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Polymorphisms of suppressor of cytokine signaling-3 associated with susceptibility to tuberculosis among Han Taiwanese

Chou-Jui Lin[a,1], Shih-Wei Lee[a,b,1], Chi-Wei Liu[a], Chih-Pin Chuu[c], Yung-Hsi Kao[b,*], Lawrence Shih-Hsin Wu[d,*]

[a] Taoyuan General Hospital, Ministry of Health and Welfare, Taoyuan, Taiwan
[b] Department of Life Sciences, National Central University, Taoyuan, Taiwan
[c] Institute of Cellular and System Medicine, National Health Research Institutes, Miaoli, Taiwan
[d] Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan

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ABSTRACT

Background: Suppressors of cytokine signaling (SOCS), particularly SOCS-3, allow discrimination of patients with active tuberculosis (TB) from healthy subjects in a gender- and age-dependent manner. However, no information is available on whether single nucleotide polymorphisms (SNPs) in the SOCS-3 gene occur in patients with TB. This study was designed to investigate SOCS-3 SNPs in association with susceptibility to TB in the Taiwanese population.

Methods: Four SNPs in the SOCS-3 gene located at rs8064821, rs4969168, rs2280148, and rs35037722 were studied by the TaqMan SNP Genotyping assay in 200 healthy and 210 TB patients enrolled in 2015–2018.

Results: Significant differences were not detected in genotype frequencies or odds ratios (ORs) between healthy and TB patients for any of the four polymorphisms. The lack of significant differences was also found when the patients were stratified by sex. However, males exhibited GG homozygous at rs35037722 in association with susceptibility to TB after the OR analysis was adjusted for age. For rs8064821, AA and AC genotypes were associated with TB susceptibility in patients ≤ 65 years old compared to CC genotype, whereas older subjects had no such association.

Conclusions: The results suggest that particular SOCS-3 SNPs are dependent on gender or age to influence TB susceptibility in the Han Taiwanese.

1. Introduction

Tuberculosis (TB), an infectious disease caused mainly by *Mycobacterium tuberculosis*, remains a leading public health problem worldwide. In 2016, > 1.3 million people died from TB, and an estimated 6.3 million new cases occurred [1]. In Taiwan, approximately 10,328 new cases and 547 deaths occurred from mycobacterial infection in 2016 [2]. Many studies have demonstrated the association between host genome and susceptibility to TB, including Taiwan [3]. For example, a previous report demonstrated that polymorphisms in *NRAMP1* are associated with susceptibility to TB among aborigines, but not among the Han Chinese population [4]. In addition, many recent studies have indicated that interferon-gamma (IFN-γ) and Toll-like receptor 1 (TLR1) gene polymorphisms are associated with an increased risk of TB susceptibility in a Han Taiwanese population and Han Chinese children, respectively [5,6]. These observations suggest a close genetic association of particular genes and their SNPs with TB susceptibility in Taiwan.

SOCS-3, located in chromosome 17, belongs to a family of cytokine-inducible inhibitors of signaling including cytokine-inducible SH2 domain containing protein (CIS) and SOCS-1 ~ 7 [7]. SOCS-3 has been shown to mediate cancer cell growth [8-10], modulate immunity and inflammation [8,11], provide negative feedback to suppress insulin and leptin signaling [8,12-14], and act as a signaling molecule for hormones (e.g., resistin and growth hormone) [15,16]. Recent studies have indicated that SOCS-3 gene expression can be induced in TB [17] and allows discrimination of active TB from healthy and latent TB infection (LTBI) [18]. In addition, the circulating levels of SOCS-3-stimulating cytokines IFN-γ and interleukin (IL)-1 are may be involved in protection against TB in TB patients undergoing drug treatment [19]. These

* Corresponding authors at: Department of Life Sciences, National Central University, Jhongli District, Taoyuan 32001, Taiwan (Y.H. Kao).
E-mail addresses: ykao@cc.ncu.edu.tw (Y.-H. Kao), shwu@mail.cmu.edu.tw (L.S.-H. Wu).
[1] These authors contributed equally to this work.

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observations suggest the importance of SOCS-3 in the regulation and development of TB.

