Am J Transl Res 2020;12(7):3613-3628
www.ajtr.org /ISSN:1943-8141/AJTR0108485

Original Article

Investigation of *Leptin* and its receptor (*LEPR*) for single nucleotide polymorphisms in colorectal cancer: a case-control study involving 2,306 subjects

Jing Lin[1,2*], Zhiqiang Xie[3*], Bin Lan[4*], Zengqing Guo[1,2,5], Wei-Feng Tang[6], Chao Liu[6], Sheng Zhang[7], Gang Chen[5,8], Fang Guo[4], Yu Chen[1,2,4]

[1]Cancer Bio-immunotherapy Center, [2]Department of Medical Oncology, Fujian Cancer Hospital & Fujian Medical University Cancer Hospital, Fuzhou, Fujian Province, China; [3]Department of Clinical Laboratory, Fujian Medical University Union Hospital, Fuzhou, Fujian Province, China; [4]Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, 800 Dong Chuan Rd, Minhang, Shanghai, China; [5]Fujian Provincial Key Laboratory of Translational Cancer Medicine, Fuzhou, Fujian Province, China; [6]Department of Cardiothoracic Surgery, Affiliated People's Hospital of Jiangsu University, Zhenjiang, Jiangsu Province, China; [7]Department of General Surgery, Changzhou, No. 3 People's Hospital, Changzhou, Jiangsu Province, China; [8]Department of Pathology, Fujian Cancer Hospital & Fujian Medical University Cancer Hospital, Fuzhou, Fujian Province, China. [*]Equal contributors.

Received January 30, 2020; Accepted June 4, 2020; Epub July 15, 2020; Published July 30, 2020

Abstract: Single nucleotide polymorphisms (SNPs) in the genes coding for leptin (*LEP*) and its receptor (*LEPR*) might regulate energy balance and be implicated in the development of colorectal cancer (CRC). In the present investigation, 1,003 CRC cases and 1,303 matched controls was compared. Five functional SNPs in *LEP* and *LEPR* genes were chosen to evaluate the correlation of these chosen SNPs with CRC susceptibility. We used the SNPscan™ genotyping assay to genotype *LEP* and *LEPR* SNPs. A significantly decreased risk of CRC was found to be associated with the *LEPR* rs6588147 polymorphism (GA *vs.* GG: crude *P*=0.007 and GA/AA *vs.* GG: crude *P*=0.018). With adjustments for risk factors (e.g. age, gender, drinking, BMI and smoking), these associations were not changed. In subgroup analyses, the association of *LEP* rs2167270 with a decreased risk of CRC was found in the ≥61 years old subgroup. For *LEPR* rs1137100, the association of this SNP with an increased susceptibility of CRC was found in the BMI <24 kg/m^2 subgroup. In subgroup analyses for *LEPR* rs6588147, we identified that this locus also decreased the susceptibility of CRC in the male subgroup, <61 years old subgroup, never smoking subgroup and never drinking subgroup. For *LEPR* rs1137101, the relationship of this polymorphism with a decreased susceptibility to CRC was found in the never drinking subgroup. In summary, the present study highlights that *LEPR* rs6588147, rs1137101 and *LEP* rs2167270 may decrease the risk of CRC. However, *LEPR* rs1137100 is associated with susceptibility to CRC. Further case-control studies with larger sample sizes should be conducted to validate our findings.

Keywords: LEP/LEPR, polymorphisms, colorectal cancer, single nucleotide polymorphisms

Introduction

Obesity and/or overweight are common public health issues all over the world [1, 2]. Several investigations have focused on the correlation of obesity and overweight with colorectal cancer (CRC) [3-7]. Some studies have reported that obesity and overweight are risk factor for the development of CRC [3, 8-10]. Among CRC cases, obesity and overweight may influence the survival of CRC patients [9, 11, 12].

The intake of excess calories contributes to the development of overweight and obesity, which is considered to be controlledby important molecular mechanisms and pathways [e.g. leptin (*LEP*), LEP receptor (*LEPR*), insulin, microRNA expression and DNA methylation] [5, 7]. *LEP* is produced by adipocytes. It has been reported that the level of *LEP* is increased in obese and overweight individuals [13]. *LEP* has been found to be associated with both appetite and body weight [14, 15]. *LEP* binds with the

LEPR and plays a significant role in energy metabolism in the body [16]. Previous investigations have reported that *LEP* and *LEPR* are associated with the development of colorectal cancer, and could be used as important therapy targetsin CRC [17]. Ho et al. reported that a high level of *LEP* conferred a susceptibility to the development of CRC [18]. Song et al. found that the level of soluble *LEPR* in the plasma was significantly associated with an increased risk of rectal cancer [19]. These previous studies showed that the *LEP/LEP*R pathway may be implicated in the occurrence of CRC.

A study has suggested that the rs1137101 A>G (Gln223Arg) single nucleotide polymorphisms (SNPs) in the *LEPR* gene are correlated with obesity [20]. Another investigation also found that *LEPR* rs1137100 G>A (Arg109Lys) and *LEP* rs7799039 G>A (-2548 G/A) polymorphisms were related to the level of *LEP* and the development of obesity [21]. Dasgupta et al. reported that the *LEP* variants rs2167270 A allele and rs7799039 A allele were independently associated with the susceptibility to obesity [22]. Nock et al. also showed an association of *LEPR* rs6588147 SNP to physical activity and food intake [23]. SNPs in *LEP* and *LEPR* genes have also been explored for their relationship to the etiology of CRC. Some case-control studies have suggested that the rs2167270 A (19A) allele of the *LEP* gene might be a protective factor for the occurrence of CRC [24, 25]. A meta-analysis indicated that *LEP* rs7799039 G>A SNP might decrease the susceptibility to CRC [26]. The *LEPR* Gln223Arg SNP was found to be associated with the tumor stage of CRC [27], and Slattery et al. reported that the combination of *LEP* rs2167270 GG and *LEPR* rs6588147 GG genotypes had a tendency to be associated with a decreased CRC risk [24]. However, these observations were not studied in Asians. In addition, the association between *LEPR* rs1137100 G>A (Arg109Lys) and the risk of CRC is unknown.

Here we report our evaluation of the correlation between *LEP* rs2167270 G>A, rs7799039 A>G, *LEPR* rs6588147 G>A, rs1137100 G>A, and rs1137101 G>A SNPs with the susceptibility to CRC. We recruited 2,306 participants from eastern China. Additionally, we assessed whether the CRC correlations with these SNPs were influenced by some risk factors [e.g. body mass index (BMI), age, gender, smoking and drinking].

Materials and methods

Subjects

This study was carried out with 1,003 CRC cases (mean age 61.10 ± 12.17 years) and 1,303 cancer-free controls (mean age 61.40 ± 9.61 years). The CRC cases were recruited from the Department of General Surgery at the Union Clinical Medical College of Fujian Medical University (Fuzhou City, China) and the Clinical Medical College of Jiangsu University (Zhenjiang City, China) between 2014-2017. CRC patients were diagnosed by two pathologists. Our investigation was performed after gaining the approval of the ethics committee of Fujian Medical University. Additionally, before recruitment, a written informed consent was also obtained from each participant. We collected the clinical data from their medical records where we also selected some important risk factors (e.g. gender, age of onset, BMI, tobacco consumption and drinking). In this study, we matched age and gender in the two groups.

DNA extraction and genotyping

We collected 2 ml blood samples from each participant and stored it at -80°C. Leukocytes was harvested to extract and purify DNA according to manual of Promega DNA Kit (Promega, Madison, USA). A NanoDrop ND-1000 spectrophotometer was used to measure the quality of obtained DNA. We used the SNPscan™ genotyping method to obtain the genotypes of the *LEP* and *LEPR* SNPs. Ninety-two (4%) of the DNA samples were randomly selected and a second technician repeated the polymerase chain reaction process. The retested genotypes were found to be accurate.

Statistical analysis

We used SAS 9.4 software (SAS Institute, Cary, NC) to analyze the data. The distribution of the *LEP* and *L*EPR genotypes in controls was evaluated to determine whether they were consistent with a Hardy-Weinberg equilibrium (HWE) by using an online calculator (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) [28-32]. Chi-square (χ^2) or a Fisher's exact test were used to compare the differences of LEP and LEPR genotypes.

Table 1. Distribution of selected characteristics in CRC cases and controls

Variable	Cases (n=1,003)		Controls (n=1,303)		*P*[a]
	n	%	n	%	
Age (years), mean (± SD)	61.10 ± 12.17		61.40 ± 9.61		0.496
Age (years)					0.605
<61	451	44.97	600	46.05	
≥61	552	55.03	703	53.95	
Sex					0.867
Male	620	61.81	801	61.47	
Female	383	38.19	502	38.53	
Smoking status					**0.002**
Never	744	74.18	1038	79.66	
Ever	259	25.82	265	20.34	
Alcohol use					**<0.001**
Never	829	82.65	1,167	89.56	
Ever	174	17.35	136	10.44	
BMI (kg/m^2)					
<24	670	66.80	688	52.80	**<0.001**
≥24	333	33.20	615	47.20	
Site of tumor					
Colon cancer	431	42.97			
Rectum cancer	572	57.03			

[a]Two-sided χ^2 test and student t-test; Bold values are statistically significant (*P*<0.05).

