TNF-α -308 G/A single nucleotide polymorphism and apical periodontitis: an updated systematic review and meta-analysis

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TITLE PAGE

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ABSTRACT:

Introduction: This study aimed to perform a more precise estimation of the association between tumor necrosis factor-alpha (TNF-α) – 308 G/A single nucleotide polymorphism (SNP) and the risk of development of AP and its phenotypes based on all available published studies.

Methods: The study was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and is registered in PROSPERO (CRD42020176190). The literature search was conducted via: Clarivate Analytics' Scopus, PubMed, Cochrane Central Register of Controlled Trials and China National Knowledge Infrastructure databases, from inception to December 2020 with no language restrictions. Two reviewers were involved independently in study selection, data extraction and appraising the studies that were included. The quality of included studies was evaluated using the Strengthening the Reporting of Genetic Association (STREGA) and the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system. Frequencies of genotypes and alleles of TNF-alpha (G>A 308, rs1800629) gene, with 95% Odds ratio was used.

Results: Four studies met the inclusion criteria with moderate risk of bias. This study revealed no significant association between TNF-α – 308 G/A SNP and AP, and the risk of AP development. Moreover, there was no significant association between genotype or allele frequency distribution and clinical manifestations (acute versus chronic) of AP. The certainty of evidence per GRADE was very low.

Conclusions: Due to very low certainty of evidence, whether there is an association between TNF-α – 308 G/A SNP and AP, warrants further well-designed multi-centric studies to adjudicate a better understanding of the role of genetic factors in the etiopathogenesis of AP.

Keywords: Apical periodontitis, heredity, tumor necrosis factor – alpha, genotype, allele**.**

INTRODUCTION

Apical periodontitis (AP) is a persistent inflammatory reaction within tooth-supporting tissues mostly caused by microbial agents from the infected root canal (1). Different microorganisms and their virulence factors induce the host's innate and adaptive immune response in the periapical region characterized by the recruitment and activation of various cells and cell-specific mediators (2, 3). The cytokines are low-molecular-weight proteins produced by several different types of immune host cells in response to inducing stimuli. Based on the current evidence, multiple pro-inflammatory cytokines (i.e. tumor necrosis factor-alpha (TNF-α), interleukin (IL) 1 beta, IL-6, etc.) have been associated with the development of AP (2, 3). These molecules establish complex interrelationships in the inflamed periapical area, resulting in direct and/or indirect (e.g. via bone resorption regulators triad) stimulation of osteoclastogenesis and consequent alveolar bone resorption (2, 3).

Tumor necrosis factor-α is one of the most potent pro-inflammatory cytokines and the modulator of immune response with a significant role in tissue injury and induced bone resorption (4, 5). It is produced by a variety of cells (e.g. macrophages, neutrophils, keratinocytes, fibroblasts, NK cells, T and B lymphocytes, etc.) after stimulation (4, 5). In 1991, for the first time, TNF-α was detected at measurable amounts in tissues of periapical lesions (6). Thereafter, several animal and human studies confirmed its role in the pathogenesis of AP (2, 3).

The expression of TNF-α is controlled at the transcriptional level, and differences in its production may be partly determined by an individual's genetic background (7, 8). The TNF-α gene is a member of the TNF superfamily located on chromosome 6p21.3,

within the major histocompatibility complex (MHC) class III region. Several polymorphisms and mutations have been identified in the TNF-α gene (9); the most common polymorphism is the single nucleotide polymorphism (SNP) at position − 308 (rs1800629), which causes guanine (G) to adenosine (A) transition in the promoter region of human TNF-α gene (10). The A allele of this SNP is associated with significantly higher transcription and TNF-α production (10), and with increased risk of various oral diseases (11-14).

Genetic association studies conducted in the past two decades have suggested that heredity may also significantly affect the development, clinical course, and severity of AP. Namely, polymorphisms in genes encoding pro-inflammatory cytokines have been proposed as potential genetic markers of AP (15). A systematic review and meta-analysis reported that the GG genotype of TNF-α – 308 G/A SNP was significantly more common in chronic compared to acute periapical lesions (16). Nevertheless, this meta-analysis did not address the AP susceptibility and therefore did not include the study by Dill et al. (17). Moreover, the most recent study demonstrated that TNF-α – 308 G/A SNP was associated with a significant increase of AP susceptibility, both in heterozygous and homozygous carriers of the variant A allele (18). Therefore, this updated systematic review and meta-analysis of all eligible studies aimed to explore a more precise understanding of the association between *TNF-α* – 308 G/A SNP and the risk for the development of AP and its phenotypes.