A recent study has demonstrated that a polymorphism in the CIS-1 gene, a member of the SOCS family, is associated with the risk of TB in China [20]. Whether relationships are present between polymorphisms in other SOCS family genes and TB susceptibility is still unknown. The present study was designed to investigate the association between SNPs in SOCS-3 and susceptibility to TB in Taiwan. The results show that genetic variants in SOCS-3 are risk factors in men and Han Taiwanese ≤ 65 years old.

2. Materials and methods

2.1. Study population

A total of 210 consecutive patients treated for active TB at General Taoyuan Hospital (Taoyuan, Taiwan) between 2015 and 2018 were surveyed. The inclusion criteria were adult patients newly diagnosed with active TB, with evident TB lesions on simple X-ray, computed tomography, and positive sputum smears and cultures for mycobacteria. In the control group, 200 volunteers without active TB or a history of TB were enrolled. We followed the method described by Rosner [21] to calculate the minimum sample size required for the experiment. When anticipated incidence was set to detect the difference between two independent study groups (case and control) with a dichotomous primary endpoint showing 40% and 35% allelic frequencies and reach, respectively, to an α(type I error rate) of 0.05 and β of 0.2 (power = 0.8), the minimum sample size needed for our study was 352 (176 for each group). The sample size estimation was performed by sample size calculator on web (https://clincalc.com/stats/samplesize.aspx). Written informed consent was obtained from each patient and volunteer enrolled in the study. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of Taoyuan General Hospital, Taoyuan, Taiwan.

2.2. DNA purification from buccal swabs

Genomic DNA was purified from oral swabs collected from the 210 TB patients and 200 non-TB controls using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Briefly, the buccal swab was placed in a 2-ml microcentrifuge tube with 400 μl PBS. We added 20 μl QIAGEN protease stock solution and 400 μl Buffer AL to each tube and incubated at 56 °C for 10 min to lyse the cells. The supernatant was collected after centrifugation at 6,000 × *g* for 1 min, precipitated out of the protein with 400 μl of absolute ethanol, and centrifuged again at 6,000 × *g* for 1 min to collect the genomic DNA. The genomic DNA was applied to the QIAamp Mini spin column for initial purification after 6,000 × *g* centrifugation for 1 min. We washed the genomic DNA-containing column with Buffer AW1 and centrifuged again at 6,000 × *g* for 1 min. The spin column was washed with Buffer AW2 and centrifuged at 20,000 × *g* for 3 min. The genomic DNA was eluted from the spin column by the addition of either Buffer AE or sterile distilled deionized water (150 μl) for the 1-minute incubation at room temperature after centrifugation at 6000 × *g* for 2 min. The purified genomic DNA was quantified by spectrophotometry using 260 nm and then stored at −80 °C until SNP analysis by TaqKey Science Co., LTD (Hualien, Taiwan).

2.3. SNP genotyping assays

The tag SNPs of the SOCS-3 genomic region, 1500 bp upstream and 1500 bp downstream, were selected according to the SeattleSNPs website (http://pga.mbt.washington.edu/education.html) [22]. The SeattleSNPs database showed five SOCS-3 tag SNPs (minor allele frequency > 0) used for the study of SOCS-3 in Han Chinese Beijing (HCB). However, this study did not indicate that any of these gene polymorphisms are related to a particular type of disease. In our study, we chose these four SNPs (one of the five SNPs failed the design of an available probe for genotyping) to study the association of the SOCS-3 gene with TB susceptibility. All SNP genotyping was performed using TaqMan SNP Genotyping Assays (Applied Biosystems, Inc. [ABI], Foster City, CA, USA) [23]. The primers and probes for the selected SNPs were from an ABI assay on demand (AOD) kit (cat. # 4351379, Thermo Fisher Scientific Inc., MA, USA). Reactions were carried out according to the manufacturer's protocol (TaqMan SNP Genotyping Assays, protocol, Part Number 4,332,856 Rev. C). The probe fluorescence signal was detected using an ABI Prism 7900 Real-Time PCR System.

