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Original Research

Association between *GLS* Gene Polymorphisms and the Susceptibility to Lung Cancer in the Chinese Han Population

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Abstract

Background: Lung cancer is one of the most serious malignant tumors endangering human health and life. This study focused on evaluating the association between single nucleotide polymorphisms (SNPs) of the glutaminase (*GLS*) and lung cancer susceptibility in the Chinese Han population. **Methods**: A total of 684 lung cancer patients and 684 healthy individuals were enrolled. Five *GLS* SNPs (rs143584207 C/A, rs117985587 T/C, rs74271715 G/T, rs2355570 G/A, and rs6713444 A/G) were screened as candidate genetic loci. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated to assess the association between *GLS* SNPs and lung cancer susceptibility. False-positive report probability (FPRP) analysis further verified whether the positive results deserved attention. Finally, the multi-factor dimensionality reduction (MDR) method was applied to analyze the interactions between SNPs. **Results**: The overall analysis revealed that *GLS* rs143584207 and rs6713444 were significantly associated with lung cancer susceptibility. The subgroup and clinical information analyses further revealed that *GLS* rs143584207 and rs6713444 could remarkably reduce lung cancer susceptibility in different subgroups (age >60, females, body mass index (BMI) <24, and lung adenocarcinoma). Rs143584207 could significantly reduce lung cancer susceptibility in non-smokers. Additionally, rs6713444 also had a protective effect on patients with advanced lung cancer. **Conclusions**: Our study indicated that *GLS* rs143584207 and rs6713444 could strikingly reduce lung cancer susceptibility in the Chinese Han population, which will give a new direction for the timely treatment of lung cancer.

Keywords: lung cancer; susceptibility; *GLS*; SNPs

1. Introduction

Lung cancer is one of the most common cancers and the main cause of cancer-related death in the world [1,2]. The Global Cancer Statistics 2020 showed that the number of newly diagnosed lung cancer cases exceeded 2.2 million (about 11.4% of all newly diagnosed cancer cases), and the number of lung cancer deaths exceeded 1.8 million (about 18.0% of all cancer deaths) in the world [2]. Additionally, the morbidity and mortality of lung cancer ranked first among all malignant tumors in males, whereas, ranked third and second in females, respectively [2]. Lung cancer mainly includes two histological subtypes, namely small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), and NSCLC includes three pathologic subtypes: lung adenocarcinoma, squamous cell lung cancer, and large cell carcinoma [3,4]. Numerous studies have revealed that lung cancer susceptibility is related to environmental factors (toxic workplaces, air pollution, smoking, etc.) and genetic factors (genetic mutation, gene polymorphisms, etc.) [5–7]. In particular, genetic factors have a vital role in the pathological mechanism of lung cancer [8]. So far, relevant studies have found that some genetic loci in certain genes are related to lung cancer susceptibility, for instance, vascular endothelial growth factor (*VEGF*), interleukin-32 (*IL-32*), and so on [9–13]. However, although the glutaminase (*GLS*) gene is closely correlated with the occurrence of various cancers, its relationship with lung cancer susceptibility has not been reported.

The *GLS* gene encodes a K-type mitochondrial glutaminase, which can catalyze the hydrolysis of glutamine to produce glutamate and ammonia. The *GLS* gene can be expressed in human cells as two subtypes, namely kidney-type glutaminase (KGA, also called *GLS1*) and liver-type glutaminase (LGA, also called *GLS2*) [14]. The expression of the *GLS* gene has an essential influence on the production of metabolic energy, the synthesis of the neurotransmitter glutamate in the brain and the maintenance of renal acid-base balance. Numerous studies have indicated that the aberrant expression of *GLS*, especially *GLS1*, plays a huge role in tumor metabolism and the development of various cancers, including hepatocellular carcinoma [15], breast cancer [16], colorectal cancer [17], intrahepatic cholangio-

