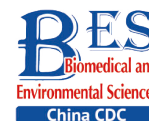## Original Article

A Comprehensive Study of the Association between *LEPR* Gene rs1137101 Variant and Risk of Digestive System Cancers[*]

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Abstract

Objective   The leptin receptor, encoded by the *LEPR* gene, is involved in tumorigenesis. A potential functional variant of *LEPR*, rs1137101 (Gln223Arg), has been extensively investigated for its contribution to the risk of digestive system (DS) cancers, but results remain conflicting rather than conclusive. Here, we performed a case–control study and subsequent meta-analysis to examine the association between rs1137101 and DS cancer risk.

Methods   A total of 1,727 patients with cancer (gastric/liver/colorectal: 460/480/787) and 800 healthy controls were recruited. Genotyping of rs1137101 was conducted using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay and confirmed using Sanger sequencing. Twenty-four eligible studies were included in the meta-analysis.

Results   After Bonferroni correction, the case–control study revealed that rs1137101 was significantly associated with the risk of liver cancer in the Hubei Chinese population. The meta-analysis suggested that rs1137101 is significantly associated with the risk of overall DS, gastric, and liver cancer in the Chinese population.

Conclusion   The *LEPR* rs1137101 variant may be a genetic biomarker for susceptibility to DS cancers (especially liver and gastric cancer) in the Chinese population.

Key words: *LEPR* gene; rs1137101; Digestive system cancers; Genetic susceptibility; Meta-analysis

INTRODUCTION

Digestive system (DS) cancers include tumors of the mouth, esophagus, liver, stomach, bile duct, colon, rectum, pancreas, and gallbladder. GLOBOCAN estimates that DS cancers accounted for approximately 19.3 million newly diagnosed cancers and 10.0 million deaths worldwide in 2020. Colorectal, gastric, liver, and esophageal cancers are the four most common DS cancers, ranked 3rd, 5th, 6th, and 7th in global incidence and 2nd, 4th, 3rd, and 6th in overall mortality, respectively[1]. Although novel therapeutic strategies and precise diagnostics have been

developed, the outcome for patients with DS cancer remains unsatisfactory[2]. More importantly, due to the largely asymptomatic nature of most types of DS cancers, patients are often diagnosed at a late stage, resulting in limited treatment options and poor prognosis.

Leptin is a 16-kD cytokine-like peptide hormone secreted primarily by the white adipose tissue[3]. It exerts its biological functions through a specific homodimeric receptor called leptin receptor (LEPR), which is expressed in multiple organs[4]. The long form of the leptin receptor, Ob-Rb, is the predominant isoform of LEPR. Interestingly, activation of Ob-Rb by leptin binding has been shown to stimulate the janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3), phosphoinositide 3-kinase/mammalian target of rapamycin (PI3K/mTOR), and mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling pathways[5-8]. Numerous lines of evidence suggest that these key signaling cascades play crucial roles in multiple aspects of tumorigenesis; thus, the *LEPR* gene is regarded as a plausible candidate gene for cancer susceptibility. Indeed, functional studies have revealed that LEPR is involved in promoting angiogenesis, facilitating cell proliferation and inhibiting epithelial cell apoptosis[9-11].

LEPR is located on chromosome 1p31 (position_38: 65420652-65637493). More than 3,117 genetic variants have been identified in the *LEPR* gene region. Current knowledge suggests that genetic variants with regulatory function are more likely to be the causative loci of complex human diseases. Matsuoka et al. reported seven common missense variants (Lys109Arg, Gln223Arg, Ser343Ser, Ser492Thr, Lys656Asn, Ala976Asp, and Pro1019Pro) that cause amino acid changes in the Ob-Rb coding region[12]. Of the seven genetic variants, Gln223Arg (rs1137101) has been extensively examined for its association with cancer risk. The interesting question is why rs1137101 has attracted the most attention. Here, we used the SNPinfo software (https://snpinfo.niehs.nih.gov/) to predict that rs1137101 may act as an exonic splicing enhancer or silencer of *LEPR* mRNA, which modifies the efficiency of RNA splicing and thereby alters the function of LEPR.

The genetic effect of rs1137101 has been investigated mainly in DS cancers (oral[13-16], esophageal[17,18], gastric[19-21], colorectal[22-27], and liver[28-31]) and breast cancer[32], as well as in a few other types (endometrial[33] and lung cancer[34]). Nevertheless, no conclusive results have been published, which calls for further investigation. To replicate and further explore the association between rs1137101 and risk of DS cancer, we conducted a case–control study to investigate the genetic effect of rs1137101 on the risk of gastric, colorectal, and liver cancers in a Hubei Chinese population.

On a different note, although the association between rs1137101 and overall cancer risk has been previously determined in several meta-analyses[35-37], a meta-analysis specific to the association between rs1137101 and DS cancer risk is lacking. Given that articles related to the relationship between rs1137101 and DS cancers are continually published and new experimental data were obtained in the present study, we performed a comprehensive meta-analysis to determine the contribution of rs1137101 to DS cancer risk.

MATERIALS AND METHODS

Participants

This hospital-based case–control study included 1,727 patients with cancer and 800 healthy controls. Of these patients, 460 were diagnosed with gastric cancer, 787 with colorectal cancer, and 480 with liver cancer; the diagnoses were confirmed histopathologically. The patients were recruited from the Hubei Cancer Hospital and Wuhan Xinzhou District People's Hospital and the healthy controls were selected from cancer-free individuals who visited the Wuhan Xinzhou District People's Hospital for medical checkups. All enrolled participants were unrelated Chinese Han individuals living in Hubei Province. Information on age, sex, smoking status, and drinking status was collected from all participants. Regular smoking was defined as smoking at least one cigarette per day on average over one year or having quit smoking for less than one year. Regular drinking was defined as drinking at least 100 mL of alcohol per day on average over one year or having quit drinking for less than one year. This study was conducted after obtaining approval from the ethical committee of Wuhan University of Technology, and signed informed consent forms were obtained from all participants.

DNA Extraction and Genotyping

Peripheral blood samples from all participants were collected into blood vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) and stored at 4 °C. Genomic DNA was extracted from the blood

samples using the TIANamp Blood DNA Kit (DP348; TianGen Biotech, Beijing, China) within one week, according to the manufacturer's instructions. The DNA samples were stored at −20 °C before use. Genotyping of the *LEPR* gene rs1137101 variant was performed using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. The DNA fragments containing rs1137101 were amplified using the primers 5'-TGTGCCAACAGCCAAACTCA-3' (forward) and 5'-CCCCAGGACTACATCTACCATCA-3' (reverse). The PCR products were digested with the restriction enzyme *Msp* I (Thermo Scientific, USA) for 4 h at 37 °C. The digested fragments were then separated on a 2.0% agarose gel and the RFLP bands were visualized under ultraviolet light using Gel-Red staining. The rs1137101 AA genotype was identified by the presence of a 243-bp band; the AG genotype by the presence of three bands of 243, 171, and 72 bp; and the GG genotype by the presence of two bands of 171 and 72 bp. To ensure the accuracy of the PCR-RFLP genotyping results, we performed Sanger sequencing on 20% of randomly selected DNA samples, and the results were 100% concordant. Supplementary Figure S1 (available in www.besjournal.com) shows the genotyping diagrams of rs1137101 obtained using PCR-RFLP and Sanger sequencing.