MATERIALS AND METHODS

Procedures and reporting of the current systematic review and meta-analysis has been conducted according to the Preferred Reporting Items for Systematic Reviews and

Meta-Analyses Protocols (PRISMA-P) statement guideline (19, 20) and the Cochrane Handbook for Systematic Reviews of Interventions (21). The protocol of the review was developed and registered under the International prospective register of systematic reviews - PROSPERO (CRD42020176190). The PRISMA checklist was added as a Supplementary Table 1.

Focused question

Using Population, Exposure, Comparison, and Outcome (PECO) the present updated systematic review and meta-analysis was conducted in order to answer the following focused question:

Population: healthy population with no systematic diseases

Intervention/Exposure: Patients with TNF-α – 308 G/A (rs1800629) SNP and AP

Comparison: The differences in the development of two clinical forms of apical periodontitis, acute and chronic.

Outcome: Genotype and allelic distribution of TNF-alpha (G>A 308, rs1800629) gene between acute and chronic apical periodontitis

Thus, the scientific question was formulated:

‘‘Is TNF-α – 308 G/A (rs1800629) SNP associated with an increase in the risk of AP?’’

Inclusion and Exclusion Criteria

The inclusion criteria were as follows:

1. Clinical trials, case-control studies, cross-sectional studies, or cohort studies,

2. Studies with diagnosed AP based on clinical and radiographic parameters in humans,

3. Studies that investigated the association between TNF-α – 308 G/A (rs1800629) SNP and AP, including a comparison between different clinical manifestations of AP (i.e. acute/chronic), and

4. Studies in which data of TNF-α – 308 G/A (rs1800629) SNP were quantified and suitable for meta-analysis.

The following exclusion criteria were applied:

1. Studies that failed to meet the above-mentioned inclusion criteria,

2. Literature and systematic reviews, meta-analyses, book chapters, guidelines, case reports and case series,

3. Studies conducted on animals or cell culture laboratory studies, and

4. Studies that reported duplicated data.

Literature Search Strategy

To identify relevant studies examining the role of TNF-α – 308 G/A (rs1800629) SNP in the development and clinical manifestations of AP, literature searches were completed up to December 2020 using Clarivate Analytics Web of Science (including Web of Science Core Collection - WoS, Korean Journal Database - KJD, Russian Science Citation Index - RSCI, SciELO Citation Index - SCIELO), Scopus, PubMed, Cochrane Central Register of Controlled Trials (CENTRAL) and China National Knowledge Infrastructure (CNKI) databases. Although the previous review (16), which

we are updating, included studies published until March 2016, we did not limit our search only to the post-2016 period, but we explored sources from each database's interception to December 2020, without language or any other restrictions. Development of the most optimal retrieval strategy involved conducting preliminary searches and identification of terms and their synonyms related to the main concepts of interest, such as "apical periodontitis" and “polymorphism". We intentionally used broad search terms to reduce the chance of missing relevant studies. The search strategy differed according to the database being searched, using the combination of the most common free keywords and associated controlled vocabularies (e.g. Medical Subject Headings - MeSH, https://www.ncbi.nlm.nih.gov/mesh), Boolean operators, truncation and proximity operators. Details of the number of articles retrieved for each database and the full search strategy are reported in Supplementary Table 2.

Furthermore, to protect against potential publication and database bias, we also explored other sources of information. To identify published papers that might not have been retrieved by the database search, as well as unpublished manuscripts, conference papers, and other grey literature, we searched Google Scholar™ and available repositories (e.g. Networked Digital Library of Theses and Dissertations, Open Access Theses and Dissertations, DART-Europe E-theses Portal - DEEP, Opening access to UK theses - EThOS). Finally, reference lists of included trials and relevant reviews (cited and citing references were collected) were scrutinized to assure the reliability of data collected. For duplicates removal and further analysis, all records obtained were imported into EndNote Online (Clarivate Analytics 2020, https://www.myendnoteweb.com) reference management software.

Study Selection

Retrieved articles were independently screened by two reviewers (A.J. and J.J.) and sequentially excluded according to the eligibility criteria of this systematic review and meta-analysis. In the first step, titles were screened to reduce the number of initial records, and then abstracts were examined to confirm appropriateness. Finally, full-texts of the remaining studies were obtained and read in order to make final decisions on whether to include or exclude them. Any disagreements over the eligibility of particular studies were resolved through discussions between these two reviewers and a third reviewer (A.A.) where necessary.

