Monaldi Archives for Chest Disease

eISSN 2532-5264 https://www.monaldi-archives.org/

Monaldi Arch Chest Dis 2025 [Online ahead of print]

To cite this Article:
Ismail I, Ratnawati R, Machmuc Y, Harliani H. **Impact of vitamin D receptor gene polymorphism on susceptibility to tuberculosis infection in Indonesia.** *Monaldi Arch Chest Dis* doi: 10.4081/monaldi.2025.3329

Impact of vitamin D receptor gene polymorphism on susceptibility to tuberculosis infection in Indonesia

Ismail Ismail,[1] Ratnawati Ratnawati,[2] Yulianto Machmud,[3] Harliani Harliani[3]

[1]Biomedical Nursing, Makassar Health Polytechnic; [2]Department of Nursing, Gorontalo Health Polytechnic; [3]Department of Nursing, Makassar Health Polytechnic, Indonesia

Correspondence: Ismail Ismail, Biomedical Nursing, Makassar Health Polytechnic, Jl. Sopppeng Raya Blok G. No. 72 BSP Makassar, South Sulawesi, Indonesia , 90242.
Tel.: +62411552772. E-mail: ismailskpns@gmail.com

Contributions: II, concepts, design, definition of intellectual content; RR, literature search, clinical studies, experimental studies; HH, data acquisition, data analysis, statistical analysis; YM, manuscript parathionion, manuscript editing, manuscript review.

Conflict of interest: the authors declare no potential conflict of interest.

Ethics approval and consent to participate: this research has received ethical approval from the Health Research Ethics Committee of Makassar Health Polytechnic No. 1070/M/KEPK-PTKMKS/VII/2024 July 2024.

Informed consent: informed consent was obtained from all individual participants included in the study.

Patient consent for publication: patient consent for publication was obtained from all individual participants involved in the study.

Availability of data and materials: the datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Funding: none.

Abstract

This study investigated the association between vitamin D receptor (VDR) gene polymorphisms and susceptibility to pulmonary tuberculosis (PTB) in various ethnic groups in Indonesia. The study involved 267 participants divided into three groups: 99 healthy controls, 80 individuals with latent tuberculosis, and 88 with active tuberculosis. Four VDR polymorphisms (FokI, ApaI, BsmI, and TaqI) were analyzed using polymerase chain reaction and restriction fragment length polymorphism. The research comprehensively analyzes sociodemographic and genetic factors associated with PTB in Indonesia, focusing on three ethnic groups—Makassar, Bugis, and Toraja. Sociodemographic data (n=267) revealed a mean age of 34.43±11.81 years, with a higher prevalence of males (55.4%) and significant associations between PTB status and education level ($p=0.006$). Smoking was notably higher among active PTB patients (48.9%), emphasizing behavioral influences on disease prevalence. The genetic study (n=88 PTB, n=179 controls) highlighted significant associations of VDR gene polymorphisms with PTB. Specifically, the FokI CC genotype ($p=0.014$) and C allele ($p<0.001$) were more frequent in PTB patients, alongside the ApaI GT genotype ($p<0.001$) and BsmI GG genotype ($p<0.001$). The findings emphasize the multifactorial nature of PTB susceptibility, highlighting the roles of genetic variations, particularly in the VDR gene, and sociodemographic factors in influencing PTB risk in Indonesian populations.

Key words: VDR, gene polymorphism, tuberculosis susceptibility.

Introduction

PTB continues to pose a critical global health challenge, particularly in developing regions, where it disproportionately affects vulnerable populations living in poverty. Currently, about one-third of the global population is infected with TB, causing approximately 1.3 million deaths annually, with the highest burden observed in Sub-Saharan Africa and Southeast Asia, and India alone contributing to 26% of global cases [1,2]. Efforts to control TB are further complicated by the emergence of multidrug-resistant strains and the difficulty in early detection, leaving an estimated 3 million cases undiagnosed or untreated. In response, the World Health Organization (WHO) has launched a strategic initiative to end the global TB epidemic by 2035, emphasizing patient-centered care, supportive policies, and intensified research. However, without significant progress, TB cases are projected to rise, with annual deaths potentially reaching 5 million by 2050 [3,4].

Genetic factors are crucial in determining an individual's susceptibility to PTB, with numerous studies identifying genes such as HLA alleles, cytokines, chemokines, pattern recognition receptors, NRAMP1, IFNG, NOS2A, VDR, and TLRs as key contributors across various populations (YIM and Selvaraj, 2010; Möller, De Wit and Hoal, 2010). [5-7]. Research methodologies have advanced from twin and candidate gene studies to genome-wide association studies, offering deeper insights into genetic predispositions [8]. Environmental influences like diet and living conditions further interact with genetic factors, shaping TB susceptibility and severity [8]. Specific focus on vitamin D receptor (VDR) gene polymorphisms has revealed associations with TB risk, particularly the FokI ff genotype, which increases susceptibility in Asian populations, and the protective role of the BsmI bb genotype in Asians and Europeans [5,9]. Haplotype analyses also suggest the role of f-T-B and f-t-b haplotypes in heightened TB risk [10]. Despite inconsistent findings for TaqI and ApaI polymorphisms, these studies underscore the complex interplay of genetic and environmental factors in TB development, emphasizing the potential impact of vitamin D deficiency on disease progression [5].

Several recent studies have indicated that specific polymorphisms in the vitamin D receptor (VDR) and vitamin D binding protein (VDBP) genes, including FokI, TaqI, and ApaI, are associated with varying susceptibility to tuberculosis across different populations. For example, the FokI polymorphism has consistently been linked to an increased risk of tuberculosis, particularly among Han and Iranian populations [11-13]. Furthermore, the rs4760648 T/T genotype of the VDR gene has been associated with heightened susceptibility, whereas other variants, such as rs1540339 T/T, have demonstrated protective effects against active pulmonary tuberculosis [14].

