CHAPTER IV

RESULTS

Cytochrome P450 2E1 (*CYP2E1*)

The present study investigated the correlation between the polymorphism of *CYP2E1* gene and NPC on a total of 255 patients and 297 controls. PCR–RFLP analysis was used to evaluate *RsaI* polymorphism in the *CYP2E1* (figure 7). The polymorphic of this gene had three type which were homozygous wild–type (+/+), heterozygous (+/-), and homologous variant (-/-) forms. The homozygous wild–type form was digested both alleles with this enzyme. The fragments of wild–type form gave two bands at 360 and 50 bp, but the last band was absent due to its short fragment. The heterozygous form had both an uncut allele and a cut allele, so the fragments of this form gave three bands at 410, 360 and 50 bp. In the last type, homozygous variant form was not digested both alleles, and its fragment gave a single undigested band at 410 bp. The distribution of alleles in all sample groups were found to be in Hardy-Weinberg equilibrium. The calculated frequencies of heterozygous using $2x(+/+)^{1/2} \times (-/-)^{1/2}$ were 0.32, 0.26, 0.42 and 0.36 from Total, Thai, Chinese and Thai-Chinese NPC patients, and 0.31, 0.26, 0.42 and 0.21 from Total, Thai, Chinese and Thai-Chinese controls, respectively. These numbers are similar to actual frequencies of heterozygous from all sample groups, 0.33, 0.28, 0.41, 0.41 from Total, Thai, Chinese and Thai-Chinese patients, respectively and 0.35, 0.28, 0.52, 0.24 from Total, Thai, Chinese and Thai-Chinese controls, respectively. As show in Table 2, there was found the relative risk of the variant form (-/-) of the *CYP2E*1 at a high risk [RR = 2.10]. However, this result had on statistical significance [95% C^{I} = 0.90–4.96]. To evaluate the relative risk from different genetic background both patients and controls were divided into three groups, Thai, Chinese, and Thai–Chinese according to the origins of their ancestors. A slightly increasing in risk of the variant from (-/-) of this gene could be demonstrated in Thai sample group [RR = 1.46; 95% C^{I} = 0.23–11.63]. Nevertheless, no statistically significance could be established. In the Chinese sample group, the variant homozygous alleles were at 2.22–fold increase in risk of developing the disease but still without statistically significance [95% CI = 0.78–6.41]. Interestingly in the Thai–Chinese sample group, the only one group had a higher RR of heterozygous (+/-) form [RR = 2.32]. Additionally, its result had statistical significance [95% CI = 1.17–4.61]. The relative risk of variant form in this group could not be calculated because variant form in control group could not be observed. This study analyzed association of the pattern of genetic *CYP2E1* and NPC phenotype by calculating the relative risk if the genotype was either Autosomal Dominant like (ADL) or Autosomal recessive like (ARL) form, contributing to phenotype by one or two alleles respectively. In ADL, the contribution of a single variant allele would show a higher RR. So that, the combination of the heterozygous (+/-) and the variant form (-/-) was computed for the RR when compare with wild–type (+/+). The association between ADL heredity and NPC risk could not be found in the total, Thai, and Chinese sample groups [RR = 1.00; 95%CI = 0.78–1.29, RR = 1.01; 95% CI = 0.66–1.54, RR = 0.81; 95% CI = 0.50–1.13, respectively] but it could be found in the Thai–Chinese sample group [RR = 2.53; 95% CI = 1.29–4.97] (Table 3). However, these results shown no statistical significance in almost sample groups exceptionally in Thai–Chinese sample group. In contrast, ARL concerns the abnormality in both alleles of *CYP2E1*. The RR were calculated by comparison between the wild type (+/+), the heterozygous (+/-) or the combination of the wild type and heterozygous (+/+ and +/-) and the variant form (-/-). The higher RR value in all comparison was concerned ARL pattern in all sample groups (Table 4). These results of ARL pattern had no statistical significance as same as ADL pattern. Exceptionally, the result of Chinese sample group when heterozygous (+/-) form compare with variant (-/-) form had statistical significance [RR = 3.19; 95% CI = 1.12–9.23, respectively]. In conclusion, all case-control data from all groups suggest the role of the mutation of this gene on NPC development as in Taiwan. The insignificant RR value from some categories should be due to lower number of samples

