

CHAPTER V

RESULT

1. Polymerase Chain Reaction-Sequence Specific Primer (PCR-SSP)

Analysis of -1557 (C/A) VEGF

1.1 PCR-SSP analysis of -1557 (C/A) VEGF

-1557 (C/A) allele was identified using the PCR-SSP method. The positive result of -1557 C VEGF allele, -1557 A VEGF allele and Interferon-alpha (IFN- α) gene showed band of 77, 95 and 274 bp fragment, respectively (Papazoglou, Galazios et al. 2004) (Figure 7).

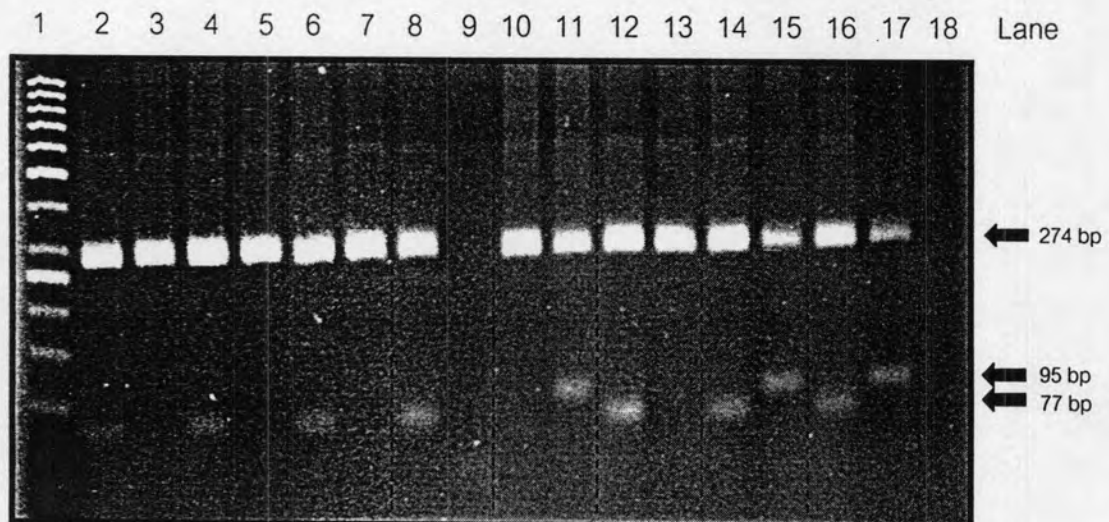


Figure 7. The representative of PCR-SSP results from samples with -1557 (C/A) VEGF specific primers amplification.

Lane 1 is 100 bp molecular markers.

Lane 2-8, 10-17 samples all show positive internal control band (274).

Lane 2, 4, 6, 8, 12, 14 and 16 are specific band that interpreted for -1557C allele band (77 bp).

Lane 11, 15 and 17 are specific band that interpreted for -1557A allele band (95 bp).

Lane 18 is negative control (no DNA sample).

2. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) Analysis of VEGF

2.1 PCR-RFLP analysis of VEGF promoter at position -460

Polymorphism at -460 C/T in the promoter region of the VEGF was identified by the PCR-RFLP method. If an C was present at this position, the *Bst*UI restriction enzyme would cut the 175 bp PCR product into two fragment; 155 and 20 bp. No digestion would occur if a T was present (Watson, Webb et al. 2000; Lin, Wu et al. 2003) (Figure 8).

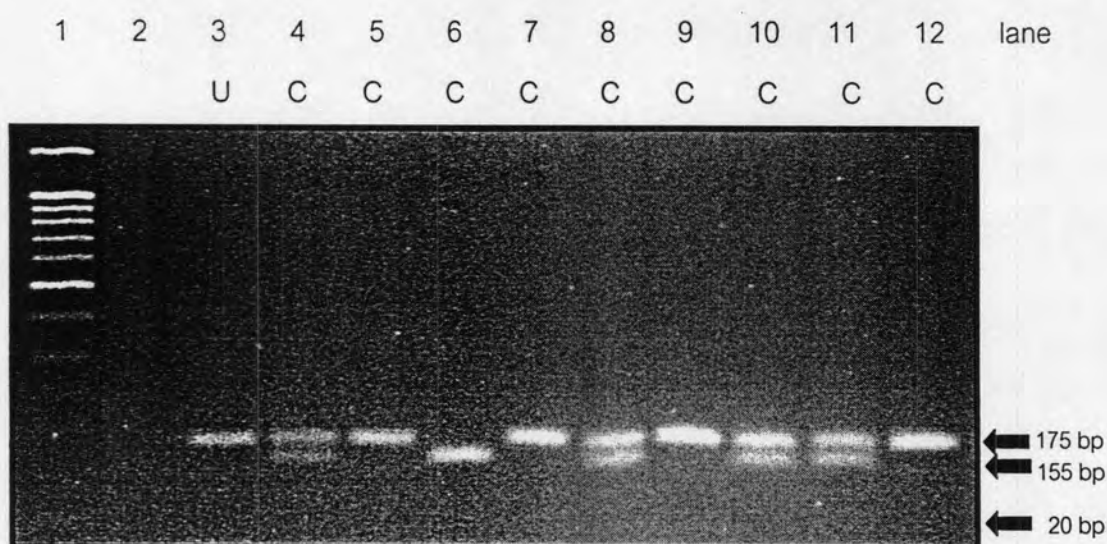


Figure 8. The representative of PCR-RFLP results from samples with homozygous of -460C, homozygous of -460T and heterozygous -460C/T.

Lane 1 is 100 bp molecular markers.

Lane 6 is homozygous of -460C.

Lane 5, 7, 9 and 12 are homozygous of -460T.

Lane 4, 8 10 and 11 are heterozygous -460C/T.

Under this electrophoresis condition the 20 bp product is not visible.

U = not add restriction enzyme, C = add restriction enzyme.

2.2 PCR-RFLP analysis of VEGF promoter at position +405

Polymorphism at +405 C/G in the promoter region of the VEGF was identified by the PCR-RFLP method. If an G was present at this position, the *BsmFI* restriction enzyme would cut the 304 bp PCR product into two fragments; 193 and 111 bp.

No digestion would occur if a C was present (Watson, Webb et al. 2000; Bhanoori, Arvind Babu et al. 2005) (Figure 9).

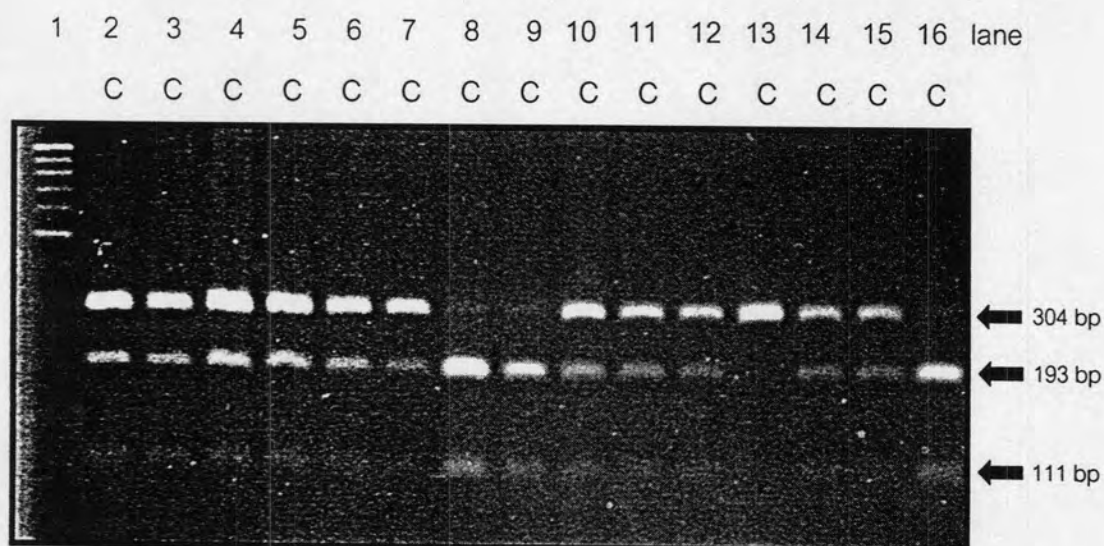


Figure 9. The representative of PCR-RFLP results from samples with homozygous of +405C, homozygous of +405G and heterozygous +405C/G.

Lane 1 is 100 bp molecular markers.

Lane 13 is homozygous of +405C.

Lane 8-9 and 16 are homozygous of +405G.

Lane 2-7, 10-12 and 14-15 are heterozygous +405C/G.

3. The association results of VEGF gene polymorphism with susceptibility to psoriasis.

We assessed the quality of the genotype data by testing for Hardy-Weinberg equilibrium in the sample, using chi-square (χ^2) test ($p < 0.05$). There are no significant deviations from Hardy-Weinberg equilibrium in all SNPs in the study.