carcinoma [18], prostate cancer [19], lung cancer [20,21], melanoma [22], and so on. Studies related to lung cancer have shown that the inhibition of tumor growth can be achieved by preventing or interfering with the metabolism of *GLS* in tumor cells. Momcilovic *et al.* [21] showed that the combined use of CB-839 and erlotinib in epidermal growth factor receptor (EGFR) mutant NSCLC may affect the utilization of glucose and glutamine by reducing the metabolism of *GLS*, and ultimately inhibit the growth of tumor cells. Galan-Cobo *et al.* [20] also found that the development of *GLS* inhibitors might be a good method in the treatment of KRAS mutant lung adenocarcinoma. The hydrolysis of glutamine promoted by the high expression of *GLS* is an important anaplerotic reaction for the proliferation and survival of many cancer cells, that is to say, cancer cells can replenish intermediates related to glutamine metabolism into the Krebs cycle, which not only provides Adenosine Triphosphate (ATP) for cancer cells but also provides precursors for the synthesis of macromolecules [23]. Relevant studies have further found that many oncogenes and tumor inhibitors are related to the expression of *GLS* and the regulation of glutamine metabolism, thus affecting the occurrence and development of cancers. For instance, selenite has been proven to inhibit the expression of *GLS* and the metabolism of glutamine, thereby inducing the dose-dependent apoptosis of a variety of cancer cells [23,24]. Van den Heuvel *et al.* [14] have found that the instantaneous knockdown of *GLS1* splice variant GAC can affect the decomposition of glutamine, thus adversely affecting the growth of lung cancer cells. In addition, relevant studies have demonstrated that gene polymorphisms may lead to changes in gene expression and the activity of cancer-related enzymes, thereby affecting the susceptibility to cancers [5,25,26]. Therefore, in this study, we speculated that *GLS* gene polymorphisms might be associated to the lung cancer susceptibility, and the specific mechanism might be that genetic variants of the *GLS* changed the activity of glutaminase and inhibited the decomposition of glutamine, thereby affecting lung cancer susceptibility.

In this study, five candidates *GLS* SNPs (rs143584207 C/A, rs117985587 T/C, rs74271715 G/T, rs2355570 G/A, and rs6713444 A/G) were successfully selected and genotyped. Among them, rs143584207 is a missense mutation, which may have a certain impact on the expression of *GLS*. The other four SNPs are located in the 3'-UTR region and do not change the amino acid sequence, but they may influence protein folding, and then, gene function, which may eventually affect the occurrence of diseases [27]. To preliminarily investigate the association between five candidate *GLS* SNPs and lung cancer susceptibility, we conducted a case-control study of 1368 Chinese Han subjects. The association between *GLS* SNPs and lung cancer susceptibility was assessed by the overall, subgroup and clinical information analyses, so as to provide a new idea for further probing into the complex pathogenesis of lung cancer and finding more effective treatments for lung cancer patients.

2. Materials and Methods

2.1 Study Subjects

There were 684 lung cancer patients (207 females, 477 males) were enrolled from the Affiliated Hospital of Xizang Minzu University. These patients were diagnosed as primary lung cancer clinically and histopathologically at the early stage, and they had no history of other cancers, and no acute or chronic pathologies. Afterwards, with the help of the tumor, node, metastasis (TNM) staging system, we determined the clinical stages of patients [28]. And the pathological types of these patients mainly included lung adenocarcinoma and squamous cell lung cancer. In addition, 684 healthy individuals (193 females, 491 males) were recruited, and they underwent health examination annually and had no any personal or family history of malignant tumors, respiratory diseases, and endocrine or metabolic nutritional diseases. This study was conducted under the standards approved by the Biomedical Ethics Committee of Xizang Minzu University (No. 20200-11), and conformed to the ethical principles of the World Medical Association Declaration of Helsinki for medical research involving humans. Before participating in this study, all participants signed informed consent forms.

2.2 SNP Selection and Genotyping

GLS SNPs were screened according to the detailed steps below: (1) The location of *GLS* was determined in the e!GRCh37 database (http://asia.ensembl.org/Homo_sapiens/Info/Index) and all mutation sites of this gene were obtained; (2) Haploview v4.2 (Daly Lab, Cambridge, Massachusetts, USA) was applied to filter SNPs (parameters: Hardy-Weinberg equilibrium (HWE) >0.01 & minor allele frequency (MAF) >0.05); (3) Primer design and random selection were performed to further select SNPs. Ultimately, five candidate SNPs of *GLS* (rs143584207 C/A, rs117985587 T/C, rs74271715 G/T, rs2355570 G/A, and rs6713444 A/G) were successfully screened. Genomic DNA was extracted from the whole blood samples and purified based on the instructions of the kit (GoldMag Co. Ltd., Xi'an, China). The target SNPs were amplified with specific PCR amplification primers (**Supplementary Table 1**) by the PCR amplification instrument ProFlex PCR System. The genotyping of SNPs was performed on the Agena MassARRAY iPLEX platform (Agena Bioscience Inc., San Diego, CA, USA).