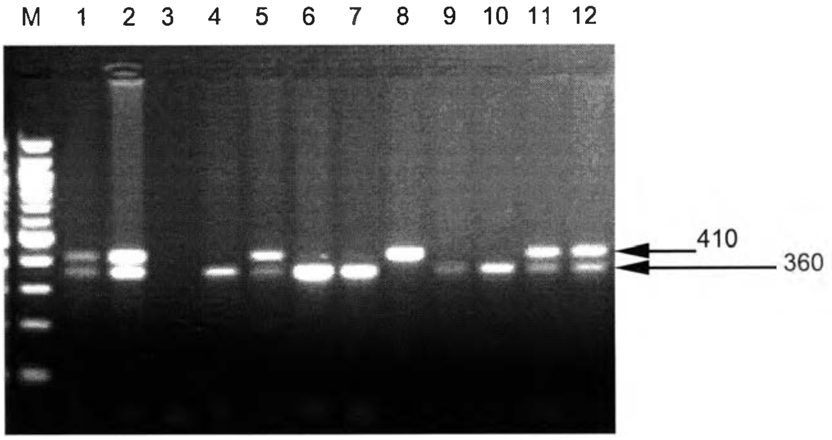

Figure7 PCR-RFLP assay to detect the polymorphism of the *CYP2E1* gene by *RsaI* enzyme digesting the 410bp PCR products into 360bp and 50bp fragments. **M**, molecular weight marker; Lanes [**1**,**2**,**5**,**11**,**12**], heterozygous (+/-); Lanes [**4**,**6**,**7**,**9**,**10**], homozygous variant (-/-); Lane **8**, homozygous wild-type (+/+); Lane **3**, non-amplify.

Table2 Frequency distribution and relative risks associated with genotype variants of *CYP2E1* detected RFLP using *RsaI*

CYP2E1	Frequency		RR	95%CI
	Cases	Controls		
Total	**255***	**297**		
+/+	162	189	1.00	
+/-	84	103	0.95	0.73-1.23
-/-	9	5	2.10	0.90-4.96
Thai	**136**	**99**		
+/+	96	70	1.00	
+/-	38	28	0.99	0.65-1.52
-/-	2	1	1.46	0.23-11.63
Chinese	**59**	**98**		
+/+	29	43	1.00	
+/-	24	51	0.70	0.42-1.16
-/-	6	4	2.22	0.78-6.41
Thai-Chinese	**27**	**100**		
+/+	15	76	1.00	
+/-	11	24	2.32	1.17-4.61
-/-	1	-	-	-

* 33 cases lack precise information regarding ethnicity

Table3 Correlation between Autosomal Dominant Like (ADL) pattern of genetic *CYP2E1* and NPC phenotype

CYP2E1	Frequency		$RR_{(ADL)}$	95%CI
	Cases	Controls		
Total				
+/+	162	189	1.00	
+/- and -/-	93	108	1.00	0.78-1.29
Thai				
+/+	96	70	1.00	
+/- and -/-	40	29	1.01	0.66-1.54
Chinese				
+/+	29	43	1.00	
+/- and -/-	30	55	0.81	0.50-1.31
Thai-Chinese				
+/+	15	76	1.00	
+/- and -/-	12	24	2.53	1.29-4.97

Table4 Correlation between Autosomal Recessive Like (ARL) pattern of genetic *CYP2E1* andNPC phenotype

CYP2E1	Frequency		$RR_{(ARL)}$	95%CI
	Cases	Controls		
Total				
+/+	162	189	1.00	
-/-	9	5	2.10	0.90-4.96
+/-	84	103	1.00	
-/-	9	5	2.21	0.94-5.29
+/+ and +/-	246	292	1.00	
-/-	9	5	2.14	0.93-5.02
Thai				
+/+	96	70	1.00	
-/-	2	1	1.46	0.23-11.63
+/-	38	28	1.00	
-/-	2	1	1.47	0.22-12.06
+/+ and +/-	134	98	1.00	
-/-	2	1	1.46	0.23-11.61
Chinese				
+/+	29	43	1.00	
-/-	6	4	2.22	0.78-6.41
+/-	24	51	1.00	
-/-	6	4	3.19	1.12-9.23
+/+ and +/-	53	94	1.00	
-/-	6	4	2.66	0.97-7.39
Thai-Chinese				
+/+	15	76		
-/-	1	-	-	-
+/-	11	24		
-/-	9	-	-	-
+/+ and +/-	26	100		
-/-	1	-	-	-

