Original Article

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Association between an Interleukin 4 Gene Polymorphism, rs2243268, and Urogenital Tuberculosis

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Purpose: Urogenital tuberculosis (UGT) is rarely reported in developed countries. This study evaluated the genetic susceptibility of Korean patients to UGT.
Materials and Methods: A total of 35 UGT patients who were confirmed pathologically, 44 intrapulmonary tuberculosis (IPT) patients who were confirmed radiologically, and 102 controls over a 6 year period were enrolled in this study. The region of rs2243268 in interleukin-4 (*IL-4*) gene was amplified from whole blood samples, and the DNA sequences were read using the Sanger method.
Results: Twenty women and 15 men were diagnosed with UGT. The occurrence of the CC, AC, and AA genotypes of rs2243268 were 26 (74.3%), 8 (22.9%), and 1 (2.9%), respectively, in UGT; 28 (63.6%), 15 (34.1%), and 1 (2.3%), respectively, in IPT; and 51 (50.0%), 45 (44.1%), and 6 (5.9%), respectively, in the control groups (p=0.115). The bivariate data of CC and AC/AA were 74.3% and 25.7% in UGT, 63.6% and 36.4% in IPT, and 50.0% and 50.0% in the control groups, respectively (p=0.029). The UGT was significantly different from the control group among the three genotypes (p=0.038, Fisher's exact test) and bivariate genotypes (p=0.017, Fisher's exact test). In addition, people carrying the CC genotype had a higher risk of UGT (odds ratios, 2.889; 95% confidence intervals, 1.233-6.770; p=0.015).
Conclusions: A single nucleotide polymorphism in the *IL-4* gene, rs2243268, is associated with the development of clinical tuberculosis. The CC type of rs2243268 increases the risk of UGT significantly compared to the CA/AA type.

Keywords: Urogenital tuberculosis; rs2243268

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INTRODUCTION

Clinical tuberculosis (TB) and its progression are usually dependent on the virulence of the TB strains, as well as environmental and host factors [1-5]. Two major environmental factors for the re-activation of latent TB are the poor general health condition and a human immunodeficiency virus (HIV)-induced immunocompromised state, which often lead to acquired immunodeficiency syndrome (AIDS) [3,4]. Therefore, non-HIV infected individuals in developed countries are relatively less prone to clinical TB in their lungs or the development of subsequent extrapulmonary TB (EPTB) lesions. Indeed, in recent years, reported cases of clinical urogenital TB (UGT) have decreased significantly and the disease is rare in Korea.

The transition from primary intrapulmonary TB (IPT) to clinical UGT takes a long time [6]. During the long incubation periods to develop clinical UGT, primary IPT lesions can be exposed on delicate host immune systems. Through the innate and adaptive immune responses, the production of various cytokines is regulated tightly during early IPT lesions, latent stage, and re-activation stage of the TB pathogenesis [4]. Among these cytokines, interleukin 4 (*IL-4*) and *IL-10* may play important roles in the progression of TB [7-9]. Consequently, numerous reports evaluating the association between the characteristics of TB patients and single-nucleotide polymorphisms (SNPs) in these representative cytokines have become available [7-9]. Nevertheless, insights into the relationship between the phenotypes of UGT in non-HIV patients in developed countries and the genotypes in these immune-deciding sites have been limited.

Among various cytokines, rs2243268-A genetic type of the *IL-4* gene is associated with a reduced risk of EPTB development, mostly lymphatic TB, in Han pediatric patients [7]. To the best of the authors' knowledge, however, the clinical significance of rs2243268 in pathologically and clinically confirmed UGT in adult patients has not been evaluated extensively.

Therefore, this study examined the significance of genetic factors that are associated with the individual susceptibility to TB lesions. Accordingly, the genetic variances in rs2243268 of the *IL-4* gene were studied to evaluate the susceptibility against TB in Korean IPT and UGT patients.

MATERIALS AND METHODS

1. Study Population

Written consent was obtained from the study participants. The Institutional Review Board of the Dankook University Hospital approved the research protocol.

