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STK15 Phe31Ile and Val57Ile Polymorphisms Increase the Risk of Gastrointestinal Cancer

(Polimorfisme *STK15* Phe31Ile dan Val57Ile Meningkatkan Risiko terhadap Kanser Gastrousus)

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ABSTRACT

*STK15 is a serine/threonine kinase that regulates chromosomal segregation during mitosis. Single nucleotide polymorphisms (SNPs) in this gene, Phe31Ile (rs2273535) and Val57Ile (rs1047972), are inconsistently associated with gastrointestinal cancer (GIC) across different populations. However, this association is unclear in Malaysian population. Therefore, this study investigated the association of STK15 Phe31Ile and Val57Ile polymorphisms to GIC risk in Malaysia. Genomic DNA was extracted from 185 GIC patients and 1110 healthy controls and was subjected to polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. SNPs were further confirmed using sequencing. We found that the 31Phe allele and 31Phe/Phe genotype in the Phe31Ile SNP significantly increased GIC risk in Malaysian population, particularly in gastric cancer (*p*<0.017). The combined analysis for both SNPs also increased the risk of GIC in this study. Etiological factors such as age, gender and ethnicity were not associated with GIC in the population. This is the first study to report the association of STK15 Phe31Ile and Val57Ile SNPs with an increased risk of GIC in Malaysians; the 31Phe allele is exclusively associated with the risk of gastric cancer. In addition, GIC incidences among Malaysians have significantly shifted to a younger age (<50 years).*

Keywords: Gastrointestinal cancer; Malaysian population; *STK*15 polymorphisms

ABSTRAK

*STK15 adalah kinase serin/treonina yang mengawal perpisahan kromosom semasa mitosis. Polimorfisme nukleotida tunggal (SNPs) pada gen ini, Phe31Ile (rs2273535) dan Val57Ile (rs1047972) adalah dikaitkan dengan kanser gastrousus (GIC) secara tidak tekal dalam populasi yang berbeza. Walau bagaimanapun, perkaitan tersebut adalah tidak jelas di dalam populasi di Malaysia. Oleh itu, penyelidikan ini mengkaji perkaitan bagi polimorfisme STK15 Phe31Ile dan Val57Ile terhadap risiko GIC di Malaysia. DNA genom diekstrak daripada 185 pesakit GIC dan 1110 kawalan yang sihat. Seterusnya, analisis tindak balas berantai polimerase pemotongan panjang cebisan (PCR-RFLP) dijalankan dan SNP turut disahkan dengan menggunakan teknik penjujukan DNA. Kami mendapati bahawa alel 31Phe dan genotip 31Phe/Phe dalam SNP Phe31Ile meningkatkan risiko terhadap GIC dalam populasi di Malaysia secara signifikan, terutamanya dalam kanser gastrik (*p*<0.017). Analisis gabungan bagi kedua-dua SNP juga meningkatkan risiko terhadap GIC dalam kajian ini. Faktor etiologi seperti umur, jantina dan etnik adalah tidak berkait dengan GIC dalam populasi ini. Kajian ini merupakan kajian pertama yang melaporkan tentang perkaitan antara SNP STK15 Phe31Ile dan Val57Ile dengan peningkatan risiko terhadap GIC di Malaysia; terutamanya alel 31Phe yang dikaitkan dengan risiko kanser gastrik. Selain itu, kejadian GIC dalam kalangan rakyat Malaysia telah beralih secara signifikan kepada usia yang lebih muda (<50 tahun).*

Kata kunci: Kanser gastrousus; polimorfisme STK15; populasi Malaysia

INTRODUCTION

STK15 is a serine/threonine kinase involved in mitotic entry, separation of centriole pairs, accurate bipolar spindle assembly, and chromosome alignment during the metaphase in a cell (Dutertre et al. 2002). *STK15* maps to chromosome 20q13.2, a common site of amplification in tumours. It has been shown to have a low penetrance cancer susceptibility gene that affects multiple cancer types (Ewart-Toland et al. 2005). Over-expression of this gene is oncogenic because it causes centrosome amplification, chromosome instability, transformation *in vitro* and tumorigenesis in mammalian cell lines (Bischoff et al. 1998; Sakakura et al. 2001). A recent study also reported a significant up-regulation of *STK15* in esophageal squamous cell carcinoma tumors when compared to normal tissues (Chen et al. 2014).