2.3 Statistical Analysis

G*Power v3.1.9.7 (Heinrich-Heine-Universität Düsseldorf, Dusseldorf, North Rhine-Westphalia, Germany) was used to calculate the sample size through independent samples *t*-test. The parameters were set as: Tail = 2, Effect size = 0.20, $\alpha = 0.05$, Power = 0.9587, Distribution

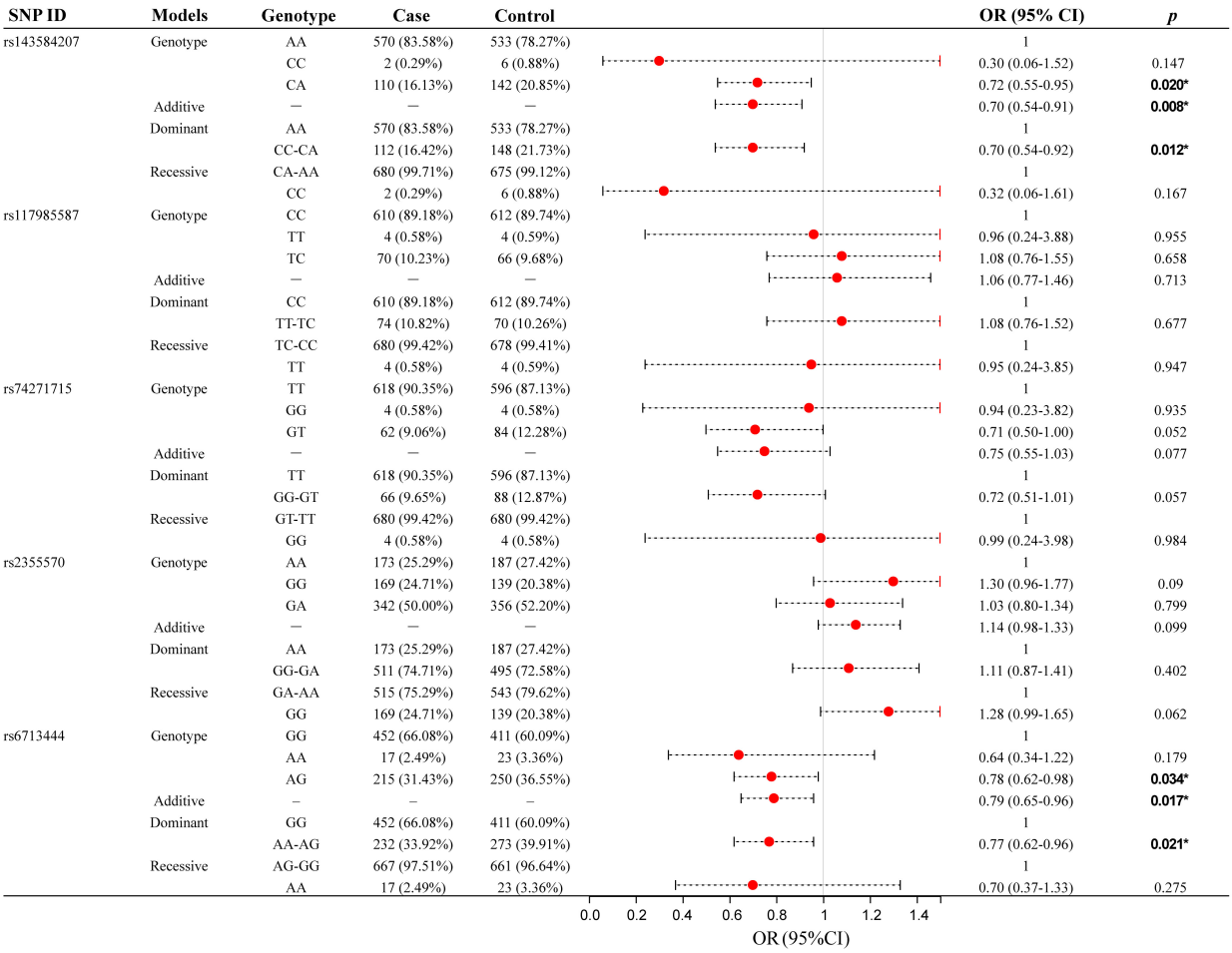

Fig. 1. Association between five candidate *GLS* SNPs and lung cancer susceptibility (overall analysis). *$p < 0.05$ shows statistically significant. All OR and 95% CIs s have been adjusted.

