CHAPTER IV

RESULTS

1.SNPs finding.

The study of the association or linkage of DNA polymorphisms was the most popular method to find genes that were resposible for multifactorial diseases. In order to prove whether *PIGR* was responsible for NPC development the SNPs were searched or selected from previously related articles or reports from genbank. The RFLP polymorphism of *PIGR* was previously published in intron 3, *PIGR*389-156G,[53] and this position was previously reported the correlation with NPC development by our group. [9]

Furthermore, several SNPs were identified by three methods. The first two SNPs, 966A→C and 2150C→T, were chosen from http://www.bioinformatics.ucla.edu/snp database. The 966A→C was a silence mutation which locating on exon 4. The 2150C→T was a missense mutation on exon 10 which change amino acid from Serine to Glycine. The second group was found by using BLAST program to explore highly frequent mismatch which methodology was presented as following: First, the *PIRG* mRNA sequence, genbank accession number s62403, was used as a template to search for homology sequences using BLAST program,http:///www.ncbi.nlm.gov. The result revealed homology of many clones with marked nucleotide mismatches in each. Mismatch positions were counted if the same position was demonstrated from at least two independent clones from each nucleotide the position would be defined as the SNP. The result of the SNPs genbank searching was shown in table 1. Six positions were found from this method. Four SNPs do not change amino acid, 549G→A, 1773C→T, 2461C→T and 2596G→C(A). Whereas two positions were missense mutation, 1093G→A and1739C→T. Lastly, one additional SNP, 1773C→T, was discovered by our preliminary sequence result. Conclusively, we have chosen 5 SNPs from three methods as the marker in this study; 1773C→T, 966A→C, 2150C→T, 1093G→A and 1739C→T.

Table1 The result of SNPs finding.

SNPs Positions	Pattern Polymorphisms
549 (silence mutation)	G 5 clone, A 4 clone
1093 (missense mutation Gly to Ser)	G 6 clone , A 4 clone
1739 (missense mutation Ala to Val)	C 15 clone , T 3 clone
1773 (silent mutation)	T 14 clone, C 3 clone
2461 (3' UTR)	C 37 clone, T 6 clone
2596 (3' UTR)	G 25 clone, C 3 clone, A 2 clone

