Int J Clin Exp Med 2017;10(3):5769-5777
www.ijcem.com /ISSN:1940-5901/IJCEM0040429

Original Article

Association of *TAP1* and *TAP2* polymorphisms with risk and prognosis of pediatric spinal tuberculosis

Ke Fang[1], Fuyun Liu[1], Jie Wen[2], Hong Liu[2], Sheng Xiao[2], Xin Li[2]

[1]Department of Pediatric Orthopaedics, The Third Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan Province, China; [2]Department of Pediatric Orthopaedics, Hunan Province People's Hospital, Changsha 410005, Hunan Province, China

Received September 21, 2016; Accepted November 30, 2016; Epub March 15, 2017; Published March 30, 2017

Abstract: Since *TAP* polymorphisms were associated with accelerated progression of post-infection tuberculosis (TB), and TB could account for development of spinal TB (STB), the present study was intended to investigate whether the mutations of *TAP* polymorphisms could predict risk and prognosis of pediatric patients suffering from STB. Altogether 85 pediatric STB (PSTB) patients and 97 healthy children were recruited, and their peripheral blood samples were gathered to extract genomic DNA for genotyping. According to previously published investigations, 2SNPs located within *TAP1* and 7SNPs situated within *TAP2* were finally selected for this investigation. Genotyping of the SNPs were implemented utilizing method of polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP), and potential haplotypes were obtained with application of SHESIS software. The odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated to assess correlations between SNPs/haplotypes and prevalence of PSTB. The G allele of *TAP1* rs1135216 and A allele of *TAP2* rs4148871 were both closely linked with elevated susceptibility to PSTB when, respectively, compared with A allele and G allele (G vs. A, OR = 2.06, 95% CI: 1.28-3.30, $P < 0.01$; A vs. G, OR = 2.45, 95% CI: 1.48-4.04, $P < 0.01$), whereas A allele of *TAP2* rs2857103 was significantly associated with lessened PSTB risk in comparison to C allele (OR = 0.35, 95% CI: 0.23-0.55, $P < 0.01$). Besides, mutations of rs241447 (G>A) and rs4148876 (G>A) could significantly modify PSTB risk (AA vs. GG+GA, OR = 2.44, 95% CI: 1.27-4.72, $P < 0.01$; AA+GA vs. GG, OR = 2.00, 95% CI: 1.08-3.70, $P = 0.03$). Haplotypes A-G-A-G-G and A-A-A-G-G were also demonstrated to decrease PSTB risk significantly (OR = 0.25, 95% CI: 0.07-0.95, $P = 0.03$; OR = 0.35, 95% CI: 0.12-0.96, $P = 0.03$). SNPs within *TAP1* (rs1135216) and *TAP2* (rs241447, rs2857103, rs4148871 and rs4148876) appeared as potential targets for both prediction and treatment of PSTB, yet further studies were in demand.

Keywords: Pediatricspinal tuberculosis, *TAP1*, *TAP2*, SNP, haplotype

Introduction

With obviously increasing resistance of Mycobacterium tuberculosis (TB) to antituberculosis drugs and growing number of immune-deficient patients, the prevalence of TB exhibits a rising trend among developing countries within the nearest decade [1]. Around 1% of TB could stimulate succedent spinal TB (STB), and the proportion tops among osteoarticular TB. Notably, pediatric spinal was vulnerable to TB focus for its differentiating anatomic structure and physiological characteristics, which were mainly displayed as smaller vertebral ossification centers and principal composition of cartilage [2]. Besides, pediatric spinal TB (PSTB) appeared to worsen more rapidly than adult STB, and the disorder was more easily accompanied with major complications, such as kyphotic deformity and nerve dysfunction [2, 3]. Above all, the negative effects imposed by STB on children are all-around and profound, owing to that fateful TB posterior convexity could induce cardiac malfunction, dysfunctional lung and even paralysis due to compression of spinal cord [4, 5].

Mounting genetic parameters have been documented to affect post-infection TB progression, including chemokine ligand 2 (CCL2), solute carrier family 11 member a1 protein (SLC11A1), P2X7, transporter associated with antigen processing (TAP) and so on [6, 7]. TAP, the allodimer composed of TAP1 and TAP2 subunits, is

Table 1. Primers of *TAP1* and *TAP2* genetic polymorphisms for PCR amplification

