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# TNF-α -308 G/A Gene Polymorphism as a Risk Factor for Pulmonary Tuberculosis in Cirebon, Indonesia

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## ABSTRACT

**Background:** Mycobacterium tuberculosis, the causative agent of pulmonary tuberculosis, spreads via droplets. The TNF-α-308 G/A gene polymorphism is one of the host genetic variables that may affect an individual's vulnerability to the disease. However, this polymorphism has not been studied in Cirebon.
**Aims:** To analyze the TNF-α -308 G/A gene polymorphism as a risk factor for the occurrence of pulmonary tuberculosis in Cirebon.
**Methods:** A total of 64 participants joined part in an analytical observational study using a case-control design at the Biomolecular and Genetics Laboratory, Faculty of Medicine, Universitas Swadaya Gunung Jati, Indonesia. DNA extraction from blood samples, ARMS-PCR genotyping, and 1.5% electrophoresis gel visualization were all part of the data gathering process. The chi-square test was used to analyze the data. This study including inclusion criteria, exclusion criteria, and sample control for the research.
**Results:** According to the study, there is no link between Cirebon's risk of pulmonary tuberculosis and the polymorphism in the TNF-α-308 G/A gene (OR = 0.462; P > 0.05). However, the study shown a protective factors which means that individuals with the TNF-α -308 G/A gene polymorphism have a lower risk of developing pulmonary tuberculosis compared to those without the polymorphism.
**Conclusion:** The TNF-α-308 G/A gene variant is not associated with an increased risk of pulmonary tuberculosis in the Cirebon community.

**Keywords:** *TNF-α -308 G/A; Gene polymorphisms; Pulmonary tuberculosis; Tumor necrosis factor-alpha*
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# 1. Introduction

Pulmonary Tuberculosis is disseminated by Mycobacterium tuberculosis droplets. Among the top ten causes of infectious disease death. (Kementerian Kesehatan Republik Indonesia, 2020). Indonesia has the second-highest number of TB cases globally, after India, with 677.464 instances in 2022, according to the country's health profile. This represents 13% of all new cases worldwide. (Kementerian Kesehatan Republik Indonesia, 2022). West Java recorded 66.756 cases with 6.289 cases in kabupaten Cirebon (P2PM Kabupaten Cirebon, 2022). Mycobacterium tuberculosis predominantly infects newly formed pulmonary parenchyma, resulting in pulmonary tuberculosis. Nonetheless, this pathogen exhibits the capacity to invade extrapulmonary sites, including the pleura, lymphatic system, and bone tissue. (Kementerian Kesehatan Republik Indonesia, 2020)

Genetic predisposition and other factors affect pulmonary TB susceptibility. The TNF-α gene, especially the TNF-α -308 G/A polymorphism significant genetic component (Adane et al., 2021). Multiple SNP variants are observed in the promoter region of the TNF-α gene, with the G/A allele position being the most frequently associated with key genetic polymorphisms. These polymorphisms influence pro-inflammatory mediator levels and are linked to the progression of infectious diseases, such as tuberculosis. (Wang et al., 2022)

Research in Ethiopia shown that TNF-α cytokine contributes to Mycobacterium tuberculosis infection by inducing nitrogen and reactive oxygen intermediates through macrophage and chemokine activity, with the -308 G/A polymorphism linked to inflammatory mediation (Adane et al., 2021). (Yinke et al., 2021) found that the A allel increased TB risk relative to the G allel (Yinke et al., 2021). Based on previous research, the current study aims to analyze the genotype and allele frequencies of the Tumor Necrosis Factor-Alpha -308 G/A gene as risk factors for pulmonary tuberculosis in Cirebon Regency. It also seeks to explore the relationship between this gene and the risk of developing the disease in the region.