Quality Assessment

The quality of included studies in the final review was evaluated using modified, specific quality assessment scales for genetic association studies (22-24) and the Strengthening the Reporting of Genetic Association (STREGA) statement (25), an extension of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) (26) for cross-sectional, case-control, and cohort studies. All of the studies were scored by one author (A.J.). Any doubts during this process were assessed in agreement with a second party (J.M.). For the first scale, scores ranged from 0 (lowest) to 20 (highest). All studies scored below or above 10 were categorized as low-quality or high-quality, respectively (22). The second scale was based on the 10-point scoring system with 0 as the lowest and 10 as the highest value. Studies presented with 4 or fewer criteria were classified as low methodological quality studies, those between 5 and 7 as moderate, while studies represented with 8 or more evaluated criteria were considered as studies of high methodological quality (23). Finally, the third scale is

presented with the 22-item STREGA checklist. Based on evaluated criteria, studies were classified as inferior (scored 0–7), medium (scored 8–14), or high (scored 15–22) quality (25). In all analyzed scales, each quality criterion or item was assessed as either positive or negative, scored 1 or 0 points, respectively. Regarding the assessed methodological quality, all studies were also reported as having high, moderate, or low evidence. This updated systematic review and meta-analysis considered only high or moderate evidence studies in the final examination. Moreover, the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system for grading evidence were used to ensure the accuracy of the data analysis in this systematic review (27).

Data Extraction

A customized data extraction form (based on the Cochrane Handbook recommendations) (21) was developed *a priori* to cover major characteristics of eligible studies, as well as all relevant information. Two independent researchers (A.J. and N.N.) extracted the required data from the included studies. For each qualified study, the following data were collected: first author's name; year of publication; study location; study design; study population including information about case and control group: ethnicity, sample size, gender, age and mean age, genotype distribution; SNP of interest; source of genomic DNA; genotyping method; main outcomes related to the investigated SNP; study limitations; Hardy-Weinberg equilibrium (HWE) and odds ratios (OR) with their corresponding 95% confidence intervals (CI) for each genotype and allele distribution between observed groups. If studies did not report sufficient data of interest, the corresponding authors were contacted to request additional material. Any

doubts were resolved through discussions between these two reviewers and a third reviewer (A.A.) where necessary.

Statistical analysis

The relevant data from the studies included in the final review were extracted and tabulated. Meta-analysis was calculated and Forest Plots generated using RevMan 5.3 Software (The Nordic Cochrane Centre, Copenhagen, Denmark). Meta-analysis was performed in order to evaluate the strength of association between AP and TNF-α – 308 G/A SNP, including the difference between AP phenotypes. The OR and 95% CI were calculated for the recessive genotypic model (GG vs. GA+AA) and the allele (G vs. A) distribution.

A random effect model was used for the meta-analysis. The Cochran-Mantel-Haenszel test was used to calculate OR and 95% CI values for individual studies. The statistical heterogeneity between the included studies was assessed using I^2 values: they indicated low, medium or high heterogeneity (25%, 50%, and 75%, respectively). The level of significance was set at .05.

RESULTS

Study Selection

The initial database search yielded a total of 934 studies and after the removal of duplicates, 401 remained. After screening the titles, 62 studies were left and after the abstract examination, only 4 studies remained for the full-text assessment for eligibility (17, 18, 28, 29). Authors were contacted for additional information (17), and after that,

all four studies met the criteria to be further included in both qualitative and quantitative analyses. The PRISMA flow diagram for the selection process is presented in Figure 1.