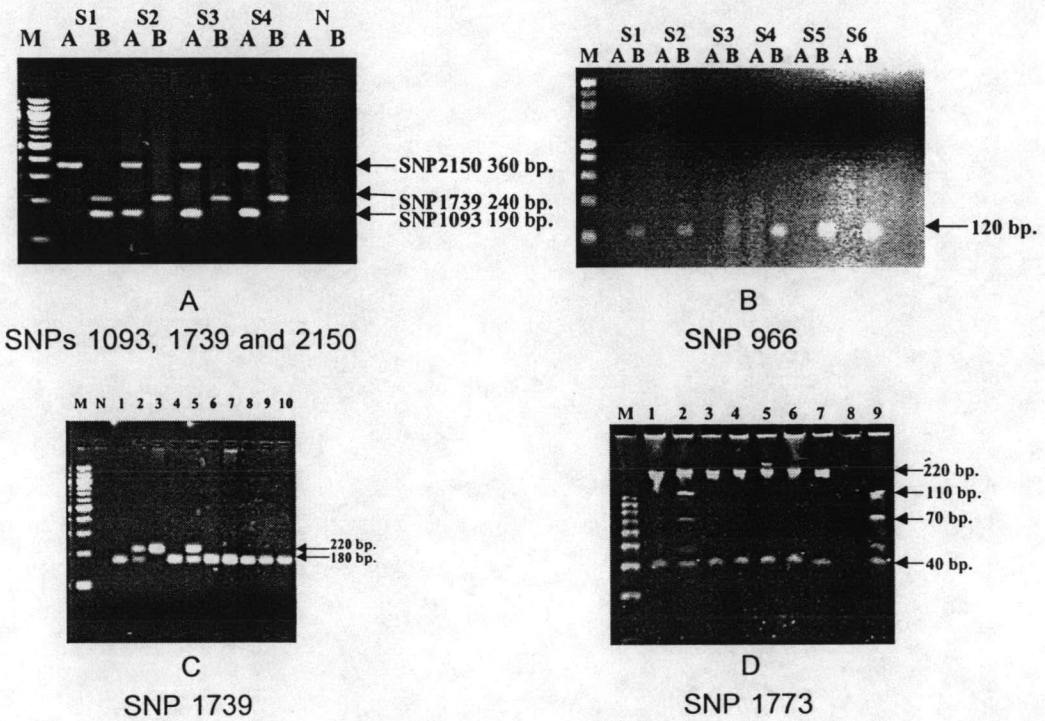

Figure 8 SNPs analysis of *PIRG* were investigated by ARMS and PCR-RFLP. (A) *PIGR*1093, 1739 and 2150 were detected by multiplex ARMS, N was the negative control for primer sets A and B. Sample S2, S3 and S4 were homozygous 1093G, 1739C and 2150G whereas S1 was homozygous 1093A, 1739C and 2150G. (B) *PIGR*966 was detected by single ARMS. Sample S1-S5 were homozygous T and 120 bp was the PCR product. (C) RFLP analysis of 220 bp *PIGR*1739 PCR products with *HgaI* digestion yielded 180 and 40 bp DNA fragments. Sample 1,4,6,7,8,9 and 10 were homozygous CC, sample 2 and 5 were heterozygous CT and sample 3 was homozygous TT, N represented negative control. (D) RFLP analysis of 220 bp *PIGR*1773 PCR products with *MspI* digestion yielded 110, 70 and 40 bp DNA fragments. Sample 1,3,4,5,6 and 7 were homozygous TT, sample 2 was heterozygous CT and sample 9 was homozygous CC. Sample 8 could not amplify.

Table 2 Genotyped of cases and controls at the SNP1093, 1739 and 1773 dividing by their ethnic.

SNPs	ETHNIC	THAI	CHINESE	THAI-CHINESE	TOTAL
1093 (ARMS)	case				
	G/G	62	27	11	100
	G/A	59	27	11	97
	A/A	13	2	5	20
	Control				
	G/G	50	60	48	158
	G/A	54	44	56	154
	A/A	13	14	17	44
1739 (*Hgal*)	case				
	C/C	98	40	24	162
	C/T	28	13	2	43
	T/T	6	1	1	8
	Control				
	C/C	76	67	59	202
	C/T	41	45	55	141
	T/T	6	10	3	19
1773 (*MspI*)	case				
	C/C	3	-	-	3
	C/T	25	11	6	42
	T/T	104	43	21	168
	Control				
	C/C	1	3	1	5
	C/T	22	33	19	74
	T/T	100	86	97	283

There were two approaches to detect the polymorphisms in this study, ARMS and RFLP. ARMS was used to genotype 4 SNPs; 966A→C, 1093G→A, 1739C→T and 2150C→T. In addition, PCR-*Hgal* digestion was used to detect 1739C→T again to

confirm the accuracy of the previous ARMS result. 1773C→T was genotyped by PCR-*MspI* digestion. (figure 8) The 966A→C and 2150C→T could not be found as the polymorphism in this Southeast Asian population whereas genotyped result of 222 cases and 368 controls, divided into three groups; Thai, Chinese and Thai-Chinese, of 1093G→A, 1739C→T and 1773C→T were reported in table 2.

2.Association between *PIGR* SNPs and NPC development.

