inadequate reporting of results as well as high risk of bias due to methodological inconsistency hampers the integration of evidence in terms of clinical applicability.

Methods: This review includes case-control studies in humans published between 1997 and July 2017. Searching was conducted through MEDLINE, EMBASE, and search handing. Specific scoring systems have been developed to evaluate the quality of methods and reporting. Each article was scored according to its adequacy, and then, the total number and the percentage of items positively qualified for both methods and reporting were calculated. The quality of methods in studies scoring 0-6, 7-12, and 13-16 was, respectively, considered poor, moderate, and good. For reporting, scores of 0-9, 10-18, and 19-26 were deemed of poor, moderate, and good quality, respectively. Pearson's correlation coefficient was calculated to explore the correlation between the year of publication and the quality in terms of methods and reporting.

Results: From the 531 screened studies, 52 met the inclusion criteria and were thus included in the study. The quality of methods and reporting of published genetic association papers on IL1 and chronic periodontitis is moderate. On a scale from 0 to 16, the mean score for methods of the reviewed studies was 8.19 ± 1.93. The items more frequently considered inadequate concerned the handling of confounders in statistical analysis, especially oral hygiene habits, socioeconomic status, subgingival colonization of specific periodontal pathogens, and stress. A significant positive correlation was found between the year of publication and the quality scores in terms of method ($r = 0.401$, $P = 0.003$). In terms of reporting, the mean score was 14.83 ± 3.04 on a scale from 0 to 26 and it was considered overall moderate. No statistically significant correlation was found between the year of publication and the quality of reporting ($P = 0.266$).

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Conclusions: The association between *IL1A −889* and *IL1B +3954* polymorphisms and chronic periodontitis is questionable due to methodological inconsistency. Evidence arising from meta-analysis is unreliable due to high risk of bias and moderate quality in terms of reporting.

KEYWORDS

chronic periodontitis, interleukin-1, methods, quality

1 | INTRODUCTION

Chronic periodontitis (CP) is a multifactorial disease which is widespread in the adult population[1] and affects approximately 40% of adults in Western industrialized countries.[1,2] The etiopathogenetic mechanisms of CP have not been fully elucidated, but it has been demonstrated that host susceptibility and in particular individuals' genetic background play a major role in its onset and development,[3-5] with a subset of genes commonly believed to be involved in the pathological processes. In order to clarify the role of each one of these genes, researches carried out genetic association studies (GAS) based on polymorphism analysis. A genetic polymorphism is defined as the occurrence in the same population of two or more alleles at one locus, each with appreciable frequency, where the minimum allele frequency (MAF) is typically taken as 1%. Being easily recognizable, it is possible to determine the frequency distribution of an established genetic polymorphism among healthy subjects and diseased patients and thus to identify the genetic variants associated with CP.

Until the middle of the last decade, the most important strategy for the identification of genes contributing to periodontal disease relied on investigations of selected candidate gene based on literature reviews and perceived pathophysiologic pathways. The major disadvantage was the requirement for an a priori hypothesis on the contribution of a particular gene to disease onset or progression.[6] Such an approach is typical of traditional scientific research, where studies were generally hypothesis-driven. Candidate genes for CP are mainly genes encoding for cytokines involved in the innate and adaptive immune response.[7] In particular, the most investigated single nucleotide polymorphisms (SNP) are

- SNP in position *IL1A −889* (in linkage with *+4845*)
- SNP in position *IL1B +3953* (also referred erroneously as *+3954*).

IL-1α and IL-1β regulate bone resorption, fibroblast proliferation, and migration of immune and inflammatory cells into the periodontal tissues. Moreover, they modulate the production of prostaglandin E2, matrix metalloproteinases, and their inhibitors. These properties make it biologically plausible to consider IL-1 a viable candidate gene for genetic studies in relation to periodontitis.

In 1997, Kornman et al[8] observed for the first time the association between the simultaneous presence of *IL1A −899* and *IL1B +3953* minor alleles, which they named "composite genotype", and an increased severity of CP in a subset of non-smoker Caucasian subjects. However, considerable variations were seen for the carriage rates of the IL1 composite genotype across populations,[9] and among studies, there were contradictory results. To clarify the role of these polymorphisms in the etiology of periodontitis, several systematic reviews and meta-analyses have been performed.[10-14] Generally, these concluded that the single polymorphism or the composite genotype are significantly associated to CP. However, the quality of methods and reporting of the included studies was poorly investigated, and thus, the results of the published meta-analysis might have been influenced by biased or unreported data. Many factors have been claimed to contribute to a high risk of bias.[15,16] The most relevant aspects derive from the use of different classification systems for periodontal diseases, the dissimilarity of selection criteria for cases and controls, the adjustment for other risk factors such as smoking, systemic conditions, age, stress, presence of specific pathogens, and oral hygiene habits.

