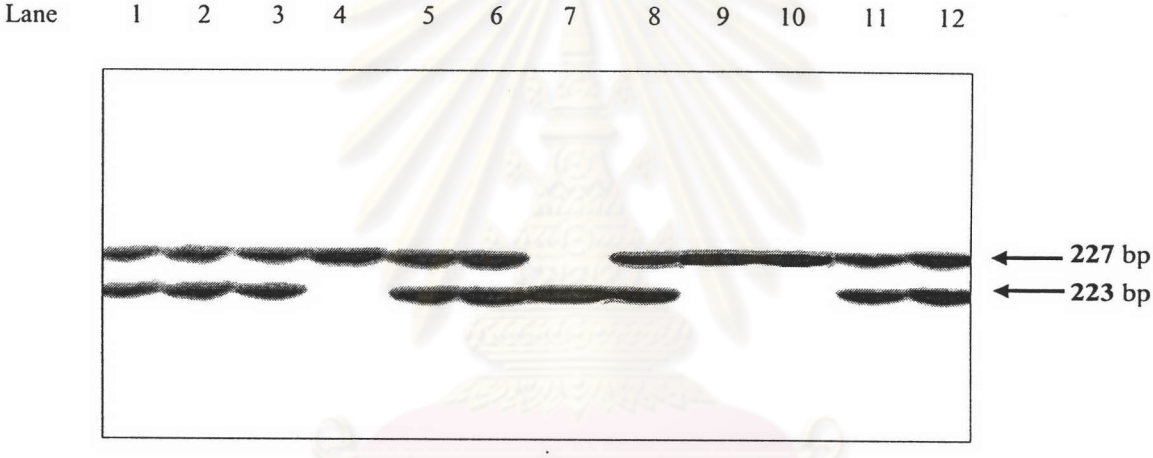
CHAPTER V

RESULTS

1. 5' – end labeling primer (kinase reaction) of TGF-β2 gene

1.1 5' – end labeling primer analysis of TGF-β2; an insertion ACAA in the 5' - untranslated region (5' UTR) at position + 71_72

Polymorphism of a 4-bp insertion +71_72 ACAA in the 5' – untranslated region (5' UTR) of the transforming growth factor beta2 (TGF-β2) gene were identified by the 5' – end labeling primer method. The patterns are indicated by arrows (FigureA).



FigureA. The representative of 5' – end labeling primer from samples with homozygous 4-bp insertion (ACAA), heterozygous of 4-bp insertion (ACAA) and homozygous for the common allele

Lane 1 is heterozygous positive control for the +71_72 insACAA

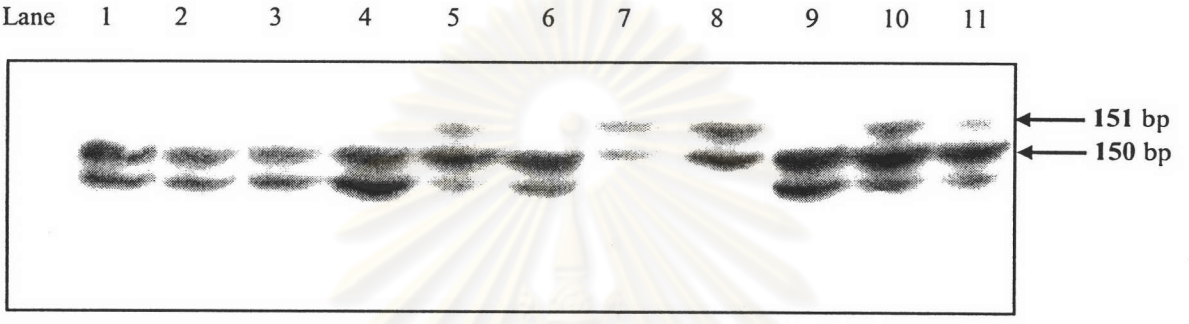
Lenes 2, 3, 5, 6, 8, 11, and 12 are heterozygous for the +71_72 insACAA

Lanes 7 is homozygous for the common allele

Lanes 4, 9 and 10 are homozygous for the +71_72insACAA

1.12 5' – end labeling primer analysis of TGF-β2; an insertion A in the intron6 region at position +94400_94401

Polymorphism of a 1-bp insertion+ 94400_94401insA in the intron6 region of the transforming growth factor beta2 (TGF-β2) gene were identified by the 5' – end labeling primer method. The patterns are indicated by arrows (FigureB).



FigureB. The representative of 5' – end labeling primer from samples with homozygous 1-bp insertion (A), heterozygous of 1-bp insertion (A) and homozygous for the common allele.

Lanes 5, 10 and 11 are heterozygous for the insertion A.

