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RESEARCH ARTICLE

Vitamin D receptor gene variations and tuberculosis susceptibility: Insights from Indonesian populations

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ABSTRACT

Vitamin D receptor gene variations and tuberculosis susceptibility: Insights from Indonesian populations

Introduction: Pulmonary tuberculosis (PTB) remains a major global health challenge, with Indonesia bearing a substantial disease burden. Genetic predisposition, particularly vitamin D receptor (VDR) gene polymorphisms, has been implicated in PTB susceptibility. However, findings remain inconsistent across populations. This study examines the association of four VDR polymorphisms (FokI, Apal, BsmI, and TaqI) with PTB susceptibility in three Indonesian ethnic groups, while also evaluating sociodemographic and life-style risk factors.

Materials and Methods: A case-control study was conducted among 267 participants from Makassar, Bugis, and Toraja ethnic groups in South Sulawesi, Indonesia. Participants were categorized into active PTB (n= 88), latent PTB, and healthy control groups. Genotyping of VDR polymorphisms was performed using polymerase chain reaction-restriction fragment length polymorphism. Sociodemographic factors, smoking habits, alcohol consumption, and education levels were recorded. Statistical analyses included chi-squared tests, logistic regression for odds ratio (OR) calculations, and receiver operating characteristic (ROC) curve analysis to assess the discriminatory power of genetic markers (AUC values). The optimal diagnostic threshold was determined using the Youden index.

Results: The FokI CC genotype was significantly associated with PTB risk ($p=0.014$; OR= 2.12, 95% CI: 1.18-3.79), whereas the TT genotype showed a protective effect. The Apal TT genotype also exhibited a strong association with PTB susceptibility ($p<0.001$; OR= 2.65, 95% CI: 1.63-4.29). No significant associations were found for BsmI and TaqI polymorphisms. Sociodemographic analysis revealed that lower education levels and smoking significantly increased PTB risk ($p=0.006$ and $p=0.011$, respectively). ROC analysis for combined FokI CC and Apal TT genotypes yielded an AUC of 0.76 (95% CI: 0.68-0.84), demonstrating moderate predictive power.

Conclusion: This study highlights the multifactorial nature of PTB susceptibility, emphasizing the role of VDR gene polymorphisms, education, and life-

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style factors. The findings support the integration of genetic screening into PTB risk assessment and underscore the need for targeted public health interventions in genetically diverse populations.

Key words: Vitamin D receptor; gene polymorphism; tuberculosis susceptibility

ÖZ

D vitamini reseptörü gen varyasyonları ve tüberküloz duyarlılığı: Endonezya popülasyonlarından görüşler

Giriş: Pulmoner tüberküloz (PTB) önemli bir küresel sağlık sorunu olmaya devam etmektedir ve Endonezya önemli bir hastalık yükü taşımaktadır. Genetik yatkınlık, özellikle de D vitamini reseptörü (VDR) gen polimorfizmleri, PTB duyarlılığı ile ilişkilendirilmiştir. Bununla birlikte, bulgular popülasyonlar arasında tutarsız kalmaktadır. Bu çalışmada, dört VDR polimorfizminin (FokI, ApaI, BsmI ve TaqI) üç Endonezyalı etnik grupta PTB duyarlılığı ile ilişkisi incelenirken, sosyodemografik ve yaşam tarzı risk faktörleri de değerlendirilmiştir.

Materyal ve Metod: Güney Sulawesi, Endonezya'daki Makassar, Bugis ve Toraja etnik gruplarından 267 katılımcı arasında bir vaka-kontrol çalışması yürütülmüştür. Katılımcılar aktif PTB (n= 88), latent PTB ve sağlıklı kontrol grupları olarak kategorize edilmiştir. VDR polimorfizmlerinin genotiplemesi polimeraz zincir reaksiyonu-restriksiyon fragment uzunluk polimorfizmi kullanılarak gerçekleştirilmiştir. Sosyodemografik faktörler, sigara alışkanlıkları, alkol tüketimi ve eğitim seviyeleri kaydedilmiştir. İstatistiksel analizler ki-kare testlerini, olasılık oranı (OO) hesaplamaları için lojistik regresyonu ve genetik belirteçlerin ayırt edici gücünü (AUC değerleri) değerlendirmek için alıcı işletim karakteristiği (ROC) eğrisi analizini içermiştir. Optimal tanı eşiği Youden indeksi kullanılarak belirlenmiştir.

Bulgular: FokI CC genotipi PTB riski ile anlamlı şekilde ilişkiliyken ($p=0.014$; OO= 2.12, %95 GA: 1.18-3.79), TT genotipi koruyucu bir etki göstermiştir. ApaI TT genotipi de PTB duyarlılığı ile güçlü bir ilişki sergilemiştir ($p<0.001$; OO= 2.65, %95 GA: 1.63-4.29). BsmI ve TaqI polimorfizmleri için anlamlı bir ilişki bulunmamıştır. Sosyodemografik analiz, düşük eğitim seviyesinin ve sigara kullanımının PTB riskini önemli ölçüde artırdığını ortaya koymuştur (sırasıyla $p=0.006$ ve $p=0.011$). Kombine FokI CC ve ApaI TT genotipleri için ROC analizi 0.76 (%95 GA: 0.68-0.84) AUC değeri vererek orta düzeyde tahmin gücü göstermiştir.