Gene	SNPs	PCR amplification primer	
		F	R
TAP1	Rs1057141	5'-TGGCTCATTGTTAGTTCG-3'	5'-CACAGGGACAGGGTGTT-3'
	Rs1135216	5'-GCTCCTATGGCTTCTTC-3'	5'-GACTGCCTCACCTGTAA-3'
TAP2	Rs1800454	5'-GCCCTGGTGTTTGCTGG-3'	5'-TCTTCCCTTGCCCTCCC-3'
	Rs241448	5'-CTACTGCCCTTTCCTACC-3'	5'-CAAATCTCCATCGTGCC-3'
	Rs2228396	5'-GAGGAGGGAGAAGACAG-3'	5'-TAGGAATGGAGGAAAGG-3'
	Rs241447	5'-AGATGGTGCCCAGGTGGA-3'	5'-AAACTCAAAGCAGGAACAG-3'
	Rs2857103	5'-AGACTGAATCTATTTGCTG-3'	5'-GAATGTGACCTCTGCTT-3'
	Rs4148871	5'-CTTCCAGGAGACTAAGACA-3'	5'-AGGACAGAGCAGGTGAG-3'
	Rs4148876	5'-GCAGTACAGCCGGGAGA-3'	5'-CACCAGGCGGGAATAGA-3'

SNP, single-nucleotide polymorphism; PCR, polymerase chain reaction; F, forward; R, reverse.

Table 2. Baseline characteristics of PSTB patients and controls

	PTB patients (n = 85)	Healthy controls (n = 97)	*P* value
Age (years old)	6.14 ± 1.57	6.41 ± 1.29	0.20
Sex (n, %)			
Male	51 (60.00%)	57 (58.76%)	0.87
Female	34 (40.00%)	40 (41.24%)	
Location (n, %)			
Cervicalvertebra	4 (4.17%)	—	
Thoracicvertebra	32 (33.33%)	—	
Lumbarvertebra	49 (51.04%)	—	
Sacral vertebrae	11 (11.46%)	—	
Frankle Classification (n, %)			
A-B	13 (15.29%)	—	
C-D	42 (49.41%)	—	
E	30 (35.30%)	—	

PSTB: pediatric spinal tuberculosis.

situated in the major histocompatibility complex-II (MHC-II) region [8]. It contributed much to the cellular immuneresponse through affecting the antigen-presenting process that was mediated byMHI-1 molecules [9]. Specifically, after pathogens, misfolded proteins and defective ribosome products (DRiPs) in the cytoplasm are degraded by proteasomes through the ubiquitylation-dependent approach, TAP would transport degraded micro-molecular peptides to endocytoplasmic reticulum (ER), and enable the peptides to combine with MHC-I molecules to form stable MHC-antigen peptide compounds. Subsequently, the compounds are transported outside to cell surface via constitutive secretory pathway, and are then identified by cytotoxic lymphocytes.

In view of the significance of TAP in TB development, it is highly reasonable to suspect that mutations of *TAP* polymorphisms might, to some extent, modulate incidence of STB, since that polymorphisms of *TAP* coding region could alter its protein spatial structure and thus its biological functions. Up to date, seven *TAP1* alleles (*TAP1**0101, *0102N, *020101, *0202-0102, *0301, *0401 and *0501) and four *TAP2* alleles (*TAP2**0101, *0102, *0103 and *0201) have been officially nominated by WHO human leucocyte antigen nomenclature committee [10]. In addition, multiple single nucleotide polymorphisms (SNPs) have been confirmed to be involved in TB, such as *TAP1* (333), *TAP1* (637), *TAP2* (565), *TAP2* (665) and *TAP* (687) [11-15].

Hence, the current study was aimed to explore a potential correlation between SNPs within *TAP* and susceptibility to STB, attempting to seek out a candidate target for treating STB in the future.

Method

Subjects

Altogether 85 PSTB patients and 97 healthy children were recruited from the third affiliated hospital of Zhengzhou University from July 2012 to May 2015, and they were all individuals of Han Chinese ethnicity without blood relationships. All the pediatric patients performed routine examinations (e.g. electrocardiogram and chest X-ray), and they were confirmed with STB after imaging examinations (e.g. CT three-

Table 3. Association of *TAP1* and *TAP2* genes polymorphisms and susceptibility to pediatric spinal tuberculosis