2.4. Real-time polymerase chain reaction for SOCS-3 mRNA

We collected peripheral blood cells (PBCs) and isolated RNA from 16 TB patients and 10 non-TB participants according to the methods published by Lee et al. [18]. First, a venous blood sample (5 ml per subject) was collected from the patient's arm in an EDTA-containing Vacutainer® Blood Collection Tube (BD Biosciences, California, USA). Second, we used the QIAamp RNA Blood Mini Kit (Qiagen, Valencia, CA, USA) to isolate RNA from PBCs. Briefly, PBCs were mixed with 25 ml Buffer EL in a 50-ml centrifuge tube and then incubated for 15 min on ice to lyse the erythrocytes. After centrifugation at 400 × *g* for 10 min at 4 °C, the collected pellet was resuspended in 2 ml β-mercaptoethanol-containing Buffer RLT in the 50-ml centrifuge tube and then vortexed to lyse the leukocytes. The lysate was pipetted into the QIAshredder spin column and the filtrate collected after maximum centrifugation for 2 min. We mixed the filtrates with 2 ml of 70% ethanol and then pipetted into a new QIAamp spin column for RNA isolation after 8000 × *g* centrifugation for 15 sec. We washed the RNA-containing column once with Buffer RW1 and centrifuged again at 8,000 × *g* for 15 sec, and then washed the column twice with Buffer RPE for centrifugation at 20,000 × *g* for 3 min. The RNA was eluted by the addition of RNase-free water (50 μl) after centrifugation at 8,000 × *g* for 1 min. The isolated RNA was quantified by spectrophotometry at 260 nm and then stored at −80 °C until real-time PCR analysis.

We used real-time PCR to determine the SOCS-3 and GAPDH mRNA levels. cDNA was synthesized from equal amounts (1 μg) of RNA using 100 units of M-MLV reverse transcriptase (Invitrogen) in the presence of 40 units of RNase inhibitor (Invitrogen) and the adapter primer 5′-GGCCACGCGTCGACTAGTAC(T)19-3′. According to the manufacturer's two-step cycling protocols, the real-time PCR analysis was performed twice in duplicate by using the power SYBR green PCR master mix (Kapa Biosystems, Boston, MA) and ABI 7300 Sequence Detection System (Applied Biosystems, Foster City, CA) under the following conditions: an initial denaturing cycle at 95 °C for 5 min, followed by 40 cycles of amplification consisting of denaturation at 95 °C for 3 s and annealing/extension/data acquisition at 60 °C for 30 s. The forward and reverse primers were 5′-GGGGAGTACCACCTGAGTCT-3′ and 5′-CGAA GTGTCCCCTGTTTGGA-3′ for human SOCS-3 (128 bp, accession no. NM_003955.4) and 5′-GGAGCCAAAAGGGTCATCAT CTC-3′ and 5′-GAGGGGCCATCCACAGTCTTCT-3′ for human GAPDH (233 bp, accession no. NM_001256799.2). Amplicons size are 128 bp and 233 bp for SOC-3 and GAPDH, respectively. Normalization was performed using GAPDH mRNA levels as controls in parallel reactions. The relative expression of SOCS-3 transcripts was calculated as described by Lee et al. [18] and then expressed as the percent of the control. Previous studies indicated the expression of SOCS-3 mRNA in PC-3 prostate cancer cells [9] and MCF-7 breast cancer cells [24]. Therefore, we used RNAs isolated from both PC-3 and MCF-7 cells as positive controls for real-time PCR analysis. In addition, we used at least two sterile water no-template controls (NTCs) per assay to demonstrate the detection of DNA contamination. The control for amplification of genomic DNA was an equivalent amount of total RNA (1 μg) without reverse transcription.

Samples determined to be free of genomic contamination were further analyzed using the forward and reverse primers for SOCS-3 and GAPDH.