Three position of SNPs were informative, 1093, 1739 and 1773. In conclusion, *PIRG* was a NPC susceptibility gene at nucleotide 1739C→T as supporting by two reasons. First, the number of alleles between the patients and the controls were compared among the same ethnic group. *PIGR*1739 showed higher relative risk and significant in all groups. *PIGR*1739 revealed OR(95%CI)=1.54(1.10-2.15) from the Thai, 2.25(1.43-3.56) from Chinese and 4.41(2.00-10.09) in Thai-Chinese. On the contrary, no significant OR could be demonstrated at position *PIGR*1093 and *PIGR*1773. When include three ethnic groups together, the significant OR of *PIGR*1739 was remarkably high with p value of less than 0.00000001.

In addition, the consequence of patients'genotypes was evaluated. (table 3) The affect of *PIGR*1739C was similar to autosomal recessive in which two alleles were required to increase the likelihood of NPC development, OR (95%) = 2.52 (1.91-3.31). Furthermore, there was no risk of deviation between heterozygous 1739 and homozygous 1739T. In contrast to *PIGR* 1739C→T, the genotype 1093 of the Chinese, but not the Thai, NPC was similar to autosomal dominant in which their significant OR was discovered when heterozygous and homozygous of lower risk allele were compared. The conflicts of genotype contribution among *PIGR* polymorphisms could be explained because of the linkage between 1093G→A and 1739C. The relative risk from this study of the Chinese with homozygous 1739C and heterozygous 1093 was more than homozygous 1093A, OR (95%CI) = 4.52 (1.39-16.30), but not the Thai, 1.47 (0.75-2.88) from this study.

Table 3 P value, odds ratio, and 95%CI of SNP at *pIgR* 1093, *pIgR* 1739 and *pIgR* 1773 calculated by comparing NPC patients and healthy blood donor.

ETHNIC	(Allele1)/(Allele2)	(Homozygous allele1)/(Total-Homozygous allele1)	(Homozygous allele1+Heterozygous)/(Homozygous allele2)	(Homozygous allele1)/ (Heterozygous)	(Heterozygous)/ (Homozygous allele2)
*PIGR*1093		Allele 1=G	Allele 2=A		
THAI	183/85,154/80	62/72, 50/67	121/13, 104/13	62/59, 50/54	59/13, 54/13
	0.85<1.12<1.47	0.80<1.15<1.67	0.63<1.16<2.15	0.77<1.13<1.67	0.57<1.09<2.08
	p=0.445	p=0.480	p=0.712	p=0.567	p=0.894
CHINESE	81/31,164/72	27/29, 60/58	54/2, 104/14	27/27, 60/44	27/2, 44/14
	0.79<1.15<1.66	0.56<0.90<1.45	1.17<3.63<12.56	0.45<0.73<1.20	1.33<4.30<15.34
	p=0.500	p=0.731	p=0.022	p=0.238	p=0.011
TC	33/21,152/90	11/16, 48/73	22/5, 1.4/17	11/11, 48/56	11/5, 56/17
	0.59<0.93<1.46	0.55<1.05<1.99	0.31<0.72<1.69	0.58<1.17<2.35	0.27<0.67<1.68
	p=0.826	p=0.993	p=0.535	p=0.766	p=0.474
TOTAL	297/137,470/242	100/117, 158/198	197/20, 312/44	100/97, 158/154	97/20, 154/44
	0.93<1.12<1.34	0.84<1.07<1.37	0.92<1.39<2.10	0.77<1.00<1.30	0.90<1.39<2.14
	p=0.250	p=0.617	p=0.123	p=0.979	p=0.150
*PIGR*1739		Allele 1=C	Allele 2=T		
THAI	224/40,193/53	98/34, 76/47	126/6, 117/6	98/28, 76/41	28/6, 41/6
	1.10<1.54<2.15	1.20<1.78<2.65	0.44<1.08<2.62	1.24<1.89<2.88	0.86<0.68<1.77
	p=0.011	p=0.0035	p=0.975	p=0.002	p=0.525
CHINESE	93/15,179/65	40/14, 67/55	53/1, 112/10	40/13, 67/45	13/1, 45/10
	1.43<2.25<3.56	1.39<2.35<3.99	1.04<4.73<29.87	1.20<2.07<3.59	0.59<2.89<19.17
	p=0.0003	p=0.001	p=0.043	p=0.008	p=0.260
TC	50/4,173/61	24/3, 59/58	26/1, 114/3	24/2, 59/55	2/1, 55/3
	2.00<4.41<10.09	3.08<7.86<21.30	0.12<0.68<5.06	5.7<11.19<37.83	0.01<0.11<1.07
	p=0.000052	p=0.00000062	p=0.999	p=0.0000002	p=0.063
TOTAL	367/59,545/179	162/51, 202/160	205/8, 343/19	162/43, 202/141	43/8, 141/19
	1.62<2.04<2.58	1.91<2.52<3.31	0.76<1.42<2.69	1.96<2.63<3.52	0.37<0.72<1.43
	p=0.00000000	p=0.0000	p=0.312	p=0.0000	p=0.406
*PIGR*1773		Allele 1=C	Allele 2=T		
THAI	31/233,24/222	3/129,1/122	28/104,23/100	3/25,1/22	25/104,22/100
	0.81<1.23<1.87	0.51<2.84<20.52	0.74<1.17<1.85	0.45<2.64<20.06	0.68<1.09<1.75
	p=0.358	p=0.333	p=0.551	p=0.417	p=0.786
CHINESE	11/97,39/205	0/54,3/119	11/43,36/86	0/11,3/33	11/43,33/86
	0.35<0.60<1.01	unidentified	0.34<0.61<1.09	unidentified	0.37<0.67<1.19
	p=0.056	p=0.233	p=0.099	p=0.373	p=0.187

