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Research Article

Association of NLRP3 rs4612666 Gene Polymorphism with Helicobacter Pylori Infection Susceptibility a Case-Control Study Using Molecular and Statistical Analysis

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Abstract

Helicobacter pylori infection is a major global health concern, associated with various gastrointestinal disorders such as peptic ulcers and gastric cancer, which result in significant morbidity and economic burden on healthcare systems. This study investigates the relationship between the NLRP3 rs4612666 gene polymorphism and susceptibility to H. pylori infection using a case-control design that involved 200 people. We employed molecular techniques and statistical analyses to evaluate the distributions of genotypes and alleles. Our findings indicated no significant difference in the distribution of NLRP3 rs4612666 genotypes between the infected group (C/C: 35.0%, C/T: 51.0%, T/T: 14.0%) and the non-infected group (C/C: 24.0%, C/T: 50.0%, T/T: 26.0%) with a p-value of 0.059. However, we did observe a statistically significant difference in allele frequencies, with allele C found in 60.5% of the infected group compared to 49.0% in the non-infected group (p=0.021), suggesting that allele C may be linked to a higher susceptibility to H. pylori infection. Despite these insights, the study has limitations, including a small sample size and a lack of clinical validation, highlighting the necessity for further research with larger cohorts to verify these associations. This research enhances our understanding of the genetic factors contributing to H. pylori infection, which could inform future diagnostic and therapeutic approaches, and emphasizes the need to explore additional genetic markers to deepen our understanding of the disease's pathogenesis.

Keywords

Association, NLRP3 rs4612666, Gene Polymorphism, Helicobacter pylori Infection

1. Introduction

Helicobacter pylori (H. pylori) infection poses a significant global health challenge, linked to various gastrointestinal disorders such as chronic gastritis, peptic ulcers, and gastric cancer. Approximately half of the world's population is believed to be infected, with prevalence rates that vary based on geographical and socio-economic conditions. The bacterium's unique ability to thrive in the stomach's acidic environment, along with its virulence factors, plays a crucial role in its pathogenicity and the clinical symptoms associated with the infection [1]. The World Health Organization has classified H. pylori as a Class I carcinogen, highlighting its involvement in the development of gastric cancer and the pressing need for

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enhanced diagnostic and therapeutic approaches [2]. Current diagnostic methods for H. pylori infection encompass non-invasive tests, including urea breath tests, stool antigen tests, and serological assays, as well as invasive procedures like endoscopy and biopsy [3]. However, each of these methods has its own limitations in terms of sensitivity, specificity, and invasiveness, which can hinder the prompt and accurate diagnosis of H. pylori infection in clinical practice [4]. Additionally, the rising rates of antibiotic resistance complicate treatment options, making it essential to gain a deeper understanding of the genetic factors that influence susceptibility to infection and the outcomes of treatment [5].

Recent studies have concentrated on the genetic variations linked to H. pylori infection, especially focusing on the NOD-like receptor family pyrin domain containing 3 (NLRP3) gene, which plays a significant role in the immune response to this pathogen [6]. One particular variant, the rs4612666 polymorphism in the NLRP3 gene, has attracted interest due to its potential influence on an individual's susceptibility to H. pylori infection and the development of related diseases. Previous research suggests that genetic factors may affect how the host responds to H. pylori, indicating that a deeper understanding of these genetic associations could pave the way for identifying new biomarkers that predict susceptibility and disease progression [4].

This study uses a case-control design to explore the link between the NLRP3 rs4612666 gene polymorphism and the risk of H. pylori infection. By employing a combination of molecular techniques and thorough statistical analyses, the research seeks to clarify the genetic factors that contribute to H. pylori infection. This work adds to the existing literature on how genetic predisposition interacts with the outcomes of infectious diseases. The main goal is to evaluate how variations in the NLRP3 rs4612666 gene are associated with the likelihood of being infected by H. pylori. The findings from this study could ultimately enhance clinical practices and inform public health strategies for managing diseases related to H. pylori.

In conclusion, this research aims to address the gaps in our understanding of the genetic factors that influence susceptibility to H. pylori infection. By identifying key associations between specific genetic polymorphisms and the risk of infection, the findings may offer important insights into the mechanisms behind disease development and suggest potential pathways for targeted prevention and treatment strategies. As the landscape of H. pylori infection evolves, ongoing investigation into genetic factors will be crucial for informing future research and clinical practices[7, 8].

