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The Association of HLA and Systemic Sclerosis (SSc) in the Northeastern Thais.

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Abstract

Aim: The majority of Thai SSc patients represent a unique ethnic group found in Northeastern Thailand. Genetic susceptibility may be found in the pathogenesis of Thai SSc patients. This study aims to identify HLA genes which correlate with clinical profiles in these SSc patients.

Methods: Peripheral blood samples were taken from 60 SSc patients diagnosed using the ACR classification 1980 criteria. HLA-A, -B, and -C were analyzed using the PCR-amplification refractory mutation system, and the PCR-sequence specific primer was used for the HLA-DR and- DQ analysis. The distribution of the alleles between SSc patients and the control was found to be significant when it was tested with the corrected P value (<0.05).

Results: Of the 60 SSc patients examined; 66.7% displayed symptoms of diffuse cutaneous SSc (dcSSc), and 33.3% showed limited cutaneous SSc (lcSSc). In the Thai SSc group, the prevalence of HLA DRB1*1502 (allele frequency: AF = 32.5%) and DQB1*0501 (AF = 30.8%) which was significantly higher when compared to the control group (AF = 18.5% and 16.3%) with corrected P value (Pc) = 0.0007 and 0.0001, respectively.

Conclusions: The majority of Thai SSc patients that actually displayed dcSSc, had a high prevalence of internal organ involvement. The results suggest a genetic susceptibility for SSc among the population of Northeastern Thailand, especially for individuals with HLA DRB1*1502. In the West, there is less HLA DRB1*1502, and this factor could explain why dcSSc is more prevalent among the Thai population.

Introduction

In 1836 scleroderma was first given its name by Giovambattista Fantonetti, a prominent physician in Milan, Italy. It is an ancient disease which can first be traced back to the writings of Hippocrates circa 400 BC. In the annals of modern medicine, the first case was fully described by Carlo Curzio, an Italian physician and dermatologist in 1753. The term systemic sclerosis (SSc) was coined by Robert Goetz in 1945 to reflect the systemic involvement of this disease. At present, it is considered to be a rare auto-immune disease which is characterized by an excessive production of connective tissue in the skin and internal organs. The etiology of the disease is not clearly understood. Many hypotheses have been made regarding the etiopathology of this disease, including its hereditary nature. Genetics are believed to play a significant part in the development of all auto-immune rheumatic diseases. Their roles are engaged in almost every step of immune response and consequently cause immune-deregulations. They can have an influence on the initiation and maintenance of the auto-reactivity to its own tissue, and as a result, causing auto-immune disease. The genetic effects of auto-immune rheumatic disease are generally described as polymorphic. Therefore, we do not know exactly to what degree they affect each disease. Various genetic associations which cause systemic sclerosis have been published in the literature. However, this disease and its association with the HLA class 2 genes has most often been described.

Although systemic sclerosis is generally considered as a rare auto-immune disease, it is fairly common in Northeastern Thai among a unique ethnic group. This may suggests that there exists a genetic susceptibility in the pathogenesis of SSc among Thai citizens. We performed the genetic study of SSc on a population of Northeastern Thai patients and compared the results to a normal healthy control group in order to identify which MHC genes are most likely to cause SSc.

Materials and methods

From each of the volunteers, twenty milliliters of peripheral blood was collected in a tube with acid citrate dextrose (ACD) before the samples were centrifuged to separate the buffy coat. The buffy coat samples from the 60 SSc patients, who had been diagnosed using the1980 ACR classification criteria, were eligible for major histocompatibility complex (MHC) analysis and then were able to be compared to the samples from the 400 healthy Northeastern Thais. Both the SSc patients and the controls had to meet the same criteria: they had to be local residents whose ancestors had lived exclusively in the Northeastern region of Thailand for more than 2 generations. HLA-A, -B, and -C were studied by PCR-amplification refractory mutation system[1] and HLA-DR and- DQ were studied by PCR-sequence specific primer.[2] The clinical data from the patients in the study were reviewed including demographic data, occupations, types of skin involvement, first clinical presentations, the duration of disease, serological testing, and the involvement of the organs.

Statistical analysis

Demographic data and clinical manifestations were done using descriptive statistics. Allele frequency calculations were performed for the SSc group and were

compared to the frequency of the control group. After performing the Chi-square methods, the P value was determined and was then corrected in order to determine the significance of the distribution of the allele's frequency. The corrected P value (Pc) for both groups was corrected for each gene. A Pc value of less than 0.05 was considered statistically significant.

Results

Of the 60 SSc patients, 66.7% showed diffuse cutaneous SSc (dcSSc), and 33.3% showed limited cutaneous SSc (lcSSc). The Male: Female ratio was 1:1.2. The mean age of onset of disease was 45.2 $\pm$10.9 years (20 - 70) and mean duration of disease was 47.8 $\pm$ 41.0 months (4 -181). The presenting symptoms were tightening of skin (49.3%), Raynaud's phenomenon (17.4%), joint pain (14.5%), and swelling of skin (10.1%). The most common internal organ involvements were the organs of the GI tract (78.3%), followed by the lungs (66.7%), the heart (25.0%), and the muscles (13.3%). In our series, renal involvement was found in only 3.3% of the patients. Involvements of the organs are shown in Table1.