# 2. Methods

## *Study design/ Research procedures*

For this study, the Department of Genetics, Faculty of Medicine, Universitas Swadaya Gunung Jati, Cirebon, Indonesia, conducted a case-control analytical observational research with following details:

### a. Materials

Blood samples were genotyped using ARMS-PCR and visualized on a 1.5% electrophoresis gel. Those who fit the requirements were questioned, given permission, and taken 3 cc of blood in EDTA tubes for DNA extraction. This research relied on questionnaires and interviews.

### b. Sample Collection

A total of 64 participants in the all participated in this study, with 32 in case group and 32 control groups. Purposive sampling is the method used for sampling. Patients with a history of tuberculosis treatment (new cases, relapse cases, dropout cases, and treatment failure cases) who have recently received a diagnosis or are presently receiving treatment, patients with drug-sensitive pulmonary tuberculosis cases, and patients with a bacteriologically confirmed diagnosis of pulmonary tuberculosis are all eligible to participate. Patients with comorbid diseases like diabetes and HIV/AIDS, those who

drink alcohol, those receiving long-term immunosuppressive treatment (such as steroids, cyclosporine, and calcium inhibitors), and those who refuse study participation are all excluded from this study. Participants in the control group must be at least 18 years old, have no history of pulmonary tuberculosis diagnosis, show no signs of the disease as determined by a screening form, and have never had intimate contact with someone who has been diagnosed with the disease.

**c. Instruments**

Through genetic analysis, the Amplification-Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) technique can be used to identify the Tumor Necrosis Factor-Alpha-308 G/A gene polymorphism. The polymorphism in the TNF-α-308 G/A gene was amplified using: primer forward G : 5'-ATAGGTTTTGAGGGGCATGG-3', primer forward A : 5'-AATAGGTTTTGAGGGGCATGA-3', and primer reverse : 5'-TCTCGGTTTCTTCTCCATCG-3'. 25 µL was used for the PCR reaction, including 2µL genomic DNA, 1µL of each primers , 7.5µL nuclease-free water , and 12.5µL 2x PCR Mater Mix. The amplification process involved five minutes of denaturation at 95°C, one minute of annealing at 52°C, and one minute of extension at 68°C to 72°C. This cycle repeated twenty-five times.

### *Statistical techniques*

A textual table is used to display the research findings. Chi-square was utilized in both univariate and bivariate statistical analysis. The study explored the link between the TNF-α -308 G/A TB history with a significance level of (0.05).

### *Ethical Clearance*

This research was conducted in accordance with the research procedure, following the approval of the research permit which was obtained after passing thesis proposal examination and ethical review by Faculty of Medicine, Universitas Swadaya Gunung Jati Ethics Committee, with the number No.90/EC/FKUGJ/VI/2024.

## 3. Results

The study included 64 participants in total. A univariate statistical analysis was used to analyze the distribution of allele's frequencies, genotype, and polymorphisms, as shown in Table 1. The TNF- α -308 G/A gene polymorphism frequency distribution in cases group had 49 A alleles (77%) and 15 G alleles (23%). The control group had 34 G and 42 A alleles (34% and 66%, respectively).

**Table 1**. Allele Frequency Distribution

| **Allele** | **Cases** | | **Control** | |
|---|---|---|---|---|
| | F | % | F | % |
| **A** | 49 | 77% | 42 | 66% |
| **G** | 15 | 23% | 22 | 34% |
| **Total** | 64 | 100% | 64 | 100% |

Table 2 displays the resulting bivariate analysis. In the case group, 15 subjects (47%) possessed the polymorphism, whereas 17 subjects (53%) did not. Eleven subjects (34%) in the control group lacked the polymorphism, while 21 subjects (66%) did. This suggests that, in comparison to the control group, a larger percentage of those in the case group lacked the polymorphism.

