The association of inflammasome and TLR2 gene polymorphisms with susceptibility to tuberculosis in the Han Taiwanese population

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Pulmonary tuberculosis (TB) caused by Mycobacterium tuberculosis (Mtb) is a global public health concern. Although inflammasome and the toll-like receptor 2 (TLR2) genes play an important role in host defense against Mtb, the associations of polymorphisms in these genes with TB risk are incompletely understood. A total of 230 TB patients and 213 individuals without TB were enrolled in this study. A significant difference in the frequencies of different AIM2 rs2276405 genotypes between the non-TB and TB groups was detected. When the patients were stratified by gender or age, significant differences in genotype frequencies at NLRP3 rs34298354 in men and in non-aged (≤65-year-old) subjects and at IFI16 rs1772408 in women were found. OR analysis showed that the TC rs34298354 genotype in NLRP3 was associated with reduced risk of TB. In women, the AG rs1772408 genotype in IFI16 was associated with decreased TB risk. Haplotype analysis showed that, in comparison with the most common haplotype (T-T) of rs3804099-rs3804100 in the TLR2 gene, the C-T haplotype was associated with an increased risk for TB. Our study indicates that rs34298354 in NLRP3 and rs1772408 in IFI16 protect individuals from TB, and that the less common TLR2 haplotype is associated with increased TB susceptibility.

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis (Mtb) that mostly affects the lungs. In their global tuberculosis report in 2019, the WHO estimated there were 10.0 million new TB cases and 1.2 million TB deaths among HIV-negative people in 2018. In Taiwan, approximately 9759 new cases and 511 deaths occurred from mycobacterial infection in 2017. About a quarter of the world's population is infected with Mtb and thus at 5–10% lifetime risk of developing TB disease. About 90% of people with a latent TB infection never develop active disease, suggesting that individual host factors (e.g., genetics, smoking, and alcohol) influence susceptibility to TB. In recent years, significant relationships between genetic variation in host immune-related genes and TB risk have been reported.

Inflammasomes are multiprotein complexes that form when cells sense invading infectious pathogens and that control antimicrobial host defenses. The five major inflammasomes (NLRP1, NLRP3, NLRC4, Pyrin, and AIM2) include cytoplasmic and nuclear sensor molecules that form a complex with the effector protein pro-caspase-1. The active caspase-1 processes pro-inflammatory interleukins (such as pro-IL-1β and IL-18) into their mature biologically active forms. ESAT-6, an Mtb protein, and transfected Mtb dsDNA can activate the NLRP3 and AIM2 inflammasomes, respectively. In addition, Mtb extracellular DNA can activate the IFI16 inflammasome, leading to the production of IFN-β. AIM2-deficient mice show high susceptibility to Mtb due to impaired production of IL-1β and IFN-γ and reduced activation of caspase-1. In recent years, associations of inflammasome gene polymorphisms with susceptibility to TB and the development of TB have been reported. NLRP3 polymorphism rs35829419 has been associated with extrapulmonary TB in Ethiopia, and IFI16 polymorphisms rs1101998 and rs1633256 have been associated with tuberculin skin test positivity in contacts of TB patients in Brazil. However, few reports have evaluated the association of AIM2 and IFI16 gene polymorphisms with TB risk. Despite the importance of inflammasomes in the immune response to tuberculosis,
it is still unknown whether inflammasome gene polymorphisms are associated with susceptibility to TB in the Han Taiwanese population.

Genetic polymorphism of toll-like receptor 2 (TLR2), a TLR family member, influences the immune response to ESAT-6 in pulmonary tuberculosis patients. TLRs are pattern recognition receptors expressed in antigen-presenting cells and are important in host immunity to infectious pathogens. They are involved in the recognition of Mtb and are linked to inflammasome activation. The associations of gene polymorphisms in other TLR (TLR1, TLR4, TLR7, TLR8, and TLR9) genes with TB risk have also been studied. In Taiwan, genetic variants of TLR2, TLR7, and TLR8 have been associated with increased risk for TB infection. However, whether other TLR2 polymorphisms are associated with genetic susceptibility to TB in the Han Taiwanese population is still unknown.

In light of the above information, we proposed that variants in inflammasome genes and TLR2 could influence the host response to Mtb infection and the development of TB. In this study, we evaluated the association of inflammasome and TLR2 gene polymorphisms with the susceptibility to TB. SNPs in inflammasome genes (NLRP3, AIM2, IFI16) and in TLR2 were analyzed using TaqMan genotyping in subjects with and without TB. Our results indicate that genetic variants of inflammasome and TLR2 genes are associated with TB risk in the Han Taiwanese population.