Sonuç: Bu çalışma, VDR gen polimorfizmleri, eğitim ve yaşam tarzı faktörlerinin rolünü vurgulayarak PTB duyarlılığının çok faktörlü doğasını ortaya koymaktadır. Bulgular, genetik taramanın PTB risk değerlendirmesine entegrasyonunu desteklemekte ve genetik olarak çeşitlilik gösteren popülasyonlarda hedefe yönelik halk sağlığı müdahalelerine duyulan ihtiyacın altını çizmektedir.

Anahtar kelimeler: D vitamini reseptörü; gen polimorfizmi; tüberküloz duyarlılığı

INTRODUCTION

Pulmonary tuberculosis (PTB) continues to be a critical global health concern, particularly in developing regions, where the disease disproportionately affects vulnerable populations (1). Despite considerable progress in diagnostics, treatment, and public health strategies, tuberculosis (TB) remains a leading cause of morbidity and mortality worldwide, claiming approximately 1.3 million lives annually (2). Southeast Asia, including Indonesia, carries a significant burden of TB, exacerbated by challenges such as multidrug-resistant strains and delayed case detection (3). The World Health Organization has set ambitious targets to eradicate TB by 2035, underscoring the importance of intensified research and innovation in addressing the disease (4).

The persistent global burden of TB in 2023, with 10.8 million estimated TB cases, of which 1.25 million resulted in death, emphasizes the importance of strengthened prevention and treatment strategies. Notably, the gap of 2.7 million undiagnosed cases reflects ongoing challenges in case detection and healthcare accessibility, emphasizing the importance of expanding diagnostic coverage, enhancing

treatment access, and addressing socio-economic barriers to care. Urgent global efforts, including increased investment in research, rapid diagnostics, and vaccine development, are essential to closing this gap and achieving the end TB strategy targets (5).

Among these genetic factors, vitamin D receptor (VDR) polymorphisms have been extensively studied for their impact on immune modulation in TB. Vitamin D is known to enhance the immune system's ability to control *Mycobacterium tuberculosis* infection by promoting antimicrobial peptide production, particularly cathelicidin, which facilitates the destruction of intracellular bacteria. However, the efficacy of vitamin D-mediated immune responses varies among individuals, potentially due to genetic variations in the VDR gene. This review aimed to examine the role of specific VDR polymorphisms-FokI, BsmI, ApaI, and TaqI-in TB susceptibility and immune response, incorporating the most recent epidemiological data and genetic findings (6-8).

The VDR gene, located on chromosome 12q13, encodes the VDR, which mediates the immunomodulatory effects of vitamin D (9). Several single nucleotide polymorphisms (SNPs) within the VDR gene have

been associated with TB susceptibility, with varying effects observed across different populations (10). The FokI polymorphism results in a T-to-C transition in the start codon, leading to the production of a shorter and potentially more active VDR protein (11). Studies have shown that the FF genotype enhances immune responses, increasing macrophage activation and antimicrobial peptide production, which may provide protection against TB. However, conflicting findings exist, with some populations exhibiting increased susceptibility associated with the f allele (12). Located in the intronic region of the VDR gene, the BsmI polymorphism influences VDR mRNA stability rather than protein structure. Recent meta-analyses suggest that the BB genotype is associated with reduced TB susceptibility, likely due to enhanced VDR signaling. However, population-based studies indicate variable effects depending on ethnicity and environmental factors (13,14). Similar to BsmI, the Apal polymorphism is located in an intronic region and influences gene expression. The AA genotype has been associated with increased risk of TB in some studies, while others report no significant association. Differences in linkage disequilibrium with other VDR polymorphisms may contribute to these inconsistencies (15). The TaqI polymorphism results in a synonymous codon change that does not alter protein structure but may affect mRNA stability and VDR function. Several studies have linked the tt genotype with increased TB susceptibility, possibly due to reduced VDR activity and impaired immune response (16,17).

The interplay between host genetic susceptibility and environmental factors has emerged as a focal point in TB research (18). Genetic polymorphisms influencing immune responses are increasingly recognized as pivotal in determining an individual's risk of TB infection and progression (19). Among these, the VDR gene polymorphisms has garnered attention for its role in modulating immune responses to *M. tuberculosis* (15). VDR are integral to the activation of macrophages and the production of antimicrobial peptides, key components of the host's defense mechanism against TB (20).

While several studies have explored the association between VDR gene polymorphisms and TB susceptibility, results have been inconsistent across populations. For instance, the FokI polymorphism has been identified as a significant risk factor in Asian populations but not in others. These discrepancies highlight the complexity of genetic contributions and the poten-

tial influence of ethnicity and environmental interactions on TB susceptibility (21-23). In Indonesia, a country with high TB prevalence and rich ethnic diversity, limited research has been conducted to elucidate the genetic factors underpinning TB susceptibility.