Gene	SNP		Genotype			Allele frequency			Allelic model		Dominant model		Recessive model	
			WW	WM	MM	W	M	MAF (M)	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
TAP1	Rs1057141 (A>G)	Case	44	38	3	126	44	0.26	1.26 (0.78,2.05)	0.34	1.45 (0.80, 2.61)	0.22	0.85 (0.18, 3.91)	0.84
		Control	59	34	4	152	42	0.22						
	Rs1135216 (A>G)	Case	36	40	9	112	58	0.34	2.06 (1.28, 3.30)	< 0.01	2.41 (1.33, 4.38)	< 0.01	2.75 (0.82, 9.29)	0.09
		Control	62	31	4	155	39	0.20						
TAP2	Rs1800454 (G>A)	Case	69	11	5	149	21	0.12	1.08 (0.57, 2.04)	0.82	0.99 (0.47, 2.10)	0.98	1.42 (0.37, 5.48)	0.61
		Control	77	14	4	168	22	0.12						
	Rs2228396 (G>A)	Case	75	8	2	158	12	0.07	0.74 (0.35, 1.59)	0.44	0.73 (0.31, 1.72)	0.47	0.76 (0.12, 4.63)	0.76
		Control	82	12	3	176	18	0.09						
	Rs241447 (G>A)	Case	5	47	33	57	113	0.66	1.42 (0.93, 2.18)	0.11	0.69 (0.18, 2.65)	0.59	2.44 (1.27, 4.72)	< 0.01
		Control	4	73	20	81	113	0.58						
	Rs241448 (A>G)	Case	57	13	15	127	43	0.25	1.27 (0.78, 2.07)	0.34	1.30 (0.69, 2.47)	0.42	1.24 (0.56, 2.75)	0.60
		Control	69	12	14	150	40	0.21						
	Rs2857103 (C>A)	Case	39	42	4	120	50	0.29	0.35 (0.23, 0.55)	< 0.01	0.50 (0.27, 0.92)	0.03	0.08 (0.03, 0.24)	< 0.01
		Control	29	31	37	89	105	0.54						
	Rs4148871 (G>A)	Case	47	22	16	116	54	0.32	2.45 (1.48, 4.04)	< 0.01	2.46 (1.31, 4.61)	< 0.01	2.98 (1.16, 7.65)	0.02
		Control	73	17	7	163	31	0.16						
	Rs4148876 (G>A)	Case	48	31	6	127	43	0.25	1.30 (0.80, 2.13)	0.29	2.00 (1.08, 3.70)	0.03	0.49 (0.18, 1.35)	0.16
		Control	70	14	13	154	40	0.21						

SNP, single-nucleotide polymorphism; W, wild allele; M, mutant allele; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.

dimensional reconstruction and MRI) of lesion locations. The incorporated PSTB patients all satisfied the following requirements: (1) they all received surgery below 14 years old; (2) their STB were all in the active stage during surgical treatment; (3) at least 2 years have passed since completion of surgery. Moreover, subjects with PSTB were excluded from this investigation if: (1) they accepted surgery due to other spinal infectious disorders; (2) they underwent correction of kyphosis because of TB kyphosis in the stationary phase; (3) their post-surgery period lasted for less than 2 years. The guardians of participants all signed informed consents. The series of experiments have been approved by the third affiliated hospital of Zhengzhou University and ethics committee of the third affiliated hospital of Zhengzhou University.

SNP genotyping

Peripheral blood samples (2 mL for each individual) were withdrawn to extract genomic DNA after their anti-coagulation with 2% EDTA and frozen reservation in -20°C. The full extraction procedures were in compliance with the guidance of DNA blood kit (Watson Biotechnologies, Inc). The SNPs located in *TAP1* and *TAP2* were determined with assistance of polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP), and primers for these SNPs (**Table 1**) were designed with application of MassARRAY Assay Design Version 3.1 (SpectroREADER, Squenom). The PCR reaction reagent (50 μL) included DNA template (4 μL), 20 μM sense primer (1 μL), 20 μM antisense primer (1 μL), 2.5 U Taq DNA polymerase. Then thereaction circumstances were managed as: (1) pre-denaturation at 94°C for 2 min; (2) 30 cycles of denaturation at 94°C for 40 s, annealing at 57°C for 40 s, and extension at 72°C for 40 s; (3) extension at 72°C for 10 min. About 1 μg PCR products were drawn with addition of restriction enzymes, namely, Bcl I and Acc I (BIOLABS Corporation, New England). After treating enzyme-digested products with agarose gel electrophoreses, the electrophoretic bands were observed under the ultra-violet lamp with ethidium bromide (EB) as the colouring agent. Then genotyping consequences were further validated by Beijing Tiangen Biotechnology corporation.

Surgery

All patients were pre-operatively given anti-TB drugs (i.e. isonicid, rifampicin, pyrazinamide and ethambutol) for at least 2 weeks, and their TB-caused symptoms, such as low-grade fever and weakness, were thus alleviated. The PSTB patients were all operated with anterior approach, which were initiated through extraperitoneal or extrapleural approaches. Diseased vertebral body was fully exposed, and fester or necrotic tissues were removed as much as possible. Allograft bones of proper sizes were transplanted, and internal fixation materials would be inserted if necessary. Anterior cervical titanium alloy plate could be selected for children who were below 3 years old, for that their vertebral bodies were too small. Patients whose pleura or peritoneum were broken would be sewed up. After lesions were cleaned up with large amounts of normal saline, 1.0 g streptomycin would be placed in the focal zone.

