Infection, Genetics and Evolution 75 (2019) 103950

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

RIPK2 polymorphisms and susceptibility to tuberculosis in a Western Chinese Han population

Jiajia Song[a], Tangyuheng Liu[a], Lin Jiao[a], Zhenzhen Zhao[a], Xuejiao Hu[a], Qian Wu[a], Hao Bai[a], Mengyuan Lv[a], Zirui Meng[a], Tao Wu[a], Hao Chen[a], Xuerong Chen[b], Xingbo Song[a,*], Binwu Ying[a,*]

[a] Department of Laboratory Medicine, West China Hospital, Sichuan University, Chengdu 610041, PR China
[b] Division of Pulmonary Disease, West China Hospital, Sichuan University, Chengdu 610041, PR China

ARTICLE INFO

Keywords:
Tuberculosis
Polymorphism
Receptor-interacting protein
Susceptibility
RIPK2

ABSTRACT

Objective: Host genetic factors play an important role in susceptibility to *Mycobacterium tuberculosis* (MTB) infection and tuberculosis (TB). Receptor interacting-serine/threonine-protein kinase 2 (RIPK2) is a critical adapter protein for signal propagation of NOD2, dysregulation of which leads to defects in bacterial detection. To investigate the role of *RIPK2* on the susceptibility of tuberculosis, we conducted a large sample size case-control study in a Western Chinese Han population.

Methods: Five single-nucleotide polymorphisms (SNPs) within or near to *RIPK2* were genotyped in 1359 TB cases and 1534 controls using the improved multiplex ligation detection reaction method in a case-control study.

Results: Of the five variants, rs39509 was observed to be associated with TB risk in the allelic effects ($P = 0.015$), additive ($P = 0.020$) and recessive model ($P = 0.005$) after Bonferroni correction. Rs39509 might fall in putative functional regions and might be eQTL for the *RIPK2* and long non-coding RNA *RP11-37B2.1* according to the Genotype-Tissue Expression (GTEx) Project.

Conclusions: Our findings firstly exhibit that the G allele of rs39509 in nearGene-3 region of *RIPK2* might serve as a hazard for TB in this Western Chinese Han population. Further validation studies on a variety of ethnic populations and function experiments are needed to confirm the roles of the variants identified.

1. Introduction

Tuberculosis (TB) is an ancient infectious disease which plagued human beings for thousands of years with approximately one billion deaths in the past two centuries and still remains to be a significantly serious public health problem in recent years (Dheda et al., 2015) with a yearly incidences of approximately 10 million cases and a mortality of 1.57 million worldwide according to the World Health Organization (WHO) 2018 Global TB report (World Health Organization Global tuberculosis report, 2018). Because of differences between individuals, one third of the world's population is infected with *Mycobacterium tuberculosis* (MTB), yet only 5% to 10% develop active clinical TB (Cantini et al., 2015). Host genetic factors play an important role in susceptibility to mycobacterial infection and tuberculosis (Fitzgerald and Caffrey, 2014). Möller et al. demonstrated that the contribution of host genetic factors to the immune response and phenotypic variation in the population infected with TB ranges up to 71% (Möller and Hoal, 2010). However, the exact mechanism remains unclear, further study of the host molecule elements involved in TB infection would considerably expanded our understanding of the genetic basis of susceptibility to TB.

Pattern recognition receptors (PRR), such as Toll like receptors (TLRs) (Akira et al., 2006) or nucleotide binding oligomerization domain -like receptors (NLRs) (Franchi et al., 2008), are important for the initial identification of bacterium. The Nucleotide-binding-oligomerization-domain-containing proteins 2 (NOD2), one of NLRs, can detect conserved cell wall features of intracellular bacteria such as MTB and *Mycobacterium leprae* and trigger a series of downstream signal transduction pathways through their adapter protein the Receptor interacting-serine/threonine-protein kinase 2 (RIPK2) (Kobayashi et al., 2002). This results in the activation and translocation of nuclear transcription factors kappa B (NFκB) which stimulates the transcription and expression of inflammatory cytokines (Inohara and Nuñez, 2003). RIPK2 is such an adapter protein that is critical for signal propagation of NOD2 (Gong et al., 2018). Dysregulation of this signaling pathway leads to defects in bacterial detection. Therefore, we hypothesized that variants within *NOD2* and *RIPK2* genes might also be associated with susceptibility to mycobacterium infection. In the aspect of leprosy disease, a genome-wide association study (GWAS) conducted by Zhang