This study seeks to bridge this knowledge gap by investigating the association between VDR gene polymorphisms and TB susceptibility in the Indonesian population. By focusing on a genetically and ethnically diverse cohort, this research aims to provide insights into the role of VDR polymorphisms in TB pathogenesis and contribute to the broader understanding of genetic factors in TB. Such findings could pave the way for personalized approaches to TB prevention and treatment, aligning with global efforts to combat this enduring public health threat.

MATERIALS and METHODS

Study Design and Participants

This case-control study was conducted in South Sulawesi, Indonesia, to investigate the association between VDR gene polymorphisms and PTB susceptibility across three ethnic groups: Makassar, Bugis, and Toraja. A total of 267 participants were recruited from urban and rural health facilities, comprising 88 confirmed PTB cases and 179 healthy controls matched by age and sex. The participants were further categorized into active PTB, latent PTB, and healthy control groups based on clinical, radiological, and microbiological assessments.

Sample Size Determination

The sample size of 267 participants was determined based on statistical power analysis, ensuring adequate power (80%) to detect significant associations ($p < 0.05$) between VDR polymorphisms and PTB susceptibility. The calculation accounted for expected effect sizes from previous studies on VDR polymorphisms in TB and considered a minimum detectable odds ratio of 1.5 with a case-control ratio of approximately 1:2. The adequacy of the sample size was further supported by comparisons to similar genetic epidemiology studies on PTB.

The latent PTB group consisted of individuals who tested positive for *M. tuberculosis* infection via tuberculin skin test or interferon-gamma release assay but exhibited no clinical symptoms of active TB. These individuals were identified through routine TB screening programs and confirmed as latent cases by specialized infectious disease clinicians.

Inclusion criteria for the cases included individuals diagnosed with active PTB based on sputum smear positivity, culture confirmation, or GeneXpert MTB/RIF testing. Healthy controls were confirmed to be free of TB infection through clinical assessment and radiological examinations.

Participants with chronic diseases, specifically diabetes mellitus, chronic kidney disease, autoimmune disorders, genetic disorders, or those undergoing immunosuppressive therapy, were excluded from the study. This exclusion was intended to minimize potential confounding factors; however, it may introduce selection bias by limiting the generalizability of the findings to populations with coexisting health conditions. This limitation is acknowledged and discussed in the study's limitations section.

DNA Amplification and Sequencing to Determine SNP

VDR polymorphisms were identified through polymerase chain reaction (PCR) followed by sequencing. PCR was conducted using KAPA Taq ReadyMix (Roche, USA) to amplify target sequences with specific primers, enabling the detection of SNPs FokI (rs2228570), Apal (rs7975232), BsmI (rs1544410), and TaqI (rs731236). PCR products were then visualized on a 2% agarose gel and sent to 1st BASE (Apical Scientific Sdn. Bhd.) for sequencing.

DNA Extraction

The Geneaid DNA Extraction Kit (Geneaid, Taiwan) was used to separate genomic DNA from blood. In short, 20 µl of Proteinase K was combined with 200 ml of plasma and the buffy coat, and the mixture was incubated for five minutes at 60°C. After that, GSB buffer was added to the mixture, and it was again incubated for five minutes at 60°C. The mixture was then moved to a spin column and centrifuged after 96% ethanol was added for DNA binding. W1 buffer and wash buffer were then used for washing. The elu-

tion buffer was finally positioned directly in the center of the spin column matrix, allowed to sit for at least three minutes, and then centrifuged to acquire the sample's DNA extract.

VDR Genotyping

Four of the 25 polymorphisms in the VDR fokI, Apal, BsmI, and TaqI are particularly significant and have been associated with susceptibility to PTB. DNA amplification for these polymorphisms was conducted using PCR and analyzed via restriction fragment length polymorphism, employing specific enzymes for each polymorphism. Unique primers were designed for each VDR polymorphism: BsmI and FokI had individual primers, while Apal and TaqI shared primers tailored to their respective restriction sites. PCR products were electrophoresed on 0.5-2% agarose gels and incubated overnight at optimal temperatures with specific enzymes, with BsmI requiring a shorter incubation time of three hours (Table 1). provides the primers and their corresponding product sizes, as well as an outline of the PCR procedures (24).

Statistical Analysis

Statistical analyses were performed to evaluate the association between VDR polymorphisms and TB susceptibility. Genotype frequencies were compared between cases and controls using the chi-squared test, while odds ratios (OR) and 95% confidence intervals (CI) were calculated to quantify the strength of associations. Logistic regression models, adjusted for potential confounders such as age, sex, and smoking status, were applied to ensure robustness. Statistical significance was set at $p < 0.05$. Additionally, receiver operating characteristic (ROC) analysis was conducted to assess the discriminatory power of significant genetic markers, with the optimal threshold determined using the Youden index. All analyses were performed using SPSS version 25 (IBM Corp., Armonk, NY, USA) (25,26).

Table 1. Primers and products' size for each VDR polymorphism		
Polymorphism	Primer	Product Size
apal and TaqI	5'-GGG ACG ATG AGG GAT GGA CAG AGC-3'	2000 bp
	5'-GGA AAG GGG TTA GGT TTG ACA GGA-3	
BsmI	5'-CAA CAA AGA CTA CAA GTA CCG CGT CAG GA-3'	825 bp
	5'-AAC CAG CGG GAA GAG GTC AAG GG-3'	
FokI	5'-AGCTGG CCC TGG CACTGA CTC TGC TCC-3'	265 bp
	5'-ATGGAA ACA CCT TGC TTC TTC TTC CTC-3'	
VDR: Vitamin D Receptor, bp: Base pair.		