Statistical Analysis

All statistical analyses were conducted using SPSS 15.0 (Chicago, IL, USA). The chi-square (χ^2) test was used to compare the distributions of age, sex, smoking status, and drinking status between the patients and healthy controls. The genotypic frequency of rs1137101 in healthy controls was examined for deviation from Hardy-Weinberg equilibrium (HWE). Unconditional logistic regression analysis under allele, carrier, homozygote, heterozygote, recessive, and dominant models was used to assess the correlation between rs1137101 and cancer risk. Statistical significance was set at P < 0.05, and an adjusted threshold of 0.0084 (0.05/6) by Bonferroni correction was used for multiple comparisons.

Meta-Analysis

A thorough literature search of the PubMed, EMBASE, ISI, Web of Science, CNKI, and Wanfang databases up to August 31, 2023 was conducted to retrieve eligible studies related to rs1137101 and DS cancer risk. The following terms and keywords were searched: "leptin receptor" OR "*LEPR*" or "obese receptor" OR "*OBR*" AND "rs1137101" OR "Q223R" OR "p. Gln223Arg" or "c. 668A>G" and "cancer" or "tumor" OR "carcinoma" OR "malignancy" OR "neoplasms". To identify additional eligible studies, we screened the references listed in the retrieved articles. The inclusion criteria were as follows: (1) case–control study, (2) rs1137101 and risk of DS cancer (oral, esophageal, gastric, liver, bile duct, colorectal, pancreatic, and gallbladder cancer), (3) human study, (4) extractable genotyping data, and (5) no departure from HWE in the controls. Supplementary Figure S2 (available in www. besjournal.com) shows a flowchart of the process for literature retrieval for this meta-analysis. Statistical analyses, including heterogeneity tests, pooled odds ratios (*ORs*), publication bias, and sensitivity analysis, were performed using STATA 14.0 (Stata Corp, College Station, Texas). P < 0.05 indicated the significance level and the Bonferroni correction was adopted for multiple comparisons. In addition, trial sequential analysis (TSA) was conducted to examine whether the statistical results were reliable and conclusive. The elucidation of TSA results was described in our previous study[38].

RESULTS

Principal Characteristics of the Participants

The principal characteristics of the participants are presented in Table 1. The distributions of age, sex, smoking status, and drinking status did not differ significantly between patients with cancer and healthy controls (P > 0.05). The average age of healthy controls and patients with gastric, colorectal, and liver cancer were 56.16 ± 10.85, 57.36 ± 10.46, 57.87 ± 10.76, and 57.57 ± 10.38 years, respectively. No statistical differences were observed in the average age between patients (with gastric, colorectal, or liver cancer) and healthy controls. These results indicate that the data from the patients with cancer and healthy controls can be compared for study purposes.

Association of rs1137101 with Susceptibility to Three DS Cancers in the Hubei Chinese Population

The association between rs1137101 and risk of gastric, colorectal, or liver cancer was examined in a Hubei Chinese population, and the statistical results are presented in Table 2. The observed genotypic distributions of rs1137101 were consistent with HWE in the healthy controls (P = 0.778), suggesting that this enrolled cohort was representative of the

population. rs1137101 was nominally associated with gastric cancer risk at a significance level of 0.05 but the association did not remain after Bonferroni correction. No association was observed between the rs1137101 polymorphism and colorectal cancer risk. Of note, the presence of rs1137101 was significantly associated with liver cancer risk, even after Bonferroni correction. Specifically, compared to the G allele, the rs1137101 A allele conferred a lower risk of liver cancer (A *vs*. G, *P* = 0.002, *OR* = 0.66, 95% confidence intervals [*CI*]: 0.50–0.85). Individuals carrying the AG genotype and those carrying the AA + AG genotypes had reduced risk of liver cancer compared to individuals carrying the GG genotype (AG *vs*. GG, *P* = 0.006, *OR* = 0.67, 95% *CI*: 0.50–0.89; AA + AG *vs*. AG, *P* = 0.003, *OR* = 0.65, 95% *CI*: 0.49–0.86).

Meta-Analysis of the Association between rs1137101 and DS Cancer Risk

A total of 24 eligible studies were included in this meta-analysis. Two separate authors (HU Wei Qiong and ZHOU Wei Guang) carefully collected essential information from the eligible studies, which is presented in Supplementary Table S1 (available in www.besjournal.com). As shown in Table 3, no significant association was identified between rs1137101 and DS cancer risk. Interestingly, the stratified analysis by ethnicity also revealed lack of any significant association between rs1137101 and DS cancer risk in the Asian or Caucasian populations. In contrast, the stratified analysis by country suggested that rs1137101 was significantly associated with DS cancer risk in the Chinese population. Chinese individuals with the AA genotype were less likely to present with DS cancers than those with the AG, GG, and AG+GG genotypes (AA *vs*. AG, *P* < 0.001, *OR* = 0.62, 95% *CI*: 0.50–0.78;

AA *vs*. GG, *P* < 0.001, *OR* = 0.53, 95% *CI*: 0.42–0.67; AA *vs*. AG + GG, *P* < 0.001, *OR* = 0.57, 95% *CI*: 0.46–0.70). Lastly, no significant association between rs1137101 and DS cancer risk was identified in the stratified analysis based on PCR-RFLP genotyping.

Meta-Analysis of the Association between rs1137101 and Risk of Specific DS Cancers

A stratified analysis according to cancer type was conducted, and the pooled results are presented in Table 4. We found that rs1137101 had no significant effect on the risk of oral, esophageal, or colorectal cancer. However, a marked effect of rs1137101 on the risk of gastric and liver cancers was observed in the total population. The AA genotype carriers were less likely to have gastric cancer than the GG (AA *vs*. GG, *P* = 0.006, *OR* = 0.44, 95% *CI*: 0.24–0.79) or AG+GG genotype carriers (AA *vs.* AG+GG, *P* = 0.008, *OR* = 0.45, 95% *CI*: 0.25–0.82). Moreover, the A allele showed a protective role compared to the G allele in liver carcinogenesis (A *vs*. G, *P* = 0.003, *OR* = 0.74, 95% *CI*: 0.61–0.91). The individuals with the AA genotype had a lower risk of presenting with liver cancer than those with the AG (AA *vs*. AG, *P* < 0.001, *OR* = 0.56, 95% *CI*: 0.42–0.75), GG (AA *vs*. GG, *P* < 0.001, *OR* = 0.41, 95% *CI*: 0.30–0.56), or AG+GG genotypes (AA *vs*. AG+GG, *P* < 0.001, *OR* = 0.50, 95% *CI*: 0.38–0.65). The individuals with the AG genotype were also less likely to have liver cancer than those with the GG genotype (AG *vs*. GG, *P* = 0.005, *OR* = 0.80, 95% *CI*: 0.69–0.94). Interestingly, the association between rs1137101 and liver cancer risk was confirmed in the Chinese population (AA *vs*. AG, AA *vs*. GG, AG *vs*. GG, and AA *vs*. AG+GG).