3.1 VEGF gene polymorphism at position -1557 (C/A)

Genotype and allele frequencies for -1557 C/A at the promoter of VEGF gene in healthy controls and chronic plaque psoriasis patients were shown in table 11 and 12. One hundred and fourteen of 234 healthy controls (48.72%) were homozygous for the common -1557CC genotype, one hundred and five (44.87%) were heterozygous for the -1557CA and fifteen (6.41%) were homozygous for the -1557AA genotype. The allele frequencies were 71.15% for common -1557C allele and 28.85% for -1557A allele. In comparison, seventy-seven of 154 psoriasis patients (50.00%) were homozygous for the common -1557CC genotype, seventy (45.45%) were heterozygous for the -1557CA and seven (4.55%) were homozygous for the -1557AA genotype. The allele frequencies were 72.73% for common -1557C allele and 27.27% for -1557A allele. There were no statistically significant difference in allele and genotype frequency of the -1557C/A polymorphism at the promoter of VEGF gene between patients with psoriasis and healthy controls.

3.2 VEGF gene polymorphism at position -460 (C/T)

Genotype and allele frequencies for -460 C/T at the promoter of VEGF gene in healthy controls and psoriasis patients were shown in table 13 and 14. One hundred and seventeen of 234 healthy controls (50.00%) were homozygous for the common -460TT genotype, ninety-seven (41.45%) were heterozygous for the -460CT and twenty (8.55%) were homozygous for the -460CC genotype. The allele frequencies were 70.73% for common -460T allele and 29.27% for -460C allele. In comparison, Seventy-

five of 154 psoriasis patients (48.70%) were homozygous for the common -460TT genotype, seventy-three (47.40%) were heterozygous for the -460CT and six (3.90%) were homozygous for the -460CC genotype. The allele frequencies were 72.40% for common -460T allele and 27.60% for -460C allele. There were no statistically significant difference in allele and genotype frequency of the -460C/T polymorphism at the promoter of VEGF gene between patients with psoriasis and healthy controls.

3.3 VEGF gene polymorphism at position +405 (C/G)

Genotype and allele frequencies for +405 C/G at the exon1 of VEGF gene in healthy controls and chronic plaque psoriasis patients were shown in table 15 and 16. Eighty-seven of 234 healthy controls (37.18%) were homozygous for the common +405GG genotype, one hundred and eighteen (50.43%) were heterozygous for the +405CG and twenty-nine (12.39%) were homozygous for the +405CC genotype. The allele frequencies were 62.39% for common +405G allele and 37.61% for +405C allele. In comparison, sixty-nine of 154 psoriasis patients (44.81%) were homozygous for the common +405GG genotype, seventy-four (48.05%) were heterozygous for the +405CG and eleven (7.14%) were homozygous for the +405CC genotype. The allele frequencies were 68.83% for common +405G allele and 31.17% for +405C allele. There were no statistically significant difference in allele and genotype frequency of the +405CG polymorphism at the exon1 of VEGF gene between patients with psoriasis and healthy controls.

4. Haplotype analysis of VEGF gene at position (-1557C/A, -460C/T, +405C/G, respectively) in psoriasis patients and normal controls.

The haplotype frequencies of the VEGF gene polymorphism were also calculated by PHASE program. The haplotype frequencies in patients with psoriasis and normal controls were shown in table 17. In haplotype analysis of 3 positions (-1557C/A, -460C/T, +405C/G) of VEGF gene, we found 8 haplotypes; -1557/-460/+405 CTC, CTG, CCC, CCG, ATC, ATG, ACC and ACG in patients with psoriasis and normal controls were shown in table 19 and also found 15 genotypes of haplotype (table 18); CCC/CCG, CCC/ACG, CCG/ACG, CTC/CTC, CTC/CTG, CTC/ACC, CTC/ACG, CTC/ATC, CTC/ATG, CTG/CTG, CTG/ACG, CTG/ATG, ACG/ACG, CTG/CCG and ATG/ACG. In this study, CTC/ACG genotype of haplotype and CTC haplotype were found the most common haplotype in normal control. In contrast, CTC/CTG genotype of haplotype and CTG haplotype were found the most common haplotype in chronic plaque psoriasis. After comparing haplotype frequencies of the 3 positions of VEGF gene polymorphism between patients with psoriasis and normal controls, the CTG haplotype was found to be significantly associated with psoriasis patients compared to normal controls ($p=0.0307$, $OR=1.40$, $95\%CI=1.03-1.91$). Moreover, the frequencies of CTG/CTG or CTG/other genotype compared to other genotypes were found to be significantly increased in psoriasis patients compared with normal controls ($p=0.0078$, $OR=1.81$, $95\%CI=1.16-2.84$) in the dominance models of inheritance (showed in table 20).

Table 11. Genotype and allele frequencies for VEGF promoter polymorphism at position -1557C/A in healthy controls and psoriasis patients.

	Psoriasis patients n = 154	Healthy controls n = 234
Genotype frequencies		
C/C	77 (50.00%)	114 (48.72%)
C/A	70 (45.45%)	105 (44.87%)
A/A	7 (4.55%)	15 (6.41%)
Allele frequencies		
C	224 (72.73%)	333 (71.15%)
A	84 (27.27%)	135 (28.85%)

No significant association

Table 12. Risk of psoriasis associated with VEGF (-1557C/A) genotype according to different models of inheritance.

	Psoriasis patients n = 154	Healthy controls n = 234
C dominance, A wild type		
C/C or C/A	147 (95.45%)	219 (93.59%)
A/A	7 (4.55%)	15 (6.41%)
C recessive, A wild type		
C/C	77 (50.00%)	114 (48.72%)
A/A or C/A	77 (50.00%)	120 (51.28%)

No significant association

Table 13. Genotype and allele frequencies for VEGF promoter polymorphism at position -460 C/T in healthy controls and psoriasis patients.

	Psoriasis patients n = 154	Healthy controls n = 234
Genotype frequencies		
C/C	6 (3.90%)	20 (8.55%)
C/T	73 (47.40%)	97 (41.45%)
T/T	75 (48.70%)	117 (50.00%)
Allele frequencies		
C	85 (27.60%)	137 (29.27%)
T	223 (72.40%)	331 (70.73%)
No significant association		

Table 14. Risk of psoriasis associated with VEGF (-460C/T) genotype according to different models of inheritance.

	Psoriasis patients n = 154	Healthy controls n = 234
C dominance, T wild type		
C/C or C/T	79 (51.30%)	117 (50.00%)
T/T	75 (48.70%)	117 (50.00%)
C recessive, T wild type		
C/C	6 (3.90%)	20 (8.55%)
T/T or C/T	148 (96.10%)	214 (91.45%)
No significant association		

Table 15. Genotype and allele frequencies for VEGF promoter polymorphisms at position +405 C/G in healthy controls and psoriasis patients.

	Psoriasis patients n = 154	Healthy controls n = 234
Genotype frequencies		
C/C	11 (7.14%)	29 (12.39%)
C/G	74 (48.05%)	118 (50.43%)
G/G	69 (44.81%)	87 (37.18%)
Allele frequencies		
C	96 (31.17%)	176 (37.61%)
G	212 (68.83%)	292 (62.39%)

No significant association

Table 16. Risk of psoriasis associated with VEGF (+405C/G) genotype according to different models of inheritance.

	Psoriasis patients n = 154	Healthy controls n = 234
C dominance, G wild type		
C/C or C/G	85 (55.19%)	147 (62.82%)
G/G	69 (44.81%)	87 (37.18%)
C recessive, G wild type		
C/C	11 (7.14%)	29 (12.39%)
G/G or C/G	143 (92.86%)	205 (87.61%)

No significant association

Table 17. Haplotype frequencies of the VEGF polymorphism (-1557C/A, -460C/T and +405C/G, respectively) between normal controls and psoriasis patients.

Haplotype frequencies	Psoriasis patients (2n=308)	Healthy controls (2n=468)
CTC	95 (30.84%)	170 (36.32%)
CTG	127 (41.23%)	156 (33.33%)
CCC	0 (0.00%)	3 (0.64%)
CCG	2 (0.65%)	4 (0.85%)
ATC	0 (0.00%)	1 (0.21%)
ATG	1 (0.32%)	4 (0.85%)
ACC	1 (0.32%)	2 (0.43%)
ACG	82 (26.62%)	128 (27.35%)

Table 18. Genotype distribution of the VEGF haplotype (-1557C/A, -460C/T and +405C/G, respectively) between normal controls and psoriasis patients.