Complement receptor type 2 (CR2)

A previous study reported the *TaqI* sites of CR2 near the exon1,2 by using the pBCR2-12.1 as DNA probe for Southern blotting and hybridization.[93] In order to locate this site, a primer set, ranged from exon1,2 to exon4b, were used for the PCR assay. Digestion of the 2.6kb PCR product with *TaqI* produced fragments at 1.7kb, 617bp and 300bp. The 1.7kb fragment was either present or absent. In otherword, the polymorphic *TaqI* should locate at 1.7kb fragment, which may be at *CR2* intron2 or 3. Accordingly, the second primer set was designed to proved the location, shorten PCR product and for improve efficiency in RFLP reading at intron2 region (Figure 8). On digestion with *TaqI*, there is a single undigested band at 1241 bp in homozygous wild-type (-/-) form and are digested products at 750 and 491 bp in homozygous variant (+/+) form. In heterozygous (+/-) form, the fragments gave three bands at 1241, 750, and 491 bp (Figure 9). The distribution of alleles in all sample groups were found to be in Hardy-Weinberg equilibrium. The calculated frequencies of heterozygous using $2x(+/+)^{1/2} \times (-/-)^{1/2}$ were 0.23, 0.24, 0.20 and 0.30 from Total, Thai, Chinese and Thai-Chinese NPC patients, and 0.20, 0.21, 0.20 and 0.20 from Total, Thai, Chinese and Thai-Chinese controls, respectively. These numbers are similar to actual frequencies of heterozygous from all sample groups, 0.24, 0.28, 0.18, 0.28 from Total, Thai, Chinese and Thai-Chinese patients, respectively and 0.21, 0.22, 0.20, 0.22 from Total, Thai, Chinese and Thai-Chinese controls, respectively. As shown in table5, a slightly increasing in risk but without statistically significant of heterozygous (+/-) and variant (+/+) form of the *CR2* could be demonstrated in all sample groups. In Chinese samples group, only one group was not found the association between heterozygous (+/-) and NPC risk [RR=0.94; 95%CI = 0.47–1.80]. Furthermore, we evaluated whether there is any correlation between *CR2* mode of inheritances and NPC phenotype. There was slight increase in NPC RR if *CR2* was calculated via ADL in total, Thai and Thai-Chinese sample groups [RR = 1.21; 95%CI = 0.90–1.63, RR = 1.34; 95% CI = 0.85–2.13, RR = 1.67; 95% CI = 0.78–3.43, respectively] but not in the Chinese sample group [RR = 0.99; 95%CI = 0.51–1.86] (Table 6). In ARL heredity, the slight higher RR value was found in only two groups, total sample group [RR = 1.33; 95%CI = 0.25–7.20, RR = 1.11; 95% CI = 0.20–6.07, RR = 1.28; 95% CI = 0.24–6.89] and Chinese sample group [RR = 1.98; 95%CI = 0.14–27.39, RR = 2.11; 95% CI = 0.14–29.75, RR = 2.00; 95% CI = 0.14–27.71] (Table7). However, all results of this gene had no statistical significance. In conclusion, our study indicates no association between *CR2* genotype and NPC phenotype. This suggests that *CR2* is not likely a susceptible gene for NPC development.

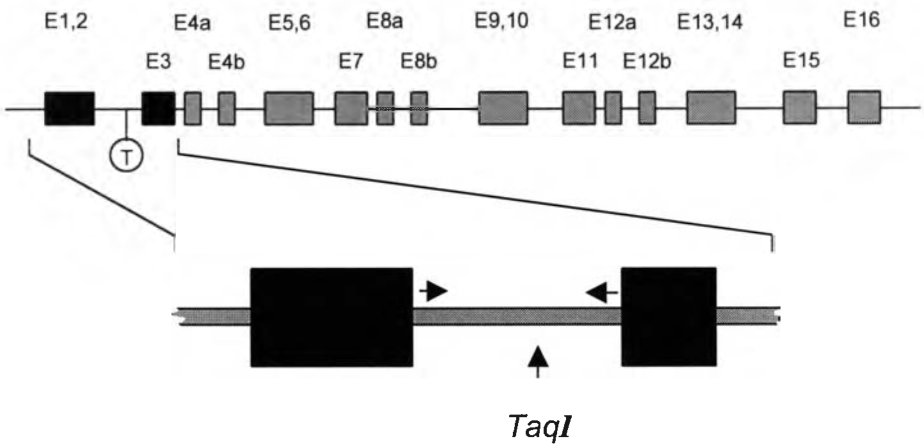

Figure8 Schematic representation of cleavage site for *TaqI* (T) on the *CR2* gene