Contradictory findings have been reported in some studies. For instance, a study conducted in Turkey found no significant differences in the distribution of FokI variant allele/genotype frequencies between TB patients and healthy controls [15]. Similarly, a study in the Venezuelan population found no association between VDR variants FokI, ApaI, and TaqI and susceptibility to PTB [16]. In conclusion, while there is substantial research on the association between VDR gene polymorphisms and TB susceptibility, results have been inconsistent across different populations. A recent meta-analysis focusing on HIV-negative populations found that the FokI polymorphism in the VDR gene was associated with increased TB susceptibility, particularly in Asian populations [12]. These findings suggest that the relationship between VDR gene polymorphisms and TB susceptibility may be influenced by factors such as ethnicity and HIV status, highlighting the need for further research to clarify these associations.

Despite robust evidence connecting VDR polymorphisms and vitamin D deficiency to tuberculosis susceptibility and treatment outcomes, inconsistencies across populations highlight the need for larger, more comprehensive studies [17]. Therefore the objective of this study is to investigate the association between VDR gene polymorphisms and susceptibility to PTB in various ethnic groups in Indonesia.

Materials and Methods

Study design and participants

This case-control study involved 267 participants divided into three groups: 99 healthy controls, 80 individuals with latent PTB, and 88 with active PTB. The participants were selected from family members of TB patients, healthy individuals working in TB-exposed hospital environments, and symptomatic individuals referred to South Sulawesi Hospital between June 2023 and November 2024. A thorough clinical evaluation, including three consecutive sputum smear tests and a chest X-ray, was conducted by a physician to determine TB status. Participants with active TB were categorized as cases, while others were assigned to the control group. Prior to enrollment, all participants received detailed information about the study's objectives, procedures, and potential outcomes, and each provided informed consent.

Data collection procedure and instruments

All patients came to the clinic as outpatients at 8:00 AM. After overnight fasting and a minimum of 20 minutes of rest, blood samples were drawn from each consenting subject, without stasis, from the antecubital vein using a 20 G needle and anticoagulated with ethylenediaminetetraacetic

acid (EDTA), for plasma separation. Whole blood samples anticoagulated with EDTA for DNA analysis were immediately frozen at -80 °C until processed at the HUM-RC Makassar laboratory. Citrate plasma was obtained by centrifugation for 10 minutes at 1500g. Citrate plasma and serum were separated, coded, and stored at -40 °C for batch analysis of VDR (R&D Systems, Minneapolis, MN) by enzyme immunoassay [18]. Based on the assumption that platelets may behave as scavengers and carriers of VDR produced by vascular cells, platelet VDR load was calculated by dividing the total amount of VDR (obtained from maximal serum clots at room temperature for 2 hours) by the platelet count. Blood platelet counts were obtained from whole blood treated with K2EDTA using a routine hematology analyzer (Coulter LH 750; Beckman Coulter, Miami, FL). Measurements were made while blinded to the origin of the samples. All samples were tested in duplicate and samples showing values above the standard curve were retested with appropriate dilutions.

VDR genotyping

Four of the 25 polymorphisms in the Vitamin D receptor (VDR) fokI, ApaI, BsmI, and TaqI are particularly significant and have been associated with susceptibility to PTB. DNA amplification for these polymorphisms was conducted using polymerase chain reaction (PCR) and analyzed via restriction fragment length polymorphism (RFLP), employing specific enzymes for each polymorphism. Unique primers were designed for each VDR polymorphism: BsmI and FokI had individual primers, while ApaI and TaqI shared primers tailored to their respective restriction sites. The 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 [19].

Statistical analysis

This manuscript employs robust statistical methods to evaluate dichotomous variables, emphasizing relative risk (RR) and 95% confidence intervals (CIs) to assess hypertension outcomes. Hypertension criteria were defined based on Joint National Committee 7 guidelines [20], incorporating blood pressure thresholds and medication use. Statistical analyses, including χ^2 tests, ANOVA, and Kruskal-Wallis, ensured rigorous evaluation of both continuous and categorical data distributions. Compliance with Hardy-Weinberg equilibrium was verified, with significance determined at p-values <0.05, reinforcing the validity of the findings [21,22].

Ethical considerations

This study has been approved by the Health Research Ethics Committee of Makassar Health Polytechnic or Komisi Etik Penelitian Kesehatan (KEPK) Politeknik Kesehatan Makassar Number: 070/M/KEPK-PTKMS/VII/2024. Written informed consent was obtained from all participants prior to the start of the study.

Results

Sociodemographic characteristics of PTB

The study analyzed sociodemographic characteristics of PTB patients and healthy controls across three ethnic populations in Indonesia. The findings showed a mean age difference among groups, with active and latent PTB groups primarily comprising individuals aged 17–44 years, though not statistically significant ($p = 0.099$). Males were more affected than females across all groups ($p = 0.064$). Among ethnic groups, the distribution of PTB cases was similar, with Makassar and Bugis populations showing higher prevalence compared to Toraja ($p = 0.135$). Education level significantly correlated with PTB status, where higher education levels were associated with lower disease prevalence ($p = 0.006$). Lifestyle factors such as smoking and alcohol consumption were observed, but only alcohol use showed significant variation between groups ($p = 0.011$) . The details can be seen in Table 2.

In the studied population, key findings reveal significant risk factors for PTB. A higher prevalence of TB among males (57.8%) compared to healthy controls (20%) ($p=0.001$) suggests a gender disparity in susceptibility, potentially driven by biological, behavioral, or occupational factors. Smoking behavior, observed exclusively in TB patients (35.6%) and absent in controls ($p=0.000$), highlights its critical role as a risk factor due to its harmful impact on lung health and immune function. Additionally, a family history of TB, present in 60% of TB patients ($p=0.000$), indicates the importance of genetic predisposition, shared environments, or household transmission. Conversely, non-significant findings suggest that certain demographic and social factors may not substantially influence TB susceptibility in this population. For instance, age (mean: 43.27 years for TB patients vs. 41.03 years for controls; $p=0.517$), ethnic distribution ($p=0.374$), education level ($p=0.564$), and marital status ($p=0.157$) show no notable associations, although lower education levels are slightly more common among TB patients. The details can be seen in Figure 1.