2.5. Statistical analysis

The quality of the genotype data was evaluated by Hardy-Weinberg equilibrium (HWE) proportion tests [5]. Intermarker linkage disequilibrium (LD) measures r^2 and D' were estimated and haplotype blocks defined using the Haploview program [25]. The association analyses were tested by the χ^2 test. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from contingency tables [26]. SNP(s) showing a significant association ($p < 0.05$) in the tests were further evaluated using logistic regression adjusted for age and gender in the OR analysis. The Mann-Whitney U-test was used to examine the differences in SOCS-3 gene expression between the HC and TB groups when both groups of subjects had the same genotype (CC or AA + AC) of rs8064821. All statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Characteristics of the study subjects

A total of 210 patients diagnosed with TB and 200 controls without a history of TB infection were enrolled. The characteristics of the study participants are presented in Table 1. We found a high men/women ratio in the TB group (2.82) compared to controls (1.47), which indicates that man were more prevalent in the TB group ($p = 0.002$, χ^2). The average age was 57 ± 19 years for TB patients and 66 ± 19 years for controls. There were significant differences in age between the TB and control groups ($p < 0.001$, t test).

3.2. SNPs in SOCS-3 are associated with susceptibility to tuberculosis

3.2.1. Genotype distributions conformed to Hardy-Weinberg equilibrium (HWE)

When the four SNPs in the SOCS-3 genomic region (rs8064821, rs2280148, rs4969168, and rs35037722) were genotyped by TaqMan SNP genotyping assays, none of the genotype distributions deviated from HWE (Table 2). The LD plot of the four SNPs is shown in Fig. 1. No haploblock was identified at the SOCS-3 gene.

Table 1
The characteristics of the study participants.

Variables	Non-TB, N (%)	TB, N (%)	p
Sex			
Man	119 (59.5)	155 (73.8)	0.002[a]
Woman	81 (40.5)	55 (26.2)	
Age (years)			
Mean ± SD (range)	66 ± 19 (20 ~ 97)	57 ± 19 (20 ~ 92)	< 0.001[b]
Man, mean ± SD (range)	70 ± 17 (20 ~ 97)	59 ± 18 (20 ~ 91)	< 0.001[b]
Woman, mean ± SD (range)	61 ± 19 (23 ~ 94)	52 ± 21 (20 ~ 92)	0.008[b]
Age group-N (%)			
≤ 65	77 (38.5)	138 (65.7)	< 0.001[a]
> 65	123 (61.5)	72 (34.3)	
Age group-gender-N (%)			
≤ 65			
Man	36 (46.8)[c]	98 (71.0)	< 0.001[a]
Woman	41 (53.2)	40 (29.0)	
> 65			
Man	83 (67.5)	57 (79.2)	0.080[a]
Woman	40 (32.5)	15 (20.8)	

SD = standard deviation; TB = tuberculosis; N = number of subjects.
[a] The statistical analysis was tested by χ^2-test.
[b] The statistical analysis was tested by t-test.
[c] The percent of each gender was calculated by its number over the total subjects at a given age (e.g., 46.8% = [36/(36 + 41)] × 100%.

3.2.2. SNPs in SOCS-3 depend on gender and age

None of the four SNPs had significantly different genotype frequencies between TB patients and controls (Table 2). We used logistic regression to test the effect of interactions age*genotype (divided into > 65 and ≤ 65 years) and gender*genotype, finding that the p values generated by logistic regression were all significant (Table 3).

The rs35037722 SNP in the SOCS-3 gene, but not the other three loci, was gender-dependent. In particular, the GG homozygous genotype of rs35037722 was a risk genotype for susceptibility to TB in male subjects adjusted for age (adjusted OR [aOR] = 2.171; 95% CI 1.125–4.193, $p = 0.021$) compared to the AA and AG genotypes (Table 4).

The rs8064821 SNP in the SOCS-3 gene, but not the other three loci, was age-dependent. For example, the A carrier (AA + AC) of rs8064821 was a risk genotype for susceptibility to TB among subjects ≤ 65 years old, but not subjects > 65 years old, before and after adjusting for gender compared to the CC genotype (OR = 1.992; 95% CI 1.088-3.645, $p = 0.025$; aOR = 2.296; 95% CI 1.218-4.326, $p = 0.010$; Table 5).

