Association of Vitamin D Receptor Polymorphism (rs2228570, rs1544410, rs7975232, and rs731236) and Macrophage Migration Inhibitory Factor -173 G/C (rs755622) with active TB

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Association of *Vitamin D Receptor* Polymorphism (rs2228570, rs1544410, rs7975232, and rs731236) and *Macrophage Migration Inhibitory Factor* -173 G/C (rs755622) with the Susceptibility of Active Pulmonary Tuberculosis in Makassar, Indonesia

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Abstract

BACKGROUND: The study of *Vitamin D Receptor* (*VDR*) and *Macrophage Migration Inhibitory Factor* (*MIF*) polymorphisms associated with active pulmonary tuberculosis (TB) (ATB) presents varying results.

AIM: This study aimed to investigate the association between *VDR* rs2228570, rs1544410, rs7975232, and rs731236 and *MIF* -173 G/C (rs755622) single-nucleotide polymorphism (SNP), with susceptibility of developing ATB, and positivity of interferon-gamma release assay (IGRA) results (in household contact).

MATERIALS AND METHODS: This study involved 83 ATB and 73 household contacts in Makassar. We checked IGRA based on ELISA in household contacts by using QuantiFERON-TB Gold Plus test, and we found that 61.64% (n = 45) of household contacts had positive IGRA. Polymorphism examination was carried out by Sanger sequencing.

RESULTS: *VDR* rs2228570 T/T and T/C-T/T were significantly associated with a higher risk of active TB. *VDR* rs7975232 G/G genotype was associated with an increased risk of developing active TB compared to T/T-T/G. Haplotype analysis of *VDR* rs2228570, rs1544410, rs7975232, and rs731236 and combination with *MIF* rs755622 demonstrated that TGGTG was observed to have a higher risk of TB.

CONCLUSIONS: The combination of *VDR* and *MIF* variants may contribute to the susceptibility of active tuberculosis disease.

Introduction

Tuberculosis (TB) remains the world health problem, where Indonesia ranks second in the largest TB incidence, and is one of the four countries that contribute to 44% of TB cases worldwide. Most of the increase in global number of TB cases since 2013, is explained by the trends occurred in Indonesia, where the number increased from 331,703 in 2015 to 562,049 in 2019 (+69%) [1].

The main etiology *Mycobacterium tuberculosis* (*Mtb*), could escape from the host immune system and remain dormant until the immunity is compromised. Some people with latent TB infection could develop active TB, and some remain latent [2]. The interpersonal variability of clinical manifestations is considered to be the effect of variations in human genes that control the body's defenses. The genetic vulnerability has been suggested as one of the most important explanations for individual TB risk. Several investigations have confirmed the involvement of genetic factors in the disease, including

twin studies, adoption studies, and population-based case–control association studies [3]. Genetic factors that play a role in the immune response may affect the development of pulmonary TB [4], [5], [6], [7].

Vitamin D receptors (VDRs), which are found on most immune cells, including macrophages, dendritic cells, neutrophils, and lymphocytes, have been reported to modulate both innate and adaptive immune responses [8]. VDR is a determinant of the biological activity of calcitriol ($1,25(OH)_2D_3$), which is the active form of Vitamin D [8]. Calcitriol must bind to VDR as a receptor to be able to actively work on target cells [9]. Its effects include increased synthesis of the innate immune system components, such as cathelicidin, which plays an important role in killing mycobacteria [10], [11]. Migration inhibitory factor (MIF) has also been investigated to be involved in the immune system, as an inflammatory mediator involved in the regulation of macrophage activity, chemotaxis, and secretion of pro-inflammatory cytokines [12]. Initially, MIF was described as a property of immune activity, isolated from supernatant T-lymphocytes, and found to function to inhibit the random migration of macrophages. MIF is released at the infection site, causing macrophages to localize and carry out antigen processing and phagocytosis [13]. VDR is encoded by the *VDR* gene which is located on chromosome 12 (12q13-14) while MIF is encoded by the *MIF* gene which is located on chromosome 22 (22q11.2). Variations in genes may affect the translation output, which can lead to changes in the structure, stability, or activity of the proteins produced [14], [15].

Various *VDR* polymorphisms have been observed in association with TB, particularly in the *FokI* single-nucleotide polymorphism (SNP) (rs2228570) in exon 2, *BsmI* (rs1544410) in intron 8, *ApaI* (rs7975232) in intron 8, and *TaqI* (rs731236) in intron 9, yet with inconsistent findings [9], [16], [17]. Besides that, the data analysis regarding the haplotype is still limited [16]. Likewise, for the *MIF* -173 G/C SNP (rs755622), existing studies linking it to the risk of TB have shown varying results [18], [19], [20]. Thus, more studies are needed to confirm the association of these polymorphisms to TB risk. The study of genetic association with TB is expected to facilitate the development of vaccinomic and immunonomic therapies for TB.

Subjects and Methods

Recruitment of research participants and sampling

This study involved 156 samples, consisting of 83 new active pulmonary TB patients and 73 household contacts. Active pulmonary TB patients were recruited from the Makassar Center for Community Lung Health (BBKPM) and were diagnosed comprehensively through anamnesis, physical examination, chest radiograph, and positive smear sputum which were further confirmed by culture using BD BACTEC MGIT 960 (BD, Sparks, MD, USA). The age of recruited patients was 15 years or more, with no previous use of anti-TB drugs, and HIV-positive TB patients were excluded. The household contacts recruited were people living in the same house for at least 6 months with targeted TB patients, have no clinical symptoms of TB, history of TB, or a history of previous anti-TB drug consumption. All research subjects have signed the consent form to be involved in the study.

This research has obtained ethical approval from the Ethics Commission of the Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.

Interferon-gamma (IFN-γ) release assay (IGRA) examination on household contact samples

IGRA examination was conducted on household contact samples using QuantiFERON-TB Gold Plus (QFT-Plus, Hilden, Germany). This assay was an *in vitro* diagnostic test using a peptide cocktail protein ESAT-6 and CFP-10 to stimulate blood cells. Detection IFN-γ with ELISA (enzyme-linked immunosorbent assay) was used to identify the antigen response associated with infection of *Mtb* [21], [22]. Blood samples from household contacts which were collected with four tubes of QFT-Plus were then further treated according to the kit instruction. Furthermore, an ELISA examination was carried out using the QFT-Plus ELISA test kit according to the manual instruction and readings were carried out using the ELISA reader and subsequently analyzed using QuantiFERON-TB Gold Plus Test analysis software (available from https://www.quantiferon.com). The results were positive, negative, and indeterminate. The indeterminate results were excluded, thus the household contact group was then divided into two groups according to the IGRA results, $IGRA^{(+)}$ and $IGRA^{(-)}$ of household contacts.

DNA extraction

Genomic DNA was isolated from blood using Geneaid DNA Extraction Kit (Geneaid, Taiwan). Briefly, as much as 200 ml of plasma and buffy coat was mixed with 20 ul of Proteinase K and then incubated for 5 min at 60° C. Then, the mixture was added with GSB buffer and re-incubated at 60°C for 5 min. After that, 96% ethanol was added for DNA binding and then the mixture was transferred to a spin column and centrifuged. Afterward, washing was conducted using W1 buffer and wash buffer. Eventually, the elution buffer was dropped right in the middle of the spin column matrix, left for at least 3 min, and then centrifuged to obtain the DNA extract of the sample.