Therefore, the aim of this systematic review was to critically evaluate the quality of reporting and methodology of scientific reports about the association between CP and *IL1A −889* and *IL1B +3954* polymorphisms in order to determine which factors contributed to such contradictory results.

2 | MATERIAL AND METHODS

2.1 | Study selection

Protocol development, eligibility criteria, and search strategy have been reported in Appendix S1. Initially, two reviewers (FC & FR) screened the articles based on their titles and abstracts independently and in duplicate. The articles selected by one of either reviewer were considered for full-text reading. Kappa scores evaluated agreement between reviewers. Subsequently, the same two reviewers obtained full-text articles for screening. Any disagreement was resolved by discussion with a third author (M.A). Inter-rater agreement was assessed with kappa.

2.2 | Articles appraisal

Extracted data have been reported elsewhere (Appendix S1). The quality of methods (Table 1) and reporting (Table 2) used in the selected studies was evaluated independently by two reviewers (FC

TABLE 1 Scoring system for methods

Methodological issue	Question	Answer
Classification of periodontal disease and conditions	Is it declared which classification system of periodontal diseases and conditions is used ? If so, which one?	NR, NO = 0; YES = 1
Periodontal disease	Is it declared which form of periodontitis is investigated?	NR, NO = 0; YES = 1
Diagnostic criteria	Appropriate diagnostic criteria have been used? (Severe Periodontitis: ≥2 interproximal sites with CAL ≥ 6 mm on different teeth; ≥1 interproximal site with PD ≥ 5 mm)	NR, NO = 0; YES = 1
Sample size: cases/controls	Is the sample size adequate?	NR, <30 = 0; >30 = 1; >1000 = 2
Periodontal status of controls	Are the controls periodontally healthy?	NR = 0; YES = 1
Origin of controls	Community or hospital controls have been used?	Community or hospital = 1; Private practice, Students, University Staff, NR = 0
Country and ethnicity	Is the ethnicity and the country of origin of the subjects described?	NR, NO = 0; YES = 1
Genotyping	Is genotyping adequate?	NR, NO = 0; YES = 1
Concealment	Is concealment performed	NR, NO = 0; YES = 1
Hardy-Weinberg equilibrium	Is the HWE respected?	NR, NO = 0; YES = 1
Risk factors		
Oral hygiene	Is the analysis adjusted for oral hygiene habits?	NR, NO = 0; YES = 1
Tobacco smoking	Is the analysis adjusted for oral smoking habits?	NR, NO = 0; YES = 1
Socioeconomic status	Is the analysis adjusted for socioeconomic status?	NR, NO = 0; YES = 1
Age	Is the analysis adjusted for age?	NR, NO = 0; YES = 1
Specific pathogens	Is the analysis adjusted for the presence of specific pathogens?	NR, NO = 0; YES = 1
Stress	Is the analysis adjusted for stress?	NR, NO = 0; YES = 1

NR, not reported

& FR) using two checklists created specifically for this purpose. To score points, reviewed articles should qualify positively in regard to the items provided in each checklist. The checklist for quality of reporting was redacted according to the indications provided by the STREGA ("Strengthening the REporting of Genetic Association studies") statement[17] (see Table 2). The checklist for methodological quality was derived from (a) the items proposed by the Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analysis and (b) the methodological criteria proposed by Nibali and coworkers[15] for GAS in relation to periodontitis (see Table 1). Finally, each item was scored according to its adequacy, and the total number and the percentage of items positively qualified for both methods and reporting were calculated.

The quality of methods could range from 0 to 16. The quality of studies scoring 0-6, 7-12, and 13-16 was considered poor, moderate, and good, respectively. The quality of reporting rate could range from 0 to 26. Studies scoring 0-9, 10-18, and 19-26 were deemed of poor, moderate, and good quality, respectively.

2.3 | Statistical analysis

Pearson's correlation coefficient was calculated to explore the correlation between the year of publication and the quality in terms of methods and reporting. Tests were considered to be of statistical significance at $P < 0.05$, and all analyses were conducted using IBM SPSS, version 24.0. The presence of outliers has been investigated creating a boxplot for each score. Eventually, outliers were excluded from the calculation of Pearson's correlation coefficient.