VEGF Genotype distribution of haplotype (-1557,-460, +405)	Genotype distribution(%) of psoriasis patients	Genotype distribution(%) of normal controls
	n = 154	n = 234
CCC, CCG	0 (0.00%)	2 (0.85%)
CCC, ACG	0 (0.00%)	1 (0.43%)
CCG, ACG	0 (0.00%)	2 (0.85%)
CTC, CTC	10 (6.49%)	26 (11.11%)
CTC, CTG	42 (27.27%)	55 (23.50%)
CTC, ACC	1 (0.65%)	2 (0.85%)
CTC, ACG	32 (20.78%)	57 (24.36%)
CTC, ATC	0 (0.00%)	1 (0.43%)
CTC, ATG	0 (0.00%)	3 (1.28%)
CTG, CTG	23 (14.94%)	31 (13.25%)
CTG, ACG	37 (24.03%)	38 (16.24%)
CTG, ATG	0 (0.00%)	1 (0.43%)
ACG, ACG	6 (3.90%)	15 (6.41%)
CTG, CCG	2 (1.30%)	0 (0.00%)
ATG, ACG	1 (0.65%)	0 (0.00%)

Table 19. Association of the VEGF polymorphism (-1557C/A, -460C/T and +405C/G, respectively) between normal controls and psoriasis patients.

Haplotype frequencies	Psoriasis patients (2n=308)	Healthy controls (2n=468)	p-value
CTC	95 (30.84%)	170 (36.32%)	0.1342
Other haplotype	213 (69.16%)	298 (63.68%)	
CTG	127 (41.23%) ^a	156 (33.33%)	0.0307
Other haplotype	181 (58.77%)	312 (66.67%)	
CCC	0 (0.00%)	3 (0.64%)	0.2809
Other haplotype	308 (100.00%)	465 (99.36%)	
CCG	2 (0.65%)	4 (0.85%)	1.000
Other haplotype	306 (99.35%)	464 (99.15%)	
ATC	0 (0.00%)	1 (0.21%)	1.000
Other haplotype	308 (100.00%)	467 (99.79%)	
ATG	1 (0.32%)	4 (0.85%)	0.6533
Other haplotype	307 (99.68%)	464 (99.15%)	
ACC	1 (0.32%)	2 (0.43%)	1.000
Other haplotype	307 (99.68%)	466 (99.57%)	
ACG	82 (26.62%)	128 (27.35%)	0.8883
Other haplotype	226 (73.38%)	340 (72.65%)	

^a $p=0.0307$, OR=1.40, 95%CI=1.03-1.91 (Compared between psoriasis patients and healthy controls)

Table 20. Association of the VEGF polymorphism (-1557C/A, -460C/T and +405C/G, respectively) between normal controls and psoriasis patients.

Genotype distribution of haplotype frequencies	Psoriasis patients (n=154)	Healthy controls (n=234)	p-value
CTG/CTG or CTG/other	104 (67.53%) ^a	125 (53.42%)	0.0078
Other genotype	50 (32.47%)	109 (46.58%)	
CTC/CTC or CTC/other	85 (55.19%)	144 (61.54%)	0.2553
Other genotype	69 (44.81%)	90 (38.46%)	
ACG/ACG or ACG/other	76 (49.35%)	113 (48.29%)	0.9199
Other genotype	78 (50.65%)	121 (51.71%)	

^a $p=0.0078$, OR=1.81, 95%CI=1.16-2.84 (Compared between psoriasis patients and healthy controls)

5. Linkage Disequilibrium (LD)

Linkage Disequilibrium coefficients ($|D|$ and r^2) among VEGF SNP at position -1557C/A, -460C/T and +405C/G. Data was shown in table 20 (see appendix D).

Table 21. Linkage disequilibrium coefficients ($|D|$ and r^2) among VEGF SNPs

$ D $ r^2	-1557 C/A	-460 C/T	+405 C/G
-1557 C/A	-	0.9468	0.8963
-460 C/T	0.8780	-	0.8365
+405 C/G	0.1963	0.1746	-

6. The association results of VEGF gene polymorphisms with age at onset of psoriasis.

We analyze the association between age at onset in patients with psoriasis and polymorphism of the position -1557C/A, -460C/T and +405C/G of VEGF gene by using Chi-square test and odds ratio.

We classified age at onset into 2 groups: early-onset (type I) psoriasis (< 40 years) and late-onset (type II) psoriasis (≥ 40 years)

Number of patients with early-onset psoriasis = 102 (66.23%)

Number of patients with late-onset psoriasis = 52 (33.77%)

6.1 VEGF gene polymorphism at position -1557 (C/A) and early-onset psoriasis

There were no statistically significant difference in allele and genotype frequency of the -1557C/A polymorphism at the promoter of VEGF gene between patients with early-onset psoriasis and healthy controls. The data were shown in table 22 and 23.

6.2 VEGF gene polymorphism at position -460 (C/T) and early-onset psoriasis

The -460 CC genotype were found to be significantly associated in patients with early-onset psoriasis compared to normal controls ($p=0.0450$, $OR=0.21$, $95\%CI=0.03-0.97$). Furthermore, the frequencies of -460 TT or CT compared to CC genotype was found to be significantly increased in early-onset psoriasis compared with normal controls ($p=0.0450$, $OR=4.67$, $95\%CI=1.03-29.52$). The data were shown in table 24 and 25.

6.3 VEGF gene polymorphism at position +405 (C/G) and early-onset psoriasis

There were no statistically significant difference in allele and genotype frequency of the +405C/G polymorphism at the exon1 of VEGF gene between patients with early-onset psoriasis and healthy controls. The data were shown in table 26 and 27.

6.4 VEGF gene polymorphism at position (-1557 C/A, -460 C/T, +405 C/G) and late-onset psoriasis

There were no statistically significant difference in allele and genotype frequency of all polymorphisms of VEGF gene between patients with late-onset psoriasis and healthy controls. The data were shown in table 22-27.

6.5 The association results of VEGF gene polymorphism at position -1557 (C/A), -460 (C/T) and +405 (C/G) were compared between early-onset and late-onset psoriasis

There were no statistically significant difference in allele, genotype and haplotype frequency of all polymorphisms of VEGF gene between patients with early-onset psoriasis and late-onset psoriasis. The data were not shown.

7. Haplotype analysis of VEGF gene at position (-1557C/A, -460C/T, +405C/G, respectively) in age at onset psoriasis and normal controls.

7.1 Haplotype analysis of VEGF gene at position (-1557C/A, -460C/T, +405C/G, respectively) in early-onset psoriasis and normal controls

The haplotype frequencies of the VEGF gene polymorphism were also calculated by PHASE program. The haplotype frequencies in patients with early-onset psoriasis and normal controls were shown in table 28. In haplotype analysis of 3 positions (-1557C/A, -460C/T, +405C/G) of VEGF gene, we found 8 haplotypes; -1557/-460/+405 CTC, CTG, CCC, CCG, ATC, ATG, ACC and ACG in patients with early-onset psoriasis and normal controls were shown in table 30 and also found 15 genotypes of haplotype (table 29); CCC/CCG, CCC/ACG, CCG/ACG, CTC/CTC, CTC/CTG, CTC/ACC, CTC/ACG, CTC/ATC, CTC/ATG, CTG/CTG, CTG/ACG, CTG/ATG, ACG/ACG, CTG/CCG and ATG/ACG. After comparing haplotype frequencies of the 3 positions of VEGF gene polymorphism between patients with early-onset psoriasis and normal controls, the CTG haplotype was found to be significantly associated with early-onset psoriasis compared

to normal controls ($p=0.0098$, $OR=1.58$, $95\%CI=1.11-2.24$). Moreover, the frequencies of CTG/CTG or CTG/other genotype compared to other genotypes were found to be significantly increased in early-onset psoriasis compared with normal controls ($p=0.0028$, $OR=2.20$, $95\%CI=1.29-3.74$) in the dominance models of inheritance (Shown in table 31).

7.2 Haplotype analysis of VEGF gene at position (-1557C/A, -460C/T, +405C/G, respectively) in late-onset psoriasis and normal controls

The haplotype frequencies of the VEGF gene polymorphism were also calculated by PHASE program. The haplotype frequencies in patients with late-onset psoriasis and normal controls were shown in table 28. In haplotype analysis of 3 positions (-1557C/A, -460C/T, +405C/G) of VEGF gene, we found 8 haplotypes; -1557/-460/+405 CTC, CTG, CCC, CCG, ATC, ATG, ACC and ACG in patients with late-onset psoriasis and normal controls were shown in table 30 and also found 15 genotypes of haplotype (table 29); CCC/CCG, CCC/ACG, CCG/ACG, CTC/CTC, CTC/CTG, CTC/ACC, CTC/ACG, CTC/ATC, CTC/ATG, CTG/CTG, CTG/ACG, CTG/ATG, ACG/ACG, CTG/CCG and ATG/ACG. After comparing haplotype frequencies of the 3 positions of VEGF gene polymorphism between patients with late-onset psoriasis and normal controls, there were no statistically significant difference in allele and genotype frequency of all haplotypes of VEGF gene between patients with late-onset psoriasis and healthy controls.

Table 22. Genotype and allele frequencies for VEGF promoter polymorphism at position -1557C/A in healthy controls, early-onset and late-onset psoriasis patients.