ETHNIC	(Allele1)/(Allele2)	(Homozygous allele1)/(Total-Homozygous allele1)	(Homozygous allele1+Heterozygous)/(Homozygous allele2)	(Homozygous allele1)/(Heterozygous)	(Heterozygous)/(Homozygous allele2)
TC	6/48,21/213	0/27,1/116	6/21,20/97	0/6,1/19	6/21,19/97
	0.61<1.27<2.61	unidentified	0.63<1.39<3.02	unidentified	0.66<1.46<3.19
	p=0.615	p=0.821	p=0.494	p=0.949	p=0.414
TOTAL	48/378,84/640	3/210,5/357	45/168,79/283	3/42,5/74	42/168,74/283
	0.73<0.97<1.27	0.33<1.02<3.07	0.71<0.96<1.30	0.33<1.06<3.31	0.70<0.96<1.30
	p=0.861	p=0.824	p=0.839	p=0.870	p=0.828

3.Association between *PIGR* haplotype and NPC.

To test the hypothesis that 1739C→T or another linked nonsynonemous mutation was responsible for NPC development, haplotype analysis was important. Regarding as SNPs association, *PIGR*1773 could not show association and was a silence mutation. Hence, haplotype *PIGR*1093-1739 was analyzed the significant between haplotype and implying the specific roles of each SNPs. Haplotype was arranged in four forms, 1093G-1739T(GT), 1093G-1739C(GC), 1093A-1739T(AT) and 1093A-1739C(AC) depending on all possible combinations of the two SNPs.

Haplotype evaluation was categorized into two parts. First, haplotype frequencies and relevant differences between each ethnic groups were calculated by EH program and presented in table 4. As shown in the table, G-C was most frequent haplotype whereas A-T was the least frequent haplotype in all groups. In addition, case and control of Thai, Thai-Chinese and total were significantly different in haplotype frequencies, p value less than 0.005, but not in Chinese group, p value=0.065. To study the significance of each haplotypes, the actual allele frequencies will be used to compare. In cases which the genotypes were homozygous at least for one marker, their exact genotypes could be directly tabulated. However, haplotype of people with compound heterozygous could not be determined because they would have two possible haplotypes from GA and CT genotypes, GC&AT and GT&AC. This formular N{(EH GC*EH AT)/[(EH GC*EH AT)+(EH GT*EH AC)]} and N{(EH GT*EH AC)/[(EH GC*EH AT)+(EH GT*EH AC)]} was designed to calculate the possibility of haplotype

frequencies of these compound heterozygous by using frequencies from EH and the estimated numbers were presented in table 5.