There are two polymorphic sites of *STK15*, namely, the Phe31Ile (rs2273535) and Val57Ile (rs1047972) that are reported to affect the pathogenesis of cancer. These two single nucleotide polymorphisms (SNPs) are in the conserved region of the NH2-terminal region that is involved in ubiquitin-based proteolysis (Honda et al. 2000). Amino acid 31Phe binds with the *E2* ubiquitin conjugating enzyme *UBE2N* during mitosis at the centrosome. Loss of

UBE2N binding function with the *STK15* variant has been reported to increase susceptibility to human cancer (Ewart-Toland et al. 2003). The function of Val57Ile SNP has not been identified. However, it might be important for kinases activity as the 57Ile homozygote genotype has 57.5% less kinases activity and showed more chromosome instability than the 57Val homozygote genotype (Dogan et al. 2008). These two SNPs are associated independently with both sporadic and hereditary cancers, especially in gastrointestinal cancer (GIC) including esophageal, gastric, pancreas, and colorectal as well as other cancers such as breast, ovarian and lung through case-control or meta-analysis studies in different populations (Chen et al. 2014, 2007; Chong et al. 2016; Dai et al. 2014; Ju et al. 2006; Kimura et al. 2005; Qin et al. 2015; Tang et al. 2013; Xu et al. 2014). We have previously reported that carriers with at least one copy of the c2 allele in the *CYP2E1* gene was associated to GIC in Malaysians (Chong et al. 2014), but the association of the Phe31Ile and Val57Ile SNPs in *STK15* to GIC in the population is still unclear. Furthermore, previous studies of these SNPs in different populations showed contradicting outcomes and inconsistent associations. Therefore, we conducted this study to screen the frequency of these *STK15* SNPs and to determine the association with the risk of getting GIC in Malaysian GIC patients.

Materials and Methods

Subjects and sample collection

A total of 185 GIC patients (gastric = 41, colorectal = 126, esophageal = 4, pancreas = 7, liver = 3 and anus = 4) who were admitted to Queen Elizabeth Hospital, Kota Kinabalu, Sabah and University Malaya Medical Centre, Kuala Lumpur and 1110 healthy volunteers randomly selected during blood donation campaigns were recruited from 2010 to 2014. This study conforms to The Code of Ethics of the World Medical Association (Declaration of Helsinki) and written consent was obtained from all subjects. Five mL of peripheral blood was obtained in BD Vacutainer® containing EDTA (Becton Dickinson, New Jersey, USA), and data including age, gender and ethnicity were collected from all subjects. Clinical characteristics for GIC and lifestyles of the subjects were not available as data collection was limited; this study solely focused on the association of the *STK15* gene polymorphisms to GIC. Overall, 68.03% of the study population were male, while 31.97% were female with age ranging from 15 to 93 years (mean age ± S.D. = 31.93 ± 14.83) and comprised of Malaysian Chinese (22.08%), Malay (9.03%), Malaysian Indian (3.71%), Kadazan-Dusun (24.40%), Bajau (9.34%) and other ethnicities (31.44%) that are indigenous in East Malaysia. This study was approved by Sabah State Health Department and Queen Elizabeth Hospital, Kota Kinabalu and the ethical approval was obtained from the University Malaya Medical Centre (UMMC) Ethical Board with Ref. 654.1.