Characteristics of Studies Included in the Final Review

All four studies were conducted under a case-control study design; however, two different approaches were used:

1. Polymorphism association with the development of AP (AP versus control) (17, 18)

2. Polymorphism association with the AP phenotype (acute AP versus chronic AP) (18, 28, 29)

All the studies have analyzed the TNF-α – 308 G/A SNP (rs1800629). Dill et al. (17) did not include their findings in the paper but kindly provided the results upon request. In addition, the findings regarding the genotype and allele frequency of the TNF-α – 308 G/A SNP in acute and chronic AP were not completely presented in a previously published study by Jakovljevic et al. (18), but are reported in the present study (Supplementary Table 3) and included in the quantitative synthesis. Dill et al. (17) have performed TaqMan based genotyping, while other studies have conducted polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) analysis for the determination of genotypes. Two studies included South American populations of mixed ethnicity (28, 29), while the other two were conducted on White Americans (17) and Caucasians from Serbia (18). From these studies, an association was found between TNF-α – 308 G/A SNP and the development of AP only by Jakovljevic et al. (18), both in heterozygous and homozygous carriers. None of the

studies has shown the association of TNF-α – 308 G/A SNP with the AP phenotype (18, 28, 29). Table 1 shows in detail the data extracted from the selected studies.

Meta-analyses of genotype and allele frequencies: Case Versus Control

The quantitative synthesis was performed using the pooled data from Dill et al. (17) and Jakovljevic et al. (18), to evaluate the potential association of TNF-α – 308 G/A SNP with the development of AP. Figure 2 presents the forest plots for the genotype (Fig.2a) and allele (Fig.2b) distributions between the groups with (cases) and without AP (controls). No significant difference was found between the groups ($P > 0.05$). The heterogeneity among the studies was high (>70%) for both analyses with serious imprecision and wide confidence interval(s).

Meta-analyses of genotype and allele frequencies: Acute AP Versus Chronic AP

Another quantitative synthesis was performed using the pooled data from de Sa et al. (28) and Amaya et al. (29), with the addition of unrepresented data by Jakovljevic et al. (18) (Table 2), in order to evaluate the association of TNF-α – 308 G/A SNP with the AP phenotype (acute or chronic AP). Figure 3 illustrates the forest plots for the genotype (Fig.3a) and allele (Fig.3b) distribution among the patients with acute AP and chronic AP. No statistically significant difference was observed between the groups ($P > 0.05$) and the heterogeneity between the studies was medium (<50%) for both genotype and allele frequencies. However, imprecision remained significant with wide confidence interval(s) for both analyses.

Subgroup Analysis

Due to high heterogeneity between the groups (Figure 2), usually a subgroup analysis would be warranted. However, in this case, subgroup analysis was not possible since only two studies were analyzed.

No subgroup analysis was required in Figure 3 since heterogeneity was not significant.

Quality Assessment

Two studies were scored as having a high quality of evidence (17, 18) and the other two (28, 29) as having a moderate quality of evidence, per STREGA. Only one study (17) used the Bonferroni's correction in the statistical analysis. Additionally, only one study (18) reported a power calculation and confirmatory testing, while all four studies reported testing for Hardy-Weinberg equilibrium.

The overall quality assessment of the studies included in the review was summarized in Table 2. There was very low certainty of evidence per GRADE (Tables 3 and 4). This indicates that the authors have low confidence in the effect estimate.

DISCUSSION

Inflammation in AP is the consequence of a complex interplay between various etiological factors and the host's immune system (1, 2). In the pathogenesis of AP, both innate and adaptive immunity are involved, with various cells that synthesize diverse immuno-modulatory mediators (1, 2). The role of pro-inflammatory cytokines in the formation, development, and progression of periapical lesions and consequent alveolar bone resorption is well established (1, 2). Moreover, experimental animal research has reported synergistic effects of a member of bone resorption triad, the receptor activator

of NF-κB ligand (RANKL), and pro-inflammatory cytokines (TNF-α, IL-1α, and IL-1β) in periapical lesion expansion (30). Although different environmental etiological factors can induce pulp necrosis, AP is mainly considered as an infectious disease (1, 2). Nevertheless, the role of genetic risk factors in the etiopathogenesis of AP remains to be clarified. In the last two decades, several genetic association studies (17, 18, 28, 29, 31, 32) were performed in order to investigate whether polymorphisms in genes encoding pro-inflammatory cytokines could be potential genetic markers of AP. So far, the results of previously published original articles are rather inconclusive, and several systematic reviews and meta-analyses (16, 24, 33) have tried to explore whether the association performed on pooled data truly exists.

