

ASAP1 gene polymorphisms are associated with susceptibility to tuberculosis in a Chinese Xinjiang Muslim population

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Abstract. Seven single-nucleotide polymorphism (SNP) sites located in ASAP1 gene have been found associated with tuberculosis (TB) susceptibility by genome-wide association studies in Russia. The case-control study was carried out to test whether these seven SNPs were associated with susceptibility to TB in a Chinese Xinjiang Muslim population. The seven SNPs were genotyped in a case-control design that included 780 Xinjiang Muslim subjects (400 TB patients and 380 controls). Multiplex PCR and direct sequencing were used to detect ASAP1 gene polymorphisms. Hardy-Weinberg equilibrium test was performed to test whether the sample was from genetic equilibrium population. The associations of SNPs with TB risk were determined by the distributions of allelic frequencies and different genetic models. Significant differences of the allelic distribution of rs4733781 and rs1017281 in ASAP1 gene were observed between control group and TB group. A allele of rs4733781 was associated with TB risk (TB vs. control, OR=1.242; 95% CI: 1.004-1.537, P=0.046); While in rs1017281 site, G allele was associated with increased risk for TB (TB vs. control, OR: 0.792, 95% CI: 0.643-0.976, P=0.028). The recessive model of rs4733781 (CC vs. AC+AA) in Xinjiang Muslim populations was associated with a lower TB risk [P=0.003, OR=0.51 (0.324-0.802)], while the recessive model of rs1017281 (GG vs. AG+AA) was associated with a higher TB risk [P=0.011, OR=1.792 (1.135-2.828)]. Using case-control analysis, we identified that two genetic polymorphism sites in the ASAP1 relate to host susceptibility of TB in a Chinese Xinjiang Muslim population.

Introduction

Tuberculosis (TB) is one of the public health emergencies all over the world and severely affects human health. TB is especially epidemic in China, which accounted for 12% of the global total cases in 2012 (1). According to 2010 National Technical Steering Group of the Epidemiological Sampling Survey for TB, the weighted prevalence of active, smear-positive, bacteriological positive pulmonary TB were 459/100,000, 66/100,000, 119/100,000, respectively (2). The geographical distribution of TB prevalence presented that it was relatively low in the eastern parts and high in the western parts, and the prevalence rates of active pulmonary TB, Mycobacterium-positive pulmonary TB and smear-positive pulmonary TB in Xinjiang Uygur autonomous region were all higher than that in other provinces (3,4), which demonstrated that Xinjiang bore a heavy burden of TB.

TB is caused by *Mycobacterium tuberculosis* (MTB), which has infected around a third of the world population (5), but only 10% of those infected progress to active disease in their lifetime, and up to 90% of infected people are asymptomatic with a latent infection (6). Susceptibility to TB varies between different people. Since 1890s, how genetic factors affect clinical outcomes with MTB infection was illustrated by a series of research. For example, several twin studies have found that concordance of TB was higher in monozygotic twins compared to dizygotic twins (7-9). Beyond that, adoption research, Genome-wide association studies, and case-control analysis have been performed to demonstrate that the associations between individual genetics and susceptibility to TB (10-12). These indicated that host genetic factors are important determinants of TB susceptibility.

The ASAP1 gene (known as AMAP1 or DDEF1), located at 8q24.1-8q24.2, encodes an Arf GTPase-activating protein (Arf GAP), which is a multifunctional scaffold protein that induces hydrolysis of GTP bound to the ADP ribosylation factor family GTP-binding (Arf) proteins (13-15). ASAP1 has been implicated in regulating cell motility and invasion (16,17). It was found to functionally link with the progression and metastasis of tumor cells, including ovary cancer (18), prostate cancer (19), and breast cancer (20,21). Curtis *et al* (14) found that the expression of ASAP1 gene in MTB-infected dendritic

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cells was dramatically reduced, which may impair DC migration, suggesting a potential mechanism that predisposes to TB.