Forest Plots, Publication Bias, Sensitivity Analysis, and TSA in this Meta-analysis

Under the genetic model of AA *vs*. GG, forest

Table 1. Principal characteristics of participants

Group	Age (years), *n* (%)		Sex, *n* (%)		Smoking status, *n* (%)		Drinking status, *n* (%)		*P*-value[1]
	≤ 60	> 60	Male	Female	Ever	Never	Ever	Never	
Healthy controls (*n* = 800)	434 (54.3)	366 (45.7)	558 (69.7)	242 (30.3)	209 (26.1)	591 (73.9)	237 (29.6)	563 (70.4)	
Patients with gastric cancer (*n* = 460)	252 (54.8)	208 (45.2)	323 (70.3)	137 (29.7)	132 (28.8)	328 (71.2)	148 (32.1)	312 (67.9)	0.855 0.862 0.323 0.344
Patients with colorectal cancer (*n* = 787)	455 (57.8)	332 (42.2)	555 (70.5)	232 (29.5)	228 (29.0)	559 (71.0)	257 (32.7)	530 (67.3)	0.153 0.737 0.204 0.192
Patients with liver cancer (*n* = 480)	280 (58.3)	200 (41.7)	343 (71.5)	137 (28.5)	140 (29.2)	340 (70.8)	158 (32.9)	322 (67.1)	0.154 0.517 0.237 0.217

Note. [1]Two-sided χ^2 test for the distributions of age (1st column), sex (2nd column), smoking status (3rd column), and drinking status (4th column) between patients with gastric/colorectal/liver cancer and healthy controls.

plots, funnel plots, sensitivity analysis, and TSA were separately performed for the meta-analysis on the association between rs1137101 and DS cancer risk in the total population (Figure 1A, 2A, 3A, and 4A) and Chinese population (Figures 1B, 2B, 3B, and 4B); and for the meta-analysis of the association of rs1137101 with gastric cancer risk in the total population (Figures 1C, 2C, 3C, and 4C) and with liver cancer risk in the total population (Figures 1D, 2D, 3D, and 4D). The Begg (Tables 3 and 4) and Egger tests (Tables 3 and 4) demonstrated no evidence of publication bias in any of the genetic models. The funnel plots of AA *vs.* GG also excluded the occurrence of publication bias. The sensitivity analysis showed that the pooled ORs did not significantly change after systematically removing individual studies. As shown in Figure 4A, the cumulative *Z*-curve did not cross *Z* = 1.96 or the trial sequential monitoring boundary (TSMB) before reaching the required information size line, providing firm evidence for the lack of association between rs1137101 and DS cancer risk in the total population. The TSA results in Figures 4B, 4C, and 4D show that the cumulative *Z*-curves crossed both *Z* = 1.96 and TSMB before reaching the required information size line, confirming the real contribution of rs1137101 to the risk of DS cancers in the Chinese population and risk of gastric and liver cancers in the total population.

DISCUSSION

DS cancers are a global health problem and a serious threat to human life. Detection at an early stage may be beneficial to patient outcome by improving the 5-year survival rate and may even lead to a complete cure. LEPR mediates key signaling pathways implicated in carcinogenesis. Specifically, the review by Rodrigues et al.[7] shows that leptin binding to Ob-Rb in the cell membrane induces homodimerization of the receptor followed by JAK-2 phosphorylation and subsequent phosphorylation of Tyr1138 in Ob-Rb. These events lead to the activation of STAT3 *via* phosphorylation, thereby regulating the expression of several cancer-associated genes[39]. Through STAT3, leptin can also trigger important cancer signaling pathways such as PI3K/mTOR. Additionally, through the phosphorylation of Tyr985 in Ob-Rb, leptin can activate Src homology 2 domain-containing phosphatase 2 (SHP2), triggering the MAPK/ERK pathway[40]. This evidence strongly suggests that *LEPR* is a plausible candidate gene for cancer susceptibility[10]. Consequently, the identification of certain causative variants in the *LEPR* gene would be of great help in the early diagnosis and treatment of DS cancers.

The *LEPR* gene rs1137101, a potential functional variant, has been extensively investigated for its contribution to the risk of DS cancers (21 studies up to August 31, 2023)[13-31], breast cancer (24 studies up to February 10, 2022), and endometrial[33] and lung cancers[34]. The findings of the studies on rs1137101 and DS cancer risk are summarized in Supplementary Table S1. rs1137101 was significantly

Table 2. Genotype and allele distributions of rs1137101 and its association with risk of gastric, colorectal and liver cancers in the Hubei Chinese population

Group	Allele, n (%)		Genotype, n (%)			HWE[1]	Genetic model [P-value, OR (95% CI)][2]					
	A	G	AA	AG	GG		A vs. G	AA vs. AG	AA vs. GG	AG vs. GG	AA vs. AG+GG	AA+AG vs. GG
Healthy controls (n = 800)	211 (0.132)	1389 (0.868)	13 (0.016)	185 (0.231)	602 (0.753)	0.778						
Patients with gastric cancer (n = 460)	92 (0.100)	828 (0.900)	1 (0.002)	90 (0.196)	369 (0.802)		0.018, 0.73 (0.56–0.95)	0.078, 0.16 (0.02–1.23)	0.046, 0.13 (0.02–0.96)	0.110, 0.79 (0.60–1.05)	0.051, 0.13 (0.02–1.01)	0.044, 0.75 (0.57–0.99)
Patients with colorectal cancer (n = 787)	216 (0.137)	1358 (0.863)	12 (0.015)	192 (0.244)	583 (0.741)		0.658, 1.05 (0.85–1.28)	0.777, 0.89 (0.40–2.00)	0.906, 0.95 (0.43–2.11)	0.558, 1.07 (0.85–1.35)	0.873, 0.94 (0.43–2.07)	0.592, 1.06 (0.85–1.33)
Patients with liver cancer (n = 480)	87 (0.091)	873 (0.909)	3 (0.006)	81 (0.169)	396 (0.825)		0.002, 0.66 (0.50–0.85)	0.328, 0.53 (0.15–1.90)	0.104, 0.35 (0.10–1.24)	0.006, 0.67 (0.50–0.89)	0.133, 0.38 (0.11–1.34)	0.003, 0.65 (0.49–0.86)

Note. [1]Genotypic frequency of rs1137101 in controls was tested for departure from Hardy-Weinberg equilibrium (HWE) using the two-sided χ^2 test. [2]The *P* value and *OR* (95% *CI*) were calculated for each genetic comparison between patients with gastric/colorectal/liver cancer and healthy controls, and adjusted for age, sex, smoking status, and drinking status. *OR*, odd ratio; *CI*, confidence interval.