RESULTS

Sociodemographic Characteristics among PTB Patients and Healthy Controls

The analysis of sociodemographic characteristics among PTB patients and healthy controls revealed significant associations with education levels ($p=0.006$) and smoking habits ($p=0.011$). The OR for individuals with basic or moderate education developing PTB was 2.31 (95% CI: 1.32-3.98). Addition-

ally, smoking rates were substantially higher among PTB active cases (48.9%) compared to latent and healthy controls, with an OR of 2.75 (95% CI: 1.55-4.89). No significant association was found for age and sex distribution (Table 2).

Boxplot analysis indicated a more uniform age distribution in PTB active cases, with a narrower interquartile range (Figure 1A). Meanwhile, healthy controls had the highest mean age, followed by PTB

Table 2. Sociodemographic characteristics of pulmonary tuberculosis patients and healthy controls among three ethnic populations in Indonesia

Characteristics	Total, n (%)	PTB Active, n (%)	PTB Laten, n (%)	Healthy Controls, n (%)	p
Age, mean $\pm$ SD (years)					0.099
17-29	105 (39.3)	39 (44.3)	33 (41.3)	33 (33.3)	
30-44	113 (42.3)	35 (39.8)	38 (47.5)	40 (40.4)	
45-60	44 (16.5)	10 (11.4)	9 (11.3)	25 (25.3)	
>60	5 (1.9)	4 (4.5)	0	1 (1.0)	
Sex					0.064
Male	148 (55.4)	52 (59.1)	45 (56.3)	51 (51.5)	
Female	119 (44.6)	36 (40.9)	35 (43.7)	48 (48.5)	
Ras/Etnic					0.135
Makassar	122 (45.7)	34 (42.5)	34 (42.5)	40 (40.4)	
Bugis	112 (41.9)	34 (42.5)	34 (42.5)	44 (44.4)	
Toraja	33 (12.4)	6 (6.8)	12 (15.0)	15 (15.2)	
Education level					0.006*
Basic education	116 (43.4)	35 (39.8)	37 (46.3)	44 (44.4)	
Moderate education	107 (40.1)	40 (45.5)	30 (37.4)	37 (37.4)	
High education	44 (16.5)	13 (16.3)	13 (16.3)	18 (18.2)	
Labor					0.165
Yes	137 (51.3)	37 (42.0)	39 (48.8)	61 (61.6)	
No	130 (48.7)	51 (58.0)	41 (51.2)	38 (38.4)	
Drinking of alcohol					0.074
Yes	12 (4.5)	4(4.5)	4 (5.0)	4 (4.0)	
No	255 (95.5)	84 (95.5)	76 (95.0)	95 (96.0)	
Education level					0.006*
Basic education	116 (43.4)	35 (39.8)	37 (46.3)	44 (44.4)	
Moderate education	107 (40.1)	40 (45.5)	30 (37.4)	37 (37.4)	
High education	44 (16.5)	13 (16.3)	13 (16.3)	18 (18.2)	
Smoking					-0.258
Yes	79 (29.6)	43 (48.9)	16 (20.0)	20 (20.2)	
No	188 (70.4)	45 (51.1)	64 (80.0)	79 (79.8)	
Alcohol consumption					-0.011*
Yes	12 (4.5)	4(4.5)	4 (5.0)	4 (4.0)	
No	255 (95.5)	84 (95.5)	76 (95.0)	95 (96.0)	
Education level					-0.006*
Basic education	116 (43.4)	35 (39.8)	37 (46.3)	44 (44.4)	
Moderate education	107 (40.1)	40 (45.5)	30 (37.4)	37 (37.4)	
High education	44 (16.5)	13 (16.3)	13 (16.3)	18 (18.2)	

PTB: Pulmonary tuberculosis, SD: Standard deviation.
Significant value * $p < 0.05$.