STK15 genotyping and direct sequencing

Genomic DNA was extracted using an alkaline phenol-chloroform lysis method from whole blood, and genotyping was carried out using a polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) analysis. Primers used were 5'-CTTTCATGAATGCCAGAAAGTT-3'/5'-CTGGGAAGAATTTGAAGGACA-3' for Phe31Ile SNP and 5'-CTTTCATGAATGCCAGAAAGTT-3'/5'-CTGCTTCTGATTCTGAACCGGCTTG-3' for Val57Ile SNP studies, respectively. The PCR reaction was carried out in a 25 µL volume containing 100 ng of extracted DNA, 0.2 µM of each primer, 0.2 mM of each dNTPs, 1.5 mM of MgCl2 and 1 unit of HotStarTaq® DNA polymerase (Qiagen, Germany). The reaction was performed with the following conditions: 1 cycle for 15 min at 95°C; 35 cycles for 30 s at 94°C, 30 s at 55°C and 30 s at 72°C; and a final elongation step for 7 min at 72°C for both primer sets. The PCR product was digested with 5 units of *Apo*I (NEB Inc., Ipswich, MA) at 50°C overnight for Phe31Ile polymorphism and 5 units of *Bst*UI (NEB Inc., Ipswich, MA) at 60°C overnight for Val57Ile polymorphism. The resulting fragments were analyzed with 3% agarose gel electrophoresis. PCR products for both polymorphisms were sequenced using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, USA) for confirmation.

Statistical analysis

The Hardy-Weinberg equilibrium (HWE) was tested for genotype distribution in controls and cases for both polymorphisms, which were represented by χ^2 value (within HWE if $\chi^2 < 3.84$, $df = 1$). By taking the 31Ile and 31Ile/Ile for Phe31Ile and 57Val and 57Val/Val for Val57Ile SNPs as reference, SPSS Software V17.0 (SPSS Inc, Chicago, Illinois, USA) was used to estimate the allelic and genotypic interactions in GIC development by calculating the odd's ratio (OR) and 95% confidence interval (95% CI). The interactions were statistically significant when the *p*-value was less than 0.05 using the Fisher's exact test. For the sub-group analysis, the association test was considered statistically significant only after Bonferroni correction.

Results

Genotype distributions were tested for Phe31Ile (cases, $\chi^2 = 0.30$; controls, $\chi^2 = 1.22$) and Val57Ile (cases, $\chi^2 = < 0.01$; controls, $\chi^2 = 0.55$) SNPs and they were within the HWE. The heterozygousity and homozygousity for both SNPs were sequenced and matched with the PCR-RFLP analysis data. For the Phe31Ile SNP, the 31Ile and 31Phe allele frequencies among all the subjects were 59.19% and 40.81%, respectively. The genotype frequency was 34.29% for 31Ile/Ile, 49.81% for 31Phe/Ile and 15.90% for 31Phe/Phe (Table 1). The 31Phe allele and 31Phe/Phe genotype showed a strong significant association with an increased risk of GIC, especially in gastric cancer with Bonferroni correction (Table 2).

TABLE 1. Risk association of *STK15* Phe31Ile and Val57Ile polymorphisms to GIC in Malaysians

	Cases, *N*	Controls, *N*	OR (95% CI)	*p*-value
Phe31Ile SNP				
Allele				
31 Ile	194	1339	1.00 (Reference)	-
31 Phe	176	881	1.38 (1.11 – 1.72)	0.005*
Genotype				
31 Ile/Ile	49	395	1.00 (Reference)	-
31 Phe/Ile	96	549	1.41 (0.98 – 2.04)	0.070
31 Phe/Phe	40	166	1.94 (1.23 – 3.06)	0.005*
31 Phe/Ile + 31 Phe/Phe	136	715	1.53 (1.08 – 2.17)	0.015*
Val57Ile SNP				
Allele				
57 Val	303	1872	1.00 (Reference)	-
57 Ile	67	348	1.19 (0.89 – 1.59)	0.251
Genotype				
57 Val/Val	124	786	1.00 (Reference)	-
57 Val/Ile	55	300	1.16 (0.82 – 1.64)	0.419
57 Ile/Ile	6	24	1.59 (0.64 – 3.95)	0.289
57 Val/Ile + 57 Ile/Ile	61	324	1.19 (0.86 – 1.66)	0.298
Phe31Ile + Val57Ile SNPs				
Allele				
31 Ile + 57 Val	497	3211	1.00 (Reference)	-
31 Phe + 57 Ile	243	1229	1.28 (1.08 – 1.51)	0.005*
Genotype				
31 Ile/Ile + 57 Val/Val	173	1181	1.00 (Reference)	-
31 Phe/Ile + 57 Val/Ile	151	849	1.21 (0.96 – 1.54)	0.116
31 Phe/Ile + 57 Ile/Ile	102	573	1.22 (0.93 – 1.58)	0.149
31 Phe/Phe + 57 Val/Ile	95	466	1.39 (1.06 – 1.83)	0.020*
31 Phe/Phe + 57 Ile/Ile	46	190	1.65 (1.15 – 2.37)	0.008*