	Early-onset n = 102	Late-onset n = 52	Healthy controls n = 234
Genotype frequencies			
C/C	55 (53.92%)	22 (42.31%)	114 (48.72%)
C/A	44 (43.14%)	26 (50.00%)	105 (44.87%)
A/A	3 (2.94%)	4 (7.69%)	15 (6.41%)
Allele frequencies			
C	154 (75.49%)	70 (67.31%)	333 (71.15%)
A	50 (24.51%)	34 (32.69%)	135 (28.85%)
No significant association			

Table 23. Risk of early-onset or late-onset psoriasis associated with VEGF (-1557C/A) genotype according to different models of inheritance.

	Early-onset n = 102	Late-onset n = 52	Healthy controls n = 234
C dominance, A wild type			
C/C or C/A	99 (97.06%)	48 (92.31%)	219 (93.59%)
A/A	3 (2.94%)	4 (7.69%)	15 (6.41%)
C recessive, A wild type			
C/C	55 (53.92%)	22 (42.31%)	114 (48.72%)
A/A or C/A	47 (46.08%)	30 (57.69%)	120 (51.28%)
No significant association			

Table 24. Genotype and allele frequencies for VEGF promoter polymorphism at position -460 C/T in healthy controls, early-onset and late-onset psoriasis patients

	Early-onset n = 102	Late-onset n = 52	Healthy controls n = 234
Genotype frequencies			
C/C	2 (1.96%)	4 (7.69%)	20 (8.55%)
C/T	46 (45.10%)	27 (51.92%)	97 (41.45%)
T/T	54 (52.94%)	21 (40.38%)	117 (50.00%)
Allele frequencies			
C	50 (24.51%)	35 (33.65%)	137 (29.27%)
T	154 (75.49%)	69 (66.35%)	331 (70.73%)
No significant association			

Table 25. Risk of early-onset or late-onset psoriasis associated with VEGF (-460C/T) genotype according to different models of inheritance.

	Early-onset n = 102	Late-onset n = 52	Healthy controls n = 234
C dominance, T wild type			
C/C or C/T	48 (47.06%)	31 (59.62%)	117 (50.00%)
T/T	54 (52.94%)	21 (40.38%)	117 (50.00%)
C recessive, T wild type			
C/C	2 (1.96%) ^a	4 (7.69%)	20 (8.55%)
T/T or C/T	100 (98.04%) ^b	48 (92.31%)	214 (91.45%)

^a $p=0.0450$, OR=0.21, 95%CI=0.03-0.97 (Compared between early-onset and healthy controls)

^b $p=0.0450$, OR=4.67, 95%CI=1.03-29.52 (Compared between early-onset and healthy controls)

Table 26. Genotype and allele frequencies for VEGF promoter polymorphisms at position +405 C/G in healthy controls, early-onset and late-onset psoriasis patients

	Early-onset n = 102	Late-onset n = 52	Healthy controls n = 234
Genotype frequencies			
C/C	7 (6.86%)	4 (7.69%)	29 (12.39%)
C/G	49 (48.04%)	25 (48.08%)	118 (50.43%)
G/G	46 (45.10%)	23 (44.23%)	87 (37.18%)
Allele frequencies			
C	63 (30.88%)	33 (31.73%)	176 (37.61%)
G	141 (69.12%)	71 (68.27%)	292 (62.39%)
No significant association			

Table 27. Risk of early-onset or late-onset psoriasis associated with VEGF (+405C/G) genotype according to different models of inheritance.

	Early-onset n = 102	Late-onset n = 52	Healthy controls n = 234
C dominance, G wild type			
C/C or C/G	56 (54.90%)	29 (55.77%)	147 (62.82%)
G/G	46 (45.10%)	23 (44.23%)	87 (37.18%)
C recessive, G wild type			
C/C	7 (6.86%)	4 (7.69%)	29 (12.39%)
G/G or C/G	95 (93.14%)	48 (92.31%)	205 (87.61%)
No significant association			

Table 28. Haplotype frequencies of the VEGF polymorphism (-1557C/A, -460C/T and +405C/G, respectively) between normal controls and early-late onset psoriasis patients.

Haplotype frequencies	Early-onset psoriasis patients (2n=204)	Late-onset psoriasis patients (2n=104)	Healthy controls (2n=468)
CTC	63 (30.88%)	32 (30.77%)	170 (36.32%)
CTG	90 (44.12%)	37 (35.58%)	156 (33.33%)
CCC	0 (0.00%)	0 (0.00%)	3 (0.64%)
CCG	1 (0.49%)	1 (0.96%)	4 (0.85%)
ATC	0 (0.00%)	0 (0.00%)	1 (0.21%)
ATG	1 (0.49%)	0 (0.00%)	4 (0.85%)
ACC	0 (0.00%)	1 (0.96%)	2 (0.43%)
ACG	49 (24.02%)	33 (31.73%)	128 (27.35%)

Table 29. Genotype distribution of the VEGF haplotype (-1557C/A, -460C/T and +405C/G, respectively) between normal controls and early-late onset psoriasis patients.

VEGF Genotype distribution of haplotype (-1557,-460, +405)	Genotype distribution(%) of early-onset psoriasis	Genotype distribution(%) of late-onset psoriasis	Genotype distribution(%) of normal controls
	n = 102	n = 52	n = 234
CCC, CCG	0 (0.00%)	0 (0.00%)	2 (0.85%)
CCC, ACG	0 (0.00%)	0 (0.00%)	1 (0.43%)
CCG, ACG	0 (0.00%)	0 (0.00%)	2 (0.85%)
CTC, CTC	7 (6.68%)	3 (5.77%)	26 (11.11%)
CTC, CTG	30 (29.41%)	12 (23.08%)	55 (23.50%)
CTC, ACC	0 (0.00%)	1 (1.92%)	2 (0.85%)
CTC, ACG	19 (18.63%)	13 (25.00%)	57 (24.36%)
CTC, ATC	0 (0.00%)	0 (0.00%)	1 (0.43%)
CTC, ATG	0 (0.00%)	0 (0.00%)	3 (1.28%)
CTG, CTG	17 (16.67%)	6 (11.54%)	31 (13.25%)
CTG, ACG	25 (24.51%)	12 (23.08%)	38 (16.24%)
CTG, ATG	0 (0.00%)	0 (0.00%)	1 (0.43%)
ACG, ACG	2 (1.96%)	4 (7.69%)	15 (6.41%)
CTG, CCG	1 (0.98%)	1 (1.92%)	0 (0.00%)
ATG, ACG	1 (0.98%)	0 (0.00%)	0 (0.00%)

Table 30. Association of the VEGF polymorphism (-1557C/A, -460C/T and +405C/G, respectively) between normal controls and early-late onset psoriasis patients.

Haplotype frequencies	Early-onset psoriasis patients (2n=204)	Late-onset psoriasis patients (2n=104)	Healthy controls (2n=468)	p-value of early-onset	p-value of late-onset
CTC	63 (30.88%)	32 (30.77%)	170 (36.32%)	0.2023	0.3376
Other haplotype	141 (69.12%)	72 (69.23%)	298 (63.68%)		
CTG	90 (44.12%) ^a	37 (35.58%)	156 (33.33%)	0.0098	0.7466
Other haplotype	114 (55.88%)	67 (64.42%)	312 (66.67%)		
CCC	0 (0.00%)	0 (0.00%)	3 (0.64%)	0.5573	1.0000
Other haplotype	204 (100%)	104 (100%)	465 (99.36%)		
CCG	1 (0.49%)	1 (0.96%)	4 (0.85%)	1.0000	1.0000
Other haplotype	203 (99.51%)	103 (99.04%)	464 (99.15%)		
ATC	0 (0.00%)	0 (0.00%)	1 (0.21%)	1.0000	1.0000
Other haplotype	204 (100%)	104 (100%)	467 (99.79%)		
ATG	1 (0.49%)	0 (0.00%)	4 (0.85%)	1.0000	1.0000
Other haplotype	203 (99.51%)	104 (100%)	464 (99.15%)		
ACC	0 (0.00%)	1 (0.96%)	2 (0.43%)	1.0000	0.4529
Other haplotype	204 (100%)	103 (99.04%)	466 (99.57%)		
ACG	49 (24.02%)	33 (31.73%)	128 (27.35%)	0.4202	0.4366
Other haplotype	155 (75.98%)	71 (68.27%)	340 (72.65%)		

^a $p=0.0098$, OR=1.58, 95%CI=1.11-2.24 (Compared between early-onset and healthy controls)

Table 31. Association of the VEGF polymorphism (-1557C/A, -460C/T and +405C/G, respectively) between normal controls and early-late onset psoriasis patients.