ratio = 1. HaploReg v4.1 (Lucas D. Ward, Amgen, Inc, Cambridge, Massachusetts, USA) (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) was used to annotate the functional elements containing five candidate *GLS* SNPs. Using SPSS v 25.0 ((SPSS Inc., Chicago, IL, USA), differences in categorical variables (gender, smoking, and drinking) and continuous variables (age and BMI) between the case and control groups were analyzed by the chi-square test and independent samples *t*-test, respectively. And the chi-square test was utilized to prove whether the genotype distribution of candidate SNPs in healthy controls conformed to HWE, and $p > 0.05$ indicated that genotype frequencies met HWE. In the overall, subgroup and clinical information analyses (age, sex, BMI, smoking, tumor type, stage, and lymph node metastasis), four genetic models (genotype, additive, dominant, and recessive models) were established using PLINK v1.9 (Shaun Purcellwhilst, Cambridge, Massachusetts, USA), and the correlation between *GLS* SNPs and lung cancer susceptibility was further assessed by calculating odds ratios (ORs) and 95% confidence intervals (95% CIs). And ORs and 95% CI were adjusted by age, sex, BMI, smoking, and drinking. The *p*-value < 0.05 indicated that the results were statistically significant. OR <1 indicated that the factor could reduce lung cancer susceptibility and vice versa. The false-positive report probability (FPRP) analysis was performed to determine whether the positive results deserved attention. At the prior probability of 0.25, FPRP value <0.2 was considered lower than the expected false positive rate of results, indicating that the correlation between *GLS* SNPs and lung cancer susceptibility deserved attention. Finally, the multifactor dimensionality reduction (MDR) method was used to analyze the interactions between SNPs. MDR analysis was determined by ten times cross-validation consistency (CVC) testing. We divided the data set into a training set and a validation set. Among them, nine tenths of the data were used as the training sets, and the rest as the validation set.

3. Results

3.1 Information about Sample Characteristics

In the case group, 684 patients with lung cancer included 207 females (30.3%) and 477 males (69.7%), and their average age was 60.18 $\pm$ 9.89 years. Among them,

Table 1. Sample characteristics information.

Characteristics		Case (n = 684)	Control (n = 684)	*p*
Age (years)	Mean ± SD	60.18 ± 9.89	59.79 ± 9.23	0.441[a]
	>60	359 (52.5%)	371 (54.2%)	
	≤60	325 (47.5%)	313 (45.8%)	
Gender	Male	477 (69.7%)	491 (71.8%)	0.405[b]
	Female	207 (30.3%)	193 (28.2%)	
Smoking	Yes	407 (59.5%)	399 (58.3%)	0.660[b]
	No	277 (40.5%)	285 (41.7%)	
Drinking	Yes	355 (51.9%)	332 (48.5%)	0.214[b]
	No	329 (48.1%)	352 (51.5%)	
BMI	≥24	388 (56.7%)	410 (59.9%)	0.641[a]
	<24	296 (43.3%)	274 (40.1%)	
Tumor types	Adenocarcinoma	314 (45.9%)	–	–
	Squamous cell carcinoma	219 (32.0%)	–	
Stages	III–IV	392 (57.3%)	–	–
	I–II	292 (42.7%)	–	
Lymph node metastasis	Yes	318 (46.5%)	–	–
	No	350 (51.2%)	–	

Notes: [a] Independent samples *t*-test; [b] Chi-square test; $p < 0.05$ shows statistically significant.

Table 2. Basic information about five candidate *GLS* SNPs.

Gene	SNP ID	Functional annotation	Chr: position	Alleles (A/B)	MAF		HWE (*p* value)	OR (95% CI)	p^a
					Cases	Controls			
GLS	rs143584207	missense_variant	2:190,881,350	C/A	0.083	0.113	0.442	0.72 (0.55–0.92)	0.010*
GLS	rs117985587	3'UTR	2:190,913,888	T/C	0.057	0.054	0.129	1.05 (0.76–1.46)	0.753
GLS	rs74271715	3'UTR	2:190,914,979	G/T	0.051	0.067	0.537	0.75 (0.54–1.03)	0.075
GLS	rs2355570	3'UTR	2:190,916,443	G/A	0.497	0.465	0.218	1.14 (0.98–1.32)	0.091
GLS	rs6713444	3'UTR	2:190,917,042	A/G	0.182	0.216	0.054	0.81 (0.67–0.97)	0.024*

Notes: [a] Chi-square test; *$p < 0.05$ shows statistically significant; Alleles (A/B), minor/major allele.