The last systematic review related to the role of TNF-α – 308 G/A SNP in the pathogenesis of AP has revealed that GG homozygotes are more prone to the chronic form of the disease (16). This analysis was based on two original studies (28, 29) published before 2016 and related only to mixed South American populations (Brazilians and Colombians). To date, the role of TNF-α – 308 G/A SNP, as a risk modifier for the development of AP, has been investigated in two more studies, including the US and Serbian populations (17, 18). Therefore, this systematic review and meta-analysis aimed to update and present all eligible data regarding this issue. Our results revealed no significant association between TNF-α – 308 G/A SNP and a risk of AP development [OR = 0.71, CI 95% (0.37 – 1.38), OR = 0.72, CI 95% (0.37 – 1.40), P= 0.32, P= 0.33, respectively]. Moreover, there was no significant association between genotype or allele frequency distribution and clinical manifestation of AP [OR =

0.69, CI 95% (0.37 – 1.31), OR = 0.81, CI 95% (0.45 – 1.41), P= 0.21, P= 0.46, respectively].

The role of TNF-α – 308 G/A gene polymorphism has been investigated in the pathogenesis of different oral diseases (11-14, 34-37). In 2013, Chen et al. (11) in the meta-analysis of eight studies have shown that the G allele and GG/GA genotypes were related to a decreased susceptibility to oral cancer. Zhou and Vieira, (12) based on the meta-analysis on seven studies, have reported a significantly increased susceptibility for oral lichen planus development in AG heterozygotes and AA homozygotes compared to individuals with the GG genotype. Ilic et al. (34) also found a strong correlation between GA/AA and an increased risk of odontogenic keratocyst development. Interestingly, with 4 meta-analyses over the past thirteen years, the most extensive research has been made in Periodontology (13, 35-37). Namely, it has been shown that this polymorphism in the TNF-α gene significantly influences the predisposition to different forms of periodontitis, particularly in Asians (13, 35-37). Finally, in the meta-analysis of 6 studies, Mo et al. (14) revealed that this SNP was not significantly associated with the risk of dental peri-implant disease which is in accordance with our findings.

Several limitations of this meta-analysis should be acknowledged. First, this updated meta-analysis included only 4 studies with relatively small sample size. This has resulted in imprecision as detailed in Figure 2 with wide CI. Second, the systematic review suffered from inconsistency (Fig 2) and heterogeneity (high I^2 and significance in p-value). Third, several methodological flaws of each study included in the final review were observed and presented in Table 1. This is mostly related to an unmatched selection of patients between study groups, undetermined study power and/or Hardy-

Weinberg Equilibrium for a given sample size, and absence of correction for a false-positive (type I) error. All these inadequacies should be taken into consideration in future investigations. Fourth, the majority of the included studies were based on South and North Americans with unknown ethnicity, and only one study included European Caucasians. Hence, further studies on other populations and ethnicities are required. Finally, previous studies were restricted to the analysis of specific polymorphisms within a single/few gene(s), failing to properly reflect the complex pathogenesis of AP. Therefore, future genome-wide association studies could be useful for identifying loci at which common variants influence disease risk, but with yet unknown role in AP. It should be emphasized that the results of this systematic review with meta-analysis should be interpreted with caution, and the aforementioned limitations of this systematic review with meta-analysis taken into consideration in future investigations.

Heredity, as a risk factor for AP, is still an under-investigated research topic in the dental community. Although different gene expression patterns, gene-gene interactions, as well as epigenetic alterations, may potentially influence AP development (15), pooled data of this updated systematic review and meta-analysis revealed no significant association of TNF-α -308 G/A SNP (rs1800629) with a risk of AP development, nor with phenotypic manifestation of the disease. In conclusion, the results of this updated systematic review did not show a significant association, but there was not enough evidence to support that there is no association. Further well-designed multi-centric studies with larger sample sizes and with different world populations/ethnic groups are needed for a better understanding of the role of genetic factors in the etiopathogenesis of AP.

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Figure Legends

Figure 1 – PRISMA flow diagram of the study search and identification. n, number of hints, WoS - Web of Science Core Collection, KJD - Korean Journal Database, RSCI - Russian Science Citation Index, SCIELO - SciELO Citation Index, CENTRAL - Cochrane Central Register of Controlled Trials, CNKI - China National Knowledge Infrastructure.

Figure 2 – Forest plots of the (a) genotype distribution under recessive model and (b) allele distribution between apical periodontitis patients (cases) and healthy individuals (controls). †based on the data not previously presented in the published study by Jakovljevic et al. (18), now presented in Table 2.

Figure 3 – Forest plots of the (a) genotype distribution under recessive model and (b) allele distribution between acute AP and chronic apical periodontitis groups. †based on the data not previously presented in the published study by Dill et al. (17), provided by the authors through personal communication.