**Results**

**Characteristics of the study subjects.** 443 adult subjects (including 213 patients with TB and 213 controls without TB infection) were enrolled in this study. In the Table 1, we found that TB patients had higher men/women ratio than controls, indicating men were more significant TB risk than women ($p=0.0001$). TB subjects had a mean age of 57 years (range 20–91 years) and controls had a mean age of 66 years (range 20–97 years). Significant differences in age between the TB and non-TB groups were found in all subjects, men, and women by $t$-test.

| Variables | Non-TB, N (%) | TB, N (%) | $p$ value |
|-----------|--------------|-----------|-----------|
| Gender    |              |           |           |
| Man       | 124 (58.2)   | 173 (75.2) | 0.0001*   |
| Woman     | 89 (41.8)    | 57 (24.8)  |           |
| Age (years) |            |           |           |
| Mean ± SD (range) | 66 ± 19 (20–97) | 57 ± 19 (20–91) | 0.0001* |
| Man, mean ± SD (range) | 70 ± 17 (20–97) | 59 ± 18 (20–91) | 0.0001* |
| Woman, mean ± SD (range) | 61 ± 19 (23–94) | 49 ± 20 (20–89) | 0.008* |
| Age group-N (%) |      |           |           |
| ≤65       | 83 (39.0)    | 151 (65.7) | 0.0001*   |
| >65       | 130 (61.0)   | 79 (34.3)  |           |

**Table 1.** The characteristics of the study participants. SD = standard deviation; TB = tuberculosis; N = number of subjects; *The statistical analysis was tested by $\chi^2$-test; bThe statistical analysis was tested by $t$-test.

**Genotype distributions conformed to Hardy-Weinberg equilibrium.** When the nine SNPs in the AIM2, NLRP3, TLR2, and IFI16 regions were genotyped, none of their allelic distributions deviated from Hardy-Weinberg equilibrium (Table 2). The LD plot of the nine SNPs is shown in Fig. 1. One haploblock was identified at NLRP3, TL1R2, and another at IFI16.

**Inflammasome and TLR2 gene polymorphisms associated with tuberculosis risk.** Our results revealed a significant difference in AIM2 SNP rs2276405 genotype frequencies between the non-TB and TB groups, whereas the other eight SNPs did not have significantly different genotype frequencies between TB patients and controls (Table 2). We used logistic regression to test the effect of interactions of age and genotype (divided into ≤65 and >65 years) and of gender and genotype, and found that the $p$ values generated by logistic regression were all significant (Table 3). When the patients were stratified by gender, significant differences in the genotype frequencies of rs34298354 in men and rs1772408 in women were found (Table 4). When the patients were stratified by age, a significant difference in genotype frequencies of rs34298354 in non-aged (≤65-year-old) subjects was found (Table 5).

The rs34298354 SNP in NLRP3, but none in the other three loci, was associated with susceptibility to TB. The TC heterozygous rs34298354 genotype was a reduced-risk genotype for susceptibility to TB, before and after adjusting for age and gender, compared with the CC genotype (aOR = 0.536; 95% CI = 0.294–0.979, $p=0.043$; Table 2).

The rs1772408 SNP in IFI16 was gender-dependent. In particular, the AG heterozygous rs1772408 genotype was associated with a reduced risk of TB in female subjects, adjusted for age (adjusted OR [aOR] = 0.397; 95% CI = 0.173–0.911, $p=0.029$), compared with the AA genotype (Table 4). However, no significant association was found in non-aged (≤65-year-old) and aged (>65-year-old) subjects with or without TB group (Table 5).
## Table 2. Genotyping frequencies of SNPs in the TB and non-TB groups and results of logistic regression. HWp: p value of Hardy-Weinberg disequilibrium test; ref: reference genotype; CI: confidence interval; OR: odds ratio.

| SNP ID | Location | Genotype | Genotype counts | Non-TB (%) | TB (%) | p-value $^a$ | Adj. odds ratio (95% CI) | p for OR $^b$ |
|--------|----------|----------|----------------|------------|--------|-------------|--------------------------|-------------|
| rs2276405 | AIM2 | Exonic | TT | 5 (2.3) | 0 (0.0) | 0.025 |
| rs34298354 | NLRP3 | TT | 0 (0.0) | 0 (0.0) | 0.079 |
| rs3806268 | NLRP3 | TT | 0 (0.0) | 0 (0.0) | 0.079 |
| rs7525979 | NLRP3 | TT | 2 (1.0) | 5 (2.2) | 0.570 | 2.119 (0.379, 11.841) | 0.392 |
| rs1772408 | IFI16 | AA | 0 (0.0) | 0 (0.0) | 0.222 |

### Haplotype and diplotype analyses.

The results of LD analysis of the four loci in NLRP3 and the three loci in TLR2 are shown in Fig. 1. Of the four possible haplotypes in NLRP3 rs7525979-rs3806268, three were detected in the non-TB and TB groups. In comparison with the most common haplotype, which includes only common alleles (C-A) of rs7525979-rs3806268, no haplotype was associated with a statistically significant increased risk of TB (Table 6). Four haplotypes of TLR2 rs3804099-rs3804100 were detected in the non-TB and TB groups (Table 6). When we compared them with the most common haplotype, which includes only common alleles (T-T) of rs3804099-rs3804100, a significant association between the C-T haplotype and TB risk was found (aOR = 3.406; 95% CI = 1.546–7.505, $p = 0.002$). In addition, the difference in distribution of the TLR2 haplotypes between the non-TB and TB groups was statistically significant ($p = 0.014$, $\chi^2$).