The study population was comprised of 35 clinically and pathologically confirmed UGT patients, 44 radiologically confirmed IPT patients, and 102 controls. Subjects with severe malnutrition, cancer, HIV/AIDS infections, and underage patients (<20 years old) were excluded. The UGT cases from six different urologists in six different hospitals in Korea over the last 6 years were collected. All 35 cases of UGT had TB-associated symptoms and specific radiological findings on the abdominal computed tomography (CT) scan or scrotal ultrasounds. Surgical operations, such as nephrectomy or epididymectomy, were performed and TB lesions on their specimens were confirmed pathologically in all cases. IPT patients and controls, who had visited hospitals with various non-infectious reasons, were enrolled in this study. The IPT patients had specific radiological IPT evidence in their posterior-anterior chest radiography but did not have any evidence of abdominal TB lesions on the simultaneous abdominal CT scans. The controls were defined as individuals who had neither radiological evidence nor medical histories of this disease.

2. Laboratory Assay

Whole blood samples were collected in tubes containing sodium ethylenediaminetetraacetic acid (EDTA) [10]. The QIAamp blood extraction kit (Qiagen, Valencia, CA, USA) was used for DNA extraction.

Polymerase chain reaction (PCR, 2720 Thermal Cycler; Applied Biosystems, Foster City, CA, USA) was performed to amplify the SNP rs2243268 site in the *IL-4* gene. The specific amplification bands from all 181 subjects were confirmed. The primer sequences were 5'-aaggggaagcttctgtagcc -3' and 5'- tccaagcagctttcaagttc -3', respectively. The genomic DNA (25-70 ng) was used as a template in a total PCR volume of 50 μl. Each tube contained 25 μl of 2×GoTaq Green Master Mix (400 μM dATP, 400 μM dGTP, 400 μM dCTP, 400 μM dTTP, and 3 mM $MgCl_2$) (Promega, Madison, WI, USA), 300 nM of each oligonucleotide primer, and 2 μl of extracted DNA template. All reaction samples were heated to 94°C for 5 minutes for activation and then subjected to 30 cycles at 95°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute. A final extension was performed at 72°C for 5 minutes. After purification (AccuPrep® Genomic DNA Extraction Kit; Bioneer, Daejon, Korea), the DNA sequences were identified with the BigDye Terminator Sequencing Kit (Applied Biosystems) using the above primers [10].

3. Statistical Methods

A Hardy-Weinberg equilibrium goodness of fit test was used to assess the deviation for the genotype frequencies of the variant. Analysis of variance was performed to test for differences in the mean ages. The Pearson chi-square test and Fisher's exact test were used to evaluate the difference in categorical data. The odds ratios (OR) and

95% confidence intervals (CI) were estimated by logistic regression analysis. Two-sided null hypotheses of no difference were rejected if the p-values were less than 0.05. All analyses were performed using SPSS software for Windows ver. 23.0 (SPSS INC., Chicago, IL, USA).

RESULTS

Thirty-five UGT blood samples were collected from 20 women and 15 men; of the 35 samples, 33 cases had TB lesions in the urinary tracts and the remaining two cases had lesions in the epididymis. The sex matched mean ages were 49.8±14.7 years in the controls, 51.7±14.6 years in the IPT patients, and 51.1±14.3 years in the UGT patients, respectively ($p>0.05$) (Table 1). An evaluation of the Hardy-Weinberg equilibrium showed that the genotype frequency of an A/C polymorphism in rs2243268 in all 181 cases was acceptable (X^2=0.53, p=0.46).

The frequencies of the CC, AC, and AA types of rs2243268 were 26 (74.3%), 8 (22.9%), and 1 (2.9%), respectively in the UGT patients; 28 (63.6%), 15 (34.1%), and 1 (2.3%), respectively, in the IPT patients; and 51 (50.0%), 45 (44.1%), and 6 (5.9%), respectively, in the control patients (p=0.115) (Fig. 1, Table 1).

