

Association of NLRP3 (rs4612666) polymorphism in gingival crevicular fluid with symptomatic irreversible pulpitis and asymptomatic apical periodontitis

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ABSTRACT

Objectives: This study investigated the association between the NLRP3 rs4612666 single-nucleotide polymorphism (SNP) and the clinical presentation of symptomatic irreversible pulpitis (SIP) and asymptomatic apical periodontitis (AAP) using gingival crevicular fluid (GCF) samples from a North Indian population.

Methods: In this observational case-control study, 48 participants were divided into three groups (n = 16 each): SIP, AAP, and healthy controls. GCF samples were collected for DNA extraction, followed by genotyping of the NLRP3 rs4612666 SNP using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). Genotypic and allelic frequencies were compared across groups, and Hardy–Weinberg equilibrium (HWE) was assessed.

Results: The heterozygous TC genotype predominated in both the SIP (62.5 %) and AAP (68.8 %) groups, whereas the control group exclusively exhibited the TT genotype. The CC genotype was absent in all groups. The C allele was more frequent in AAP (43.8 %) than in SIP (18.8 %). Genotypic and allelic distributions differed significantly between the disease groups and controls (p < 0.001). Deviation from HWE in the AAP group (p = 0.0361) suggests potential genetic susceptibility to chronic inflammation.

Conclusion: These findings suggest an association between the C allele of the NLRP3 rs4612666 SNP and increased inflammatory response in endodontic disease. Genetic screening may aid in early risk assessment and support personalized treatment planning.

Clinical relevance: Identifying NLRP3 rs4612666 polymorphism in patients may help predict susceptibility to chronic pulpal and periapical inflammation. This genetic insight can aid in risk-based diagnosis and guide personalized endodontic treatment planning.

1. Introduction

Inflammatory pathologies of the dental pulp and periapical tissues represent a significant global health burden affecting many adults

globally. Epidemiological studies have long demonstrated the high prevalence of untreated dental caries and pulpal inflammation.¹ These conditions result from complex interactions of microbial challenges and host immune responses. Recent high-throughput genomic and

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transcriptomic analyses have further refined our understanding of these processes by uncovering specific genetic and molecular pathways that regulate the inflammatory cascade.² Recent studies emphasize cellular stress responses, including hypoxia and oxidative stress, in modulating inflammatory signaling in dental pulp tissues.³

The NLRP3 inflammasome is a key mediator of the host's inflammatory response. As a cytosolic multiprotein complex, the NLRP3 inflammasome is composed of the sensor protein NLRP3, the adaptor protein ASC, and pro-caspase-1. Under normal conditions, NLRP3 expression is maintained at low levels; however, when stimulated by pathogenic signals or endogenous stressors such as hypoxia and reactive oxygen species (ROS), its expression is upregulated through NF- κ B pathways.⁴ Emerging evidence indicates that the activation of the NLRP3 inflammasome occurs via a well-defined two-signal mechanism: a priming phase, which upregulates the transcription of NLRP3 and pro-IL-1 β , followed by an activation phase that promotes the assembly of the inflammasome complex and subsequent cleavage of pro-caspase-1.⁵ In addition, recent proteomics studies have detailed how post-translational modifications—including phosphorylation, ubiquitination, acetylation, and SUMOylation—finely control the activity of NLRP3 in dental pulp cells, thus influencing the magnitude of the inflammatory response.^{6,7}

Genetic polymorphisms in the NLRP3 gene have also been implicated in modulating the threshold for inflammasome activation and the downstream production of pro-inflammatory cytokines. Single-cell RNA sequencing investigations have provided compelling evidence by demonstrating differential NLRP3 expression in subpopulations of dental pulp fibroblasts, correlating specific risk alleles with heightened expression and an exacerbated inflammatory phenotype.⁸ Furthermore, studies focusing on dental pulp mesenchymal stem cells suggest that genetic background may affect not only basal levels of inflammation but also the reparative capacity of the pulp through modulating inflammasome activity and cytokine release.⁹