The association observed in the haplotype analyses was also found in the diplotype analyses (Table 6). In NLRP3, no significant association between any diplotype of NLRP3 rs7525979-rs3806268 was found when compared with the homozygous C-A/C-A diplotype. In TLR2, we observed that carriers of at least one C-T haplotype of rs3804099-rs3804100 had increased TB risk in comparison with the individuals carrying the homozygous T-T/T-T diplotype (aOR = 3.513; 95% CI = 1.550–7.962, $p = 0.003$).

### Discussion

Previous studies have found associations of NLRP3 and TLR2 polymorphisms with susceptibility to TB. In our study of the Taiwanese population, we found that the TC genotype of NLRP3 rs34298354 was associated with decreased risk of TB. In addition, we found that the AG genotype of IFI16 rs1772408 was gender-dependently associated with reduced risk for TB. In haplotype analysis, we found that the C-T haplotype of TLR2 rs3804099-rs3804100 was associated with increased susceptibility to TB. This indicates that NLRP3, IFI16, and TLR2 play an important role in protection against TB infection in the Han Taiwanese population.

We found no significant association between TB risk and rs3804099 and rs3804100 in TLR2, similar to the finding of no significant association of these SNPs with TB risk in a Western Chinese population. However, they have been associated with TB risk in the Tibetan Chinese population, suggesting a possible ethnicity-specific
effect of rs3804099 and rs3804100 on susceptibility to TB. Using Haploview v.4.2 to evaluate linkage disequilibrium, one haploblock (rs3804099-rs3804100) in TLR2 was found. This haploblock has been reported in multiple studies\textsuperscript{26–29}. The rs3804099-rs3804100 haplotype has been reported to be associated with risks of tuberculosis\textsuperscript{27},

Table 3. Interaction of genetic variation and gender contribute to tuberculosis risk. df: degree of freedom.
\textsuperscript{a}rs2276405 TT genotype is only five subjects, none in male subjects and five in female subjects, so the df=4;
\textsuperscript{b}rs34298354 TT genotype, rs6689545 CC genotype, and rs5743705 CC genotype are not detected in case and control groups, so the df=3. p value was calculated by logistic regression.

| gender* genotype | \(\chi^2\) | df  | \(p\) value |
|------------------|-----------|-----|-------------|
| rs2276405        | 22.167    | 4\(^a\) | <0.0001     |
| rs34298354       | 19.257    | 3\(^b\) | <0.0001     |
| rs3806268        | 17.004    | 5   | 0.004       |
| rs7525979        | 16.712    | 5   | 0.005       |
| rs6689545        | 16.705    | 3\(^b\) | 0.001       |
| rs3804099        | 18.653    | 5   | 0.002       |
| rs3804100        | 15.271    | 5   | 0.009       |
| rs5743705        | 15.009    | 3\(^b\) | 0.002       |
| rs1772408        | 22.028    | 5   | 0.001       |

| age*genotype | \(\chi^2\) | df  | \(p\) value |
|--------------|-----------|-----|-------------|
| rs2276405    | 40.480    | 5   | <0.0001     |
| rs34298354   | 36.157    | 3\(^b\) | <0.0001     |
| rs3806268    | 32.769    | 5   | <0.0001     |
| rs7525979    | 34.750    | 5   | <0.0001     |
| rs6689545    | 33.305    | 3\(^b\) | <0.0001     |
| rs3804099    | 33.294    | 5   | <0.0001     |
| rs3804100    | 33.205    | 5   | <0.0001     |
| rs5743705    | 32.012    | 3\(^b\) | <0.0001     |
| rs1772408    | 34.462    | 5   | <0.0001     |