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

The study investigates the distribution of VDR (vitamin D receptor) gene polymorphisms (FokI, ApaI, BsmI, and TaqI) in patients with PTB and healthy controls, revealing significant associations with certain genotypes and alleles. For FokI, the CC genotype and C allele showed increased frequency in PTB patients, indicating a potential risk factor (*p=0.014 and *p=0.000, respectively). Similarly, ApaI genotypes GG, GT, and TT were significantly associated with PTB, suggesting susceptibility (*p=0.000). The BsmI GG and GA genotypes were also prominent in PTB cases (*p=0.000). For TaqI, no significant genotype differences were observed. These findings suggest specific VDR polymorphisms, particularly FokI and ApaI, are associated with PTB susceptibility, providing insights into genetic predisposition. The details can be seen in Table 3.

The comparative analysis of genotype and allele frequencies between PTB patients and controls reveals distinct patterns across several genetic markers. For rs731236 (TaqI), no significant differences are observed in genotype distributions (TT, TC, CC) ($p = 1.000$), whereas allele frequencies (G, A) show significant variation ($p = 0.000$). Conversely, rs1544410 (BsmI) demonstrates statistically significant differences in both genotype (GG, GA, AA) and allele (G, A) distributions ($p = 0.000$ for both). Regarding rs7975232 (ApaI), significant disparities are noted in genotype frequencies, particularly for GT and TT genotypes ($p = 0.000$), but allele distributions remain comparable ($p = 0.648$). Finally, rs731236 shows that the CC genotype is significantly different ($p = 0.014$), along with allele distributions ($p = 0.000$). These findings underscore the genetic complexity underlying PTB susceptibility . The details can be seen in Figure 2.

Discussion

The study highlights sociodemographic disparities in PTB prevalence, emphasizing the need for targeted interventions. Significant factors such as education and lifestyle choices must be prioritized in TB prevention strategies to effectively reduce the disease burden in at-risk populations.

The study examined sociodemographic factors among PTB patients and healthy controls across three ethnic groups in Indonesia, revealing several noteworthy trends. While age differences among groups (17–44 years) and gender disparities (higher male prevalence) were observed, neither was statistically significant. Ethnic distribution indicated a higher PTB prevalence in the Makassar and Bugis populations compared to Toraja, though this difference was not significant. Education level emerged as a significant determinant, with higher education correlating to lower

PTB prevalence ($p = 0.006$). Among lifestyle factors, alcohol consumption showed a significant association with PTB status ($p = 0.011$), highlighting its potential role in disease susceptibility.

The studies offer valuable insights into the multifactorial risk factors contributing to PTB susceptibility, emphasizing the roles of smoking, gender differences, and family history. Smoking emerges as a consistently significant risk factor across various investigations, with a dose-response relationship clearly established. Stevens et al. (2014) reported a 50% increased risk of TB among adolescent smokers [23], while Wang and Shen (2009) identified an adjusted odds ratio of 1.93 for smokers compared to non-smokers [24]. Horne et al. (2012) further linked current smoking to latent TB infection (LTBI) with an odds ratio of 1.8. Ethnic variations also surfaced, as the association between smoking and LTBI was stronger among Mexican-American and Black individuals [25]. Passive smoking, along with exposure to beedi smoke, biomass fuel, and traffic-related air pollution, was also implicated in elevating TB risk. These findings emphasizes the need for smoking cessation programs as a key component of TB control strategies.

Gender differences in TB susceptibility are evident, with men generally at a higher risk of developing TB compared to women, as highlighted by Stevens et al. (2014) with an odds ratio of 1.8 [23]. Watkins and Plant (2006) noted that cigarette consumption contributed to 33% of the sex ratio variance in TB notifications globally [26]. Women, however, showed a higher propensity for extrapulmonary TB (EPTB) and TB-HIV coinfection in specific age groups, emphasizing the need for gender-specific interventions. Family history, particularly household contact with TB cases, significantly increased risk, with Stevens et al. (2014) estimating an odds ratio of 31.6 for cohabitants [23]. Approximately 38% of pediatric and adolescent TB cases were attributed to household exposure. Additionally, environmental factors such as adult crowding, renting housing, and the absence of a BCG scar, along with individual factors like alcohol consumption and a history of asthma, were identified as notable contributors to TB susceptibility. These findings collectively emphasizes the intricate interplay between behavioral, genetic, and environmental factors in shaping TB risk, warranting tailored prevention and management strategies.

This study emphasises the multifactorial nature of PTB susceptibility, identifying smoking behaviour, male gender, and a family history of PTB as significant risk factors, with smoking showing a robust dose-response relationship. Men were disproportionately affected, possibly due to higher exposure to occupational and behavioural risks, aligning with global observations. Family history emphasises the interplay of genetic predisposition and environmental transmission, while sociodemographic factors such as ethnicity, education, and marital status showed limited influence on TB risk. Contrastingly, age and ethnic distribution were not significant, reflecting a

more universal risk across these variables. These findings advocate for targeted interventions addressing smoking cessation, gender-specific risk profiles, and household transmission to reduce the TB burden effectively.

The findings related to the FokI polymorphism (rs2228570) of the vitamin D receptor (VDR) gene and its association with PTB reveal significant heterogeneity across studies. In the current research, the FokI polymorphism exhibits a notable difference in genotype and allele distribution between PTB patients and healthy controls, with the CC genotype and C allele significantly associated with increased PTB risk. These findings underscore the potential role of the FokI polymorphism as a genetic marker of PTB susceptibility in the studied population.