3.3. The SOCS-3 polymorphism contributed to differential mRNA levels

The rs8064821 SNP is located in the SOCS-3 gene promoter [22]. In parallel, we observed that TB patients had a higher proportion of AA + AC genotypes (42.8% vs 27.3%) and a lower proportion of CC genotype (57.2% vs. 72.7%) at rs8064821 compared to controls (Table 5). To further look at the relationship of genotypes at rs8064821 with the SOCS-3 gene, we used real-time PCR to detect mRNA levels in 26 subjects (Fig. 2). The SOCS mRNA levels in the TB subjects with CC genotype were approximately 151.7% of those in the control subjects with CC genotype. Although TB subjects with AA and AC genotypes exhibited SOCS-3 mRNA expression that was not significantly different from controls with AA and AC genotypes, they had approximately 287.1% higher levels.

4. Discussion

Gene polymorphisms in the association of the SOCS family with susceptibility to TB are emerging even though the expression of SOCS genes has been reported to be associated with the development of TB [17,27]. In particular, a recent report indicated that CIS-1 gene polymorphisms are associated with susceptibility to TB in the Chinese Han population [20]. In our study of the Taiwanese population, the SNPs rs8064821, rs2280148, rs4969168, and rs35037722 in the SOCS-3 gene region did not show any different genotype frequencies between TB patients and controls. This suggests a SOCS family-dependent effect of SNPs on TB susceptibility. This notion is supported by the OR analysis that a difference in the OR of the four SOCS-3 SNPs was absent between TB patients and non-TB controls. When the OR analysis was adjusted for gender and age using logistic regression, we also found that no SNPs in the SOCS-3 gene were risk genotypes for TB susceptibility.

We recently found a difference in SOCS-3 mRNA levels between non-TB and TB patients, both men and women [18]. In rs35037722, major allele homozygous (GG) was a risk genotype for TB in men, but not women, because the OR was significantly different between controls and TB patients. The rs35037722 polymorphism is located in the 3′ region of the SOCS-3 gene. However, no report has indicated that the presence of the rs35037722 major allele is related to regulation of SOCS-3 gene expression and the development of disease. Similar to previous studies, the rs4331426 SNP was associated with TB in the Han Taiwanese population, especially in women [28]. In addition, our recent study found a significant difference in SOCS-3 mRNA levels

Table 2
Genotyping frequencies of SNPs in the TB and non-TB groups and results of logistic regression.

SNP ID	Location	Genotype	Genotype counts		p value	Odds ratio (95% CI)	p for OR
			Non-TB (%)	TB (%)			
rs8064821	5′near gene (−1231, from transcription start site)	AA	14 (7.0)	11 (5.2)	0.532	1.188 (0.488. 2.888)	0.704
(HWp = 0.393)		AC	64 (32.0)	74 (35.2)		0.804 (0.517, 1.250)	0.332
		CC (ref.)	122 (61.0)	125 (59.6)			
rs4969168	3′UTR	AA	30 (15.0)	39 (18.6)	0.167	0.554 (0.297, 1.031)	0.062
(HWp = 0.115)		AG	99 (49.5)	104 (49.5)		0.862 (0.543, 1.368)	0.528
		GG (ref.)	71 (35.5)	67 (31.9)			
rs2280148	3′UTR	GG	9 (4.5)	5 (2.4)	0.413	1.683 (0.511, 5.540)	0.392
(HWp = 0.795)		TG	69 (34.5)	84 (40.0)		0.827 (0.540, 1.269)	0.385
		TT (ref.)	122 (61.0)	121 (57.6)			
rs35037722	3′near gene	AA	1 (0.5)	1 (0.5)	0.476	2.703 (0.165, 44.234)	0.486
(HWp = 0.791)		AG	36 (18.0)	31 (14.8)		1.343 (0.766, 2.335)	0.303
		GG (ref.)	163 (81.5)	178 (84.7)			

HWp: p value of Hardy-Weinberg disequilibrium test; ref: reference genotype; p value was adjusted by age and gender, age divided into two groups, > 65 and ≤ 65 years; CI: confidence interval; OR: odds ratio.