Figure 1. Linkage disequilibrium (LD) plot in \(D'\) demonstrating adjacent strength between single nucleotide polymorphisms (SNPs) in the AIM2, IFI16, TLR2, and NLRP3 genes. Patterns of LD between the TLR2 SNPs in (A) and NLRP3, AIM2, and IFI16 SNPs in (B). \(D'\) and \(r^2\) values were multiplied by 100.
| SNP ID | Genotype | Genotype counts | Non-TB | TB | p value | Adj. OR (95% CI) | p for OR |
|--------|----------|----------------|--------|----|---------|-----------------|---------|
|        |          |                |        |    |         |                 |         |
| rs2276405 |          |                |        |    |         |                 |         |
| Men    | TT       | 0 (0.0)        | 0 (0.0)| 0.136 |         |                 |         |
|        | TC       | 12 (9.7)       | 27 (15.6)| \(2^*2 \chi^2\) test | 1.541 (0.728, 3.260) | p = 0.258 |
| Women  | TT       | 5 (5.6)        | 0 (0.0) | 0.166 |         |                 |         |
|        | TC       | 12 (13.5)      | 10 (17.5)| 1.071 (0.406, 2.822) | p = 0.490 |
|        | CC (ref.)| 72 (80.9)      | 47 (82.5) |   |         |                 |         |
| rs34298354 |          |                |        |    |         |                 |         |
| Men    | TT       | 0 (0.0)        | 0 (0.0) | 0.30 |         |                 |         |
|        | TC       | 23 (18.5)      | 17 (9.8)| \(2^*2 \chi^2\) test | 0.540 (0.267, 1.090) | p = 0.085 |
| Women  | TT       | 101 (81.5)     | 156 (90.2)|  |         |                 |         |
|        | TC       | 9 (10.1)       | 5 (8.8)| \(2^*2 \chi^2\) test | 0.539 (0.162, 1.794) | p = 0.314 |
|        | CC (ref.)| 80 (89.9)      | 52 (91.2) |   |         |                 |         |
| rs3806368 |          |                |        |    |         |                 |         |
| Men    | GG       | 31 (25.0)      | 37 (21.4)| 0.742 | 0.826 (0.418, 1.631) | p = 0.582 |
|        | AG       | 61 (49.2)      | 87 (50.3)| 0.916 (0.514, 1.631) | p = 0.766 |
|        | AA (ref.)| 32 (25.8)      | 49 (28.3) |   |         |                 |         |
| Women  | GG       | 17 (19.1)      | 15 (26.3)| 0.393 | 1.356 (0.531, 3.461) | p = 0.524 |
|        | AG       | 44 (49.4)      | 22 (38.6)| 0.727 (0.327, 1.617) | p = 0.434 |
|        | AA (ref.)| 28 (31.5)      | 20 (35.1) |   |         |                 |         |
| rs7525979 |          |                |        |    |         |                 |         |
| Men    | TT       | 2 (1.6)        | 4 (2.3)| 0.872 | 1.518 (0.249, 9.250) | p = 0.651 |
|        | TC       | 27 (21.8)      | 40 (23.1)| 1.120 (0.626, 2.004) | p = 0.703 |
| Women  | TT       | 1 (1.8)        | 1 (1.8) | 0.455 |         |                 |         |
|        | TC       | 21 (23.6)      | 13 (22.8)| 0.872 (0.381, 1.994) | p = 0.746 |
|        | CC (ref.)| 68 (76.4)      | 43 (75.4) |   |         |                 |         |
| rs6689545 |          |                |        |    |         |                 |         |
| Men    | CC       | 0 (0.0)        | 0 (0.0) | 0.291 |         |                 |         |
|        | TC       | 12 (9.7)       | 11 (6.4)| \(2^*2 \chi^2\) test | 0.587 (0.239, 1.440) | p = 0.245 |
| Women  | CC       | 0 (0.0)        | 0 (0.0) | 0.294 |         |                 |         |
|        | TC       | 4 (4.5)        | 5 (8.8)| \(2^*2 \chi^2\) test | 3.175 (0.717, 14.054) | p = 0.128 |
|        | TT (ref.)| 85 (95.5)      | 52 (91.2) |   |         |                 |         |
| rs3804099 |          |                |        |    |         |                 |         |
| Men    | CC       | 9 (7.2)        | 15 (8.7)| 0.584 | 1.257 (0.501, 3.156) | p = 0.626 |
|        | TC       | 43 (34.7)      | 68 (39.3)| 1.234 (0.740, 2.060) | p = 0.420 |
| Women  | TT (ref.)| 72 (58.1)      | 90 (52.0)|  |         |                 |         |
|        | CC       | 6 (6.7)        | 9 (15.8)| 0.210 | 2.053 (0.636, 6.624) | p = 0.229 |
|        | TC       | 36 (40.5)      | 20 (35.1)| 0.954 (0.453, 2.011) | p = 0.902 |
|        | TT (ref.)| 47 (52.8)      | 28 (49.1) |   |         |                 |         |
| rs3804100 |          |                |        |    |         |                 |         |
| Men    | CC       | 5 (4.0)        | 9 (5.2)| 0.805 | 1.134 (0.351, 3.668) | p = 0.834 |
|        | TC       | 46 (37.1)      | 59 (34.1)| 0.836 (0.502, 1.392) | p = 0.491 |
| Women  | CC       | 6 (6.7)        | 3 (5.3)| 0.863 | 0.671 (0.145, 3.097) | p = 0.609 |
|        | TC       | 34 (38.2)      | 24 (42.1)| 1.144 (0.557, 2.350) | p = 0.715 |
|        | TT (ref.)| 49 (55.1)      | 30 (52.6) |   |         |                 |         |