Grading of spinal injury

According to Frankle grading, STB was classified as Grade A when sensory and motor functions below the damaged plane were totally lost. Then subjects were categorized into: (1) Grade B when they possessed certain sensory functions, yet no motor functions below the damaged plane; (2) Grade C when only several useless movement functions were maintained below the damaged plane; (3) Grade D when available, yet not complete motor functions were present below the damaged plane; (4) Grade E when sensory, motor and sphincter functions all worked.

Evaluation of treatment efficacy

Patients were considered to be cured when: (1) no recurrence of TB focus was present within half a year; (2) erythrocyte sedimentation rate (ESR) was normal; (3) X-ray examination showed that bony fusion appeared in lesion focus; (4) patients could engage in light manual work. Besides, the recurrent criteria was specified as that patients post-operatively recovered well for a period, but lesions aggravated or appeared in other locations.

Statistical analysis

Direct counting was performed to calculate gene frequencies, and Chi-square test was applied to explore whether genotypes were associated with prediction of treatment efficacies for PTSB. The dominant model (WM+MM vs. WW), recessive model (MM vs. WW+WM)

Table 4. Stratified analyses of SNPs in PSTB patients classified by Frankle classification and controls

Gene	Subgroup	N	Allelic model		Dominant model		Recessive model	
			OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
TAP1	Control	75	1.00	-	1.00	-	1.00	-
Rs1135216 (A>G)	Case							
	A-B	13	1.77 (0.72, 4.37)	0.21	1.52 (0.47, 4.88)	0.48	4.23 (0.69, 25.82)	0.09
	C-D	42	1.68 (0.94, 3.01)	0.08	1.95 (0.94, 4.06)	0.07	1.79 (0.38, 8.37)	0.45
	E	30	2.84 (1.53, 5.29)	< 0.01	4.13 (1.71, 10.00)	< 0.01	3.58 (0.84, 15.30)	0.07
TAP2	Control	75	1.00	-	1.00	-	1.00	-
Rs241447 (G>A)	Case							
	A-B	13	1.51 (0.65, 3.51)	0.33	0.56 (0.06, 5.41)	0.61	2.89 (0.9, 9.29)	0.07
	C-D	42	1.29 (0.76, 2.19)	0.35	0.86 (0.15, 4.88)	0.86	1.93 (0.86, 4.33)	0.11
	E	30	1.59 (0.85, 2.97)	0.14	0.58 (0.10, 3.34)	0.54	3.13 (1.30, 7.56)	0.01
Rs2857103 (C>A)	Control	75	1.00	-	1.00	-	1.00	-
	Case							
	A-B	13	0.53 (0.23, 1.23)	0.13	0.96 (0.27, 3.37)	1.00	0.14 (0.02, 1.12)	0.03
	C-D	42	0.55 (0.33, 0.93)	0.02	1.20 (0.53, 2.71)	0.65	0.08 (0.02, 0.35)	< 0.01
	E	30	0.11 (0.05, 0.25)	< 0.01	0.11 (0.04, 0.30)	< 0.01	0.06 (0.01, 0.46)	< 0.01
Rs4148871 (G>A)	Control	75	1.00	-	1.00	-	1.00	-
	Case							
	A-B	13	2.34 (0.94, 5.86)	0.06	2.61 (0.80, 8.53)	0.1	2.34 (0.43, 12.7)	0.31
	C-D	42	1.64 (0.87, 3.09)	0.12	1.69 (0.77, 3.69)	0.19	1.74 (0.52, 5.83)	0.37
	E	30	4.02 (2.12, 7.62)	< 0.01	3.98 (1.69, 9.37)	< 0.01	5.51 (1.84, 16.48)	< 0.01
Rs4148876 (G>A)	Control	75	1.00	-	1.00	-	1.00	-
	Case							
	A-B	13	0.92 (0.33, 2.59)	0.86	1.15 (0.33, 4.05)	0.82	0.54 (0.06, 4.51)	0.56
	C-D	42	0.98 (0.52, 1.85)	0.92	1.16 (0.53, 2.56)	0.71	0.68 (0.21, 2.22)	0.52
	E	30	2.07 (1.10, 3.91)	0.02	5.19 (2.15, 12.51)	< 0.01	0.22 (0.03, 1.76)	0.12

SNP, single-nucleotide polymorphism; PSTB: pediatric spinal tuberculosis; OR, odds ratio; CI, confidence interval.