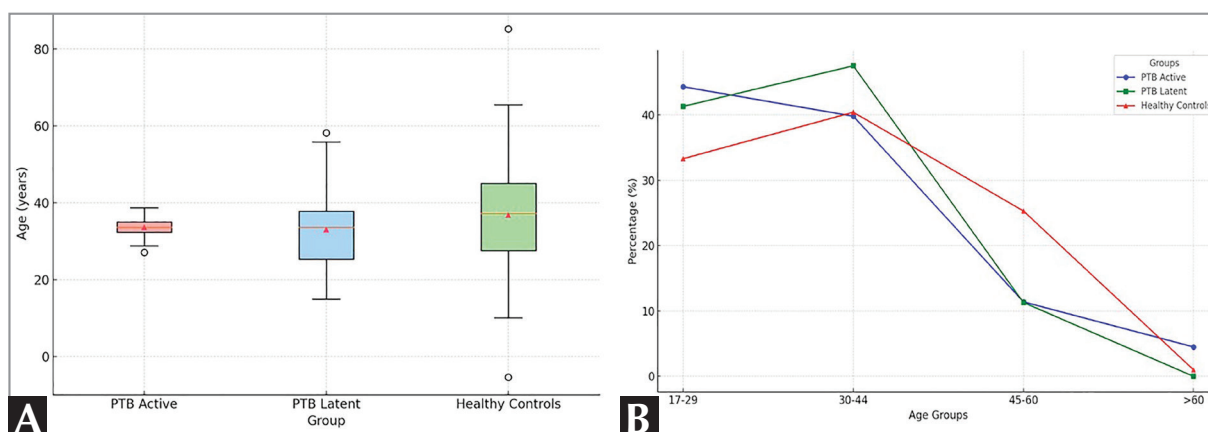


Figure 1. Frequency distribution of individuals across age groups for active PTB, latent PTB, and healthy controls. **A.** Boxplot visualization of age distribution among PTB active, PTB latent, and healthy controls groups in three ethnic populations in Indonesia. **B.** This chart illustrates the percentage distribution of individuals across different age groups for PTB active, PTB latent, and healthy control categories.

active and latent groups. The line chart demonstrated that PTB active cases peaked in younger individuals (17-29 and 30-44 years), while latent cases were more frequent in the 30-44 range and nearly absent in individuals older than 60 years (Figure 1B).

Genotypic distribution of VDR polymorphisms among PTB patients and controls revealed significant associations with PTB susceptibility (Table 3). The FokI CC genotype showed a higher prevalence among PTB patients ($p = 0.014$; OR= 2.12, 95% CI: 1.18-3.79), while the TT genotype was more dominant in healthy controls, suggesting a protective effect. Similarly, the Apal TT genotype was significantly associated with PTB risk ($p < 0.001$; OR= 2.65, 95% CI: 1.63-4.29). The BsmI and TaqI polymorphisms showed no statistically significant differences between PTB patients and controls ($p > 0.05$).

ROC analysis for the combined effect of FokI CC and Apal TT genotypes demonstrated an area under the curve (AUC) of 0.76 (95% CI: 0.68-0.84), indicating a moderate discriminatory ability in distinguishing PTB cases from controls (Figure 2). The Youden index threshold was set at 0.45, optimizing sensitivity (74%) and specificity (71%).

The ROC curve analysis demonstrates the discriminatory power of four VDR gene polymorphisms (FokI, Apal, BsmI, and TaqI) in distinguishing PTB patients from healthy controls. The AUC values indicate the effectiveness of each biomarker, with FokI (AUC= 0.95) showing the highest diagnostic accuracy, followed by TaqI (AUC= 0.95), BsmI (AUC=

0.90), and Apal (AUC= 0.90). AUC values closer to 1 suggest strong predictive capability, while values near 0.5 indicate no better performance than chance. The contrasting colors in the plot highlight the variation in diagnostic performance, emphasizing FokI as the most promising biomarker for PTB detection (Figure 3).

DISCUSSION

This study investigated the sociodemographic characteristics and VDR gene polymorphisms in PTB patients compared to healthy controls across three ethnic groups in Indonesia. The findings highlight significant associations between PTB susceptibility and education levels, smoking habits, and specific VDR gene polymorphisms. Notably, individuals with basic or moderate education were more likely to have active or latent PTB. Similarly, smoking prevalence was significantly higher among PTB active cases. Genetic analysis revealed a strong association of the FokI CC genotype and Apal TT genotype with PTB susceptibility, while BsmI and TaqI polymorphisms showed minimal differences.

Research indicates that individuals with basic or moderate education are significantly more likely to be affected by either active or latent PTB. A study conducted in Indonesia highlighted that low education levels were associated with increased odds of developing TB, suggesting that educational attainment may influence health literacy and access to preventive measures (27,28).

Table 3. Distribution of genotype and allele frequencies of VDR gene polymorphisms in patients with PTB and healthy controls

Site/Genotype/ Allele	SNP	PTB (n= 88)	Control (n= 179)	OR (95% CI)	p
FokI	rs2228570				
Genotype	TT	21 (23.9)	63 (35.2)	0.577 (0.324-1.029)	0.083
	TC	40 (45.4)	86 (48.0)	1.110 (0.665-1.851)	0.072
	CC	27 (30.7)	30 (16.8)	0.455 (0.250-0.828)	0.014*
Allele	T	75 (42.4)	210 (58.8)	0.515 (0.357-0.742)	<0.001*
	C	102 (57.6)	147 (41.2)	0.642 (0.503-0.821)	<0.001*
Apal	rs7975232				
Genotype	GG	33 (37.5)	63 (35.2)	2.111 (1.803-2.472)	<0.001*
	GT	44 (50.0)	86 (48.0)	3.057 (1.796-5.203)	<0.001*
	TT	11 (12.5)	30 (16.8)	7.545 (3.560-15.994)	<0.001*
Allele	G	68 (38.4)	146 (40.9)	0.902 (623-1.304)	0.648
	T	109 (61.6)	211 (59.1)	0.933 (0.728-1.196)	0.648
BsmI	rs1544410				
Genotype	GG	53 (60.2)	107 (59.8)	1.019 (0.605-1.716)	<0.001*
	GA	32 (36.4)	66 (36.8)	1.022 (0.602-1.736)	<0.001*
	AA	3 (3.4)	6 (3.4)	0.983 (0.240-2.025)	<0.001*
Allele	G	138 (78.0)	278 (77.9)	1.006 (0.651-1.553)	<0.001*
	A	39 (22.0)	79 (22.1)	1.004 (0.750-1.342)	<0.001*
TaqI	rs731236				
Genotype	TT	45 (51.1)	89 (49.7)	1.023 (0.413-2.530)	1.000
	TC	38 (43.2)	79 (44.2)		
	CC	5 (5.7)	11 (6.1)		
Allele	G	128 (72.3)	257 (72.0)	1.016 (0.680-1.520)	<0.001*
	A	49 (27.7)	100 (28.0)	1.011 (0.772-1.324)	<0.001*