Genome-wide association study is an effective way (9) to screen for the genes exerting the best population-wide impact on susceptibility to a multifactorial disease (22). By using this method, Curtis *et al* identified a novel ASAP1 gene which was associated with susceptibility to TB (14). Eleven single-nucleotide polymorphisms (SNPs) were identified with significant association with susceptibility to MTB ($P < 5 \times 10^{-8}$). Seven out of the most significantly associated ASAP1 SNPs were individually genotyped to replicate their discovery, the most significant association was at rs4733781 ($P = 2.6 \times 10^{-11}$). Then the associations between ASAP1 SNPs and susceptibility to MTB were studied in Chinese population, but not in Xinjiang minorities (23,24). As far as we know, it is the first study which explored association between ASAP1 SNPs and susceptibility to MTB in Xinjiang Muslim populations.

Owing to population heterogeneity, different races have different causative polymorphisms (25). In this study, we selected a set of SNPs within the entire ASAP1 gene and used case-control analysis in Xinjiang Muslim populations to investigate whether ASAP1 SNP was associated with TB risk.

Materials and methods

Subject. In this study all the participants, including 400 TB patients and 380 control subjects, were of Xinjiang Muslim ethnicity. For TB patients, there were 322 Uyghur patients, 46 Kazak patients and 32 Hui nationality patients. Correspondingly, the Uyghur patients, Kazak patients and Hui nationality patients were 306, 44 and 30, respectively in the control group.

Eligible cases were adult patients who were newly diagnosed with active TB. These patients have evident lesions of TB through simple computed tomography, X-ray, and positive results of sputum smears and cultures for mycobacteria. Patients with HIV-infection, hepatitis virus infection, immunodeficiency disease, and other lung diseases were excluded from this cohort. Healthy controls were nationality-, age- and sex-matched Xinjiang minorities from Department of Respiratory Medicine, Xinjiang Uygur Autonomous Region Chest Hospital, the Xinjiang Uygur Autonomous Region, Urumqi, Xinjiang, China. The controls were negative both for history of TB and T-SPOT assay. All participants were BCG vaccinated.

Each patient and control enrolled in this study provided a written informed consent. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of Department of Respiratory Medicine, Xinjiang Uygur Autonomous Region Chest Hospital.

Blood sample collection, DNA isolation, purification and quality test. Peripheral blood samples (10 ml) was collected from each participants and stored at -80°C . Genomic DNA was extracted from peripheral blood collected from 400 TB patients and 380 non-TB controls using a Genomic DNA Mini Preparation kit (Beyotime, Shanghai, China) according to the manufacturer's instructions. Then a reference gene ASAP1 was used for qualifying the extracted samples by polymerase chain reaction (PCR) and electrophoresis. Obvious imaging

Table I. Clinical characteristics of patients and controls.

Characteristics	TB patients	Control	χ^2	P-value
Number	400	380		
Age (years)	55.4±12.4	55.7±12.1		0.675
Nationality			0.281	0.869
Uyghur	322 (79.8%)	306 (81.2%)		
Kazakhs	46 (11.5%)	44 (11.0%)		
Hui	32 (8.8%)	30 (7.9%)		
Sex			0.666	0.415
Male	237 (59.2%)	236 (62.1%)		
Female	163 (40.8%)	144 (37.9%)		
Family history of TB			1.319	0.251
Yes	38 (9.5%)	29 (7.2%)		
No	362 (90.5%)	371 (92.8%)		

TB, tuberculosis.

was regarded as qualified, or genomic DNA would be extracted again.

Gene polymorphism detection

SNP selection. According to Curtis *et al* (14), 7 polymorphic sites in ASAP1 gene were adopted in our study, including rs1017281, rs10956514, rs1469288, rs17285138, rs2033059, rs4733781, rs12680942. The details of primers are presented in Table I.

Primer design. After searching the whole ASAP1 gene sequence in Genbank, 7 primers pairs were designed and synthesized by Shanghai Biological Engineering Company (Table II).