The bivariate genotypes of CC and AC/AA were 74.3% and 25.7% in UGT, respectively; 63.6% and 36.4% in IPT, respectively; and 50.0% and 50.0% in the control groups, respectively (p=0.029) (Table 1). Significant differences in the CC, AC, and AA genotypes (p=0.038, Fisher's exact test) and CC and AC/AA genotypes (p=0.017, Fisher's exact test) were observed between the UGT and control group (Fig. 2). In addition, people carrying the CC genotype of rs2243268 had an increased risk of IPT (OR, 1.750; 95% CI, 0.846-3.619; p=0.131) and UGT (OR, 2.889; 95% CI, 1.233-6.770; p=0.015) compared with those with the CA/AA genotypes.

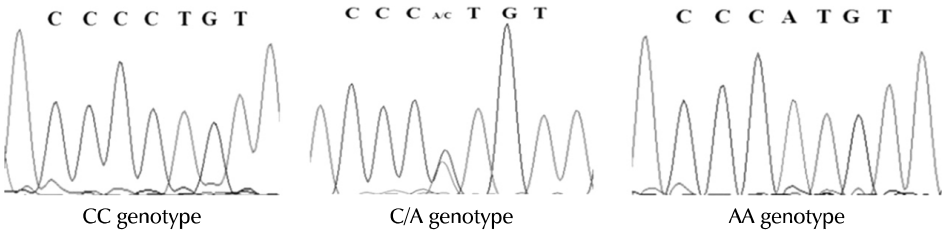
DISCUSSION

Clinical TB lesions can be classified into IPT or EPTB lesions. EPTB lesions refer to a manifestation of TB in organs other than the lungs [11]. The epidemiological characteristics of EPTB are significantly different from that of IPTB [12,13]. In terms of frequency, cases of EPTB in Korea are mostly reported as lymphatic TB, followed by abdominal, bone-joint, central nerve system, and finally

Table 1. Clinical characteristics and genotype distribution of the rs2243268 polymorphism among urogenital tuberculosis (UGT), intrapulmonary tuberculosis (IPT), and control groups

Parameter	Patient group			p-value
	UGT	IPT	Control	
No. of subjects	35	44	102	
Sex (male/female)	15/20	20/24	46/56	
Age (y)	51.1±14.3	51.7±14.6	49.8±14.7	
Urogenital tuberculosis location				
Urinary tract	33	None	None	
Genital tract	2	None	None	
Genetic polymorphism				0.115
CC	26 (74.3)	28 (63.6)	51 (50.0)	
CA	8 (22.9)	15 (34.1)	45 (44.1)	
AA	1 (2.9)	1 (2.3)	6 (5.9)	
CC	26 (74.3)	28 (63.6)	51 (50.0)	
CA & AA	9 (25.7)	16 (36.4)	51 (50.0)	0.029

Values are presented as number only, mean±standard deviation, or number (%).



CC genotype C/A genotype AA genotype

Fig. 1. Single nucleotide polymorphism in rs2243268. Sequencing chromatogram revealed the CC genotype (left), C/A genotype (middle), and AA genotype (right).

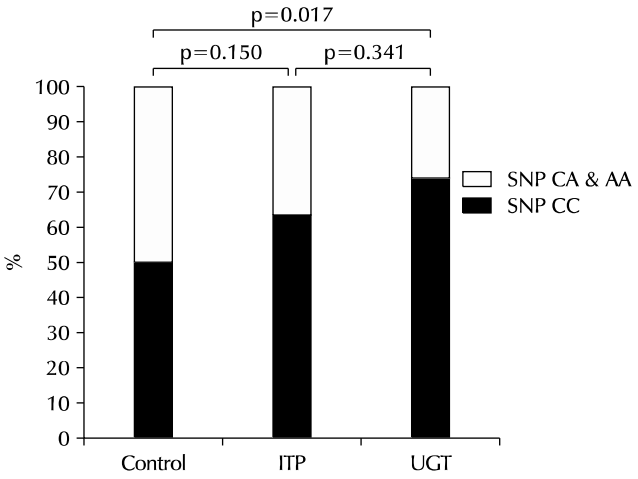


Fig. 2. Significant differences in bivariate genotypes were observed between the urogenital tuberculosis (UGT) and the control group (p=0.017, Fisher's exact test). People carrying the CC genotype of rs2243268 had an increased risk of UGT (odds ratios, 2.889; 95% confidence intervals, 1.233-6.770; p=0.015) compared to those with the CA/AA genotypes. SNP: single-nucleotide polymorphism, IPT: intrapulmonary tuberculosis.