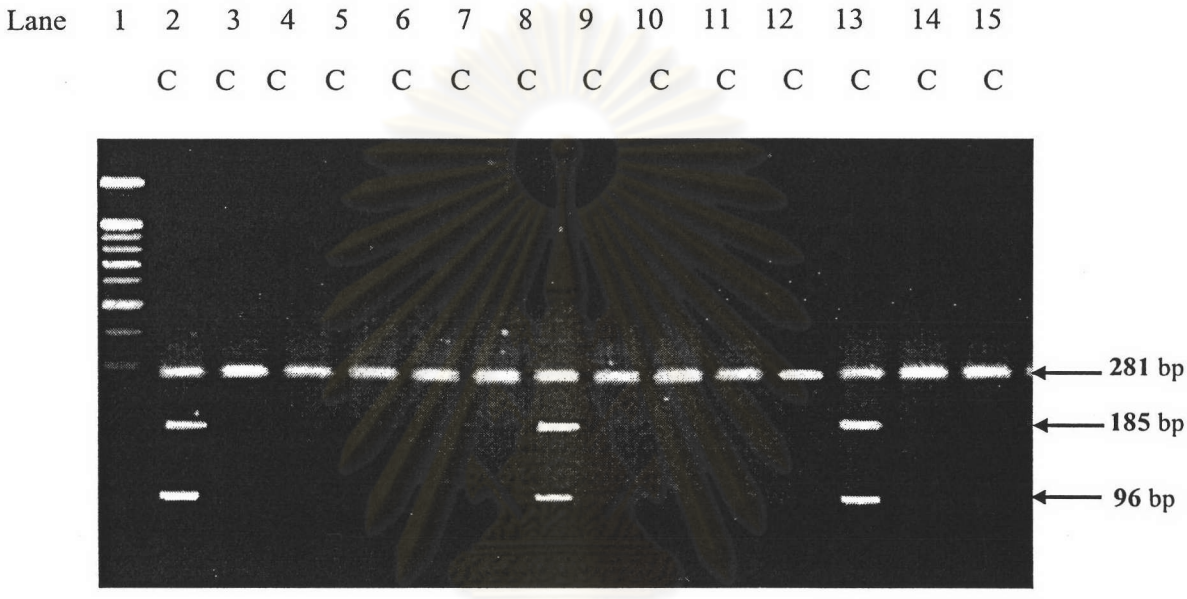
Lanes 1, 2, 3, 4, 6 and 9 are homozygous for the common allele.

Lanes 7 and 8 are homozygous for the insertion A.

2. Polymerase Chain Reaction - Restriction Fragment Length Polymorphism analysis of TGF-β2 gene

2.1 PCR-RFLP analysis of TGF-β2 at position + 720

Polymorphism at +720T/G in the intron1 region of the TGF-β2 were identified by the PCR-RFLP method. If a G was present at this position, the TaaI (Tsp4CI) restriction enzyme would cut the 281 bp PCR product into two fragments; 185 and 96 bp. No digestion would occur if a T was present. (FigureC).



FigureC. The representative of PCR-RFLP results from samples with homozygous of + 720T and heterozygous + 720T/G.

Lane 1 is 100 bp molecular marker.

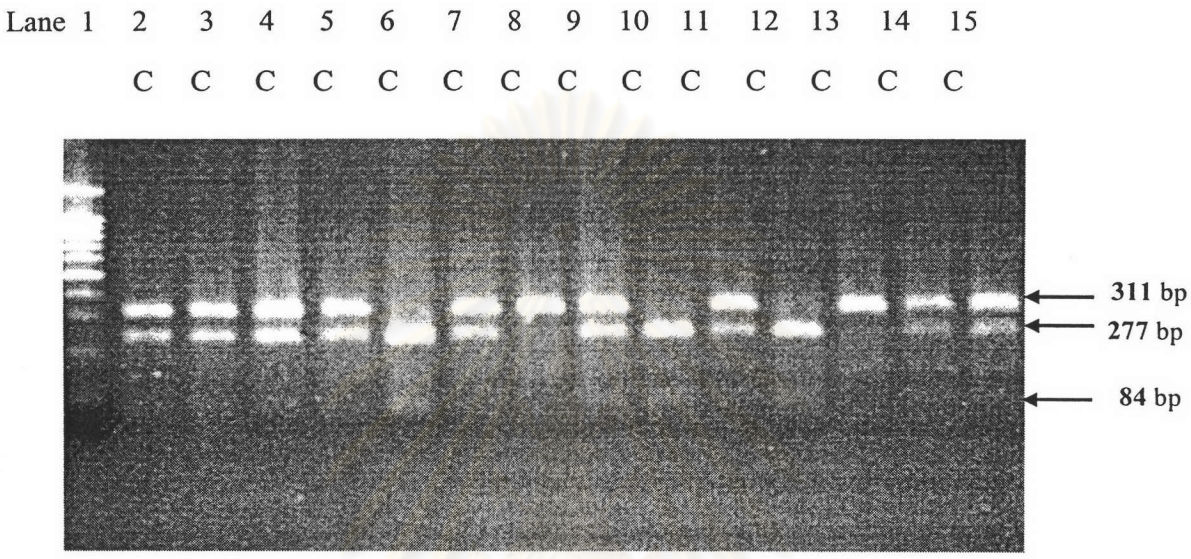
Lane 2, 8 and 13 are heterozygous of + 720T/G.