Table 4 Haplotype frequencies of *PIGR*1093-1739 from EH calculation.

	THAI		CHINESE		THAI-CHINESE		TOTAL	
Haplotype	case	control	case	control	case	control	case	control
G-C	0.539849	0.489836	0.592374	0.461543	0.555556	0.447597	0.553212	0.463242
G-T	0.135723	0.169647	0.119164	0.230764	0.055556	0.176335	0.122979	0.195329
A-C	0.307479	0.281716	0.263395	0.273499	0.388889	0.291719	0.308693	0.285329
A-T	0.016948	0.058801	0.025066	0.034193	0.000000	0.084349	0.015116	0.056100
Case-cont p-value	<0.005		0.065		<0.005		<0.005	

Table 5 Haplotype frequencies of *PIGR*1093-1739

ETHNIC	GGCC	GGCT	GGTT	GACC	GATT	AACC	AACT	GACT *	1	2
	G/C G/C	G/C G/T	G/T G/T	G/C A/C	G/T A/T	A/C A/C	A/C A/T		G/C A/T *	G/T A/C *
THAI										
CASE	36	18	5	50	1	11	2	8	1	7
CONTROL	26	22	2	34	4	9	4	15	6	9
CHINESE										
CASE	16	8	-	20	1	2	-	5	2	3
CONTROL	22	31	6	31	3	11	3	10	2	8
TC										
CASE	8	3	-	11	-	5	-	-	-	-
CONTROL	20	24	1	31	2	8	8	23	10	13
TOTAL										
CASE	60	29	5	81	2	18	2	13	3	10
CONTROL	68	77	9	96	9	28	15	48	18	30

* There are two possible combinations of haplotypes. The estimate number was calculated from probability of haplotype frequency from EH calculation using the following formular N{(EH GC*EH AT)/[(EH GC*EH AT)+(EH GT*EH AC)]} and N{(EH GT*EH AC)/[(EH GC*EH AT)+(EH GT*EH AC)]} for GC, AT (1) and GT, AC (2) combination, respectively

Second part, comparison among the haplotypes implied that it was unlikely to have additional susceptible mutation linked to particular *PIGR* allele but 1739C was the NPC SNP. Whereas 1739C→T was, none of each haplotype revealed considerable risk from both the Thai and the Chinese populations (table 6). The fluctuation of the significant relative risk of each haplotype might be due to the present of the same 1739 polymorphism from another haplotype. To test the hypothesis, the relative risk of each haplotype was reevaluated by excluding the other haplotype with the same 1739 or 1093 SNPs from the comparison (table 6). The value of 1739C→T was confirmed by the method. Both GC and AC became susceptible alleles in which significant OR could be determined from the Thai, the Chinese and the total population. GT, in the Chinese and the total, and AT, in the Thai and the total, were displayed as protective alleles, OR below 1. However, the impact of 1093G→A was not demonstrated. Neither GC and GT nor AC and AT showed the same orientations of relative risk. Finally, the relationship between each haplotype was measured and the data reinforced the importance of 1739C→T (table 6). Whereas the OR between GC and AC was not statistically significant, both haplotypes had higher relative risk than GT or AT. Interestingly, the risk contribution of haplotypes within 1739T subset, GT and AT, was discriminated in the Thai and the total, OR (95%CI) = 3.23 (1.30-8.28) and 2.28(1.20-4.41), respectively. This data suggested that, in addition to 1739C→T, there was a possible functional significant of 1093G→A.

4.*PIGR*1739C→T and gender & age in NPC patients.