* Corresponding authors.
E-mail addresses: songxingbo1981@163.com (X. Song), docbwy@126.com (B. Ying).

https://doi.org/10.1016/j.meegid.2019.103950
Received 12 February 2019; Received in revised form 23 June 2019; Accepted 1 July 2019
Available online 03 July 2019

et al. has demonstrated that variants of genes in the NOD2-mediated signaling pathway included *RIPK2* gene are correlated with predisposition to infection with *Mycobacterium leprae* (Zhang et al., 2009). In the aspect of TB disease, the relationship between *NOD2* gene polymorphisms have been discussed in many studies and significant differences have been observed in several single nucleotide polymorphisms (SNPs) within *NOD2* gene [Arg587Arg (Zhao et al., 2012), rs7194886 (Pan et al., 2012), rs2066842, rs2066844, and rs5743278 (Austin et al., 2008)]. However, little studies have examined the correlations between polymorphisms within *RIPK2* gene and tuberculosis predisposition.

In the present study, we aimed to firstly investigate five SNPs of *RIPK2* to explore the association between them and susceptibility to TB in a Western Chinese Han population.

2. Methods

2.1. Subjects

All participants were consecutively recruited between October 2014 and October 2017 from West China Hospital of Sichuan University for our case-control study. All of the TB patients included were confirmed according to the following criteria: 1) for the Han population; 2) clinically diagnosed by two independent experienced respiratory physicians according to standard criteria recommended by WHO (World Health Organization, 2009); 3) without evidence of immunodeficiency diseases and other lung problems. Finally, cases group included 1359 TB patients in the same population of cases. At the same time, we recruited the 1534 control subjects from healthy blood donors who have no positive TB-related examinations, no history of TB, and absent symptoms of active TB disease. Healthy individuals with evidence of immunodeficiency diseases and other lung problems were also excluded.

The study was approved by the Committee on Human Research, Publications, and Ethics, West China Hospital, Sichuan University (Reference no. 198; 2014), and informed consent was obtained from all participants before blood sampling.

2.2. Genetic molecular analyses

We obtained genetic variation data of the entire *RIPK2* gene and its upstream and downstream intergenic regions from the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/) and comprehensively searched candidate SNPs for this study. We selected SNPs with a minor allele frequency > 0.20 in the East Asian population according to the 1000 Genomes Project. Finally, a total of five SNPs (rs10956271, rs39509, rs7015630, rs2697668, and rs989384) were chosen for the next analyses. A 2 to 3 mL EDTA-anticoagulated peripheral blood sample was taken from each participant in the morning before breakfast. Genomic DNA was extracted from whole blood samples by the QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany) and stored at -80× for subsequent genotyping. The genotype used was an improved multiplex ligation detection reaction (iMLDR) method (Genesky Biotechnologies Inc., Shanghai, China) (Liu et al., 2013). Experienced technicians who didn't know the status of cases and controls performed genotyping. After genotyping, we randomly selected 10% of the total samples for re-genotyping as quality controls and the concordance rate is 100%.

2.3. Statistical analysis

For the demographic characteristics, differences between two groups were evaluated by the chi-square test (for categorical variables) and the Student *t*-test or Mann-Whitney *U* test (for continuous variables) using SPSS20.0(IBM, Chicago, USA). The PLINK version 1.07 was used for polymorphisms analysis (Purcell et al., 2007). The Hardy-Weinberg equilibrium (HWE) of each SNP was tested using the goodness-of-fit chi-square test for controls. Associations between TB susceptibility and candidate genetic allele using the chi-square test. Unconditional logistic regression models were used to examine the association between the genetic models (additive, dominant, and recessive model) and TB risk while adjusting for gender and age. We calculated the sample size using Quanto Version 1.2.4. The sample size with a minimum of 1400 cases and 1400 controls had a statistical power of > 80% to identify an association with an OR of 1.20 or greater at a p value of < 0.05 for variants with a MAF of no < 0.20 under an additive model. The linkage disequilibrium (LD) was estimated by calculating the pairwise r^2 coefficient using Haploview software version 4.2, which employed the expectation-maximization clustering algorithm. All tests were two-sided, and a P value of < 0.05 was considered to be statistical significance; a Bonferroni P was canculated to correct for multiple testing.

