CHAPTER 4

RESULTS

One hundred and sixty-two subjects were enrolled in this study. Their blood samples were collected and gDNA was extracted from the buffy coat layers. The geography distribution residence of the 162 subjects are demonstrated in Table 7.

Table 7 The distribution residence of the subjects enrolled in this study according to their living places (provinces)

Provinces	Number of subject
Songkhla	77
Chumporn	29
Nakhonsrithammarat	17
Yala	5
Pattani	5
Trang	3
Pattalung	3
Suratthani	3
Satun	3
Krabi	2
Phang Nga	2
Phuket	2
Narathiwat	2

The characteristics such as age, body weight, height and body mass index are shown in Table 8. These characteristics are not different among the homozygous EM (homEM), heterozygous EM (hetEM) and PM groups.

Table 8 Demographic data of the 162 study subjects

Variable	homEM (n=82)	hetEM (n=65)	PM (n=12)
Age (y), mean ± S.D	31.6 ± 12.3	32.6 ± 12.4	28.6 ± 14.4
Body weight (kg), mean ± S.D	59.2 ± 8.6	59.6 ± 9.9	56.1 ± 9.0
Sex (male/female),	49/33	35/33	8/4
Height (cm), mean ± S.D	160.8 ± 8.0	159.5 ± 7.3	153.6 ± 7.1
BMI, mean ± S.D	22.4± 3.3	22.9 ± 3.8	22 ± 2.8

BMI; body mass index = Body weight (kg)/ Height ^{2}(m)

n = number of subjects

hetEM = heterozygous extensive metabolizer

homEM = homozygousextensive metabolizer

After amplification of gDNA by using specific primer for exon 5 of CYP2C19, a 321 base pair (bp) DNA fragment was successfully amplified (Figure 4).

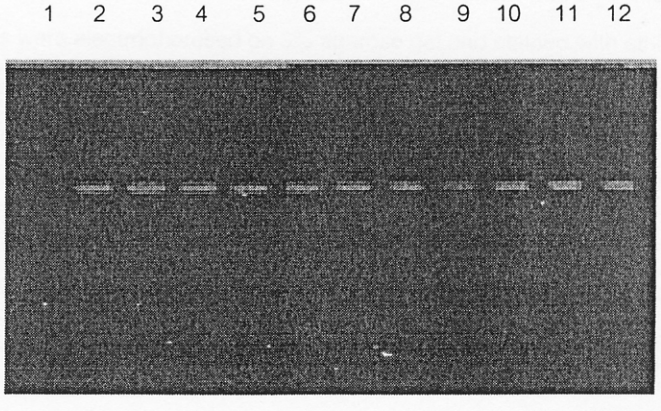

Figure 4 The PCR product amplified for *CYP2C19*2* digestion. Lane 1 represents for negative control.

The 321 bp PCR product was then digested with SmaI endonuclease. A 321 bp PCR product obtained from individual who has homozygous *CYP2C19*1/*1* can be digested with this endonuclease to yeild 212 and 109 bp DNA fragment because it contained *Sma*I recognition site. In contrast, the $G_{681}A$ transition which due to a *Sma*I

recognition site was disapeared in a 321 bp PCR product obtained from individual who has homozygous *CYP2C19*2/*2* and thus could not be digested with *Sma*I. Whereas a 321 bp DNA fragment from individual who was heterozygous *CYP2C19*1/*2* could be digested by *Sma*I to 109, 212 and 321 bp DNA fragment (Figure 5).

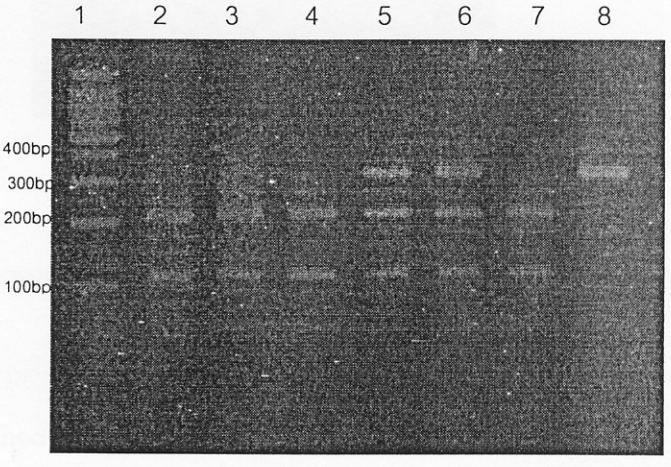

Figure 5 The PCR-based diagnostic test for *CYP2C19*2* mutation. Genomic DNA was isolated and amplified with specific primers. The amplified 271 bp fragment was digested with *Sma*I. The samples were electrophoresed on 3% agarose gel and stained with ethidium bromide. Lane 1 represents 100bp molecular weigh markers. lanes 8 represents homozygous *CYP2C19*2/CYP2C19*2*. Lanes 2, 3,4, and 7 represents homozygous *CYP2C19*1/CYP2C19*1*. Lanes 5 and 6 represents heterozygous *CYP2C19*1/CYP2C19*2*.