Lane 3, 4, 5, 6, 7, 9, 10, 11, 12, 14 and15 are homozygous of + 720T.

No homozygous of G in this position.

2.2 PCR-RFLP analysis of TGF-β2 at position + 89835

Polymorphism at + 89835A/G in the intron5 region of the TGF-β2 were identified by the PCR-RFLP method. If a G was present at this position, the FspBI (MaeI) restriction enzyme would cut the 311 bp PCR product into two fragments; 227 and 84 bp. No digestion would occur if an A was present. (FigureD).



FigureD. The representative of PCR-RFLP results from samples with homozygous + 89835A, heterozygous+ 89835 A/G and homozygous + 89835G

Lane 1 is 100 bp molecular marker.

Lane 5 and 13 are homozygous of + 89835 A.

Lane 2, 3, 4, 5, 7, 9, 11, 14 and15 are heterozygous of + 89835 A/G.

Lane 6, 10 and12 are homozygous of + 89835 G.

3. The association results of TGF-β2 gene polymorphisms with SLE

We assessed the quality of the genotype data by testing for Hardy-Weinberg equilibrium in the sample, using Fisher's exact test ($p < 0.05$). There are three significant deviations from Hardy-Weinberg equilibrium in all SNPs in this study.

3.1 TGF-β2 gene polymorphism at position +71_72insACAA

Genotype and allele frequencies for +71_72insACAA at the 5' UTR of TGF-β2 gene in healthy controls and SLE patients were shown in table 9 and 10. Thirty-nine of 133 healthy controls (29.3%) were homozygous for the common _/_ genotype, sixty-nine (51.9%) were heterozygous for the _/insACAA and twenty-five (18.8%) were homozygous for the insACAA/insACAA genotype. The allele frequencies were 55.3% for common _ allele and 44.7% for ACAA allele. In comparison, sixty-six of 155 SLE patients (43.1%) were homozygous for the common _/_ genotype, sixty-five (42.5%) were heterozygous for the _/insACAA and twenty-two (14.4%) were homozygous for the insACAA/insACAA genotype. The allele frequencies were 64.4% for common _ allele and 35.6% for ACAA allele. The +71_72 allele at the 5' UTR of TGF-β2 gene was found to be significantly increased in SLE patients compared to healthy controls ($p = 0.048$, OR = 1.43, 95%CI = 1.00-2.03). The effect of +71_72 allele at the 5' UTR of TGF-β2 gene was similar to autosomal recessive mode of inheritance. The presence of one common _/_ genotype conferred with the significant OR of 1.83 ($p = 0.02$, OR = 1.83, 95%CI = 1.09-3.08).

3.2 TGF-β2 gene polymorphism at position +720 (T/G)

Genotype and allele frequencies for +720 (T/G) at the intron1 of TGF-β2 gene in healthy controls and SLE patients were shown in table 11 and 12. Xero of 133 healthy controls (0.0%) were homologous for the G/G genotype, twenty-nine (21.8%) were heterozygous for the G/T and one-hundred and four (78.2%) were homozygous for the T/T genotype. The allele frequencies were 10.9% for G allele and 89.1% for T allele. In comparison, xero of 155 SLE patients (0.0%) were homozygous for the G/G genotype, fifteen (9.8%) were heterozygous for the G/T and one-hundred and thirty- eight (90.2%) were homozygous for the T/T genotype. The allele frequencies were 4.9% for G allele and 95.1% for T allele. The +720 T allele at the intron1 of TGF-β2 gene was found to be significantly increased in SLE patients compared to healthy controls ($p = 0.01$, OR = 2.37,

95%CI = 1.19-4.77). The effect of +720 T allele at the intron1 of TGF-β2 gene was similar to autosomal recessive mode of inheritance. The presence of one genotype (T/T) conferred with the significant OR of 2.57 (p =0.008, OR = 2.57, 95%CI = 1.25-5.32).