2.4. Functional annotation

We identified the SNPs ($r^2 > 0.9$) in strong linkage disequilibrium (LD) with the SNPs associated with TB risk in our correlation analysis according to data from the 1000 Genomes Project (The 1000 Genomes Project Consortium, 2012).We analyzed positive loci and their linked SNPs about regions promoter or enhancer activity, DNAse I hypersensitivity, proteins bound and transcription factor binding motifs using the Encyclopedia of DNA Elements tool HaploReg (v4.1) (Ward and Kellis, 2012). In addition, we examined genome-wide eQTL data in multiple tissues from the Genotype-Tissue Expression (GTEx) Project (Carithers et al., 2015) and the Multiple Tissue Human Expression Resource Project (Grundberg et al., 2012). eQTL is a region of the chromosome that can specifically regulate mRNA or protein expression levels. eQTL can serve as a bridge between genetic mutations and phenotypes, providing new ideas for explaining the association between mutations and phenotypes.

3. Results

Ultimately, 1359 TB patients for cases group and 1534 healthy individuals for controls group were included in our case-control study. The mean age (± standard deviation) of cases group (42.90 ± 18.12) was higher than control group (37.96 ± 11.07) in Table 1 ($P = 0.001$). Among 1359 TB cases, 809 (59.5%) were males and 550 (40.5%) were females while control group was composed of 821 (53.5%) males and 713 (46.5%) females in Table 1 ($P < 0.001$).

We typed 1359 TB patients and 1534 healthy controls from the same region and ethnic group for five selected SNPs (rs10956271, rs39509, rs7015630, rs2697668, and rs989384) within *RIPK2* gene or its upstream and downstream intergenic regions. We summarized the detailed information of candidate SNPs, including chromosomal locations, functional annotations, minor allele frequencies (MAFs), and P values for Hardy-Weinberg equilibrium (HWE) test in control subjects in

Table 1
The demographic data of all study subjects.

Variables	Cases		Controls		*P*
	N = 1359		N = 1534		
	N	%	N	%	
Gender					
Male	809	59.5	821	53.5	0.001
Female	550	40.5	713	46.5	
Age, years					
Mean ± SD	42.90 ± 18.115		37.96 ± 11.074		< 0.001
Median(Quartile)	42(27, 57)		36(29, 45)		< 0.001

Table 2
Genotype distributions of polymorphisms of TB cases and healthy controls.

SNP	Case n(%)	Control n(%)	OR (95% CI)	P*	P[b]†		Case n(%)	Control n(%)	P*	P[b]†
rs10956271	609(22.47)	728(23.76)	0.93(0.82,1.05)	0.247	1.000	AA	73(5.39)	82(5.35)	0.326	1.000
G > A	2101(77.53)	2336(76.24)				AG	463(34.17)	564(36.81)		
						GG	819(60.44)	886(57.83)		
rs39509	1348(49.74)	1404(45.85)	1.17(1.05,1.30)	0.003	0.015	GG	356(26.27)	324(21.16)	0.005	0.025
A > G	1362(50.26)	1658(54.15)				GA	636(46.94)	756(49.38)		
						AA	363(26.79)	451(29.46)		
rs7015630	608(23.00)	761(25.52)	0.87(0.77,0.99)	0.028	0.140	GG	75(5.67)	102(6.84)	0.092	0.460
A > G	2036(77.00)	2221(74.48)				GA	458(34.64)	557(37.36)		
						AA	789(59.68)	832(55.8)		
rs2697668	1319(48.67)	1550(50.59)	0.93(0.84,1.03)	0.146	0.730	GG	329(24.28)	390(25.46)	0.259	1.000
A > G	1391(51.33)	1514(49.41)				GA	661(48.78)	770(50.26)		
						AA	365(26.94)	372(24.28)		
rs989384	810(29.85)	899(29.36)	1.02(0.91,1.15)	0.687	1.000	AA	128(9.43)	133(8.69)	0.780	1.000
G > A	1904(70.15)	2163(70.64)				AG	554(40.83)	633(41.35)		
						GG	675(49.74)	765(49.97)		

SNP = single-nucleotide polymorphism; OR = odds ratio; CI = confidence interval.
* P was calculated by Chi-square test.
† P was calculated after Bonferroni correction.