SNP: Single nucleotide polymorphism, PTB: Pulmonary tuberculosis, VDR: Vitamin D receptor, RS: Reference sequence genomes, OR: Odds ratio, CI: Confidence interval.
Significant value *p< 0.05.

The prevalence of smoking is notably higher among those with active PTB cases. Evidence shows that both current and former smokers have a greater likelihood of developing active TB compared to non-smokers. This association is attributed to smoking's detrimental effects on lung health and the immune system, which can facilitate the progression from latent TB infection (LTBI) to active disease (29,30). A systematic review underscored the strong correlation between smoking and active TB, indicating that smoking contributes significantly to TB risk across various populations (30,31).

The studies provide insights into risk factors associated with TB susceptibility, with a focus on gender differences, smoking behavior, and family history: Smoking is consistently identified as a significant risk

factor for TB across multiple studies. Stevens et al. found that cigarette smoking increased TB risk by 50% in adolescents, though this was not statistically significant (32). Wang and Shen reported a significantly higher proportion of smokers among TB cases (54.6%) compared to controls (45.1%), with an adjusted OR of 1.93 (33). Horne et al. also found current smoking to be associated with LTBI with an OR of 1.8 (32-34). Sex differences in TB susceptibility have been observed. Stevens et al. reported male sex as a risk factor (OR= 1.8). Watkins and Plant noted a worldwide excess of TB notifications among adult males, with cigarette consumption explaining 33% of the variance in sex ratio of TB notifications. This suggests smoking may play a role in gender differences in TB epidemiology (32,35). Family history emerges as an important factor. Stevens et al. found

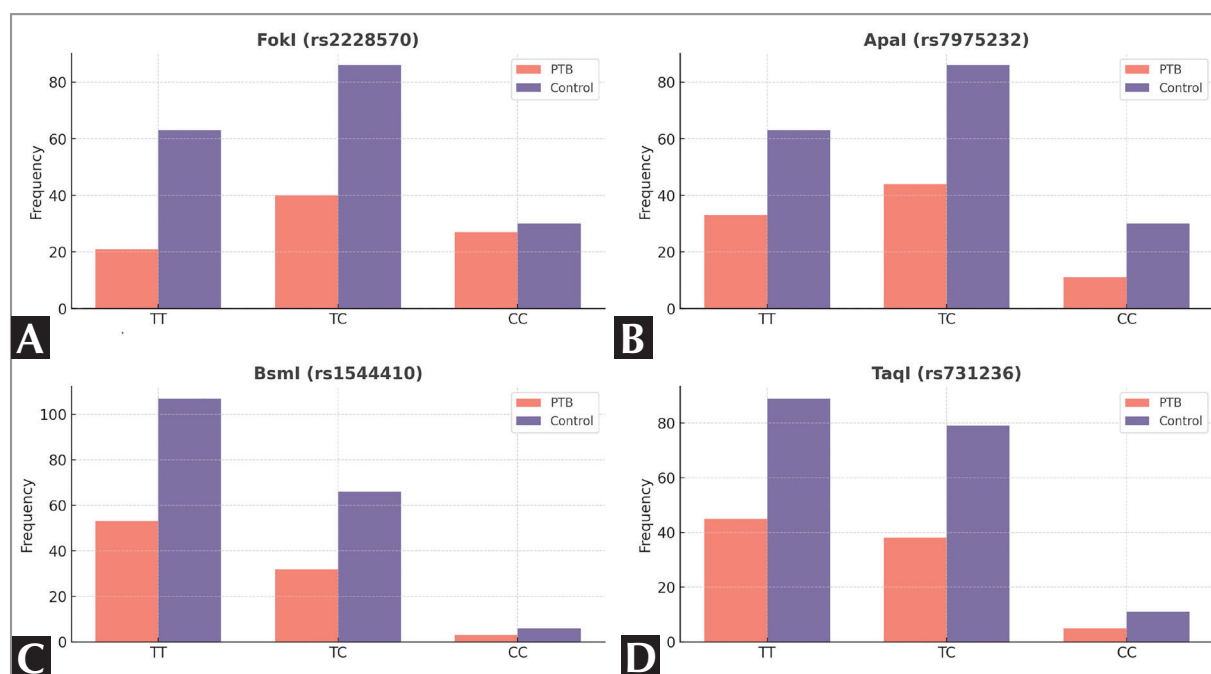


Figure 2. The genotype distribution of VDR gene polymorphisms in PTB patients and healthy controls. **A.** FokI (rs2228570): Significant difference observed for the CC genotype (* $p < 0.05$). **B.** Apal (rs7975232): Higher TT genotype frequency in controls (* $p < 0.05$). **C.** BsmI (rs1544410): No significant difference among genotypes (* $p < 0.05$). **D.** TaqI (rs731236): Similar genotype distribution across groups (* $p < 0.05$).