DNA amplification and sequencing to determine SNP

VDR and *MIF* polymorphism was detected by PCR followed by sequencing. PCR was performed using KAPA Taq ReadyMix (Roche, USA) to amplify the targeted sequence using specific primers, to detect rs2228570, rs1544410, rs7975232, rs731236, and rs755622 SNPs (Table 1). PCR product was visualized on 2% agarose gel and sent to 1st BAse (Apical Scientific Sdn. Bhd.) to be sequenced.

Statistical analysis

This study uses the IBM SPSS Statistics for Windows, version 25.0 (IBM Corp. Armonk, NY, USA) application to perform statistical analysis. Categorical data were analyzed using the Chi-square to determine the significances between groups. While numerical data with normal distribution were analyzed using the t-test and one-way ANOVA for comparisons in two and three groups, respectively. A p-value under 0.05 was considered as a statistically significant value. The deviation of polymorphisms from the Hardy–Weinberg equilibrium was examined using $\chi 2$ model, by comparing the observed and expected genotype frequencies in the two groups. Individual genotype analysis was performed using age- and sex-corrected logistic regression model for genotypes that were significantly detected using the SNPStats online software (https://www.snpstats.net) [25]. Haplotype analysis was also carried out by using SNPStats online software and p-value below 0.05 was considered statistically significant. Linkage disequilibrium (LD) was analyzed using the SNPStats online software with D' and r^2 as the measurement values.

Results

Sample characterization

A total of 83 samples of pulmonary TB patients and 73 household contacts participated in this study. All samples were homogeneous by age (p = 0.333). Based on the IGRA examination with the QuantiFERON-Gold Plus TB kit, it was found that 45 household contacts (61.64%) had positive IGRA results and the rest (38.35%) had negative IGRA results. The characterization of the participa is described in Table 2. Gender has a significant relationship with the incidence of TB and the positivity of IGRA results (p = 0.001). There was a significant difference in body mass index (BMI) between the three groups in this study (p < 0.0001). In addition, more positive IGRA samples (62.2%) were found in household contacts' spouses (husband or wife), compared to the other household contacts such as parents, children, and siblings. Moreover, about 55.4% of the pulmonary TB patient group were smokers, and this result was statistically different when compared to the other group (p < 0.0001).

Single SNP analysis

The genotype frequencies across all SNPs were consistent with Hardy–Weinberg equilibrium. The sequencing chromatogram results are represented in Figures 1 and 2 on Supplementary Materials for VDR and MIF SNPs, respectively. Table 3 shows the distribution of *VDR* genotype of each SNP in ATB patients as the case group and household contacts as the control group. The frequencies of *VDR* rs2228570 T/T were more in ATB patients than in control, and this genotype was significantly associated with a higher risk of ATB.

Table 1: Primers and PCR conditions used in this study

SNP	Primer	Target	PCR conditions						Primer Reference
			P	C	D	A	E	F	
VDR rs2228570	F 5'-AGC TGG CGC TGG CAC TGA CTC TGC TCT-3' R 5'-ATG GAA CCT TGC TTC TTC TCC CTC-3'	267 bp	94°C 5 m	35 x	94°C 30 s	58°C 30 s	72°C 20 s	72°C 7 m	[10]
VDR rs1544410	F 5'-AGT GTG CAG GCG ATT CGTA G-3' R 5'-ATA GGC AGA ACC ATC TCTC AG-3'	191 bp	94°C 5 m	35x	94°C 30 s	55°C 30 s	72°C 20 s	72° C 5 m	[23]
VDR rs7975232 and *VDR* rs731236	F 5'-CAG AGC ATG GAC AGG GAG CAA-3' R 5'-ACT TCG AGC ACA AGG GGC GTT AC	500 bp	94°C 5 m	35x	94°C 30 s	50°C 30 s	72°C 1 m	72° C 5 m	HUM-RC Laboratory
MIF -173 G/C rs755622	F 5'-CTG ACT TCT CGG ACA CCA CT-3' R 5'-AAG GGT AAG GGG CCA TCT TC-3'	352 bp	95°C 5 m	40x	95°C 30 s	60°C 30 s	72°C 1 m	72° C 5 m	[24]

P: Predenaturation; C: Cycles; D: Denaturation; A: Annealing; F: Final extension; M: minutes; s: Seconds

Table 2: Characterization of participants in this study

Variables	Active pulmonary tuberculosis n = 83	IGRA(+) household contacts n = 45	IGRA(-) household contacts n = 28	p-value
Sex				
• Male	44 (53%)	14 (31.1%)	5 (17.9%)	0,001[a]
• Female	39 (47%)	31 (68.9%)	23 (82.1%)	
Age (years)	41 ± 14.17 (44)	39.6 ± 13.81 (40)	36.57 ± 11.63 (37.5)	0.333[b]
BMI (kg/m^2)	19.03 ± 3.42 (18.75)	22.95 ± 4.5 (22.49)	26.44 ± 5.66 (25.55)	<0.0001[b]
Relationship to index cases (household contacts only)				
• Parent/son/daughter		11 (24.4%)	9 (32.1%)	0.025[a]
• Spouse		28 (62.2%)	9 (32.1%)	
• Others		6 (13.3%)	10 (35.7%)	
History of smoking habits				
• Yes	46 (55.4%)	11 (24.4%)	4 (14.3%)	<0.0001[a]
• No	37 (44.6%)	34 (75.6%)	24 (85.7%)	

[a]Chi-square test. [b]One-way ANOVA test.

Table 3: Distribution of genotype and their association in pulmonary tuberculosis compared to control (household contacts)