Table 3
Comparison of polymorphisms in relation to TB risk in Chinese Han population in the genetic models (additive, dominant, and recessive model).

SNP	Additive model			Dominant model			Recessive model		
	OR (95% CI)	P^a*	P^b†	OR (95% CI)	P^a*	P^b†	OR (95% CI)	P^a*	P^b†
rs10956271G > A	0.92(0.82,1.05)	0.211	1.000	0.89(0.77,1.04)	0.142	0.710	0.98(0.71,1.36)	0.911	1.000
rs39509A > G	1.16(1.05,1.29)	0.004	0.020	1.14(0.96,1.34)	0.134	0.670	1.33(1.12,1.59)	0.001	0.005
rs7015630A > G	0.88(0.77,0.99)	0.036	0.180	0.85(0.73,0.99)	0.042	0.210	0.83(0.61,1.14)	0.250	1.000
rs2697668A > G	0.93(0.84,1.03)	0.162	0.810	0.87(0.73,1.03)	0.112	0.560	0.94(0.79,1.12)	0.487	1.000
rs989384G > A	1.01(0.90,1.13)	0.877	1.000	1.01(0.90,1.13)	0.877	4.385	1.06(0.82,1.37)	0.663	1.000

SNP = single-nucleotide polymorphism; OR = odds ratio; CI = confidence interval.
* P was adjusted by age and gender.
† P was calculated after Bonferroni correction.

Appendix A Table A.$_1$. The genotype distributions of the tested SNPs did not deviate from in the control group (HWE-P > 0.05). The association between the five SNPs and TB risk was studied. Initially, two polymorphisms (rs39509 and rs7015630) were observed to be significantly associated with susceptibility to TB before Bonferroni correction (P = 0.003 and 0.028 respectively) in the allelic effects (Table 2). After Bonferroni correction, the correlation between rs39509 and tuberculosis susceptibility was still statistically significant (P = 0.015, OR = 1.17, 95%CI = 1.05–1.30), while rs7015630 was not (P = 0.140). The G frequency of rs39509 among cases (49.74%) was higher than among controls (45.85%). We then estimated the correlation of polymorphisms and TB risk under the three genetic models (additive, dominant, and recessive model). Rs39509 was significantly associated with TB susceptibility both in additive (P = 0.020, OR = 1.16, 95%CI = 1.05–1.29) and recessive model (P = 0.005, OR = 1.33, 95%CI = 1.12–1.59) after adjusted by age and gender and Bonferroni correction (Table 3). The results suggested that the GG genotype of rs39509 might serve as a hazard for TB. Moreover, there was a weak correlation observed between rs7015630 and the susceptibility of TB in the additive (P = 0.036) and dominant (P = 0.042) model adjusted by age and gender. However, after Bonferroni correction, the correlation was no longer statistically significant. All five variants in/near to the *RIPK2* gene were analyzed whether were in a linkage disequilibrium block according to the threshold of pairwise r^2 > 0.5. As presented in Appendix A Figure A.$_1$, the LD between rs2697668 and rs989384 in our studies is weak (r^2 = 0.42), and the other three SNPs selected were considered independent (r^2 < 0.3).

Rs39509 which was associated with TB susceptibility is in a nearGene-3 region, 873 bp 3′ of *RIPK2*. Eight SNPs were identified strong linked (r^2 > 0.9) with rs39509 according to the data form the 1000 Genomes Project (Appendix A Table A.$_2$). Three of them map to intronic region of the *RIPK2* gene and the remaining five SNPs are in intergenic region near to 3′ of *RIPK2*. According to data from the Encyclopedia of DNA Elements Project, rs40247, rs40453, rs400411, rs39509, rs383592, rs201720542, rs411279, and rs51331 might be located in a region with strong promoter or/and enhancer activity; rs400411, rs39509, rs383592, rs411279, and rs51331 might be located in a region with DNAse I hypersensitivity site; rs51331 might be located in a region of transcription factor binding; rs40247, rs40453, rs400411, rs39509, rs383592, rs201720542, rs411279, and rs416324 might be in the regulatory motif (Appendix A Table A.$_2$). Except for rs201720542, the GTEx Project shows that the eight SNPs are eQTLs for the *RIPK2* and *RP11-37B2.1* and associated with an increase in *RIPK2* and a decrease in *RP11-37B2.1* (Appendix A Table A.$_3$).

