3’UTR polymorphisms in NRAMP1 are associated with the susceptibility to pulmonary tuberculosis: A MOOSE-compliant meta-analysis

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Abstract
Many studies have investigated the association between the 3’UTR polymorphism in natural resistance-associated macrophage protein 1 (NRAMP1) and the risk of pulmonary tuberculosis (PTB). Revealing inconclusive results, this study aimed to investigate the correlation between the NRAMP1 3’UTR polymorphism and the risk of PTB.

This meta-analysis included 29 case–control studies to better and comprehensively assess this correlation. Pooled odds ratios (ORs) and 95% confidence interval (95% CIs) were calculated to assess the strength of the association.

These 29 case–control studies included 4672 cases and 6177 controls. The NRAMP1 3’UTR polymorphism displayed a significant positive correlation with the risk of PTB in 3 models (for del/del vs ins/ins: OR = 1.22, 95% CI = 1.01–1.47; for ins/del vs ins/ins: OR = 1.19, 95% CI = 1.08–1.30; for ins/del + del/del vs ins/ins: OR = 1.25, 95% CI = 1.08–1.45). A stratified analysis by ethnicity revealed that the NRAMP1 3’UTR polymorphism was associated with an increased risk of PTB in the Asian population, but not in Caucasian, African, and South American populations. The present results indicate that the NRAMP1 3’UTR polymorphism may be considered a risk factor for PTB in the Asian population.

Abbreviations: 95% CI = 95% confidence interval, GWAS = genome-wide association studies, HIV = human immunodeficiency virus, HWE = Hardy–Weinberg equilibrium, NOS = Newcastle–Ottawa scale, NRAMP1 = natural resistance associated macrophage protein 1, OR = odds ratios, PTB = pulmonary tuberculosis, SLC11A1 = solute carrier family 11A member 1, TB = tuberculosis.

Keywords: meta-analysis, NRAMP1, polymorphism, pulmonary tuberculosis

1. Introduction
Tuberculosis (TB), a chronic infectious disease caused by Mycobacterium tuberculosis, is a major cause of worldwide mortality and morbidity. In 2016, 10.4 million new cases were reported, and 1.7 million individuals died of TB. Most of the individuals with TB reportedly reside in India China, Philippines, Pakistan, Nigeria, and South Africa.[1] The precise mechanisms underlying TB pathogenesis remain unknown. Weakened immunity, human immunodeficiency virus (HIV) infections, alcohol abuse, advanced age, chronic corticosteroid therapy, diabetes, socio-economic status, and malnutrition are the prominent risk factors of TB.[2] Not all individuals exposed to the similar risk factors develop tuberculosis, thus suggesting the involvement of genetic factors, including single-nucleotide polymorphisms, in TB pathogenesis.

When M tuberculosis infects the body, the immune system produces a barrage of defensive protein molecules, recruiting phagocytic cells, which eliminate the pathogen. Natural resistance-associated macrophage protein 1 (NRAMP1) is also referred to as the solute carrier family 11 proton-coupled divalent metal ion transporter membrane 1 (SLC11A1), which plays an important role in the immune response to mycobacterial infections. NRAMP1 encodes a divalent transition metal (Fe and Mn) transporter that localizes on the lysosomal membrane.[3] Iron is an essential mycobacterial nutrient that also influences the generation of reactive oxygen and nitrogen intermediates. Therefore, NRAMP1 is involved in resistance to intracellular pathogens, including Leishmania, Salmonella, and Mycobacteria. NRAMP1 is associated with various infectious diseases and inflammatory diseases. It primarily contains 4 polymorphisms, rs17235416 (3’UTR), rs17235409 (D543N), rs3731865 (INT4), and rs3448891 (5’UTR). Among these polymorphisms, 3’UTR polymorphisms have been widely investigated for their association with TB. A functionally significant TGTG del allele in NRAMP1 leads to reduced production of NRAMP1 when compared to the TGTG+ allele and may be correlated with the risk of TB.

Numerous studies have investigated the association between the NRAMP1 3’UTR polymorphism and the risk of pulmonary tuberculosis (PTB) in different regions,[4–29] revealing inconclusive results. A case–control study by Medapati et al.[29] reported that the NRAMP1 3’UTR polymorphism is significantly associated with the susceptibility to TB among Indian
individuals. However, Jafari[28] reported no significant association between these 2 aspects. Hence, the present meta-analysis aimed to better and comprehensively assess the correlation between the NRAMP1 3’UTR polymorphism and the risk of PTB.