SNP	Genotype	Control (household contacts) n (%)	Active pulmonary tuberculosis patients n (%)	OR (95% CI)	p-value
rs2228570 (*VDR FokI*)	Codominant				
	C/C	29 (39.7)	20 (24.1)	1.00	0.04
	T/C	38 (52)	46 (55.4)	1.84 (0.85–3.99)	
	T/T	6 (8.2)	17 (20.5)	4.16*(1.29–13.42)	
	Dominant				
	C/C	29 (39.7)	20 (24.1)	1.00	0.041
	T/C-T/T	44 (60.3)	63 (75.9)	2.16* (1.02–4.55)	
	Recessive				
	C/C-T/C	67 (91.8)	66 (79.5)	1.00	0.045
	T/T	6 (8.2)	17 (20.5)	2.82 (0.98–8.12)	
rs1544410 (*VDR BsmI*)	Codominant				
	G/G	51 (69.9)	63 (75.9)	1.00	0.12
	G/A	22 (30.1)	18 (21.7)	0.59 (0.27–1.30)	
	A/A	0 (0)	2 (2.4)	NA (0.00–NA)	
	Dominant				
	G/G	51 (69.9)	63 (75.9)	1.00	0.29
	G/A-A/A	22 (30.1)	20 (24.1)	0.66 (0.30–1.43)	
	Recessive				
	G/G-G/A	73 (100)	81 (97.6)	1.00	0.12
	A/A	0 (0)	2 (2.4)	NA (0.00–NA)	
rs7975232 (*VDR ApaI*)	Codominant				
	A/A	21 (28.8)	38 (45.8)	1.00	0.13
	T/G	38 (52)	31 (37.4)	0.49 (0.22–1.05)	
	T/T	14 (19.2)	14 (16.9)	0.46 (0.17–1.26)	
	Dominant				
	G/G	21 (28.8)	38 (45.8)	1.00	0.044
	G/T-T/T	52 (71.2)	45 (54.2)	0.48* (0.23–0.99)	
	Recessive				
	G/G-G/T	59 (80.8)	69 (83.1)	1.00	0.41
	T/T	14 (19.2)	14 (16.9)	0.69 (0.28–1.70)	
rs731236 (*VDR TaqI*)	Codominant				
	T/T	61 (83.6)	72 (86.8)	1.00	0.47
	T/C	11 (15.1)	11 (13.2)	0.91 (0.35–2.41)	
	C/C	1 (1.4)	0 (0)	0.00 (0.00–NA)	
	Dominant				
	T/T	61 (83.6)	72 (86.8)	1.00	0.71
	T/C-C/C	12 (16.4)	11 (13.2)	0.83 (0.32–2.16)	
	Recessive				
	T/T-T/C	72 (98.6)	83 (100)	1.00	0.22
	C/C	1 (1.4)	0 (0)	0.00 (0.00–NA)	
rs755622 (*MIF -173 G/C*)	Codominant				
	G/G	38 (52)	50 (60.2)	1.00	0.84
	G/C	33 (45.2)	31 (37.4)	1.05 (0.47–2.39)	
	C/C	2 (2.7)	2 (2.4)	0.05 (0.07–4.49)	
	Dominant				
	G/G	38 (52)	50 (60.2)	1.00	0.99
	G/C-C/C	35 (48)	33 (39.8)	1.00 (0.45–2.20)	
	Recessive				
	G/G-G/C	71 (97.3)	81 (97.6)	1.00	0.56
	C/C	2 (2.7)	2 (2.4)	0.54 (0.07–4.29)	

OR and p value are adjusted by age, sex, and history of smoking. NA: not applicable. Asterisk means significative OR.

The dominant model showed that T/C-T/T was also significantly associated with increased risk of ATB and C/C was associated with reduced risk of ATB. Furthermore, a significant association was observed in T/T genotype when ATB group was compared to IGRA$^{(+)}$ household contact (Table 4). *VDR* rs1544410 showed no association between genotypic differences and increased ATB risk, however, specifically, observed genotype G/A was associated with a reduced risk of ATB compared to G/G in men (data not shown). Using the codominant model, no relationship was discovered between the *VDR* rs7975232 and ATB. An association was only observed using dominant model, where G/G genotype was associated with about two-fold increased risk of contracted TB compared to T/T-T/G. A significant association was also found when ATB was compared to IGRA$^{(+)}$ household contact group, where T/T was associated with a lower risk of ATB. This association is further clarified by using the dominant model where G/T-T/T is also associated with reduced risk of ATB, and this means that G/G is associated with an increased risk of TB. While polymorphisms *VDR* rs731236 and *MIF* -173 G/C rs755622 were not associated with an increased risk of TB in this study. We also examined

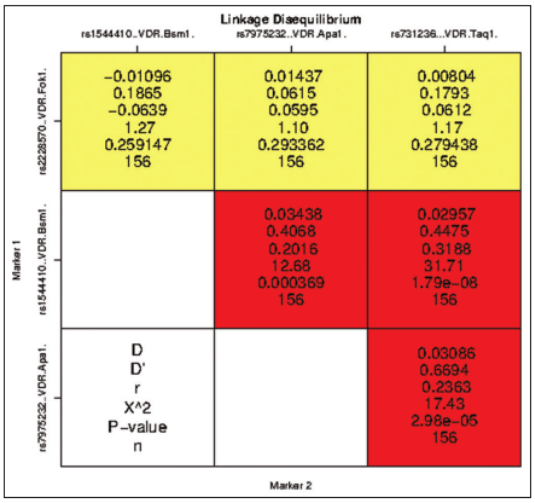

Figure 1: The graph demonstrates the linkage disequilibrium (LD) on VDR rs1544410, rs7975232, and rs731236. The square shows the association of genes listed on the horizontal curve and those listed on the vertical curve. The statistical parameters of LD were listed on each square. A darker color indicates that the genes are in strong LD. A strong LD relationship was also shown with a significant ($p < 0.05$)

Table 4: Distribution of genotype and their association in pulmonary tuberculosis compared to IGRA(+) household contacts

SNP	Genotype	IGRA(+) household contacts n (%)	Active pulmonary tuberculosis patients n (%)	OR (95% CI)	p-value
rs2228570 (*VDR FokI*)	Codominant				
	C/C	17 (37.8)	20 (24.1)	1.00	0.097
	T/C	24 (53.3)	46 (55.4)	1.74 (0.73–4.13)	
	T/T	4 (8.9)	17 (20.5)	3.94* (1.05–14.82)	
	Dominant				
	C/C	17 (37.8)	20 (24.1)	1.00	0.091
	T/C-T/T	28 (62.2)	63 (75.9)	2.05 (0.89–4.73)	
	Recessive				
	C/C-T/C	41 (91.1)	66 (79.5)	1.00	0.079
	T/T	4 (8.9)	17 (20.5)	2.75 (0.83–9.13)	
rs1544410 (*VDR BsmI*)	Codominant				
	G/G	32 (71.1)	63 (75.9)	1.00	0.28
	G/A	13 (28.9)	18 (21.7)	0.68 (0.31–1.66)	
	A/A	0 (0)	2 (2.4)	NA (0.00–NA)	
	Dominant				
	G/G	32 (71.1)	63 (75.9)	1.00	0.55
	G/A-A/A	13 (28.9)	20 (24.1)	0.76 (0.32–1.83)	
	Recessive				
	G/G-G/A	45 (100)	81 (97.6)	1.00	0.17
	A/A	0 (0)	2 (2.4)	NA (0.00–NA)	
rs7975232 (*VDR ApaI*)	Codominant				
	A/A	11 (24.4)	38 (45.8)	1.00	0.081
	G/T	23 (51.1)	31 (37.4)	0.45 (0.18–1.12)	
	T/T	11 (24.4)	14 (16.9)	0.32* (0.11–0.97)	
	Dominant				
	G/G	11 (24.4)	38 (45.8)	1.00	0.032
	G/T-T/T	34 (75.6)	45 (54.2)	0.41* (0.18–0.94)	
	Recessive				
	G/G-G/T	34 (75.6)	69 (83.1)	1.00	0.16
	T/T	11 (24.4)	14 (16.9)	0.50 (0.19–1.31)	
rs731236 (*VDR TaqI*)	Codominant				
	T/T	36 (80)	72 (86.8)	1.00	0.37
	T/C	8 (17.8)	11 (13.2)	0.80 (0.28–2.27)	
	C/C	1 (2.2)	0 (0)	0.00 (0.00–NA)	
	Dominant				
	T/T	36 (80)	72 (86.8)	1.00	0.52
	T/C-C/C	9 (20)	11 (13.2)	0.71 (0.26–1.98)	
	Recessive				
	T/T-T/C	44 (97.8)	83 (100)	1.00	0.18
	C/C	1 (2.2)	0 (0)	0.00 (0.00–NA)	
rs755622 (*MIF -173* G/C)	Codominant				
	G/G	24 (53.3)	50 (60.2)	1.00	0.9
	G/C	19 (42.2)	31 (37.4)	0.83 (0.37–1.84)	
	C/C	2 (4.4)	2 (2.4)	0.87 (0.11–6.83)	
	Dominant				
	G/G	24 (53.3)	50 (60.2)	1.00	0.64
	G/C-C/C	21 (46.7)	33 (39.8)	0.83 (0.38–1.81)	
	Recessive				
	G/G-G/C	43 (95.6)	81 (97.6)	1.00	0.95
	C/C	2 (4.4)	2 (2.4)	0.93 (0.12–7.17)	