Continuous variables were expressed as mean ± standard deviation. The differences in continuous variables were analyzed by a Student's t-test. The distribution of categorical variables [e.g. age, sex, body mass index (BMI), smoking status, alcohol consumption, and *LEP* and *LEPR* genotypes] was compared by χ^2 or a Fisher's exact test. A *P*<0.05 was consider significant.

Results

Characteristics

There were 2,306 participants (1,003 CRC cases and 1,303 cancer-free controls) included in this investigation. **Table 1** summarizes the distribution of age, gender, BMI, smoking status, and alcohol consumption between the two groups. In the current study, the distribution by age [number of CRC cases/controls (<61 years *vs.* ≥61 years): 451/552 *vs.* 600/703, *P*=0.605], gender [number of cases *vs.* controls (male/female): 620/383 *vs.* 801/502, *P*=0.867] were not significantly different. However, the distribution by BMI [number of cases *vs.* controls (<24 kg/m^2/≥24 kg/m^2): 670/333 *vs.* 688/615, *P*<0.001], smoking status [number of cases *vs.* controls (never/ever): 774/259 *vs.* 1,038/265, *P*=0.002] and alcohol consumption [number of cases *vs.* controls (never/ever): 829/174 *vs.* 1,167/136, *P*<0.001] were significantly different. **Table 2** contains the information in the database and our results for *LEP* and *LEPR* SNPs. The success rate for identifying *LEP* and *LEPR* SNPs genotyping was excellent (>98%). The minor allele frequency (MAF) of *LEP* and *LEPR* SNPs is also shown in **Table 2**. For the included *LEP* and *LEPR* loci in controls, the distribution of variants was consistent with the HWE. The sequencing results of each of the SNPs were showed in **Figures 1**-**6**.

Association of LEP and LEPR polymorphisms with the development of CRC

The occurrence of the genotypes of the *LEPR* rs6588147 locus were 735 (GG), 229 (GA) and 16 (AA) in the CRC cases and 917 (GG), 362 (GA) and 21 (AA) in controls. When we compared *LEPR* rs6588147 GA to GG, a significantly decreased occurence of the *LEPR* rs6588147 GA genotype was associated with the development of CRC (crude OR=0.77, 95% CI, 0.63-0.93, *P*=0.007). We also compared the *LEPR* rs6588147 GA/AA genotype to the GG genotype and found a protective role for the GA/AA genotype against the development of CRC (crude OR=0.80, 95% CI, 0.66-0.96, *P*=0.018). When we made adjustments for included risk factors (e.g. age, gender, drinking, BMI, and smoking), these associations were not changed (GA *vs.* GG: adjusted OR, 0.77; 95% CI, 0.63-0.93; *P*=0.007 and AA/GA *vs.* GG: adjusted OR, 0.79; 95% CI, 0.66-0.96; *P*=0.018; **Table 3**).

When we focused on the potential correlation of the rs2167270, rs7799039, rs1137100 and rs1137101 loci with the occurrence of CRC, we found a null association between them (**Table 3**).

Table 2. Primary information for *LEP* rs2167270 G>A, rs7799039 A>G, *LEPR* rs6588147 G>A, rs1137100 G>A and rs1137101 G>A polymorphisms

Genotyped SNPs	Chromosome	Chr Pos (NCBI Build 37)	Region	MAF[a] for Chinese in database	MAF in our controls (n=1,303)	*P* value for HWE[b] test in our controls	Genotyping method	Genotyping value (%)
LEP rs7799039 A>G	7	127878783	Promoter	0.201	0.271	0.520	SNPscan	98.87
LEP rs2167270 G>A	7	127881349	5'UTR	0.175	0.228	0.185	SNPscan	98.87
LEPR rs1137100 G>A	1	66036441	Exon 4	0.169	0.155	0.852	SNPscan	98.87
LEPR rs1137101 G>A	1	66058513	Exon 6	0.111	0.124	0.783	SNPscan	98.83
LEPR rs6588147 G>A	1	65935494	Intron 2	0.150	0.155	0.028	SNPscan	98.87

[a]MAF: minor allele frequency. [b]HWE: Hardy-Weinberg equilibrium.

Association of LEP and LEPR polymorphisms with the development of CRC in subgroup analysis

The *LEP* rs7799039 genotype frequency in subgroup analysis are shown in **Table 4**. No association of rs7799039 with the risk of CRC was found in any subgroup.

Table 5 shows the *LEP* rs2167270 genotype frequency in subgroup analysis. When we adjusted the potential risk factors (e.g. age, gender, drinking, BMI and smoking), the association of *LEP* rs2167270 with a decreased risk of CRC was found in the ≥61 year old subgroup (GA *vs.* GG adjusted OR=0.78, 95% CI, 0.61-0.99, *P*=0.042).

For *LEPR* rs1137100, the association of this SNP with an increased susceptibility of CRC was found in the BMI <24 kg/m^2 subgroup (GA/AA *vs.* GG adjusted OR=1.29, 95% CI, 1.02-1.63, *P*=0.036, **Table 6**).

In subgroup analysis for *LEPR* rs6588147, we identified that this locus also decreased the susceptibility of CRC (male subgroup: GA *vs.* GG adjusted OR=0.69, 95% CI, 0.54-0.89, *P*=0.004 and GA/AA *vs.* GG adjusted OR=0.72, 95% CI, 0.57-0.92, *P*=0.010; <61 years old subgroup: GA *vs.* GG adjusted OR=0.68, 95% CI, 0.50-0.91, *P*=0.009 and GA/AA *vs.* GG adjusted OR=0.70, 95% CI, 0.53-0.94, *P*=0.016; never smoking subgroup: GA *vs.* GG adjusted OR=0.59, 95% CI, 0.39-0.89, *P*=0.012 and GA/AA *vs.* GG adjusted OR=0.64, 95% CI, 0.43-0.96, *P*=0.030 and never drinking subgroup: GA *vs.* GG adjusted OR=0.33, 95% CI, 0.19-0.57, *P*<0.001 and GA/AA *vs.* GG adjusted OR=0.37, 95% CI, 0.22-0.63, *P*<0.001, **Table 7**).

For *LEPR* rs1137101, the relationship of this polymorphism with a decreased susceptibility to CRC was found in the never drinking subgroup (GA *vs.* GG adjusted OR=0.47, 95% CI, 0.27-0.80, *P*=0.006 and GA/AA *vs.* GG adjusted OR=0.54, 95% CI, 0.32-0.90, *P*=0.019, **Table 8**).

Discussion

Obesity/overweight is a contemporary common public health issue worldwide. A number of investigations have suggested that obesity and/or overweight may be associated with the occurrence of CRC [3, 8]. Thus, any obesity/overweight related genes may also be implicated in the development and survival of CRC patients [9, 11, 12]. Here, we recruited 2,306 participants (1,003 CRC cases and 1,303 cancer-free controls) to assess the correlation between *LEP/LEPR* SNPs and the susceptibility of CRC. We found a significant association between the *LEPR* rs6588147 locus and the decreased risk of CRC. In subgroup analysis for *LEPR* rs6588147 and rs1137101 and *LEP* rs2167270, the association of these SNPs with the decreased risk of CRC was found in some subgroups. For example, we found that the *LEPR* rs1137100 locus might increase the susceptibility of CRC in the BMI <24 kg/m^2 subgroup.

The rs6588147 site is an intron locus in the *LEPR* gene and Zhang *et al.* had reported that the *LEPR* rs6588147 A allele is implicated in the occurence of hepatocellular carcinoma [33]. Another study in a mixed population has also indicated that the presence of the rs6588147 A allele tended to decrease the risk of colon cancer [24]. However, Nyante *et al.* suggested that this locus might promote the occurrence of breast cancer in some subtypes [34]. In the current study the correlation of the rs6588147 A allele to the decreased risk of

