การศึกษาการกระจายตัวของ TGF-β1 C-509T ในประชากรไทย

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บทคัดย่อ

ความหลากหลายของยีน Transforming growth factor beta 1 (TGF-β1) C-509T มีความสัมพันธ์กับความรุนแรงของโรคและความไวต่อการเป็นโรคหลายชนิด การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาความหลากหลายของยีน TGF-β1 C-509T (rs1800469) ในประชากรไทย โดยศึกษาในคนไทยปกติจำนวน 56 คน ด้วยวิธี polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) จากการศึกษาพบว่ามีความหลากหลายของ TGF-β1 C-509T ดังนี้ CC 12.5%, CT 39.3% และ TT 48.2 % ซึ่งความถี่จีโนไทป์เหล่านี้ในประชากรไทยมีค่าไม่แตกต่างจากกลุ่มประชากรจีน (p=0.390), อิตาลี (p=0.06) เซอเบียร์ (p=0.55) และบราซิล (p=0.442) อย่างมีนัยสำคัญทางสถิติ แต่พบว่ามีความแตกต่างจากประชากรอินเดีย (p=0.005) รัสเซีย (p=0.048) อังกฤษ (p=0.006) เยอรมนี (p=0.014) และอียิปต์ (p<0.0001) อย่างมีนัยสำคัญทางสถิติ เมื่อศึกษาความถี่ของอัลลีล C และ T พบว่า อัลลีลที่พบมากในประชากรไทยคือ T ในขณะที่ในประชากรอื่นๆ พบอัลลีล C มากกว่า (p<0.05) การศึกษานี้น่าจะบ่งชี้ให้เห็นว่าความหลากหลายของยีน TGF-β1 C-509T ในประชากรไทยมีความแตกต่างจากประชากรกลุ่มอื่น และอาจนำข้อมูลนี้ไปใช้ในการพัฒนาการพยากรณ์โรคหรือการรักษาโรคได้ต่อไปในอนาคต อย่างไรก็ตามการศึกษาความหลากหลายของยีน เพื่อเป็นการยืนยันผลการศึกษาในครั้งนี้ควรทำการศึกษาในกลุ่มตัวอย่างที่มีขนาดใหญ่ขึ้นต่อไป

คำสำคัญ: TGF-β1 C-509T, ความหลากหลายของยีน, ประชากรไทย

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Distribution of TGF-β1 C-509T polymorphism in Thai population

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Abstract

Transforming growth factor beta 1 (TGF-β1) C-509T gene polymorphism has been found to be associated with severity and susceptibility of many diseases. To examine the distribution of TGF-β1 C-509T (rs1800469) gene polymorphism in Thai population, 56 samples from unrelated healthy Thai volunteers were analyzed by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The genotype frequencies of TGF-β1 C-509T polymorphisms were as follows: CC 12.5%, CT 39.3% and TT 48.2%, which showed no statistically significant differences to those of Chinese (Han) (p=0.390), Italian (p=0.057), Serbian (p=0.554) and Brazilian (p=0.442) population. However, a statistically significant difference was observed between the genotype frequencies found in this study and those of Indian (p=0.005), Russian (p=0.048), English (p=0.006), German (p=0.014), and Egyptian (p<0.0001) population. In addition, statistically significant differences were also observed between allele frequencies in Thai population in this study and those of other reported groups; including, Chinese (Han), Indian, Russian, Italian, English, German, Serbian, Egyptian, and Brazilian (p<0.05). The frequency of T allele in Thai was significantly higher than that of C allele, whereas in other reported groups, the C allele frequencies were significantly higher than T allele frequencies (p<0.05). From the above data, it is likely that the distribution of TGF-β1 C-509T gene polymorphisms in Thai population is different from those of Asian, Caucasian, Egyptian, and Brazilian population. This observation may be useful and could be applied for treatment and prognostic of disease in the future. However, further studies in larger numbers of Thai subjects are required for confirmation.