3.3 TGF-β2 gene polymorphism at position +89835 A/G

Genotype and allele frequencies for +89835 A/G at the intron5 of TGF-β2 gene in healthy controls and SLE patients were shown in table 13 and 14. Thirteen of 133 healthy controls (9.8%) were homozygous for the A/A genotype, sixty-nine (51.9%) were heterozygous for the A/G and fifty-one (38.3%) were homozygous for the G/G genotype. The allele frequencies were 35.7% for A allele and 64.3% for G allele. In comparison, twenty-eight of 155 SLE patients (18.3%) were homozygous for the A/A genotype, sixty-nine (45.1%) were heterozygous for the A/G and fifty-six (36.6%) were homozygous for the G/G genotype. The allele frequencies were 40.9% for A allele and 59.1% for G allele. There were no significant differences in allele and genotype frequency of the +89835 (A/G) polymorphism at the intron5 of TGF-β2 gene between patients with SLE and healthy controls.

3.4 TGF-β2 gene polymorphism at position +94400_944001insA

Genotype and allele frequencies for +94400_944001insA at the intron6 of TGF-β2 gene in healthy controls and SLE patients were shown in table 15 and 16. Eighty-eight of 133 healthy controls (66.2%) were homologous for the common _/_ genotype, fourty-one (30.8%) were heterozygous for the _/insA and four (3.0%) were homozygous for the insA/insA genotype. The allele frequencies were 81.6% for common _ allele and 18.4% for A allele. In comparison, one-hundred and thirty-two of 155 SLE patients (86.2%) were homozygous for the common _/_ genotype, twenty (13.1%) were heterozygous for the _/insA and one (0.7%) were homozygous for the insA/insA genotype. The allele frequencies were 92.8% for wild type _ allele and 7.2% for A allele. The +94400_944001 _(common) allele at the intron6 of TGF-β2 gene was found to be significantly increased in SLE patients compared to healthy controls (p = 0.00008, OR = 2.91, 95%CI = 1.66-5.15). The effect +94400_944001 _ allele at the intron6 of TGF-β2 gene was similar to autosomal recessive mode of inheritance. The presence of one common (_/_) genotype conferred with the significant OR of 1.83 (p = 0.0001, OR = 3.21, 95%CI = 1.73-6.02).

4. Haplotype analysis of TGF-β2 gene at position (+71_72insACAA, +720T/G, +89835A/G, +94400_94401insA), respectively

The haplotype frequencies of the TGF-β2 gene polymorphism were also calculated by PHASE program. The haplotype frequencies in patients with SLE and normal controls were shown in table 17. In haplotype analysis of 4 positions (+71_72insACAA, +720T/G, +89835A/G , +94400_94401insA) of TGF-β2 gene, we found 11 haplotypes; _/T/G/_, insACAA/T/A/_, insACAA/T/G/_, _/T/G/insA, _/T/A/_, insACAA/G/A/_, insACAA/G/G/_, insACAA/T/G/insA, insACAA/T/A/insA, _/G/G/_ and _/T/A/insA in patients with SLE and normal concrols were shown in table 18. After comparing haplotype frequencies of the 4 positions of TGF-β2 gene polymorphism between patients with SLE and normal controls, the _/T/G/insA, _/T/A/_ and insACAA/G/A/_ were found to be significantly increased in patient with SLE compared to normal controls (p = 0.01, OR = 0.49, 95%CI = 0.27-0.89 , p = 0.001, OR = 2.64, 95%CI = 1.58-4.42 and p = 0.00005, OR = 0.05, 95%CI = 0.02-0.32) , respectively.

Table9. Genotype and allele frequencies for TGF-β2 polymorphism at position +71_72insACAA in healthy controls and SLE patients.

	SLE patients n = 153	Healthy controls n =133
Genotype frequencies		
_/_	66(43.1%)	39(29.3%)
_/ACAA(ins)	65(42.5%)	69(51.9%)
ACAA/ACAA(ins)	22(14.4%)	25(18.8%)
Allele frequencies		
_	197(64.4%)[a]	147(55.3%)
ACAA(ins)	109(35.6%)	116(44.7%)

[a] p = 0.048, OR = 1.43, 95%CI = 1.00-2.03

Table10. Risk of SLE associated with TGF-β2 (insACAA) genotype according to different models of inheritance.

	SLE patients n = 153	Healthy controls n =133
_ dominance, A(ins) wild type		
_/_ or _/ACAA(ins)	131(85.6%)[a]	108(81.2%)
ACAA/ACAA(ins)	22(14.4%)	25(18.8%)
_ recessive, ACAA(ins) wild type		
_/_	66(43.1%)[b]	39(29.3%)
_/ACAA or ACAA/ACAA(ins)	87(56.9%)	94(70.7%)

[a] p =0.40

[b] p = 0.02, OR = 1.83, 95%CI = 1.09-3.08

Table11. Genotype and allele frequencies for TGF-β2 polymorphism at position +720 (T/G) in healthy controls and SLE patients.

	SLE patients n = 153	Healthy controls n =133
Genotype frequencies		
G/G	0(0.0%)	0(0.0%)
G/T	15(9.8%)	29(21.8%)
T/T	138(90.2%)	104(78.2%)
Allele frequencies		
T	291(95.1%)[a]	237(89.1%)
G	15(4.9%)	29(10.9%)

[a] $p = 0.01$, OR = 2.37, 95%CI = 1.19-4.77

Table12. Risk of SLE associated with TGF-β2 (T/G) genotype according to different models of inheritance.