The analysis of gingival crevicular fluid (GCF) offers a non-invasive window into the underlying molecular events of endodontic pathologies. Advanced metabolomic and proteomic profiling of GCF has led to the identification of over 150 molecular signatures that are associated with pulpal inflammation. These biomarkers include specific damage-associated molecular patterns (DAMPs) and microRNA profiles that have been validated as reliable indicators of disease severity and progression.¹⁰ Moreover, recent studies integrating machine learning techniques have demonstrated that combinations of these biomarkers can predict treatment outcomes and disease progression with high accuracy, highlighting the potential for personalized therapeutic approaches in endodontics.¹¹

The convergence of genetic, proteomic, and metabolomic insights has paved the way for the development of targeted interventions in endodontic therapy. For example, small-molecule modulators that target the NLRP3 inflammasome and its regulatory pathways are under active investigation, with preclinical models showing reduced inflammatory cytokine production and improved pulp tissue repair.^{12,13} Integrating molecular diagnostics enables precision-based therapies and better outcomes.

Despite these significant advances in our understanding of NLRP3 inflammasome biology and its role in pulpal inflammation, several critical gaps remain in our knowledge. While genetic polymorphisms in NLRP3 have been associated with various inflammatory conditions, their specific impact on pulpal and periapical disease progression remains poorly understood. Furthermore, the relationship between these genetic variations and the clinical manifestation of endodontic pathologies has not been fully elucidated. This study evaluates the role of NLRP3 (rs4612666) polymorphism in patients with SIP and AAP. Therefore, this study aims to investigate the association between NLRP3 genetic polymorphisms and the severity of pulpal inflammation, using GCF analysis non-invasively. By linking genetic variants to GCF mediator profiles and outcomes, this research seeks to establish a foundation

for personalized therapeutic approaches in endodontic treatment. Understanding these relationships could potentially lead to the development of targeted interventions based on individual genetic profiles, ultimately improving treatment predictability and patient outcomes in endodontic practice.

2. Materials and methodology

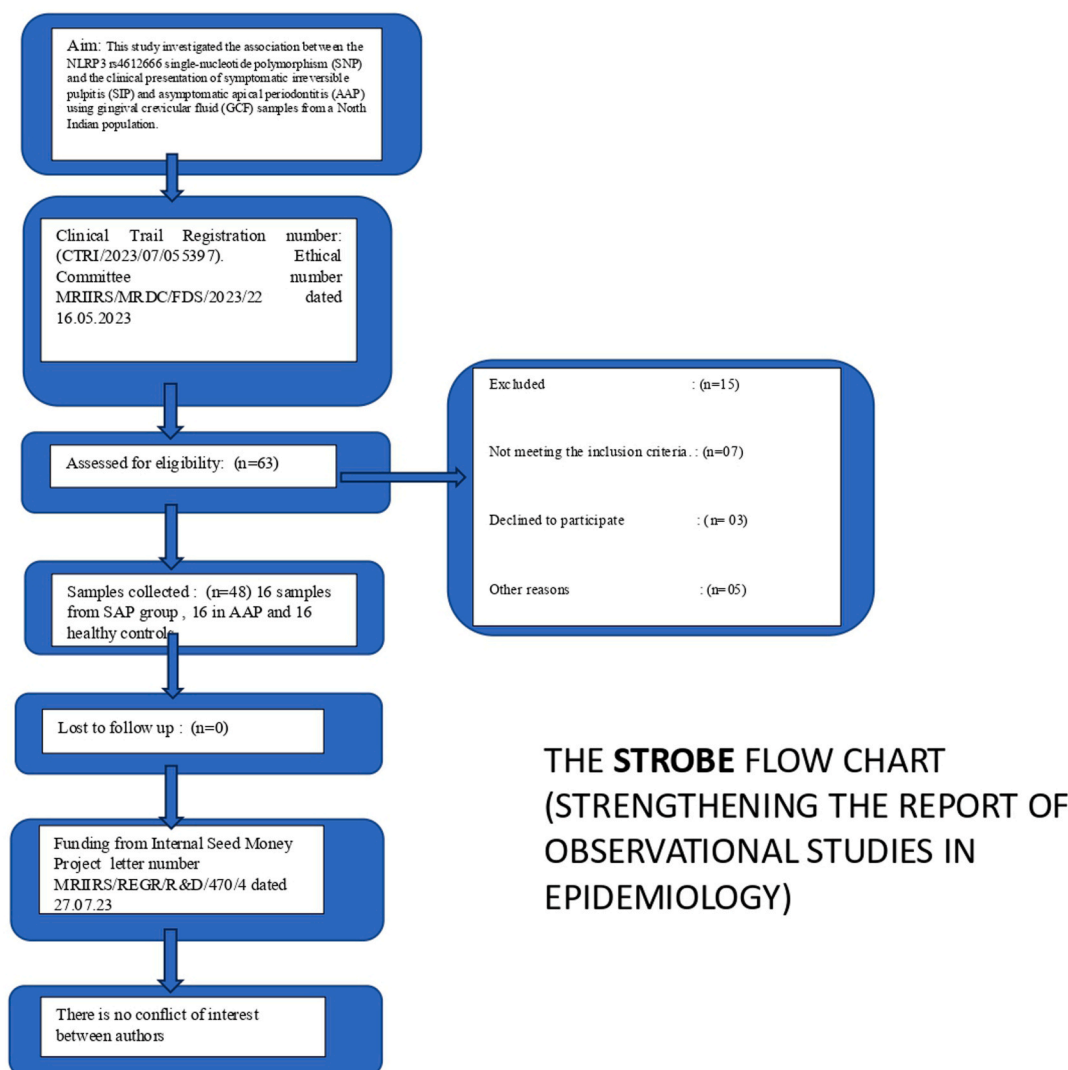
2.1. Study design, subject recruitment, and inclusion/exclusion criteria