3 | RESULTS

3.1 | Studies selection

After duplicate removal, a total of 531 studies were identified, 523 from the electronic search and 8 from the manual search and screening of references in the selected papers. After screening titles and abstracts, the reviewers rejected 467 papers (97.93% agreement between reviewers; kappa = 0.894) and retained 64 papers for a full-text evaluation. After full-text reading, 12 papers were rejected: 3 included patients with systemic diseases,[18-21] 2 due to language,[22,23] 1 was the abstract of a conference paper,[24] and 5 because of study design.[25-29] One paper was a review[30] (100.00% agreement between reviewers; kappa = 1.00). Finally, a total of 52 papers were included into the systematic review (Figure 1). Inter-examiner agreement during data extraction was strong (86.23% agreement between reviewers; kappa = 0.725).

3.2 | Quality of methods

The present systematic review observed that the quality of methods of GAS in periodontal research is generally moderate. Table S2 presents an overview of the results. The mean score of the reviewed

TABLE 2 Scoring system for reporting

Study Phase	Question	Answer
Title and Abstract	(A) Is the study design properly described in the title with a keyword (case-control, association, prevalence, etc)	NO = 0, YES = 1
	(B) Is the abstract properly redacted? does it describes the material and methods, results and main conclusions in summary?	NO = 0, YES = 1
Introduction	(C) Is there a scientific rationale?	NO = 0, YES = 1
	(D) Is the aim of the study declared?	NO = 0, YES = 1
	(E) Is it declared if the study is a first report or a replication of previous findings?	NO = 0, YES = 1
Material and Methods	(F) Is the study design described in its key elements (selection of participants, DNA sampling etc)?	NO = 0, YES = 1
	(G) Are the date and setting of the study declared?	NO = 0, YES = 1
	(H) Are the diagnostic criteria for the selection of cases and control specified?	NO = 0, YES = 1
	(I) Is the primary outcome declared?	NO = 0, YES = 1
	(J) Is it described how to deal with possible bias?	NO = 0, YES = 1
	(K) Is sample size calculated?	NO = 0, YES = 1
	(L) Are the laboratory methods properly described including the allele calling algorithm, error rates and call rates?	NO = 0, YES = 1
	(M) Is the statistical analysis described?	NO = 0, YES = 1
	(N) Is it reported if HWE is assessed?	NO = 0, YES = 1
	(O) Is it reported how it is intended to deal with population stratification?	NO = 0, YES = 1
	(P) Is information provided about haplotype?	NO = 0, YES = 1
Results	(Q) Is the participant selection properly described (how many screened, how many included, how many didn't sign the informed consent etc)	NO = 0, YES = 1
	(R) Are the demographic characteristic of cases and control reported?	NO = 0, YES = 1
	(S) Is it reported what factors could act as possible confounders?	NO = 0, YES = 1
	(T) Is the distribution of polymorphisms among cases and controls reported? (possibly in terms of %, OR and 95% IC)	NO = 0, YES = 1
	(U) Is the distribution of polymorphisms reported after adjustment for at least 3 confounders?	NO = 0, YES = 1
	(V) Is the distribution of polymorphisms reported according to the severity of periodontitis?	NO = 0, YES = 1
Conclusions	(W) Are the main results properly summarized?	NO = 0, YES = 1
	(X) Are the limits of the study discussed?	NO = 0, YES = 1
	(Y) Are the results interpreted properly, also in comparison with previously published findings?	NO = 0, YES = 1
	(Z) Is the generalizability of the results discussed?	NO = 0, YES = 1

papers for methods was 8.19 ± 1.93. Eight papers classified as poor, 1 as good, and the remaining 43 papers were of moderate quality (Figure 2). The lowest score reached by a paper was 3,[31] while the best score was 13.[32] The items most frequently deemed inadequate involved the handling of confounders, especially oral hygiene habits, socioeconomic status, presence of specific periodontal pathogens, and stress. On the other hand, analysis was properly adjusted for smoking habits in the majority of the studies (73.08%).

Different methods to adjust for confounders were used. Seven studies reported adjustment for oral hygiene between cases and controls. Six used regression analysis,[32-37] and 1 did not observe any statistically significant differences between cases and controls.[38] Thirty-eight studies made some efforts to control the effects of smoking habits. Twenty-two studies excluded smokers,[33,39-53] 10 articles used regression analysis,[32,34,35,37,54,55] 1 adjusted the analysis of variance for smoking,[60] 3 papers performed a stratified analysis,[61,62] and the 2 remaining studies did not observe any statistically significant differences between cases and controls. Smoking habits were self-reported by means of questionnaires.