Genotype distribution of haplotype frequencies	Early-onset psoriasis patients (n=102)	Late-onset psoriasis patients (n=52)	Healthy controls (n=234)	p-value early	p-value late
CTG/CTG or CTG/other	73 (71.57%) ^a	30 (57.69%)	125 (53.42%)	0.0028	0.5107
Other genotype	29 (28.43%)	22 (42.31%)	109 (46.58%)		
CTC/CTC or CTC/other	56 (55.45%)	29 (55.77%)	144 (61.54%)	0.3084	0.5399
Other genotype	46 (44.55%)	23 (44.23%)	90 (38.46%)		
ACG/ACG or ACG/other	47 (46.08%)	29 (55.77%)	113 (48.29%)	0.7991	0.4109
Other genotype	55 (53.92%)	23 (44.23%)	121 (51.71%)		

^a $p=0.0028$, OR=2.20, 95%CI=1.29-3.74 (Compared between early-onset and healthy controls)

8. The association results of VEGF gene polymorphisms with clinical severity of psoriasis

We analyze the association between clinical severity in patients with psoriasis and polymorphism of the position -1557C/A, -460C/T and +405C/G of VEGF gene by using Chi-square test and odds ratio. We classified the severity into 3 groups including mild, moderate and severe psoriasis, decided by Psoriasis Area and Severity Index (PASI score) (Louden, Pearce et al. 2004). The criterion was incorporated in this study.

Mild: Psoriasis Area and Severity Index (PASI score), 0 to 10

Moderate: PASI score, > 10 to < 15

Severe: PASI score, ≥ 15

Number of severity: Mild = 90 (58.44%)

Moderate = 27 (17.53%)

Severe = 37 (24.03%)

8.1 VEGF gene polymorphism at position -1557 (C/A), -460 (C/T) and +405 (C/G) and psoriasis severity

There were no statistically significant difference in allele and genotype frequency of the -1557 (C/A), -460 (C/T) and +405 (C/G) polymorphism at the promoter and exon1 of VEGF gene between psoriasis severity of psoriasis patients and healthy controls. The data were shown in table 32 and 33.

8.2 The association results of VEGF gene polymorphism at position -1557 (C/A), -460 (C/T) and +405 (C/G) were compared between the groups of mild, moderate and severe of psoriasis

There were no statistically significant difference in allele, genotype and haplotype frequency of all polymorphism of VEGF gene between the groups of mild, moderate and severe of psoriasis. The data were not shown.

9. Haplotype analysis of VEGF gene at position (-1557C/A, -460C/T, +405C/G, respectively) in psoriasis severity and normal controls.

9.1 Haplotype analysis of VEGF gene at position (-1557C/A, -460C/T, +405C/G, respectively) in severe psoriasis and normal controls

The haplotype frequencies in patients with the severe psoriasis and normal controls were shown in table 34. In haplotype analysis of 3 positions (-1557C/A, -460C/T, +405C/G) of VEGF gene, we found 8 haplotypes; -1557/-460/+405 CTC, CTG, CCC, CCG, ATC, ATG, ACC and ACG in patients with severe psoriasis and normal controls were shown in table 35. After comparing haplotype frequencies of the 3 positions of VEGF gene polymorphism between patients with severe psoriasis and normal controls, the CTG haplotype was found to be significantly associated in patients with the severe

psoriasis compared to normal controls ($p=0.0475$, $OR=1.70$, $95\%CI=1.01-2.87$). The data were shown in table 35.

Table 32. Allele frequencies for VEGF promoter and exon1 polymorphism at -1557C/A, -460C/T and +405C/G positions in healthy controls and psoriasis severity.

Position (-1557, -460,+405)	Allele	Allele frequencies(%)	Allele frequencies(%)	Allele frequencies(%)	Allele frequencies(%)
		Mild	Moderate	Severe	Control
		1015 (2n=180)	1015 (2n=54)	1015 (2n=74)	Control (2n=468)
-1557	C	128 (71.11%)	41 (75.93%)	55 (74.32%)	333 (71.15%)
	A	52 (88.89%)	13 (24.07%)	19 (25.68%)	135 (28.85%)
-460	C	52 (28.89%)	15 (27.78%)	18 (24.32%)	137 (29.27%)
	T	128 (71.11%)	39 (72.22%)	56 (75.68%)	331 (70.73%)
+405	C	58 (32.22%)	17 (31.48%)	21 (28.38%)	176 (37.61%)
	G	122 (67.78%)	37 (68.52%)	53 (71.62%)	292 (62.39%)

No significant association

Table 33. Genotype frequencies for VEGF promoter and exon1 polymorphism at -1557C/A, -460C/T and +405C/G positions in healthy controls and psoriasis severity.

Position (-1557, -460,+405)	Genotype	Genotype distribution(%)	Genotype distribution(%)	Genotype distribution(%)	Genotype distribution(%)
		Mild	Moderate	Severe	
		1015 (n=90)	1015 (n=27)	1015 (n=37)	Control (n=234)
-1557	CC	43 (47.78%)	15 (55.56%)	19 (51.35%)	114 (48.72%)
	CA	42 (46.67%)	11 (40.74%)	17 (45.95%)	105 (44.87%)
	AA	5 (5.56%)	1 (3.70%)	1 (2.70%)	15 (6.41%)
-460	CC	5 (5.56%)	1 (3.70%)	0 (0.00%)	20 (8.55%)
	CT	42 (46.67%)	13 (48.15%)	18 (48.65%)	97 (41.45%)
	TT	43 (47.78%)	13 (48.15%)	19 (51.35%)	117 (50.00%)
+405	CC	6 (6.67%)	2 (7.41%)	3 (8.11%)	29 (12.39%)
	CG	46 (51.11%)	13 (48.15%)	15 (40.54%)	118 (50.43%)
	GG	38 (42.22%)	12 (44.44%)	19 (51.35%)	87 (37.18%)

No significant association

Table 34. Haplotype frequencies for VEGF promoter and exon1 polymorphism at -1557C/A, -460C/T and +405C/G positions in healthy controls and psoriasis severity.

VEGF Haplotype (-1557, -460,+405)	Haplotype frequencies(%) Mild	Haplotype frequencies(%) Moderate	Haplotype frequencies(%) Severe	Haplotype frequencies(%)
	1015 (2n=180)	1015 (2n=54)	1015 (2n=74)	Control (2n=468)
CTC	57 (31.67%)	17 (31.48%)	21 (28.38%)	170 (36.32%)
CTG	71 (39.44%)	22 (40.74%)	34 (45.95%)	156 (33.33%)
CCC	0 (0.00%)	0 (0.00%)	0 (0.00%)	3 (0.64%)
CCG	0 (0.00%)	2 (3.70%)	0 (0.00%)	4 (0.85%)
ATC	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.21%)
ATG	0 (0.00%)	0 (0.00%)	1 (2.50%)	4 (0.85%)
ACC	1 (0.56%)	0 (0.00%)	0 (0.00%)	2 (0.43%)
ACG	51 (28.33%)	13 (24.07%)	18 (24.32%)	128 (27.35%)

Table 35. Association of the VEGF polymorphism (-1557C/A, -460C/T and +405C/G, respectively) between normal controls and severe psoriasis patients.

Haplotype frequencies	Severe psoriasis patients (2n=74)	Healthy controls (2n=468)	p-value
CTC	21 (28.38%)	170 (36.32%)	0.2306
Other haplotype	53 (71.62%)	298 (63.68%)	
CTG	34 (45.95%) ^a	156 (33.33%)	0.0475
Other haplotype	40 (54.05%)	312 (66.67%)	
CCC	0 (0.00%)	3 (0.64%)	1.0000
Other haplotype	74 (100%)	465 (99.36%)	
CCG	0 (0.00%)	4 (0.85%)	1.0000
Other haplotype	74 (100%)	464 (99.15%)	
ATC	0 (0.00%)	1 (0.21%)	1.0000
Other haplotype	74 (100%)	467 (99.79%)	
ATG	1 (1.35%)	4 (0.85%)	1.0000
Other haplotype	73 (98.65%)	464 (99.15%)	
ACC	0 (0.00%)	2 (0.43%)	1.0000
Other haplotype	74 (100%)	466 (99.57%)	
ACG	18 (24.32%)	128 (27.35%)	0.6860
Other haplotype	56 (75.68%)	340 (72.65%)	

^a p=0.0475, OR=1.70, 95%CI=1.01-2.87 (Compared between severe psoriasis and healthy controls)

Table 36. Genotype distributions of haplotype for VEGF promoter and exon1 polymorphism at position -1557C/A, -460C/T and +405C/G in healthy controls and psoriasis severity.