This observational case-control study, with a cross-sectional analytical design, was conducted in the Department of Conservative Dentistry and Endodontics. The study protocol adhered to the Declaration of Helsinki guidelines and received approval from the institutional ethics committee. The study received institutional funding. The trial was registered with the Clinical Trial Registry of India (CTRI/2023/07/055397). The study spanned from August 2023 to February 2025, post the CTRI registration. The study was registered to ensure transparency and methodological rigor, despite its observational nature. The manuscript preparation followed the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) checklist. Fig. 1 describes the same.

The study's power and sample size estimation were calculated using Genetic Power software (version 3.0), based on chi-square tests for goodness-of-fit in contingency tables. (version 3.0), with calculations based on a chi-square test for goodness-of-fit tests (contingency tables). A minimum sample size of 48 participants was sufficient, distributed equally as 16 in each group (SIP patients, AAP patients, and healthy controls) (Fig. 2).

Before enrollment, written informed consent was obtained from each participant. Demographic and medical information were collected through interviews and a clinical examination conducted simultaneously by 2 trained clinicians. This examination aimed to exclude any inflammatory conditions of the oral mucosa and to carry out a thorough periodontal assessment. Data collected included participants' gender, age, medication history, systemic health status, and smoking habits. Non-smokers had never smoked or quit over five years ago. Healthy controls were age- and gender-matched with the SIP and AAP groups and shared similar sociodemographic characteristics, including non-smoking status and absence of systemic disease.

A study population comprised of 16 patients with untreated AAP, 16 patients with untreated SIP, and 16 healthy controls. Only North Indian participants were eligible. The inclusion criteria for the study required participants to be diagnosed with symptomatic irreversible pulpitis or asymptomatic apical periodontitis, as defined by the American Association of Endodontists (AAE) diagnostic guidelines. Participants had no significant medical/systemic diseases to reduce systemic confounders. Exclusion criteria were established to minimize confounding factors related to oral health and body comorbidities. To control for oral health confounding factors, individuals with multiple carious teeth were excluded. To address body comorbidity confounding factors, individuals with systemic health conditions such as diabetes, cardiovascular diseases, or immunocompromised states were also excluded. Participants who declined to provide informed consent were excluded from the study. Additional exclusion criteria help minimize sociodemographic confounders. Individuals with a history of smoking (less than 5 years), alcohol consumption, poor oral hygiene awareness, pregnancy or lactation, or systemic conditions affecting the structural integrity of oral hard and soft tissues (excluding dental caries and periodontal diseases) were excluded. Subjects wearing orthodontic appliances, those with chronic systemic illnesses affecting periodontal health or bone metabolism, or individuals requiring pre-medication for dental procedures were also excluded. Furthermore, participants who had received periodontal therapy in the past six months or had used anti-inflammatory medications, antiseptic mouthwashes, or antimicrobial agents within the previous three months were not included. To ensure sample



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Fig. 1. STROBE flow chart.