Four studies controlled for socioeconomic status. One study[32] performed a multiple regression analysis using the educational level as a proxy of the socioeconomic status. Another research controlled for socioeconomic status assessed this in terms of annual income, educational level, type of residence, and job.[62] One study observed non statistically significant differences among cases and controls in terms of years of education,[64] while the last paper featured a selection of patients of similar socioeconomic status without providing more details.[57]

Twenty-one studies analyzed the possible effects of age as a confounder by testing the differences between cases and controls, using regression analysis or age-matched groups.
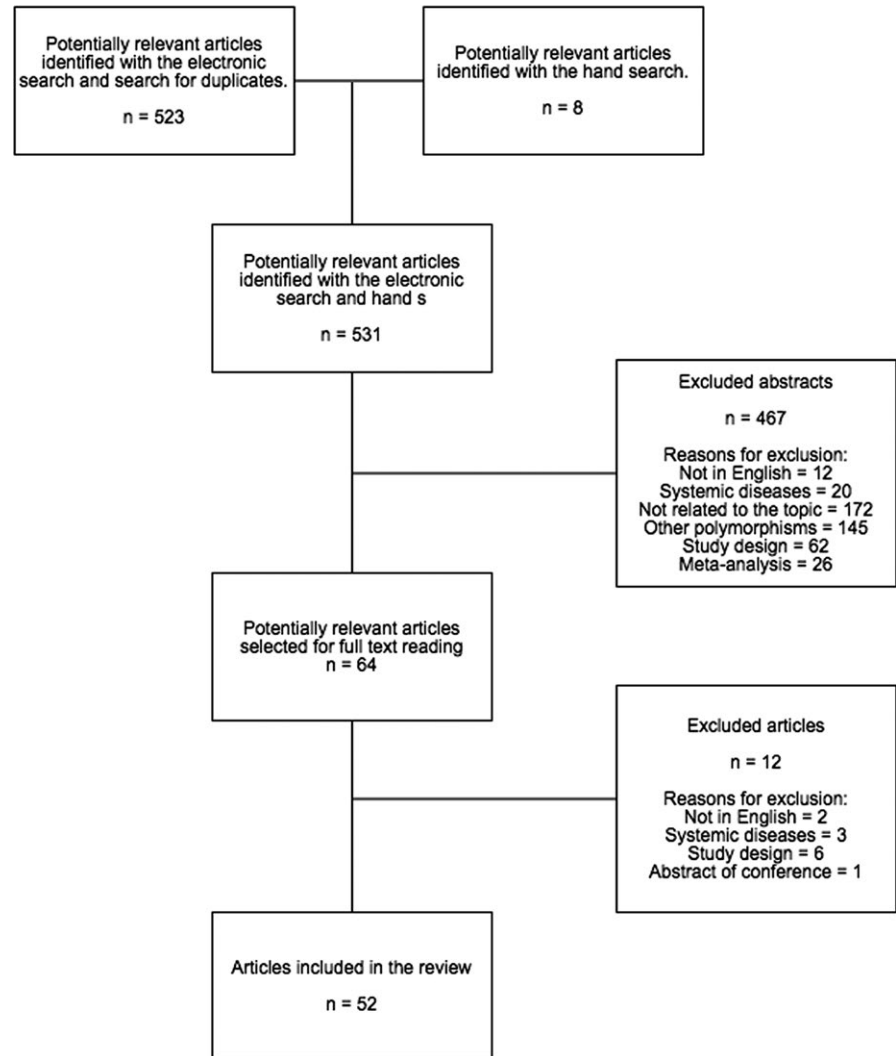
FIGURE 1 Flowchart of selection

Four studies performed microbiological examination. One study used two-way ANOVA to assess the impact of the possible interaction between genotype and subgingival colonization by periodontopathogens on IL-1 mRNA levels in gingival crevicular fluid,[43] and another study assessed the possible correlation between genotypes and the presence of *Porphyromonas gingivalis* (PG) and *Aggregatibacter actinomycetemcomitans* (AA) by cultural methods[61] with a Fisher's exact test. The remaining studies used multivariate linear regression[36,38] to associate microbial loads to genotype.

Appropriate criteria for diagnosis of a conclamated case of periodontitis[65] were adopted sparsely (32.00%). Case definition for periodontitis varied consistently among studies. The variables used to assess the presence of periodontitis were mainly clinical parameters.

In all papers, the overall population included at least 30 individuals, with two of the studies enrolling more than 1000 subjects.[32,38].

Hardy-Weinberg equilibrium (HWE) was properly respected in approximately half of the papers (53.8%).