After amplification of gDNA by using specific primer for exon 4 of CYP2C19, a 271bp DNA fragment was successfully amplified (Figure 6).

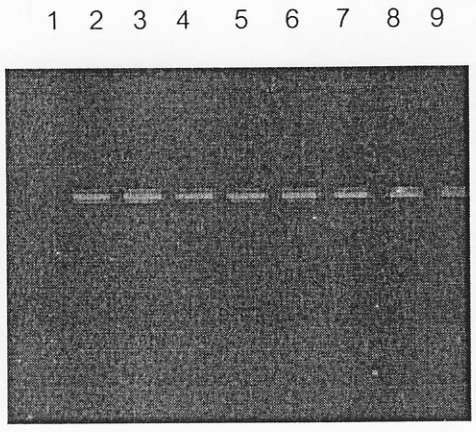

Figure 6 The PCR product amplified for *CYP2C19*3* digestion. Lane 1 represent for negative control.

The 271bp PCR product was then digested with *Bam*HI endonuclease. A 271 bp PCR product obtained from homozygous *CYP2C19*1/*1* individual can be digested with this endonuclease to yeild 95 and 175 bp DNA fragment because its contained *Bam*HI recognition site. In contrast, the $G_{636}A$ transition due to a *Bam*HI recognition site was disappeared in a 271 bp PCR product obtained from homozygous *CYP2C19*2/*2* individual and thus could not be digested with *Bam*HI. Whereas a 271 bp DNA fragment from individual who has heterozygous *CYP2C19*1/*2* could be digested by *Bam*HI to 95 and 175 bp DNA fragment (Figure 7).

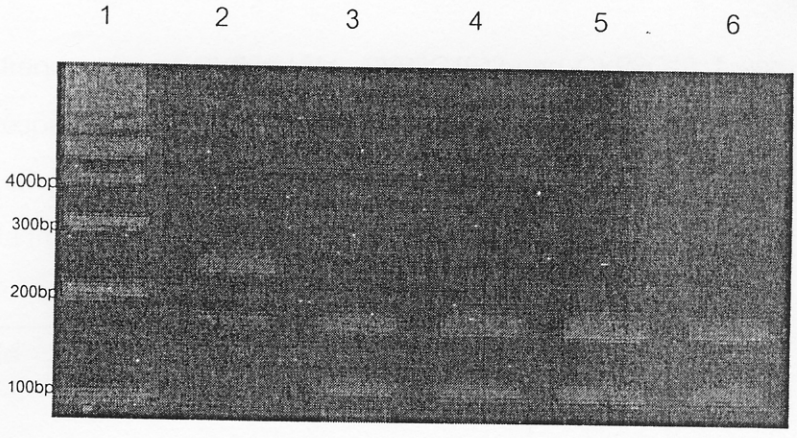

Figure 7 The PCR-based diagnostic test for *CYP2C19*3* mutation. Genomic DNA was isolated and amplified with specific primers. The amplified 271 fragment was digested with *BamH* I. The sample were electrophoresis on 3% agarose gel and stained with ethidium bromide. Lane 1 represents 100bp molecular weigh markers. Lane 2 represents heterozygous *CYP2C19*1/CYP2C19*3*. Other lanes represent homozygous *CYP2C19*1/CYP2C19*1*.

Genotyping of 162 unrelated healthy Southern Thai subjects demonstrated that 82 subjects (50.62%) were homozygous wild-type (**1/*1*), 65 subjects (40.12%) were heterozygous **1/*2*, 10 subjects (6.17%) were homozygous **2/2**, 3 subjects (1.85%) were heterozygous **1/*3*, 2 subjects (1.23%) were heterozygous **2/*3*. Homozygous *CYP2C19*3/*3* were not detected in this study. The results of the CYP2C19 genotype analysis are summarized in Table 9.

Table 9 CYP2C19 genotypes of 162 Southern Thai subjects

Genotype CYP2C19	n	% (95% CI)
**1/*1*	82	50.62 (42.97-58.27)
**1/*2*	65	40.12 (34.79-45.45)
**1/*3*	3	1.85 (0.39-3.31)
**2/*2*	10	6.17 (3.55-8.79)
**2/*3*	2	1.23 (0.03-2.43)
**3/*3*	0	0

The allele frequencies of *CYP2C19*1*, *CYP2C19*2* and *CYP2C19*3* were 0.716, 0.268 and 0.015, respectively (Table 10).

Table 10 The allele frequencies of CYP2C19 in Southern Thai subjects

Allele	Allele frequency (95% CI)
*1	0.716 (0.677-0.765)
*2	0.269 (0.221-0.317)
*3	0.154 (0.002-0.028)

Observed and calculated of the CYP2C19 genotype analysis in Southern Thai populations based on the Hardy-Weinberg equilibrium are shown in Table 11.