292 patients (42.7%) were in clinical stage I–II and 392 patients (57.3%) were in clinical stage III–IV. In the control group, 684 healthy individuals included 193 females (28.2%) and 491 males (71.8%), and their average age was 59.79 ± 9.23 years (Table 1). There was no statistical difference in age ($p = 0.441$), gender ($p = 0.405$), smoking ($p = 0.660$), drinking ($p = 0.214$), and BMI ($p = 0.641$) between the case and control groups.

3.2 Basic Information about Five Candidate GLS SNPs

As shown in Table 2, five *GLS* SNPs (rs143584207 C/A, rs117985587 T/C, rs74271715 G/T, rs2355570 G/A, and rs6713444 A/G) were screened and genotyped. The functional prediction results revealed that the *GLS* SNPs were associated with the promoter and enhancer histone marks, DNAase, changed motifs, GRASP QTL hits, and bound proteins, suggesting *GLS* SNPs might produce biological effects in lung cancer patients through these ways. The chi-square test indicated that the genotype frequencies of these candidate SNPs met HWE ($p > 0.05$). Among these SNPs, the minor C allele of rs143584207 (OR = 0.72, 95% CI 0.55–0.92, $p = 0.010$) and the minor A allele of rs6713444 (OR = 0.81, 95% CI 0.67–0.97, $p = 0.024$) both significantly reduced lung cancer susceptibility.

3.3 Association between GLS SNPs and Lung Cancer Susceptibility (Overall Analysis)

In the case and control groups, the genotypes with the highest frequency distribution of rs143584207, rs117985587, rs74271715, rs2355570, and rs6713444 were AA, CC, TT, GA, and GG, respectively (Fig. 1). Rs143584207 could significantly reduce lung cancer susceptibility under the heterozygote (CA *vs*. AA: OR = 0.72, 95% CI 0.55–0.95, $p = 0.020$), additive (OR = 0.70, 95% CI 0.54–0.91, $p = 0.008$) and dominant (CC-CA *vs*. AA: OR = 0.70, 95% CI 0.54–0.92, $p = 0.012$) models. Rs6713444 could also significantly reduce lung cancer susceptibility under the heterozygote (AG *vs*. GG: OR = 0.78, 95% CI 0.62–0.98, $p = 0.034$), additive (OR = 0.79, 95% CI 0.65–0.96, $p = 0.017$) and dominant (AA-AG *vs*. GG: OR = 0.77, 95% CI 0.62–0.96, $p = 0.021$) models. There was no evidence of a marked correlation between the other three candidate SNPs (rs117985587, rs74271715, and rs2355570) and lung cancer susceptibility.