2. Materials and methods

2.1. Search strategy

We performed a comprehensive search in PubMed, Elsevier, and the Cochrane Library (update till January 1, 2019), using the following keywords ("tuberculosis" or “TB” or “mycobacteria”), (“polymorphism” or “mutation” or “variant”), and (“Natural resistance associated macrophage protein 1” or “NRAMP1” or “Solute carrier family 11A member 1 or SLC11A1” or “rs17235416”). Articles in all languages were included. Furthermore, additional related articles were identified through screening of the references of relevant articles. In case of a duplicate publication, the largest study was selected. The present study was conducted in accordance with the PRISMA guidelines for systematic reviews and meta-analyses.[10] Ethical approval was not necessary since this study is a meta-analysis.

2.2. Inclusion and exclusion criteria

Following were the inclusion criteria of this meta-analysis:

1. case–control or cohort studies on the NRAMP1 3’UTR polymorphism and the risk of PTB;
2. sufficient data estimating odds ratios (ORs) with 95% confidence intervals (95% CIs).

The major exclusion criteria were as follows:

1. meta-analyses, letters, reviews, or editorial comments;
2. studies were not irrelevant to TGTG ins/del;
3. not PTB;
4. studies with insufficient data;
5. other than case–control study;
6. genotype distributions were not in accordance with Hardy–Weinberg equilibrium (HWE) in the control group.

2.3. Data extraction and quality assessment

For all studies, EZ and LZ extracted the data independently and reached a consensus. Any disagreement was resolved through discussion among the 3 authors. The following information was collected from all eligible studies: the first author, year of publication, country of origin, ethnicity, source of controls, number of case and control subjects, HIV status, genotype frequencies in case and control groups, and HWE. We evaluated the quality of all of the studies included according to Newcastle–Ottawa scale (NOS).[11] The NOS contains 3 categories which are selection (0–4 points), comparability (0–2 points), and exposure (0–3 points). The total scores ranged from 0 to 9.

2.4. Statistical analysis

The association between the NRAMP1 3’UTR polymorphism and the risk of PTB was evaluated using crude ORs with 95% CIs. To assess heterogeneity, the Cochrane Q test and the I-squared (I²) metric were used. If the I² metric was >50%, indicating a lack of heterogeneity among studies, A fixed-effects model (the Mantel–Haenszel method) was used. Otherwise, the random-effects model (the DerSimonian and Laird method) was applied.[12] In addition, subgroup analyses based on ethnicity (Asian, Caucasian, African, and South American) were carried out. Funnel plot analysis and Egger test were conducted to identify a publication bias of the meta-analysis.[13] All statistical analyses were performed using STATA 11.0 software (STATA Corp., College Station, TX) and P values were 2-tailed.

3. Results

3.1. Characteristics of eligible studies

In accordance with the flowchart shown in Figure 1, 211 studies were retrieved through our initial literature search. After excluding duplicate articles, reviews, letters, comments, and irrelevant studies, 50 studies remained for further evaluation. After reading full-text articles, 24 studies were excluded. Finally, 26 eligible studies including 29 case–control studies, including 4672 cases and 6177 controls were included (Fig. 1). The major characteristics of identified studies were summarized in Table 1. These studies included 18 studies on Asians, 4 studies on Caucasians, 5 studies on Africans, and 2 studies on South American populations.

3.2. Quantitative synthesis

Table 2 summarizes the results of this meta-analysis. Overall, the NRAMP1 3’UTR polymorphism was significantly associated with the risk of PTB in 3 models (for del/del vs ins/ins: OR = 1.22, 95% CI = 1.01–1.47; for Ins/del vs ins/ins: OR = 1.19, 95% CI = 1.08–1.30; for Ins/del + del/del vs ins/ins: OR = 1.25, 95% CI = 1.08–1.45); however, the recessive model did not reveal this significant association (for del/del vs ins/ins + Ins/del: OR = 1.18, 95% CI = 0.98–1.42). In a stratified analysis by ethnicity, the present meta-analysis revealed that the NRAMP1 3’UTR polymorphism was associated with an increased risk of PTB in the Asian population (for del/del vs ins/ins: OR = 2.08, 95% CI = 1.45–2.98; for Ins/del vs ins/ins: OR = 1.49, 95% CI = 1.29–1.73, Figure 2; for Ins/del + del/del vs ins/ins: OR = 1.57, 95% CI = 1.36–1.81; for del/del vs ins/ins + Ins/del: OR = 1.89, 95% CI = 1.33–2.69), but not in the Caucasian, African, and South American populations. In a stratified analysis by HIV status, the NRAMP1 3’UTR polymorphism was significantly associated with the risk of PTB in HIV- individuals in 2 models (for Ins/del vs ins/ins: OR = 1.26, 95% CI = 1.06–1.49; for Ins/del + del/del vs ins/ins: OR = 1.28, 95% CI = 1.07–1.54), but not in the HIV+ individuals.