Keywords: TGF-β1 C-509T, gene polymorphism, Thai population

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Introduction

Transforming growth factor beta 1 (TGF-β1), a multifunctional cytokine, is a member of the transforming growth factor beta superfamily[1]. Mainly, TGF-β1 protein has been associated with cell growth, cell proliferation, cell differentiation and apoptosis[2-5] leading to regulation of tissue repair and extracellular matrix production. TGF-β1 can be found throughout the body and plays a role in almost every biological process, including the development before birth, the formation of blood vessels, the regulation of muscle tissue, the development of body fat and wound healing[4]. In immunity, TGF-β1 has an important role in modulating immunity[6].

From the earlier evidence, TGF-β1 was associated with fibrosis development[7,8], stimulating of collagen-producing cardiomyofibroblast in heart fibrosis[9] and inducing or facilitating vascular stenosis and thrombogenesis[10]. Moreover, TGF-β1 was involved in pathologic states such as inflammation processes, atherosclerosis, and restenotic lesions[11]. TGF-β1 was also found to possibly be a critical risk factor of genetic susceptibility to cerebral infarction[12] and one of the most promising candidates for chronic obstructive pulmonary disease (COPD)[13]. However, increase in the levels of TGF-β1 was seen with reduced risk of Graft-versus-host-disease (GVHD)[14].

The TGF-β1 gene is located on chromosome 19 (q13.1-13.3). Six commonly known polymorphisms were found within this gene, including C-988A (rs1800820), G-800A (rs1800468), C-509T (rs1800469), T-869C (rs1982073), G-915C (rs1800471), and C-11929T (rs1800472)[15-17]. A number of studies have been attempted to determine whether naturally occurring single-nucleotide polymorphisms (SNPs) in the TGF-β1 gene affect TGF-β1 expression and TGF-β1 production[16]. A C-to-T SNP at position -509 relative to the first major transcription starting site (rs1800469) was found to be differentially related with transcription factor binding to the TGF-β1 C-509T promoter, transcriptional activity of TGF-β1, and TGF-β1 plasma concentration[16,18].

Recently, TGF-β1 C-509T polymorphism has been implicated in several diseases in different population, for example: myocardial infarction (MI) in German white population[3] and young Italian population[19], asthma occurrence in American, Indian and Chinese adults[18,20,21], modulation of asthma severity in Brazilian[22], as well as rheumatic heart disease (RHD) in Taiwanese[23]. The exploration of the distribution of TGF-β1 C-509T polymorphism in Thai population is limited. So far only one study has been reported that the genotype frequency of CC was revealed separately, while those of CT and TT were reported in combination (CT+TT)[24]. Therefore, to get more information about genotype frequency of TGF-β1 C-509T polymorphisms in Thai population, the present study was undertaken to identify the distribution of CC, CT and TT genotype frequencies separately in healthy unrelated Thai individuals. Additionally, the TGF-β1 C-509T genotype and allele frequencies

in Thai population were compared to those of previously reported groups.

Objectives

1. To identify the TGF-β1 C-509T polymorphism in healthy unrelated Thai individuals.

2. To compare TGF-β1 C-509T genotype and allele frequencies of Thai population with data from other published population.

Materials and Methods

Subjects

Gene polymorphisms were analyzed in 56 healthy unrelated people, including staffs and students of Faculty of Medicine, Srinakarinwirot University. These volunteers had no history or sign of immunodeficiency diseases. This study was approved by the Faculty of Medicine Srinakharinwirot University Ethical Review Committee and all subjects gave written informed consent (SWUEC/E-026/2558).

DNA Extraction

Five microliters of saliva were collected in tubes. Genomic DNA was extracted from saliva using a commercial extraction kit (Roche, Germany) according to the manufacturer's instructions. After extraction, DNA was stored at -20°C until analysis.