4. Dissusion

RIPK2 is an adapter protein that is critical for signal propagation of NOD2 (Inohara and Nuñez, 2003). The NOD2 system is specialized in identifying bacteria with actively disrupt host membranes and is especially sensitive to mycobacteria (Pandey et al., 2009). The relationship between *NOD2* gene polymorphisms and TB susceptibility has been discussed in many studies and significant associations have been observed in several single nucleotide polymorphisms (SNPs) within *NOD2* gene [Arg587Arg (Zhao et al., 2012), rs7194886 (Pan et al., 2012), rs2066842, rs2066844, and rs5743278 (Austin et al., 2008)]. However, little studies have examined the correlations between polymorphisms within *RIPK2* gene and tuberculosis predisposition. Therefore, we carry out the first association study to clarify the effect of polymorphisms within or near to *RIPK2* gene on TB risk in a Western

Chinese Han population.

In our study, rs39509 was observed to be associated with TB risk and showed an eQTL effect on the *RP11-37B2.1* and *RIPK2* according to the data from The GTEx Project (Appendix A Table A.$_1$). *RP11-37B2.1*, located at chromosome8: 89,609,409-89,757,727, adjoining *RIPK2*, is a newly discovered long noncoding RNA. A genome-wide association study (GWAS) of TB disease conducted by Thye T et al. (Thye et al., 2010) has demonstrated that the SNP rs160441 within the lncRNA *RP11-37B2.1* gene is significantly associated with genetic predisposition to TB in a Ghanaian population as well as in an independent Gambian population. Rs160441 also showed an eQTL effect on the *RP11-37B2.1* and *RIPK2* according to the data from the GTEx Project. We speculated that rs39509 and rs160441 may affect *Mycobacterium tuberculosis* infection through similar mechanisms. However, in our previous study, rs160441 was not found to be associated with tuberculosis susceptibility in a Western Chinese Han population (Song et al., 2019). *RIPK2*, a protein-coding gene, encodes a member of the receptor-interacting protein (RIP) family of serine/threonine protein kinases which is a part of signaling complexes in both the innate and adaptive immune pathways. SNPs of *RIPK2* gene has been observed associated with relevant immunological diseases, such as systemic lupus erythematosus (SLE) (Li et al., 2012) and with the severity of childhood atopic asthma (Nakashima et al., 2006), and neoplastic diseases, such as gastric cancer (Ota et al., 2018) and urothelial bladder cancer (Guirado et al., 2012). On the other hand, RIPK2 is one of adapter proteins for PRRs which play a key role in fighting infection of intracellular bacteria. Rs383592 linked with rs39509 ($r^2 = 1$) was observed to be associated with leprosy ($P = 1.56 \times 10^{-35}$) (Liu et al., 2015). In addition, a GWAS has demonstrated that the SNP rs42490 within the *RIPK2* gene is significantly associated with predisposition to leprosy (Zhang et al., 2009) and a replicate study conducted in a Vietnamese population showed that rs42490 was significantly associated with leprosy which also is a strong link locus with rs39509 ($r^2 = 0.75$, D' = 0.99) according to the 1000 Genomes Project in Chinese Southern Han population. Moreover, a research suggests that deficiency of NOD2 and RIPK2 increase the vulnerability to M. tuberculosis infection of mice (Divangahi et al., 2015). *RIPK2* polymorphisms were previously associated with leprosy, which is also a mycobacterium infection, we speculate that *RIPK2* may has a similar mechanism for affecting tuberculosis and leprosy susceptibility or tolerance.

There are several limitations to this study. First, the control samples are from healthy people. However, TB is an infectious disease and pathogens are the important factors that affect an individual's susceptibility to TB. Adding a latent TB group might show more clues on how polymorphisms of *RIPK2* affects *Mycobacterium tuberculosis* infection. Second, RIPK2 is critical for signal propagation of NOD1 and NOD2[9]. The genetic variants in or near to *RIPK2* we studied are not independent risk factors for susceptibility to TB, and we did not further perform the gene-gene or gene-environment interaction analyses in our study due to the uncorrelated-TB risk among these SNPs.