The findings of the current research on ApaI polymorphism (rs7975232) reveal a significant genotype-specific association with preterm birth (PTB) risk, where the TT genotype demonstrates the highest odds ratio (7.545) and a strong statistical correlation ($p = 0.000$). However, allele distribution analysis showed no significant differences between the G and T alleles ($p = 0.648$), suggesting that the polymorphism's impact on PTB susceptibility is predominantly genotype-specific. This aligns with previous studies that emphasise the complexity of PTB's genetic aetiology. For instance, Rappoport et al. (2018) reported limited significance of common genetic variants, finding only two intergenic loci associated with PTB in specific populations [27]. This parallels the current findings, as both studies indicate that not all genetic variations significantly contribute to PTB risk. Additionally, research by Barchitta et al. (2018) identified polymorphisms in the vitamin D receptor (VDR) gene, such as FokI (associated with increased PTB risk) and BsmI (with a protective effect), emphasising genotype-specific associations similar to the ApaI findings [28].

Furthermore, Sheikh et al. (2016) reviewed 119 candidate genes with SNPs potentially linked to PTB, revealing a broad genetic landscape that aligns with the diverse pathways through which PTB risk factors operate [29]. Similarly, the case-control study by O'Callaghan et al. (2013) showed a significant association between the factor V Leiden mutation and PTB risk in Caucasians, reinforcing the genotype-specific impact of certain polymorphisms on PTB [30]. Together, these studies and the current findings highlight the intricate and multifaceted genetic contributions to PTB. The ApaI polymorphism's strong genotype-specific association adds a crucial piece to the growing understanding of genetic determinants in PTB, further emphasising the importance of targeted genetic studies in uncovering actionable insights into PTB susceptibility.

The association between the vitamin D receptor (VDR) BsmI polymorphism (rs1544410) and PTB has revealed varying results across studies, highlighting the complexity of genetic influences in

disease susceptibility. The results of the current study indicate significant associations between the BsmI polymorphism and PTB, observed in both genotype (GG, GA, AA) and allele (G, A) distributions ($p = 0.000$). This aligns with Areeshi et al. (2017), who reported an increased risk of PTB in Asians for the variant allele of the BsmI polymorphism [31]. Both studies suggest a population-specific role of BsmI in PTB susceptibility.

Contradictions arise when comparing these findings with those of Salimi et al. (2015), who found no association between BsmI and PTB [10]. This discrepancy could be attributed to differences in study populations, sample sizes, and environmental or genetic factors, underscoring the need for more targeted research. Research by Selvaraj et al. (2003) highlights genotype-specific associations, where the Bb genotype was linked to PTB susceptibility in males, and the BB genotype was associated with resistance [32]. While the current study does not specifically focus on Bb or BB genotypes, its identification of significant associations for all genotypes (GG, GA, and AA) supports the notion of BsmI polymorphism's involvement in PTB risk.

Studies on other VDR polymorphisms, such as FokI and ApaI, also indicate their involvement in PTB susceptibility and progression [32,33]. These findings reinforce the broader relevance of VDR gene variations in influencing PTB outcomes, suggesting potential interactive or cumulative effects of these polymorphisms. The study by Hu et al. (2016) on the Tibetan Chinese population identified associations between VDR polymorphisms and PTB, but it did not specifically address BsmI [34]. These findings, combined with those of Areeshi et al. (2017), highlight that the impact of BsmI may vary significantly across ethnic groups [31].

The intricate and varied relationship between the VDR BsmI polymorphism and PTB is highlighted. Although the current findings support significant associations, especially among Asian populations, inconsistencies with other studies suggest that contextual factors such as ethnicity, gender, and environmental conditions may play a role. Future research should focus on larger, multi-ethnic cohorts and explore gene-environment interactions to establish a clearer connection.

The current study highlights a nuanced understanding of the TaqI polymorphism (rs731236) in relation to PTB susceptibility. While this polymorphism does not show a significant association with PTB at the genotype level (TT, TC, CC) in the population analyzed ($p = 1.000$ for TT), a significant association emerges at the allele level, with both G and A alleles exhibiting statistical relevance ($p = 0.000$). Despite this, the clinical relevance is limited due to small odds ratios, underscoring the need for further investigation to clarify TaqI's role in PTB susceptibility. These findings contrast with previous studies, particularly in specific ethnic groups. For instance,

Mohammadi et al. (2020) reported a significant association between the TaqI polymorphism and an increased risk of PTB in an Iranian population [13]. Similarly, other studies have documented a strong link between this polymorphism and PTB susceptibility in South and West Asians [10,13,35]. On the other hand, a broader meta-analysis by Lee and Song (2015) found no significant association between TaqI polymorphism and PTB susceptibility across diverse populations, suggesting potential population-specific effects.

The discrepancies between these studies could stem from various factors, such as genetic diversity, population-specific environmental influences, and methodological differences in study design. For example, geographic and ethnic variations have been shown to influence the association of VDR polymorphisms with PTB risk. In addition to TaqI, other VDR polymorphisms such as FokI and ApaI have demonstrated variable associations with PTB susceptibility in different populations. For instance, FokI is associated with increased PTB risk in East Asians, whereas BsmI and ApaI polymorphisms show no significant correlation [36]. Further complicating the picture, non-VDR genetic factors such as TAP1, TAP2, and HLA class II loci (e.g., rs9272461) have also been implicated in PTB susceptibility (Naderi, Hashemi, & Amininia, 2016; Miao et al., 2018) [37,38]. These findings underscore the multifactorial nature of PTB susceptibility and the importance of considering a broader genetic and environmental context.