A positive significant correlation was found between the year of publication and the quality scores in terms of method ($r = 0.401$, $P = 0.003$—Figure 3).

3.3 | Quality of reporting

The results on the quality of reporting are summarized in Table S3. The mean score of the reviewed papers was 14.83 ± 3.04. One paper classified as poor, 46 papers classified as moderate, and 5 as good (Figure 4). The items that lacked description accuracy usually referred to the date and setting of patients recruitment and the laboratory methods (1.92%). No significant correlation was found between the year of publication and the quality scores in terms of reporting ($P = 0.266$).

4 | DISCUSSION

The objective of this systematic review was to evaluate the quality of reporting and methods of scientific reports about the association
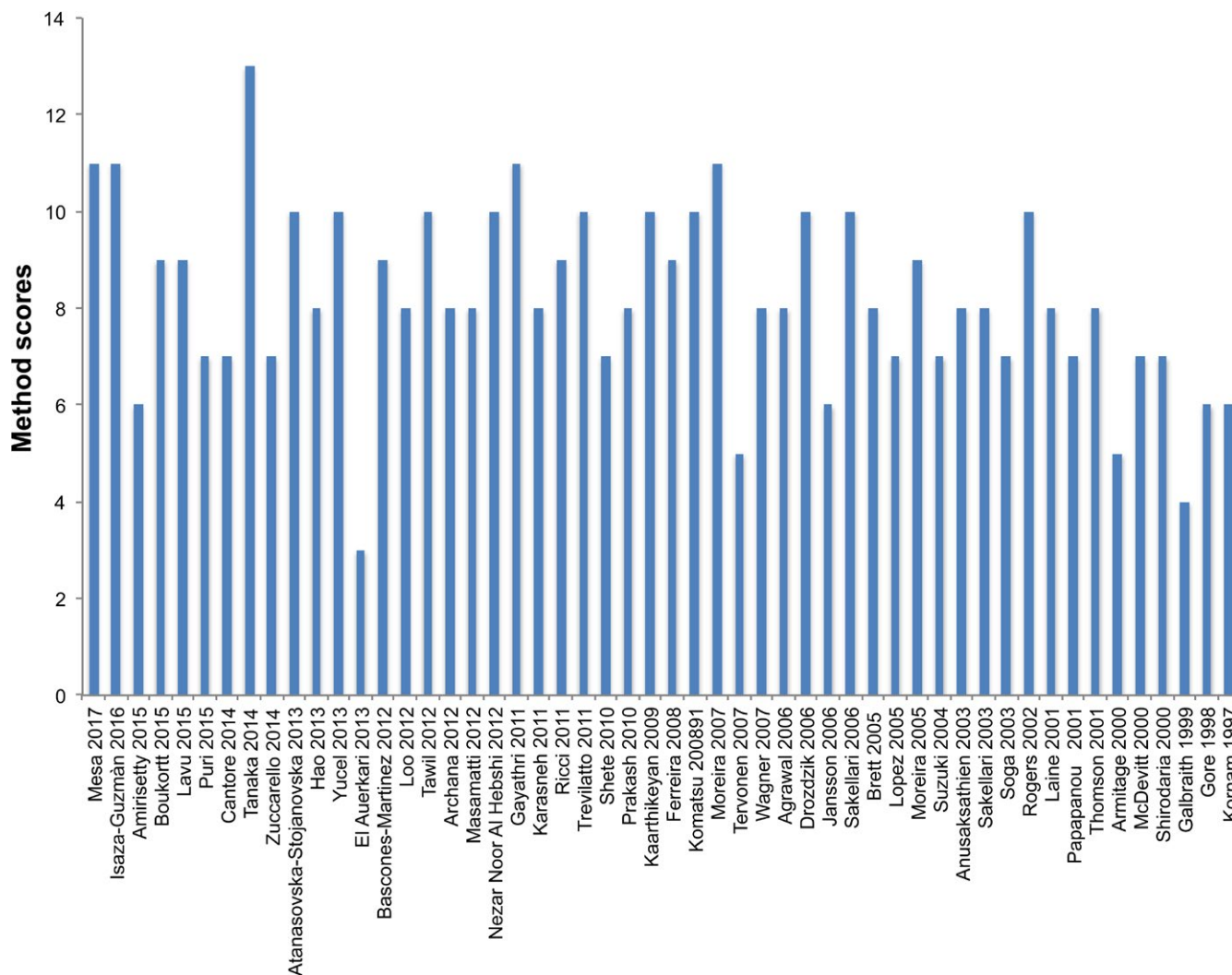
FIGURE 2 Score in methods per each paper

between CP and *IL1A −889* and *IL1B +3954* polymorphisms. Overall, the quality of the case-control studies included in the review was moderate in terms of both methods and reporting. In addition, the strength of the association is quite weak. Thus, solid scientific evidence from GAS is still lacking to support the clinical applicability of specific genetic risk factors for CP.[66]