OR and P value are adjusted by age, sex, and history of smoking. NA: Not applicable. Asterisk means significative OR

Table 5: Haplotype analysis of *VDR* gene (rs2228570, rs1544410, rs7975232, and rs731236)

Haplotype				Control (household contact)	Active tuberculosis patients	OR (95% CI)	p-value
rs2228570-rs1544410-rs7975232							
C	G	G		0.3156	0.3118	1.00	---
T	G	G		0.125	0.2834	1.84 (0.79–4.28)	0.16
C	G	T		0.2525	0.1208	0.43 (0.16–1.16)	0.097
T	G	T		0.1562	0.1515	1.73 (0.57–5.19)	0.33
C	A	G		0.046	0.0493	0.80 (0.21–3.04)	0.75
C	A	T		0.0433	0.0361	0.83 (0.09–7.75)	0.87
rs2228570-rs1544410-rs731236							
C	G	T		0.4549	0.5369	1.00	---
T	G	T		0.3673	0.2734	2.66* (1.18–5.98)	0.02
C	A	T		0.0903	0.0716	1.11 (0.36–3.46)	0.85
A	A	C		0.0261	0.023	0.57 (0.04–9.15)	0.7
C	G	C		0.024	0.022	1.79 (0.19–16.99)	0.61
C	A	C		0.0141	0.0271	1.44 (0.11–18.00)	0.78
T	G	C		0.0127	0.017	0.61 (0.04–10.08)	0.25
T	A	T		0.0105	0.0291	0.68 (0.01–43.98)	0.86
rs1544410-rs7975232-rs731236							
C	G	T		0.401	0.5971	1.00	---
T	T	T		0.4099	0.236	0.51* (0.27–0.94)	0.034
A	T	T		0	0.0532	0.55 (0.12–2.61)	0.45
A	G	T		0.1	0.0475	0.81 (0.17–3.73)	0.78
A	T	C		0.0273	0.0319	0.69 (0.11–4.19)	0.69
C	T	C		0.0149	0.0344	7.07 (0.01–6619.79)	0.58
rs2228570-rs1544410-rs7975232-rs731236							
C	G	G	T	0.3073	0.3171	1.00	---
T	G	G	T	0.0968	0.2802	1.96 (0.83–4.61)	0.13
C	G	T	T	0.2064	0.0951	0.30* (0.10–0.93)	0.039
T	G	T	T	0.2045	0.142	2.03 (0.61–6.71)	0.25
C	A	G	T	0.0959	0.0473	1.28 (0.27–6.07)	0.76
T	G	T	C	0.0137	0.0121	0.50 (0.02–11.63)	0.67

OR and p value are adjusted by age, sex, and history of smoking. Asterisk means significative OR.

the association between IGRA positivity in household contacts based on the QFT-Plus results. Of all the SNPs analyzed with various models, there were no relationship between these polymorphisms and an increased risk of positive QFT-Plus (Supplementary Table 1 and Supplementary Figures 1 and 2).

Table 6: Haplotype analysis of *VDR* gene (rs2228570, rs1544410, rs7975232, and rs731236) and combination with *MIF* -173 (rs755622)

rs2228570	rs1544410	rs7975232	rs731236	rs755622	Control (Household contact)	Active tuberculosis patients	OR (95% CI)	p-value
C	G	G	T	G	0.2397	0.2151	1.00	---
T	G	G	T	G	0.046	0.2604	11.01*(1.91–63.50)	0.0082
C	G	T	T	G	0.1351	0.1043	0.78 (0.21–2.87)	0.7
T	G	T	T	G	0.1535	0.0788	4.18 (0.77–22.68)	0.099
C	G	G	T	C	0.0679	0.0958	3.77 (0.78–18.24)	0.1
T	G	T	T	C	0.0521	0.0521	3.24 (0.44–24.03)	0.25
T	G	G	T	C	0.0502	0.026	0.43 (0.05–3.84)	0.45
C	A	G	T	G	0.0957	0.0335	2.22 (0.19–26.55)	0.53
T	G	T	C	G	0.0132	0.0228	1.10 (0.12–9.94)	0.93

Asterisk means significative OR frequency threshold for rare haplotypes: 0.01.

Multiple SNP analysis

VDR rs1544410, rs7975232, and rs731236 were in LD (Figure 1). The analysis compiled of *VDR* gene rs2228570, rs1544410, rs7975232, and rs731236 is described in Table 5. Haplotype of TG-T on rs2228570-rs1544410-rs731236 was found to be associated with a higher risk of ATB, and -GTT rs1544410-rs7975232-rs731236 was observed to be associated with a lower risk of ATB. Haplotype analysis using four SNPs of *VDR* found that CGTT was associated with a significantly reduced risk of ATB. The analysis of association between haplotype of *VDR* gene (rs2228570, rs1544410, rs7975232, and rs731236) in combination with *MIF* -173 (rs755622) and TB is presented in Table 6. Using the logistic regression model, the TGGTG was observed to have a higher risk of TB (OR: 11.01 [1.91–63.50], p = 0.0082).

Discussion

Our result on *VDR* rs2228570 (*VDR FokI*) signified a consistent result to prior study in Beijing, Iran, as well as New Delhi, and Shimla India, which uncovered that recessive homozygous (TT) genotype was associated with the increased risk of TB pulmonary [11], [26], [27], [28]. The change in C to T is known to create alternative codons (ACG-ATG), making the protein is three amino acids longer, namely methionine-glutamine-alanine. It is suspected that this longer protein variant has lower activity than the short VDR protein variant (generated by the C allele) [29].

As for the *VDR* polymorphism rs7975232 (*VDR ApaI*), our analysis showed significant results only for the dominant comparison model, where the G/G genotype had a two-fold risk of having TB than the T/G-T/T genotypes. Our finding was similar to research in West Africa [30] but in contrast to research in Romania which showed that aa (GG) might serve as a protective allele on TB development [31]. The other studies from Beijing (China), Taiwan, and Venezuela did not find an association between *VDR* polymorphism rs7975232 and TB [11], [32], [33]. *VDR* rs7975232 is an intronic variant considered to affect VDR translation through changes of the splices site [34].