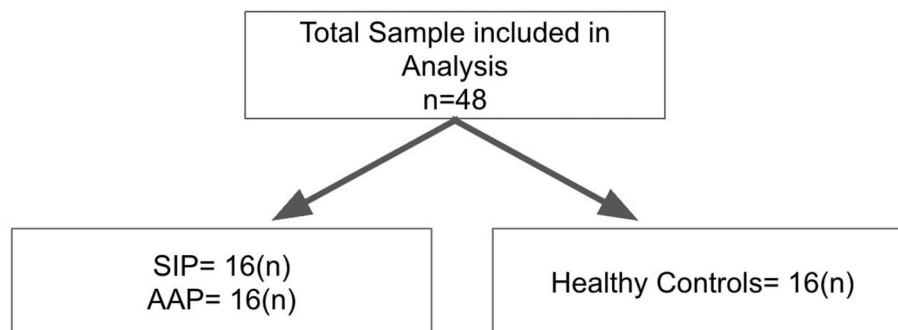


Fig. 2. Participant Flow diagram.

homogeneity, individuals over the age of 40 were excluded. These strict criteria improved reliability.

2.2. Gingival crevicular fluid sample and processing technique

A 5- μ L sample of gingival crevicular fluid (GCF) was collected from the target site using a microcapillary tube immediately prior to the initiation of root canal treatment. To prevent salivary contamination,

the tooth was isolated, and the area was dried with cotton pellets to remove any debris and residual saliva. The tooth was then air-dried, and the GCF sample was carefully collected around the sulcus from the specific carious tooth. Blood-contaminated samples were discarded and recollected. The GCF samples were subsequently transferred into 1.5 mL Eppendorf tubes and stored at -80°C until further processing.

3. Genotyping

3.1. DNA extraction

After the sample was prepared, 200 µL was used for DNA extraction using the QIAamp DNA Mini Kit (Qiagen Sciences®, Germantown, Maryland, USA), following the manufacturer's spin protocol for DNA purification from body fluids. The final elution was performed in 25 µL of AE buffer provided in the kit. The isolated DNA was stored at −20 °C.

3.2. Detection of NLRP3 sites

All PCR reactions were carried out in a total volume of 25 µL 1X PCR master mix (GeNei™ Laboratories, Bengaluru, Karnataka, India). The PCR master included premixed and ready-to-use reagents such as Taq DNA Polymerase, dNTPs, standard reaction buffer, stabilizers, an easy-to-track blue loading dye, Magnesium chloride, additives like BSA, DMSO, and glycerol.

PCR amplification was performed in a 96-well thermal cycler (MiniAmp™ Thermal Cycler, Thermofisher Scientific, Waltham, Massachusetts, USA). PCR included denaturation (95 °C), 35 cycles (56 °C), and final extension (72 °C, 5 min). The annealing temperatures were optimized using gradient PCR, and the optimal temperature was selected based on agarose gel electrophoresis, ensuring optimal resolution. PCR products were run on a 2 % agarose gel, stained with 0.5 mg/mL ethidium bromide (Promega®), and visualized using an ultraviolet transilluminator (RabroTech, New Delhi, India). The size of the expected fragments before enzymatic digestion was 260bp for NLRP3. For negative control, the DNA sample was replaced with sterile water. The samples were then purified using a DNA purification kit (Qiagen Sciences®, Germantown, Maryland, USA) to remove impurities and a high-yielding DNA product. Primers used for determining *BpiI* polymorphic sites in the NLRP3 are 5'- TGC TTA AGG CCA TTA ATT GTG-3' 5' -CTC CAC CAT GGA CAA GGA AG -3', size of the expected fragments length before enzymatic digestion expected was 260 bp.