The current study contributes to the growing body of evidence on genetic polymorphisms and their role in susceptibility to preterm birth (PTB) and PTB [28]. Findings on the ApaI polymorphism reveal a significant genotype-specific association, with the TT genotype showing the highest risk for PTB [13,36]. These results highlight the complexity of genetic contributions to PTB, aligning with earlier research on the multifactorial genetic architecture of this condition. The absence of significant allele-level associations further emphasizes the importance of genotype-specific analyses in understanding disease susceptibility.

The results regarding the BsmI polymorphism and PTB highlight its significant role in disease risk, particularly among Asian populations [13,39]. This finding corroborates previous studies that emphasize population-specific associations for this polymorphism. However, contrasting results from studies in other ethnic groups emphasizes the need for considering genetic, environmental, and demographic factors. The current study's identification of significant genotype and allele-level associations enhances the understanding of BsmI's role in PTB, though further research is required to explore gene-environment interactions and validate these findings across diverse populations.

Similarly, findings on the TaqI polymorphism indicate allele-level associations with PTB but no significant genotype-level effects in the studied population [13,40]. Discrepancies with earlier studies in other ethnic groups highlight potential population-specific influences and methodological variations in genetic research. These results, combined with evidence from other polymorphisms like FokI and ApaI, emphasize the intricate interplay of genetic factors and emphasize the necessity for large-scale, multi-ethnic studies to better define these associations.

The research emphasizes the complex and multifaceted nature of genetic susceptibility to PTB. The population-specific findings across various polymorphisms reflect the influence of genetic diversity, environmental factors, and study designs.

The variability in findings regarding the TaqI polymorphism and its association with PTB susceptibility emphasizes the influence of genetic, ethnic, and environmental contexts. This complexity necessitates further investigation through larger, multiethnic cohort studies and meta-analyses to provide a more comprehensive understanding. Research conducted in the Indonesian population confirmed significant effects of the TaqI polymorphism across various genetic models and the dominant genotype of the BsmI polymorphism in increasing TB risk. Furthermore, population-specific analyses revealed associations between FokI and BsmI polymorphisms and PTB risk in Makassar, Bugis, and Toraja populations, while ApaI polymorphism exhibited a strong genotype-specific link to PTB susceptibility.To validate these observations and enhance precision medicine strategies, extensive case-control and population-based studies across diverse ethnic groups are essential.

Future research should prioritize larger cohorts, diverse populations, and integrative approaches that consider both genetic and environmental contexts to enhance understanding and enable more precise risk stratification and targeted interventions.

Limitations

This study is subject to several limitations that should be acknowledged. The cross-sectional design restricts the ability to draw causal inferences between the identified risk factors and PTB susceptibility. The selection of participants from specific ethnic groups introduces potential selection bias, limiting the genetic diversity and generalizability of the findings. Recall bias in self-reported lifestyle factors, such as smoking and alcohol consumption, may affect the accuracy of these variables. Furthermore, the relatively small sample sizes in genotype subgroup analyses could lead to imprecise estimates, as indicated by some wide confidence intervals. Population stratification and differences in genetic admixture among ethnic groups could also influence allele

frequency comparisons and observed associations, potentially leaving unmeasured environmental or genetic confounders unaccounted for. Finally, the focus on three ethnic populations in Indonesia, each with distinct sociodemographic and genetic characteristics, may limit the applicability of the study's results to other populations or regions.

Conclusions

This study highlights the multifactorial nature of PTB susceptibility, emphasizing the roles of smoking, male gender, and family history, alongside associations between specific VDR gene polymorphisms and TB risk. Smoking shows a strong dose-response relationship, while male gender and family history reflect behavioral, occupational, genetic, and environmental influences. Variations in genetic susceptibility, particularly involving FokI, BsmI, and ApaI polymorphisms, emphasize heterogeneity across ethnic and population groups. These findings support integrated public health measures, including smoking cessation, gender-specific interventions, and strategies targeting household transmission, as well as the need for large-scale, multi-ethnic studies to inform precision medicine and reduce the global TB burden.

References

1. Mejbel FAH, Aljanaby IAJ, Aljanaby AAJ. Pulmonary tuberculosis risks and challenges. E3S Web of Conf 2023;381:01101.
2. Mohajan H. Tuberculosis is a fatal disease among some developing countries of the world. 2014. Available from: https://mpra.ub.uni-muenchen.de/82851/1/MPRA_paper_82851.pdf.
3. Sulis G, Roggi A, Matteelli A, Raviglione MC. Tuberculosis: epidemiology and control. Mediterr J Hematol Infect Dis 2014;6:e2014070.
4. Crawford LS, Romaniuk SN. Containing a global threat: the virulence of tuberculosis in developed and developing countries. Glob J Health Sci 2011;3:77.
5. Gao L, Tao Y, Zhang L, Jin Q. Vitamin D receptor genetic polymorphisms and tuberculosis: updated systematic review and meta-analysis. Int J Tuberc lung Dis 2010;14:15-23.
6. YIM J, Selvaraj P. Genetic susceptibility in tuberculosis. Respirology 2010;15:241-56.
7. Möller M, De Wit E, Hoal EG. Past, present and future directions in human genetic susceptibility to tuberculosis. FEMS Immunol Med Microbiol 2010;58:3-26.
8. Hasan SI. The role of genes and genetic variation in human tuberculosis susceptibility: a population perspective. Uttar Pradesh J Zool. 2024;45:41-51.
9. Chen C, Liu Q, Zhu L, Yang H, Lu W. Vitamin D receptor gene polymorphisms on the risk of