There was no association between *VDR* rs1544410 (*VDR BsmI*) polymorphism and TB in this study. *VDR* rs1544410 can be in disequilibrium with other functional polymorphisms that regulate *VDR* gene expression [35]. Considering the research results which investigated the links between the *VDR* rs1544410 and risk of TB showed inconsistent results, for example, studies in Kolkata (India), Zahedan (Iran), and Beijing (China) did not find an association between the *VDR* rs1544410 and risk of TB [11], [26], [36]. Contrarily, other studies in Taiwan, Shimla (India), and even in Medan (Indonesia), a different province from our study site, revealed different results [28], [33], [37]. Using the meta-analysis method, some researchers were used to conclude various publications. Some of them concluded that there was no relationship between *VDR* rs1544410 and the risk of TB, while some concluded that the association was only found in the Asian population [9], [16], [38].

MIF gene promoter polymorphisms have been associated with overproduction of MIF and found to provide an increased risk of susceptibility to chronic inflammatory disease [39], [40]. Several studies have reported that the C allele of MIF -173 G/C (rs755622) was linked to TB in Colombia, South Africa, and the East China population [19], [15], [41], [42]. However, this study did not found an association between the genotype of *MIF* -173 G/C with the risk of pulmonary TB, and this result was similar to a study in the Brazilian population [18]. The diversity in different ethnicities or populations and diverse clinical features may contribute to the genotype distribution of *VDR* and *MIF* polymorphisms and different results explaining this correlation to TB. The single SNP alone may not explain much of the genetic association to TB susceptibility, hence several SNPs may be considered for analysis. Haplotype association can be an alternative in this regard and maybe a more useful mapping method than SNP analysis alone.

Based on haplotype analysis of the *VDR* gene (rs2228570, rs1544410, rs7975232, and rs731236) in combination with *MIF* -173 G/C (rs755622), the TGGTG was observed to have a higher risk of TB (OR: 11.01 [1.91–63.50], p = 0.0082). We can see that the other combinations of TG---, i.e., TGTTG, TGTTC, and TGTCG have OR value >1, are also likely to be associated with TB risk. However, the value of 95% CI range exceeds the value 1, indicating a nonsignificant value. Thus, we can assume that TG--- (the combination

of allele T on rs2228570 and G on rs1544410) may be a potential predictor to develop TB disease, but it should be in series with --GTG on rs7975232, rs731236, and rs755622, respectively.

A study in Iran showed that f-B-T (TGT) and f-b-t (TAC) on rs2228570-rs1544410-rs731236 may contribute to an increased risk of TB. This result indicated that G and A allele on rs1544410 and T and C on rs731236 could be a potential risk allele in ATB development if they were together with T allele on rs2228570. Our study showed a similar result on TGT, but not on TAC. The association of the TGT genotype decreased when compiled with rs7975232, whereas TGGT (G on rs7975232) only showed a marginal association with an increased risk of TB and TGTT (T on rs7975232) did not show a significant association with TB. The lack of association with TGTT may be explained by the combination rs1544410-rs7975232-rs731236 analysis, which showed that haplotype -GTT significantly reduces the risk of TB. However, when TGGT was combined with the G allele at MIF -173 G/C rs755622, the risk of susceptibility increased. This suggests that more SNPs involved in the analysis may further clarify the association of genetic variants to susceptibility to TB.

IGRA indicated the presence of specific antibodies in the body of a patient whose immune system has been exposed to TB, and the levels of IFN-specific *Mtb* produced have reached the threshold of positive IGRA results. The principle of IGRA is that when individual T-cells infected with TB infection restimulated with the *Mtb* antigen, they release IFN-γ cytokines [43]. A positive result can indicate the presence of *Mtb* bacteria, both in active and dormant status. IGRA is often used to screen as well as to diagnose latent TB, although IGRA is not the only method used to identify a person with latent TB. Confirmation of latent TB requires clinical examination as well as a chest radiograph that is clear of TB spots. There is another method that can be used to detect TB latency other than the IGRA, called TST. Yet, IGRA is considered to have higher sensitivity and specificity values [44]. The results of this study indicated that *VDR* polymorphisms may be associated with a person's susceptibility to developing active TB, but not with IGRA positivity. Thus, we might consider the possibility that the *VDR* genotype does not affect either person infected with TB or not (because the VDR genotype is not associated with an increased risk of positive IGRA). Nevertheless, this *VDR* polymorphism may predispose a person to develop active TB disease. Our findings showed that the T allele on *VDR* rs2228570 was associated with an increased risk of active TB, but it was not associated with the positivity of IGRA. However, when he is later infected with TB, the risk may be more likely to develop into active TB. This presumption can be supported by the finding of a significant association which was observed in T/T genotype when ATB group was compared to IGRA$^{(+)}$ household contact. Surely, this argument requires further research, whether there are certain polymorphisms in household contacts who are susceptible to developing latent TB to active TB, by following their disease course.

The authors are aware of the limitations of this study. The small number of samples may cause certain genotypes to be unidentified in the population. Furthermore, IGRA was performed only once, without a follow-up upon the IGRA results. Hence, the possible transition from negative to positive IGRA results may occur and influence the results. In addition, this study did not identify the genotype or species of *Mycobacterium*, which may need to be investigated, as a factor in influencing human genetic susceptibility to certain genotypes or strains of *Mycobacterium*. Previous studies have shown genetic diversity of Mtb isolates in a similar city where our study was conducted [45]. However, the sequencing method was applied in this study, considerably more accurate and feasible than the widely used RFLP method [46] of previous studies. The results of this study further strengthen the previous research that there is an association between the combination of VDR with MIF and TB. It is expected that these findings would provide more insight into the link between VDR and a person's susceptibility to active TB. Since Indonesia is a country with the second-highest number of TB cases, it may be difficult for its population to avoid exposure to TB. However, being aware of the higher risk in a particular population, certain efforts can be increased to prevent the development of the disease.

Conclusion

VDR rs2228570 T/T was significantly associated with a higher risk of active TB. In addition, the dominant model showed that T/C-T/T was also significantly associated with an increased risk of active TB. *VDR* rs7975232 G/G genotype was associated with an increased risk of developing active TB compared to T/T-T/G. Haplotype analysis of *VDR* rs2228570, rs1544410, rs7975232, and rs731236 and combination with *MIF* -173 (rs755622) demonstrated that TGGTG was observed to have a higher risk of TB. In summary, the combination of *VDR* and *MIF* variants may contribute to the susceptibility to active tuberculosis. Further research is needed to include more samples and more combinations of other polymorphism sites so that the role of genetics in the susceptibility or resistance of TB can be better understood.