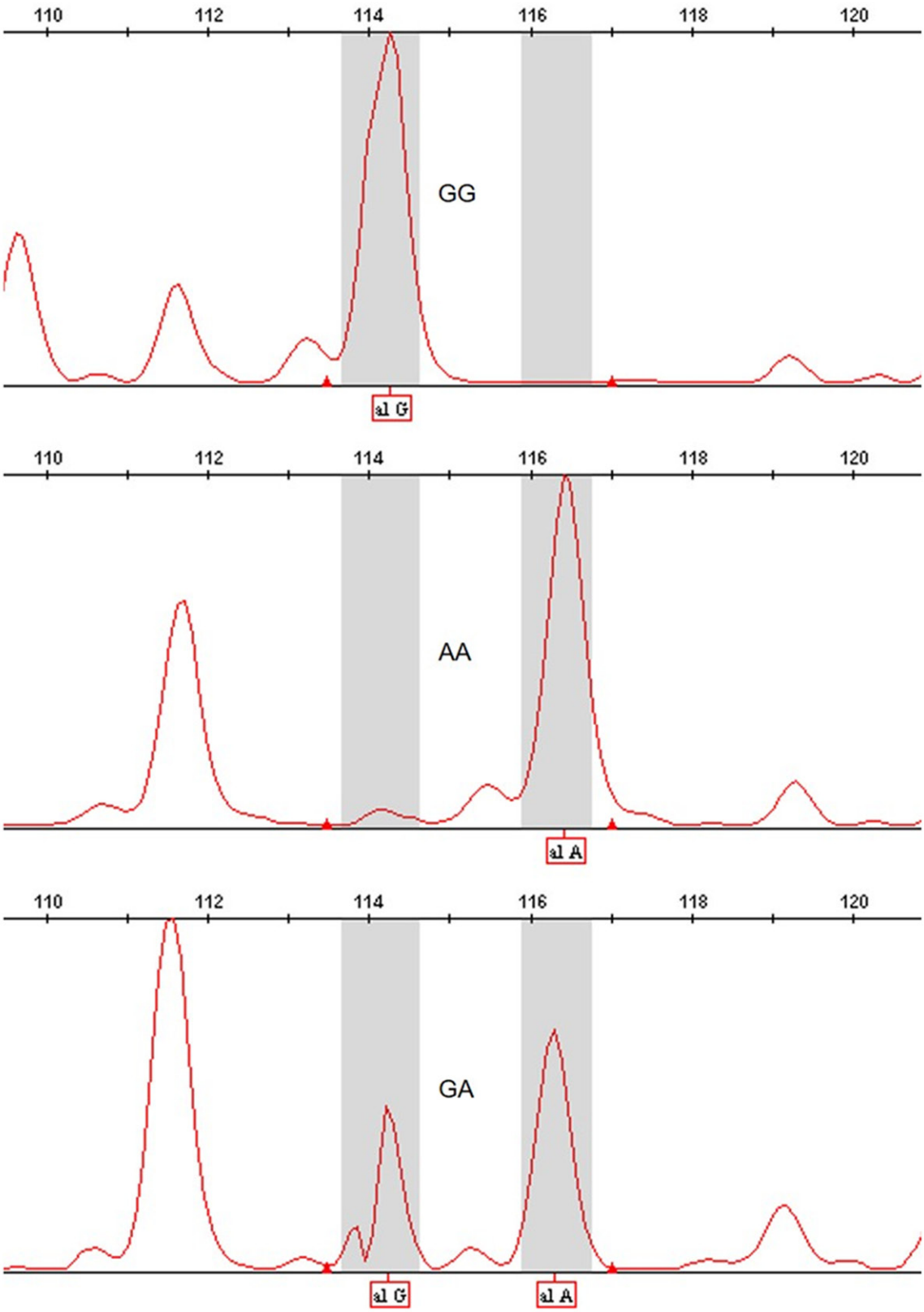

Figure 1. *LEPR* rs1137101 G>A SNPs, GG, AA, GA from top to bottom.

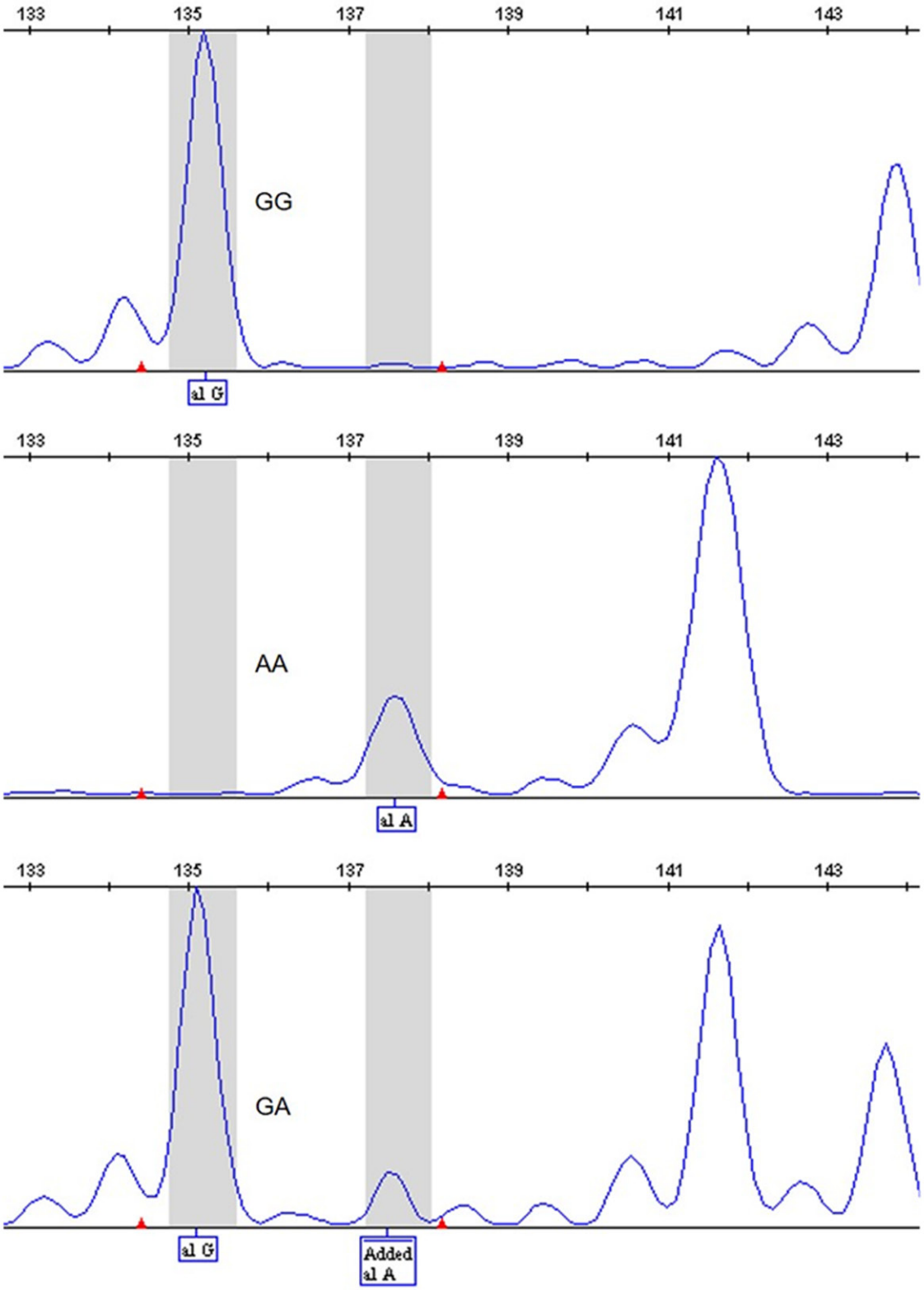

Figure 2. *LEPR* rs2167270 G>A SNPs, GG, AA, GA from top to bottom.

CRC was significant. In subgroup analysis we identified that this locus also decreased the susceptibility of CRC in male, <61 years, never smoking and never drinking subgroups. Few