5. Conclusions

In conclusion, we firstly identified that rs39509 873 bp 3′ of *RIPK2* might be a predisposing factor for TB risk, and G alleles of rs39509 might serve as a hazard for TB. Rs39509 and its strong LD SNPs associate an increase in *RIPK2* and a decrease in *RP11-37B2.1*. The results of our research strongly suggest that the studied loci around 3′ of *RIPK2* may be novel molecules which plays a decisive role in susceptibility or resistance TB to development in a Western Chinese Han population. Therefore, replications in other independent populations and function experiments are urgently needed.

Funding

This work was supported by grants from the National Natural Science Foundation of China [grant numbers 81472026, 81202375], the Projects of the Health and family planning commission in Sichuan Province [grant number 16ZD004, and the National Science and Technology Major Project of the Ministry of Science and Technology of China [grant number 2018ZX10715003-001]

Acknowledgements

We thank all the study participants and research staff for their contributions and commitment to this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.103950.

References

Akira, S., Uematsu, S., Takeuchi, O., 2006. Pathogen recognition and innate immunity. Cell 124, 783–801. https://doi.org/10.1016/j.cell.2006.02.015.

Austin, C.M., Ma, X., Graviss, E.A., 2008. Common nonsynonymous polymorphisms in the NOD2 gene are associated with resistance or susceptibility to tuberculosis disease in African Americans. J. Infect. Dis. 197, 1713–1716. https://doi.org/10.1086/588384.

Cantini, F., Nannini, C., Niccoli, L., Iannone, F., Delogu, G., Garlaschi, G., Sanduzzi, A., Matucci, A., Prignano, F., Conversano, M., Goletti, D., 2015. Guidance for the management of patients with latent tuberculosis infection requiring biologic therapy in rheumatology and dermatology clinical practice. Autoimmun. Rev. 14, 503–509. https://doi.org/10.1016/j.autrev.2015.01.011.

Carithers, L.J., Ardlie, K., Barcus, M., Branton, P.A., Britton, A., Buia, S.A., Compton, C.C., Deluca, D.S., Peter-demchok, J., Gelfand, E.T., Guan, P., Korzeniewski, G.E., Lockhart, N.C., Robinson, K.L., Roche, N.V., Sawyer, S.J., Shive, C.E., Smith, A.M., Sobin, L.H., Undale, A.H., Valentino, K.M., Vaught, J., Young, T.R., 2015. A novel approach to high-quality Postmortem tissue procurement: the GTEx project. Biopreserv Biobank 13, 311–319. https://doi.org/10.1089/bio.2015.0032.

Dheda, K., Barry, C.E., Maartens, G., Town, C., Town, C., 2015. Tuberculosis. Lancet 387, 1211–1226. https://doi.org/10.1016/S0140-6736(15)00151-8.

Divangahi, M., Mostowy, S., Coulombe, F., Kozak, R., Guillot, L., Veyrier, F., Koichi, S., Flavell, R.A., Gros, P., Marcel, A., Kobayashi, K.S., Flavell, R.A., Gros, P., Behr, M.A., 2015. NOD2-deficient mice have impaired resistance to Mycobacterium tuberculosis infection through defective innate and adaptive immunity. J. Immunol. 181, 7157–7165. https://doi.org/10.4049/jimmunol.181.10.7157.

Fitzgerald, K.A., Caffrey, D.R., 2014. Long noncoding RNAs in innate and adaptive immunity. Curr. Opin. Immunol. 26, 140–146. https://doi.org/10.1016/j.coi.2013.12.001.

Franchi, L., Park, J., Shaw, M.H., Marina-garcia, N., Chen, G., Kim, Y., Núñez, G., 2008. Microreview intracellular NOD-like receptors in innate immunity, infection and disease. Cell. Microbiol. 10, 1–8. https://doi.org/10.1111/j.1462-5822.2007.01059.x.

Gong, Q., Long, Z., Zhong, F.L., Teo, D.E.T., Jin, Y., Yin, Z., Boo, Z.Z., Zhang, Y., Zhang, J., Yang, R., Bhushan, S., Reversade, B., Li, Z., Wu, B., 2018. Structural basis of RIP2 activation and signaling. Nat. Commun. 9, 4993. https://doi.org/10.1038/s41467-018-07447-9.