Genotyping

Genotyping was carried out by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique as previous report[25]. Briefly, to determine the genotype of the TGF-β1 C-509T polymorphism, PCR was done using the forward primer (5′-GTC CCT CTG GGC CCA GTT TC-3′) and the reverse primer (5′-GAG GGG GCA ACA GGA CAC CTT A-3′). Amplification was performed in a 8 μL reaction mix containing 0.1-1 μg of genomic DNA, 10 μM of each oligonucleotide primer, 2.5 mM each deoxyribonucleotide triphosphates (dNTPs), and 2.5 units of *i-Taq* DNA polymerase (iNtRON Master Mix, Korea). Amplification conditions were as follows: an initial activation step of 94°C for 2 min followed by 34 cycles of denaturation at 94°C for 20 s, annealing at 62°C for 10 s, extension at 72°C for 30 s, and a final extension step at 72°C for 5 min. After amplification, the PCR products were digested at 37 °C with five units of *Afl* II restriction enzyme (New England Biolabs, USA) for 1 hour. DNA fragments were analyzed by 3% agarose gel containing ethidium bromide.

The interpretations were as follows: the presence of TGF-β1 C-509T allele was represented by 178 bp product, while the presence of TGF-β1 C-509T allele was detected as 159 and 19 bp fragments. However, the 19 bp fragment could not be seen in the agarose gel because of its small size (Figure 1) The samples were tested in duplicate, and the results were 100 percent concordant.

Statistical analysis

Allele and genotype frequencies were calculated. The statistical significance of the difference was tested by chi-square test. The $p<0.05$ were considered statistically significant.

Statistical analysis was performed using the Vassarstats[26].

Results

The genotype distribution of TGF-β1 C-509T (rs1800469) was consistent with the assumption of Hardy-Weinberg equilibrium (data not shown). From this study, the genotype frequencies of 3 genotypes: CC, CT and TT of TGF-β1 C-509T polymorphisms were 12.5%, 39.3% and 48.2%, respectively. The frequency of CC genotype was found to be lower than those of CT and TT in this population, which differed from other published data where the TT genotype frequencies were lower than CT and CC genotype frequencies. The allele frequency of C and T were 32.1% and 67.9%, respectively.

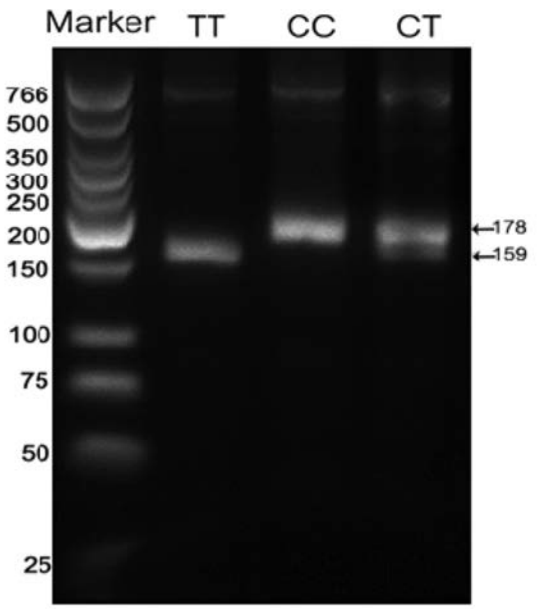

Figure 1 3% agarose gel with EtBr electrophoresis of TGF-β1 C-509T polymorphism

When genotype frequencies from this study were compared with those of other populations, no statistically significant differences with data from Chinese (Han) (p=0.390), Italian (p=0.057), Serbian (p=0.554), and Brazilian (p=0.442) were seen, whereas statistically significant differences were seen in data from Indian (p=0.005), Russian (p=0.048), English (p=0.006), German (p=0.014), Egyptians (p<0.001). In contrast, when compared allele frequencies from this study with other published data, statistically significant differences were found with all previously reported groups (p<0.05). (Table 1. and 2.)

Discussion

This study aimed to explore the SNPs of TGF-β1 C-509T in Thai population. The major findings of our study were as follows: (1), All types of genotypes (CC, CT and TT) were seen

Table 1 Distribution of TGF-β1 C-509T gene polymorphism:The genotype and allele frequencies in 56 unrelated healthy Thai individuals.

Genotype	n (%)
CC	7 (12.5%)
CT	22 (39.3%)
TT	27 (48.2%)
Allele	**n (%)**
C	36 (32.1%)
T	76 (67.9%)

in Thai population, which were similar to previous reports. (2), The genotype frequencies between Thais, and Indian, Russian, English, German, Egyptian population revealed statistically significant differences. (3), The genotype frequencies between Thai, and Chinese (Han), Italian, Serbian, Brazilian population revealed no statistically significant difference.