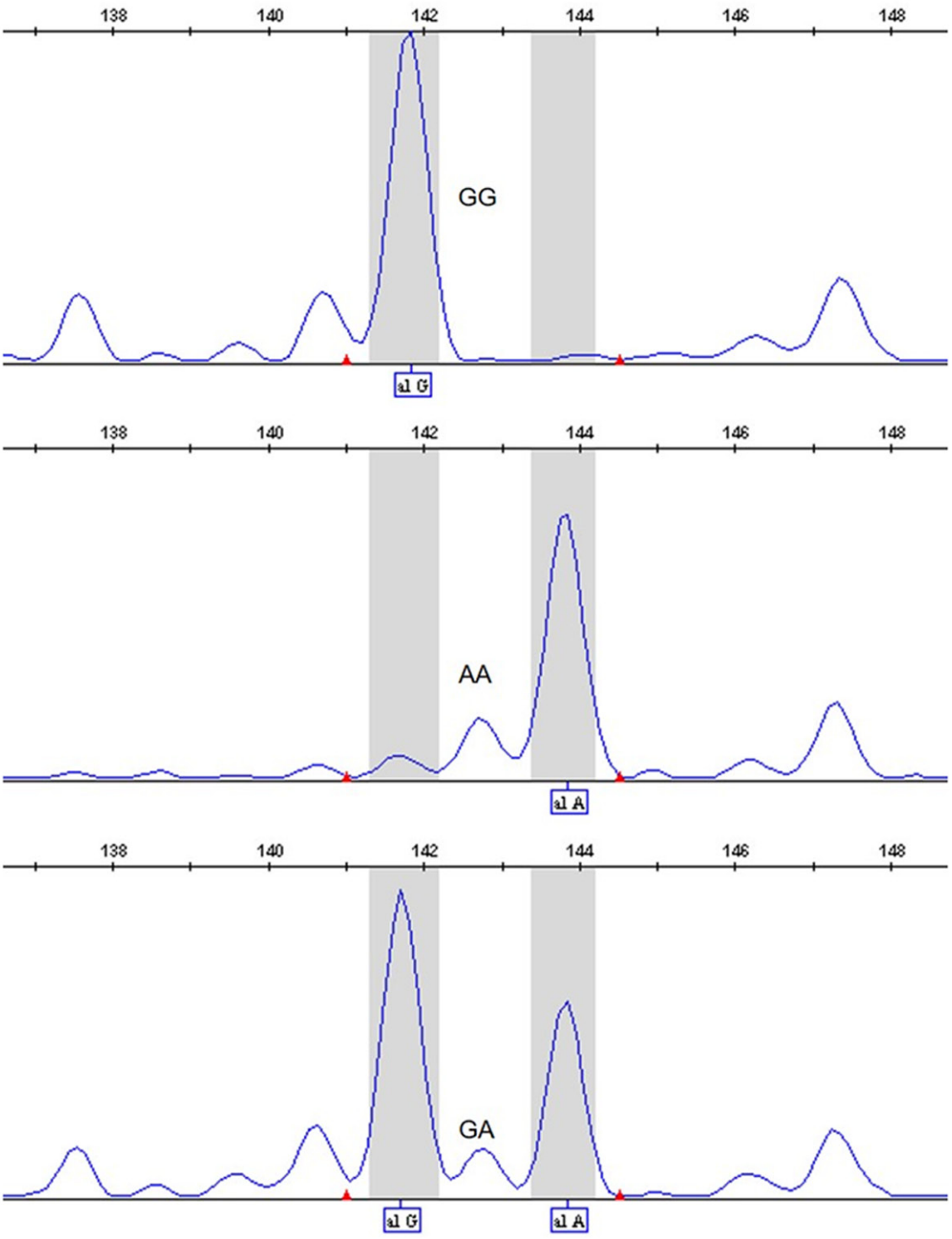

Figure 3. *LEPR* rs7799039 A>G SNPs, GG, AA, GA from top to bottom.

studies have explored the relationship of *LEPR* rs6588147 polymorphisms with the development of cancer. And the function of this locus was also unknown. In the future, the role of this locus should be further studied to explore the correlation to the development cancer. Additionally, a functional study should also be conducted.

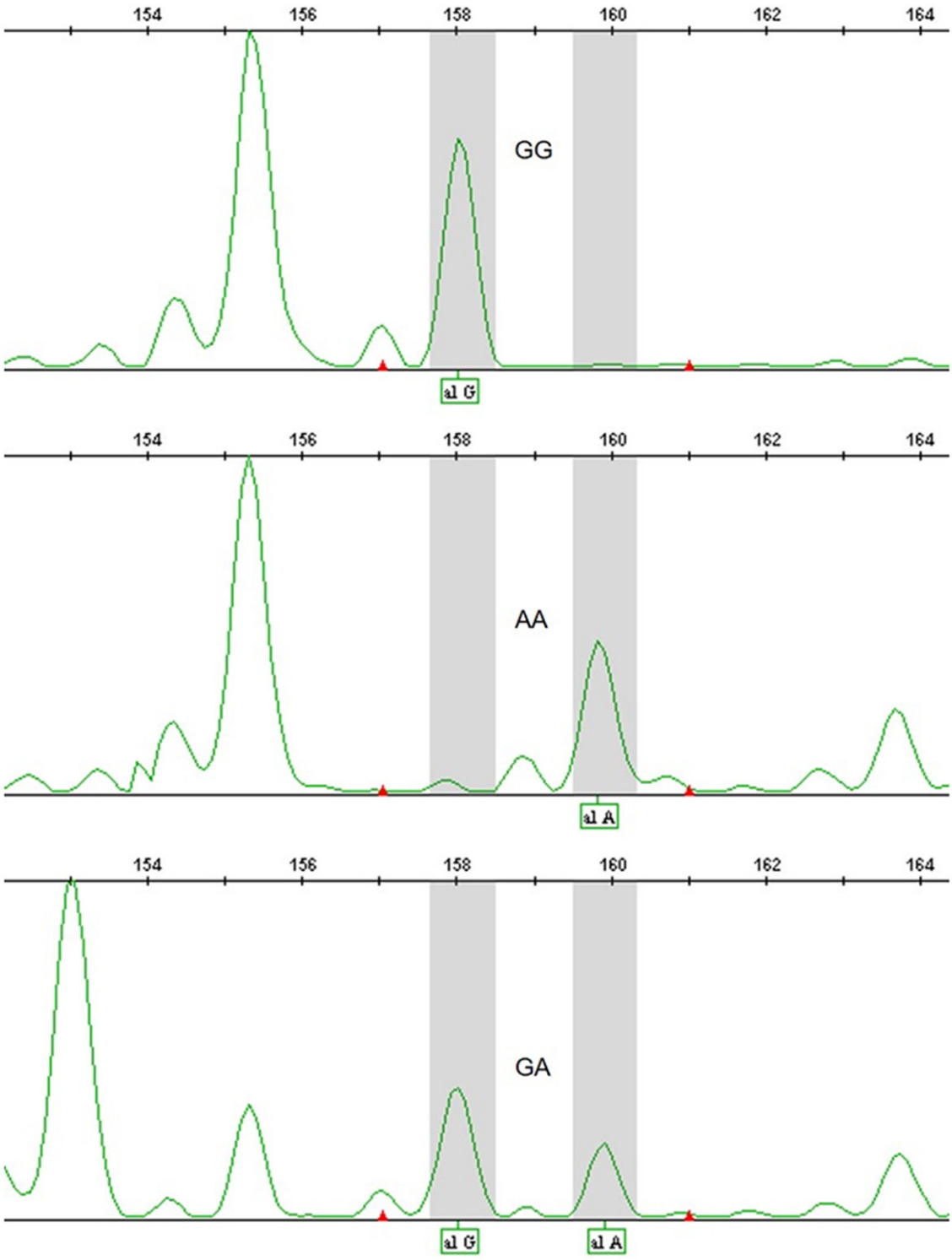

Figure 4. *LEPR* rs6588147 G>A SNPs, GG, AA, GA from top to bottom.

Rs2167270 is located in the 5'-utr of the *LEP* gene and a 5'-utr SNP might affect the mRNA translation process. For example, it has been suggested that the rs2167270 locus in the *LEP* gene could be implicated in the development of diabetes in a post-transplant populations [35]. A previous investigaton indicated that the serum *LEP* level in individuals who carry the GA

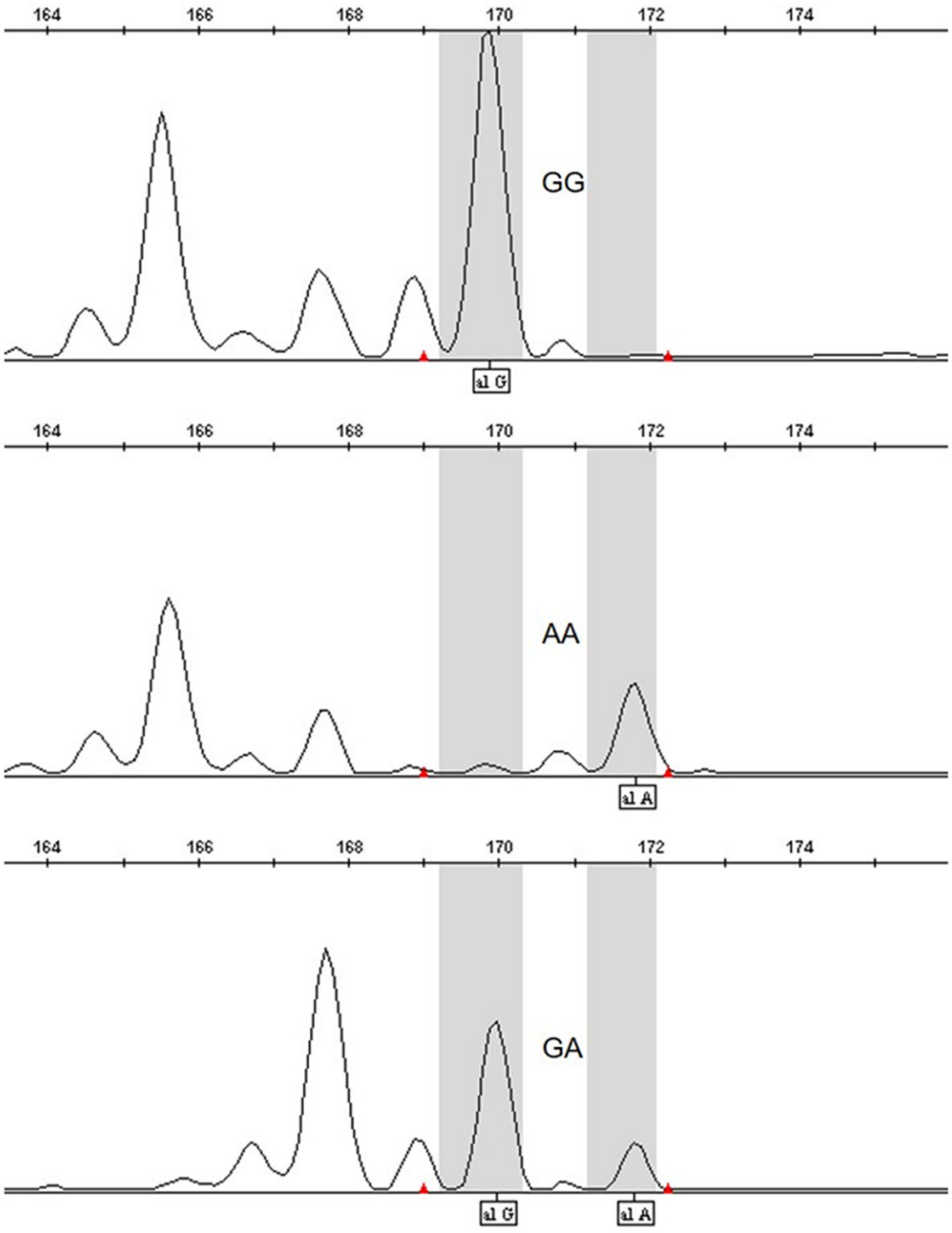

Figure 5. *LEPR* rs1137100 G>A SNPs, GG, AA, GA from top to bottom.

genotype of the *LEP* rs2167270 was higher than in those who carried with GG genotype [36]. In a more recent meta-analysis, Yang et al. found that *LEP* rs2167270 variants were associated with a decreased risk of cancer [37]. In this case-control study, we did not find any association between *LEP* rs2167270 and the overall risk of CRC. However, in subgroup analysis for *LEP* rs2167270, the association of this SNP with the decreased risk of CRC was

Figure 6. Using the SNPscanTM genotyping method to obtain the genotypes of LEP and LEPR SNPs. the sequencing results of each of the SNPs. *; number of miss cases in Cases =23, **; number of miss cases in Controls =3.