Table 3. Meta-analysis of the association between rs1137101 and digestive system cancers risk

Genetic model	Heterogeneity test			Pooled OR (95% CI)[1]	Hypothesis test		Begg's test		Egger's test		Studies (n)
	Q value	P value	I^2 (%)		Z value	P value	Z value	P value	Z value	P value	
In the total population (8,279 cases/14,225 controls)											
A vs. G	86.03	< 0.001	74.400	0.95 (0.85–1.06)	0.93	0.352	0.00	1.000	0.40	0.695	23
AA vs. AG	39.22	0.013	43.900	0.90 (0.77–1.07)	1.18	0.237	1.16	0.245	−0.72	0.477	23
AA vs. GG	63.79	< 0.001	65.500	0.86 (0.67–1.11)	1.16	0.248	1.85	0.064	−0.42	0.682	23
AG vs. GG	38.11	0.018	42.300	0.97 (0.87–1.07)	0.65	0.514	0.53	0.597	0.55	0.588	23
AA vs. AG+GG	58.04	< 0.001	60.400	0.89 (0.74–1.08)	1.17	0.244	1.46	0.143	−0.72	0.479	24
AA+AG vs. GG	58.91	< 0.001	62.700	0.95 (0.84–1.08)	0.78	0.434	0.21	0.833	0.57	0.574	23
In the Asian population (6,105 cases/8,710 controls)											
A vs. G	48.98	< 0.001	73.500	0.90 (0.79–1.03)	1.52	0.129	0.11	0.913	0.56	0.588	14
AA vs. AG	21.30	0.067	39.000	0.80 (0.61–1.06)	1.58	0.115	1.42	0.155	−1.09	0.298	14
AA vs. GG	28.84	0.007	54.900	0.69 (0.48–0.99)	2.00	0.045	1.20	0.228	−0.07	0.942	14
AG vs. GG	22.30	0.051	41.700	0.92 (0.82–1.03)	1.49	0.137	0.44	0.661	0.25	0.808	14
AA vs. AG+GG	31.75	0.003	59.100	0.74 (0.54–1.03)	1.79	0.074	1.20	0.228	−0.87	0.400	14
AA+AG vs. GG	33.86	0.001	61.600	0.89 (0.78–1.03)	1.61	0.106	0.33	0.743	0.28	0.784	14
In the Chinese population (5,671 cases/8,198 controls)											
A vs. G	39.19	< 0.001	71.900	0.86 (0.74–0.99)	2.10	0.036	0.75	0.451	0.32	0.757	12
AA vs. AG	7.36	0.769	< 0.001	0.62 (0.50–0.78)	4.14	< 0.001	0.89	0.373	−0.12	0.905	12
AA vs. GG	15.34	0.168	28.300	0.53 (0.42–0.67)	5.33	< 0.001	0.62	0.537	0.40	0.699	12
AG vs. GG	21.77	0.026	49.500	0.91 (0.80–1.03)	1.50	0.133	0.89	0.373	−0.03	0.978	12
AA vs. AG+GG	11.49	0.403	4.200	0.57 (0.46–0.70)	5.20	< 0.001	0.62	0.537	0.15	0.882	12
AA+AG vs. GG	31.78	0.001	65.400	0.87 (0.75–1.01)	1.85	0.064	1.17	0.244	−0.22	0.833	12
In the Caucasian population (2,174 cases/5,515 controls)											
A vs. G	30.01	< 0.001	73.300	1.04 (0.88–1.23)	0.44	0.663	−0.10	1.000	0.25	0.808	9
AA vs. AG	16.63	0.034	51.900	0.98 (0.79–1.22)	0.17	0.864	0.52	0.602	0.67	0.524	9
AA vs. GG	26.00	0.001	69.200	1.08 (0.77–1.52)	0.46	0.644	0.94	0.348	0.43	0.680	9
AG vs. GG	11.84	0.159	32.400	1.09 (0.95–1.25)	1.17	0.242	−0.10	1.000	−0.20	0.851	9
AA vs. AG+GG	23.11	0.006	61.100	1.01 (0.81–1.26)	0.09	0.931	0.18	0.858	0.56	0.591	10
AA+AG vs. GG	19.16	0.014	58.300	1.08 (0.86–1.36)	0.68	0.497	0.10	0.917	−0.13	0.904	9
In the Australian population (774 cases/4,056 controls)											
A vs. G	0.27	0.874	< 0.001	0.96 (0.86–1.07)	0.80	0.424	0.00	1.000	1.31	0.415	3
AA vs. AG	0.66	0.718	< 0.001	0.85 (0.71–1.02)	1.76	0.078	1.04	0.296	4.51	0.139	3
AA vs. GG	0.28	0.871	< 0.001	0.94 (0.75–1.18)	0.56	0.575	0.00	1.000	1.32	0.413	3
AG vs. GG	0.51	0.775	< 0.001	1.10 (0.90–1.35)	0.93	0.355	0.00	1.000	−0.27	0.831	3
AA vs. AG+GG	0.52	0.772	< 0.001	0.87 (0.74–1.04)	1.55	0.122	1.04	0.296	28.33	0.022	3
AA+AG vs. GG	0.36	0.833	< 0.001	1.04 (0.86–1.26)	0.38	0.707	0.00	1.000	0.12	0.921	3
In the total population based on PCR–RFLP[2] assay (3,990 cases/4,949 controls)											
A vs. G	84.79	< 0.001	84.700	0.94 (0.77–1.16)	0.57	0.571	0.55	0.584	0.55	0.593	14
AA vs. AG	34.66	0.001	62.500	0.92 (0.67–1.27)	0.49	0.626	0.77	0.443	−0.36	0.723	14
AA vs. GG	60.46	< 0.001	78.500	0.83 (0.51–1.35)	0.76	0.447	1.09	0.274	0.11	0.913	14
AG vs. GG	33.89	0.001	61.600	0.94 (0.78–1.14)	0.64	0.522	0.22	0.827	0.52	0.615	14
AA vs. AG+GG	54.49	< 0.001	76.100	0.88 (0.61–1.27)	0.68	0.495	0.77	0.443	−0.37	0.717	14
AA+AG vs. GG	55.80	< 0.001	76.700	0.94 (0.74–1.18)	0.55	0.581	0.00	1.000	0.60	0.563	14

Note. [1]OR, odd ratio; CI, confidence interval. [2]PCR–RFLP, polymerase chain reaction–restriction fragment length polymorphism.

Table 4. Meta-analysis of the association between rs1137101 and risk of specific digestive system cancers