	SLE patients n = 153	Healthy controls n =133
T dominance, G wild type		
T/T or G/T	153(100.0%)	133(100.0%)
G/G	0(0.0%)	0(0.0%)
T recessive, G wild type		
T/T	138(90.2%)[b]	104(78.2%)
G/G or G/T	15(9.8%)	29(21.8%)

[b] p =0.008, OR = 2.57, 95%CI = 1.25-5.32

Table13. Genotype and allele frequencies for TGF-β2 polymorphism at position +89835 (A/G) in healthy controls and SLE patients.

	SLE patients n = 153	Healthy controls n =133
Genotype frequencies		
A/A	28(18.3%)	13(9.8%)
A/G	69(45.1%)	69(51.9%)
G/G	56(36.6%)	51(38.3%)
Allele frequencies		
A	125(40.9%)[a]	95(35.7%)
G	181(59.1%)	171(64.3%)

[a] $p = 0.20$

Table14. Risk of SLE associated with TGF-β2 (A/G) genotype according to different models of inheritance.

	SLE patients n = 153	Healthy controls n =133
A dominance, G wild type		
AA or A/G	97(63.4%)[a]	82(61.7%)
G/G	56(36.6%)	51(38.3%)
A recessive, G wild type		
A/A	28(18.3%)[b]	13(9.8%)
A/G or G/G	125(81.7%)	120(90.2%)

[a] p =0.86

[b] p =0.06

Table15. Genotype and allele frequencies for TGF-β2 polymorphism at position +94400_94401insA in healthy controls and SLE patients.

	SLE patients n = 153	Healthy controls n =133
Genotype frequencies		
_/_	132(86.2%)	88(66.2%)
_/A(ins)	20(13.1%)	41(30.8%)
A/A(ins)	1(0.7%)	4(3.0%)
Allele frequencies		
_	284(92.8%)[a]	217(81.6%)
A(ins)	22(7.2%)	49(18.4%)

[a] p = 0.00008, OR = 2.91, 95%CI = 1.66-5.15

Table16. Risk of SLE associated with TGF-β2 (insA) genotype according to different models of inheritance.

	SLE patients n = 153	Healthy controls n =133
_ dominance, A(ins) wild type		
_/_ or _/A(ins)	152(99.3%)[a]	129(97.0%)
A/A(ins)	1(0.7%)	4(3.0%)
_ recessive, A(ins) wild type		
_/_	132(86.3%)[b]	88(66.2%)
_/A or A/A(ins)	21(13.7%)	45(33.8%)

[a] p =0.29

[b] p = 0.0001, OR = 3.21, 95%CI = 1.73-6.02

Table17. Haplotype frequencies of the TGF-β2 polymorphism (+71_72insACAA, +720T/G, +89835 A/G, +94400_94401insA respectively) between normal controls and SLE patients.

Haplotype frequencies	SLE patients (n=306)	Healthy controls (n=266)
_/T/G/_	107 (35.0%)	79 (29.7%)
insACAA/T/A/_	55 (18.0%)	44 (16.5%)
insACAA/T/G/_	36 (11.8%)	39 (14.7%)
_/T/G/insA	22 (7.2%)	36 (13.5%)
_/T/A/ _	68 (22.2%)	26 (9.8%)
insACAA/G/A/_	1 (0.3%)	18 (6.8%)
insACAA/G/G/_	13 (4.2%)	9 (3.4%)
insACAA/T/G/insA	2 (0.7%)	6 (2.3%)
insACAA/T/A/insA	1 (0.3%)	3 (1.1%)
_/G/G/_	1 (0.3%)	3 (1.1%)
_/T/A/insA	0 (0%)	3 (1.1%)

Table18. Association of the TGF-β2 polymorphism (+71_72insACAA, +720T/G, +89835 A/G, +94400_94401insA respectively) between normal controls and SLE patients.