Table 3. Logistic regression analyses of association between *LEP* rs2167270 G>A, rs7799039 A>G, *LEPR* rs6588147 G>A, rs1137100 G>A and rs1137101 G>A SNPs and risk of CRC

Genotype	Cases (n=1,003)		Controls (n=1,303)		Crude OR (95% CI)	*P*	Adjusted OR[a] (95% CI)	*P*
	n	%	n	%				
LEP rs7799039 A>G								
AA	521	53.16	686	52.77	1.00		1.00	
AG	394	40.20	523	40.23	0.95 (0.80-1.13)	0.594	0.96 (0.81-1.15)	0.658
GG	65	6.63	91	7.00	0.91 (0.65-1.27)	0.562	0.87 (0.62-1.23)	0.432
AG+GG	459	46.84	614	47.23	0.98 (0.83-1.16)	0.852	0.99 (0.83-1.17)	0.872
AA+AG	915	93.37	1209	93.00	1.00		1.00	
GG	65	6.63	91	7.00	0.94 (0.68-1.31)	0.732	0.91 (0.65-1.27)	0.565
G allele	524	26.73	705	27.12				
LEP rs2167270 G>A								
GG	589	60.10	767	59.00	1.00		1.00	
GA	340	34.69	474	36.46	0.90 (0.76-1.08)	0.251	0.91 (0.76-1.09)	0.300
AA	51	5.20	59	4.54	1.09 (0.74-1.61)	0.673	1.06 (0.71-1.57)	0.787
GA+AA	391	39.90	533	41.00	0.96 (0.81-1.13)	0.596	0.96 (0.81-1.14)	0.641
GG+GA	929	94.80	1241	95.46	1.00		1.00	
AA	51	5.20	59	4.54	1.16 (0.79-1.70)	0.463	1.12 (0.76-1.66)	0.574
A allele	442	22.55	592	22.77				
LEPR rs6588147 G>A								
GG	735	75.00	917	70.54	1.00		1.00	
GA	229	23.37	362	27.85	**0.77 (0.63-0.93)**	**0.007**	**0.77 (0.63-0.93)**	**0.007**
AA	16	1.63	21	1.62	0.93 (0.48-1.79)	0.816	0.89 (0.46-1.74)	0.740
GA + AA	245	25.00	383	29.46	**0.80 (0.66-0.96)**	**0.018**	**0.79 (0.66-0.96)**	**0.018**
GG+GA	964	98.37	1279	98.38	1.00		1.00	
AA	16	1.63	21	1.62	1.01 (0.53-1.95)	0.974	0.98 (0.50-1.90)	0.944
A allele	261	13.32	404	15.54				
LEPR rs1137100 G>A								
GG	667	68.06	914	70.91	1.00		1.00	
GA	289	29.49	351	27.23	1.09 (0.91-1.32)	0.338	1.09 (0.90-1.31)	0.379
AA	24	2.45	35	1.86	0.91 (0.54-1.55)	0.731	0.88 (0.52-1.50)	0.642
GA+AA	313	31.94	375	29.09	1.11 (0.93-1.33)	0.250	1.10 (0.92-1.32)	0.293
GG+GA	956	97.55	1265	98.14	1.00		1.00	
AA	24	2.45	35	1.86	0.91 (0.54-1.54)	0.719	0.88 (0.52-1.50)	0.638
A allele	337	17.19	399	15.48				
LEPR rs1137101 G>A								
GG	760	76.85	995	76.60	1.00		1.00	
GA	205	20.73	285	21.94	0.92 (0.75-1.12)	0.407	0.92 (0.75-1.13)	0.435
AA	15	2.43	19	1.46	1.01 (0.51-2.00)	0.983	1.01 (0.50-2.02)	0.983
GA+AA	229	2.43	304	23.40	0.95 (0.78-1.15)	0.593	0.95 (0.78-1.16)	0.630
GG+GA	965	23.15	1280	98.54	1.00		1.00	
AA	15	2.43	19	1.46	1.05 (0.53-2.07)	0.894	1.05 (0.52-2.10)	0.894
A allele	253	12.79	323	12.43				

[a]Adjusted for age, sex, smoking status, alcohol use and BMI. Bold values are statistically significant ($P<0.05$).

Table 4. Stratified analyses between *LEP* rs7799039 A>G polymorphism and CRC risk by sex, age, smoking status and alcohol consumption

Variable	*LEP* rs7799039 A>G (case/control)[a]			Adjusted OR[b] (95% CI); *P*				
	AA	AG	GG	AA	AG	GG	AG/GG	GG vs. (AG/AA)
Sex								
Male	322/415	238/328	44/56	1.00	0.91 (0.73-1.14); *P*: 0.398	0.95 (0.62-1.46); *P*: 0.814	0.96 (0.77-1.19); *P*: 0.694	1.02 (0.67-1.54); *P*: 0.944
Female	199/271	156/195	21/35	1.00	1.02 (0.77-1.36); *P*: 0.871	0.73 (0.41-1.31); *P*: 0.294	1.01 (0.77-1.33); *P*: 0.960	0.74 (0.42-1.30); *P*: 0.295
Age								
<61	229/329	182/228	32/41	1.00	1.16 (0.89-1.51); *P*: 0.266	1.11 (0.67-1.85); *P*: 0.678	1.19 (0.92-1.53); *P*: 0.183	1.06 (0.65-1.74); *P*: 0.810
≥61	292/357	212/295	33/50	1.00	0.83 (0.66-1.05); *P*: 0.128	0.71 (0.44-1.14); *P*: 0.158	0.85 (0.68-1.07); *P*: 0.174	0.79 (0.50-1.26); *P*: 0.321
Smoking status								
Never	391/548	291/414	46/73	1.00	0.96 (0.79-1.18); *P*: 0.723	0.87 (0.58-1.29); *P*: 0.478	0.98 (0.81-1.19); *P*: 0.873	0.90 (0.61-1.32); *P*: 0.585
Ever	130/138	103/109	19/18	1.00	0.96 (0.67-1.38); *P*: 0.830	0.92 (0.45-1.84); *P*: 0.803	1.01 (0.71-1.43); *P*: 0.974	0.96 (0.48-1.89); *P*: 0.899
Alcohol consumption								
Never	433/609	326/474	51/81	1.00	0.93 (0.77-1.12); *P*: 0.459	0.85 (0.58-1.24); *P*: 0.391	0.96 (0.80-1.15); *P*: 0.621	0.89 (0.62-1.29); *P*: 0.548
Ever	88/77	68/49	14/10	1.00	1.17 (0.72-1.90); *P*: 0.525	1.05 (0.43-2.53); *P*: 0.916	1.20 (0.76-1.90); *P*: 0.441	1.01 (0.43-2.38); *P*: 0.989
BMI (kg/m^2)								
<24	338/373	271/261	47/52	1.00	1.10 (0.88-1.38); *P*: 0.391	0.92 (0.61-1.41); *P*: 0.715	1.11 (0.90-1.38); *P*: 0.332	0.90 (0.60-1.37); *P*: 0.633
≥24	183/313	123/262	18/39	1.00	0.76 (0.58-1.01); *P*: 0.061	0.79 (0.44-1.43); *P*: 0.439	0.80 (0.61-1.06); *P*: 0.115	0.91 (0.51-1.63); *P*: 0.756

[a]For *LEP* rs7799039 A>G, the genotyping was successful in 980 (97.71%) CRC cases, and 1,300 (99.77%) controls; [b]Adjusted for multiple comparisons [age, sex, BMI, smoking status and alcohol consumption (besides stratified factors accordingly)] in a logistic regression model.