Continued
hepatocellular carcinoma\(^{28}\), and allergic asthma\(^{29}\). In this study, haplotype analysis of two variations in TLR2 (rs3804099 and rs3804100) showed significant association of the C-T haplotype with increased risk of TB (Table 6). A previous study found that peripheral blood leukocytes from trauma patients with the TLR2 rs3804099 CC genotype produce greater amounts of IL-10, IL-8, and TNF-\(\alpha\) than those having the TT genotype, after bacterial lipopolysaccharide stimulation\(^{30}\). In another study, TB patients with the rs3804100 CC genotype had significantly higher blood absolute NK cell counts at diagnosis than those carrying the T allele\(^{24}\). Therefore, the C-T haplotype of TLR2 rs3804099-rs3804100 may influence TB infection by affecting cytokine production or NK cell counts.

In this study, we found that NLRP3 rs34298354 and the TLR2 rs3804099-rs3804100 haplotype were associated with susceptibility to TB. However, rs34298354, rs3804099, and rs3804100 are exonic, synonymous SNPs. In the GTExPortal database, rs3804099 and rs3804100 are reported to be associated with TLR2 expression (according to expression quantitative trait loci analysis) in whole blood and some tissues (https://gtexportal.org/home/snp/rs3804099; https://gtexportal.org/home/snp/rs3804100). The synonymous genetic variants alter mRNA splicing, mRNA stability, mRNA structure, and protein folding\(^{31}\). In MDR1, the synonymous SNP C3435T is reported to alter protein activity/substrate specificity\(^{32}\). Peripheral blood mononuclear cells from subjects with the NLRP3 rs34298354 CC genotype had higher IL-1\(\beta\) levels than those from subjects with the CT genotype after stimulation with dead mycobacterium avium complex (MAC) bacilli and lipopolysaccharide\(^{33}\). In this study, we did not investigate whether the associations of rs34298354, rs3804099, and rs3804100, with TB risk were due to respective changes in NLRP3 or TLR2 gene expression or protein activity/substrate specificity, but this should be done in the future.

The TB statistics of the Taiwan CDC showed that TB prevalence in men is higher than in women, with a ratio of 2.2:1 in 2017\(^{2}\). Accordingly, we separated men from women to examine the possibility of a difference in TB prevalence between non-TB and TB subjects (Table 4). Our results indicate that the IFI16 rs1772408 AG genotype is associated with a reduced risk of TB in women. However, no report has indicated that this genotype is related to regulation of IFI16 gene expression and the development of disease. Similar to previous studies in the Han Taiwanese population, SOC53 SNPs rs4331426 in women and rs5037722 in men were associated with TB\(^{25,26}\) and NLRP3 SNPs rs3806268 and rs34298354 in women and TLR2 SNP rs3804100 in men were associated with MAC\(^{25}\). In addition, bisphenol A, an environmental estrogen, stimulates IFI16 protein expression in human peripheral blood mononuclear cells\(^{26}\), suggesting a possible gender-dependent association of IFI16 gene polymorphism with TB risk. rs1772408 is located in the seventh intron of IFI16. Intronic polymorphisms can act as enhancers or silencers that regulate mRNA splicing\(^{36}\). In addition, the database GTExPortal reports a significant association between different IFI16 rs1772408 genotypes and IFI16 expression in skin (https://gtexportal.org/home/snp/rs1772408). Thus, rs1772408 may exert a gender-dependent effect on susceptibility to TB by regulating IFI16 expression. A future study that involves a larger number of TB and non-TB subjects and compares IFI16 expression levels among different genotypes will help solidify the association of rs1772408 with susceptibility to TB.

Our study has some limitations: First, the sample size was relatively small and some significance may have been under-estimated. According to the method of Levine et al.\(^{37}\) by using the Post-hoc Power Calculator on web (https://clincalc.com/stats/Power.aspx), the statistical powers for ORs ratio analysis of rs34298354 TC genotype in total subjects, rs1772408 AG genotype in women, and rs3804099-rs3804100 C-T haplotype in total subjects were 0.41, 0.70, and 0.90, respectively. Except haplotype analysis, the total number of subjects provided low statistical power (<0.8) in this study. Thus, a future study to further increase the number of each grouped subjects will help solidify our finding. Second, the selected participants were enroll in Taiwan, which means our study