Genetic model	Heterogeneity test			Pooled OR (95% CI)[1]	Hypothesis test		Begg's test		Egger's test		Studies (n)
	Q value	P value	I^2 (%)		Z value	P value	Z value	P value	Z value	P value	
Oral cancer (1,027 cases/1,063 controls)											
A vs. G	14.05	0.003	78.600	1.28 (0.88–1.86)	1.30	0.194	−0.34	1.000	−0.35	0.761	4
AA vs. AG	7.05	0.070	57.400	1.39 (0.76–2.51)	1.07	0.284	0.34	0.734	−0.42	0.714	4
AA vs. GG	15.35	0.002	80.500	1.42 (0.50–4.06)	0.66	0.508	1.02	0.308	−4.18	0.053	4
AG vs. GG	7.33	0.062	59.100	1.22 (0.80–1.86)	0.91	0.365	−0.34	1.000	−0.24	0.836	4
AA vs. AG+GG	10.14	0.017	70.400	1.42 (0.73–2.78)	1.03	0.302	1.02	0.308	−0.73	0.539	4
AA+AG vs. GG	12.11	0.007	75.200	1.31 (0.78–2.20)	1.02	0.309	−0.34	1.000	−0.02	0.985	4
Esophageal cancer (1,278 cases/5,545 controls)											
A vs. G	0.28	0.963	< 0.001	0.96 (0.87–1.06)	0.83	0.404	−0.34	1.000	1.25	0.337	4
AA vs. AG	0.66	0.882	< 0.001	0.85 (0.71–1.02)	1.80	0.073	1.02	0.308	0.19	0.867	4
AA vs. GG	0.33	0.955	< 0.001	0.93 (0.75–1.16)	0.64	0.523	−0.34	1.000	−0.41	0.721	4
AG vs. GG	0.97	0.809	< 0.001	1.05 (0.90–1.23)	0.64	0.519	0.34	0.734	1.05	0.403	4
AA vs. AG+GG	0.52	0.914	< 0.001	0.87 (0.74–1.03)	1.59	0.112	0.34	0.734	0.02	0.986	4
AA+AG vs. GG	0.51	0.917	< 0.001	1.01 (0.87–1.18)	0.17	0.862	0.34	0.734	0.90	0.461	4
Gastric cancer in the Chinese population (1,212 cases/1,633 controls)											
A vs. G	14.43	0.002	79.200	0.83 (0.56–1.24)	0.91	0.363	0.34	0.734	0.32	0.778	4
AA vs. AG	2.84	0.418	< 0.001	0.51 (0.28–0.95)	2.11	0.035	0.34	0.734	−0.55	0.638	4
AA vs. GG	5.78	0.123	48.100	0.44 (0.24–0.79)	2.74	0.006	0.34	0.734	−0.27	0.812	4
AG vs. GG	10.17	0.017	70.500	0.90 (0.61–1.31)	0.56	0.577	0.34	0.734	0.41	0.718	4
AA vs. AG+GG	4.94	0.177	39.200	0.45 (0.25–0.82)	2.62	0.008	0.34	0.734	−0.33	0.774	4
AA+AG vs. GG	12.98	0.005	76.900	0.86 (0.57–1.30)	0.73	0.467	0.34	0.734	0.37	0.750	4
Colorectal cancer (3,041 cases/3,517 controls)											
A vs. G	4.74	0.448	< 0.001	1.01 (0.92–1.10)	0.15	0.882	0.38	0.707	0.43	0.689	6
AA vs. AG	5.72	0.335	12.500	1.04 (0.88–1.24)	0.50	0.614	0.38	0.707	−0.70	0.522	6
AA vs. GG	2.98	0.703	< 0.001	1.00 (0.81–1.23)	0.04	0.968	0.75	0.452	0.37	0.731	6
AG vs. GG	1.71	0.888	< 0.001	0.99 (0.87–1.12)	0.21	0.833	0.75	0.452	1.07	0.346	6
AA vs. AG+GG	5.96	0.427	< 0.001	1.03 (0.88–1.20)	0.40	0.691	0.30	0.764	−0.64	0.553	7
AA+AG vs. GG	1.83	0.872	< 0.001	0.99 (0.88–1.12)	0.15	0.885	0.38	0.707	1.17	0.306	6
Liver cancer in the total population (1,721 cases/2,467 controls)											
A vs. G	9.01	0.061	55.600	0.74 (0.61–0.91)	2.93	0.003	0.24	0.806	0.66	0.556	5
AA vs. AG	0.07	0.999	< 0.001	0.56 (0.42–0.75)	3.96	<0.001	−0.24	1.000	−0.62	0.581	5
AA vs. GG	0.38	0.984	< 0.001	0.41 (0.30–0.56)	5.45	<0.001	1.22	0.221	1.08	0.360	5
AG vs. GG	5.37	0.251	25.600	0.80 (0.69–0.94)	2.78	0.005	−0.24	1.000	0.03	0.980	5
AA vs. AG+GG	0.28	0.991	< 0.001	0.50 (0.38–0.65)	5.06	< 0.001	0.73	0.462	−0.46	0.679	5
AA+AG vs. GG	8.70	0.069	54.000	0.74 (0.58–0.94)	2.43	0.015	0.24	0.806	0.03	0.980	5
Liver cancer in the Chinese population (1,621 cases/2,417 controls)											
A vs. G	8.96	0.030	66.500	0.75 (0.60–0.95)	2.42	0.015	0.34	0.734	0.93	0.450	4
AA vs. AG	0.07	0.995	< 0.001	0.56 (0.42–0.75)	3.88	< 0.001	0.34	0.734	−0.67	0.574	4
AA vs. GG	0.38	0.944	< 0.001	0.41 (0.29–0.57)	5.30	< 0.001	1.70	0.089	0.96	0.436	4
AG vs. GG	5.30	0.151	43.400	0.81 (0.69–0.95)	2.66	0.008	−0.34	1.000	0.22	0.843	4
AA vs. AG+GG	0.27	0.966	< 0.001	0.50 (0.38–0.66)	4.94	< 0.001	0.34	0.734	−0.30	0.789	4
AA+AG vs. GG	8.60	0.035	65.100	0.75 (0.57–0.99)	2.04	0.042	0.34	0.734	0.21	0.854	4