3.3. Publication bias

Funnel plot analysis and Egger’s test were conducted to assess publication bias. Egger test did not reveal any evidence of publication bias in the Ins/del versus ins/ins models (t = 0.14, P = 0.888) and in the dominant model (t = 0.81, P = 0.423); however, publication bias was detected in the del/del vs. ins/ins model (t = 2.98, P = 0.004).
ins models \( t = 2.65, P = .014 \) (Fig. 3) and in the recessive model \( t = 2.43, P = .023 \).

### 4. Discussion

The innate immune response activates early events during a *M. tuberculosis* infection. NRAMP1 plays an important role in the immune response to a mycobacterial infection. Therefore, this meta-analysis aimed to better and comprehensively assess the correlation between the NRAMP1 3'UTR polymorphism and the risk of PTB. The present study reports a significant correlation between the NRAMP1 3'UTR polymorphism and the risk of PTB in overall and Asian populations.

Many epidemiological studies have investigated the association between the NRAMP1 3'UTR polymorphism and the risk of PTB. In 2000, the association between the NRAMP1 3'UTR polymorphism and the risk of PTB was first investigated by Ryu et al. They revealed a significant relationship of the ins/del genotype variant with the risk of PTB among Koreans, but not in the del/del genotype. A case-control study by Medapati et al. reported that the NRAMP1 3'UTR polymorphism is significantly associated with the susceptibility to TB in the Indian population. However, Taype et al. reported did not report an association in extra-PTB reported that 3'UTR polymorphisms increased the risk of TB in comparison with their corresponding common alleles. Furthermore, stratified analyses by ethnicity, which assessed the forms of TB, confirmed the results of Li et al. This meta-analysis includes 29 recent case-control studies including 4672 cases and 6177 controls to better and comprehensively assess the correlation between the NRAMP1 3'UTR polymorphism and the risk of PTB. The present results are similar to those of 2 previous meta-analyses. The 3'UTR del/del and ins/del genotypes were significantly associated with a higher risk than the 3'UTR ins/ins genotype.

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**Figure 1.** Flow chart of included studies in the current meta-analysis.
Table 1
Main characteristics of studies included in this meta-analysis.

| First author | Yr | Country   | Ethnicity | Source of controls | No. of pat/con | HIV status of cases | NRAMP1 | P for HWE | Results of NOS |
|--------------|----|-----------|-----------|--------------------|----------------|-------------------|--------|----------|----------------|
| Ryu          | 2009 | Korea     | Asian     | healthy individuals | 192/192        | NR                |        |          |                |
| Li et al     | 2005 | China     | Asian     | healthy individuals | 49/48          | negative          |        |          |                |
| Li et al     | 2002 | China     | Asian     | healthy individuals | 29/31          | positive          |        |          |                |
| Abe et al    | 2003 | Japan     | Asian     | healthy individuals | 95/90          | negative          |        |          |                |
| Liu          | 2003 | China     | Asian     | healthy individuals | 120/240        | negative          |        |          |                |
| Fitness et al| 2004 | Malawi    | African   | healthy individuals | 435/709        | positive          |        |          |                |
| Fitness et al| 2004 | Malawi    | African   | healthy individuals | 218/709        | negative          |        |          |                |
| Tayebi       | 2006 | Peru      | South American | healthy individuals | 507/513        | negative          |        |          |                |
| Hidaka et al | 2006 | China     | Asian     | healthy individuals | 85/79          | NR                |        |          |                |
| Velez et al  | 2007 | Venezuela | South American | staff of the hospital | 218/709        | negative          |        |          |                |
| Saha et al   | 2007 | Netherlands | Caucasian | healthy individuals | 214/363        | negative          |        |          |                |
| Asal         | 2008 | Japan     | Asian     | healthy individuals | 57/51          | negative          |        |          |                |
| Farzaneh     | 2008 | Iran      | Asian     | staff of the hospital | 71/39          | negative          |        |          |                |
| Chen         | 2009 | China     | Asian     | healthy individuals | 140/139        | positive          |        |          |                |
| Mercaz et al | 2009 | Sweden    | Caucasian | staff of the hospital | 117/60         | NR                |        |          |                |
| Hatta et al  | 2010 | Indonesia | Asian     | healthy individuals | 58/198         | positive          |        |          |                |
| de Wit et al | 2011 | South Africa | African | healthy individuals | 492/312        | negative          |        |          |                |
| Solgun et al | 2011 | Turkey    | Caucasian | healthy individuals | 49/50          | positive          |        |          |                |
| Nugrah et al | 2011 | Indonesia | Asian     | staff of the hospital | 78/43          | NR                |        |          |                |
| Ben Selma et al | 2012 | Tunisia   | Caucasian | healthy individuals | 168/127        | positive          |        |          |                |
| Sarkadi et al | 2012 | Japan     | Asian     | healthy individuals | 111/211        | negative          |        |          |                |
| Tiko et al   | 2013 | Indonesia | Asian     | healthy individuals | 123/123        | NR                |        |          |                |
| Wu et al     | 2013 | China     | Asian     | healthy individuals | 213/211        | negative          |        |          |                |
| Fernandez et al | 2015 | Venezuela | South American | staff of the hospital | 89/50          | negative          |        |          |                |
| Wu L et al   | 2015 | China     | Asian     | healthy individuals | 151/453        | negative          |        |          |                |
| Jafari et al | 2016 | Iran      | Asian     | healthy individuals | 96/122         | NR                |        |          |                |
| Modapat et al | 2017 | India     | Asian     | healthy individuals | 91/88          | negative          |        |          |                |