**Table 2**. Genotypic Frequency Distribution

| No | Group | Genotype | N | Percent |
|---|---|---|---|---|
| 1 | Cases | AA | 17 | 53.1% |
| | | GA | 15 | 46.9% |
| | | GG | 0 | 0% |
| | | Total | 32 | 100% |
| 2 | Control | AA | 11 | 34.4% |
| | | GA | 20 | 62.5% |
| | | GG | 1 | 3.1% |
| | | Total | 32 | 100% |

The statistical analysis revealed an odds ratio of 0.462 (as shown in Table 3), indicating that the TNF-α -308 G/A polymorphism does not constitute a significant risk factor for pulmonary tuberculosis in the Cirebon population. Moreover, this polymorphism confers a 54% greater protective effect compared to individuals without the polymorphism, as reflected by the 95% confidence interval (CI) of 0.169-1.265. Figure 1 shows the genotype frequency TNF-α -308 G/A by ARMS-PCR analysis: GG homozygous mutant (±161 bp), heterozygous GA (±186 bp), and wild-type AA (±184 bp) genotypes.

**Table 3**. Association of Polymorphism with pulmonary tuberculosis

| Polymorphisms | Cases | | Control | | p | OR | CI (95%) |
|---|---|---|---|---|---|---|---|
| | F | % | F | % | | | |
| Yes (GA+GG) | 15 | 47 | 21 | 66 | 0.131 | 0.462 | 0.169-1.265 |
| No (AA) | 17 | 53 | 11 | 34 | | | |
| Total | 32 | 100 | 32 | 100 | | | |

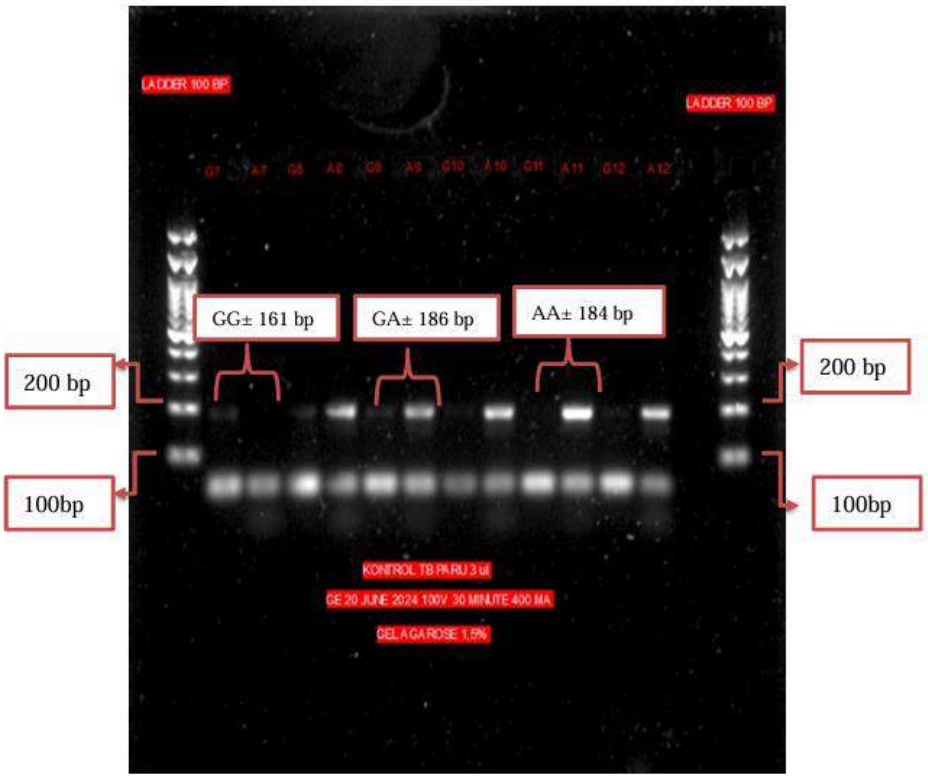


**Figure 1**. Visualization of 1,5% agarose gel of TNF-α -308 gene polymorphisms ARMS-PCR product,

result (GG: ±161 bp; GA: ±186 bp; AA: ± 184 bp), DNA Marker 100 bp.