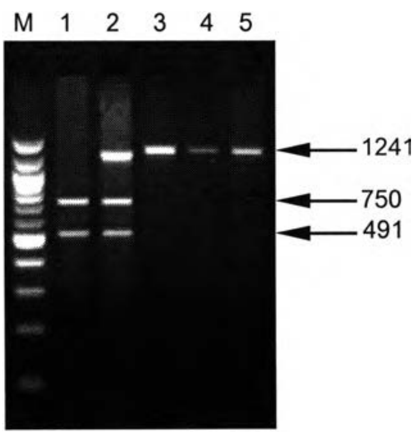

Figure9 PCR-RFLP assay to detect the polymorphism of the *CR2* gene by *TaqI* enzyme digesting the 1241bp PCR products into 750bp and 491bp fragments. M, molecular weight marker; Lane 1, homozygous variant (+/+); Lane 2, heterozygous (+/-); Lanes 3-5, homozygous wild-type (-/-).

Table5 Frequency distribution and relative risks associated with genotype variants of *CR2* detected RFLP using *TaqI*

CR2	Frequency		RR	95%CI
	Cases	Controls		
Total	**232**	**296**		
-/-	174	232	1.00	
+/-	56	62	1.20	0.89-1.63
+/+	2	2	1.33	0.25-7.20
Thai	**124**	**97**		
-/-	89	75	1.00	
+/-	35	21	1.40	0.89-2.24
+/+	-	1	0.00	0.00-14.91
Chinese	**50**	**99**		
-/-	40	79	1.00	
+/-	9	19	0.94	0.47-1.80
+/+	1	1	1.98	0.14-27.39
Thai-Chinese	**25**	**100**		
-/-	17	78	1.00	
+/-	7	22	1.46	0.66-3.08
+/+	1	-	-	-

* 33 cases lack precise information regarding ethnicity

Table6 Correlation between Autosomal Dominant Like (ADL) pattern of genetic *CR2* and NPC phenotype

CR2	Frequency		$RR_{(ADL)}$	95%CI
	Cases	Controls		
Total				
-/-	174	232	1.00	
+/- and +/+	58	64	1.21	0.90-1.63
Thai				
-/-	89	75	1.00	
+/- and +/+	35	22	1.34	0.85-2.13
Chinese				
-/-	40	79	1.00	
+/- and +/+	10	20	0.99	0.51-1.86
Thai-Chinese				
-/-	17	76	1.00	
+/- and +/+	8	22	1.67	0.78-3.43

Table7 Correlation between Autosomal Recessive Like (ARL) pattern of genetic *CR2* and NPC phenotype

CR2	Frequency		$RR_{(ARL)}$	95%CI
	Cases	Controls		
Total				
-/-	174	232	1.00	
+/+	2	2	1.33	0.25-7.20
+/-	56	62	1.00	
+/+	2	2	1.11	0.20-6.07
+/- and -/-	230	294	1.00	
+/+	2	2	1.28	0.24-6.89
Thai				
-/-	89	75	1.00	
+/+	-	1	0.00	0.00-14.91
+/-	35	21	1.00	
+/+	-	1	0.00	0.00-3.35
+/- and -/-	124	96	1.00	
+/+	-	1	0.00	0.00-4.17
Chinese				
-/-	40	79	1.00	
+/+	1	1	1.98	0.14-27.39
+/-	9	19	1.00	
+/+	1	1	2.11	0.14-29.75
+/- and -/-	49	98	1.00	
+/+	1	1	2.00	0.14-27.71
Thai-Chinese				
-/-	17	76		
+/+	1	-	-	-
+/-	7	22		
+/+	1	-	-	-
+/- and -/-	24	100		
+/+	1	-	-	-

Polymeric immunoglobulin receptor (pIgR)