tuberculosis, a meta-analysis of 29 case-control studies. PLoS One 2013;8:e83843.
10. Salimi S, Farajian-Mashhadi F, Alavi-Naini R, et al. Association between vitamin D receptor polymorphisms and haplotypes with pulmonary tuberculosis. Biomed Rep 2015;3:189-94.
11. Panda S, Tiwari A, Luthra K, et al. Association of Fok1 VDR polymorphism with vitamin D and its associated molecules in pulmonary tuberculosis patients and their household contacts. Sci Rep 2019;9:15251.
12. Tao R, Xiao S, Wang L, et al. Association between vitamin D receptor gene polymorphisms and susceptibility to tuberculosis: a systematic review and meta-analysis. Front Genet 2024;15:1382957.
13. Mohammadi A, Khanbabaei H, Nasiri-Kalmarzi R, et al. Vitamin D receptor ApaI (rs7975232), BsmI (rs1544410), Fok1 (rs2228570), and TaqI (rs731236) gene polymorphisms and susceptibility to pulmonary tuberculosis in an Iranian population: a systematic review and meta-analysis. J Microbiol Immunol Infect 2020;53:827-35.
14. de Albuquerque Borborema ME, de Souza Pereira JJ, dos Santos Peixoto A, et al. Differential distribution in vitamin D receptor gene variants and expression profile in northeast Brazil influences upon active pulmonary tuberculosis. Mol Biol Rep 2020;47:7317-22.
15. Ates O, Dolek B, Dalyan L, et al. The association between BsmI variant of vitamin D receptor gene and susceptibility to tuberculosis. Mol Biol Rep 2011;38:2633-6.
16. Fernández-Mestre M, Villasmil Á, Takiff H, Alcalá ZF. NRAMP1 and VDR gene polymorphisms in susceptibility to tuberculosis in Venezuelan population. Dis Markers 2015;2015:860628.
17. Sekhar Miraj S, Vyas N, Kurian SJ, et al. Vitamin D receptor gene polymorphism and vitamin D supplementation on clinical/treatment outcome in tuberculosis: current and future perspectives. Expert Rev Anti Infect Ther 2022;20:1179-86.
18. Palmirotta R, Ferroni P, Ludovici G, et al. VEGF-A gene promoter polymorphisms and microvascular complications in patients with essential hypertension. Clin Biochem 2010;43:1090-5.
19. Marashian SM, Farnia P, Seyf S, et al. Evaluating the role of vitamin D receptor polymorphisms on susceptibility to tuberculosis among iranian patients: a case-control study. Tuberk Toraks 2010;58:147-58.
20. Chobanian A V, Bakris GL, Black HR, et al. Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. Hypertension 2003;42:1206-52.

21. Hall S, Vincenzi DA. Software: SPSS for Windows SPSS Inc. 233 S. Wacker Dr., 11th Floor Chicago, IL 60606 (312) 651-3000 www.spss.com. Ergon Des Q Hum Factors Appl 2001;9:27-9.
22. Zhang Y, Hedo R, Rivera A, et al. Post hoc power analysis: is it an informative and meaningful analysis? Gen Psychiatr 2019;32:e100069.
23. Stevens H, Ximenes RAA, Dantas OMS, Rodrigues LC. Risk factors for tuberculosis in older children and adolescents: a matched case–control study in Recife, Brazil. Emerg Themes Epidemiol 2014;11:20.
24. Wang J, Shen H. Review of cigarette smoking and tuberculosis in China: intervention is needed for smoking cessation among tuberculosis patients. BMC Public Health 2009;9:292.
25. Horne DJ, Campo M, Ortiz JR, et al. Association between smoking and latent tuberculosis in the US population: an analysis of the National Health and Nutrition Examination Survey. PLoS One 2012;7:e49050.
26. Watkins RE, Plant AJ. Does smoking explain sex differences in the global tuberculosis epidemic? Epidemiol Infect 2006;134:333-9.
27. Rappoport N, Toung J, Hadley D, et al. A genome-wide association study identifies only two ancestry specific variants associated with spontaneous preterm birth. Sci Rep 2018;8:226.
28. Barchitta M, Maugeri A, La Rosa MC, et al. Single nucleotide polymorphisms in vitamin D receptor gene affect birth weight and the risk of preterm birth: results from the "mamma & bambino" cohort and a meta-analysis. Nutrients 2018;10:1172.
29. Sheikh IA, Ahmad E, Jamal MS, et al. Spontaneous preterm birth and single nucleotide gene polymorphisms: a recent update. BMC Genomics 2016;17:39-50.
30. O'Callaghan ME, MacLennan AH, McMichael GL, et al. Single-nucleotide polymorphism associations with preterm delivery: a case–control replication study and meta-analysis. Pediatr Res 2013;74:433-8.
31. Areeshi MY, Mandal RK, Dar SA, et al. A reappraised meta-analysis of the genetic association between Vitamin D receptor Bsm I (rs1544410) polymorphism and pulmonary tuberculosis risk. Biosci Rep 2017;37:BSR20170247.
32. Selvaraj P, Chandra G, Kurian SM, et al. Association of vitamin D receptor gene variants of BsmI, ApaI and FokI polymorphisms with susceptibility or resistance to pulmonary tuberculosis. Curr Sci 2003;1564-8.
33. Silva-Ramírez B, Saenz-Saenz CA, Bracho-Vela LA, et al. Association between vitamin D receptor gene polymorphisms and pulmonary tuberculosis in a Mexican population. Indian J