HWE = P value for Hardy–Weinberg equilibrium in controls; NOS = Newcastle–Ottawa scale. NR = not reported.

Table 2
Summary ORs and 95% CI of Association between 3’UTR polymorphism and pulmonary tuberculosis risk.

| Subgroup | n | OR95% CI | OR95% CI | OR95% CI | OR95% CI |
|-----------|---|----------|----------|----------|----------|
|           | del/del vs ins/ins | del/del vs ins/ins | del/del + del/del vs ins/ins | del/del vs ins/ins + ins/del |
| Total     | 29 | 1.22 (1.01–1.47) | 1.19 (1.08–1.30) | 1.25 (1.08–1.45) | 1.18 (0.98–1.42) |
| Ethnicity |   | 35.5% | 45.1% | 54.3% | 32.3% |
| Asian     | 18 | 2.08 (1.45–2.98) | 1.49 (1.29–1.71) | 1.57 (1.36–1.81) | 1.89 (1.33–2.69) |
| Caucasian | 4  | 0.78 (0.45–1.36) | 0.93 (0.70–1.23) | 0.90 (0.69–1.18) | 0.81 (0.47–1.38) |
| African   | 5  | 0.97 (0.74–1.27) | 1.01 (0.87–1.17) | 1.01 (0.86–1.16) | 0.97 (0.75–1.27) |
| South American | 2  | 1.42 (0.72–2.78) | 1.05 (0.58–1.89) | 1.23 (0.95–1.58) | 1.34 (0.69–2.62) |
| HIV status of cases |   |   |   |   |
| Positive | 2  | 0.68 (0.39–1.19) | 1.03 (0.42–2.13) | 0.95 (0.42–2.13) | 0.77 (0.44–1.33) |
| Negative | 19 | 1.25 (0.98–1.59) | 1.26 (1.06–1.49) | 1.28 (1.07–1.54) | 1.18 (0.93–1.50) |

OR = odds ratio, CI = confidence interval.
GWAS scanning entire genomes for genetic variation include with a candidate gene strategy. Genome-wide association studies potential gene-gene and gene-environment interactions because considered with caution. Third, PTB is a complex disease; detected in the del/del versus ins/ins models and the recessive establish an actual association. Second, publication bias was detected in the del/del versus ins/ins models and the recessive model in overall analysis; hence, the present results should be considered with caution. Third, PTB is a complex disease; however, we did not carry out subgroup analysis to analyze potential gene-gene and gene-environment interactions because of insufficient data. Fourth, this meta-analysis was performed with a candidate gene strategy. Genome-wide association studies (GWAS) scanning entire genomes for genetic variation include immense amounts of SNPs and have been designed for the same ethnic background and study design. A GWAS by Zheng et al.\(^{[16]}\) reported that 2 loci 14q24.3 (rs12437118, Pcombined = 1.72 × 10^-11, OR = 1.277, ESRRB) and 20p13 (rs6114027, Pcombined = 2.37 × 10^-11, OR = 1.339, TGM6) were significantly associated with TB in Han Chinese individuals. Miao et al.\(^{[17]}\) reported that rs9272461 is significantly associated with the risk of PTB in various genetic models. Hence, GWAS are required to further validate the present results.

In conclusion, the present results indicate that the NRAMP1 3’UTR polymorphism may be associated with an increased risk of PTB in the Asian population, but not in the Caucasian, African, and South American populations. Well-designed epidemiological studies with larger sample sizes are needed to verify the present findings.
Figure 3. Funnel plot of the association 3’UTR polymorphism and pulmonary tuberculosis risk for the del/del genotype compared with the ins/ins genotype in overall populations. OR = odds ratio, se = standard error.

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