### Table 4. Odds ratio analysis of AIM2 SNP (rs2276405), NLRP3 SNPs (rs34298354, rs3806268, rs7525979, rs6689545), TLR2 SNPs (rs3804099, rs3804100, rs5743705), and IFI16 SNP (rs1772408) in men and women with or without TB. \(p\) values were determined by the \(\chi^2\) test. \(^{a}\)Adj. = adjusted for age by logistic regression; ref. = reference genotype.
| SNP ID | Genotype | Genotype counts | p value<sup>a</sup> | Adj. OR (95% CI)<sup>b</sup> | p for OR |
|--------|----------|----------------|------------------|--------------------------|----------|
|        |          | Non-TB | TB |                   |           |           |
| rs2276405 |   ≤65   | TT      | 2 (2.4) | 0 (0.0) | 0.134 |          |
|         |          | TC      | 11 (13.3) | 25 (16.6) | 1.401 (0.632, 3.106) | p = 0.407 |
|         |          | CC (ref.) | 70 (84.3) | 126 (83.4) |           |           |
|         |          | >65   | TT      | 3 (2.3) | 0 (0.0) | 0.226 |          |
|         |          | TC      | 13 (10.0) | 12 (15.2) | 1.573 (0.670, 3.689) | p = 0.298 |
|         |          | CC (ref.) | 114 (87.7) | 67 (84.8) |           |           |
| rs34298354 |   ≤65   | TT      | 0 (0.0) | 0 (0.0) | 0.038 |          |
|         |          | TC      | 14 (16.9) | 12 (7.9) | 0.662 (0.196, 1.087) | p = 0.077 |
|         |          | CC (ref.) | 69 (83.1) | 139 (92.1) |           |           |
|         |          | >65   | TT      | 0 (0.0) | 0 (0.0) | 0.807 |          |
|         |          | TC      | 18 (13.8) | 10 (12.7) | 0.720 (0.309, 1.680) | p = 0.447 |
|         |          | CC (ref.) | 112 (86.2) | 69 (87.3) |           |           |
| rs3806268 |   ≤65   | GG      | 14 (16.9) | 32 (21.2) | 0.727 | 1.274 (0.563, 2.882) | p = 0.561 |
|         |          | AG      | 42 (50.6) | 72 (47.7) | 0.898 (0.476, 1.691) | p = 0.738 |
|         |          | AA (ref.) | 27 (32.5) | 47 (31.1) |           |           |
|         |          | >65   | GG      | 34 (26.2) | 20 (25.3) | 0.926 | 0.855 (0.390, 1.875) | p = 0.696 |
|         |          | AG      | 63 (48.5) | 37 (46.8) | 0.847 (0.426, 1.684) | p = 0.636 |
|         |          | AA (ref.) | 33 (25.3) | 22 (27.9) |           |           |
| rs7525979 |   ≤65   | TT      | 1 (1.2) | 3 (2.0) | 0.716 | 1.370 (0.130, 14.396) | p = 0.793 |
|         |          | TC      | 21 (25.3) | 32 (21.2) | 0.818 (0.424, 1.577) | p = 0.549 |
|         |          | CC (ref.) | 61 (73.5) | 116 (76.8) |           |           |
|         |          | >65   | TT      | 1 (0.8) | 2 (2.5) | 0.340 | 2.953 (0.261, 33.461) | p = 0.382 |
|         |          | TC      | 27 (20.8) | 21 (26.6) | 1.406 (0.722, 2.739) | p = 0.317 |
|         |          | CC (ref.) | 102 (78.4) | 56 (70.9) |           |           |
| rs6689545 |   ≤65   | CC      | 0 (0.0) | 0 (0.0) | 0.337 |          |
|         |          | TC      | 3 (3.6) | 10 (6.6) | (2*2 χ² test) | 1.774 (0.456, 6.903) | p = 0.409 |
|         |          | TT (ref.) | 80 (96.4) | 141 (93.4) |           |           |
|         |          | >65   | CC      | 0 (0.0) | 0 (0.0) | 0.558 |          |
|         |          | TC      | 13 (10.0) | 6 (7.6) | (2*2 χ² test) | 0.733 (0.263, 2.042) | p = 0.553 |
|         |          | TT (ref.) | 117 (90.0) | 73 (92.4) |           |           |
| rs3804099 |   ≤65   | CC      | 7 (8.4) | 19 (12.6) | 0.574 | 1.975 (0.741, 5.263) | p = 0.173 |
|         |          | TC      | 30 (36.1) | 56 (37.1) | 1.105 (0.607, 2.011) | p = 0.743 |
|         |          | TT (ref.) | 46 (55.5) | 76 (50.3) |           |           |
|         |          | >65   | CC      | 8 (6.3) | 5 (6.3) | 0.913 | 1.010 (0.306, 3.332) | p = 0.987 |
|         |          | TC      | 49 (37.7) | 32 (40.5) | 1.286 (0.664, 2.192) | p = 0.539 |
|         |          | TT (ref.) | 73 (56.1) | 42 (53.2) |           |           |
| rs3804100 |   ≤65   | CC      | 5 (6.0) | 10 (6.6) | 0.968 | 1.186 (0.367, 3.838) | p = 0.775 |
|         |          | TC      | 30 (36.1) | 56 (37.1) | 1.127 (0.624, 2.035) | p = 0.691 |
|         |          | TT (ref.) | 48 (57.9) | 85 (56.3) |           |           |
|         |          | >65   | CC      | 6 (4.6) | 2 (2.5) | 0.566 | 0.539 (0.102, 2.847) | p = 0.467 |
|         |          | TC      | 50 (38.5) | 27 (34.2) | 0.823 (0.452, 1.498) | p = 0.524 |
|         |          | TT (ref.) | 74 (56.9) | 50 (63.3) |           |           |
| rs5743705 |   ≤65   | CC      | 0 (0.0) | 0 (0.0) | 0.844 |          |
|         |          | TC      | 6 (7.2) | 12 (7.9) | (2*2 χ² test) | 0.959 (0.333, 2.757) | p = 0.937 |
|         |          | TT (ref.) | 77 (92.8) | 139 (92.1) |           |           |
|         |          | >65   | CC      | 0 (0.0) | 0 (0.0) | 0.941 |          |