3.3. Restriction endonuclease digestion (RFLP)

Polymorphic sites were determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method using the *BpiI* restriction enzyme. PCR products from NLRP3 sites were digested with the restriction enzyme (Molecular Biology, Thermofisher Scientific, Waltham, Massachusetts, USA) in a volume of 20 µL containing 0.1 µL of 1X restriction buffer and 10 µL of PCR product. The reaction was performed in a digital dry bath at 65 °C for 2 h. Fragments were separated in 3 % agarose in TBE (Tris base -Boric acid-EDTA) buffer, stained with 0.5 mg/ml ethidium bromide using a 50–100 bp DNA ladder (Thermo Scientific®) as a molecular size marker (Table 1). Genotypes were determined at TT (260bp), TC (260, 102, 158bp), or CC (102, 158bp) for NLRP3 (rs4612666) polymorphism. The mutation was designated following the nomenclature guidelines set by the HUGO Gene Nomenclature Committee (HGNC).

3.4. Statistical analysis and data management

Sociodemographic profiles, clinical parameters, and genetic variant analysis were analyzed Descriptive statistics were used to summarize participant characteristics. Age was reported as mean with standard

deviation (SD), while categorical variables such as gender was expressed as counts and percentages.

All study participants were non-smokers and had no systemic illnesses, ensuring sample homogeneity. Normality was assessed using the Shapiro-Wilk test, and as the data followed a normal distribution, parametric tests were employed.

Between-group comparisons were conducted using one-way analysis of variance (ANOVA) for continuous variables (age), and Pearson's Chi-square test for categorical variables (gender, genotype, and allele distribution). Bonferroni correction was applied for pairwise comparisons to control for type I error. p-value <0.05 was considered statistically significant.

Genotype and allele frequencies of the NLRP3 rs4612666 polymorphism were analyzed across the three study groups: healthy controls (n = 16), SIP patients (n = 16), and AAP patients (n = 16). The SIP and AAP groups displayed significant differences in genotype distributions compared to healthy controls (p < 0.001). However, the difference between SIP and AAP was not statistically significant. Similarly, the allele frequency analysis showed a significantly higher frequency of the C allele in AAP compared to both SIP and healthy groups.

Deviation from Hardy-Weinberg equilibrium (HWE) was assessed using Chi-square goodness-of-fit tests. The SIP group was found to be in HWE (p = 0.069), while the AAP group deviated significantly (p = 0.0361). The association between disease status (SIP/AAP) and NLRP3 rs4612666 genotypes and alleles was evaluated using comparative frequency analysis.

4. Results

4.1. Genetic power analysis and assay consistency

An a priori genetic power analysis was conducted using GPower software (version 3.0) to determine the required sample size for detecting differences in NLRP3 rs4612666 polymorphism across groups. Based on a chi-square test for goodness-of-fit, with an alpha level of 0.05, an assumed effect size of 0.5, and a power of 80 %, the analysis determined that a total sample size of 48 (16 subjects per group) was sufficient (actual power = 0.802). The critical χ^2 value was 9.4877290, and the non-centrality parameter (λ) was 12.0000000.

All GCF samples used for DNA extraction were processed and analyzed with standardized methods. Sample integrity was ensured through double evaluations and gel electrophoresis confirmation.

4.2. Participant demographics and clinical characteristics

The study included a total of 48 participants, evenly distributed across three groups: symptomatic irreversible pulpitis (SIP, n = 16), asymptomatic apical periodontitis (AAP, n = 16), and healthy controls (n = 16), as mentioned in Table 2.

The mean age ($\pm$ SD) of participants was 25.38 $\pm$ 5.52 years in the healthy control group, 24.31 $\pm$ 4.60 years in the SIP group, and 22.12 $\pm$ 2.66 years in the AAP group. There was no statistically significant difference in age across the groups (p = 0.106, one-way ANOVA). Gender distribution also did not differ significantly between groups (p = 0.706, Chi-square test).

All participants were non-smokers and free from systemic conditions such as diabetes, cardiovascular disease, or immunosuppressive disorders. These inclusion criteria ensured a demographically and clinically

Table 1
Nomenclature of the NLRP3 according to NCBI reference sequence genes.

Gene	SNP id	Restriction Enzyme	Localization	Reference sequence: position	Base change	Genotype	Restriction enzyme digest fragment size (bp)
NLRP3	rs4612666	<i>BpiI</i>	Intron 7	NG007509.2: g.24596	T > C	CC(mut) TC(ht) TT(wt)	102, 158 260, 102, 158 260

Table 2

Summary of patient demographics.