NPC is a tumor with 2.5 times higher prevalence in male and has a wide range of onset from very young to old age[95] Distinction threshold was observed in difference of sex and age. Table 7 were presented to evaluate whether 1739C→T could contribute differently among these distinct characteristic of these patients. The NPC patients were divided into two groups, according to their gender or age of onset prior to or after age 40. Comparing 1739C and T in male cases and higher or equal to 40 years cases with controls showed strong association with NPC in all group. Female cases and less than 40 years cases presented significant in some ethnic group. When comparing male with

female and less than 40 years with more than or 40 years were no statistical significance. This data indicated that, gender and age had not affect significantly in the frequency of *PIGR*1739C→T.

Table 6 Haplotype analysis of *PIGR*1093 and *PIGR*1739 in Thai and Chinese groups. Four haplotypes consisting of the GC, GT, AC and AT and excluding interference haplotype.

			THAI	CHINESE	THAI-CHINESE	TOTAL
	Allele	VS Allele	95%CI<OR<95%CI	95%CI<OR<95%CI	95%CI<OR<95%CI	95%CI<OR<95%CI
Haplotype vs Haplotype	GC	AC+GT+AT	141/121,114/118	62/42,108/126	30/24,105/129	233/187,327/373
			0.93<1.21<1.56	1.22<1.72<2.43	0.99<1.54<2.39	1.19<1.42<1.69
			p=0.160	p=0.0017	p=0.058	p=0.00007
	AC	GC+GT+AT	81/181,65/157	27/77,64/170	21/33,68/166	129/291,197/503
			0.87<1.15<1.53	0.63<0.93<1.37	0.98<1.55<2.46	0.93<1.13<1.37
			p=0.630	p=0.778	p=0.061	p=0.212
	GT	GC+AC+AT	36/226,39/193	12/92,54/180	3/51,41/193	51/369,134/566
			0.55<0.79<1.13	0.26<0.43<0.72	0.11<0.28<0.68	0.45<0.58<0.75
			p=0.216	p=0.0007	p=0.0031	p=0.00002
	AT	GC+AC+GT	4/258,14/218	3/101,8/226	0/54,20/214	7/413,42/658
			0.10<0.24<0.56	0.29<0.84<2.32	unidentified	0.14<0.27<0.48
			p=0.0003	p=0.900	p=0.003	p=0.000002
Nucleotide 1739C	GC	GT+AT	141/40,114/53	62/15,108/62	30/3,105/61	233/58,327/176
			1.15<1.64<2.33	1.47<2.37<3.84	2.33<5.81<15.41	1.69<2.16<2.77
			p=0.0054	p=0.0002	p=0.000023	p=0.00000000
	AC	GT+AT	81/40,65/53	27/15,64/62	21/3,68/61	129/58,197/176
			1.12<1.65<2.43	1.02<1.74<3.00	2.45<6.28<17.06	1.52<1.99<2.61
			p=0.010	p=0.044	p=0.000016	p=0.00000033
Nucleotide 1739T	GT	GC+AC	36/222,39/179	12/89,54/172	3/51,41/173	51/362,134/524
			0.52<0.74<1.07	0.26<0.43<0.71	0.09<0.25<0.61	0.43<0.55<0.71
			p=0.116	p=0.0006	p=0.0011	p=0.0000024
	AT	GC+AC	4/222,14/179	3/89,8/172	0/51,20/173	7/362,42/524
			0.10<0.23<0.54	0.25<0.72<2.01	unidentified	0.13<0.24<0.44
			p=0.0002	p=0.666	p=0.0015	p=0.00000029
Nucleotide 1093G	GC	AC+AT	141/85,114/79	62/30,108/72	30/21,105/88	233/136,327/239
			0.89<1.15<1.53	0.93<1.38<2.04	0.75<1.20<1.91	1.03<1.25<1.52
			p=0.363	p=0.112	p=0.493	p=0.023
	GT	AC+AT	36/85,39/79	12/30,54/72	3/21,41/88	51/136,134/239
			0.57<0.86<1.29	0.30<0.53<0.94	0.11<0.31<0.79	0.50<0.67<0.89
			p=0.498	p=0.029	p=0.011	p=0.0046