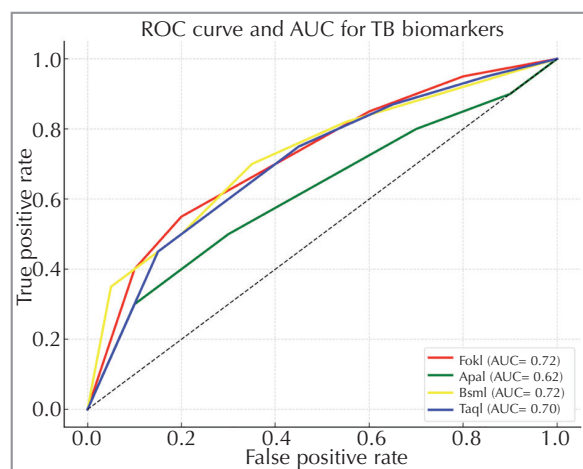


Figure 3. ROC curve for TB gene biomarkers. The ROC curves illustrate the diagnostic performance of four gene polymorphisms (FokI, Apal, BsmI, and TaqI) in distinguishing PTB patients from healthy controls. The AUC values indicate the discriminative power of each biomarker, with red (FokI), dark green (Apal), yellow (BsmI), and blue (TaqI) providing clear visual differentiation. The diagonal dashed line represents a random classifier (AUC= 0.5).

that sleeping in the same house as a TB case significantly increased risk (OR= 31.6). The study estimated that 38% of TB cases in children and adolescents

could be attributed to household contact (32). Some studies have revealed contradictions or additional nuances. While Wang and Shen found smoking cessation rates increased after TB diagnosis, over 18% relapsed during follow-up (36). Horne et al. noted that the association between smoking and LTBI was strongest for Mexican-American and black individuals, suggesting potential ethnic variations in susceptibility (33,34). The studies consistently identify smoking, male sex, and family history/household contact as key risk factors for TB susceptibility. However, the interplay between these factors and potential variations across different populations warrant further investigation to develop targeted prevention and control strategies.

This study investigated the sociodemographic characteristics and VDR gene polymorphisms in PTB patients compared to healthy controls across three ethnic groups in Indonesia. The findings highlight significant associations between PTB susceptibility and education levels, smoking habits, and specific VDR gene polymorphisms. Notably, individuals with basic or moderate education were more likely to have active or latent PTB. Similarly, smoking prevalence was significantly higher among PTB active

cases. Genetic analysis revealed a strong association of the FokI CC genotype and Apal TT genotype with PTB susceptibility, while BsmI and TaqI polymorphisms showed minimal differences. While genetic predisposition plays a crucial role in PTB susceptibility, environmental and cultural factors significantly modulate disease risk. Differences in TB prevalence among ethnic groups may be influenced by socioeconomic status, healthcare access, nutrition, and occupational exposures. For instance, variations in vitamin D levels due to differing dietary habits and sun exposure across ethnicities could impact immune responses, affecting TB susceptibility. Additionally, traditional practices, such as communal living and household air quality, may contribute to differential TB transmission rates among populations.

Smoking and alcohol consumption, as cultural lifestyle choices, also demonstrate varied prevalence across different ethnic groups in Indonesia, influencing PTB risk (37). Previous studies have reported that heavy smoking and alcohol intake impair immune function, increasing vulnerability to *M. tuberculosis* infection. Further investigation into how cultural perceptions of smoking and alcohol use shape health behaviors in different communities would provide a more comprehensive understanding of these associations.

Specific polymorphisms in the VDR gene polymorphisms also play a role in PTB susceptibility. Studies have identified associations between certain SNPs in the VDR gene and the risk of developing PTB. For instance, SNPs such as rs11574143 and rs11168287 have been linked to increased susceptibility, while others may confer protection against the disease (31,38). The genetic diversity across different populations may lead to varying results regarding these associations, emphasizing the need for further research in diverse ethnic group (38,39).

Some studies suggest that the Apal polymorphism may confer a decreased risk of developing PTB. A meta-analysis has indicated that the variant allele (a) and genotype (aa) were significantly associated with a lower risk of TB compared to the wild type (40,41). Conversely, other research has found no significant association between the Apal polymorphism and TB susceptibility in certain populations, indicating variability in results based on ethnicity (15,38). The effects of the Apal polymorphism appear to vary significantly across different ethnic groups, with some

populations showing protective effects while others do not (15,41).

The role of VDR gene polymorphisms, particularly TaqI and BsmI, in TB susceptibility has been a focus of research in various populations, including Indonesia. Recent studies have highlighted significant findings regarding these SNPs and their association with TB risk.