2. Materials and Methods

2.1. Study Participants

This study selected 200 participants who underwent health examinations or visited our hospital between January 2023 and March 2025. Based on the results of H. pylori infection testing, they were divided into an infected group (n=100 participants) and a non infected group (n=100 participants). The diagnostic criterion for H. pylori infection was a positive result on the ^{13}C or ^{14}C urea breath test, with comprehensive clinical evaluation. Participants in the non-infection group tested negative for H. pylori and had no history of taking antibiotics, proton pump inhibitors, gastric mucosal protective agents, or other medications that may affect H. pylori test results within the past 6 months. Additionally, individuals with severe systemic diseases (e.g., cardiovascular, hepatic, or renal disorders) or malignancies were excluded. The study was approved by the Institutional Ethics Committee of our hospital, and all participants provided written informed consent.

2.2. Reagents and Instruments

Genomic DNA Extraction Kit purchased from Shanghai Sangon Biotech Co., Ltd. rs4612666 Genotyping Detection Kit purchased from Shanghai Tsingke Biotech Co., Ltd. Real-time Fluorescence Quantitative PCR Instrument (Model:ETC811) purchased from Dongsheng. DNA Sequencer (Model:3730XL) purchased from Applied Biosystems, USA)

2.3. Experimental Methods

2.3.1. Sample Collection and DNA Extraction

Peripheral venous blood (5 mL) was collected from each participant using EDTA anticoagulant tubes and stored at 4 ℃. Genomic DNA was extracted within 24 hours according to the instructions of the DNA extraction kit. The purity (A260/A280 ratio between 1.8 and 2.0) and concentration of the extracted DNA were measured using a UV spectrophotometer. Qualified DNA samples were stored at -20 ℃ for subsequent experiments.

2.3.2. NLRP3 rs4612666 Genotyping

Genotyping of NLRP3 rs4612666 was performed using real-time fluorescence quantitative PCR (qPCR), strictly following the instructions of the genotyping detection kit. The reaction system (total volume: 20 μL) consisted of: 2×PCR Master Mix: 10 μL, Forward and Reverse Primers: 0.5 μL each, Probe: 0.5 μL, DNA Template: 2 μL, Nuclease-Free Water: 6.5 μL, The PCR conditions were as follows: Initial denaturation: 95 ℃ for 10 min, 40 cycles of: Denaturation: 95 ℃ for 15 s, Annealing/Extension: 60 ℃ for 60 s, Genotypes (C/C, C/T, T/T) were determined based on fluorescence signals detected by the qPCR instrument. Each experiment included positive controls, negative controls, and blank controls to ensure accuracy and reliability.

2.4. Statistical Analysis

Data were processed and analyzed using SPSS version 26.0. Categorical data were presented as frequencies (n). Comparisons of genotype and allele frequency distributions between the two groups were performed using the Chi-square test, with a significance level of $\alpha = 0.05$. A p-value < 0.05 was considered statistically significant.

3. Result

1. Comparison of General Clinical Data between Two Groups

Statistical analysis revealed no significant differences in gender, age, BMI, smoking history, or alcohol consumption history between the two groups (p>0.05), as illustrated in Table 1.

Table 1. *Comparison of General Clinical Characteristics Between the Two Groups*

Indicator	Infection Group (n=100)	Non-Infection Group (n=100)	χ^2/t Value	p Value
Gender (Male/Female, n)	52/48	50/50	0.080	0.777
Age (Years, x±s)	45.6±10.2	46.2±10.5	0.392	0.695
BMI (kg/m², x±s)	23.5±2.1	23.8±2.3	0.987	0.325
Smoking History (Yes/No, n)	28/72	26/74	0.107	0.744
Alcohol History (Yes/No, n)	22/78	20/80	0.162	0.687

2. Comparison of NLRP3 rs4612666 Genotype Distribution between Two Groups

Chi-square analysis showed that there was no statistically significant difference in the distribution of NLRP3 rs4612666 genotypes between the two groups (p>0.05), as illustrated in Table 2.

Table 2. *Comparison of rs4612666 Genotype Distributions Between the Two Groups*

Genotype	Infection Group (n=100)	Composition Ratio (%)	Non-Infection Group (n=100)	Composition Ratio (%)	χ^2 Value	Degrees of Freedom	p Value
C/C	35	35.0	24	24.0	5.661	2	0.059
C/T	51	51.0	50NLRP3	50.0			
T/T	14	14.0	26	26.0			

3. Comparison of NLRP3 rs4612666 Allele Distribution between Two Groups

Chi-square analysis indicated a statistically significant difference in the distribution of NLRP3 rs4612666 alleles between the two groups,(p<0.05), as shown in Table 3.