Haplotype frequencies	SLE patients (n=306)	Healthy controls (n=266)	p-value
_/T/G/_ other haplotype	107 (35.0%) 199 (65.0(%)	79 (29.7%) 187 (70.3%)	0.21
insACAA/T/A/_ other haplotype	55 (18.0%) 251(82.0%)	44 (16.5%) 222(83.5%)	0.73
insACAA/T/G/_ other haplotype	36 (11.8%) 270(88.2%)	39 (14.7%) 227(85.3%)	0.37
_/T/G/insA other haplotype	22 (7.2%) [a] 284(92.8%)	36 (13.5%) 230(86.5%)	0.02
_/T/A/ _ other haplotype	68 (22.2%) [b] 238(77.8%)	26 (9.8%) 240(%)	0.0001
insACAA/G/A/_ other haplotype	1 (0.3%) [c] 305(99.7%)	18 (6.8%) 248(90.2%)	0.00005
insACAA/G/G/_ other haplotype	13 (4.2%) 293(95.8%)	9 (3.4%) 257(%)	0.75
insACAA/T/G/insA other haplotype	2 (0.7%) 304(99.3%)	6 (2.3%) 260(96.6%)	0.20
insACAA/T/A/insA other haplotype	1 (0.3%) 305(99.7%)	3 (1.1%) 263(%)	0.52
_/G/G/_ other haplotype	1 (0.3%) 305(99.7%)	3 (1.1%) 263(98.9%)	0.52
_/T/A/insA other haplotype	0 (0%) 306(100.0%)	3 (1.1%) 263(98.9%)	0.20

[a] $p = 0.01$, OR = 0.49, 95%CI = 0.27-0.89

[b] $p = 0.0001$, OR = 2.64, 95%CI = 1.58-4.42

[c] $p = 0.00005$, OR = 0.05, 95%CI = 0.02-0.32

5. Linkage Disequilibrium (LD)

Linkage Disequilibrium coefficients ($|D'|$ and r^2) among TGFß2 SNP at position + 71_72 ins(ACAA), + 720 (T/G), + 89835 (A/G) and + 94400_94401ins(A). Data was shown in table 19 (see appendix F).

Table19. Linkage disequilibrium coefficients ($|D'|$ and r^2) among TGFß2 SNPs

$\|D'\|$ / r^2	+ 71_72 ins	+ 720	+ 89835	+ 94400_94401ins
+ 71_72 ins	-	0.8467	0.0788	0.4610
+ 720	0.0649	-	0.6597	1.0000
+ 89835	0.0051	0.0155	-	0.7360
+ 94400_94401ins	0.0094	0.0042	0.0290	-

6. The association results of TGF-β2 gene polymorphisms with clinical manifestation SLE

We analyze the association between clinical manifestation in patients with SLE and polymorphism of the position +71_72insACAA, +720T/G, +89835 A/G and +94400_94401insA of TGF-β2 gene by using chi-square test and odds ratio.

6.1 Clinical manifestation of SLE patients

The clinical expression of SLE is tremendously varied among individuals. In this study, we obtained clinical data of 127 patients, as shown in table20.

6.2 The 4 positions (+71_72insACAA, +720T/G, +89835 A/G and +94400_94401insA) of TGF-β2 gene polymorphisms and clinical presentation of SLE

There is one significant association between insA allele at position +94400_94401insA. The association is with cellular cast (p = 0.025, OR = 3.34, 95%CI = 1.05-10.46).

Table20. Clinical manifestation of patients with SLE in this study

Clinical manifestation	No. of patients with SLE (%)
1. Malar rash	72 (56.7%)
2. Discoid rash	36 (28.3%)
3. Photosensitivity	47 (37.0%)
4. Oral ulcers	51 (40.1%)
5. Arthritis	84 (66.1%)
6. Proteinuria	82 (64.6%)
7. Cellular cast	26 (20.5%)
8. Anemia	64 (50.4%)
9. Leukopenia	44 (34.6%)
10. Lymphopenia	47 (37.0%)
11. Thrombocytopenia	6 (4.7%)
12. Anti-DNA antibodies	29 (22.8%)

Table21. Genotype and allele frequencies for TGF-β2 polymorphism at position +94400_94401insA in SLE patients with malar rash.

	SLE patients with malar rash n = 72	Healthy controls without malar rash n =55
Genotype frequencies		
_/_	65(90.3%)	47(85.5%)
_/A(ins)	7(9.7%)	7(12.7%)
A/A(ins)	0(0.0%)	1(1.8%)
Allele frequencies		
_	137(95.1%)	101(91.8%)
A(ins)	7(4.9%)	9(8.2%)

Table22. Risk of malar rash associated with TGF-β2 (insA) genotype according to different models of inheritance.

	SLE patients with malar rash n = 72	Healthy controls without malar rash n =55
_ dominance, A(ins) wild type		
_/_ or _/A(ins)	72(100.0%)[a]	54(98.2%)
A/A(ins)	0(0.0%)	1(1.8%)
_ recessive, A(ins) wild type		
_/_	65(90.3%)[b]	47(85.5%)
_/A or A/A(ins)	7(9.7%)	8(14.5%)

[a] p =0.43

[b] p = 0.58

Table23. Genotype and allele frequencies for TGF-β2 polymorphism at position +94400_94401insA in SLE patients with discoid rash.