Table 5. Stratified analyses between *LEP* rs2167270 G>A polymorphism and CRC risk by sex, age, smoking status and alcohol consumption

Variable	*LEP* rs2167270 G>A (case/control)[a]			Adjusted OR[b] (95% CI); *P*				
	GG	GA	AA	GG	GA	AA	GA / AA	AA vs. (GA/GG)
Sex								
Male	362/467	207/294	35/38	1.00	0.88 (0.70-1.11); *P*: 0.274	1.13 (0.69-1.84); *P*: 0.624	0.95 (0.76-1.18); *P*: 0.634	1.21 (0.75-1.97); *P*: 0.429
Female	227/300	133/180	16/21	1.00	0.93 (0.70-1.25); *P*: 0.641	0.92 (0.46-1.82); *P*: 0.803	0.96 (0.72-1.26); *P*: 0.751	0.96 (0.49-1.88); *P*: 0.898
Age								
<61	261/369	158/204	24/25	1.00	1.12 (0.86-1.47); *P*: 0.394	1.39 (0.76-2.53); *P*: 0.282	1.18 (0.91-1.53); *P*: 0.205	1.35 (0.75-2.44); *P*: 0.315
≥61	328/398	182/270	27/34	1.00	**0.78 (0.61-0.99); *P*: 0.042**	0.86 (0.50-1.46); *P*: 0.569	0.82 (0.65-1.04); *P*: 0.097	0.97 (0.57-1.64); *P*: 0.899
Smoking status								
Never	437/609	255/379	36/47	1.00	0.93 (0.76-1.14); *P*: 0.476	1.05 (0.67-1.66); *P*: 0.824	0.97 (0.80-1.19); *P*: 0.790	1.11 (0.70-1.74); *P*: 0.665
Ever	152/158	85/95	15/12	1.00	0.87 (0.60-1.26); *P*: 0.452	1.10 (0.49-2.44); *P*: 0.823	0.93 (0.65-1.34); *P*: 0.711	1.19 (0.54-2.62); *P*: 0.670
Alcohol consumption								
Never	489/685	282/425	39/54	1.00	0.91 (0.75-1.10); *P*: 0.325	0.97 (0.63-1.50); *P*: 0.891	0.95 (0.79-1.14); *P*: 0.565	1.03 (0.67-1.58); *P*: 0.901
Ever	100/82	58/49	12/5	1.00	0.96 (0.59-1.56); *P*: 0.857	1.78 (0.59-5.30); *P*: 0.304	1.07 (0.67-1.71); *P*: 0.768	1.85 (0.63-5.43); *P*: 0.265
BMI (kg/m^2)								
<24	390/	230/	36/34	1.00	0.98 (0.78-1.23); *P*: 0.852	1.06 (0.65-1.73); *P*: 0.819	1.02 (0.82-1.27); *P*: 0.860	1.09 (0.67-1.77); *P*: 0.729
≥24	199/354	110/235	15/25	1.00	0.81 (0.61-1.08); *P*: 0.144	1.05 (0.54-2.06); *P*: 0.879	0.87 (0.66-1.14); *P*: 0.310	1.17 (0.61-2.27); *P*: 0.633

[a]For *LEP* rs2167270 G>A,the genotyping was successful in 980 (97.71%) CRC cases, and 1,300 (99.77%) controls; [b]Adjusted for multiple comparisons [age, sex, BMI, smoking status and alcohol consumption (besides stratified factors accordingly)] in a logistic regression model. Bold value is statistically significant ($P<0.05$).

Table 6. Stratified analyses between *LEPR* rs1137100 G>A polymorphism and CRC risk by sex, age, smoking status and alcohol consumption

Variable	*LEPR* rs1137100 G>A (case/control)[a]			Adjusted OR[b] (95% CI); *P*				
	GG	GA	AA	GG	GA	AA	GA/AA	AA vs. (GA/GG)
Sex								
Male	409/561	180/221	15/17	1.00	1.07 (0.85-1.36); *P*: 0.568	1.12 (0.54-2.30); *P*: 0.761	1.12 (0.89-1.41); *P*: 0.354	1.13 (0.55-2.31); *P*: 0.744
Female	258/353	109/130	9/18	1.00	1.09 (0.81-1.49); *P*: 0.570	0.76 (0.33-1.74); *P*: 0.509	1.07 (0.80-1.45); *P*: 0.617	0.75 (0.33-1.72); *P*: 0.495
Age								
<61	310/425	127/162	6/11	1.00	1.04 (0.79-1.38); *P*: 0.782	0.77 (0.27-2.18); *P*: 0.623	1.05 (0.79-1.38); *P*: 0.745	0.77 (0.28-2.18); *P*: 0.628
≥61	357/489	162/189	18/24	1.00	1.11 (0.86-1.42); *P*: 0.437	1.04 (0.55-1.97); *P*: 0.897	1.14 (0.90-1.46); *P*: 0.284	1.04 (0.55-1.95); *P*: 0.907
Smoking status								
Never	496/730	216/272	16/33	1.00	1.14 (0.92-1.41); *P*: 0.229	0.69 (0.37-1.28); *P*: 0.235	1.12 (0.91-1.38); *P*: 0.274	0.68 (0.37-1.25); *P*: 0.214
Ever	171/184	73/79	8/2	1.00	0.94 (0.64-1.38); *P*: 0.739	4.20 (0.87-20.38); *P*: 0.075	1.06 (0.73-1.55); *P*: 0.768	4.43 (0.92-21.42); *P*: 0.064
Alcohol consumption								
Never	551/825	240/309	19/30	1.00	1.13 (0.92-1.38); *P*: 0.246	0.96 (0.53-1.73); *P*: 0.882	1.15 (0.94-1.40); *P*: 0.171	0.95 (0.52-1.71); *P*: 0.853
Ever	116/89	49/42	5/5	1.00	0.84 (0.51-1.39); *P*: 0.493	0.85 (0.23-3.09); *P*: 0.803	0.87 (0.53-1.41); *P*: 0.568	0.91 (0.25-3.28); *P*: 0.886
BMI (kg/m^2)								
<24	439/497	203/174	14/15	1.00	1.27 (1.00-1.62); *P*: 0.051	1.00 (0.47-2.10); *P*: 0.990	**1.29 (1.02-1.63); *P*: 0.036**	0.95 (0.45-1.99); P: 0.890
≥24	228/417	86/177	10/20	1.00	0.85 (0.63-1.15); *P*: 0.291	0.86 (0.39-1.90); *P*: 0.716	0.88 (0.66-1.18); *P*: 0.401	0.93 (0.42-2.03); *P*: 0.849

[a]For *LEPR* rs1137100 G>A, the genotyping was successful in 980 (97.71%) CRC cases, and 1,300 (99.77%) controls; [b]Adjusted for multiple comparisons [age, sex, BMI, smoking status and alcohol consumption (besides stratified factors accordingly)] in a logistic regression model. Bold value is statistically significant (*P*<0.05).

Table 7. Stratified analyses between *LEPR* rs6588147 G>A polymorphism and CRC risk by sex, age, smoking status and alcohol consumption

Variable	*LEPR* rs6588147 G>A (case/control)[a]			Adjusted OR[b] (95% CI); *P*				
	GG	GA	AA	GG	GA	AA	GA/AA	AA vs. (GA/GG)
Sex								
Male	460/559	135/228	9/12	1.00	**0.69 (0.54-0.89); *P*: 0.004**	0.82 (0.34-1.98); *P*: 0.652	**0.72 (0.57-0.92); *P*: 0.010**	0.91 (0.38-2.22); *P*: 0.842
Female	275/358	94/134	7/9	1.00	0.87 (0.63-1.18); *P*: 0.365	1.05 (0.38-2.92); *P*: 0.928	0.89 (0.66-1.21); *P*: 0.474	1.10 (0.40-3.06); *P*: 0.850
Age								
<61	339/421	96/166	8/11	1.00	**0.68 (0.50-0.91); *P*: 0.009**	0.91 (0.35-2.32); *P*: 0.838	**0.70 (0.53-0.94); *P*: 0.016**	1.01 (0.40-2.58); *P*: 0.982
≥61	396/496	131/196	8/10	1.00	0.82 (0.63-1.07); *P*: 0.140	0.85 (0.33-2.23); *P*: 0.747	0.85 (0.66-1.10); *P*: 0.225	0.92 (0.36-2.40); *P*: 0.869
Smoking status								
Never	539/732	179/286	10/17	1.00	0.83 (0.66-1.03); *P*: 0.089	0.80 (0.36-1.78); *P*: 0.589	0.85 (0.68-1.05); *P*: 0.130	0.86 (0.39-1.90); *P*: 0.704
Ever	196/185	50/76	6/4	1.00	**0.59 (0.39-0.89); *P*: 0.012**	1.16 (0.32-4.23); *P*: 0.826	**0.64 (0.43-0.96); *P*: 0.030**	1.36 (0.37-4.93); *P*: 0.644
Alcohol consumption								
Never	597/832	200/312	13/20	1.00	0.86 (0.70-1.06); *P*: 0.156	0.85 (0.41-1.73); *P*: 0.647	0.88 (0.72-1.08); *P*: 0.234	0.90 (0.44-1.83); *P*: 0.764
Ever	138/85	29/50	3/1	1.00	**0.33 (0.19-0.57); *P*: <0.001**	1.89 (0.19-19.06); *P*: 0.588	**0.37 (0.22-0.63); *P*: <0.001**	2.50 (0.25-25.07); *P*: 0.435
BMI (kg/m^2)								
<24	489/483	156/190	11/13	1.00	0.79 (0.62-1.02); *P*: 0.068	0.82 (0.36-1.86); *P*: 0.637	0.82 (0.64-1.04); *P*: 0.099	0.89 (0.39-2.01); *P*: 0.776
≥24	246/434	73/172	5/8	1.00	**0.71 (0.52-0.98); *P*: 0.035**	1.15 (0.37-3.60); *P*: 0.809	0.75 (0.55-1.03); *P*: 0.076	1.28 (0.41-3.98); *P*: 0.674

[a]For *LEPR* rs6588147 G>A, the genotyping was successful in 980 (97.71%) CRC cases, and 1,300 (99.77%) controls; [b]Adjusted for multiple comparisons [age, sex, BMI, smoking status and alcohol consumption (besides stratified factors accordingly)] in a logistic regression model. Bold values are statistically significant (*P*<0.05).