Identification of polymorphic *pIgR* gene was analyzed with *PvuII* nuclease in altogether 224 cases and 296 controls. In a previous study, Southern blot analysis of *PvuII*-digested genomic DNA hybridized with the 0.67kb *PvuII* cDNA probe, indicating the presence of the polymorphic cleavage site located in intron3.[93] Hence, in this study, the PCR products of this gene were confirmed that the cleavage sites of this enzyme located in intron3 by using designed new primers (Figure 10). As shown in the figure 11, the fragments of homozygous wild–type (+/+) form gave bands of digestion products at 1163 and 229 bp and homozygous variant (-/-) form gave only a single undigested band at 1392 bp. Heterozygous (+/-) form had all three bands. The distribution of alleles in all sample groups were found to be in Hardy-Weinberg equilibrium. The calculated frequencies of heterozygous using $2x(+/+)^{1/2} \times (-/-)^{1/2}$ were 0.49, 0.49, 0.50 and 0.48 from Total, Thai, Chinese and Thai-Chinese NPC patients, and 0.48, 0.49, 0.48 and 0.47 from Total, Thai, Chinese and Thai-Chinese controls, respectively. These numbers are similar to actual frequencies of heterozygous from all sample groups, 0.55, 0.55, 0.58, 0.56 from Total, Thai, Chinese and Thai-Chinese patients, respectively and 0.50, 0.59, 0.44, 0.48 from Total, Thai, Chinese and Thai-Chinese controls, respectively. The result showed association between *pIgR* gene and NPC development (Table8). The Chinese sample group not only had a higher RR value but also statistical significance of both heterozygous (+/-) form and variant (-/-) form [RR = 2.77; 95%CI = 1.63–4.73, RR = 2.90; 95% CI = 1.30–6.39, respectively]. Including, the result of heterozygous (+/-) form from total sample group was also statistical significance but the RR value was lower than Chinese sample group [RR = 1.37; 95%CI = 1.03–1.83]. The relative risk of Thai and Thai-Chinese sample group had a slightly increasing risk and not statistical significance. Interestingly, the higher RR value was concerned ADL heredity in Chinese sample group [RR = 2.81; 95%CI = 1.52–5.34] as the same result in total sample group [RR = 1.38; 95%CI = 1.05–1.82] (Table9). Thai-Chinese sample group showed also result but not statistical significance [RR = 1.36; 95%CI = 0.68–2.81]. Exceptionally, Thai sample group was not found risk of NPC development [RR = 0.91; 95%CI = 0.59–1.40]. Interestingly, ARL pattern of this gene was not agreed since there is no distinction between heterozygous and homozygous variant (Table10). This data suggests that the *pIgR* RFLP may link to an ancient functional variant allele, especially from Chinese population and having heterozygous or homozygous of this allele can contribute to NPC susceptibility.

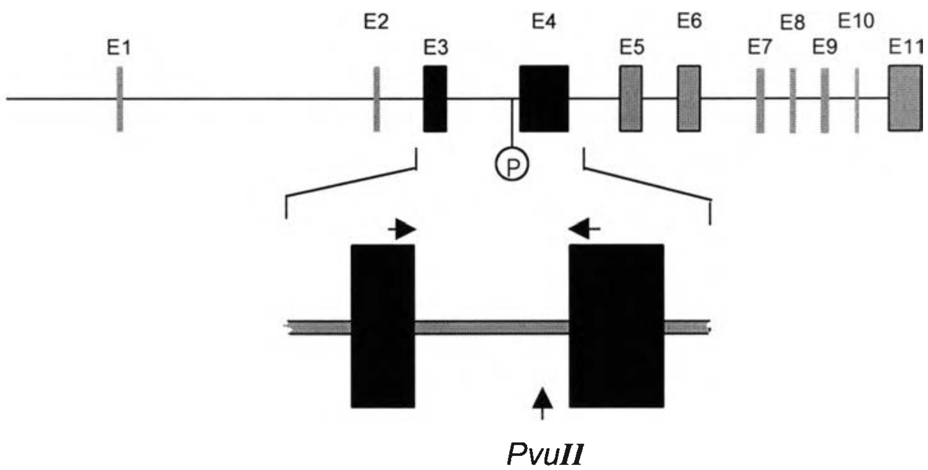

Figure10 Schematic representation of cleavage site for *PvuII* ((P)) on the *pIgR* gene