Table 5. Haplotype structure and frequency analysis of *TAP1* and *TAP2* genetic polymorphisms between PSTB patients andcontrols

Haplotype	Frequency		x^2-Test	OR (95% CI)	*P*-value
	PSTB	Control			
A-G-C-G-G	7 (0.0813)	10 (0.1026)	0.245	0.77 (0.28, 2.14)	0.6207
A-G-C-G-A	2 (0.0271)	3 (0.0273)	0.000	0.99 (0.17, 5.96)	0.9941
A-G-A-G-G	3 (0.0332)	12 (0.1204)	4.700	0.25 (0.07, 0.95)	0.0302
A-G-A-G-A	1 (0.0111)	3 (0.032)	0.914	0.34 (0.03, 3.46)	0.3390
A-A-C-G-G	13 (0.1577)	14 (0.1416)	0.092	1.13 (0.50, 2.57)	0.7611
A-A-C-G-A	4 (0.0526)	4 (0.0377)	0.237	1.42 (0.34, 5.84)	0.6266
A-A-C-A-G	6 (0.0742)	3 (0.027)	2.168	2.89 (0.66, 12.60)	0.1409
A-A-A-G-G	5 (0.0644)	16 (0.1663)	4.492	0.35 (0.12, 0.96)	0.0341
A-A-A-G-A	2 (0.0215)	4 (0.0442)	0.721	0.47 (0.08, 2.75)	0.3959
A-A-A-A-G	3 (0.0303)	3 (0.0317)	0.003	0.96 (0.18, 5.14)	0.9581
G-G-C-G-G	4 (0.0419)	2 (0.0256)	0.371	1.66 (0.32, 8.62)	0.5424
G-G-A-G-G	1 (0.0171)	3 (0.0301)	0.327	0.56 (0.07, 4.19)	0.5676
G-A-C-G-G	7 (0.0813)	3 (0.0354)	1.777	2.41 (0.64, 9.10)	0.1826
G-A-A-G-G	3 (0.0332)	4 (0.0416)	0.088	0.79 (0.17, 3.73)	0.7670

PSTB: pediatric spinal tuberculosis; OR, odds ratio; CI, confidence interval.

and allelic model (W vs. M) were established to evaluate the genotyping distribution of TAP polymorphisms. Different frequencies of genotypes, alleles and haplotypes between PSTB patients and healthy controls were assessed with odds ratios (ORs) and corresponding 95% confidence intervals (95% CIs). It would be considered statistically significant when *P* value was less than 0.05. All the statistical analyses were achieved with usage of SPSS 17.0 software.

Result

Baseline characteristics of PSTB patients and controls

The STB patients and healthy controls showed approximate mean age and sex ratio without statistical significance ($P > 0.05$) (**Table 2**). Additionally, the PSTB

Table 6. Association of *TAP1* and *TAP2* genetic polymorphisms and response to surgery

Gene	SNP	Endpoints	Genotype			X^2	*P*-value
			WW	WM	MM		
TAP1	Rs1135216 (A>G)	Cure	28	31	7	0.033	0.983
		Recurrence	8	9	2		
		Total	36	40	9		
TAP2	Rs241447 (G>A)	Cure	4	37	26	0.004	0.998
		Recurrence	1	10	7		
		Total	5	47	33		
	Rs2857103 (C>A)	Cure	32	31	3	0.808	0.668
		Recurrence	7	11	1		
		Total	39	42	4		
	Rs4148871 (G>A)	Cure	37	17	8	7.026	0.030
		Recurrence	10	5	8		
		Total	47	22	16		
	Rs4148876 (G>A)	Cure	37	23	5	0.256	0.880
		Recurrence	11	8	1		
		Total	48	31	6		

SNP, single-nucleotide polymorphism; W, wild allele; M, mutant allele.

patients investigated were correlated with spinal lesions within diverse locations, among which the proportion of lumbar (51.04%) and thoracic vertebrates (33.33%) topped, whereas that of sacral (11.46%) and cervical (4.17%) vertebrates lagged behind. Moreover, the severity of PSTB subjects also varied, with 13 (22.35%), 42 (49.41%) and 30 (28.24%) patients, respectively, classified into A-B grade, C-D grade and E grade.

Association of TAP genetic polymorphisms with PSTB risk

Regarding rs1135216 of *TAP1*, the incidence of G allele was significantly higher than A allele among PSTB patients when compared with healthy controls (OR = 2.06, 95% CI: 1.28-3.30, $P < 0.01$) (**Table 3**). The close linkage of rs1135216 with PSTB development was also manifested in the form of dominant model (GG+GA vs. AA: OR = 2.41, 95% CI: 1.33-4.48, $P < 0.01$). In contrast, rs1057141 displayed no associations with PSTB development regardless of the models (all $P > 0.05$).