*, statistical significant ($p < 0.05$)
SNP = single nucleotide polymorphism, *N* = number of samples, OR = odd ratio, CI = confidence interval, Ile = isoleucine, Phe = phenylalanine, Val = valine

In the Val57Ile SNP analysis, 83.98% of allele frequency was the 57Val, while for 57Ile, it was 16.02% in this study. The genotype distributions for 57Val/Val, 57Val/Ile and 57Ile/Ile were 70.27%, 27.41%, and 2.32%, respectively. There was no significant evidence for this SNP to increase the risk of GIC, but the combined analysis of the Phe31Ile and Val57Ile SNPs showed that the 31Phe + 57Ile alleles as well as the 31Phe/Phe + 57Val/Ile and 31Phe/Phe + 57Ile/Ile genotypes increased the risk to GIC significantly in the Malaysian population. Etiological factors including age, gender and ethnicity were not associated with GIC for both SNPs in this study (Tables 3 and 4).

DISCUSSION

To the best of our knowledge, this is the first study to report a significant risk association of the *STK15* Phe31Ile and Val57Ile SNPs with GIC in the Malaysian population by including indigenous ethnics in East Malaysia. By using MuPro software (Cheng et al. 2006), the presence of the Phe31Ile SNP in the *STK15* gene has been predicted to decrease the stability of the protein structure (confidence score = -0.577) whereas the Val57Ile SNP appears to increase the stability of the gene (confidence score = 0.038). The conflicting predictions of both SNPs towards the stability of the protein structure making them worth to be studied, especially their associations to the risk of GIC that encounter approximately 2.9 million of deaths globally in 2012 (IARC 2014) and mortality rate of 4.71 per 0.1 million of populations in Malaysia (Department of Health Statistics Malaysia 2013) and the mortality rate for GIC is projected to increase continuously in the future. In this study, we mainly focused on gastric and colorectal cancers due to our sample limitation.

A previous study reported that the 31Ile allele in Phe31Ile SNP was more common in Asian (Ewart-Toland et al. 2005) and therefore we took it as the reference allele in this study. We found that the 31Phe allele and the 31Phe/Phe genotype elevated the risk of GIC in our Malaysian sample, mainly in gastric cancer even after the Bonferroni correction; suggesting that the 31Phe allele is highly associated with increased risk of GIC and may act as a biomarker for early detection of gastric cancer in the Malaysian population. Although the presence of 31Phe was not associated with esophageal cancer in this study, contradicting results were previously reported where Kimura et al. (2005) claimed that 31Phe/Phe genotype

TABLE 2. Risk association of *STK15* Phe31Ile and Val57Ile polymorphisms to different types of GIC in Malaysians