Continued
Taiwanese population. NLRP3 haplotype. We conclude that haplotype at showed a gender-dependent influence on TB susceptibility. Through haplotype analysis, we found that the C-T rs34298354 showed reduced risk of TB, and AG heterozygosity at rs1772408 NLRP3 IFI16 rs34298354 and without TB. In addition, there were significant differences in the genotype frequencies of IFI16 rs3804100; and tag SNPs rs3804099, rs3804100, rs5743705, and IFI16 SNP (rs1772408) in non-aged (≤ 65-year-old) subjects with or without TB. After the OR was adjusted for age and gender, in men and non-aged subjects and of IFI16 rs34298354 and without TB. In addition, there were significant differences in the genotype frequencies of NLRP3 rs34298354, TLR2 SNPs (rs3804099, rs3804100, rs5743705), and IFI16 SNP (rs1772408) in women. After the OR was adjusted for age and gender, AG heterozygotes at NLRP3 rs34298354 showed reduced risk of TB, and AG heterozygosity at IFI16 rs1772408 showed a gender-dependent influence on TB susceptibility. Through haplotype analysis, we found that the C-T haplotype at TLR2 rs3804099-rs3804100 was associated with an increased risk for TB compared with the T-T haplotype. We conclude that NLRP3, IFI16, and TLR2 polymorphisms are associated with TB risk in the Han Taiwanese population.

### Subjects and methods

#### Study population.

In this prospective study, all participants (297 men, 146 women) were recruited from General Taoyuan Hospital (Taoyuan, Taiwan) from January 2016 to December 2019. The inclusion criteria for TB group were as follows: adult patients (20 to 99 years old) diagnosed with active TB, with evident TB lesions and with TB infection diagnosis in our study. The influence of these factors on TB development has been suggested, and this may affect the assay results.

Our result showed a significant difference in AIM2 rs2276405 genotype frequencies between subjects with and without TB. In addition, there were significant differences in the genotype frequencies of NLRP3 rs34298354 in men and non-aged subjects and of IFI16 rs1772408 in women. After the OR was adjusted for age and gender, AG heterozygotes at NLRP3 rs34298354 showed reduced risk of TB, and AG heterozygosity at IFI16 rs1772408 showed a gender-dependent influence on TB susceptibility. Through haplotype analysis, we found that the C-T haplotype at TLR2 rs3804099-rs3804100 was associated with an increased risk for TB compared with the T-T haplotype. We conclude that NLRP3, IFI16, and TLR2 polymorphisms are associated with TB risk in the Han Taiwanese population.

#### DNA purification from buccal swabs.

According to the methods of Wu et al., genomic DNA was purified from oral swabs collected from the 443 subjects using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA). Briefly, the buccal swab placed in a 2-ml microcentrifuge tube with 400 µl PBS, 20 µl QIAGEN protease stock solution and 400 µl Buffer AL and then incubated at 56 °C for 10 min to lyse the cells. After mixing the cell lysate with 400 µl of absolute ethanol, the supernatant after centrifugation at 6,000 × g for 1 min was applied to a QIAamp Mini spin column for DNA purification and washed twice with buffer AW1 and AW2. DNA was eluted from the spin column with Buffer AE or sterile distilled deionized water (150 µl) for a 1-min incubation at room temperature before centrifugation at 6,000 × g for 2 min. The purified DNA concentration was quantified by spectrophotometry at 260 nm and then stored at −80 °C until SNP genotyping by TaqKey Science Co., LTD (Hualien, Taiwan).