Note. [1]*OR*, odd ratio; *CI*, confidence interval.
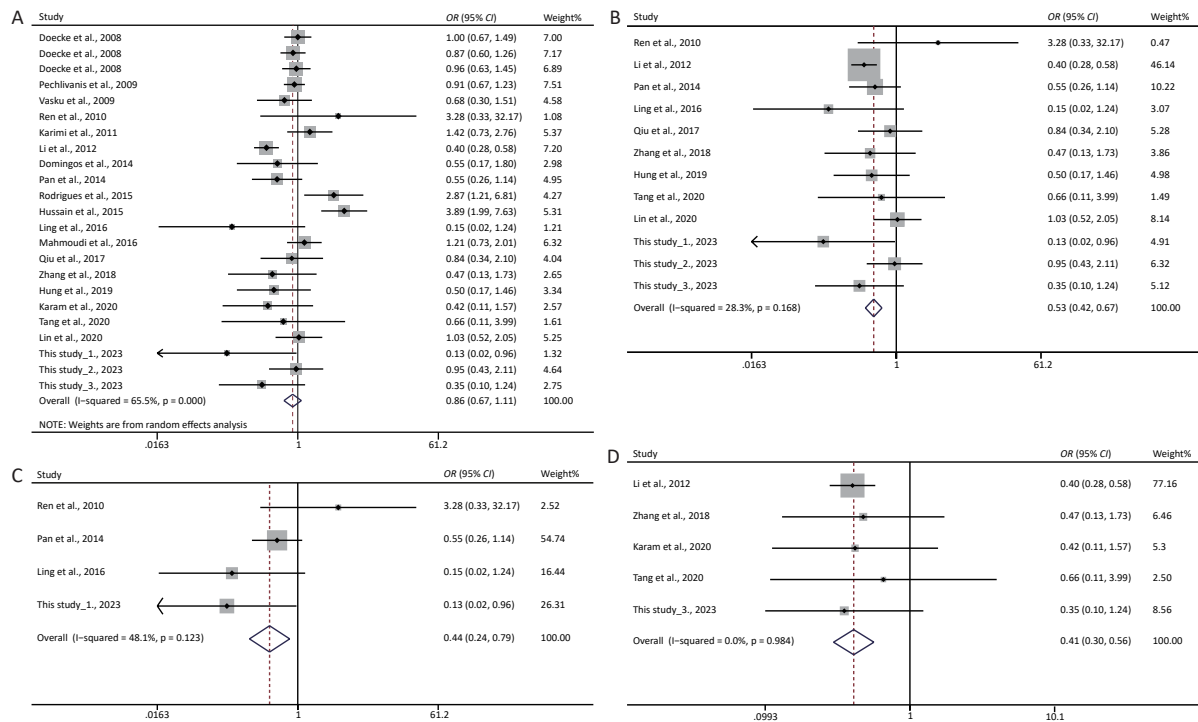
Figure 1. Forest plots of AA *vs.* GG for the meta-analysis of the association of rs1137101 with digestive-system cancer risk in the total population (A) and the Chinese population (B); and for the meta-analysis of the association of rs1137101 with gastric cancer risk (C) and liver cancer risk (D) in the total population. *OR*, odd ratio; *CI*, confidence interval.
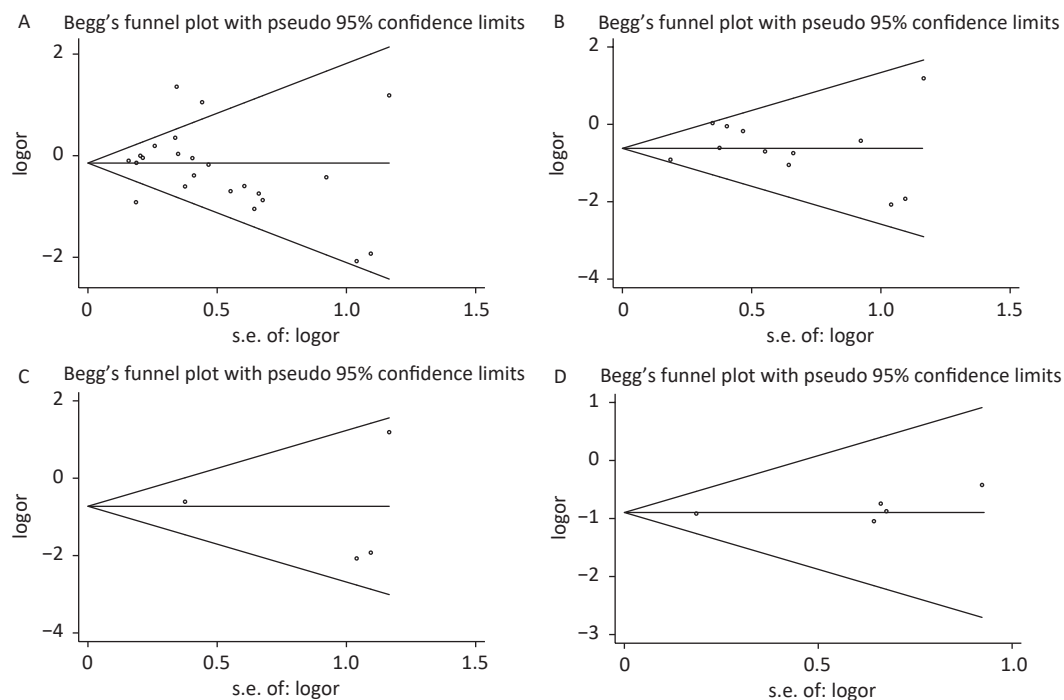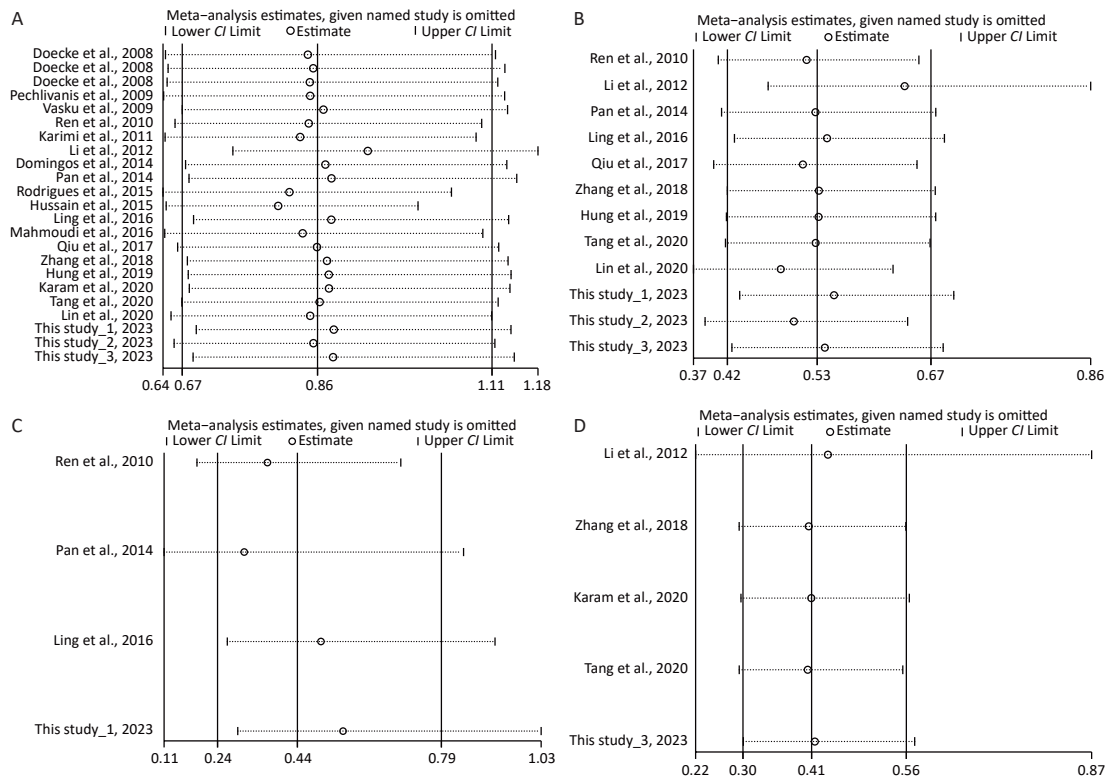

Figure 2. Funnel plots of AA *vs.* GG for the meta-analysis on the relationship between rs1137101 and digestive-system cancer risk in the total population (A) and the Chinese population (B); and for the meta-analysis of the association of rs1137101 with gastric cancer risk (C) and liver cancer risk (D) in the total population.