Table 1. Overall characteristics of studies included in the final review

Author, Year (reference)	Country (City)	Population /Ethnicity	Study design	Sample size (cases/ controls)	Gender (F/M)	Age (mean ± SD)	SNP of interest (ID)	Source of genomic DNA	Genotype method	Main outcomes related to investigate SNP	Limitations
De Sá et al. (28)	Brazil (Minas Gerais)	Brazilian/ Mixed	Case-control	45 cases 53 controls	24/21 26/27	-	TNF-α -308 G/A (rs1800629)	Oral mucosal swabs	PCR-RFLP	No significant differences between investigated groups	Power calculation was not provided. Correction for type I error was not calculated.
Amaya et al. (29)	Colombia (Bucaramanga City)	Columbian/ Mixed	Case-control	63 cases 57 controls	37/26 40/17	32.8±10.4 35.4±11.6	TNF-α -308 G/A (rs1800629)	Blood	PCR-RFLP	No significant differences between investigated groups	Unmatched groups. Power calculation was not provided. Correction for type I error was not calculated.
Dill et al. (17)	USA (Houston, Pittsburgh)	American/ White	Case-control	136 cases 180 controls	75/61 97/83	55±3 58±8	TNF-α -308 G/A (rs1800629)	Saliva	TaqMan probes	No significant differences between investigated groups	Unmatched groups. Power calculation was not provided.
Jakovljevic et al. (18)	Serbia (Belgrade)	Serbian/ White	Case-control	120 cases 200 controls	65/55 110/90	35±14.2 34.9±13.2	TNF-α -308 G/A (rs1800629)	Periapical lesion and buccal swabs	PCR-RFLP	Significant increase of AP susceptibility, both in heterozygous and homozygous carriers.	Correction for type I error was not calculated.

F: female. M: male. SD: standard deviation. SNP: single nucleotide polymorphism. ID: identification. DNA: deoxyribonucleic acid. TNF-α: tumour necrosis factor – alpha. G: guanine. A: adenine. PCR-RFLP: polymerase chain reaction – restriction fragment length polymorphism.

Supplementary Table 4. Quality assessment of studies included in the final review

Evaluated criteria	Studies			
	De Sá et al. [28]	Amaya et al. [29]	Dill et al. [17]	Jakovljevic et al. [18]
*				
Selection	4	4	4	4
Comparability	1	0	0	0
Exposure	2	1	2	2
Study methodology/ design	1	2	3	3
Genetic analysis	6	6	6	7
Score	14	13	15	16
Quality	High-quality	High-quality	High-quality	High-quality
**				
Control group	1	1	1	1
Hardy-Weinberg equilibrium	1	1	1	1
Case group	1	1	1	1
Primer	1	1	1	1
Reproducibility	1	1	1	1
Blinding	1	1	1	1
Power calculation	0	0	0	1
Statistics	1	1	1	1
Corrected statistics	0	0	1	0

Independent replication	0			
Score	7	7	8	9
Quality	Moderate	Moderate	High	High

***

Title and abstract	1	1	1	1
Background rationale	1	1	1	1
Objectives	1	1	1	1
Study design	1	1	1	1
Setting	1	1	1	1
Participants	1	1	1	1
Variables	0	0	0	0
Data sources/ measurement	1	1	1	1
Bias	0	0	0	0
Study size	0	0	0	0
Quantitative variables	1	1	1	1
Statistical methods	0	0	0	0
Participants	1	1	1	1
Descriptive data	0	0	0	0
Outcome data	1	1	1	1
Main results	0	1	1	1
Other analyses	0	0	0	0

Key results	1			
Limitations	0	0	0	0
Interpretation	1	1	1	1
Generalizability	1	1	1	1
Funding	1	0	1	1
Score	14	14	15	15
Quality	Medium	Medium	High	High

*Quality of studies assessed by guidelines published by Nibali[22] high-quality (presenting ≥ 10 criteria), low-quality (presenting < 10 criteria).

** Quality of studies assessed by guidelines published by Clark & Baudouin;[23] high methodological quality (presenting 8 or more criteria), moderate methodological quality (presenting 5 to 7 criteria), and low methodological quality (presenting 4 or fewer criteria).

*** Quality of studies assessed by guidelines published by Little et al.[25] high quality (presenting 15–22 criteria), medium quality (presenting 8–14 criteria), and inferior quality (presenting 0–7 criteria).