VEGF Genotype distribution of haplotype (-1557,-460,+405)	Genotype distribution(%) Mild	Genotype distribution(%) Moderate	Genotype distribution(%) Severe	Genotype distribution(%)
	1015 (n=90)	1015 (n=27)	1015 (n=37)	Control (n=234)
CCC, CCG	0 (0.00%)	0 (0.00%)	0 (0.00%)	2 (0.85%)
CCC, ACG	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.43%)
CCG, ACG	0 (0.00%)	0 (0.00%)	0 (0.00%)	2 (0.85%)
CTC, CTC	5 (5.56%)	2 (7.41%)	3 (8.11%)	26 (11.11%)
CTC, CTG	25 (27.78%)	9 (33.33%)	8 (21.62%)	55 (23.50%)
CTC, ACC	1 (1.11%)	0 (0.00%)	0 (0.00%)	2 (0.85%)
CTC, ACG	21 (23.33%)	4 (14.81%)	7 (18.92%)	57 (24.36%)
CTC, ATC	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.43%)
CTC, ATG	0 (0.00%)	0 (0.00%)	0 (0.00%)	3 (1.28%)
CTG, CTG	13 (14.44%)	2 (7.41%)	8 (21.62%)	31 (13.25%)
CTG, ACG	20 (22.22%)	7 (25.93%)	10 (27.03%)	38 (16.24%)
CTG, ATG	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.43%)
ACG, ACG	5 (5.56%)	1 (3.70%)	0 (0.00%)	15 (6.41%)
CTG, CCG	0 (0.00%)	2 (7.41%)	0 (0.00%)	0 (0.00%)
ATG, ACG	0 (0.00%)	0 (0.00%)	1 (2.70%)	0 (0.00%)

No significant association

10. The distribution of promoter and exon1 polymorphisms (-1557C/A, -460C/T and +405C/G, respectively) of VEGF gene.

Besides, association study of VEGF polymorphisms with psoriasis, this study also presents the basic knowledge of the frequencies of VEGF polymorphisms (-1557C/A, -460C/T and +405C/G, respectively) in Thai population. The distributions of the three positions polymorphisms between Thai population and Caucasian or some Asian population previous reports were compared.

Pattern of VEGF gene polymorphisms (-1557C/A, -460C/T and +405C/G)

10.1 Pattern of VEGF at position -1557 (C/A)

Genotype and allele frequencies for the polymorphism at -1557C/A in promoter region of VEGF gene were analyzed. The analysis showed significant differences in genotype and allele frequencies between Thai and Caucasian population. The data were shown in table 37. Beside, the analysis showed no significant differences in genotype and allele frequencies between Thai and Asian population. The data were shown in table 38

10.2 Pattern of VEGF at position -460 (C/T)

Genotype and allele frequencies for the polymorphism at -460C/T in promoter region of VEGF gene were analyzed. The analysis showed significant differences in genotype and allele frequencies between Thai and Caucasian or some Asian population. The data were shown in table 39 and 40.

10.3 Pattern of VEGF at position +405 (C/G)

Genotype and allele frequencies for the polymorphism at +405C/G in exon1 region of VEGF gene were analyzed. The analysis showed no significant differences in

genotype and allele frequencies between Thai and Caucasian or some Asian population. The data were shown in table 41-42.

Table 37. Comparison between genotype and allele frequencies of VEGF (-1557C/A, rs699947) gene polymorphism in the different population.

Cytokine	Position	Genotypes	Control groups					
			Author	Barile et al.	Papazoglou et al.	Breunis et al.	Bo et al.	This study
			Year	2006	2004	2006	2006	2006
			Ethnic	Italy	Greece	Natherland	Italy	Thai
			N	215	73	293	222	234
VEGF	-1557	C/C		^a 61 (28.4%)	^b 23 (31.5%)	^c 65 (22.2%)	^d 63 (32.8%)	114 (48.7%)
		C/A		117 (54.4%)	30 (41.1%)	145 (49.5%)	119 (53.6%)	105 (44.9%)
		A/A		37 (17.2%)	20 (27.4%)	83 (28.3%)	30 (13.5%)	15 (6.4%)
		Allele						
		C		239 (56.0%)	76 (52%)	275 (46.9%)	265 (59.7%)	333 (71.2%)
		A		191 (44.0%)	70 (47.9%)	311 (53.1%)	179 (40.3%)	135 (28.9%)

^a The genotype distribution is significantly different when compared with Thai ($\chi^2 = 18.66$, $p = 0.000016$).

^b The genotype distribution is significantly different when compared with Thai ($\chi^2 = 5.99$, $p = 0.01$).

^c The genotype distribution is significantly different when compared with Thai ($\chi^2 = 18.66$, $p = 0.000000$).

^d The genotype distribution is significantly different when compared with Thai ($\chi^2 = 18.66$, $p = 0.000834$).

Table 38. Comparison between genotype and allele frequencies of VEGF (-1557C/A, rs699947) gene polymorphism in the different population.

Cytokine	Position	Genotypes	Control groups				
			Author	HapMap-CEU	HapMap-HCB	HapMap-JPT	This study
			Year	-	-	-	2006
			Ethnic	European	Han Chinese	Japanese	Thai
			<i>N</i>	60	45	44	234
VEGF	-1557	C/C	^a 21 (35.0%)	^b 23 (51.1%)	^c 20 (45.5%)	114 (48.7%)	
		C/A	29 (48.3%)	19 (42.2%)	20 (45.5%)	105 (44.9%)	
		A/A	10 (16.7%)	3 (6.7%)	4 (9.1%)	15 (6.4%)	
		Allele					
		C	71 (59.2%)	65 (72.2%)	60 (68.2%)	333 (71.2%)	
		A	49 (40.8%)	25 (27.8%)	28 (31.8%)	135 (28.9%)	

^a The genotype distribution is no significantly different when compared with Thai.

^b The genotype distribution is no significantly different when compared with Thai.

^c The genotype distribution is no significantly different when compared with Thai.

Table 39. Comparison between genotype and allele frequencies of VEGF (-460C/T, rs833061) gene polymorphism in the different population.

Cytokine	Position	Genotypes	Control groups					
			Author	Young et al.	Barile et al.	Ku et al.	Liu et al.	This study
			Year	2004	2006	2005	2003	2006
			Ethnic	UK	Italy	Taiwan	Taiwan	Thai
			N	101	215	230	119	234
VEGF	-460	C/C		21 (20.8%)	39 (18.1%)	0 (0.0%)	4 (3.4%)	20 (8.6%)
		C/T		60 (59.4%)	117 (54.4%)	192 (83.4%)	72 (60.5%)	97 (41.4%)
		T/T		^a 20 (19.8%)	^b 59 (27.4%)	^c 38 (16.6%)	^d 43 (36.1%)	117 (50.0%)
		Allele						
		C		102 (50.5%)	195 (45.4%)	192 (41.7%)	80 (33.6%)	137 (29.3%)
		T		100 (49.5%)	235 (54.6%)	268 (58.3%)	158 (66.4%)	331 (70.7%)

^a The genotype distribution is significantly different when compared with Thai ($\chi^2 = 25.38$, $p = 0.0000005$).

^b The genotype distribution is significantly different when compared with Thai ($\chi^2 = 22.99$, $p = 0.000002$).

^c The genotype distribution is significantly different when compared with Thai ($\chi^2 = 56.94$, $p = 0.0000$).

^d The genotype distribution is no significantly different when compared with Thai ($\chi^2 = 5.57$, $p = 0.02$).

Table 40. Comparison between genotype and allele frequencies of VEGF (-460C/T, rs833061) gene polymorphism in the different population.

Cytokine	Position	Genotypes	Control groups				
			Author	HapMap-CEU	HapMap-HCB	HapMap-JPT	This study
			Year	-	-	-	2006
			Ethnic	European	Han Chinese	Japanese	Thai
			N	58	45	44	234
VEGF	-460	C/C		11 (19.0%)	3 (6.7%)	4 (9.1%)	20 (8.6%)
		C/T		27 (46.6%)	19 (42.2%)	20 (45.5%)	97 (41.4%)
		T/T		^a 20 (34.5%)	^b 23 (51.1%)	^c 20 (45.5%)	117 (50.0%)
		Allele					
		C		49 (42.2%)	25 (27.8%)	28 (31.8%)	137 (29.3%)
		T		67 (57.8%)	65 (72.2%)	60 (68.2%)	331 (70.7%)

^a The genotype distribution is significantly different when compared with Thai ($\chi^2 = 3.89$, $p = 0.049$).

^b The genotype distribution is no significantly different when compared with Thai.

^c The genotype distribution is no significantly different when compared with Thai.

Table 41. Comparison between genotype and allele frequencies of VEGF (+405C/G, rs2010963) gene polymorphism in the different population.

Cytokine	Position	Genotypes	Control groups					
			Author	Young et al.	Barile et al.	Papazoglou et al.	Breunis et al.	This study
			Year	2004	2006	2004	2006	2006
			Ethnic	UK	Italy	Greece	Natherland	Thai
			<i>N</i>	102	215	73	297	234
VEGF	+405	C/C		7 (7.0%)	28 (13.0%)	16 (21.9%)	35 (11.8%)	29 (12.4%)
		C/G		48 (47.0%)	99 (46.1%)	31 (42.5%)	130 (43.8%)	118 (50.4%)
		G/G		^a 47 (46.0%)	^b 88 (40.9%)	^c 26 (35.6%)	^d 132 (44.4%)	87 (37.18%)
		Allele						
		C		62 (30.4%)	155 (36.0%)	63 (43.1%)	200 (33.7%)	176 (37.6%)
		G		142 (69.6%)	275 (64.0%)	83 (56.8%)	394 (66.3%)	292 (62.4%)

^a The genotype distribution is no significantly different when compared with Thai.

^b The genotype distribution is no significantly different when compared with Thai.

^c The genotype distribution is no significantly different when compared with Thai.