Multiplex PCR amplification and product purification. SNPs were genotyped by multiplex PCR reaction using AmpliTaq Gold® 360 Master Mix (Applied Biosystems, Carlsbad, CA, USA), according to its protocol. The PCR reaction was designed to amplify fragments covering all 7 SNP loci. The PCR product was purified using PCR Clean Up kit (Beyotime) according to the protocol.

SNP detection. Polymorphic loci genotypes were detected using Sanger sequencing.

Statistical analysis. Statistical power was calculated by a post hoc power analysis by G*Power 3.1.9.2 software (Program written, conceptualized and designed by Franz, Universitat Kiel, Germany; freely available windows application software). The data were analyzed by SPSS 18.0 software (SPSS Inc., Chicago, IL). Continuous variables and categorical data were compared by χ^2 test. Hardy-Weinberg equilibrium test was used to detect whether the two groups were in genetic equilibrium. Genotype frequency comparisons between groups were presented as odds ratio (OR) and 95% confidence interval (CI). Haploview 4.2 (26) was used to performed linkage disequilibrium analysis. The tests were 2-sided and

Table II. SNPs with their primers.

SNP ID	Gene	SNP	Primer sequence (5'-3')	Annealing temperature (°C)	Fragment size (bp)
rs10956514	ASAP1	A/G	GGCCACTGGCAAAAATAAGC AGTTGTCCAACCTGCGGATAC	55	320
rs4733781	ASAP1	A/C	CAAATGAACCCCCATAAAGG CCAGTGGCTGCATCCTACAT	55	238
rs1017281	ASAP1	C/T	TATCTAATGTGCAGGGGATTG TCTCCCTTTTGCAGCTCACA	55	298
rs1469288	ASAP1	C/T	TCCACACTGCTGAAAAATCTG AAGGATGTGGGGAGTTGAGG	55	521
rs2033059	ASAP1	C/T	ACATACGTGGTGGTTGACTG TCCCAAAGCACAGAGGAAGA	54	393
rs12680942	ASAP1	A/G	GCTGCTATAAAGACCCAGAAG GGCCATTTCTCCAAAGCCTCT	56	207
rs17285138	ASAP1	A/T	CTGACTTGGTGCCAGCCTAC TGCTTTCCAGAGCTTTCAG	54	319

SNP, single-nucleotide polymorphism; ASAP1, Arf GTPase-activating protein-1.

Table III. Genotyping of rs1469288, rs2033059, rs12680942, Rs17285138 in TB and control group in Xinjiang Muslin population.

Site/genotype/allele	SNP	TB patients	Control	OR (95% CI)	P-value
rs1469288	HWE(P)				0.20
Genotype	AA	203 (50.8%)	194 (51.1%)		
	AG	169 (42.4%)	160 (42.2%)		
	GG	28 (6.8%)	26 (6.7%)		0.994
Allele	A	575 (71.9%)	548 (72.1%)		
	G	225 (28.1%)	212 (27.9%)	0.989 (0.793-1.233)	0.919
rs2033059	HWE(P)				0.45
Genotype	CC	130 (32.5%)	108 (28.5%)		
	CT	192 (48.0%)	203 (53.3%)		
	TT	78 (19.5%)	69 (18.2%)		0.293
Allele	C	452 (56.5%)	419 (55.1%)		
	T	348 (43.5%)	341 (44.9%)	1.507 (0.866-1.291)	0.586
rs12680942	HWE(P)				0.99
Genotype	AA	132 (33.0%)	110 (29.0%)		
	AG	183 (45.9%)	202 (53.0%)		
	GG	85 (21.2%)	68 (17.9%)		0.116
Allele	A	447 (55.9%)	422 (55.5%)		
	G	353 (44.1%)	338 (44.5%)	1.014 (0.83-1.239)	0.89
rs17285138	HWE(P)				0.09
Genotype	AA	126 (31.6%)	105 (27.7%)		
	AT	204 (51.0%)	204 (53.6%)		
	TT	70 (17.4%)	71 (18.7%)		0.496
Allele	A	456 (57.0%)	414 (54.5%)		
	T	344 (43.0%)	346 (45.5%)	1.108 (0.907-1.353)	0.315

HWE(P), Hardy-Weinberg equilibrium P-value; OR, odds ratio; CI, confidence intervals.