Supplementary Table 3: Is TNF-α – 308 G/A (rs1800629) SNP associated with an increase in the risk of AP (Acute compared to chronic)?

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	acute	chronic	Relative (95% CI)	Absolute (95% CI)		
genotypes												
3	observational studies	not serious	serious	not serious	serious	strong association	108/159 (67.9%)	129/179 (72.1%)	**OR 0.69** (0.37 to 1.31)	**80 fewer per 1,000** (from 232 fewer to 51 more)	⨁◯◯◯ VERY LOW	
alleles												
3	observational studies	not serious	serious	not serious	serious	strong association	264/318 (83.0%)	300/358 (83.8%)	**OR 0.81** (0.45 to 1.44)	**31 fewer per 1,000** (from 139 fewer to 44 more)	⨁◯◯◯ VERY LOW	

CI: Confidence interval; OR: Odds ratio

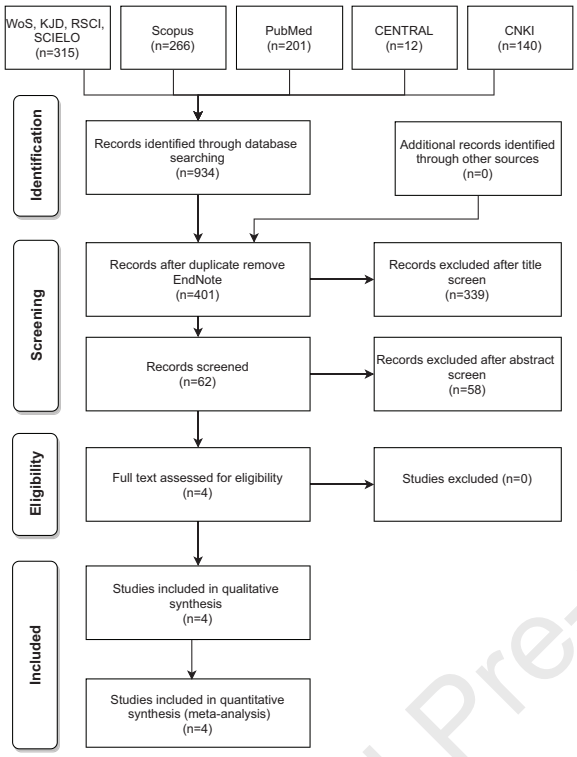
Grade	**Definition**
High	Further research is very unlikely to change our confidence in the estimate of effect.
Moderate	Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low	Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low	Any estimate of effect is very uncertain.

Supplementary Table 4: Is TNF-α – 308 G/A (rs1800629) SNP associated with an increase in the risk of AP: Cases compared to controls

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	cases	controls	Relative (95% CI)	Absolute (95% CI)		
genotypes												
2	observational studies	not serious	serious	not serious	serious	strong association	162/250 (64.8%)	267/371 (72.0%)	**OR 0.71** (0.37 to 1.38)	**74 fewer per 1,000** (from 233 fewer to 60 more)	⨁◯◯◯ VERY LOW	
alleles												
2	observational studies	not serious	serious	not serious	serious	strong association	403/500 (80.6%)	634/742 (85.4%)	**OR 0.72** (0.37 to 1.40)	**46 fewer per 1,000** (from 170 fewer to 37 more)	⨁◯◯◯ VERY LOW	

CI: Confidence interval; OR: Odds ratio

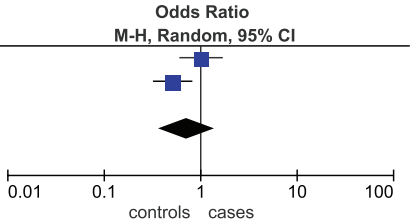
Grade	**Definition**
High	Further research is very unlikely to change our confidence in the estimate of effect.
Moderate	Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low	Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low	Any estimate of effect is very uncertain.



a

Study or Subgroup	cases Events	Total	controls Events	Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
Dill et al. 2015†	96	130	126	171	48.7%	1.01 [0.60, 1.69]	2015
Jakovljevic et al. 2020	66	120	141	200	51.3%	0.51 [0.32, 0.82]	2020
Total (95% CI)		**250**		**371**	**100.0%**	**0.71 [0.37, 1.38]**	
Total events	162		267				