## 4. Discussion

Mycobacterium tuberculosis causes chronic pulmonary tuberculosis (Kementerian Kesehatan Republik Indonesia, 2020). The disease's risk is influenced by various variables, including genetics. Important genetic factors include the TNF-α -308 G/A gene (Adane et al., 2021). The MHC class III region houses the TNF-α gene, which creates cytokine. TNF-α is essential for development (Xia et al., 2022). A study by (Yinke et al., 2021) found that TNF-α is essential for granuloma development by recruiting immune cells and enhancing macrophage regulation.

The TNF-α -308 G/A is located in the MHC Class III gene on chromosome 6p21 encodes TNF-α, an anti-inflammatory cytokine that activates immunological responses (Wang et al., 2022). The 26 kDa transmembrane precursor of TNF-α is synthesised (mTNF-α), which is subsequently processed by TNF-α converting enzyme (TACE), a metalloproteinase (Xia et al., 2022). The enzyme cleavage generates a soluble form ( sTNF-α) which is released into circulation, enhancing endocrine activity across various tissues (Xia et al., 2022). The membrane-bound TNF-α intracts with two specific receptors, TNFR1 and TNFR2, initating pro-inflammatory responses critical to immune regulation (Varljen et al., 2019).

This research found that 15 case group participants (47%) had GA and GG genotype polymorphisms, indicated by restriction bands of approximately 186 base pairs 161 (bp) and 161 base pairs(bp), while 17 subjects (53%) did not have polymorphisms, shown by the appearance of the AA (wild-type homozygous) genotype with a restriction band of approximately 184 base pairs (bp). According to the statistical test, the TNF-α -308 G/A gene polymorphism is not a significant risk factor for pulmonary tuberculosis in Cirebon, as evidenced by the odds ratio of 0.462. As opposed to those who do not have the TNF-α -308 G/A gene polymorphism, it offers a 54% protective factor, with a 95% CI of 0.169–1.265. In this study, a protective factor means that individuals with the TNF-α -308 G/A gene polymorphism have a lower risk of developing pulmonary tuberculosis compared to those without the polymorphism.

The TNF-α -308 G/A gene polymorphism was not significantly correlated with prevalence pulmonary tuberculosis in the Cirebon Population, according the study's findings ($p>0.05$). However, it is possible that this polymorphism contributes to pulmonary tuberculosis as a result of population variations and other factors. Both the genetic component and the incidence of pulmonary tuberculosis affect the distribution of gender, age, smoking, and ethnicity. The differences in results compared to previous studies are due to variations in ethnic groups within the populations studied. Certain genes remain within specific ethnic groups and circulate within those groups. People within an ethnic group share particular versions of their genes, inherited from common ancestors. This explains the genetic differences among ethnic groups, which may account for the differing results found in this study compared to others.

This is supported by a meta-analysis conducted by Qin Wang et al., which concluded that the TNF-α-308 gene polymorphism is not associated with tuberculosis risk in the overall population. However, there is a significant risk associated with the A allele of the TNF-α-308 gene in Asian populations.

Previous studies on TNF-α-308 G/A gene polymorphism and pulmonary tuberculosis incidence in Indian population yielded insignificant by (Joshi et al., 2018) evaluated 490 participants, including 150 APTB, 190 HCC, and 150 HC with p-value >0.05. A study by (Varahram et al., 2014) found no significant TNF-α -308 G/A gene this TB risk in Iranian population ($p>0.05$). In contrast, (Torres et al., 2018) found a strong correlation TNF-α -308 G/A gene polymorphism, TB in Mexican population. The research found p-value of 0.04 ($p<0.05$), an OR of 0.16, and a 95% CI of 0.01 to 0.92.

## 5. Conclusion

In Cirebon populations, The TNF- α -308 G/A polymorphism does not substantially affect pulmonary TB (p= 0.131 OR=0.462); however, among those who do not have the gene polymorphism, it offers a 54% protective factor, with a 95% CI of 0.169–1.265. Although the TNF-α -308 G/A polymorphism in this study shows potential as a protective factor against pulmonary tuberculosis, further studies with a larger sample size are needed to confirm the association statistically. This study does not assess other risk factors or genetic variants that may affect to pulmonary tuberculosis therapeutic responses. For future research, it is suggested to investigate the impact of the Tumor Necrosis Factor-Alpha -308 G/A gene polymorphism on the treatment of pulmonary tuberculosis patients.