	Allele	VS Allele	THAI 95%CI<OR<95%CI	CHINESE 95%CI<OR<95%CI	THAI-CHINESE 95%CI<OR<95%CI	TOTAL 95%CI<OR<95%CI
Nucleotide 1093A	AC	GC+GT	81/177,65/153 0.81<1.08<1.43 p=0.64	27/74,64/162 0.63<0.92<1.36 p=0.747	21/33,68/146 0.86<1.37<2.17 p=0.198	129/284,197/461 0.88<1.06<1.29 p=0.558
	AT	GC+GT	4/177,14/153 0.10<0.25<0.58 p=0.0005	3/74,8/162 0.28<0.82<2.29 p=0.866	0/33,20/146 unidentified p=0.006	7/284,42/461 0.15<0.27<0.49 p=0.00000362
Haplotype vs Haplotype	GC	AC	141/70,114/65 0.85<1.15<1.56 p=0.400	62/27,108/64 0.91<1.36<2.04 p=0.144	30/21,105/68 0.58<0.93<1.49 p=0.824	233/129,327/197 0.89<1.09<1.33 p=0.429
	GC	AT	141/70,4/14 2.97<7.05<17.29 p=0.00000028	62/27,3/8 2.10<6.1218.66 p=0.00024	30/0,105/20 unidentified p=0.002	233/129,51/134 5.86<10.84<20.39 p=0.00000000
	GC	GT	141/70,36/39 1.47<2.18<3.25 p=0.000071	62/27,12/54 5.79<10.33<18.56 p=0.00000000	30/3,105/41 1.55<3.90<10.46 p=0.002	233/129,51/134 3.57<4.75<6.30 p=0.00000000
	AC	AT	114/65,4/14 2.58<6.14<15.11 p=0.00000334	108/64,3/8 1.60<4.50<13.25 p=0.002	21/0,68/20 unidentified p=0.0015	327/197,7/42 5.42<9.96<18.60 p=0.00000000
	AC	GT	114/65,36/39 1.27<1.90<2.85 p=0.0015	108/64,12/54 4.52<7.59<12.84 p=0.00000000	21/3,68/41 1.63<4.22<11.58 p=0.0015	327/197,51/134 3.34<4.36<5.71 p=0.00000000
	GT	AT	36/39,4/14 1.30<3.23<8.28 p=0.0092	12/54,3/8 0.19<0.59<1.90 p=0.483	3/0,41/20 unidentified p=0.218	51/134,7/42 1.20<2.28<4.41 p=0.010

(95%CI)<OR<(95%CI) = odd ratios and 95% confidence interval between allele and compared allele,

(case:control) = number of alleles of case and control haplotype respectively.

GC, AC, GT, and AT are 1093G-1739C, 1093A-1739C, 1093G-1739T, and 1093A-1739T haplotypes respectively.

+ = plus number of haplotype alleles.

5.Haplotype frequency of *PIGR*1093-1739 in low risk ethnic group.

On the contrary to the Oriental people, Caucasian is a low risk group in NPC disease. It would be of great interest to investigate *PIGR* genotype between these two populations. The haplotype frequency of *PIGR*1093-1739 of fifty-two Caucasian normal control were genotyped and compared with the normal Thais and Chinese populations. Interestingly, Caucasian's haplotype frequencies were significantly different from the

other ethnic groups. The p values between the Caucasian and Thai, Chinese or Thai-Chinese were 0.035, 0.010 and 0.0062. This data led to an interesting speculation that even though the 1739C susceptibility was not the specific mutation that explained the unique endemic distribution of this disease, the *PIGR* evolution process was different between ethnic with high and low risk in NPC.