Figure 3. Sensitivity analysis of AA *vs.* GG for the meta-analysis of the association of rs1137101 with digestive-system cancer risk in the total population (A) and the Chinese population (B); and for the meta-analysis of the association of rs1137101 with gastric cancer risk (C) and liver cancer risk (D) in the total population. *CI*, confidence interval.
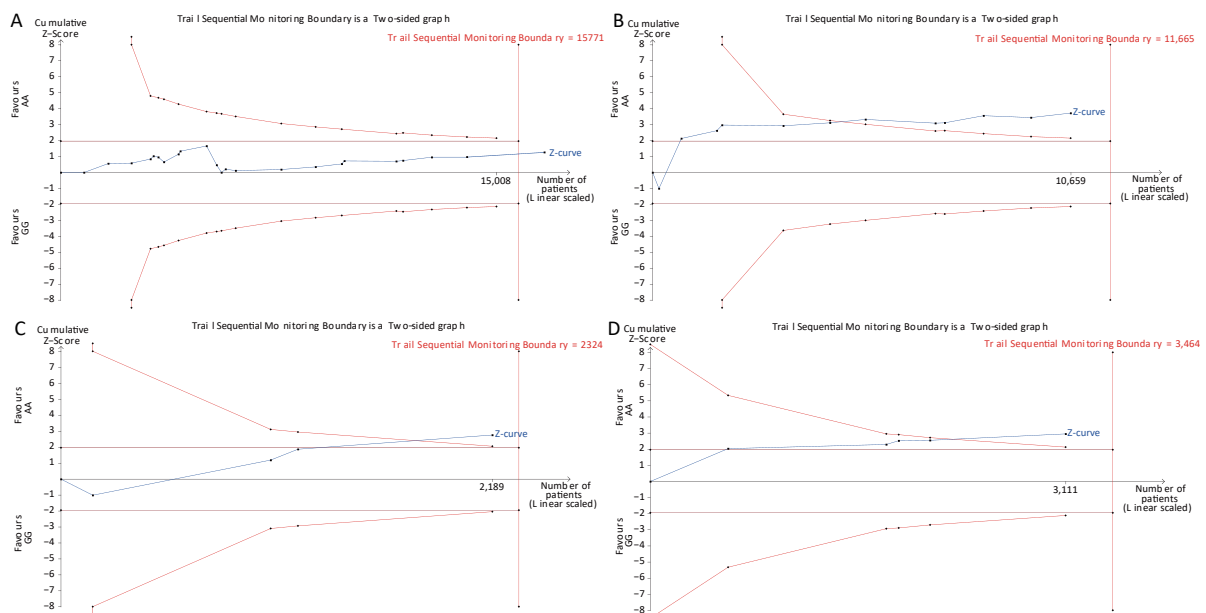

Figure 4. Trial sequential analysis of AA *vs.* GG for the meta-analysis on the relationship between rs1137101 and digestive-system cancer risk in the total population (A) and the Chinese population (B); and for the meta-analysis of the association of rs1137101 with gastric cancer risk (C) and liver cancer risk (D) in the total population.

associated with oral cancer risk in the Caucasian but not in the Asian population, and with esophageal cancer risk in the Asian but not in the Caucasian population. A significant association between rs1137101 and gastric cancer risk has been previously identified in the Chinese population[19-21] but the present study failed to confirm this finding. Lin et al. previously reported that the presence of rs1137101 may decrease colorectal cancer risk[27], although no such association was noted in other studies[22-26] or in the present study. A significant association between rs1137101 and liver cancer risk was observed in the Egyptian population[30] and in Chinese populations from Henan and Hubei provinces[28], but not in the Chinese population from Fujian or Guangxi provinces[29,31]. These discrepancies may be attributed to the following reasons. First, a tissue-specific pattern may exist for the regulatory network of rs1137101 in LEPR function in different types of DS cancers. Second, the interaction of rs1137101 with different genetic backgrounds may lead to different susceptibilities to cancer among different ethnic populations. Third, the recruited Chinese participants are from different geographical areas of China and differ in their living environments and lifestyles. Fourth, the insufficient sample size may have resulted in inadequate statistical strength.

Several meta-analyses have attempted to estimate the association between rs1137101 and cancer risk more accurately. A recent meta-analysis of 44 case–control studies by Rong et al. revealed no significant association between rs1137101 and overall cancer risk[37]. Another recent meta-analysis by Zhu et al., which included 20 case-control studies, revealed a significant correlation between rs1137101 and breast cancer risk in African populations[41]. Since the studies related to rs1137101 and breast cancer make up nearly half of all related studies, they likely affect the estimation of the association between rs1137101 and overall cancer risk. Similarly, the studies related to rs1137101 and DS cancers account for a high proportion of all related studies. Thus, meta-analyses are necessary to determine the effect of rs1137101 on DS cancer risk. After pooling the data from previous related studies and the present study, a significant association between rs1137101 and DS cancer risk was only identified in the Chinese population. In the stratified analysis by cancer type, rs1137101 was significantly associated with the risk of gastric and liver cancer but not oral, esophageal, or colorectal cancer.

Several limitations should be considered when interpreting these results. First, the recruitment of participants was hospital-based, which may potentially have contributed to a selection bias. Second, the association of rs1137101 with gastric cancer risk involved only a Chinese population; confirmatory research in other ethnic groups is needed. Third, considering the small number of included studies for each specific DS cancer in this meta-analysis, replication studies and updated meta-analyses are required for a more accurate estimation of the effect of rs1137101 on DS cancer risk. Lastly, no evidence is currently available to show that rs1137101 is functionally significant and contributes to cancer development *in vitro* and *in vivo*. Future functional studies are thus needed to gain a better understanding of the role that *LEPR* gene rs1137101 plays in cancer pathogenesis.

In conclusion, our case–control study suggests that the *LEPR* gene rs1137101 variant is associated with liver cancer risk in the Hubei Chinese population. The subsequent meta-analysis revealed that rs1137101 may contribute to susceptibility to DS cancers (especially gastric and liver cancer) in the Chinese population. Additional well-designed studies with larger sample sizes and involving different ethnic groups are required to confirm the present findings.

DATA AVAILABILITY STATEMENT

The data are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

None of the authors has a financial interest related to this study to disclose.

AUTHORS' CONTRIBUTIONS

All authors contributed significantly to this work. FENG Xian Hong collected the blood samples. CHEN Bi Feng and GU Xiu Li designed the study. HU Wei Qiong and ZHOU Wei Guang performed the study and collected the data. LIAO Jia Xi and SHI Jia Xing analyzed the data. HU Wei Qiong, ZHOU Wei Guang, XIE Feng Yang, and LI Shou Heng performed the literature search, data collection, and statistical analysis for the meta-analysis. WANG Yong and FENG Xian Hong reviewed and interpreted the data. CHEN Bi Feng wrote the manuscript. GU Xiu Li critically revised the manuscript. CHEN Bi Feng made the final approval of the manuscript. ZHOU Guang

REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 2021; 71, 209–49.

2. Washington MK, Goldberg RM, Chang GJ, et al. Diagnosis of digestive system tumours. Int J Cancer, 2021; 148, 1040–50.

3. Zhang YY, Proenca R, Maffei M, et al. Positional cloning of the mouse obese gene and its human homologue. Nature, 1994; 372, 425–32.

4. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol, 2006; 6, 772–83.

5. Abella V, Scotece M, Conde J, et al. Leptin in the interplay of inflammation, metabolism and immune system disorders. Nat Rev Rheumatol, 2017; 13, 100–9.

6. Friedman JM. Leptin and the endocrine control of energy balance. Nat Metab, 2019; 1, 754–64.

7. Marques V, Arella F, Afonso MB, et al. Decoding the role of leptin and adiponectin in obesity-related gastrointestinal cancer. Clin Sci, 2023; 137, 1095–114.