In recent years, to overcome the need for large sample size, researchers performed meta-analyses and found a positive correlation between IL1 SNPs and CP.[10-12] However, only reliable and comparable data should be pooled together,[68,69] and thus, publication bias,[71] quality of methods, and quality of reporting of included studies[15] should be addressed. Publication bias is a widespread problem that may seriously distort attempts to evaluate the theory under investigation. It occurs when the outcome of a study influences the decision whether to publish it or not. Authors, editors, and reviewers all play a role in selecting studies for publication. Proposals for detecting and correcting publication bias include statistical methods which have been usually implemented in meta-analysis design. Conversely, quality of methods and reporting has been assessed sparsely by mean of scoring systems.

It should be emphasized that although quality of methods and quality of reporting have been assessed and scored separately in the present study, they are closely related. In fact, it is often unclear whether a study is fraught with methodological inconsistencies or if the authors did not report properly some items.

Genetic association studies present several specific challenges including an unprecedented volume of new data and the likelihood of very small individual effects.[72] As a consequence, achieving adequate statistical power is one of the most common problems of GAS across different fields of science. A recent meta-analysis estimated the median statistical power of neuroscience studies to range between ~8% and ~31%, when in general, statistical power is considered adequate when over 80%.[73] Low statistical power could be due to small sample sizes of studies and/or small effect size. It is difficult to assess the statistical power of GAS in periodontology, but these studies have traditionally been claimed to be underpowered[3,7,74] by increased chance of false-positive or false-negative findings. Furthermore, it has to be noticed that the estimated magnitude of a true effect may be inflated. This is often referred to as the 'winner's curse',[75,76] and its main consequence is that
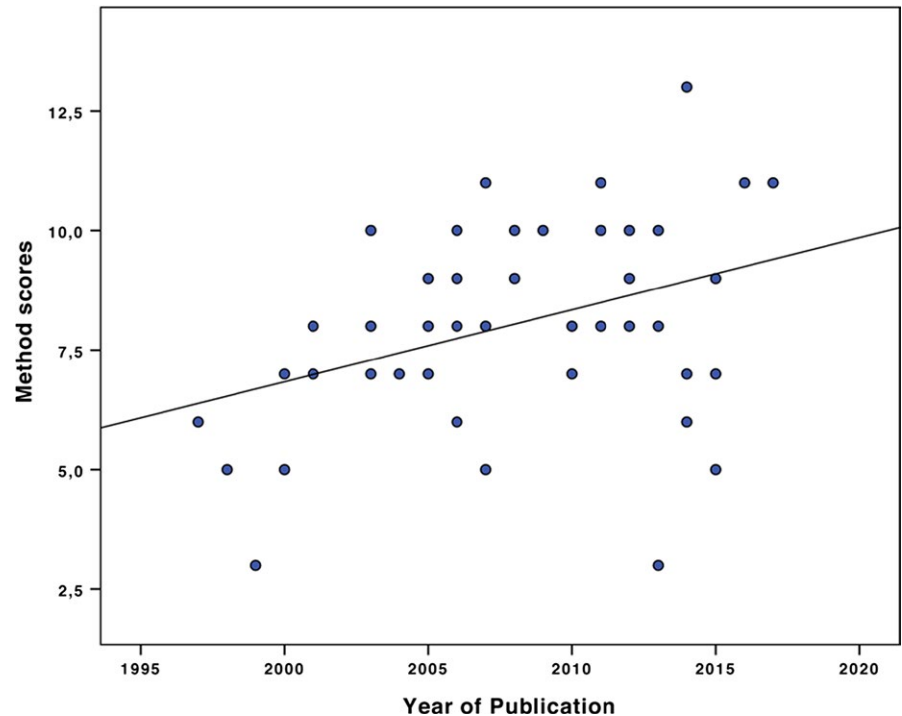
FIGURE 3 Relationship between methods scores and the year of publication

replication studies will be tailored on an inflated effect size and will probably fail to confirm the findings, fueling uncertainty. The candidate gene approach in periodontal GAS requires specific strategies to achieve sufficient statistical power. Sample size should be adequate to small effect size and minor allele frequencies, requiring thousands of cases and controls. This was seldom the case in the reviewed papers. Larger samples would also allow investigating different polymorphisms at the same time. Periodontitis is in fact a polygenic disease and depends on the simultaneous presence of several alleles, which have traditionally been considered to be between 10 and 20. Additionally, combination of SNPs may have greater effect size, which in turn will favor statistical power.