Table 3. *Comparison of rs4612666 Allele Frequencies Between the Two Groups*

Allele	Infection Group (n=200)	Composition Ratio (%)	Non-Infection Group (n=200)	Composition Ratio (%)	χ^2 Value	Degrees of Freedom	p Value
C	121	60.5	98	49.0	5.338	1	0.021
T	79	39.5	102	51.0			

4. Discussion

H. pylori infection poses a significant global health challenge, being a primary cause of chronic gastritis, peptic ulcers, and gastric cancer. This gram-negative bacterium infects nearly half of the world's population, with its prevalence influenced by geographical and socio-economic factors [1, 2]. H. pylori's ability to survive in the stomach's acidic environment is due to its unique virulence factors, which enhance its pathogenic potential and lead to various gastrointestinal diseases [3, 9]. The considerable impact of H. pylori-related illnesses highlights the pressing need for effective diagnostic and treatment strategies, especially given the rising antibiotic resistance observed in H. pylori strains, which complicates treatment efforts [10, 6].

This study explores the genetic variation of the NLRP3 rs4612666 gene and its connection to H. pylori infection. Previous studies have indicated a possible genetic predisposition associated with this polymorphism, which could affect an individual's likelihood of contracting the infection[6, 4]. Utilizing a case-control study design, this research seeks to clarify the relationship between different NLRP3 rs4612666 genotypes and the risk of H. pylori infection, thereby deepening our understanding of the genetic factors that contribute to the development of this common infection. The discussion will emphasize the implications of the findings in terms of genetic diversity, allele distribution, and their overall importance for future diagnostic and therapeutic strategies [2, 11].

The findings of this study provide important insights into the genetic factors linked to H. pylori infection, with a particular emphasis on the NLRP3 rs4612666 polymorphism. While the analysis of genotype distribution did not show statistically significant differences between the infected and non-infected groups, the examination of allele frequencies revealed a noteworthy association. Specifically, the C allele was found to be more prevalent in the infection group at 60.5%, compared to 49.0% in the non-infection group. This observation suggests that the C allele may contribute to an increased susceptibility to H. pylori infection, indicating the potential utility of genetic screening in diagnostic strategies for populations at risk. This association is consistent with previous research that has identified genetic variations influencing the host's response to H. pylori and its related health outcomes, underscoring the intricate interplay between genetic predispositions and environmental factors in the development of this disease [1, 3].

The absence of notable differences in genotype frequency suggests that other genetic factors or polymorphisms might play a role in susceptibility to H. pylori infection. This finding emphasizes the need for further research into additional genetic markers that could be significant across various populations. Such an approach is essential, considering the wide range of genetic backgrounds and environmental factors that can affect disease outcomes[12, 13]. Furthermore, these results point to the necessity for larger, multi-center studies to confirm these findings and investigate the potential for creating targeted therapeutic strategies based on genetic predispositions[14, 15].

5. Conclusion

This study highlights a significant link between the C allele of the NLRP3 rs4612666 polymorphism and H. pylori infection. However, it also underscores the necessity for further research to explore the complex genetic factors that contribute to this widespread infection. Gaining insights into these genetic elements could lead to personalized medicine strategies for preventing and treating diseases related to H. pylori, which would ultimately enhance patient outcomes [16, 17].

The limitations of this study are significant and deserve careful attention. First, the relatively small sample size may restrict the statistical power and generalizability of the findings, potentially masking the true relationships between NLRP3 rs4612666 polymorphisms and the risk of H. pylori infection. Additionally, the lack of clinical validation analyses limits our ability to establish a causal relationship between the genetic variant being studied and disease susceptibility. There is also the possibility of batch effects due to the use of multiple testing kits, which could introduce variability in the results and complicate data interpretation. Together, these limitations highlight the need for larger, multi-center studies that include diverse populations and robust experimental designs to validate and expand upon the findings presented here. In conclusion, this study offers strong evidence of a significant association between the allele distribution of NLRP3 rs4612666 and susceptibility to H. pylori infection. The higher frequency of allele C in the infection group suggests it may serve as a genetic marker for increased infection risk, which could inform diagnostic and therapeutic strategies. However, the identified limitations call for further research to confirm these findings and investigate additional genetic factors that might influence susceptibility to H. pylori infection, ultimately enhancing our understanding and management of this widespread public health issue.

Abbreviations

H. pylori	Helicobacter Pylori
NLRP3	NOD-like receptor Family Pyrin Domain Containing 3
BMI	Body Mass Index
qPCR	Quantitative Real-time Polymerase Chain Reaction

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Conflicts of Interest

The authors declare no conflicts of interest.

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