UGT [11]. In addition, as lymphatic TB is mostly associated with pediatric patients, the clinical characteristics of lymphatic TB must be different from other EPTB lesions because they usually manifest later in life. Owing to the rarity of UGT, only a handful of studies have characterized UGT independently from other EPTB diseases.

IL-4 is located in a gene cluster at chromosome 5q31, and acts as an anti-inflammatory cytokine during the immune responses against TB infections [7-9]. The recent report of a potential association between the rs2243268-A genetic type of the *IL-4* gene and a reduced risk of EPTB development in Han pediatric patients suggest that genetic factors may be involved in the development of EPTB lesions [7]. Nevertheless, its clinical implication may be limited because the majority of enrollments were pediatric patients with lymphatic TB. To the best of the authors' knowledge, this study is the first to examine the association between rs2243268 genetic polymorphisms and their potential susceptibility in pathologically confirmed UGT patients in the Korean population.

These results showed that genotype CC increases the risk of a TB infection. In addition, genotype CC significantly increased the risk of UGT (OR, 2.889; 95% CI, 1.233-6.770; p=0.015). Furthermore, while the statistical significance was not established, genotype CC was also associated with an increased risk of IPT (OR, 1.750; 95% CI, 0.846-3.619). Accordingly, the relationship between genotype CC and an increased risk of infection was particularly pronounced in UGT than IPT. Although an explanation for this finding remains inconclusive, some factors may play roles in this development. First, all cases of UGT were symptomatic and patients had severe destructive lesions on their urogenital tracts; all patients must take the appropriate surgical interventions, such as nephrectomy or epididymectomy because of the uncontrolled UGT symptoms and signs or complete functional loss of infected organs. In contrast, enrolled IPT patients are usually asymptomatic with inactive TB lesions based on posterior-anterior chest radiography. *Mycobacterium tuberculosis* can infect the organs in the urinary tracts, such as kidney, ureter and urinary bladder through urination, and their long incubation periods allow them to be simultaneously spread into the male genital tract, such as epididymis and prostate, through the blood vessels. Elevated *IL-4* levels usually are observed during the advanced stages of TB [14]. Therefore, various immune systems may have more chances to interact with the urogenital organs than intrapulmonary lesions because of their severity and long incubation periods.

The bivariate genotypes of CC and AC/AA were 74.3% and 25.7% in cases of UGT, and 63.6% and 36.4% in cases of IPT (Fisher's exact test, p=0.341). This suggests that the risk-associated with genotype CC in rs2243268 is not limited to UGT but may be generalized with severity in TB lesions [7].

Antimycobacterial immune responses may be distinct between TB patients of different ethnic origins. After adjusting for socioeconomic factors, significant differences in the risk of TB among the Caucasian (northern and western European ancestry), African-American, Hispanic, Asian, and Native American population have been observed [5,15]. Accordingly, Asians are most vulnerable to TB infections. The reference cluster report for rs2243268 in ss19645261 revealed very interesting results. In Caucasians, the most dominate genotype of rs2243268 is the AA type. In contrast, the most dominant genotype in Asians is the CC type. Such variances in the genotypes of rs2243268 may account the differences in the TB patterns in various ethnic origins, but this hypothesis must be supported by ongoing studies in the near future.

An early diagnosis of UGT is quite difficult because the clinical symptoms or signs are usually vague and asymptomatic. Indeed, a delayed diagnosis of UGT is

frequent and it entails an increase in mortality and morbidity. Therefore, such genetic information may have additional advantages for the early detection of UGT.

CONCLUSIONS

A SNP in the *IL-4* gene, rs2243268, plays a role in the development of clinical TB. People with genotype CC of the rs2243268 have an increased risk of UGT compared to those with genetic types CA/AA, but an examination of only one polymorphism and a lack of biochemical markers in the blood still remain as challenges.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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