	Cases, *N*	Controls, *N*	OR (95% CI)	*p*-value
Phe31Ile SNP				
Gastric				
31 Ile/Ile	6	395	1.00 (Reference)	-
31 Phe/Ile	18	549	2.16 (0.85 – 5.49)	0.106
31 Phe/Phe	17	166	6.74 (2.61 – 17.40)	<0.001*
31 Phe/Ile + 31 Phe/Phe	35	715	3.22 (1.34 – 7.73)	0.009*
Colorectal				
31 Ile/Ile	40	395	1.00 (Reference)	-
31 Phe/Ile	69	549	1.24 (0.82 – 1.87)	0.302
31 Phe/Phe	17	166	1.01 (0.56 – 1.83)	0.971
31 Phe/Ile + 31 Phe/Phe	86	715	1.19 (0.80 – 1.76)	0.393
Others**				
31 Ile/Ile	3	395	1.00 (Reference)	-
31 Phe/Ile	9	549	2.16 (0.58 – 8.02)	0.251
31 Phe/Phe	6	166	4.76 (1.18 – 19.25)	0.029
31 Phe/Ile + 31 Phe/Phe	15	715	2.76 (0.79 – 9.60)	0.110
Val57Ile SNP				
Gastric				
57 Val/Val	25	786	1.00 (Reference)	-
57 Val/Ile	15	300	1.57 (0.82 – 3.02)	0.175
57 Ile/Ile	1	24	1.31 (0.17 – 10.07)	0.795
57 Val/Ile + 57 Ile/Ile	16	324	1.55 (0.82 – 2.95)	0.178
Colorectal				
57 Val/Val	89	786	1.00 (Reference)	-
57 Val/Ile	32	300	0.94 (0.62 – 1.44)	0.783
57 Ile/Ile	5	24	1.84 (0.68 – 4.94)	0.227
57 Val/Ile + 57 Ile/Ile	37	324	1.01 (0.67 – 1.52)	0.967
Others**				
57 Val/Val	10	786	1.00 (Reference)	-
57 Val/Ile	8	300	2.10 (0.82 – 5.36)	0.123
57 Ile/Ile	0	24	-	-
57 Val/Ile + 57 Ile/Ile	8	324	1.94 (0.76 – 4.96)	0.166

*, statistical significant with Bonferroni correction ($p < 0.017$); **, including esophageal ($N = 4$), pancreas ($N = 7$), liver ($N = 3$), and anus ($N = 4$)
SNP = single nucleotide polymorphism, *N* = number of samples, OR = odd ratio, CI = confidence interval, Ile = isoleucine, Phe = phenylalanine, Val = valine

increased the risk of esophageal cancer (OR = 1.93, 95% CI = 0.90-4.16) but Miao et al. (2004) showed that 31Ile/Ile genotype was significantly associated with increased risk of esophageal cancer (OR = 1.97, 95% CI = 1.36-2.85). Interestingly, Pan et al. (2012) reported that 31Phe/Ile genotype was significantly associated with recurrence of esophageal cancer with OR = 4.39 (95% CI = 2.12-8.94). Therefore, SNP association studies targeting specific type of GIC cancers are needed in the future for more precise risk estimation and healthcare approaches given to each cancer should be distinguished empirically in the Malaysian population.

We had no evidence to associate the variant allele and genotype in Val57Ile SNP alone to an increase in the risk of GIC, but past studies showed that the 57Ile/Ile genotype was protective against lung cancer in Caucasians (OR = 0.72, 95% CI = 0.41-1.26) and breast cancer in Han Chinese (OR = 0.8, 95% CI = 0.4-1.6) (Dai et al. 2004; Gu et al. 2007), indicating that the Val57Ile SNP affects different cancer development across different populations. However, combined analysis of both SNPs in this study showed an increase risk to GIC significantly. Since *STK15* is a low penetrance gene, it is discrepant to claim that the gene alone is the significant genetic factor to GIC regardless of the influence of the environmental factors. Therefore, additional work on gene-environmental interactions to GIC development should be conducted for validation.

In this study, age and *STK15* polymorphisms were not interacting factors that contributed to GIC, but we noticed that around 26.49% of GIC patients in our database were less than 50 years old. This statistic was much higher than those previously reported by the Malaysian Gastro-Intestinal Registry (MGIR) with only 18.14% (MGIR 2009); this suggests that GIC cases are becoming common among younger individuals and therefore screening for GIC should be performed before 50 years old among Malaysians to obtain better survival rates from GIC.