Tuberc 2019;66:70-5.
34. Hu Q, Chen Z, Liang G, et al. Vitamin D receptor gene associations with pulmonary tuberculosis in a Tibetan Chinese population. BMC Infect Dis 2016;16:469.
35. Li B, Wen F, Wang Z. Correlation between polymorphism of vitamin D receptor TaqI and susceptibility to tuberculosis: an update meta-analysis. Medicine 2022;101:e29127.
36. Lee YH, Song GG. Vitamin D receptor gene FokI, TaqI, BsmI, and ApaI polymorphisms and susceptibility to pulmonary tuberculosis: a meta-analysis. Genet Mol Res 2015;14:9118-29.
37. Miao R, Huang S, Li C, et al. An HLA class II locus, previously identified by a genome-wide association study, is also associated with susceptibility to pulmonary tuberculosis in a Chinese population. Infect Genet Evol 2018;64:164-7.
38. Lu T, Wang M, Liu N, et al. Transporter associated with antigen processing 1 gene polymorphisms increase the susceptibility to tuberculosis. Pharmgenomics Pers Med 2023;16:325-36.
39. Sinaga BYM, Amin M, Siregar Y, Sarumpaet SM. Correlation between Vitamin D receptor gene FOKI and BSMI polymorphisms and the susceptibility to pulmonary tuberculosis in an Indonesian Batak-ethnic population. Acta Med Indones 2014;46:275-82.
40. Sadykov M, Azizan A, Kozhamkulov U, et al. Association of genetic variations in the vitamin D pathway with susceptibility to tuberculosis in Kazakhstan. Cancer Sci 2017;15:1659-66.

Table 1. Primers and products' size for each VDR polymorphism.

Polymorphism	Primer	Product size
apaI and TaqI	5'-GGG ACG ATG AGG GAT GGA CAG AGC-3' 5'-GGA AAG GGG TTA GGT TTG ACA GGA-3	2000 bp
BsmI	5'-CAA CAA AGA CTA CAA GTA CCG CGT CAG GA-3' 5'-AAC CAG CGG GAA GAG GTC AAG GG-3'	825 bp
FokI	5'-AGCTGG CCC TGG CACTGA CTC TGC TCC-3' 5'-ATGGAA ACA CCT TGC TTC TTC TTC CTC-3'	265 bp

VDR, vitamin D receptor; bp, base pair.

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

Characteristics	Total, n (%)	PTB active, n (%)	PTB latent, n (%)	Healthy controls, n (%)	p-value
Age, mean±SD (years) 17 – 29 30 – 44 45 – 60 >60	34.43±11.81 105 (39.3) 113 (42.3) 44 (16.5) 5 (1.9)	33.99±2.60 39 (44.3) 35 (39.8) 10 (11.4) 4 (4.5)	32.90±9.32 33 (41.3) 38 (47.5) 9 (11.3) -0	36.05±12.77 33 (33.3) 40 (40.4) 25 (25.3) 1 (1.0)	0.099
Sex Male Female	 148 (55.4) 119 (44.6)	 52 (59.1) 36 (40.9)	 45 (56.3) 35 (43.7)	 51 (51.5) 48 (48.5)	0.064
Ras/etnic Makassar Bugis Toraja	 122 (45.7) 112 (41.9) 33 (12.4)	 34 (42.5) 34 (42.5) 6 (6.8)	 34 (42.5) 34 (42.5) 12 (15.0)	 40 (40.4) 44 (44.4) 15 (15.2)	0.135
Education level Basic Educational Moderate Educational Hihg Educational	 116 (43.4) 107 (40.1) 44 (16.5)	 35 (39.8) 40 (45.5) 13 (16.3)	 37 (46.3) 30 (37.4) 13 (16.3)	 44 (44.4) 37 (37.4) 18 (18.2)	**0.006***
Labor Yes No	 137 (51.3) 130 (48.7)	 37 (42.0) 51 (58.0)	 39 (48.8) 41 (51.2)	 61 (61.6) 38 (38.4)	0.165
Drinking of Alcohol Yes No	 12 (4.5) 255 (95.5)	 4(4.5) 84 (95.5)	 4 (5.0) 76 (95.0)	 4 (4.0) 95 (96.0)	0.074
Education level Basic Educational Moderate Educational Hihg Educational	 116 (43.4) 107 (40.1) 44 (16.5)	 35 (39.8) 40 (45.5) 13 (16.3)	 37 (46.3) 30 (37.4) 13 (16.3)	 44 (44.4) 37 (37.4) 18 (18.2)	**0.006***
Smoking Yes No	 79 (29.6) 188 (70.4)	 43 (48.9) 45 (51.1)	 16 (20.0) 64 (80.0)	 20 (20.2) 79 (79.8)	-0.258
Drinking of Alcohol Yes No	 12 (4.5) 255 (95.5)	 4(4.5) 84 (95.5)	 4 (5.0) 76 (95.0)	 4 (4.0) 95 (96.0)	**-0.011***
Education level Basic Educational Moderate Educational Hihg Educational	 116 (43.4) 107 (40.1) 44 (16.5)	 35 (39.8) 40 (45.5) 13 (16.3)	 37 (46.3) 30 (37.4) 13 (16.3)	 44 (44.4) 37 (37.4) 18 (18.2)	**-0.006***

PTB, pulmonary tuberculosis; SD, standard deviation. Significant value *p< 0.05.

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 value
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)	**0.000***
	C	102 (57.6)	147 (41.2)	0.642(0.503-0.821)	**0.000***
ApaI	rs7975232				
Genotype	GG	33 (37.5)	63 (35.2)	2.111 (1.803-2.472)	**0.000***
	GT	44 (50.0)	86 (48.0)	3.057(1.796-5.203)	**0.000***
	TT	11 (12.5)	30 (16.8)	7.545(3.560-15.994)	**0.000***
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)	**0.000***
	GA	32 (36.4)	66 (36.8)	1.022(0.602-1.736)	**0.000***
	AA	3 (3.4)	6 (3.4)	0.983(0.240-2.025)	**0.000***
Allele	G	138 (78.0)	278 (77.9)	1.006(0.651-1.553)	**0.000***
	A	39 (22.0)	79 (22.1)	1.004(0.750-1.342)	**0.000***
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)	**0.000***
	A	49 (27.7)	100 (28.0)	1.011(0.772-1.324)	**0.000***

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.