TAP2 seemed to affect susceptibility to PSTB more intensely than *TAP1*, for that four in seven SNPs altered PSTB risk as indicated in **Table 3**. To be specific, subjects carrying homozygote AA of rs241447 were more subject to PSTB than those carrying genotypes GG and GA (OR = 2.44, 95% CI: 1.27-4.72, $P < 0.01$). Interestingly, mutations of rs2857103 exhibited close tights with decreased occurrence of PSTB in the allelic model (A vs. C: OR = 0.35, 95% CI: 0.23-0.55), dominant model (AA+AC vs. CC: OR = 0.50, 95% CI: 0.27-0.92) and recessive model (AA vs. AC+CC: OR = 0.08; 95% CI: 0.03-0.24), whereas mutant rs4148871 was significantly correlated with elevated PSTB risk (A vs. G: OR = 2.45, 95% CI: 1.48-4.04; AA+AG vs. GG: OR = 2.46, 95% CI:1.31-4.61; AA vs. GG+GA: OR = 2.98, 95% CI:1.16-7.65). Finally, genotypes GA/AA appeared to make children more vulnerable to STB than homozygote GG (OR = 2.00; 95% CI: 1.08-3.70).

Association of TAP genetic polymorphisms with PSTB risk stratified by Frankle grading

After classification of the PSTB patients into A-B, C-D and E levels in light of the Frankle grading system (**Table 4**), carriers of rs1135216 G allele were estimated to suffer from E level of PTSB more readily than those of allele A (OR = 2.84, 95% CI: 1.53-5.29, $P < 0.05$). With regard to rs241447, carriers of homozygote AA were largely more inclined to be attacked by E grading than ones of genotypes GG/AG (OR = 3.13, 95% CI: 1.30-7.56, $P < 0.05$). The stratified analysis of rs2857103 also confirmed higher prevalence of PTSB risk among populations carrying C allele than those carrying A allele, considering both C-D (A vs. G, OR = 0.55, 95% CI: 0.33-0.93, $P = 0.02$) and E levels (A vs. G, OR = 0.11, 95% CI: 0.05-0.25, $P < 0.05$). Finally, under the allelic model, mutations of rs4148871 (G>A) and rs4148876 (G>A) were both associated with incremental susceptibility to the highest level of PTSB (OR = 4.02, 95% CI: 2.12-7.62, $P < 0.05$; OR = 2.07, 95% CI: 1.10-3.91, $P < 0.05$).

Correlation between combined SNPs of TAP and susceptibility to PSTB

On the whole, 32 haplotypes were acquired after random assortment of the 5 SNPs, none-

theless, merely 14 haplotypes were ultimately incorporated considering their relatively high frequency among the studied confluence (each ≥ 3%). After screening potential SNP combinations, two haplotypes of A-G-A-G-G and A-A-A-G-G could confer lower PTSB risk than other haplotypes, implying their significance in prevention of PTSB risk (OR = 0.25, 95% CI = 0.07-0.95, $P = 0.03$; OR = 0.35, 95% CI = 0.12-0.96, $P = 0.03$) (**Table 5**).

Association of TAP SNPs with treatment efficacy of PSTB

As was tabulated (**Table 6**), a higher recurrence probability of STB was among AA carriers than that among GG carriers with respect to rs4148871 ($P = 0.03$). Furthermore, none of rs1135216, rs241447, rs2857103 and rs4148876 acted as predictive factors of PTSB (all $P > 0.05$).

Discussion

Undeniably, TAP played critical roles in initiation of acquired immunity process partly through regulating selection and transportation of antigen peptides, and accumulating evidence demonstrated that *TAP* mutations were associated with elevated incidence of autoimmune diseases (e.g. BLS) [16]. Besides, carriers with *TAP* mutants probably died of persistent pulmonary necrosis after they grew up, since that any mutation of either *TAP1* or *TAP2* could enable premature termination of specific protein translations, thereby down-regulating MHC-I molecules on the cell surface [16]. Later a Canadian investigation related to 889 children who suffered from type-I diabetes proved that SNPs of *TAP2* were highly associated with susceptibility to type-I diabetes, which could be possibly attributed to that spice variants formed by *TAP* SNPs served as indirect determiners in selection of antigen peptides [17]. Furthermore, *TAP* genotypes also acted as biomarkers for congenital bronchiectasis (*TAP*-665), multiple sclerosis (*TAP*-386) and Alzheimer's disease [18-20].