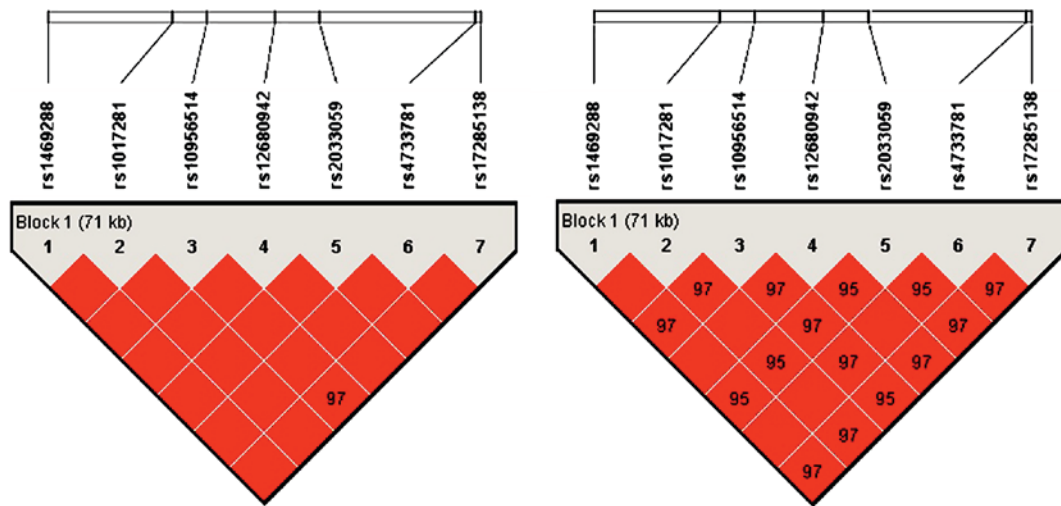


Figure 1. Linkage disequilibrium (LD) patterns of seven SNPs among CHB (n=103). The left displays Pairwise D' and the right displays r^2 . Strong linkage disequilibrium is represented by a high percentage. The red squares without a number indicate 100%. The whole area was in strong linkage disequilibrium, can be used as a block.

$P < 0.05$ was considered to indicate a statistically significant difference.

Results

General characteristic of TB patients and healthy controls. The clinical characteristics of the total TB patients and healthy controls are summarized in Table III. TB patients were age matched with healthy controls. The age distribution between the patients (55.4 ± 12.4 years) and controls (55.7 ± 12.1 years) was not significantly different based on Mann-Whitney U test ($P = 0.675$), as the data were non-normally distributed (Table I). Patients were also gender-matched with controls, no significant difference of gender was found between the two groups ($\chi^2 = 0.666$, $P = 0.415$). Nationality of objects display no difference between patients and controls ($\chi^2 = 0.281$, $P = 0.869$). Among 400 TB patients, 38 persons had family history of TB, while there were 29 persons with family history of TB in control group, no significance was found between two groups ($\chi^2 = 1.319$, $P = 0.251$). A post hoc power analysis showed that Power value equals 0.8620, meaning a high statistical effect (Fig. 1). The parameters used in power analysis were as follows: cases, 400; controls, 380; prevalence, 0.00178; odds ratio, 1.5070; minor allele frequency, 0.3536.