Table 8. Stratified analyses between *LEPR* rs1137101 G>A polymorphism and CRC risk by sex, age, smoking status and alcohol consumption

Variable	*LEPR* rs1137101 G>A (case/control)[a]			Adjusted OR[b] (95% CI); *P*				
	GG	GA	AA	GG	GA	AA	GA/AA	AA vs. (GA/GG)
Sex								
Male	467/608	127/183	10/7	1.00	0.87 (0.67-1.13); *P*: 0.304	1.74 (0.65-4.70); *P*: 0.272	0.93 (0.72-1.21); *P*: 0.598	1.85 (0.69-4.97); *P*: 0.226
Female	293/387	78/102	5/12	1.00	0.99 (0.70-1.38); *P*: 0.935	0.57 (0.19-1.65); *P*: 0.296	0.96 (0.69-1.33); *P*: 0.810	0.58 (0.20-1.68); *P*: 0.312
Age								
<61	340/458	99/133	4/6	1.00	1.01 (0.75-1.37); *P*: 0.947	0.92 (0.25-3.42); *P*: 0.903	1.03 (0.76-1.38); *P*: 0.867	0.93 (0.25-3.45); *P*: 0.916
≥61	420/287	106/152	11/13	1.00	0.84 (0.63-1.10); *P*: 0.215	1.08 (0.47-2.45); *P*: 0.861	0.88 (0.67-1.16); *P*: 0.374	1.14 (0.50-2.60); *P*: 0.750
Smoking status								
Never	561/799	157/216	10/19	1.00	1.03 (0.81-1.30); *P*: 0.829	0.69 (0.31-1.52); *P*: 0.356	1.02 (0.81-1.29); *P*: 0.853	0.70 (0.32-1.54); *P*: 0.376
Ever	199/196	48/69	5/0	1.00	**0.65 (0.42-0.99); *P*: 0.045**	-	0.75(0.49-1.13); *P*: 0.165	-
Alcohol consumption								
Never	625/903	174/242	11/18	1.00	1.03 (0.83-1.29); *P*: 0.792	0.87 (0.40-1.86); *P*: 0.712	1.05 (0.84-1.30); *P*: 0.686	0.88 (0.41-1.89); *P*: 0.742
Ever	135/92	31/43	4/1	1.00	**0.47 (0.27-0.80); *P*: 0.006**	2.95 (0.32-27.37); *P*: 0.342	**0.54 (0.32-0.90); *P*: 0.019**	3.59 (0.39-33.19); *P*: 0.261
BMI (kg/m^2)								
<24	512/534	136/141	8/11	1.00	0.97 (0.74-1.27); *P*: 0.831	0.70 (0.28-1.78); *P*: 0.457	0.98 (0.75-1.26); *P*: 0.847	0.72 (0.29-1.82); *P*: 0.491
≥24	248/461	69/144	7/8	1.00	0.85 (0.61-1.18); *P*: 0.318	1.64 (0.58-4.60); *P*: 0.350	0.91 (0.67-1.26); *P*: 0.583	1.73 (0.62-4.86); *P*: 0.296

[a]For *LEPR* rs1137101 G>A, the genotyping was successful in 980 (97.71%) CRC cases, and 1,299 (99.69%) controls; [b]Adjusted for multiple comparisons [age, sex, BMI, smoking status and alcohol consumption (besides stratified factors accordingly)] in a logistic regression model. Bold values are statistically significant (*P*<0.05).

found in the ≥61 years old subgroup, which was similar to the results from a meta-analysis [37]. The vital relationship between *LEP* rs2167270 and the risk of CRC should be more carefully considered.

The *LEPR* rs1137101 G/A (Arg223Gln), a missense SNP, has been widely investigated for its correlation between this locus and cancer. This SNP leads to a G→A variant in exon 6 and results in a Arg→Gln substitution in the extracellular region of the *LEPR* [38]. Recently, some case-control studies have reported that the rs1137101 A allele is a protective factor against cancer development [39, 40]. In the current study, we found that the rs1137101 G>A SNP was associated with a decreased risk for CRC in the never drinking subgroup, a result consistent with the studies mentioned above.

The rs1137100 G/A SNP in the *LEPR* gene is a missense variant that might influence the *LEPR* structure and its function. In a meta-analysis, Shi *et al.* suggested that the rs1137100 A allele was a risk factor for gastric cancer [41]. In this study, we found that the rs1137100 A allele increased the risk of CRC in the BMI <24 kg/m^2 subgroup as well. In controls, the MAF of rs1137100 (A allele) was 0.155, which was similar to the database.

Some limitations in this study should be addressed. Firstly, although the number of participants was relatively large, the sample size in certain subgroups was moderate. Thus, the power in these subgroups might be insufficient. Secondly, our study is designed as hospital-based and a potential bias cannot be ignored. Thirdly, we only foucus on the five risk factors (e.g. age, gender, BMI, smoking status and alcohol consumption). Other vital environmental carcinogen exposure factors were not considered. Finally, we only focused on five SNPs in the *LEP/LEPR* pathways and other functional SNPs should be considered in the future.

In conclusion, this study highlights that polymorphisms in the *LEPR* rs6588147, rs1137101 and *LEP* rs2167270 sites may decrease the risk of CRC. However, polymorphisms in the *LEPR* rs1137100 may increase the susceptibility of CRC. Further case-control studies with larger sample sizes should be conducted to valid our findings.

Acknowledgements

We appreciate all subjects who participated in this study. We wish to thank Dr. Yan Liu (Genesky Biotechnologies Inc., Shanghai, China) for technical support. This project was supported in part by the National Natural Science Foundation of China (Grant No. U17-05282), Natural Science Foundation of Fujian Province (Grant No. 2017J01259, 2018J01267), Fujian provincial health and family planning research talent training program (Grant No. 2018-ZQN-13, 2019-CX-4), Joint Funds for the innovation of science and Technology, Fujian province (Grant No. 2017Y9077), Fujian Provincial Science and Technology Department Planning Project (Grant No. 2018Y2003) and the National Clinical Key Specialty Construction Program.

Disclosure of conflict of interest

None.

Address correspondence to: Yu Chen and Fang Guo, Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, 800 Dong Chuan Rd, Minhang, Shanghai 200240, China. E-mail: chenyu1980@sjtu.edu.cn (YC); fguo@sjtu.edu.cn (FG)

References

[1] Marcelino LF and Patricio ZM. The complexity of obesity and life after bariatric surgery: a public health issue. Cien Saude Colet 2011; 16: 4767-4776.

[2] Mello MM. Obesity--personal choice or public health issue? Nat Clin Pract Endocrinol Metab 2008; 4: 2-3.

[3] Hamaguchi M, Hashimoto Y, Obora A, Kojima T and Fukui M. Non-alcoholic fatty liver disease with obesity as an independent predictor for incident gastric and colorectal cancer: a population-based longitudinal study. BMJ Open Gastroenterol 2019; 6: e000295.

[4] Akay S, Urkan M, Balyemez U, Ersen M and Tasar M. Is visceral obesity associated with colorectal cancer? The first volumetric study using all CT slices. Diagn Interv Radiol 2019; 25: 338-345.

[5] Dong L, Ma L, Ma GH and Ren H. Genome-wide analysis reveals DNA methylation alterations in obesity associated with high risk of colorectal cancer. Sci Rep 2019; 9: 5100.