### Conflict of Interest

There is no conflict of interest. Nothing to disclosure.

### References


Adane, G., Lemma, M., Geremew, D., Sisay, T., Tessema, M. K., Damtie, D., & Ayelign, B. (2021). *Genetic Polymorphism of Tumor Necrosis Factor-Alpha, Interferon-Gamma and Interleukin-10 and Association With Risk of Mycobacterium Tuberculosis Infection*. *26*, 1–7. https://doi.org/10.1177/2515690X211006344

Joshi, L., Kiran, L., Valluri, V., & Gaddam, S. (2018). Comparative Immunology , Microbiology and Infectious Diseases Association of TNF- α , IL-10 and IL-6 promoter polymorphisms in pulmonary tuberculosis patients and their household contacts of younger age group. *Comparative Immunology, Microbiology and Infectious Diseases*, *56*(December 2017), 20–26. https://doi.org/10.1016/j.cimid.2017.12.001

Kementerian Kesehatan Republik Indonesia. (2020). *PEDOMAN NASIONAL PELAYANAN KEDOKTERAN TATALAKSANA TUBERKULOSIS*. Kementerian Kesehatan Republik Indonesia.

Kementerian Kesehatan Republik Indonesia. (2022). *Profil kesehatan indonesia 2022*.

P2PM Kabupaten Cirebon. (2022). *Laporan Tahunan Seksi Pencegahan dan Pengendalian Penyakit Menular Bidang Pengendalian dan Pemberantasan Penyakit Tahun 2022*.

Torres, I., Arellano, R. De, Lara, C. S., María, L., Espíndola, T., Jesús, M. De, López, C., Prado, A. J., Cruz, R. V., Camacho, J. L. G., Patiño, N., Bojórquez, J. D. R., Lilia, M., & Suck, T. (2018). *Basic research Exposure to biomass smoke , cigarettes , and alcohol modifies the association between tumour necrosis and tuberculosis in Mexican carriers*.

Varahram, M., Farnia, P., Nasiri, M. J., Karahrudi, M. A., & Kazempour, M. (2014). *Association of Mycobacterium Tuberculosis Lineages with IFN- γ and TNF - α Gene Polymorphisms among Pulmonary Tuberculosis Patient*. https://doi.org/10.4084/MJHID.2014.015

Varljen, T., Rakic, O., Sekulovic, G., Jekic, B., Maksimovic, N., Janevski, M. R., & Novakovic, I. (2019). *Association between Tumor Necrosis Factor- α Promoter -308 G / A Polymorphism and Early Onset Sepsis in Preterm Infants*. 259–264. https://doi.org/10.1620/tjem.247.259

Wang, Z., Kong, L., Zhang, H., Sun, F., Guo, Z., & Zhang, R. (2022). *Tumor Necrosis Factor Alpha -308G / A Gene Polymorphisms Combined with Neutrophil-to-Lymphocyte and Platelet-to-Lymphocyte Ratio Predicts the Ef fi cacy and Safety of Anti-TNF- α Therapy in Patients with Ankylosing Spondylitis , Rheumatoid Arthritis , and*. *12*(January), 1–11. https://doi.org/10.3389/fphar.2021.811719

Xia, Z., Wang, Y., Liu, F., Shu, H., & Huang, P. (2022). *Association Between TNF- α -308 , Susceptibility : An Updated Meta-Analysis and Trial Sequential Analysis*. *12*(January). https://doi.org/10.3389/fgene.2021.772032

Yinke, B., Sinaga, M., & Amir, Z. (2021). *Tumor Necrosis Factor-alpha -308G / A Polymorphism Associated with Increased Risk for Pulmonary Tuberculosis in Medan City , Indonesia*. *9*, 7–11.



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