Patients Characteristics		Healthy Control [n = 16]	SIP [n = 16]	AAP [n = 16]	F-value/P-value
Age (Years)	Mean (SD)	25.38(5.52)	24.31(4.60)	22.12(2.66)	F = 2.36/P = 0.106
Gender	Male	11(68.8)	9(56.2)	9(56.2)	One-way ANOVA P = 0.706
	Female	5(31.2)	7(43.8)	7(43.8)	
Smoking		16(100.0)	16(100.0)	16(100.0)	Chi-square

comparable sample for evaluating genetic and inflammatory markers across groups.

4.3. Identification of NLRP3 gene variant and its association with SIP and AAP

Genotyping of the NLRP3 rs4612666 single-nucleotide polymorphism was successfully performed in 48 samples, 16 each with SIP, AAP, and HC. Samples with non-amplifiable DNA or incomplete genotyping results were excluded from the final analysis. No imputation was performed. The genotypes were identified based on three distinct banding patterns produced during restriction digestion, corresponding to the presence or absence of restriction enzyme recognition sites. Specifically, the TT genotype yielded a single 260 bp band (wild-type), the TC genotype showed three bands at 260 bp, 158 bp, and 102 bp (heterozygote), and the CC genotype exhibited two distinct bands at 158 bp and 102 bp (mutant homozygote) (Fig. 3). In contrast, none of the samples from the healthy control group (n = 16) exhibited visible band amplification following PCR-RFLP analysis. This may reflect low baseline NLRP3 expression or lack of polymorphisms.

In the SIP group, the TT genotype (wild-type homozygote) was observed in 6 individuals (37.5 %), while the remaining 10 (62.5 %) exhibited the heterozygous TC genotype. No CC genotype was found. Similarly, in the AAP group, 5 individuals (31.2 %) presented with the TT genotype, and 11 (68.8 %) with the TC genotype. The CC genotype was absent in this group as well (Table 3).

Allelic frequency analysis revealed a predominance of the T allele in the SIP group (81.2 %) compared to the AAP group (56.2 %). Conversely, the C allele was more frequent in the AAP group (43.8 %) than in SIP (18.8 %). While this trend suggests a possible shift in allele distribution with disease progression, the difference between the SIP and AAP groups was not statistically significant ($p = 1.000$). However, comparisons between either disease group and the healthy controls were statistically significant ($p < 0.001$), consistent with the monomorphic expression (TT genotype) observed in all control samples.

Assessment of Hardy–Weinberg equilibrium (HWE) demonstrated that genotype distribution in the SIP group did not deviate significantly from expected frequencies ($\chi^2 = 3.388$, $p = 0.069$). In contrast, a significant deviation from HWE was observed in the AAP group ($\chi^2 = 4.388$, $p = 0.0361$), indicating disease-related selection pressure or underlying genetic predisposition influencing NLRP3 gene regulation in asymptomatic apical periodontitis.

5. Discussion

The present investigation provides compelling evidence for the association between NLRP3 rs4612666 polymorphism and endodontic inflammatory conditions in a North Indian population, with particular emphasis on SIP and AAP. Our findings reveal several critical insights into the genetic basis of endodontic pathologies and their potential implications for clinical practice.

A key finding of our study was the predominance of the heterozygous TC genotype in both SIP (62.5 %) and AAP (68.8 %) groups, contrasting sharply with healthy controls who did not exhibit any polymorphic bands. This distinct distribution pattern suggests that the variant C allele may contribute to endodontic disease susceptibility.¹⁴ The higher frequency of the C allele in the AAP group (43.8 %) compared to the SIP group (18.8 %) further indicates its potential role in chronic inflammatory progression.

The deviation from Hardy–Weinberg equilibrium observed in the AAP group is noteworthy, suggesting selective pressure on this genetic variant in chronic inflammatory conditions. This aligns with research showing that NLRP3 polymorphisms can significantly influence inflammatory response patterns through altered cytokine production.¹⁵ These results offer insights into the molecular mechanisms underlying endodontic pathologies. The NLRP3 inflammasome's role as a critical regulator of inflammatory responses, particularly through IL-1 β activation, has been well-documented.¹⁶ The differential distribution of the rs4612666 polymorphism between acute (SIP) and chronic (AAP) conditions suggests that this genetic variant may influence disease