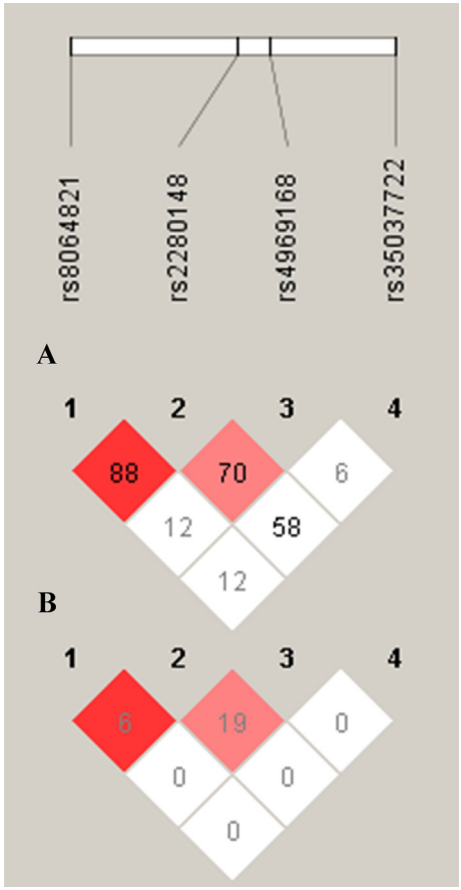

Fig. 1. Linkage disequilibrium plot in D' demonstrating adjacent strength between SNP pairs in the SOCS-3 gene. D' (A) and r^2 (B) values were multiplied by 100. In (A), squares without a number have a value of 100, equal to a D' value of 1. When two SNPs are completely linked, the D' value is 1. In (B), squares without a number have a value of 80, equal to an r^2 value of 0.8. The r^2 values ≥0.8 were considered significant. The four SNPs were not in linkage disequilibrium.

between control and TB subjects in the ≤ 65 years age group [18]. When the whole data were stratified according to age, the OR analysis indicated that the A carrier (AA + AC) genotype of rs8064821 is significantly associated with susceptibility to TB in the younger group only. Previous studies have indicated that rs8064821 is associated with the risk of childhood obesity [29] and head and neck squamous cell cancer [30]. Similarly, in a recent study, the *LEP* G-2548A polymorphism affected age- and gender-specific development of obesity [31]. The gender- and age-dependent effect of SOCS-3 SNPs on susceptibility to TB still requires further study with a large sample size of each group of subjects.

Table 3
Interaction of Genetic variation and gender/age contribute to tuberculosis risk.

	χ^2	df	p
age*genotype			
rs8064821	48.903	5	< 0.0001
rs4969168	33.767	5	< 0.0001
rs2280148	34.768	5	< 0.0001
rs35037722	34.821	4	< 0.0001
sex*genotype			
rs8064821	11.796	5	0.038
rs4969168	11.231	5	0.047
rs2280148	16.995	5	0.005
rs35037722	13.624	4	0.009

df: degree of freedom, rs35037722 AA genotype is only two subjects, one in case and another in control, so the df = 4; p value was calculated by logistic regression

Previous studies have indicated that SOCS-3 can regulate Th1, Th2, and Th17 differentiation [32,33]. Overexpression of regulatory SOCS proteins in TB patients suggest favored Th2-type development and attenuated Th1-type responses that could promote intracellular persistence of *Mycobacterium tuberculosis* [27]. In this study, OR analysis showed that the GG homozygous genotype of rs35037722 is a risk genotype for susceptibility to TB in men, but not women. Whether the rs35037722 regulatory mechanism explains a gender-dependent difference in SOCS-3 gene expression between TB and non-TB subjects was not demonstrated in this study. Previous studies indicated that sex hormones up-regulate SOCS-3 gene expression in prostate [34] and hepatoma [35] cancer cells. Patients with TB have lower levels of testosterone and higher levels of IL-6, IFN-γ, and TGF-β compared to healthy controls [36]. Estrogen levels are significantly higher in postmenopausal women with TB than in those without TB [37]. In a *Listeria monocytogenes*-infected mouse model, the expression of Th1 cytokines (IFN-γ, TGF-α, and IL-2) was higher and the expression of Th2 cytokines (IL-4, IL-10, and TNF-β) lower in mice treated with β-estradiol than in mice not treated with β-estradiol [38]. Given this information, sex hormones are a factor of TB susceptibility and can regulate many immune-related cytokines against TB infection. Future studies are needed on the effect of sex hormones on the association between rs35037722 and TB susceptibility.