Previously, we have reported that females had a nearly two-fold higher risk to GIC in the Malaysian population, but we did not analyze the association of different genotypes to

TABLE 3. Association of age, gender and ethnicity in *STK15* Phe31Ile SNP to GIC

	Cases, *N*	Controls, *N*	OR (95% CI)	*p*-value
Age				
< 50 years				
Ile/Ile	14	379	1.00 (Reference)	-
Phe/Ile	23	531	1.17 (0.60 – 2.31)	0.645
Phe/Phe	12	160	2.03 (0.92 – 4.49)	0.080
Phe/Ile + Phe/Phe	35	691	1.37 (0.73 – 2.58)	0.328
≥ 50 years				
Ile/Ile	35	16	1.00 (Reference)	-
Phe/Ile	73	18	1.85 (0.85 – 4.06)	0.123
Phe/Phe	28	6	2.13 (0.74 – 6.17)	0.162
Phe/Ile + Phe/Phe	101	24	1.92 (0.92 – 4.03)	0.083
Gender				
Male				
Ile/Ile	23	295	1.00 (Reference)	-
Phe/Ile	50	383	1.67 (1.00 – 2.81)	0.061
Phe/Phe	17	113	1.93 (0.99 – 3.75)	0.066
Phe/Ile + Phe/Phe	67	496	1.73 (1.06 – 2.84)	0.028
Female				
Ile/Ile	26	100	1.00 (Reference)	-
Phe/Ile	46	166	1.07 (0.62 – 1.83)	0.891
Phe/Phe	23	53	1.67 (0.87 – 3.21)	0.131
Phe/Ile + Phe/Phe	69	219	1.21 (0.73 – 2.02)	0.526
Ethnicity				
Chinese				
Ile/Ile	18	97	1.00 (Reference)	-
Phe/Ile	33	100	1.78 (0.94 – 3.37)	0.084
Phe/Phe	12	26	2.49 (1.06 – 5.81)	0.057
Phe/Ile + Phe/Phe	45	126	1.93 (1.05 – 3.53)	0.041
Malay				
Ile/Ile	2	38	1.00 (Reference)	-
Phe/Ile	5	50	1.90 (0.35 – 10.33)	0.695
Phe/Phe	0	22	-	-
Phe/Ile + Phe/Phe	5	72	1.32 (0.24 – 7.12)	1.000
Indian				
Ile/Ile	0	5	1.00 (Reference)	-
Phe/Ile	2	21	-	-
Phe/Phe	0	20	-	-
Phe/Ile + Phe/Phe	2	41	-	-
Kadazan-Dusun				
Ile/Ile	14	95	1.00 (Reference)	-
Phe/Ile	27	133	1.38 (0.69 – 2.77)	0.393
Phe/Phe	14	33	2.88 (1.24 – 6.67)	0.021
Phe/Ile + Phe/Phe	41	166	1.68 (0.87 – 3.23)	0.160
Bajau				
Ile/Ile	6	41	1.00 (Reference)	-
Phe/Ile	10	46	1.49 (0.50 – 4.45)	0.589
Phe/Phe	6	12	3.42 (0.93 – 12.56)	0.077
Phe/Ile + Phe/Phe	16	58	1.89 (0.68 – 5.23)	0.239
Others				
Ile/Ile	9	119	1.00 (Reference)	-
Phe/Ile	19	199	1.26 (0.55 – 2.88)	0.685
Phe/Phe	8	53	2.00 (0.73 – 5.46)	0.183
Phe/Ile + Phe/Phe	27	252	1.42 (0.65 – 3.11)	0.455

N = number of samples, OR = odd ratio, CI = confidence interval, Ile = isoleucine, Phe = phenylalanine

TABLE 4. Association of age, gender, and ethnicity in *STK15* Val57Ile SNP to GIC