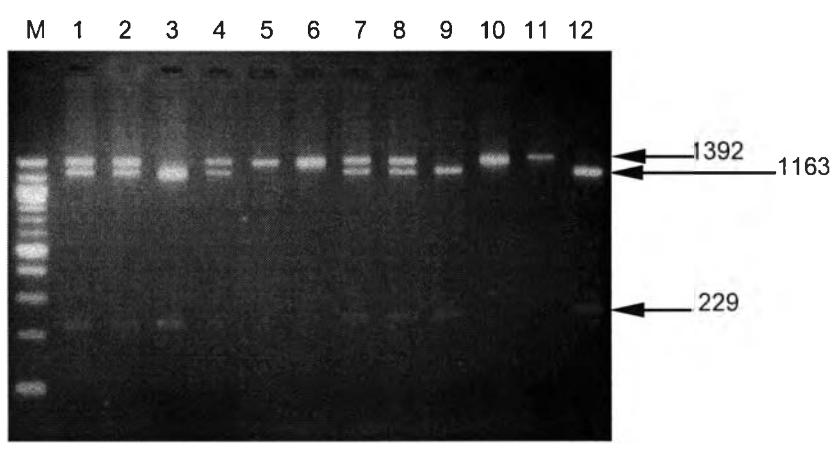

Figure11 PCR-RFLP assay to detect the polymorphism of the *pIgR* gene by *PvuII* enzyme digesting the 1392bp PCR products into 1163bp and 229bp fragments. M, molecular weight marker; Lanes [1,2,4,7,8], heterozygous (+/-); Lanes [3,9,12], homozygous wild-type (+/+); Lanes [5,6,10,11], homozygous variant (-/-).

Table8 Frequency distribution and relative risks associated with genotype variants of *pIgR* detected RFLP using *PVUII*

pIgR	Frequency		RR	95%CI
	Cases	Controls		
Total	**224**	**296**		
+/+	63	104	1.00	
+/-	124	149	1.37	1.03-1.83
-/-	37	43	1.42	0.95-2.12
Thai	**122**	**99**		
+/+	37	28	1.00	
+/-	67	58	0.87	0.56-1.37
-/-	18	13	1.05	0.54-2.03
Chinese	**50**	**97**		
+/+	9	37	1.00	
+/-	29	43	2.77	1.63-4.73
-/-	12	17	2.90	1.30-6.39
Thai-Chinese	**25**	**100**		
+/+	8	39	1.00	
+/-	14	48	1.42	0.69-3.01
-/-	3	13	1.13	0.33-3.42

* 27 cases lack precise information regarding ethnicity

Table9 Correlation between Autosomal Dominant Like (ADL) pattern of genetic *pIgR* and NPC phenotype

pIgR	Frequency		$RR_{(ADL)}$	95%CI
	Cases	Controls		
Total				
+/+	63	104	1.00	
+/- and -/-	161	192	1.38	1.05-1.82
Thai				
+/+	37	28	1.00	
+/- and -/-	85	71	0.91	0.59-1.40
Chinese				
+/+	9	37	1.00	
+/- and -/-	41	60	2.81	1.52-5.34
Thai-Chinese				
+/+	8	39	1.00	
+/- and -/-	17	61	1.36	0.68-2.81

Table10 Correlation between Autosomal Recessive Like (AR) pattern of genetic *pIgR* and NPC phenotype

pIgR	Frequency		$RR_{(ARL)}$	95%CI
	Cases	Controls		
Total				
+/+	63	104	1.00	
-/-	37	43	1.42	0.95-2.12
+/-	124	149	1.00	
-/-	37	43	1.03	0.71-1.49
+/+ and +/-	187	253	1.00	
-/-	37	43	1.16	0.82-1.65
Thai				
+/+	37	28	1.00	
-/-	18	13	1.05	0.54-2.03
+/-	67	58	1.00	
-/-	18	13	1.20	0.66-2.20
+/+ and +/-	104	86	1.00	
-/-	18	13	1.14	0.64-2.06
Chinese				
+/+	9	37	1.00	
-/-	12	17	2.90	1.30-6.39
+/-	29	43	1.00	
-/-	12	17	1.05	0.54-2.03
+/+ and +/-	38	80	1.00	
-/-	12	17	1.49	0.78-2.77
Thai-Chinese				
+/+	8	39	1.00	
-/-	3	13	1.13	0.33-3.42
+/-	14	48	1.00	
-/-	3	13	0.79	0.24-2.23
+/+ and +/-	22	87	1.00	
-/-	3	13	0.91	0.29-2.45