The rs8064821 SNP is located in the 5′ region of the human SOCS-3 gene. A recent report indicated that the presence of the minor allele of

Table 4
Odds ratio analysis of SOCS-3 SNPs: rs8064821, rs4969168, rs2280148, and rs35037722 in men and women with or without TB groups.

SNP ID	Genotype	Genotype counts		*p* value[a]	OR (95% CI)	Adj.OR (95% CI)[b]
		Non-TB	TB			
rs8064821						
Men	AC + CC	112	148	0.611	1.321 (0.451, 3.876)	1.500 (0.483, 4.658)
	AA (ref.)	7	7		$p = 0.612$	$p = 0.483$
Women	AC + CC	74	51	0.774	1.206 (0.339, 4.334)	1.100 (0.294, 4.111)
	AA (ref.)	7	4		$p = 0.774$	$p = 0.888$
rs4969168						
Men	AA	16	27	0.370	1.358 (0.694, 2.655)	1.689 (0.830, 3.436)
	AG + GG (ref.)	103	128		$p = 0.371$	$p = 0.148$
Women	AA	14	12	0.509	1.336 (0.565, 3.159)	1.483 (0.608, 3.621)
	AG + GG (ref.)	67	43		$p = 0.510$	$p = 0.386$
rs2280148						
Men	TG + GG	44	70	0.173	1.404 (0.861, 2.288)	1.574 (0.938, 2.640)
	TT (ref.)	75	85		$p = 0.174$	$p = 0.086$
Women	TT	47	36	0.383	1.371 (0.674, 2.787)	1.524 (0.731, 3.177)
	TG + GG (ref.)	34	19		$p = 0.384$	$p = 0.261$
rs35037722						
Men	GG	91	132	0.067	1.766 (0.957, 3.259)	2.171 (1.125, 4.193)
	AA + AG (ref.)	28	23		$p = 0.069$	$p = 0.021$
Women	GG	72	46	0.375	0.639 (0.236, 1.728)	0.590 (0.210, 1.658)
	AG (ref.)	9	9		$p = 0.378$	$p = 0.317$

[a] *p* values were determined by the χ^2 test.
[b] Adj. = adjusted for age by logistic regression; ref. = reference genotype.

rs8064821 may result in a new transcription binding site for the transcription factor nuclear-factor 1 (NF-1) [39]. In addition, a previous study indicated that NF-1 acts as a promoter-binding transcription activator [40], suggesting that rs8064821 may influence TB susceptibility by altering SOCS-3 gene expression in subjects ≤ 65 years of age. We recently demonstrated that TB patients have higher levels of SOCS-3 mRNA than non-TB subjects in a group ≤ 65 years of age [18]. In this study, there were no significant differences in SOCS-3 mRNA level between controls and TB patients with CC and AA + AC genotypes at rs8064821. However, TB patients with the AA + AC genotype seemed to have higher levels of SOCS-3 mRNA than controls with the AA + AC genotype. In the future, more factors need to be considered to explore TB susceptibility and development combined with genetic factors with regulatory effects on age.

In Taiwan, the incidence of new TB cases in elderly adults (≥65 years old) was more than half (~52%) of that of young adults (< 65 years old) [41]. The difference in the incidence of TB between non-elderly and elderly subjects is due to the effect of underlying chronic diseases and the biological changes associated with aging. In addition, some studies have indicated the association of declined immune responses with age [42,43]. As age is a risk factor for TB infection, the TB susceptibility in elderly subjects is more likely due to the aging effect rather than direct genetic effects. Young adults have stronger immune systems to protect against TB infection; therefore, the association with TB susceptibility in young patients is more likely due to reasonable factors other than age. The information may explain the

Table 5
Odds ratio analysis of SOCS-3 SNPs: rs8064821, rs4969168, rs2280148, and rs35037722 in non-aged (≤65-year-old) and aged (> 65-year-old) subjects with or without TB groups.