Hardy-Weinberg equilibrium test. The rs10956514, rs1469288, rs2033059, rs4733781, rs1017281, rs17285138, rs12680942 SNPs were investigated in 400 pulmonary TB cases and 380 healthy controls in the Xinjiang Muslim population. rs10956514 site was not in Hardy-Weinberg equilibrium ($P < 0.05$), so it was excluded from our research. The last six SNPs were in Hardy-Weinberg equilibrium in the control group and the pulmonary TB group ($P > 0.05$) (Tables III and IV).

Genotype frequency distribution of ASAP1 gene SNPs in the Xinjiang Muslim population. We used the case-control analysis to examine whether 7 polymorphisms in the ASAP1 gene were associated with susceptibility to TB in Xinjiang

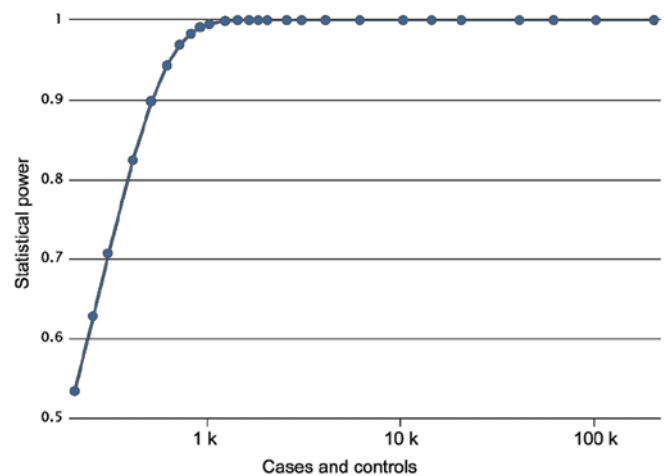


Figure 2. Linkage disequilibrium analysis.

Muslim population. The genotype and allelic frequencies of ASAP1 7 SNPs are summarized in Tables III and IV. Two polymorphisms were associated with TB ($P < 0.05$), while the other five SNPs showed no significance. For SNP rs4733781 the frequency of allele A in the pulmonary TB group was higher than that in the control group, and there was a significant difference between the two groups ($P = 0.046$). While SNP rs1017281 was lower than that in the control group (Tables III and IV). The direction of effect for the associated alleles of rs4733781 and rs1017281 was the same as in Curtis *et al* (14).

Associations between genetic model of SNPs and TB risk. In order to find the optimal genetic model, we built codominant, dominant, recessive and overdominant model of ASAP1 gene polymorphisms. rs4733781 site was found related to the occurrence of TB in the recessive model (CC vs. AA+AC: OR, 0.51; 95% CI: 0.324-0.802; $P = 0.003$) and co-dominant model (AA vs. CC: OR, 1.926; 95% CI: 1.198-3.097; $P = 0.006$). rs1017281 site was associated with TB in the recessive model (GG vs. AA+AG: OR, 1.792; 95% CI: 1.135-2.828; $P = 0.011$)

Table IV. Genotyping of rs10956514, rs4733781, rs1017281 in TB and control group in Xinjiang Muslim population.

Site/genotype/allele	SNP	TB patients	Control	OR (95% CI)	P-value
rs10956514	HWE(P)				0.013
Genotype	GG	136 (34.0%)	133 (35.0%)		
	GA	207 (51.8%)	199 (52.4%)		
	AA	57 (14.2%)	48 (12.6%)		0.799
Allele	G	479 (59.9%)	465 (61.2%)		
	A	321 (40.1%)	295 (38.8%)	0.947 (0.773-1.160)	0.597
rs4733781	HWE(P)				0.12
Genotype	AA	194 (48.6%)	174 (45.8%)		
	AC	173 (43.2%)	149 (39.2%)		
	CC	33 (8.2%)	57 (15%)		0.012
Allele	A	561 (70.1%)	497 (65.4%)		
	C	239 (29.9%)	263 (34.6%)	1.242 (1.004-1.537)	0.046
rs1017281	HWE(P)				0.56
Genotype	AA	165 (41.2%)	171 (45.0%)		
	AG	187 (46.8%)	177 (46.6%)		
	GG	58 (12.0%)	32 (8.4%)		0.034
Allele	A	517 (64.6%)	519 (67.3%)		
	G	283 (35.4%)	241 (31.7%)	0.792 (0.643-0.976)	0.028

HWE(P), Hardy-Weinberg equilibrium P-value; OR, odds ratio; CI, confidence intervals. Bold text, statistically significant.