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References

1. World Health Organization. Global Tuberculosis Report 2020. Geneva: World Health Organization, 2020. Available from: https://www.apps.who.int/iris/rest/bitstreams/1312164/retrieve [Last accessed on 2021 Jul 17].
2. World Health Organization. Guidelines on the Management of Latent Tuberculosis Infection; 2015. Available from: https://apps.who.int/iris/bitstream/10665/136471/1/9789241548908_eng.pdf [Last accessed on 2021 Jul 16].
3. Moller M, Hoal EG. Current findings, challenges and novel approaches in human genetic susceptibility to tuberculosis. Tuberculosis. 2010;90(2):71-83. https://doi.org/10.1016/j.tube.2010.02.002
PMid:20206579
4. Aravindan PP. Host genetics and tuberculosis: Theory of genetic polymorphism and tuberculosis. Lung India. 2019;36(3):244-52. https://doi.org/10.4103/lungindia.lungindia_146_15
PMid:31031349
5. van Tong H, Velavan TP, Thye T, Meyer CG. Human genetic factors in tuberculosis: An update. Trop Med Int Health. 2017;22(9):1063-1071. https://doi.org/10.1111/tmi.12923
PMid:28685916
6. Harishankar M, Selvaraj P, Bethunaickan R. Influence of genetic polymorphism towards pulmonary tuberculosis susceptibility. Front Med. 2018;5:213. https://doi.org/10.3389/fmed.2018.00213
PMid:30167433
7. Leandro AC, Rocha MA, Cardoso CS, Bonecini-Almeida MG. Genetic polymorphisms in Vitamin D receptor, Vitamin D-binding protein, toll-like receptor 2, nitric oxide synthase 2, and interferon-γ genes and its association with susceptibility to tuberculosis. Braz J Med Biol Res. 2009;42(4):312-22. https://doi.org/10.1590/s0100-879x2009000400002
PMid:19330258
8. Pike JW, Meyer MB. The Vitamin D receptor: New paradigms for the regulation of gene expression by 1,25-dihydroxyvitamin D_3. Rheum Dis Clin. 2012;38(1):13-27. https://dx.doi.org/10.1016%2Fj.ecl.2010.02.007
PMid:20511050
9. 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(1):15-23.
PMid:20003690
10. Medapati RV, Suvvari S, Godi S, Gangisetti P. NRAMP1 and VDR gene polymorphisms in susceptibility to pulmonary tuberculosis among Andhra Pradesh population in India: A case-control study. BMC Pulm Med. 2017;17(1):89. https://doi.org/10.1186/s12890-017-0431-5
PMid:28583097
11. Zhang Y, Zhu H, Yang X, Guo S, Liang Q, Lu Y, *et al*. Serum Vitamin D level and Vitamin D receptor genotypes may be associated with tuberculosis clinical characteristics A case-control study. Medicine (Baltimore). 2018;97(30):e11732. https://doi.org/10.1097/MD.0000000000011732
PMid:30045341
12. Lue H, Kleemann R, Calandra T, Roger T, Bernhagen J. Macrophage migration inhibitory factor (MIF): Mechanisms of action and role in disease. Microbes Infect. 2002;4(4):449-60. https://doi.org/10.1016/s1286-4579(02)01560-5
PMid:11932196
13. Kok T, Wasiel AA, Cool RH, Melgert BN, Poelarends GJ, Dekker FJ. Small-molecule inhibitors of macrophage migration inhibitory factor (MIF) as an emerging class of therapeutics for immune disorders. Drug Discov Today. 2018;23(11):1910-8. https://doi.org/10.1016/j.drudis.2018.06.017
PMid:29936245
14. Johnson AD, Zhang Y, Papp AC, Pinsonneault JK, Lim JE, Saffen D, *et al*. Polymorphisms affecting gene transcription and mRNA processing in pharmacogenetic candidate genes: Detection through allelic expression imbalance in human target tissues. Pharm Genom. 2008;18(9):781-91. https://doi.org/10.1097/fpc.0b013e3283050107
PMid:18698231
15. Robert F, Pelletier J. Exploring the impact of single-nucleotide polymorphisms on translation. Front Genet. 2018;9:1-11. https://doi.org/10.3389/fgene.2018.00507
PMid:30425729
16. 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(3):9118-29. https://doi.org/10.4238/2015.august.7.21
PMid:26345844
17. Xu X, Shen M. Associations between Vitamin D receptor genetic variants and tuberculosis: A meta-analysis. Innate Immun. 2019;25(5):305-13. https://doi.org/10.1177/1753425919842643
PMid:30987490
18. Milano M, Moraes MO, Rodenbusch R, Carvalho CX, Delcroix M, Gehlen M, Mousquer G, *et al*. Macrophage migration inhibitory factor -173 G>C single nucleotide polymorphism and its association with active pulmonary tuberculosis. PLoS One. 2020;15(6):e0234565. https://doi.org/10.1371/journal.pone.0234565
PMid:32525926
19. Go LM, Sa E, Lo MA. Macrophage migration inhibitory factor gene influences the risk of developing tuberculosis in northwestern Colombian population. Tissue Antigens. 2007;70(1):28-33. https://doi.org/10.1111/j.1399-0039.2007.00843.x
PMid:17559578
20. Li Y, Yuan T, Lu W, Chen M, Cheng X, Deng S. Association of tuberculosis and polymorphisms in the promoter region of macrophage migration inhibitory factor (MIF) in a Southwestern China Han population. Cytokine. 2012;60(1):64-7. https://doi.org/10.1016/j.cyto.2012.06.010
PMid:22742858
21. Barcellini L, Borroni E, Brown J, Brunetti E, Campisi D, Castellotti PF, *et al*. First evaluation of QuantiFERON-TB gold plus performance in contact screening. Eur Respir J. 2016;48(5):1411-9. https://doi.org/10.1183/13993003.00510-2016
PMid:27390280
22. Qiagen. QuantiFERON ® TB Gold Plus (QFT ® -Plus) ELISA Package Insert 2; 2016. Available from: http://www.quantiferon.com/wp-content/uploads/2017/04/

English_QFTPlus_ELISA_R04_022016.pdf [Last accessed on 2021 May 20].