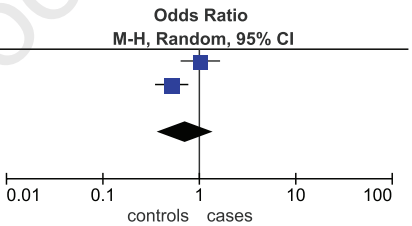
Heterogeneity: Tau² = 0.17; Chi² = 3.61, df = 1 (P = 0.06); I² = 72%
Test for overall effect: Z = 1.00 (P = 0.32)



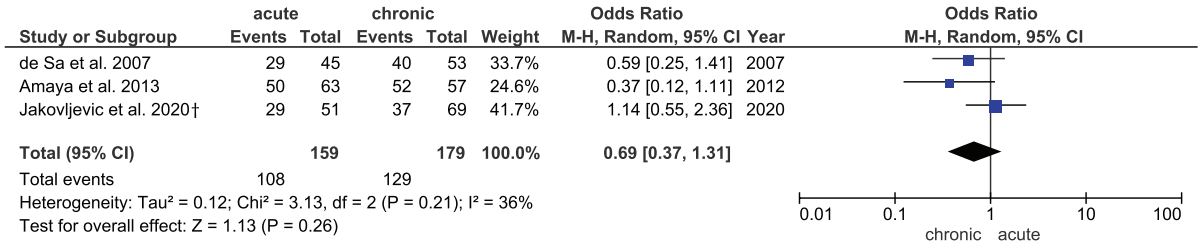
b

Study or Subgroup	cases Events	Total	controls Events	Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
Dill et al. 2015†	225	260	295	342	48.2%	1.02 [0.64, 1.64]	2015
Jakovljevic et al. 2020	178	240	339	400	51.8%	0.52 [0.35, 0.77]	2020
Total (95% CI)		**500**		**742**	**100.0%**	**0.72 [0.37, 1.40]**	
Total events	403		634				

Heterogeneity: Tau² = 0.18; Chi² = 4.74, df = 1 (P = 0.03); I² = 79%
Test for overall effect: Z = 0.97 (P = 0.33)



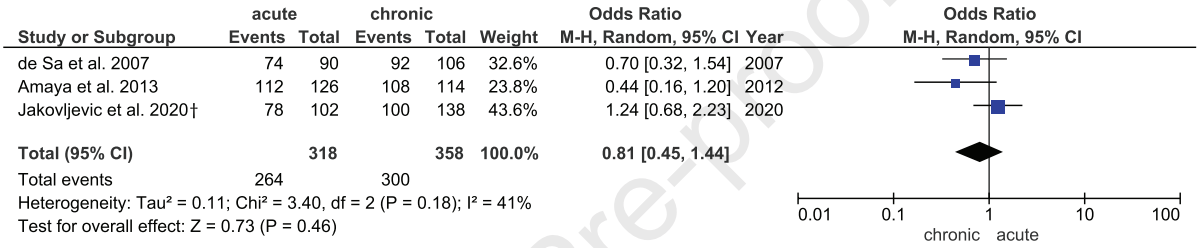
a



Study or Subgroup	acute Events	acute Total	chronic Events	chronic Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
de Sa et al. 2007	29	45	40	53	33.7%	0.59 [0.25, 1.41]	2007
Amaya et al. 2013	50	63	52	57	24.6%	0.37 [0.12, 1.11]	2012
Jakovljevic et al. 2020†	29	51	37	69	41.7%	1.14 [0.55, 2.36]	2020
Total (95% CI)		**159**		**179**	**100.0%**	**0.69 [0.37, 1.31]**	
Total events	108		129				

Heterogeneity: Tau² = 0.12; Chi² = 3.13, df = 2 (P = 0.21); I² = 36%
Test for overall effect: Z = 1.13 (P = 0.26)

b



Study or Subgroup	acute Events	acute Total	chronic Events	chronic Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
de Sa et al. 2007	74	90	92	106	32.6%	0.70 [0.32, 1.54]	2007
Amaya et al. 2013	112	126	108	114	23.8%	0.44 [0.16, 1.20]	2012
Jakovljevic et al. 2020†	78	102	100	138	43.6%	1.24 [0.68, 2.23]	2020
Total (95% CI)		**318**		**358**	**100.0%**	**0.81 [0.45, 1.44]**	
Total events	264		300				

Heterogeneity: Tau² = 0.11; Chi² = 3.40, df = 2 (P = 0.18); I² = 41%
Test for overall effect: Z = 0.73 (P = 0.46)