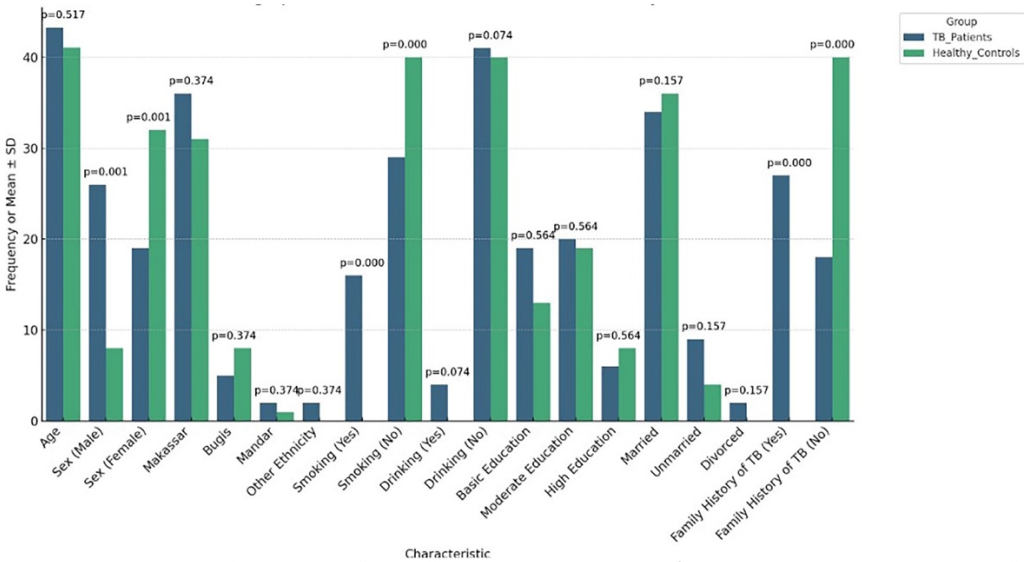

Figure 1. Sociodemographic characterization of PTB patients compared to healthy controls in the Kaluku Bodoa population Indonesia. The bar heights represent the frequencies or mean values of each characteristic, while the p-values above the bars indicate the statistical significance of the differences. Significant factors include sex (male predominance), smoking status, and family history of TB. Non-significant variables include age, ethnicity, education level, and marital status.

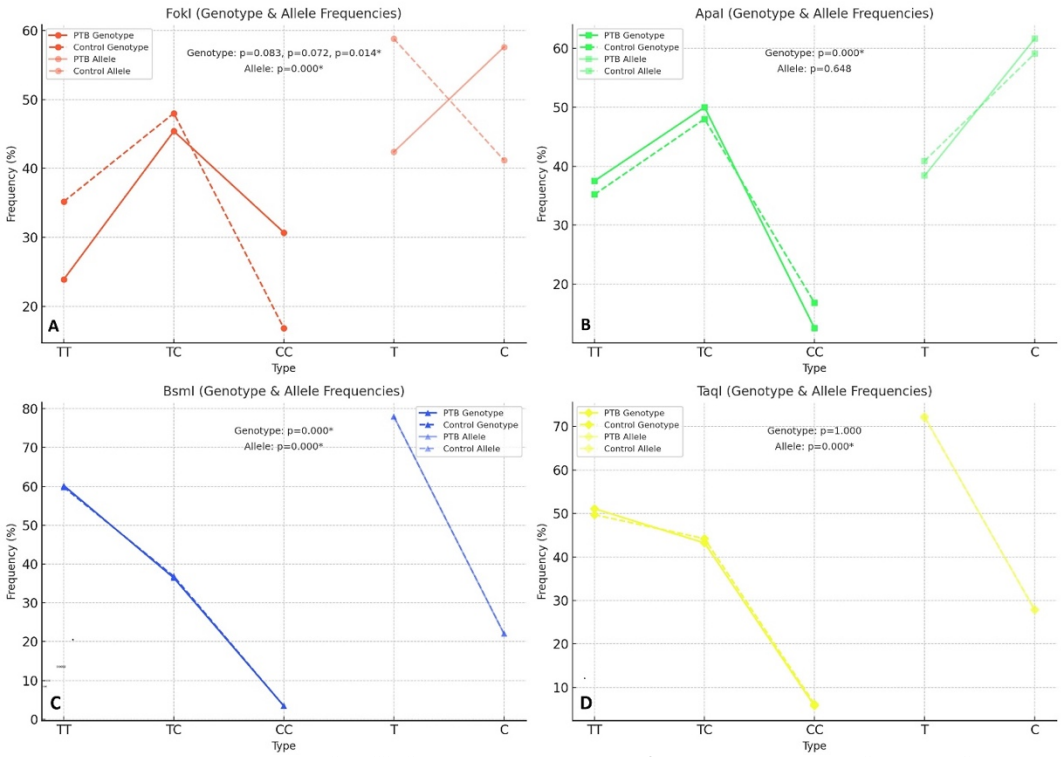

Figure 2. Genotype and Allele Frequencies of VDR Gene Polymorphisms in PTB and Control Groups. A. FokI (rs2228570): The frequencies of the TT, TC, and CC genotypes, as well as the T and C alleles, are compared between PTB patients and controls. Statistically significant differences are observed for the CC genotype (p = 0.014) and allele distributions (p = 0.000). B. ApaI (rs7975232): Genotype and allele frequencies display significant differences, particularly with the GT and TT genotypes (p = 0.000). However, allele distributions do not show significant differences (p = 0.648). C. BsmI (rs1544410): Genotype distributions (GG, GA, AA) and allele distributions (G, A) show statistically significant differences between PTB patients and controls (p = 0.000 for both). D. TaqI (rs731236): The genotype (TT, TC, CC) and allele (G, A) frequencies exhibit no significant differences between PTB patients and controls, as indicated by the p-values (p = 1.000 for genotype and p = 0.000 for allele).