Table 7 Sex and age comparison of 1739C /1739T between NPC patients and controls.

	THAI	CHINESE	TC	TOTAL
male case/control	152/26,179/53	50/6,172/62	31/1,173/61	233/33,524/176
	1.18<1.73<2.54	1.54<3.00<5.97	2.57<10.93<65.62	1.77<2.37<3.19
	p=0.004	p=0.0006	p=0.0000935	p=0.00000000
female case/control	68/14,179/53	39/9,172/62	20/2,173/61	127/25,524/176
	0.89<1.44<2.34	0.87<1.56<2.82	1.18<3.53<11.86	1.22<1.71<2.40
	p=0.151	p=0.144	p=0.021	p=0.0016
Male case/female case	152/26,68/14	50/6,39/9	31/1,20/2	233/33,127/25
	0.71<1.20<2.05	0.82<1.92<4.55	0.46<3.10<25.75	0.92<1.39<2.11
	p=0.554	p=0.150	p=0.371	p=0.128
<40 case/control	80/10,179/53	14/2,172/62	13/1,173/61	107/13,524/176
	1.38<2.37<4.09	0.82<2.52<8.67	1.04<4.58<28.38	1.78<2.76<4.33
	p=0.001	p=0.123	p=0.044	p=0.00000177
>=40 case/control	127/25,179/53	70/12,172/62	34/2,173/61	231/39,524/176
	1.02<1.50<2.22	1.27<2.10<3.50	2.05<5.99<19.75	1.51<1.99<2.63
	p=0.039	p=0.0029	p=0.00024	p=0.00000051
<40 case/>=40 case	80/10,127/25	14/2,70/12	13/1,34/2	107/13,231/39
	0.88<1.57<2.85	0.36<1.20<4.44	0.11<0.76<6.45	0.85<1.39<2.29
	p=0.140	p=0.969	p=0.867	p=0.210

Table 8 Fifty two Caucasian controls were calculated haplotype frequencies and comparing frequencies between controls in Thai,Chinese and Thai-Chinese ethnics by EH program.

	Haplotype frequencies				p-value of control-control		
	CAUCASIAN	THAI	CHINESE	THAI-CHINESE	THAI	CHINESE	THAI-CHINESE
G-C	0.501139	0.489836	0.461543	0.447597			
G-T	0.104630	0.169647	0.230764	0.176335	0.035	0.010	0.0062
A-C	0.383476	0.281716	0.273499	0.291719			
A-T	0.010754	0.058801	0.034193	0.084349			

6.Other mutation in *PIGR* gene.

6.1 Polymorphism finding by DNA sequencing.

SNPs 1739C→T showed higher relative risk than haplotype. This data suggested that 1739C→T was the most important position of *PIRG* in association with NPC. To confirm this result, DNA samples obtained from eight patients were amplified and directly sequenced to find others polymorphism in all exons of the *PIGR*, except exon 6. As the result, no additional mutation was found in this region.

6.2 3'UTR polymorphism.

From the study of Fabregat and colleague reporting that 3'UTR of rat Pigr had the important role in transcription.[93] 3'UTR poly-dispersed simple tandemly repeated microsatellites (STMSs) was composed of an R-Y element, purine-rich, that consisted of a 60-nt G-rich tract, followed by two neighboring GGA and GAA triplet repeat motifs, $(GGA)_{n=11-15}$, $(GAA)_{n=39-60}$. This element was conserved in mouse Pigr gene. The results

showed that functional pleitropy of this fragment depends on the DNA context of its purine-rich microsatellite strand and an DNA supercoiling. Intramolecular triplexes stabilized by supercoiling and secondary structures of purine repeat-rich mRNAs may also confer regulatory properties to similar genomic elements. To identify if there was the polymorphisms responsible to NPC development, a PCR protocol was designed to characterize human 3'UTR. Twenty patients were amplified and compared the length of this region. As the result, there was no polymorphism in human 3' UTR in any studied patients.