Grundberg, E., Small, K.S., Hedman, Å.K., Nica, A.C., Buil, A., Keildson, S., Bell, J.T., Yang, T., Meduri, E., Barrett, A., Nisbett, J., Sekowska, M., Wilk, A., Shin, S., Glass, D., Travers, M., Min, J.L., Ring, S., Ho, K., Thorleifsson, G., Kong, A., Thorsteindottir, U., Ainali, C., Dimas, A.S., Hassanali, N., Ingle, C., Knowles, D., Krestyaninova, M., Lowe, C.E., Meglio, P. Di, Montgomery, S.B., Parts, L., Potter, S., Surdulescu, G., Tsaprouni, L., Tsoka, S., Bataille, V., Durbin, R., Nestle, F.O., Rahilly, S.O., Soranzo, N., Lindgren, C.M., Zondervan, K.T., Ahmadi, K.R., Schadt, E.E., Stefansson, K., Smith, G.D., Mccarthy, M.I., Deloukas, P., Dermitzakis, E.T., Spector, T.D., Multiple, T., Human, T., 2012. Articles mapping cis- and trans -regulatory effects across multiple tissues in twins. Nat. Genet. 44, 1084–1089. https://doi.org/10.1038/ng.2394.

Guirado, M., Gil, H., Saenz-lopez, P., Reinboth, J., Garrido, F., Manuel, J., Ruiz-cabello, F., Carretero, R., 2012. Association between C13ORF31, NOD2, RIPK2 and TLR10 polymorphisms and urothelial bladder cancer. Hum. Immunol. 73, 668–672. https://doi.org/10.1016/j.humimm.2012.03.006.

Inohara, N., Nuñez, G., 2003. NODs: intracellular proteins involved in inflammation and apoptosis. Nat. Rev. Immunol. 3, 371–382. https://doi.org/10.1038/nri1086.

Kobayashi, K., Inohara, N., Hernandez, L.D., 2002. RICK/Rip2/CARDIAK mediates signalling for receptors of the innate and adaptive immune systems. Nature 416, 194–199. https://doi.org/10.1038/416194a.

Li, J., Tian, J., Ma, Y., Cen, H., 2012. Association of RIP2 gene polymorphisms and systemic lupus erythematosus in a Chinese population. Mutagenesis 27, 319–322. https://doi.org/10.1093/mutage/ger081.

Liu, Q., Yu, J., Miao, D., Ma, X., Wang, H., Wang, W., Tan, L., 2013. Neurobiology of aging an exploratory study on STX6, MOBP, MAPT, and EIF2AK3 and late-onset Alzheimer's disease. Neurobiol. Aging 34, 1519.e13–1519.e17. https://doi.org/10.1016/j.neurobiolaging.2012.10.004.

Liu, H., Irwanto, A., Fu, X., Yu, G., Yu, Y., Sun, Y., Wang, C., Wang, Z., Okada, Y., Low, H., Li, Y., Liany, H., Chen, M., Bao, F., Li, J., You, J., Zhang, Q., Liu, J., Chu, T.,

Andiappan, A.K., Wang, N., Niu, G., Liu, D., Yu, X., Zhang, L., Tian, H., Zhou, G., Rotzschke, O., 2015. Discovery of six new susceptibility loci and analysis of pleiotropic effects in leprosy. Nat. Genet. 47, 267–271. https://doi.org/10.1038/ng.3212.

Möller, M., Hoal, E.G., 2010. Current findings, challenges and novel approaches in human genetic susceptibility to tuberculosis. Tuberculosis (Edinb) 90, 71–83. https://doi.org/10.1016/j.tube.2010.02.002.

Nakashima, K., Hirota, T., Suzuki, Y., Akahoshi, M., Shimizu, M., Jodo, A., Doi, S., Fujita, K., Ebisawa, M., Yoshihara, S., Enomoto, T., 2006. Association of the RIP2 gene with childhood atopic asthma. Allergol. Int. 55, 77–83. https://doi.org/10.2332/allergolint.55.77.

Ota, M., Tahara, T., Otsuka, T., 2018. Association between receptor interacting serine/threonine kinase 2 polymorphisms and gastric cancer susceptibility. Oncol. Lett. 15, 3772–3778. https://doi.org/10.3892/ol.2018.7785.

Pan, H., Dai, Y., Tang, S., Wang, J., 2012. Polymorphisms of NOD2 and the risk of tuberculosis: a validation study in the Chinese population. Int. J. Immunogenet. 39, 233–240. https://doi.org/10.1111/j.1744-313X.2011.01079.x.