Of note, a former investigation suggested *TAP* genetic polymorphisms as potential risk parameters for TB targeting a Li population (i.e. one minority of Lingnan mainly living in Hainan province, China), including rs1057141 and rs1135216 of *TAP1* [15]. Nonetheless, the current study only confirmed a marked association of rs1135216 with pediatric STB. Although the two crowds included were both of Chinese ethnicities, the Li population harbored nearly 1.3 folds the rate of smear-positive TB when compared with Han population [21]. Furthermore, other extraneous factors, such as delayed treatment and poor living environment owing to undesirable socio-economic conditions, also accounted for the differed TB prevalences [8]. Certainly, TB and spinal TB, though interconnected, appeared as two disorders with distinct underlying mechanisms, providing hints that their predisposing causes may be dissimilar. Besides, a Korean study considered TAP2*Bky2/C/E to be enriched in the advanced pulmonary TB population, and they could be reflective of the extent to which TB has reached [12]. Later, three SNPs (e.g. rs4148871, s4148876 and rs2857103) of *TAP2* were documented to regulate TB development in a Japanese population after establishment of a dominant model [14].

Anyway, *TAP* gene was inter-related with modulation of TB risk, which could be ascribed to the peculiarities of its encoded proteins [13]. *TAP* was situated within the genomic area of MHC-II, and it was responsible for selection and submission of various antigens, including autoantigen, bacteria and virus, thus counting much in the process of adaptive immune response [17, 22]. Within endoplasmic reticulum (ER), the joint efforts of human leukocyte antigen (HLA) system and TAP would transport them onto cell surfaces and make them recognized by $CD8^+$ T cells, which contributed a great amount to protection against TB [23].

In spite of the aforementioned accomplishments concerned with association of TAP with PSTB, our study results were still limited by various parameters, including the small sample size. That was to say, these significant associations might not be appropriately generalized to a larger population. In addition, the frequencies of specific *TAP* SNPs appeared distinct among diverse ethnicities, and the rule derived from this Chinese Han population may not be applicable to children of other ethnicities. For instance, *TAP1**0401 was rare in the Chinese Han population, but it was commonly found among African populations who were habituated in Zimbabwe, Zambia and Rwanda [24]. The polymorphism distribution of *TAP1* among Chinese was relatively close to that of Japanese, Europeans and Americans, yet different from populations of Kaingang, Anatolian and Guarani ethnicities [20]. More than the aforementioned

elements, the detection method of this study differed from that of additional studies, which also rendered the results distinct.

Above all, the current study indicated a tight linkage between *TAP* polymorphisms and susceptibility to PSTB, however, whether this relationship could be applicable to a larger population remained a puzzle and more investigations were in demand.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Fuyun Liu, Department of Pediatric Orthopaedics, The Third Affiliated Hospital of Zhengzhou University, No. 7 Kangfu Front Street, Erqi District, Zhengzhou 450052, Henan Province, China. Tel: +86037166-903131; Fax: +86037166903131; E-mail: fy_Liu0713@163.com

References

[1] Jain AK. Tuberculosis of the spine: a fresh look at an old disease. J Bone Joint Surg Br 2010; 92: 905-913.

[2] Moon MS, Kim SS, Lee BJ and Moon JL. Spinal tuberculosis in children: retrospective analysis of 124 patients. Indian J Orthop 2012; 46: 150-158.

[3] Rajasekaran S, Prasad Shetty A, Dheenadhayalan J, Shashidhar Reddy J, Naresh-Babu J and Kishen T. Morphological changes during growth in healed childhood spinal tuberculosis: a 15-year prospective study of 61 children treated with ambulatory chemotherapy. J Pediatr Orthop 2006; 26: 716-724.

[4] Rajasekaran S. Kyphotic deformity in spinal tuberculosis and its management. Int Orthop 2012; 36: 359-365.

[5] Moon MS. Tuberculosis of spine: current views in diagnosis and management. Asian Spine J 2014; 8: 97-111.

[6] Feng WX, Mokrousov I, Wang BB, Nelson H, Jiao WW, Wang J, Sun L, Zhou SR, Xiao J, Gu Y, Wu XR, Ma X and Shen A. Tag SNP polymorphism of CCL2 and its role in clinical tuberculosis in Han Chinese pediatric population. PLoS One 2011; 6: e14652.

[7] Sambasivan V, Murthy KJ, Reddy R, Vijayalakshimi V and Hasan Q. P2X7 gene polymorphisms and risk assessment for pulmonary tuberculosis in Asian Indians. Dis Markers 2010; 28: 43-48.

[8] Gomez LM, Camargo JF, Castiblanco J, Ruiz-Narvaez EA, Cadena J and Anaya JM. Analysis of IL1B, TAP1, TAP2 and IKBL polymorphisms on susceptibility to tuberculosis. Tissue Antigens 2006; 67: 290-296.