Table 1. Prevalence of Organ involvements in SSc subsets

Organ involvement	DcSSc (n=40) (%)	LcSSc (n=20) (%)	Overall (%)
Raynaud's phenomenon	38 (95.0)	18(90.0)	56(93.3)
Digital pitting scar	35(87.5)	16(80.0)	51(85.0)
GI tract involvement	34(85.5)	13(65.0)	47(78.3)
Lung ifbrosis	26(65.5)	14(70.0)	40(66.7)
Arthralgia/arthritis	25(62.5)	2(10.0)	27(45.0)
Hand contracture/deformity	15(37.5)	2(10.0)	17(28.3)
Telangiectasia	6(15.0)	3(15.0)	9(15.0)
Myositis	3(7.5)	5(25.0)	8(13.3)
Tendon friction rub	4(10.0)	0	4(6.7)
Renal involvement	2(5.0)	0	2(3.3)
Calcinosis cutis	1(2.5)	0	1(1.7)
Neurological involvement	0	1(5.0)	1(1.7)

The detection of alleles in SSc patients as compared to healthy controls is shown in Table2. The corrected P value (Pc) was calculated by the following formula; Pc = P-value x number of specifically examined alleles. (For HLA-DR = 19 alleles, HLA-DQ = 12 alleles, and HLA-B = 19 alleles).

Table 2. Alleles detection in SSc patients and control comparison

Allele	Number in SSc (total 60 persons)	Number in HC (total 400 persons)	P-value	Odd ratio	Pc
DRB1*1106	9	21	0.00930	3.1849	0.176700
DRB1*1502	39	148	0.00004	3.1622	0.000728*
DRB1*0301	1	49	0.01400	0.1214	0.266930
DQB1*0201	6	87	0.03458	0.3997	0.414970
DQB1*0501	37	130	0.00001	3.3411	0.000142*
B*5801	3	63	0.02676	0.2815	0.050840
B*40(02,04,06)	7	19	0.03051	2.6485	0.579603

The prevalence of HLA DRB1*1502 (allele frequency: AF = 32.5%) and DQB1*0501 (AF = 30.8%) was significantly higher in SSc patients as compared to the control group (AF = 18.5% and 16.3%) with Pc = 0.0007 and 0.0001 respectively.

Discussion

Although SSc is not an uncommon disease among Northeastern Thais, the exact prevalence of SSc has not been known. In the majority of cases of SSc among Thai patients consisted of a dcSSc phenotype and most of them presented with anti-topoisomerase I antibody. When compared to studies done on Caucasians, dcSSc subset was found only in a minority of the population.[3] Genetic differences were hypothesized, including HLA and non-HLA genes. Among HLA gene studies, the allele frequency of HLA DRB1*1502 was significantly higher in Koreans,[4] in the Japanese[5] and in ThaiSSc[6] patients when these three groups were compared to the controls.[4,5,6] However, the allele frequency of HLA DRB1*1502 was not significantly higher in western countries.[1,2,8] In the Korean, Japanese, and Thai studies, one distinct feature was found: patients showed a greater prevalence of anti-topoisomerase I and expressed the clinical features of dcSSc. In a single report from a center of systemic sclerosis in Northern Thailand, they have been able to establish a greater prevalence of both HLA DRB1*1502 and HLA DRB5*0102 in their population of SSc patients.[6] Similar to the characters of patients in our study, they reported that most patients exhibited a dcSSC subset and had the anti-topoisomerase I antibody. The fact that their research from another part of Thailand shows the discovery of a greater prevalence of HLA DRB1*1502 as compared to the control is a factor that has been supported by our study. These findings were confirmed by the high prevalence of SSc in these two regions when compared to other regions in Thailand. This may explain why Thai SSc patients show a greater prevalence of the dcSSc phenotype as compared to Caucasian populations. In our study, SSc patients also have a greater prevalence of allele frequency HLA DQB1*0501, but its clinical significance is not yet known. In the other series, the findings showed that HLA DQB1*0501 is

a protective allele in anti-topoisomerase I antibody (ATA) patients[9]and it is associated with anti-centromere antibody production.[10] Another HLA gene that has been shown to be more prevalent in ATA positive is the HLA DPB1.[11,12] The non-HLA genes that were found to be more prevalent in the dcSSc phenotype included the macrophage migration inhibitory factor gene (MIF-173)[13] and the Toll-like receptor 2 (TLR2) gene. [14] However, these two genes have not been included in our report.

Conclusions

Results from our HLA study suggest that HLA DRB1*1502 and DQB1*0501 are associated with Northeastern Thai patients exhibiting symptoms of SSc. From another study conducted at Chiang Mai University, it was found that among the patients examined that the presence of HLA DRB1*1502 makes patients genetically susceptible to SSc. In addition, it has been discovered that the presence of HLA DRB1*1502 can be correlated with the production of ATA and the clinical manifestation of dcSSc. Among Asians populations, the dcSSc subtype is more common. However, a higher prevalence of HLA DQB1*0501 was also determined in Northeastern Thai patients exhibiting SSc. Even though this allele has been found to be correlated with ACA production in Chinese populations, our SSc database shows a low prevalence of ACA. For SSc patients in Northeastern Thailand, a further exploration needs to be completed which can correlate the dimension of genetic susceptibility and clinical manifestations of the disease.

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Potential conflicts of interest

None.

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