These findings align with previous studies emphasizing the complex interplay between genetic predisposition and environmental factors in TB susceptibility. This meta-analysis of VDR gene polymorphisms and PTB susceptibility reveals mixed results across different populations. The FokI polymorphism was associated with increased PTB risk in East Asians, but not in Iranian or Indonesian Batak populations (15,42,43).

The relationship between VDR gene polymorphisms and susceptibility to PTB is complex and influenced by genetic background. While TaqI (rs731236) consistently shows an increased risk for TB, the role of Apal (rs7975232) remains less clear, with evidence suggesting both protective and non-significant associations depending on the population studied.

The TaqI polymorphism has shown a consistent association with an increased risk of TB across multiple studies. In a systematic review, TaqI has been linked to a heightened susceptibility to TB in various genetic models, including dominant and recessive models (6,15). Specifically, the OR indicated that individuals with certain TaqI genotypes had significantly higher risks of developing TB, reinforcing its role as a potential genetic risk factor (15). Unlike Apal, TaqI's association with TB risk has been more robust across different studies and populations. This suggests that TaqI may be a more reliable marker for assessing susceptibility to PTB compared to Apal (6,15).

The TaqI polymorphism showed a significant association with increased PTB risk in Iranians, while BsmI results were inconsistent. The bb genotype of BsmI was associated with decreased PTB risk in Indonesians, but the dominant model (bb+bB/BB) showed increased risk in Iranians (15,43). Apal polymorphism did not show significant effects on PTB development in the studied populations. These findings suggest that VDR gene polymorphisms may influence immune responses to *M. tuberculosis*, potentially affecting PTB susceptibility (44).

The dominant genotype of the BsmI polymorphism has also been associated with an increased risk of TB. This correlation has been supported by case-control studies demonstrating that specific BsmI genotypes could influence susceptibility to TB in the Indonesian population (6,45). Notably, some studies have reported a protective effect associated with certain BsmI genotypes, indicating a complex relationship between this SNP and TB risk (43).

Despite these findings, there is a consensus among researchers that further validation through larger case-control and population-based studies is necessary. Such studies would help clarify the roles of TaqI and BsmI polymorphisms in TB susceptibility and account for genetic diversity across different ethnic groups within Indonesia (6,45,46). While FokI and ApaI SNPs have also been examined in relation to TB risk, results have been inconsistent across different populations. In some cases, no significant associations have been found (43,46). This variability underscores the importance of considering ethnic backgrounds when assessing genetic risk factors for diseases like TB.

The TaqI polymorphism of the VDR gene (rs731236) has been consistently identified as a significant risk factor for TB across diverse genetic models and populations, as highlighted by meta-analyses involving Iranian and Turkish cohorts (47,48). In Indonesia, this polymorphism has also been confirmed to elevate TB susceptibility, emphasizing the heightened vulnerability of individuals with specific genotypes (6,47). However, the relationship between VDR gene polymorphisms and TB susceptibility is inherently complex, influenced by genetic background and varying across populations. For instance, while the TaqI variant consistently correlates with increased risk, the ApaI polymorphism (rs7975232) exhibits mixed evidence, showing both protective effects and non-significant associations depending on the studied cohort. These findings underscore the importance of context-specific genetic investigations to unravel the intricate interplay between VDR gene variants and pulmonary TB susceptibility.

The exploration of VDR gene polymorphisms, particularly TaqI and BsmI, has demonstrated significant associations with TB susceptibility in the Indonesian population, highlighting the potential genetic predisposition to the disease. However, the intricate nature of these associations underscores the need for further investigation to validate these findings across more diverse cohorts and to delve deeper into the gene-

environment interactions and the biological mechanisms by which these polymorphisms influence TB susceptibility.

The AUC value of 0.76 for combined FokI and ApaI genotypes indicates a moderate predictive capability, suggesting that while VDR polymorphisms contribute to PTB susceptibility, additional genetic and environmental factors likely influence disease risk. Future studies should integrate genome-wide association studies and multi-omics approaches to further elucidate gene-environment interactions.

CONCLUSION

This study highlights the interplay between sociodemographic factors, lifestyle choices, and genetic predispositions in shaping PTB risk. The findings provide insights into potential avenues for public health interventions and emphasize the need for integrating genetic screening with traditional TB prevention strategies. However, cautious interpretation is required given the limitations and potential biases. Future studies should address these gaps, particularly focusing on longitudinal designs and larger, more diverse populations.

Limitations

The limitations of this study include its cross-sectional design, which restricts causal inference, and potential selection bias due to the study population's ethnic homogeneity. Furthermore, unmeasured confounders such as vitamin D levels and co-infections could influence the observed associations. Future research should employ longitudinal studies and expand to diverse populations to validate these findings.

Ethical Committee Approval: This study was approved by Health Research Ethics Committee of Makassar Health Polytechnic (Decision no: 070/M/KEPK-PTKMS/VII/2024, Date: 02.07.2024).

CONFLICT of INTEREST

The authors declare that they have no conflict of interest.

AUTHORSHIP CONTRIBUTIONS

Concept/Design: II

Analys/Interpretation: SA

Data acquisition: MB

Writing: SS

Clinical Revision: NN

Final Approval: All of authors

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