SNP ID	Genotype	Genotype counts		*p* value[a]	OR (95% CI)	Adj.OR (95% CI)[b]
		Non-TB	TB			
rs8064821						
≤ 65	AA + AC	21	59	0.024	1.992 (1.088, 3.645)	2.296 (1.218, 4.326)
	CC (ref.)	56	79		$p = 0.025$	$p = 0.010$
> 65	AA + AC	57	26	0.163	0.654 (0.360, 1.189)	
	CC (ref.)	66	46		$p = 0.164$	
rs4969168						
≤ 65	AA	8	21	0.320	1.548 (0.651, 3.684)	1.918 (0.778, 4.729)
	AG + GG (ref.)	69	117		$p = 0.323$	$p = 0.157$
> 65	AA	22	18	0.235	1.530 (0.756, 3.097)	
	AG + GG (ref.)	101	54		$p = 0.237$	
rs2280148						
≤ 65	TT + TG	74	135	0.462	1.824 (0.359, 9.267)	1.988 (0.373, 10.606)
	GG (ref.)	3	3		$p = 0.468$	$p = 0.421$
> 65	TT + TG	117	70	0.475	1.795 (0.353, 9.138)	
	GG (ref.)	6	2		$p = 0.481$	
rs35037722						
≤ 65	GG	66	114	0.554	0.792 (0.365, 1.719)	0.951 (0.427, 2.119)
	AA + AG (ref.)	11	24		$p = 0.555$	$p = 0.902$
> 65	GG	97	64	0.075	2.144 (0.914, 5.032)	
	AG (ref.)	26	8		$p = 0.080$	

[a] *p* values were determined by the χ^2 test.
[b] Adj. = adjusted for gender by logistic regression; ref. = reference genotype.

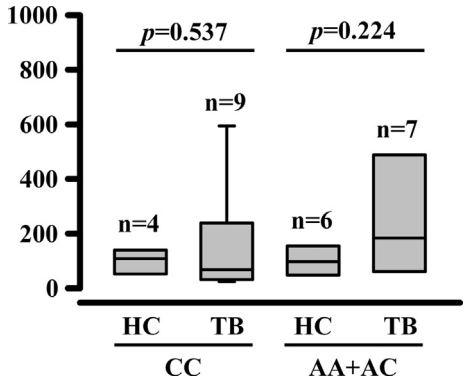

Fig. 2. Differences in SOCS-3 mRNA levels between healthy controls (HCs) and TB patients with CC or AA + AC genotypes at rs8064821. Mann-Whitney U-test was used to examine differences between the groups.

positive association of the rs8064821 SNP in the SOCS-3 gene in non-elderly patients rather than elderly patients with TB. Notably, we observed higher SOCS-3 mRNA levels in non-elderly, but not elderly, TB patients compared to those healthy subjects [18]. Other studies have shown indirect support for the age-dependent association as reported for the relationship between *NRAMP1* 3′UTR and D543N polymorphisms and susceptibility to TB infection in the Chinese [44] and Tunisian populations [45].

Expression of SOCS-3 can be regulated by SNPs [46]. It was evident from our previous study that the level of SOCS-3 mRNA expression is higher in active TB subjects than non-TB groups [18]. However, we observed no significant differences in the genotype frequencies of the four SNPs in the SOCS-3 gene between non-TB and TB groups. The results make it difficult to explain the differences in SOCS-3 mRNA levels between TB and non-TB groups. Potentially functional polymorphisms have also been reported at −1044 (C/A) in the promoter [47] and −920 (C/T; rs12953258) in the 5′ UTR [48] of the SOCS-3 gene. Accordingly, future studies should explore whether any of these promoter regions and other SNPs in the SOCS-3 gene are involved in the gene regulation associated with TB.

We conclude that rs8064821, rs2280148, rs4969168, and rs35037722 in the SOCS-3 gene do not have different genotype frequencies between TB patients and non-TB individuals. However, male subjects GG homozygous at rs35037722 had demonstrated susceptibility to TB after the OR was adjusted for age. The A carrier (AA and AC) genotype at rs8064821 was associated with susceptibility to TB in subjects ≤ 65 years old compared to CC genotype. These results suggest that particular SOCS-3 SNPs are dependent on gender or age to influence TB susceptibility in Han Taiwanese.

Conflicts of interest

The authors have declared that no competing interests exist.

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