Table 11 Observed and Expected frequency of CYP2C19 genotypes in a Southern Thai population.

Genotype	% Observed frequency (95% CI)	%Expected frequency
**1/*1*	50.62 (42.97-58.27)	50.26
**1/*2*	40.12 (34.79-45.45)	38.45
**1/*3*	1.85 (0.39-3.31)	2.20
**2/*2*	6.17 (3.55-8.79)	7.21
**2/*3*	1.23 (0.03-2.43)	0.83
**3/*3*	0	0.02

The prevalence of *CYP2C19*1/*1*, *CYP2C19*1/*2* *CYP2C19*1/*3* *CYP2C19*2/*2* and *CYP2C19*2/*3* from our study are 50.6%, 40.1%, 1.9%, 6.2%, and 1.2% respectively. There was the absence of *CYP2C19*3/*3* in this study. The calculated genotype frequency values based on Hardy-Weinberg law, $p^2 + 2pq + q^2 = 1$, were similar with the observed values. The number in parenthesis represents the actual observed percentage of each genotype. The genotype frequency of homozygous EMs, heterozygous EMs and

homozygous PMs of Southern Thai populations were compared with Asians, Caucasians and Africans population (Table 12).

Table 12 Comparison of the genotype frequency of homozygous EMs, heterozygous EMs and homozygous PMs of Southern Thai populations with other Asians, Caucasians and Africans populations.

Population	n	Genotype frequency%)			
		homo EM	heter EM	homo PM	Reference
Thais					
Southern (present study)	162	51	42	7	**Present study**
North-Eastern	107	48	46	6	Tassaneeyakul.,2002
Filipinos	52	31	46	23	Goldstein.,1997
Chinese-Taiwanese	118	41	43	16	Joyce,1997
Jawish Israeli	140	71	26	3	Nan,2002
Koreans	103	47	42	12	Roh,1996
Saudi Arabians	97	72	26	2	Goldstein,1997
European-Americans	105	76	23	2	Goldstein,1997
African-Americans	108	56	37	6	Goldstein,1997

Homozygous EMs genotype = **1/*1*

Heterozygous EMs genotype = **1/*2, *1/*3*

The genotype frequencies of homozygous EMs, heterozygous EMs and homozygous PMs in Southern Thai populations were about 51%, 42% and 7% respectively which are very similar to the North-Eastern Thai populations. The prevalence of homozygous PMs (*CYP2C19*2/*2* and *CYP2C19*2/*3*) from our study are 7.4% which were significantly higher than Saudi Arabians and European-Americans and much lower than Filipinos and Chinese-Taiwanese ($p<0.05$) but similar to that found in African-Americans.

The comparative prevalence of allele frequencies among Oriental populations are summarized in Table 13.

Table 13 Comparative frequencies of CYP2C19 alleles in various populations.

population	n	Allele frequencies			References
		*CYP2C19*1*	*CYP2C19*2*	*CYP2C19*3*	
Thais					
Southern	162	0.71	0.27	0.02	**Present study**
North-eastern	107	0.72	0.27	0.02	Tassaneeyakul.,2002
Chinese-Dai	193	0.66	0.30	0.03	Nan,2002
Chinese-Han	101	0.56	0.37	0.07*	Xiao,1997
Chinese-Taiwanese	118	0.63	0.32	0.055	Joyce,1997
Filipinos	52	0.54	0.39	0.07*	Goldstein.,1997
North Indians	100	0.76	0.24	0*	Lamba *et al.*, 2000
Japaneses	186	0.58	0.29	0.13**	Kubuta,1996
Koreans	103	0.68	0.21	0.11**	Roh,1996
Black Tanzanians	195	0.90	0.10*	0.00*	Bathum *et al.*, 1999
Turkisks	404	0.88	0.12*	0.004*	Aynacioglu *et al.*, 1999
Saudi Arabians	97	0.85	0.15*	0*	Goldstein.,1997
European-Americans	105	0.87	0.13*	0*	Goldstein.,1997
African-Americans	108	0.75	0.25	0*	Goldstein.,1997

* significantly different from a Southern Thai populations with $p<0.05$.

** significantly different from a Southern Thai populations with $p<0.001$.

The frequency of *CYP2C19*1*, *CYP2C19*2*, *CYP2C19*3* of Southern and North-Eastern Thai populations were most similar. The frequency of *CYP2C19*1* allele were slightly different among the Asians populations (0.54-0.72). The frequency of *CYP2C19*2* allele were slightly different among other Asians populations (0.21-0.39). *CYP2C19*2* allele in Southern Thai populations was but being significantly lower than Chinese-Han, Chinese-Taiwanese and Filipinos ($p<0.05$). Similar to *CYP2C19*2*, the frequency of *CYP2C19*3* allele in Southern Thai populations were significantly lower than other Asians populations ($p<0.05$).