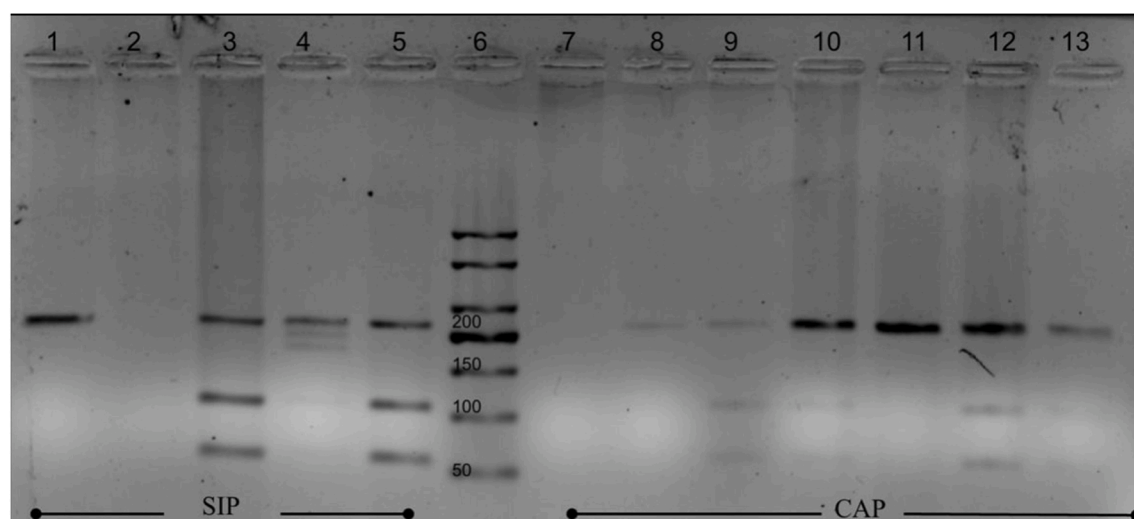


Fig. 3. SNP image.

Table 3

Genotype distribution and allele frequency of the NLRP3 among the three groups.

Gene (NLRP3)	Healthy Control [n = 16]	SIP [n = 16]	AAP [n = 16]	F-value/P-value	Pairwise comparison with Bonferroni correction
Genotype					
TT	16(100.0)	6(37.5)	5(31.2)	P < 0.001 (Exact Significance)	HC vs SIP: p < 0.001
TC	0(0.0)	10(62.5)	11(68.8)		HC vs AAP = p < 0.001
Allele frequency					
T	32(100.0)	22(81.2)	21(56.2)	P < 0.001 (Exact Significance)	HC vs SIP: p = 0.003
C	0(0.0)	10(18.8)	11(43.8)		HC vs AAP = p < 0.001 SIP vs AAP = 1.00
Hardy-Weinberg equilibrium Chi-square value	Cannot be computed	3.388	0.4388		
Chi-square P-value		0.069	0.0361		

progression through modulation of the inflammatory cascade.

Endodontic diseases are inherently multifactorial, arising from microbial challenge, local tissue factors, host immune response, and environmental influences such as oral hygiene and caries burden. While stringent exclusion criteria were applied to minimize these confounding factors, their contribution cannot be entirely eliminated. The present findings therefore suggest that NLRP3 rs4612666 polymorphism may act as a genetic modifier of inflammatory response rather than an independent etiological factor. Previous studies evaluating NLRP3 polymorphisms in different populations have reported variable genotype and allele distributions, underscoring the influence of ethnic and geographic factors on inflammasome-related inflammatory responses. Studies from East Asian and European cohorts have demonstrated population-specific differences in NLRP3 variant frequencies and their association with chronic inflammatory conditions. The present findings in a North Indian population add to this growing evidence, emphasizing the need for region-specific genetic evaluation in endodontic diseases.

The population-specific nature of our findings in the North Indian cohort highlights the importance of genetic studies across different ethnic groups in endodontics. Other populations have shown varying distributions of NLRP3 polymorphisms, emphasizing the need for population-specific genetic screening approaches.¹⁷

These findings inform clinical care. The identification of genetic susceptibility markers could enable more personalized approaches to endodontic treatment, potentially allowing for early intervention in high-risk patients.¹⁸ Furthermore, understanding the role of NLRP3 polymorphisms in disease progression could lead to the development of targeted therapeutic strategies.