23. Marozik P, Mosse I, Alekna V, Rudenko E, Tamulaitienė M, Ramanau H, *et al*. Association between polymorphisms of VDR, COL1A1, and LCT genes and bone mineral density in belarusian women with severe postmenopausal osteoporosis. Medicina (Kaunas, Lithuania). 2013;49(4):177-84. https://doi.org/10.3390/medicina49040028
PMid:23985982
24. Wang J, Zhan X, Liu C, Zhang D, Meng L, Deng L. MIF, TGF-β1, IFN-γ and NRAMP1 gene polymorphisms in relation to the clinicopathological profile of spinal tuberculosis in Chinese Han population. Int J Clin Exp Pathol. 2016;9(4):4438-47. http://www.ijcep.com/files/ijcep0021163.pdf
25. Solé X, Guinó E, Valls J, Iniesta R, Moreno V. SNPStats: A web tool for the analysis of association studies. Bioinformatics. 2006;22(15):1928-29. https://doi.org/10.1093/bioinformatics/btl268
PMid:16720584
26. Salimi S, Farajian-masshadi F, Alavi-Naini R, Talebian G, Narooie-Nejad M. Association between Vitamin D receptor polymorphisms and haplotypes with pulmonary tuberculosis. Biomed Reports 2015;3(2):189-94. https://doi.org/10.3892/br.2014.402
PMid:26075071
27. Panda S, Tiwari A, Luthra K, Sharma SK, Singh A. Association of Fok1 VDR polymorphism with Vitamin D and its associated molecules in pulmonary tuberculosis patients and their household contacts. Sci Rep. 2019;9(1):1-10. https://doi.org/10.1038/s41598-019-51803-8
PMid:31649297
28. Singla N, Gupta D, Birbian N, Singh J. Association of VDR gene polymorphism and haplotype with tuberculosis susceptibility, treatment outcome, bacillary load and extrapulmonary site. J Mol Biol Tech. 2015;1(1):101.
29. Jurutka PW, Remus LS, Whitfield GK, Thompson PD, Hsieh JC, Zitzer H, *et al*. The polymorphic N terminus in human Vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB. Mol Endocrinol. 2000;14(3):401-20. https://doi.org/10.1210/mend.14.3.0435
PMid:10707958
30. Olesen R, Wejse C, Bisseye C, Sodemann M, Aaby P, Rabna P, *et al*. DC-SIGN (CD209), pentraxin 3 and Vitamin D receptor gene variants associate with pulmonary tuberculosis risk in West Africans. Genes Immun. 2007;8(6):456-67. https://doi.org/10.1038/sj.gene.6364410
PMid:17611589
31. Alexandra SG, Georgiana DC, Nicoleta C, Daniela PM, Traian S, Veronica S. Apa I and taq I polymorphisms of CDR (Vitamin D receptor) gene in association with susceptibility to tuberculosis in the Romanian population. Rom Biotechnol Lett. 2013;18(1):7956-62.
32. 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. https://doi.org/10.1155/2015/860628
PMid:26578819
33. Lee SW, Chuang TY, Huang HH, Liu CW, Kao YH, Wu LS. VDR and VDBP genes polymorphisms associated with susceptibility to tuberculosis in a Han Taiwanese population. J Microbiol Immunol Infect. 2016;49(5):783-7. https://doi.org/10.1016/j.jmii.2015.12.008
PMid:26869016
34. Hussain T, Naushad SM, Ahmed A, Alamery S, Mohammed AA, Abdelkader MO, *et al*. Association of Vitamin D receptor TaqI and ApaI genetic polymorphisms with nephrolithiasis and end stage renal disease: A meta-analysis. BMC Med Genet. 2019;20(1):193. https://doi.org/10.1186/s12881-019-0932-6
PMid:31822280
35. Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, *et al*. Prediction of bone density from Vitamin D receptor alleles. Nature. 1994;367(6460):284-7. https://doi.org/10.1038/367284a0
PMid:8161378
36. Singh A, Gaughan JP, Kashyap VK. SLC11A1 and VDR gene variants and susceptibility to tuberculosis and disease progression in East India. Int J Tuberc Lung Dis. 2011;15(11):1468-75. https://doi.org/10.5588/ijtld.11.0089
PMid:22008758
37. Sinaga BY, 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(4):275-82. http://www.inaactamedica.org/archives/2014/25633543.pdf
PMid:25633543
38. Areeshi MY, Mandal RK, Dar SA, Alshahrani AM, Ahmad A, Jawed A, *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(3):BSR20170247. https://doi.org/10.1042/bsr20170247
PMid:28533426
39. De Benedetti F, Meazza C, Vivarelli M, Rossi F, Pistorio A, Lamb R, *et al*. Functional and prognostic relevance of the -173 polymorphism of the macrophage migration inhibitory factor gene in systemic-onset juvenile idiopathic arthritis. Arthritis Rheum. 2003;48(5):1398-407. https://doi.org/10.1002/art.10882
PMid:12746913
40. Fei B, Lv HX, Yang JM, Ye ZY. Association of MIF-173 gene polymorphism with inflammatory bowel disease in Chinese Han population. Cytokine. 2008;41(1):44-7. https://doi.org/10.1016/j.cyto.2007.10.010
PMid:18054247
41. Reid D, Shenoi S, Singh R, Wang M, Patel V, *et al*. Low expression macrophage migration inhibitory factor (MIF) alleles and tuberculosis in HIV infected South Africans. Cytokine X. 2019;1(1):100004. https://doi.org/10.1016/j.cytox.2019.100004
PMid:33604547
42. Kuai SG, Ou QF, You DH, Shang ZB, Wang J, Liu J, *et al*. Functional polymorphisms in the gene encoding macrophage migration inhibitory factor (MIF) are associated with active pulmonary tuberculosis. Infect Dis (Auckl). 2016;48(3):222-8. https://doi.org/10.3109/23744235.2015.1107188
PMid 26542751
43. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: An update. Ann Intern Med. 2008;149(3):177-84. https://doi.org/10.7326/0003-4819-149-3-200808050-00241
PMid:18593687
44. Trajman A, Steffen RE, Menzies D. Interferon-gamma release assays versus tuberculin skin testing for the diagnosis of latent tuberculosis infection: An overview of the evidence. Pulm Med. 2013;2013:601737. https://doi.org/10.1155/2013/601737
PMid:23476763
45. Sasmono RT, Massi MN, Setianingsih TY, Wahyuni S, Anita, Halik H, *et al*. Heterogeneity of *Mycobacterium tuberculosis* strains in Makassar, Indonesia. Int J Tuberc Lung Dis 2012;16(11):1441-8. https://doi.org/10.5588/ijtld.12.0055
PMid:23044445
46. Davis AH, Wang J, Tsang TC, Harris DT. Direct sequencing is more accurate and feasible in detecting single nucleotide polymorphisms than RFLP: Using human vascular endothelial growth factor gene as a model. Biol Res Nurs. 2007;9:170-8. https://doi.org/10.1177/1099800407308083
PMid:17909169