^d The genotype distribution is no significantly different when compared with Thai.

Table 42. Comparison between genotype and allele frequencies of VEGF (+405C/G, rs2010963) gene polymorphism in the different population.

Cytokine	Position	Genotypes	Control groups			
			Author	Lee, S.J. et al.	Kim, S.H. et al.	This study
			Year	2005	2005	2006
			Ethnic	Korea	Korea	Thai
			N	432	219	234
VEGF	+405	C/C		92 (21.3%)	29 (13.2%)	29 (12.4%)
		C/G		232 (53.7%)	116 (53.0%)	118 (50.4%)
		G/G		^a 108 (25.0%)	^b 74 (33.8%)	87 (37.18%)
		Allele				
		C		416 (48.1%)	174 (39.7%)	176 (37.6%)
		G		448 (51.9%)	264 (60.3%)	292 (62.4%)

^a The genotype distribution is significantly different when compared with Thai ($\chi^2 = 10.29$, $p = 0.001$).

^b The genotype distribution is no significantly different when compared with Thai.

11. The results of VEGF expression levels in plasma by ELISA technique.

We measured VEGF levels in plasma of 37 psoriasis patients. Normal distribution was not observed by the test of normality in these samples. Wilcoxon Mann-Whitney test was used to analyze the VEGF concentration in different groups of genotype distributions of haplotype including: -1557/-460/+405; homozygous CTG/CTG, heterozygous CTG/other and Non-CTG. We could not observe any significant relationship. This difference between groups was not statistically significant (Wilcoxon Mann-Whitney test, $p>0.3$).

Moreover, plasma from 12 psoriasis patients containing different haplotype but with the same condition including early-onset, mild, stopped systemic treatments were assayed for VEGF protein expression by ELISA. Wilcoxon Mann-Whitney test was used to analyze the VEGF concentration in different groups of genotype distributions of haplotype including: -1557/-460/+405; homozygous CTG/CTG, heterozygous CTG/CTC and Non-CTG. We could not observe any significant relationship. This difference between groups was not statistically significant (Wilcoxon Mann-Whitney test, $p>0.3$).

Plasma samples were classified from 27 psoriasis patients into 2 different haplotype groups including haplotype containing +405C and +405G. Wilcoxon Mann-Whitney test was used to analyze the VEGF concentration in different groups of genotype distributions of haplotype. We could not observe any significant relationship. This difference between groups was not statistically significant (Wilcoxon Mann-Whitney test, $p>0.2$). The data of VEGF concentration were shown in table 43-45. The statistical data were not shown. The graph analyses of VEGF concentration in different groups of haplotype were shown in Figure 10-12.

Table 43. The data showed VEGF plasma concentration in each groups of genotype distribution of haplotype (Non Classified group).

Haplotype No. Sample	VEGF concentration CTG/CTG (pg/ml)	VEGF concentration CTG/other (pg/ml)	VEGF concentration Non-CTG (pg/ml)
1	34.597 ± 8.8	49.262 ± 17.77	187.69 ± 2.97
2	6.2613 ± 4.47	17.817 ± 11.87	113.17 ± 22.23
3	11.522 ± 2.97	91.157 ± 0.0	231.89 ± 17.88
4	412.86 ± 4.51	106.88 ± 13.34	88.023 ± 42.97
5	105.83 ± 5.93	72.303 ± 0.0	56.592 ± 34.07
6	50.310 ± 16.29	23.068 ± 4.45	96.435 ± 87.45
7	3.1018 ± 0.0	63.925 ± 2.96	10.468 ± 7.43
8	50.310 ± 10.37	79.634 ± 1.48	26.212 ± 8.90
9	6.264 ± 1.49	77.541 ± 19.26	117.36 ± 10.38
10	190.84 ± 7.43	67.068 ± 51.84	17.823 ± 0.0
11	81.729 ± 4,44	28.309 ± 8.89	98.491 ± 1.48
12	-	135.19 ± 14.84	47.169 ± 0.0
13	-	-	9.4201 ± 2.97
14	-	-	69.161 ± 7.41

Table 44. The data showed VEGF plasma concentration in each groups of genotype distribution of haplotype (Classified group by onset and severity).

Haplotype No. Sample	VEGF concentration CTG/CTG (pg/ml) Early/Mild	VEGF concentration CTG/CTC (pg/ml) Early/Mild	VEGF concentration Non-CTG (pg/ml) Early/Mild
1	6.2613 ± 4.47	17.817 ± 11.87	231.89 ± 17.88
2	105.83 ± 5.93	91.157 ± 0.0	56.592 ± 34.07
3	50.310 ± 10.37	106.88 ± 13.34	10.468 ± 7.43
4	6.264 ± 1.49	-	17.823 ± 0.0
5	81.729 ± 4,44	-	-

Table 45. The data showed VEGF plasma concentration in each groups of genotype distribution of haplotype (Classified group by haplotype containing +405G or +405C).

Haplotype No. Sample	VEGF concentration Haplotype containing +405G (pg/ml)	VEGF concentration Haplotype containing +405C (pg/ml)
1	34.597 ± 8.8	187.69 ± 2.97
2	6.2613 ± 4.47	113.17 ± 22.23
3	11.522 ± 2.97	231.89 ± 17.88
4	412.86 ± 4.51	88.023 ± 42.97
5	105.83 ± 5.93	56.592 ± 34.07
6	50.310 ± 16.29	9.4201 ± 2.97
7	3.1018 ± 0.0	
8	50.310 ± 10.37	
9	6.264 ± 1.49	
10	190.84 ± 7.43	
11	81.729 ± 4.44	
12	63.925 ± 2.96	
13	79.634 ± 1.48	
14	77.541 ± 19.26	
15	67.068 ± 51.84	
16	28.309 ± 8.89	
17	135.19 ± 14.84	
18	96.435 ± 87.45	
19	10.468 ± 7.43	
20	26.212 ± 8.90	
21	69.161 ± 7.41	

Figure 10. The graph showed VEGF concentration in different groups of haplotype (Non Classified group).

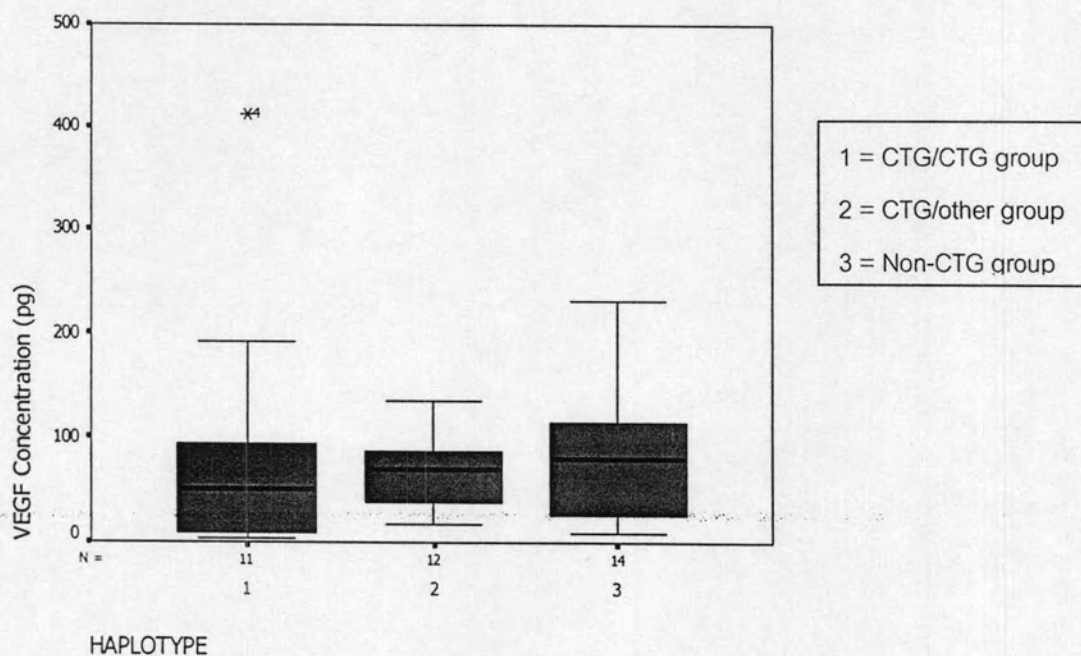


Figure 11. The graph showed VEGF concentration in different groups of haplotype (Classified group by onset and severity).