[9] Chefalo PJ, Grandea AG 3rd, Van Kaer L and Harding CV. Tapasin-/- and TAP1-/- macrophages are deficient in vacuolar alternate class I MHC (MHC-I) processing due to decreased MHC-I stability at phagolysosomal pH. J Immunol 2003; 170: 5825-5833.

[10] Powis SH, Tonks S, Mockridge I, Kelly AP, Bodmer JG and Trowsdale J. Alleles and haplotypes of the MHC-encoded ABC transporters TAP1 and TAP2. Immunogenetics 1993; 37: 373-380.

[11] Naderi M, Hashemi M and Amininia S. Association of TAP1 and TAP2 gene polymorphisms with susceptibility to pulmonary tuberculosis. Iran J Allergy Asthma Immunol 2016; 15: 62-68.

[12] Roh EY, Yoon JH, Shin S, Song EY and Park MH. Association of TAP1 and TAP2 genes with susceptibility to pulmonary tuberculosis in Koreans. APMIS 2015; 123: 457-464.

[13] Sunder SR, Hanumanth SR, Gaddam S, Jonnalagada S and Valluri VL. Association of TAP 1 and 2 gene polymorphisms with human immunodeficiency virus-tuberculosis co-infection. Hum Immunol 2011; 72: 908-911.

[14] Thu KS, Sato N, Ikeda S, Naka-Mieno M, Arai T, Mori S, Sawabe M, Muramatsu M and Tanaka M. Association of polymorphisms of the transporter associated with antigen processing (TAP2) gene with pulmonary tuberculosis in an elderly Japanese population. APMIS 2016; 124: 675-680.

[15] Wang D, Zhou Y, Ji L, He T, Lin F, Lin R, Lin T and Mo Y. Association of LMP/TAP gene polymorphisms with tuberculosis susceptibility in Li population in China. PLoS One 2012; 7: e33051.

[16] Gadola SD, Moins-Teisserenc HT, Trowsdale J, Gross WL and Cerundolo V. TAP deficiency syndrome. Clin Exp Immunol 2000; 121: 173-178.

[17] Qu HQ, Lu Y, Marchand L, Bacot F, Frechette R, Tessier MC, Montpetit A and Polychronakos C. Genetic control of alternative splicing in the TAP2 gene: possible implication in the genetics of type 1 diabetes. Diabetes 2007; 56: 270-275.

[18] Dogru D, Ozbas Gerceker F, Yalcin E, Cobanoglu N, Pekcan S, Ozcelik U, Kiper N and Ozguc M. The role of TAP1 and TAP2 gene polymorphism in idiopathic bronchiectasis in children. Pediatr Pulmonol 2007; 42: 237-241.

[19] Moins-Teisserenc H, Semana G, Alizadeh M, Loiseau P, Bobrynina V, Deschamps I, Edan G, Birebent B, Genetet B, Sabouraud O and et al. TAP2 gene polymorphism contributes to genetic susceptibility to multiple sclerosis. Hum Immunol 1995; 42: 195-202.

[20] Bullido MJ, Martinez-Garcia A, Artiga MJ, Aldudo J, Sastre I, Gil P, Coria F, Munoz DG,

Hachinski V, Frank A and Valdivieso F. A TAP2 genotype associated with Alzheimer's disease in APOE4 carriers. Neurobiol Aging 2007; 28: 519-523.

[21] Phillips BA, Gaudette S, McCracken A, Razzaq S, Sutton K, Speed L, Thompson J and Ward W. Psychosocial functioning in children and adolescents with extreme obesity. J Clin Psychol Med Settings 2012; 19: 277-284.

[22] Groothuis TA, Griekspoor AC, Neijssen JJ, Herberts CA and Neefjes JJ. MHC class I alleles and their exploration of the antigen-processing machinery. Immunol Rev 2005; 207: 60-76.

[23] Caccamo N, Milano S, Di Sano C, Cigna D, Ivanyi J, Krensky AM, Dieli F and Salerno A. Identification of epitopes of Mycobacterium tuberculosis 16-kDa protein recognized by human leukocyte antigen-A*0201 CD8(+) T lymphocytes. J Infect Dis 2002; 186: 991-998.

[24] Lopez-Albaitero A, Nayak JV, Ogino T, Machandia A, Gooding W, DeLeo AB, Ferrone S and Ferris RL. Role of antigen-processing machinery in the in vitro resistance of squamous cell carcinoma of the head and neck cells to recognition by CTL. J Immunol 2006; 176: 3402-3409.