Table V. Association of ASAP1 SNP genotypes with pulmonary TB under different genotype models.

	Model	Genotype	Case	Control	OR (95% CI)	P-value
rs4733781	Codominant	AA	194 (48.6%)	174 (45.8%)	1	
		AC	173 (43.2%)	149 (39.2%)	0.96 (0.712-1.296)	0.791
		CC	33 (8.2%)	57 (15%)	1.926 (1.198-3.097)	0.006
	Dominant	AA	194 (48.5%)	174 (45.8%)		
		AC+CC	206 (51.5%)	206 (54.2%)	1.115 (0.841-1.477)	0.448
	Recessive	CC	33 (8.2%)	57 (15.0%)		
AC+AA		367 (91.8%)	323 (85.0%)	0.51 (0.324-0.802)	0.003	
Overdominant	AA+CC	227 (56.8%)	231 (60.8%)			
	AC	173 (43.2%)	149 (39.2%)	0.846 (0.636-1.126)	0.252	
rs1017281	Codominant	AA	165 (41.2%)	171 (45.0%)	1	
		AG	187 (46.8%)	177 (46.6%)	0.913 (0.679-1.229)	0.549
		GG	58 (12.0%)	32 (8.4%)	0.532 (0.329-0.862)	0.01
	Dominant	AA	165 (41.2%)	171 (45.0%)		
		AG+GG	235 (58.8%)	209 (55.0%)	0.858 (0.646-1.140)	0.29
	Recessive	GG	48 (12.0%)	32 (8.4%)		
		AA+AG	352 (88.0%)	348 (91.0%)	1.792 (1.135-2.828)	0.011
	Overdominant	AA+GG	213 (53.2%)	203 (53.4%)		
	AG	187 (46.8%)	177 (46.6%)	1.04 (0.786-1.375)	0.785	

Bold text, statistically significant.

and co-dominant model (AA vs. GG: OR, 0.532; 95% CI: 0.329-0.826; P=0.01) (Tables V and VI).

Linkage disequilibrium analysis. Haploview 4.2 was used to performed linkage disequilibrium analysis. As shown in Fig. 2,

Table VI. The data of D' and r² among 7 SNPs of ASPAP1 gene in the Chinese Xinjiang Muslim population.

L1	L2	D'	r ²
rs1469288	rs1017281	1	1
rs1469288	rs10956514	1	0.979
rs1469288	rs12680942	1	1
rs1469288	rs2033059	1	0.959
rs1469288	rs4733781	1	1
rs1469288	rs17285138	1	0.979
rs1017281	rs10956514	1	0.979
rs1017281	rs12680942	1	1
rs1017281	rs2033059	1	0.959
rs1017281	rs4733781	1	1
rs1017281	rs17285138	1	0.979
rs10956514	rs12680942	1	0.979
rs10956514	rs2033059	1	0.979
rs10956514	rs4733781	1	0.979
rs10956514	rs17285138	0.979	0.958
rs12680942	rs2033059	1	0.959
rs12680942	rs4733781	1	1
rs12680942	rs17285138	1	0.979
rs2033059	rs4733781	1	0.959
rs2033059	rs17285138	1	0.979
rs4733781	rs17285138	1	0.979

linkage disequilibrium patterns for the cluster of 7 SNPs in the ASAP1 gene genotyped in a Chinese Xinjiang Muslim population. The results showed that the whole areas were strong linkage disequilibrium and they can be used as a block. Therefore, rs4733781 and rs1017281 were linked together, they should be considered as one locus.