Supplementary Materials

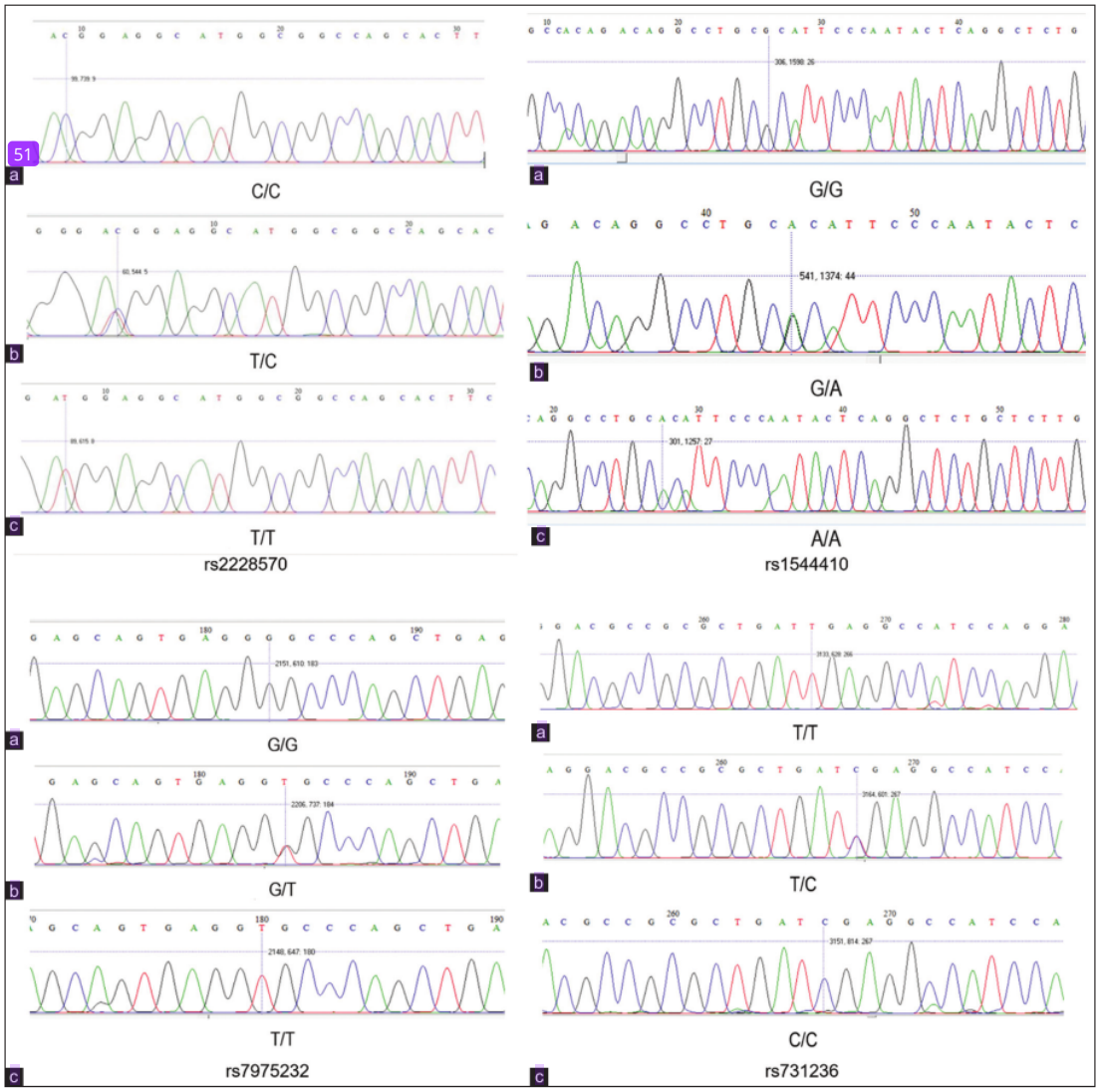

Figure 1: (a-c) Sequencing result of VDR gene shows rs2228570, rs1544410, rs7975232, and rs731236 SNP

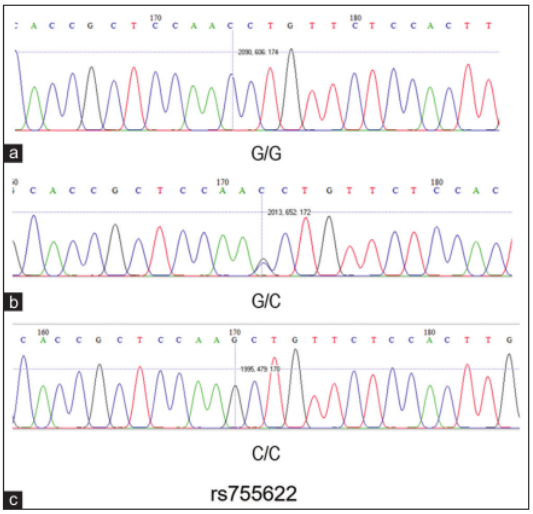

Figure 2: (a-c) Sequencing result of MIF gene shows rs755622 SNP (minus strand)

Table 1: Distribution of genotype and their association in IGRA$^{(-)}$ and IGRA$^{(+)}$ household contacts

SNP	Genotype	IGRA$^{(-)}$ household contacts n ()	IGRA$^{(+)}$ household contacts n ()	OR (95 CI)	p-value
rs2228570 (*VDR FokI*)	Codominant				
	C/C	12 (42.9)	17 (37.8)	1.00	0.94
	T/C	14 (50)	24 (53.3)	1.19 (0.42–3.36)	
	T/T	2 (7.1)	4 (8.9)	1.23 (0.18–8.37)	
	Dominant				
	C/C	12 (42.9)	17 (37.8)	1.00	0.73
	T/C-T/T	16 (57.1)	28 (62.2)	1.19 (0.44–3.25)	
	Recessive				
	C/C-T/C	26 (92.9)	41 (91.1)	1.00	0.89
	T/T	2 (7.1)	4 (8.9)	1.13 (0.18–7.16)	
rs1544410 (*VDR BsmI*)	G/G	19 (67.9)	32 (71.1)	1.00	0.6
	G/A	9 (32.1)	13 (28.9)	0.75 (0.26–2.18)	
rs7975232 (*VDR ApaI*)	C[illegible]minant				
	G/G	3 (10.7)	11 (24.4)	1.00	0.28
	T/G	15 (53.6)	23 (51.1)	0.46 (0.10–1.99)	
	T/T	10 (35.7)	11 (24.4)	0.29 (0.06–1.40)	
	Dominant				
	G/G	10 (35.7)	11 (24.4)	1.00	0.24
	G/T-T/T	18 (64.3)	34 (75.6)	1.93 (0.65–5.75)	
	Recessive				
	G/G-G/T	25 (89.3)	34 (75.6)	1.00	0.16
	T/T	3 (10.7)	11 (24.4)	2.60 (0.64–10.58)	
rs731236 (*VDR TaqI*)	Codominant				
	T/T	25 (89.3)	36 (80)	1.00	0.55
	T/C	3 (10.7)	8 (17.8)	1.74 (0.41–7.47)	
	C/C	0 (0)	1 (2.2)	NA (0.00–NA)	
	Dominant				
	T/T	25 (89.3)	36 (80)	1.00	0.38
	T/C-C/C	3 (10.7)	9 (20)	1.88 (0.44–7.98)	
	Recessive				
	T/T-T/C	28 (100)	44 (97.8)		0.44
	C/C	0 (0)	1 (2.2)	NA (0.00–NA)	
rs755622 (*MIF* -173 G/C)	Codominant				
	G/G	14 (50)	24 (53.3)	1.00	0.4
	G/C	14 (50)	19 (42.2)	0.94 (0.35–2.54)	
	C/C	0 (0)	2 (4.4)	NA (0–NA)	
	Dominant				
	G/G	14 (50)	24 (53.3)	1.00	0.94
	G/C-C/C	14 (50)	21 (46.7)	1.04 (0.39–2.78)	
	Recessive				
	G/G-G/C	28 (100)	43 (95.6)	1.00	0.17
	C/C	0 (0)	2 (4.4)	NA (0–NA)	

OR and p value are adjusted by age, sex, and history of smoking. NA: Not applicable. rs1544410 is only available in the G/G and G/A genotype comparison models.

Association of Vitamin D Receptor Polymorphism (rs2228570, rs1544410, rs7975232, and rs731236) and Macrophage Migration Inhibitory Factor -173 G/C (rs755622) with active TB

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