Key limitations include, the sample size, while adequate for initial association studies, could be expanded in future investigations to validate these findings in larger cohorts. Additionally, functional studies are needed to elucidate the precise mechanisms by which the rs4612666 polymorphism influences inflammatory responses in pulpal and periapical tissues. Although strict inclusion and exclusion criteria were applied to minimize confounding, local factors such as microbial composition, caries burden, and oral hygiene status, as well as environmental influences including dietary habits and socioeconomic factors, were not quantitatively assessed. These factors may interact with genetic susceptibility and influence the clinical expression of pulpal and periapical inflammation.

Hence the authors conclude by stating that this study shows an association between the NLRP3 rs4612666 polymorphism and endodontic inflammatory conditions, with the heterozygous (TC) genotype occurring more frequently in patients with symptomatic irreversible pulpitis and asymptomatic apical periodontitis, and absent in healthy individuals. This suggests individuals carrying the C allele may exhibit a heightened inflammatory response to pulpal and periapical insults, contributing to disease progression. The deviation from Hardy-Weinberg equilibrium in the AAP group further implies a possible genetic susceptibility in chronic inflammatory lesions. These insights offer potential translational relevance, as early genotyping may aid personalized

treatment strategies, improve risk assessment, and enhance prognostic accuracy in endodontic care. From a translational perspective, identifying genetic variants associated with exaggerated inflammatory responses may allow early risk stratification of patients prone to chronic periapical disease. Such insights could support personalized recall intervals, closer monitoring, and future host-modulatory therapeutic strategies in endodontic care.

While the present study identified statistically significant differences in NLRP3 (rs4612666) genotype and allele frequencies between symptomatic irreversible pulpitis (SIP), asymptomatic apical periodontitis (AAP), and healthy controls, the clinical significance of these findings warrants careful consideration. In the absence of an established minimum clinically important difference (MCID) for genetic polymorphisms in endodontic pathology, it is difficult to directly translate these findings into diagnostic or therapeutic thresholds. However, the observed trend of higher C allele frequency in AAP patients suggests a potential genetic predisposition to disease chronicity and progression, possibly aiding biomarker development. Larger studies with clinical metrics (lesion size, pain, treatment response) are essential to determine whether NLRP3 genotyping can meaningfully guide patient-specific diagnosis or management in clinical practice.

6. Conclusion

In conclusion, our study strongly supports the link between NLRP3 rs4612666 polymorphism and endodontic inflammatory conditions in the North Indian population. These findings contribute to our understanding of the genetic basis of endodontic pathologies and may inform future approaches to personalized endodontic treatment strategies.

Informed consent

Written informed consent form were signed and taken from all the participants.

Ethical approval statement and document

This cross-sectional study was approved by the Institutional Internal Ethical Committee (IEC) of the School of Dental Sciences, MRIIRS, Faridabad, Haryana, India (MRDC/IEC/2023/22).

Artificial intelligence

No artificial intelligence tools were used in writing this manuscript.

Authors' contribution/credit author statement

Dax Abraham: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing- original draft, Writing-review and editing. Anjana Goyal:

Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing- original draft, Writing-review and editing. Alpa Gupta: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing- original draft, Writing-review and editing. Saumya Verma: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing- original draft, Writing-review and editing. Vineeta Sharma: Formal Analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing- original draft, Writing-review and editing. Rajeev Kumar Malhotra: Formal Analysis, Methodology, Resources, Software, Validation, Writing- original draft, Writing-review and editing. Sucheta Jala: Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing- original draft, Writing-review and editing. Gurjot Singh: Formal Analysis, Investigation, Methodology, Resources, Validation, Visualization.

Ethical clearance

Ethical clearance was obtained from the Institutional Review Board.

Clinical trial registration

The clinical study was registered under the Clinical Trial Registry-India, www.ctri.nic.in (CTRI) on July 20, 2023 (Ref no. CTRI/2023/07/055397)

Relevant Reporting Guidelines paperwork: The manuscript has been prepared in accordance with 'Strengthening the Reporting of Observational studies in Epidemiology' (STROBE) checklist. A Flow chart and a checklist for the same have been prepared and uploaded.

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Declaration of competing interest

We certify that We have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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