Discussion

Xinjiang Uygur Autonomous Region bears heavier tuberculosis (TB) burden than other areas in China (3). The selection of candidate genes and detection of their polymorphism sites have been considered breakthroughs of TB prevention and treatment. Curtis *et al* (14) found single-nucleotide polymorphisms of ASAP1 gene was associated with the susceptibility to TB in the Russian population (14), while Hu *et al* (23) and Miao *et al* (24) found no associations. This may be partly due to differences between populations and small statistical power of the follow-up studies to detect weak genetic effects. However, no case-control analysis has been reported in Xinjiang Muslim population to date. Hence, investigating the susceptibility genes in Xinjiang Muslim population may provide access to control TB in Xinjiang Uygur autonomous region.

In the present study, we tested association of ASAP1 gene polymorphism and TB susceptibility in Xinjiang Muslim population by SNP genotyping. Our data suggested that ASAP1 rs4733781 and rs1017281 polymorphisms were associated with TB susceptibility in Xinjiang Muslim population

(P=0.046 and P=0.028). While no significant associations were found in rs10956514, rs1469288, rs2033059, rs12680942 and rs17285138. In contrast to our findings, Hu *et al* (23) and Miao *et al* (24) found that ASAP1 gene polymorphism was not associated with TB susceptibility in Chinese population and Tibetan population. As known, there are ethnic variations of the allelic frequency distribution in the investigated polymorphism markers (25). In addition, numerous gene studies indicated that the risk variants of genetic heterogeneity and ethnicity specificity was associated with TB (27,28). An explanation for these divergent results may involve whether polymorphism itself acts functionally and confers a truly altered susceptibility to TB disease or the associated allele was an unknown disease susceptibility allele in linkage disequilibrium. In this regard, it is necessary to note that excessive reduction of ASAP1 expression results in the impaired migration of *Mycobacterium tuberculosis* (MTB)-infected DCs, which may contribute to TB pathogenesis (14). Other co-variables may not be excluded, such as socio-economic factors, nutritional status, and interactions between genes (29). Therefore, in-depth study on the function of these sites is needed. In addition, in light of marginal P-values of associations between ASAP1 SNPs and TB susceptibility (P=0.046 and P=0.028), multiple corrections should be considered in our later work, such as nationality, age, sex, smoking and family history. An independent Uyghur sample will be a good option to get converse results.

Regarding different genotype models, our results demonstrated that a trend of higher rs4733781 A allele and rs1017281 G allele in TB group compared to controls. Different genotype models revealed that subjects with rs4733781 A and rs1017281 G could be more susceptible to TB while subjects with rs4733781 T and rs1017281 A could be more resistant. The recessive model of rs4733781 (CC vs. AC+AA) in Xinjiang Muslim populations tended to be related with a lower TB risk [P=0.003, OR=0.51 (0.324-0.802)], while the recessive model of rs1017281 (GG vs. AA+AG) seemed to be related with a higher TB risk [P=0.011, OR=1.792 (1.135-2.828)]. Additionally, the linkage disequilibrium analysis showed that rs4733781 and rs1017281 were linked together, they should be considered one locus. Next, profound studies will be performed to explore the potential functional roles of these two SNPs to help understand major findings. Do they participate in the immunoreaction caused by TB? What is the exact mechanism? Much work remains to be done.

In conclusion, ASAP1 rs4733781 and rs1017281 polymorphism may be a genetic factor for susceptibility to MTB among the Xinjiang Muslim populations. Further investigations of the functional role of SNP rs4733781 and rs1017281 and the genomic surrounding region are warranted.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

XW contributed to the conception of the study; AM and XH contributed significantly to analysis and manuscript preparation; AL performed the data analyses and wrote the manuscript; FX helped perform the analysis with constructive discussions. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of Department of Respiratory Medicine, Xinjiang Uygur Autonomous Region Chest Hospital. Each patient and control enrolled in this study provided a written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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