In GAS, sorting subjects according exclusively to their disease status may be incorrect. First, periodontal diagnosis refers to the disease's clinical signs and not directly to a patient's susceptibility, which is instead related to genetic risk factors. Second, periodontitis is a multifactorial disease, and patients with similar clinical conditions may have different genetic backgrounds. To overcome these limitations, tailored strategies for case and control selection may be implemented in GAS. An approach based on highly susceptible versus resistant genotypes, rather than on diseased versus clinically healthy, increases statistical power and the odds of identification of genetic factors.[25,77] Thus, ideal cases should be subjects suffering from extremely severe forms of periodontitis early in life in the absence of other true risk factors. Specific patterns of disease progression, severity, and extent may further help to clean out the selection. Resistant subjects rather than healthy subjects are more suitable as controls. Indeed, oral hygiene procedures contrast the microbial challenges, and consequently, a periodontally healthy population is theoretically composed of both susceptible and resistant subjects.[25]

Patients with poor plaque control and minimal attachment loss at an advanced stage of life are the most suitable as controls.

To avoid spurious associations, test and control groups should be balanced for variables which supposedly have an effect on the outcome.[78,79] Alternatively, statistical methods should be applied to adjust for potential confounders. Smoking and poor oral hygiene are major risk factors, which may overcome by themselves the effect of SNPs on the periodontal status. Stress is a putative risk factor. Age and socioeconomic status are risk indicators. The subgingival colonization of specific periodontal pathogens such as PG, AA, *Tannerella forsythia*, and *Treponema denticola* (TD) over established counts is associated with increased odds for the onset and/or progression of periodontitis. Importantly, there is plenty of evidence supporting the implication of periodontal pathogens in the modulation of epigenetic mechanisms related to biological interaction between hosts and pathogens.[80] For all these reasons, smoking, oral hygiene, the burden of periodontal pathogens, stress, age, and socioeconomic status should be taken into account during statistical analyses, since all of them may directly or indirectly affect the susceptibility profile of the subjects.

Population stratification is another cause of false-positive findings. It occurs when cases and controls have different allele frequencies attributable to diversity in the population background, unrelated to health status. This is due to non-random mating between ethnic groups, mainly caused by geographical separation which is then followed by genetic drift of allele frequencies. In the reviewed papers, population stratification has been often addressed selecting cases and controls from the same country, same ethnicity, and in some sparse case asking for ancestry. However, these methods have proven to be inconsistent, especially in areas cohabited by different ethnicities. Although the potential effect of population stratification
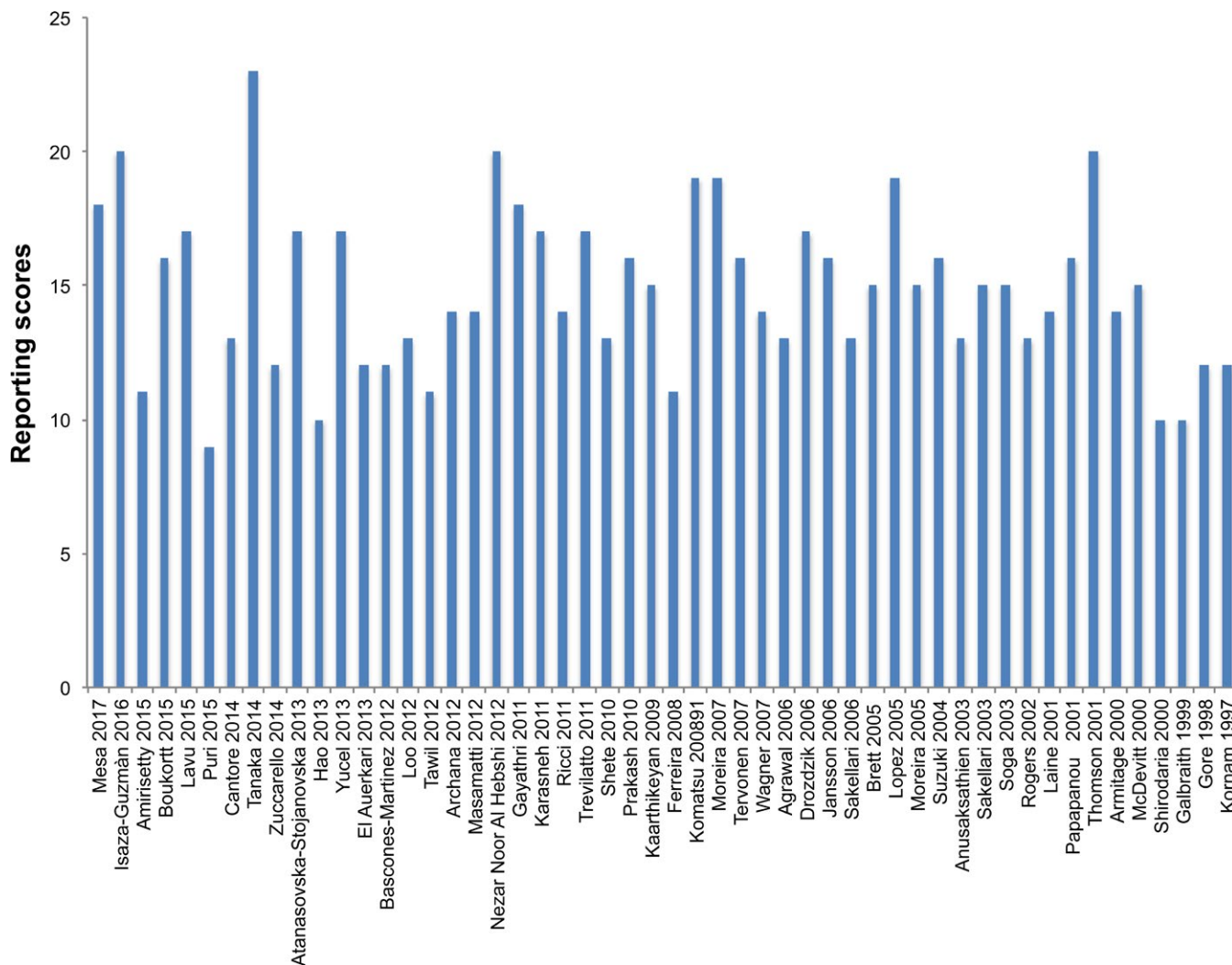
FIGURE 4 Score in reporting per each paper