#### SNP genotyping assays.

We chose nine SNPs to study the association of inflammasome genes and TLR2 with TB susceptibility. The tag SNPs of the AIM2, NLRP3, IFI16, and TLR2 genomic regions were selected according to the SeattleSNPs website (https://gvs.gs.washington.edu/GVSN150). The SeattleSNPs database showed AIM2 tag SNP rs2276405; NLRP3 tag SNPs rs3806268, rs7525979, and rs689545; TLR2 tag SNPs rs3804099 and rs3804100; and IFI16 tag SNP rs1772408 (all tag SNPs with minor allele frequency >0) used for the study in Han Chinese Beijing (HCB). In addition, rs34298354 in NLRP3 and rs5743705 in TLR2 were selected on the basis of a prior report. All SNP genotyping was performed using TaqMan SNP Genotyping Assays. The primers and probes for the selected SNPs were from an ABI assay on demand (AOD) kit (cat. #4351379, Thermo Fisher Scientific).
| SNP                      | Frequency (%), (n) | Non-TB (n) | TB (n) | p value<sup>a</sup> | Adj. OR (95% CI)<sup>b</sup> | p value for OR |
|--------------------------|--------------------|------------|--------|---------------------|-----------------------------|----------------|
| rs7525979-rs3806268      |                    |            |        |                     |                             |                |
| Haplotype                |                    |            |        |                     |                             |                |
| C-A (ref.)               | 53.3               | 225        | 247    | 0.678               |                             |                |
| C-G                     | 33.7               | 149        | 150    | 0.937 (0.689, 1.274) | 0.679                       |                |
| T-G                     | 13.0               | 52         | 63     | 1.083 (0.702, 1.670) | 0.720                       |                |
| Diploype                 |                    |            |        |                     |                             |                |
| C-A/C-A (ref.)           | 60                 | 60         | 60     |                     |                             |                |
| C-G/any                 | 124                | 121        | 123    | 0.865 (0.546, 1.369) | 0.535                       |                |
| T-G/any                 | 50                 | 58         | 66     | 0.969 (0.565, 1.662) | 0.910                       |                |
| rs3804099-rs3804100      |                    |            |        |                     |                             |                |
| Haplotype                |                    |            |        |                     |                             |                |
| T-T (ref.)               | 72.0               | 315        | 323    | 0.014               |                             |                |
| C-C                     | 23.2               | 100        | 106    | 1.011 (0.724, 1.411) | 0.949                       |                |
| C-T                     | 4.4                | 9          | 30     | 3.406 (1.546, 7.505) | 0.002                       |                |
| T-C                     | 0.4                | 2          | 1      | 0.810 (0.066, 9.988) | 0.870                       |                |
| Diploype                 |                    |            |        |                     |                             |                |
| T-T/T-T (ref.)           | 118                | 117        | 119    |                     |                             |                |
| C-C/any                 | 90                 | 94         | 94     | 1.042 (0.691, 1.572) | 0.843                       |                |
| C-T/any                 | 9                  | 30         | 39     | 3.513 (1.550, 7.962) | 0.003                       |                |
| T-C/any                 | 2                  | 1          | 3      | 0.834 (0.067, 10.402) | 0.888                       |                |

Table 6. Haplotype and diplotype distribution of the two investigated NLRP3 and TLR2 polymorphisms in control subjects and TB patients. <sup>a</sup>p values were determined by the χ² test; <sup>b</sup>Adj. = adjusted for age and gender by logistic regression; ref. = reference genotype; TB = tuberculosis; OR = odds ratio.

Scientific reports. Reactions were carried out according to the manufacturer’s protocol (TaqMan SNP Genotyping Assays, protocol, Part Number 4332856 Rev. C). The probe fluorescence signal was detected using an ABI Prism 7900 Real-Time PCR System.

Statistical analysis. All statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Genotype deviations from Hardy-Weinberg equilibrium were assessed and the differences in genotype frequencies between the non-TB and TB groups were tested using the χ² test<sup>6</sup>. Intermarker linkage disequilibrium (LD) measures r² and D² were estimated and haplotype blocks were defined using Haploview v.4.2<sup>41</sup>. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from contingency tables<sup>42</sup>. The associations between different genotypes and TB were estimated by odds ratios (ORs) from univariate and multivariate logistic regression analyses adjusted by age and gender.

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Author contributions
C.W. and L.S.W. performed the analysis and prepared the manuscript. L.S.W. and S.W.L. designed the project. C.J.L., H.C.H., H.J.L., Y.C.C., and S.W.L. the experiments. L.S.W. supervised the project and revised the manuscript. S.W.L. reviewed the manuscript. All authors read and approved the final version of the manuscript.

Competing interests
The authors declare no competing interests.

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