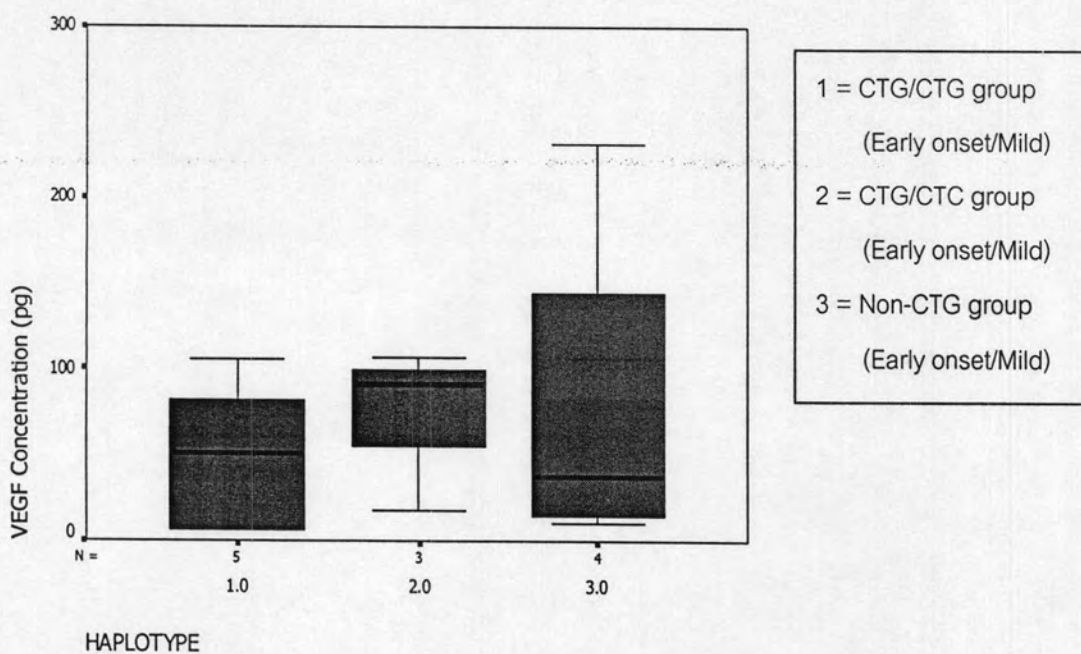
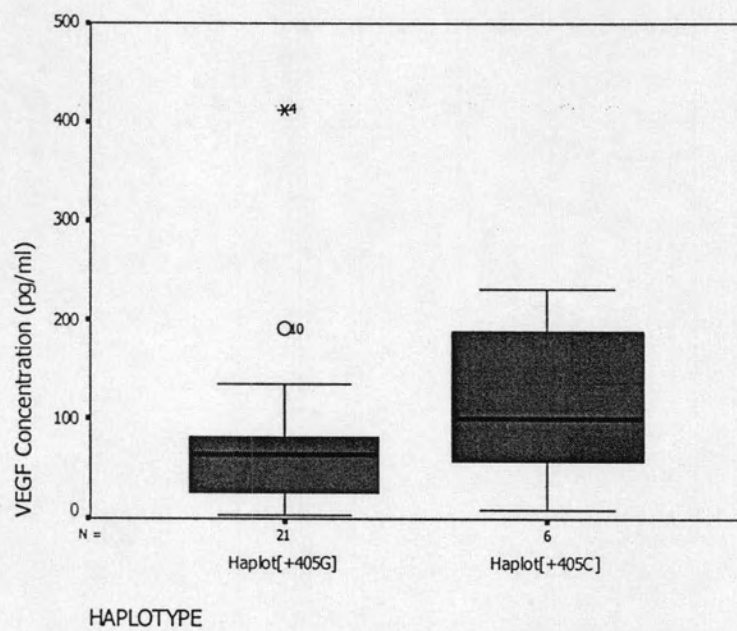


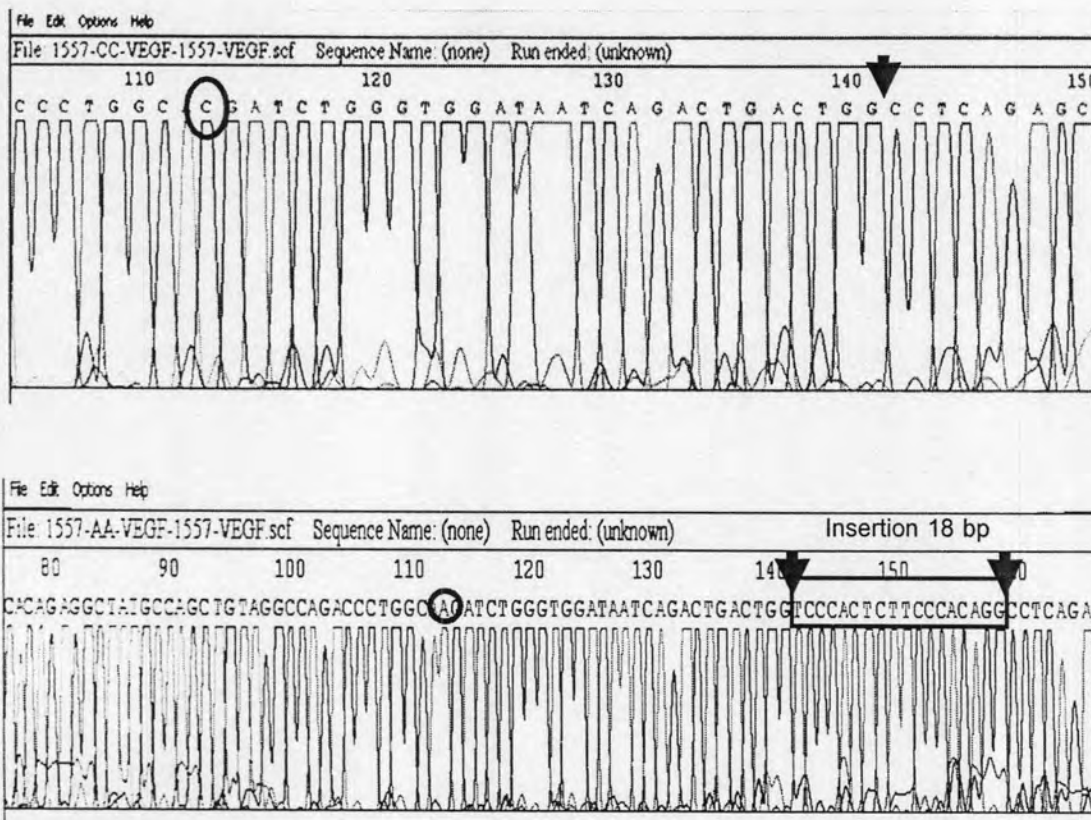
Figure 12. The graph showed VEGF concentration in different groups of haplotype (Classified group by haplotype containing +405G or +405C).



12. The schematic of DNA sequencing at 3 positions

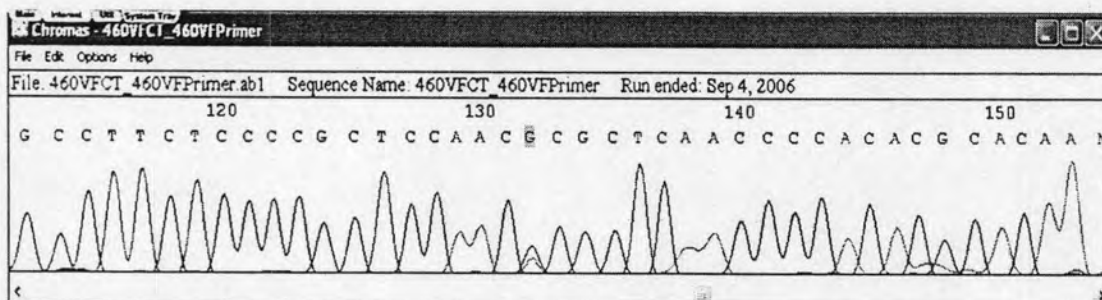
12.1 Position -1557 (C/A)

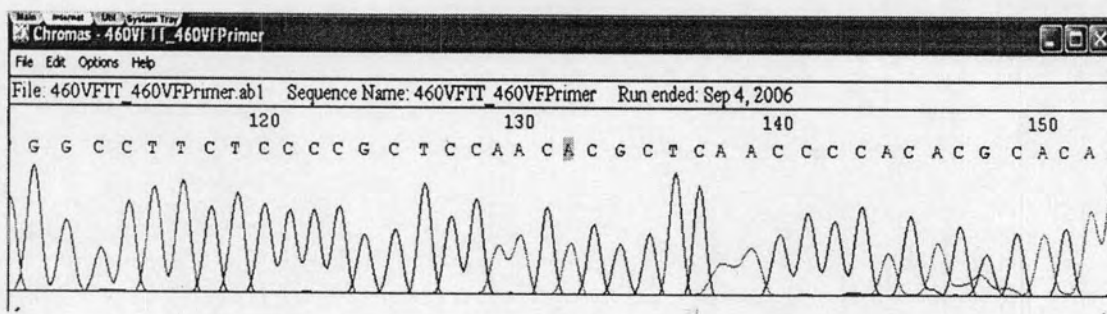
Figure 13. The schematic of -1557C/A VEGF sequencing



12.2 Position -460 (C/T), reverse direction sequencing

Figure 14. The schematic of -460C/T VEGF sequencing





12.3 Position +405 (C/G)

Figure 15. The schematic of +405C/G VEGF sequencing