is small in most situations,[81] population stratification should definitely be assessed statistically,[82] as studies of genetic association typically address small effects.

Genotyping errors can affect the results of GAS. It has been reported that the magnitude of genotyping errors varies between 0.5% and 30%, and these errors are addressed by observing deviations from the HWE. HWE is assessed by comparing the difference between observed genotype frequencies among controls and the corresponding expected frequencies. However, when the disease of interest is common, controls might not represent the general population, as cases account for a relatively large portion of the general population.[83] In these situations, using the HWE only in controls might lead to discarding important SNPs that could potentially be causal SNPs of the disease. The most marked bias due to genotyping errors occurs when specificity is poor and genotype prevalence is low (<15%), which is the case of periodontitis. Furthermore, unblinded assessment may lead to differential misclassification, and hence, blinding of the operators who perform genotype analysis is a fundamental requisite in GAS.[15,17]

In dentistry, as already noticed, the quality of the published materials appears often insufficient to allow readers to assess the validity of the trials.[84] Taking into account the high number of non-reported items, the literature examined in this review clearly indicates a still inappropriate reporting quality in GAS about CP and the investigated SNPs. The fact that no significant correlation was found between the year of publication and the quality of reporting suggests that the scientific dental community has not embraced the STREGA recommendations yet. Further efforts should be made to increase the awareness about reporting guidelines.

A limitation of the present review is that only two databases (MEDLINE and EMBASE) were searched, no gray literature search was conducted and studies other than in English were excluded. This in turn may have reduced the number of included papers.

5 | CONCLUSIONS

Beside being weak, the association between IL1A −889 and IL1B +3953/4 SNPs and chronic periodontitis in literature is questionable due to methodological inconsistencies[85]; this is essentially due to the moderate quality of design and reporting of studies.

In the future, improved quality of methods and reporting would allow to draw definitive conclusions on this topic. Multicentric researches, which allow the sampling of sufficiently powered and well-designed case-control populations, should be performed in order to draw reliable conclusions. Stricter criteria for case and control selection, including a susceptible vs resistant approach, and adequate statistical adjustments for confounders, population stratification and genotyping errors, must be implemented.

Importantly, the design of genome-wide association (GWA) studies, which are observational studies of a genome-wide set of genetic variants in different individuals to see if any variant is associated with a trait without an a priori hypothesis, should also be encouraged.

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