Pandey, A.K., Yang, Y., Jiang, Z., Fortune, S.M., Coulombe, F., Behr, M.A., Fitzgerald, K.A., Sassetti, C.M., Kelliher, M.A., 2009. NOD2, RIP2 and IRF5 play a critical role in the type I interferon response to Mycobacterium tuberculosis. PLoS Pathog., e1000500. https://doi.org/10.1371/journal.ppat.1000500.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81, 559–575. https://doi.org/10.1086/519795.

Song, J., Liu, T., Zhao, Z., Hu, X., Wu, Q., Peng, W., Chen, X., Ying, B., 2019. Associate with susceptibility of tuberculosis and adverse events of antituberculosis drugs in West China. J. Clin. Lab. Anal., e22880. https://doi.org/10.1002/jcla.22880.

The 1000 Genomes Project Consortium, 2012. An integrated map of genetic variation from 1,092 human genomes. Nature 491, 56–65. https://doi.org/10.1038/nature11632.

Thye, T., Vannberg, F.O., Wong, S.H., Owusu-Dabo, E., Osei, I., Gyapong, J., Sirugo, G., Sisay-Joof, F., Enimil, A., Chinbuah, M.A., Floyd, S., Warndorff, D.K., Sichali, L., Malema, S., Crampin, A.C., Ngwira, B., Teo, Y.Y., Smal, K., Rocket, K., Kwiatkowski, D., Fine, P.E., Hill, P.C., Newport, M., Lienhardt, C., Adegbola, R.A., Corrah, T., Ziegler, A., Morris, A.P., Meyer, C.G., Horstmann, R.D., Hill, A.V.S., 2010. Genome-wide association analyses identifies a susceptibility locus for tuberculosis on chromosome 18q11.2. Nat. Genet. 42, 739–741. https://doi.org/10.1038/ng.639.

Ward, L.D., Kellis, M., 2012. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res. 40, D930–D934. https://doi.org/10.1093/nar/gkr917.

World Health Organization, 2009. The Treatment of Tuberculosis: Guidelines, 4th ed. . http://www.who.int/tb/publications/tb_treatmentguidelines/en/.

World Health Organization Global tuberculosis report, 2018. http://wwwwhoint/tb/publications/global_report/en/, Accessed date: 18 September 2018.

Zhang, F.R., Huang, W., Chen, S.M., Sun, L.D., Liu, H., Li, Y., Cui, Y., Yan, X.X., Yang, H.T., Yang, R.D., Chu, T.S., Zhang, C., Zhang, L., Han, J.W., Yu, G.Q., Quan, C., Yu, Y.X., Zhang, Z., Shi, B.Q., Zhang, L.H., Cheng, H., Wang, C.Y., Lin, Y., Zheng, H.F., Fu, X.A., Zuo, X.B., Wang, Q., Long, H., Sun, Y.P., Cheng, Y.L., Tian, H.Q., Zhou, F.S., Liu, H.X., Lu, W.S., He, S.M., Du, W.L., Shen, M., Jin, Q.Y., Wang, Y., Low, H.Q., Erwin, T., Yang, N.H., Li, J.Y., Zhao, X., Jiao, Y.L., Mao, L.G., Yin, G., Jiang, Z.X., Wang, X.D., Yu, J.P., Hu, Z.H., Gong, C.H., Liu, Y.Q., Liu, R.Y., Wang, D.M., Wei, D., Liu, J.X., Cao, W.K., Cao, H.Z., Li, Y.P., Yan, W.G., Wei, S.Y., Wang, K.J., Hibberd, M.L., Yang, S., Zhang, X.J., Liu, J.J., 2009. Genomewide association study of leprosy. N. Engl. J. Med. 361, 2609–2618. https://doi.org/10.1056/NEJMoa0903753.

Zhao, M., Jiang, F., Zhang, W., Li, F., Wei, L., Liu, J., Xue, Y., Deng, X., Wu, F., Zhang, L., Zhang, X., Zhang, Y., Fan, D., Sun, X., Jiang, T., 2012. A novel single nucleotide polymorphism within the NOD2 gene is associated with pulmonary tuberculosis in the Chinese Han, Uygur and Kazak populations. BMC Infect. Dis. 12, 91. https://doi.org/10.1186/1471-2334-12-91.