	Cases, *N*	Controls, *N*	OR (95% CI)	*p*-value
Age				
< 50 years				
Val/Val	31	753	1.00 (Reference)	-
Val/Ile	15	293	1.24 (0.66 – 2.34)	0.498
Ile/Ile	3	24	3.04 (0.87 – 10.63)	0.082
Val/Ile + Ile/Ile	18	317	1.38 (0.76 – 2.50)	0.290
≥ 50 years				
Val/Val	93	33	1.00 (Reference)	-
Val/Ile	40	7	2.03 (0.83 – 4.97)	0.122
Ile/Ile	3	0	-	-
Val/Ile + Ile/Ile	43	7	2.18 (0.89 – 5.32)	0.087
Gender				
Male				
Val/Val	63	574	1.00 (Reference)	-
Val/Ile	25	202	1.13 (0.69 – 1.84)	0.612
Ile/Ile	2	15	1.22 (0.27 – 5.43)	0.682
Val/Ile + Ile/Ile	27	217	1.13 (0.70 – 1.83)	0.620
Female				
Val/Val	61	212	1.00 (Reference)	-
Val/Ile	30	98	1.06 (0.65 – 1.75)	0.800
Ile/Ile	4	9	1.55 (0.46 – 5.19)	0.501
Val/Ile + Ile/Ile	34	107	1.10 (0.68 – 1.78)	0.712
Ethnicity				
Chinese				
Val/Val	45	186	1.00 (Reference)	-
Val/Ile	16	34	1.95 (0.99 – 3.83)	0.060
Ile/Ile	2	3	2.76 (0.45 – 16.98)	0.260
Val/Ile + Ile/Ile	18	37	2.01 (1.05 – 3.85)	0.045
Malay				
Val/Val	6	85	1.00 (Reference)	-
Val/Ile	1	25	0.57 (0.07 – 4.93)	1.000
Ile/Ile	0	0	-	-
Val/Ile + Ile/Ile	1	25	0.57 (0.07 – 4.93)	1.000
Indian				
Val/Val	2	35	1.00 (Reference)	-
Val/Ile	0	11	-	-
Ile/Ile	0	0	-	-
Val/Ile + Ile/Ile	0	11	-	-
Kadazan-Dusun				
Val/Val	30	166	1.00 (Reference)	-
Val/Ile	23	87	1.46 (0.80 – 2.67)	0.270
Ile/Ile	2	8	1.38 (0.28 – 6.84)	0.656
Val/Ile + Ile/Ile	25	95	1.46 (0.81 – 2.62)	0.224
Bajau				
Val/Val	15	72	1.00 (Reference)	-
Val/Ile	6	23	1.25 (0.44 – 3.60)	0.781
Ile/Ile	1	4	1.20 (0.13 – 11.51)	1.000
Val/Ile + Ile/Ile	7	27	1.24 (0.46 – 3.38)	0.794
Others				
Val/Val	26	186	1.00 (Reference)	-
Val/Ile	9	34	1.89 (0.82 – 4.39)	0.146
Ile/Ile	1	3	2.39 (0.24 – 23.79)	0.416
Val/Ile + Ile/Ile	10	37	1.93 (0.86 – 4.35)	0.159

N = number of samples, OR = odd ratio, CI = confidence interval, Ile = isoleucine, Val = valine

risk of GIC in both genders (Chong et al. 2014). We included the genotype analysis of the Phe31Ile and Val57Ile SNPs for both genders in this study and found no significant association of gender in both SNPs with GIC risk. Besides, Malaysia is a multi-ethnic country including a majority of Malay, Chinese and Indians in West Malaysia and indigenous ethnics such as Kadazan-Dusun and Bajau in East Malaysia and SNP association to GIC might be different across ethnicities. We observed no significant association of different ethnicities in both SNPs towards risk of GIC. Taken together, our study showed that etiological factors including age, gender, and ethnicity are not the risk factors for GIC in the Malaysian population.

Conclusion

We hereby conclude that the 31Ile allele and the 31Ile/Ile genotype in the *STK15* Phe31Ile SNP significantly elevated the risk to GIC, particularly in gastric cancer. Despite that age and *STK15* SNPs were not interacting factors associated with GIC, we recommend that screening for GIC should be done before 50 years old as GIC incidences have been alarmingly shifted to younger populations among Malaysians. As etiological factors such as age, gender and ethnicity are not risk factors for GIC in the Malaysian population, gene-gene and gene-environmental interactions should be taken into the account in future study with larger sample sizes for better risk estimation.

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