	SLE patients with malar rash n = 36	Healthy controls without malar rash n =91
Genotype frequencies		
_/_	32(88.9%)	80(87.9%)
_/A(ins)	4(11.1%)	10(11.0%)
A/A(ins)	0(0.0%)	1(1.1%)
Allele frequencies		
_	68(94.4%)	170(93.4%)
A(ins)	4(5.6%)	12(6.6%)

Table24. Risk of malar rash associated with TGF-β2 (insA) genotype according to different models of inheritance.

	SLE patients with malar rash n = 36	Healthy controls without malar rash n =91
_ dominance, A(ins) wild type		
_/_ or _/A(ins)	36(100.0%)[a]	90(98.9%)
A/A(ins)	0(0.0%)	1(1.1%)
_ recessive, A(ins) wild type		
_/_	32(88.9%)[b]	80(87.9%)
_/A or A/A(ins)	4(11.1%)	11(12.1%)

[a] p =0.72

[b] p = 0.57

Table25. Genotype and allele frequencies for TGF-β2 polymorphism at position +94400_94401insA in SLE patients with photosensitivity.

	SLE patients with photosensitivity n = 47	Healthy controls without photosensitivity n =80
Genotype frequencies		
_/_	41(87.2%)	71(88.8%)
_/A(ins)	6(12.8%)	8(10.0%)
A/A(ins)	0(0.0%)	1(1.2%)
Allele frequencies		
_	88(93.6%)	150(93.8%)
A(ins)	6(6.4%)	10(6.2%)

Table26. Risk of photosensitivity associated with TGF-β2 (insA) genotype according to different models of inheritance.

	SLE patients with photosensitivity n = 47	Healthy controls without photosensitivity n =80
_ dominance, A(ins) wild type		
_/_ or _/A(ins)	47(100.0%)[a]	79(98.8%)
A/A(ins)	0(0.0%)	1(1.2%)
_ recessive, A(ins) wild type		
_/_	41(87.2%)	71(88.8%)
_/A or A/A(ins)	6(12.8%)[b]	9(11.2%)

[a] p =0.63

[b] p = 0.98

Table27. Genotype and allele frequencies for TGF-β2 polymorphism at position +94400_94401insA in SLE patients with oral ulcers.

	SLE patients with oral ulcers n = 51	Healthy controls without oral ulcers n =76
Genotype frequencies		
_/_	45(88.2%)	67(88.2%)
_/A(ins)	6(11.8%)	8(10.5%)
A/A(ins)	0(0.0%)	1(1.3%)
Allele frequencies		
_	96(94.1%)	142(93.4%)
A(ins)	6(5.9%)	10(6.6%)

Table28. Risk of oral ulcers associated with TGF-β2 (insA) genotype according to different models of inheritance.

	SLE patients with oral ulcers n = 51	Healthy controls without oral ulcers n =76
_ dominance, A(ins) wild type		
_/_ or _/A(ins)	51(00.0%)[a]	75(98.7%)
A/A(ins)	0(0.0%)	1(1.3%)
_ recessive, A(ins) wild type		
_/_	45(86.3%)[b]	67 (88.2%)
_/A or A/A(ins)	6(13.7%)	9 (11.8%)

[a] p =0.60

[b] p = 0.79

Table29. Genotype and allele frequencies for TGF-β2 polymorphism at position +94400_94401insA in SLE patients with persistent proteinurie.

	SLE patients with persistent proteinurie n = 82	Healthy controls without persistent proteinurie n =45
Genotype frequencies		
_/_	73(89.0%)	39(86.7%)
_/A(ins)	8(9.8%)	6(13.3%)
A/A(ins)	1(1.2%)	0(0.0%)
Allele frequencies		
_	154(93.9%)	84(93.3%)
A(ins)	10(6.1%)	6(6.7%)

Table230. Risk of persistent proteinurie associated with TGF-β2 (insA) genotype according to different models of inheritance.

	SLE patients with persistent proteinurie n = 82	Healthy controls without persistent proteinurie n =45
_ dominance, A(ins) wild type		
_/_ or _/A(ins)	81(98.8%)[a]	45(100.0%)
A/A(ins)	1(1.2%)	0(0.0%)
_ recessive, A(ins) wild type		
_/_	73(89.0%)[b]	39(86.7%)
_/A or A/A(ins)	9(11.0%)	6(13.3%)

[a] p =0.65

[b] p = 0.92

Table31. Genotype and allele frequencies for TGF-β2 polymorphism at position +94400_94401insA in SLE patients with cellular cast.

	SLE patients with cellular cast n = 26	Healthy controls without cellular cast n =101
Genotype frequencies		
_/_	20(76.9%)	92(91.1%)
_/A(ins)	5(19.2%)	9(8.9%)
A/A(ins)	1(3.8%)	0(0.0%)
Allele frequencies		
_	45(86.5%)	193(95.5%)
A(ins)	7(13.5%)	9(4.5%)

Table32. Risk of cellular cast associated with TGF-β2 (insA) genotype according to different models of inheritance.