[6] Ahechu P, Zozaya G, Marti P, Hernandez-Lizoain JL, Baixauli J, Unamuno X, Fruhbeck G and Catalan V. NLRP3 inflammasome: a pos-

sible link between obesity-associated low-grade chronic inflammation and colorectal cancer development. Front Immunol 2018; 9: 2918.
[7] Cirillo F, Catellani C, Sartori C, Lazzeroni P, Amarri S and Street ME. Obesity, insulin resistance, and colorectal cancer: could miRNA dysregulation play a role? Int J Mol Sci 2019; 20.
[8] Garcia H and Song M. Early-life obesity and adulthood colorectal cancer risk: a meta-analysis. Rev Panam Salud Publica 2019; 43: e3.
[9] Ayers D, Boughanem H and Macias-Gonzalez M. Epigenetic Influences in the obesity/colorectal cancer axis: a novel theragnostic avenue. J Oncol 2019; 2019: 7406078.
[10] Dong Y, Zhou J, Zhu Y, Luo L, He T, Hu H, Liu H, Zhang Y, Luo D, Xu S, Xu L, Liu J, Zhang J and Teng Z. Abdominal obesity and colorectal cancer risk: systematic review and meta-analysis of prospective studies. Biosci Rep 2017; 37.
[11] Billy M, Sholihah H, Andanni K, Anggraeni MI, Siregar SM and Mirtha LT. Obesity as predictor of mortality of colorectal cancer: an evidence-based case report. Acta Med Indones 2016; 48: 242-246.
[12] Shah MS, Fogelman DR, Raghav KP, Heymach JV, Tran HT, Jiang ZQ, Kopetz S and Daniel CR. Joint prognostic effect of obesity and chronic systemic inflammation in patients with metastatic colorectal cancer. Cancer 2015; 121: 2968-2975.
[13] Viesti ACR, Salgado W Jr, Pretti da Cunha Tirapelli D and dos Santos JS. The expression of LEP, LEPR, IGF1 and IL10 in obesity and the relationship with microRNAs. PLoS One 2014; 9: e93512.
[14] Kucuk N, Orbak Z, Karakelloglu C and Akcay F. The effect of therapy on plasma ghrelin and leptin levels, and appetite in children with iron deficiency anemia. J Pediatr Endocrinol Metab 2019; 32: 275-280.
[15] Liu J, Yang X, Yu S and Zheng R. The leptin signaling. Adv Exp Med Biol 2018; 1090: 123-144.
[16] Perez-Perez A, Vilarino-Garcia T, Fernandez-Riejos P, Martin-Gonzalez J, Segura-Egea JJ and Sanchez-Margalet V. Role of leptin as a link between metabolism and the immune system. Cytokine Growth Factor Rev 2017; 35: 71-84.
[17] Bartucci M, Svensson S, Ricci-Vitiani L, Dattilo R, Biffoni M, Signore M, Ferla R, De Maria R and Surmacz E. Obesity hormone leptin induces growth and interferes with the cytotoxic effects of 5-fluorouracil in colorectal tumor stem cells. Endocr Relat Cancer 2010; 17: 823-833.
[18] Ho GY, Wang T, Gunter MJ, Strickler HD, Cushman M, Kaplan RC, Wassertheil-Smoller S, Xue X, Rajpathak SN, Chlebowski RT, Vitolins MZ, Scherer PE and Rohan TE. Adipokines linking obesity with colorectal cancer risk in postmenopausal women. Cancer Res 2012; 72: 3029-3037.
[19] Song M, Zhang X, Wu K, Ogino S, Fuchs CS, Giovannucci EL and Chan AT. Plasma adiponectin and soluble leptin receptor and risk of colorectal cancer: a prospective study. Cancer Prev Res (Phila) 2013; 6: 875-885.
[20] Hassan NE, El-Masry SA, Zarouk W, El Banna RA, Mosaad RM, Al-Tohamy M and Salamah AR. Obesity phenotype in relation to gene polymorphism among samples of Egyptian children and their mothers. Genes Dis 2018; 5: 150-157.
[21] Nesrine Z, Haithem H, Imen B, Fadoua N, Asma O, Fadhel NM and Ali B. Leptin and Leptin receptor polymorphisms, plasma Leptin levels and obesity in Tunisian volunteers. Int J Exp Pathol 2018; 99: 121-130.
[22] Dasgupta S, Salman M, Siddalingaiah LB, Lakshmi GL, Xaviour D and Sreenath J. Genetic variants in leptin: determinants of obesity and leptin levels in South Indian population. Adipocyte 2015; 4: 135-140.
[23] Nock NL, Li L and Elston RC. Modeling genetic and environmental factors in biological systems using structural equation modeling: an application to energy balance. Proc Ohio Collab Conf Bioinform 2009; 3-8.
[24] Slattery ML, Wolff RK, Herrick J, Caan BJ and Potter JD. Leptin and leptin receptor genotypes and colon cancer: gene-gene and gene-lifestyle interactions. Int J Cancer 2008; 122: 1611-1617.
[25] Partida-Perez M, de la Luz Ayala-Madrigal M, Peregrina-Sandoval J, Macias-Gomez N, Moreno-Ortiz J, Leal-Ugarte E, Cardenas-Meza M, Centeno-Flores M, Maciel-Gutierrez V, Cabrales E, Cervantes-Ortiz S and Gutierrez-Angulo M. Association of LEP and ADIPOQ common variants with colorectal cancer in Mexican patients. Cancer Biomark 2010; 7: 117-121.
[26] Tang W, Kang M, Liu C and Qiu H. Leptin rs7799039 (G2548A) polymorphism is associated with cancer risk: a meta-analysis involving 25,799 subjects. Onco Targets Ther 2019; 12: 2879-2890.
[27] Vasku A, Vokurka J and Bienertova-Vasku J. Obesity-related genes variability in Czech patients with sporadic colorectal cancer: preliminary results. Int J Colorectal Dis 2009; 24: 289-294.
[28] Qiu H, Cheng C, Wang Y, Kang M, Tang W, Chen S, Gu H, Liu C and Chen Y. Investigation of cyclin D1 rs9344 G>A polymorphism in colorectal cancer: a meta-analysis involving 13,642 subjects. Onco Targets Ther 2016; 9: 6641-6650.

[29] Tang W, Chen Y, Chen S, Sun B, Gu H and Kang M. Programmed death-1 (PD-1) polymorphism is associated with gastric cardia adenocarcinoma. Int J Clin Exp Med 2015; 8: 8086-8093.
[30] Yan P, Xia M, Gao F, Tang G, Zeng H, Yang S, Zhou H, Ding D and Gong L. Predictive role of miR-146a rs2910164 (C>G), miR-149 rs2292832 (T>C), miR-196a2 rs11614913 (T>C) and miR-499 rs3746444 (T>C) in the development of hepatocellular carcinoma. Int J Clin Exp Pathol 2015; 8: 15177-15183.
[31] Tang W, Chen Y, Chen S, Sun B, Gu H and Kang M. Programmed death-1 (PD-1) polymorphism is associated with gastric cardia adenocarcinoma. Int J Clin Exp Med 2015; 8: 8086-8093.
[32] Tang W, Wang Y, Chen S, Lin J, Chen B, Yu S, Chen Y, Gu H and Kang M. Investigation of cytotoxic T-lymphocyte antigen 4 polymorphisms in gastric cardia adenocarcinoma. Scand J Immunol 2016; 83: 212-218.
[33] Zhang S, Jiang J, Chen Z, Wang Y, Tang W, Liu C, Liu L and Chen Y. Investigation of LEP and LEPR polymorphisms with the risk of hepatocellular carcinoma: a case-control study in Eastern Chinese Han population. Onco Targets Ther 2018; 11: 2083-2089.
[34] Nyante SJ, Gammon MD, Kaufman JS, Bensen JT, Lin DY, Barnholtz-Sloan JS, Hu Y, He Q, Luo J and Millikan RC. Common genetic variation in adiponectin, leptin, and leptin receptor and association with breast cancer subtypes. Breast Cancer Res Treat 2011; 129: 593-606.
[35] Romanowski M, Dziedziejko V, Maciejewska-Karlowska A, Sawczuk M, Safranow K, Domanski L and Pawlik A. Adiponectin and leptin gene polymorphisms in patients with post-transplant diabetes mellitus. Pharmacogenomics 2015; 16: 1243-1251.
[36] Marcello MA, Calixto AR, de Almeida JF, Martins MB, Cunha LL, Cavalari CA, Etchebehere EC, da Assumpcao LV, Geloneze B, Carvalho AL and Ward LS. Polymorphism in LEP and LEPR may modify leptin levels and represent risk factors for thyroid cancer. Int J Endocrinol 2015; 2015: 173218.
[37] Yang J, Zhong Z, Tang W and Chen J. Leptin rs2167270 G > A (G19A) polymorphism may decrease the risk of cancer: A case-control study and meta-analysis involving 19 989 subjects. J Cell Biochem 2019; 120: 10998-11007.
[38] Gotoda T, Manning BS, Goldstone AP, Imrie H, Evans AL, Strosberg AD, McKeigue PM, Scott J and Aitman TJ. Leptin receptor gene variation and obesity: lack of association in a white British male population. Hum Mol Genet 1997; 6: 869-876.
[39] Mohammadzadeh G, Ghaffari MA, Bafandeh A and Hosseini SM. Effect of leptin receptor Q223R polymorphism on breast cancer risk. Iran J Basic Med Sci 2014; 17: 588-594.
[40] Li Z, Yuan W, Ning S, Li J, Zhai W and Zhang S. Role of leptin receptor (LEPR) gene polymorphisms and haplotypes in susceptibility to hepatocellular carcinoma in subjects with chronic hepatitis B virus infection. Mol Diagn Ther 2012; 16: 383-388.
[41] Shi H, Shu H, Huang C, Gong J, Yang Y, Liu R, Yang Y and Liu P. Association of LEPR K109R polymorphisms with cancer risk: a systematic review and pooled analysis. J BUON 2014; 19: 847-854.