8. Ghilardi N, Ziegler S, Wiestner A, et al. Defective STAT signaling by the leptin receptor in diabetic mice. Proc Natl Acad Sci USA, 1996; 93, 6231–5.

9. Ghasemi A, Saeidi J, Azimi-Nejad M, et al. Leptin-induced signaling pathways in cancer cell migration and invasion. Cell Oncol, 2019; 42, 243–60.

10. Spyrou N, Avgerinos KI, Mantzoros CS, et al. Classic and novel adipocytokines at the intersection of obesity and cancer: diagnostic and therapeutic strategies. Curr Obes Rep, 2018; 7, 260–75.

11. Philips RL, Wang YX, Cheon H, et al. The JAK-STAT pathway at 30: much learned, much more to do. Cell, 2022; 185, 3857–76.

12. Matsuoka N, Ogawa Y, Hosoda K, et al. Human leptin receptor gene in obese Japanese subjects: evidence against either obesity-causing mutations or association of sequence variants with obesity. Diabetologia, 1997; 40, 1204–10.

13. Domingos PLB, Farias LC, Pereira CS, et al. Leptin receptor polymorphism Gln223Arg (rs1137101) in oral squamous cell carcinoma and potentially malignant oral lesions. Springerplus, 2014; 3, 683.

14. Hussain SR, Naqvi H, Gupta S, et al. A study on oncogenic role of leptin and leptin receptor in oral squamous cell. Tumour Biol, 2015; 36, 6515–23.

15. Rodrigues PRS, Maia LL, Santos M, et al. Leptin receptor expression and Gln223Arg polymorphism as prognostic markers in oral and oropharyngeal cancer. Genet Mol Res, 2015; 14, 14979–88.

16. Hung WC, Tsai CM, Lin CW, et al. Leptin -2548 G/A polymorphisms are associated to clinical progression of oral cancer and sensitive to oral tumorization in nonsmoking population. J Cell Biochem, 2019; 120, 15145–56.

17. Doecke JD, Zhao ZZ, Stark MS, et al. Single nucleotide polymorphisms in obesity-related genes and the risk of esophageal cancers. Cancer Epidemiol Biomarkers Prev, 2008; 17, 1007–12.

18. Qiu H, Lin XT, Tang WF, et al. Investigation of TCF7L2, LEP and LEPR polymorphisms with esophageal squamous cell carcinomas. Oncotarget, 2017; 8, 109107–19.

19. Ren XH, Jiang YB. Study on relationship between Gln223Arg variant in leptin receptor gene and gastric cancer. Chin J Health Lab Technol, 2010; 20, 806–7. (In Chinese)

20. Pan H. Studies on association of the overweight/obesity, helicobacter pylori infection and gastric cancer. Lanzhou University, 2014. (In Chinese)

21. Ling W. The study on leptin receptor gene polymorphsims and environmental factors interaction associated with the susceptibility of gastric cancer. Wuhan University, 2016. (In Chinese)

22. Chia VM, Newcomb PA, Lampe JW, et al. Leptin concentrations, leptin receptor polymorphisms, and colorectal adenoma risk. Cancer Epidemiol Biomarkers Prev, 2007; 16, 2697–703.

23. Pechlivanis S, Bermejo JL, Pardini B, et al. Genetic variation in adipokine genes and risk of colorectal cancer. Eur J Endocrinol, 2009; 160, 933–40.

24. Vašků A, Vokurka J, Bienertová-Vašků J. Obesity-related genes variability in Czech patients with sporadic colorectal cancer: preliminary results. Int J Colorectal Dis, 2009; 24, 289–94.

25. Karimi K, Arkani M, Safaei A, et al. Association of leptin receptor gene Gln223Arg polymorphism with susceptibility to colorectal cancer. Gastroenterol Hepatol Bed Bench, 2011; 4, 192–8.

26. Mahmoudi T, Farahani H, Nobakht H, et al. Genetic Variations in Leptin and Leptin receptor and Susceptibility to colorectal cancer and obesity. Iran J Cancer Prev, 2016; 9, e7013.

27. Lin J, Xie ZQ, Lan B, et al. Investigation of Leptin and its receptor (LEPR) for single nucleotide polymorphisms in colorectal cancer: a case-control study involving 2, 306 subjects. Am J Transl Res, 2020; 12, 3613–28.

28. Li Z, Yuan WT, Ning SJ, et al. 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, 383–8.

29. Zhang S, Jiang JK, Chen Z, et al. 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, 2083–9.

30. Karam HA, Bessa SS, Ali EMM, et al. The Inter-Relation between leptin receptor (Q223R) Gene Polymorphism and the risk of Egyptian patients with HCC. Asian Pac J Cancer Prev, 2020; 21, 3557–65.

31. Tang YZ, Zhan LL, Lu Y, et al. Association of LEPR gene polymorphisms with the risk of hepatitis B virus-related liver disease in Guangxi Chinese: a case-control study. Infect Genet Evol, 2020; 84, 104366.

32. Li L, Meng XC, Liu LY, et al. Single-nucleotide polymorphisms in LEP and LEPR associated with breast cancer risk: results from a multicenter case-control study in Chinese females. Front Oncol, 2022; 12, 809570.

33. Bieńkiewicz J, Romanowicz H, Wilczyński M, et al. Association of single nucleotide polymorphism LEP-R c. 668A>G (p. Gln223Arg, rs1137101) of leptin receptor gene with endometrial cancer. BMC cancer, 2021; 21, 925.

34. Unsal M, Kara N, Karakus N, et al. Effects of leptin and leptin receptor gene polymorphisms on lung cancer. Tumour Biol,

2014; 35, 10231−6.

35. He J, Xi B, Ruiter R, et al. Association of *LEP* G2548A and *LEPR* Q223R polymorphisms with cancer susceptibility: evidence from a meta-analysis. PLoS One, 2013; 8, e75135.

36. Liu PC, Shi H, Liu R, et al. Lack of association between LEPR Q223R polymorphisms and cancer susceptibility: evidence from a meta-analysis. J BUON, 2014; 19, 855−62.

37. Rong GX, Tang WF, Wang YF, et al. Investigation of *leptin receptor* rs1137101 G>A polymorphism with cancer risk: evidence from 35936 subjects. Biosci Rep, 2019; 39, BSR20182240.

38. Lu XL, Liu MT, Liao YX, et al. Meta-analysis of the association between mTORC1-related genes polymorphisms and cancer risk. Pathol Res Pract, 2022; 229, 153696.

39. Johnson DE, O'Keefe RA, Grandis JR. Targeting the IL-6/JAK/STAT3 signalling axis in cancer. Nat Rev Clin Oncol, 2018; 15, 234−48.

40. Bjørbæk C, Buchholz RM, Davis SM, et al. Divergent roles of SHP-2 in ERK activation by leptin receptors. J Biol Chem, 2001; 276, 4747−55.

41. Zhu SL, Tang ZY, Tang Y, et al. Correlation of Q223R and K109R polymorphisms in leptin receptor gene with susceptibility of breast cancer: a systematic review and meta-analysis. J Chin Med Assoc, 2023; 86, 549−56.