	SLE patients with cellular cast n = 26	Healthy controls without cellular cast n =101
_ dominance, A(ins) wild type		
_/_ or _/A(ins)	25(96.2%)	101(97.0%)
A/A(ins)	1(3.8%)[a]	0(3.0%)
_ recessive, A(ins) wild type		
_/_	20(76.9%)	92(91.1%)
_/A or A/A(ins)	6(23.1%)[b]	9(8.9%)

[a] p =0.20

[b] p = 0.08, OR = 3.07, 95%CI = 0.85-10.92

Table33. Genotype and allele frequencies for TGF-β2 polymorphism at position +94400_94401insA in SLE patients with arthritis.

	SLE patients with arthritis n = 84	Healthy controls without arthritis n =43
Genotype frequencies		
_/_	76(90.5%)	36(83.7%)
_/A(ins)	8(9.5%)	6(14.0%)
A/A(ins)	0(0.0%)	1(2.3%)
Allele frequencies		
_	160(95.2%)	78(90.7%)
A(ins)	8(4.8%)	8(9.3%)

Table34. Risk of arthritis associated with TGF-β2 (insA) genotype according to different models of inheritance.

	SLE patients with arthritis n = 84	Healthy controls without arthritis n =43
_ dominance, A(ins) wild type		
_/_ or _/A(ins)	84(100.0%)[a]	42(97.0%)
A/A(ins)	0(0.0%)	1(3.0%)
_ recessive, A(ins) wild type		
_/_	76(90.5%)[b]	36(83.7%)
_/A or A/A(ins)	8(9.5%)	7(16.3%)

[a] p =0.34

[b] p = 0.41

7. The distribution of 5'UTR and Introns polymorphisms (+71_72insACAA, +720T/G, +89835 A/G and +94400_94401insA, respectively)

Besides association study of TGFß2 polymorphisms with SLE disease, this study also provides the basic knowledge of the frequencies of TGFß2 polymorphisms (position +71_72insACAA, +720T/G and +89835 A/G and, respectively) in Thai population. The distributions of the three positions polymorphisms between Thai population and Caucasian population previous reports were compared.

Pattern of TGFß2 gene polymorphisms (+71_72insACAA, +720T/G and +89835 A/G)

7.1 Pattern of TGFß2 at position +71_72insACAA

Allele frequencies for the polymorphism at +71_72insACAA in 5'UTR region of the TGFß2 gene were analyzed. The analyzed showed significant differences in allele frequencies between Thai and Caucasians population ($^{a}\chi^{2}$ = 19.35 , p<0.001).

7.2 Pattern of TGFß2 at position +720T/G

Allele frequencies for the polymorphism at +720T/G in intron1 region of the TGFß2 gene were analyzed. The analyzed showed no significant differences in allele frequencies between Thai and Caucasians population.

7.3 Pattern of TGFß2 at position 89835 A/G

Allele frequencies for the polymorphism at 89835 A/G in intron5 region of the TGFß2 gene were analyzed. The analyzed showed no significant differences in allele frequencies between Thai and Caucasians population.

Table35. Allele and genotype frequencies of the TGFß2 promoter polymorphisms in healthy Thais individuals compared Caucasian population.

Gene/Allele/Genotype	This study (Thai)	Caucasian
+71_72insACAA [a]	N=133	N=187
- _ allele	147 (55%)	273 (73%)
- ACAAins allele	116 (45%)	101 (27%)
- _/_ genotype	39 (29%)	102 (54%)
- _/ACAAins genotype	69 (52%)	16 (9%)
- ACAAins/ACAAins genotype	25 (19%)	69 (37%)
+720T/G [b]	N=133	N=12
- T allele	237 (89%)	11 (91%)
- G allele	29 (11%)	1 (9%)
- T/T genotype	104 (78%)	-
- T/G genotype	29 (22%)	-
- G/G genotype	0 (0%)	-
+89835 A/G [c]	N=133	N=60
- A allele	95 (36%)	97 (81%)
- G allele	171 (64%)	23 (19%)
- A/A genotype	13 (10%)	40 (67%)
- A/G genotype	69 (52%)	17 (28%)
- G/G genotype	51 (38%)	3 (5%)
+94400_94401insA	N=133	
- _ allele	217 (82%)	NON - REPORT
- Ains allele	48 (18%)	
- _/_ genotype	88 (66%)	
- _/Ains genotype	41 (31%)	
- Ains/Ains genotype	4 (3%)	

[a] χ^2 =19.35 , p<0.00001; compare between allele frequencies in Thai with Caucasian population (Alansari, Hajeer et al. 2001).

[b] Not significant ; compare between genotype and allele frequencies in Thai with Caucasian population.

[c] χ^2 =65.55 , p<0.00000; compare between allele frequencies in Thai with Caucasian population.