TGF-β1 C-509T, promoter, located on 19th chromosome plays a role in transcription starting site of TGF-β1 gene. The polymorphism of this region can cause 3 types of genotypes as follows: CC, CT, and TT. This polymorphism has been found to influence the TGF-β1 production levels, affecting the outcome of immune balance. In addition, this polymorphism has been implicated in many inflammatory and autoimmune diseases, including thrombogenesis[14], atherosclerosis[15], rheumatic heart disease[23].

Statistically significant difference in allele frequencies in Thai population in this study compared with previous reports in several populations[22,27-31] was noted, which revealed that the C allele frequency in Thai population in this study was lower than T allele frequency. While in other population the C allele frequencies were higher than T allele frequencies. For genotype frequency, the CC genotype found at 12.5% in Thai population of this study was the lowest and is consistent with the earlier study (4.2%)[24]. However, the genotype frequencies of CT and TT could not be compared with data from this earlier study since the frequencies of CT and TT were reported in combination (CT+TT), instead of separately as in this study. The TT genotype frequency of this study was the highest (48.2%), followed by the CT genotype frequency (39.3%). In addition, we found that the TGF-β1 C-509T genotype frequencies in Thai population were similar to

Table 2 Genotype and allele frequencies of the TGF-β1 C-509T gene polymorphism in healthy Thai individuals compared with data from other groups

Genotype/ Allele	Thai n=56 n(%)	Chinese n=1015 n(%)	Indian n=100 n(%)	Russian n=212 n(%)	English n=2143 n(%)	Italian n=201 n(%)	German n=1211 n(%)	Serbian n=49 n(%)	Egyptian n=40 n(%)	Brazilian n=202 n(%)
References	This study	[27]	[28]	[29]	[29]	[29]	[29]	[30]	[31]	[22]
Genotype										
CC	7(12.5)	284(27.9)	52(52)	90(42.4)	1090(50.9)	80(39.8)	564(46.6)	13(26.5)	33(82.5)	58(28.7)
CT	22(39.3)	508(50.1)	42(42)	103(48.6)	885(41.3)	92(45.8)	508(41.9)	27(55.1)	4(10)	112(55.4)
TT	27(48.2)	223(22.0)	6(6)	19(9)	168(7.8)	29(14.4)	139(11.5)	9(18.4)	3(7.5)	32(15.8)
p-value*		0.390	0.005	0.048	0.006	0.057	0.014	0.554	<0.001	0.442
Allele										
C	36(32.1)	1076(53.0)	146(73)	283(66.8)	3065(70.1)	272(67.7)	1636(67.6)	53(54.1)	70(87.5)	228(56.4)
T	76(67.9)	954(47.0)	54(27)	141(33.2)	1309(29.9)	130(32.3)	786(32.4)	45(45.9)	10(12.5)	176(43.6)
p-value*		0.004	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	0.001

*Chi-square test was used for the analysis (calculated from the percentage).

those of Chinese (Han), Italian, Serbian and Brazilian population, but were different from those of Indian, Russian, English, German and Egyptian population. Earlier studies reported the ethnic difference in distribution pattern of TGF-β1 C-509T gene polymorphism and the severity and susceptibility of many diseases. Therefore, the polymorphism of TGF-β1 C-509T gene observed in Thai population in this study may be used potentially as genetic prediction of disease susceptibility or clinical outcome of diseases in the future. However for more information, the study with larger sample size would be needed.

Conclusion

This study showed that 3 genotypes (CC, CT, and TT) of TGF-β1 C-509T polymorphisms were found in Thai population. The TT genotype showed the highest frequency (48.2%), followed by CT (39.3%) and CC genotype (12.5%). The C allele frequency of Thai population was lower than T allele frequency. These polymorphisms may affect the level of TGF-β1 production in different individuals. Further investigation is underway to elucidate the role of TGF-β1-C-509T promoter polymorphism in susceptibility to various diseases.

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