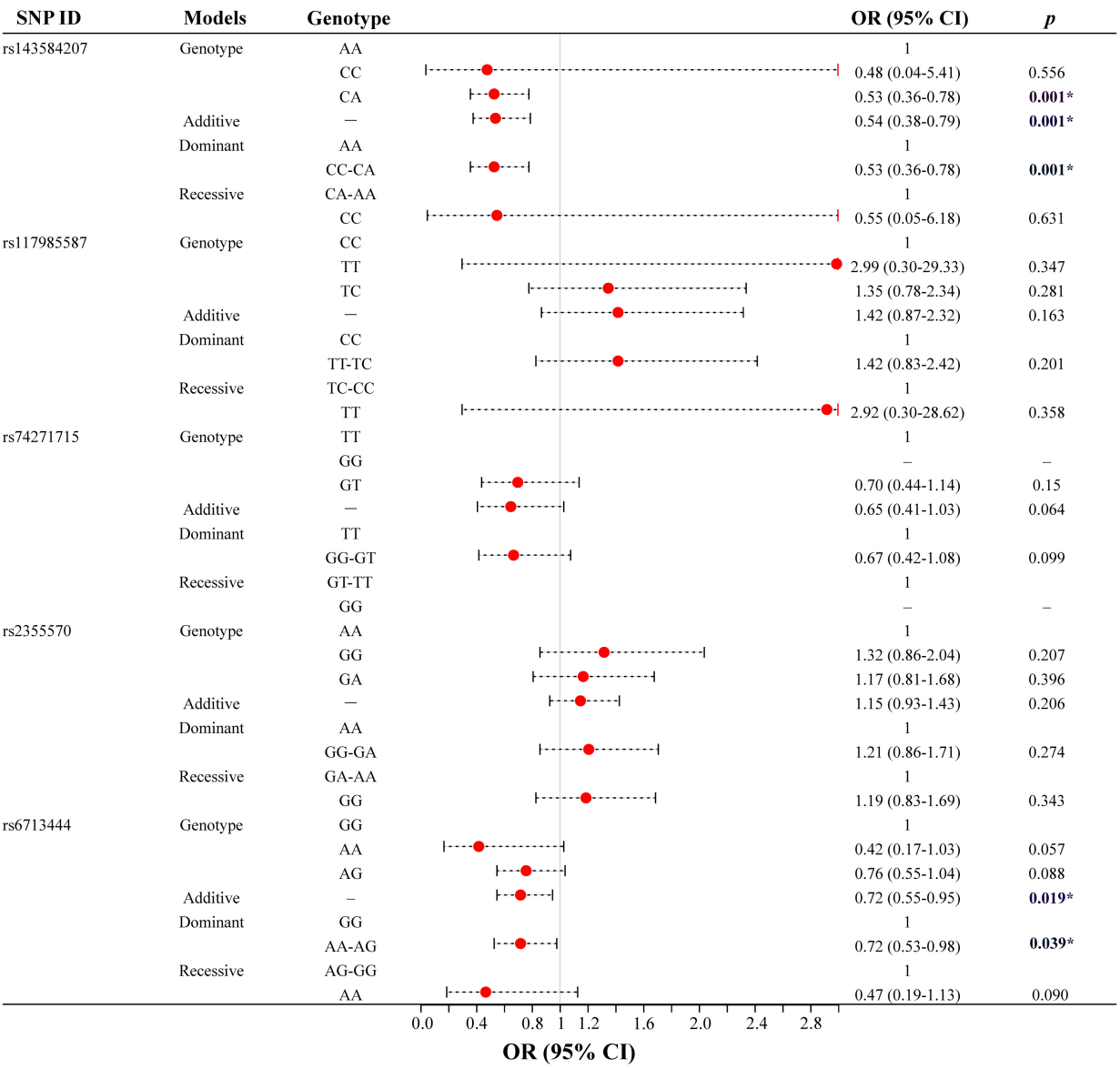

Fig. 2. Association between five candidate *GLS* SNPs and lung cancer susceptibility (age-stratified analysis, age >60 years, N = 730). *$p < 0.05$ shows statistically significant. All OR and 95% CIs have been adjusted.

3.4 Association between GLS SNPs and Lung Cancer Susceptibility (Subgroup and Clinical Information Analyses)

Age-stratified analysis (Fig. 2, **Supplementary Table 2**) indicated that there was no significant correlation between *GLS* SNPs and lung cancer susceptibility in participants aged ≤60 years. Whereas in participants aged >60 years, rs143584207 had a risk-decreasing effect on lung cancer under the heterozygote (CA *vs.* AA: OR = 0.53, 95% CI 0.36–0.78, $p = 0.001$), additive (OR = 0.54, 95% CI 0.38–0.79, $p = 0.001$) and dominant (CC-CA *vs.* AA: OR = 0.53, 95% CI 0.36–0.78, $p = 0.001$) models. Rs6713444 could also significantly reduce lung cancer susceptibility under the additive (OR = 0.72, 95% CI 0.55–0.95, $p = 0.019$) and dominant (AA-AG *vs.* GG: OR = 0.72, 95% CI 0.53–0.98, $p = 0.039$) models.

Gender-stratified analysis (**Supplementary Table 2**) indicated that in female participants, rs143584207 could significantly reduce lung cancer susceptibility under the heterozygote (CA *vs.* AA: OR = 0.53, 95% CI 0.31–0.91, $p = 0.021$), additive (OR = 0.50, 95% CI 0.30–0.84, $p = 0.008$) and dominant (CC-CA *vs.* AA: OR = 0.51, 95% CI 0.30–0.86, $p = 0.013$) models, and rs6713444 could also significantly reduce lung cancer susceptibility under the heterozygote (AG *vs.* GG: OR = 0.58, 95% CI 0.38–0.89, $p = 0.011$), additive (OR = 0.67, 95% CI 0.47–0.95, $p = 0.025$) and dominant (AA-AG *vs.* GG: OR = 0.60, 95% CI 0.40–0.89, $p = 0.012$) models.

BMI-stratified analysis (**Supplementary Table 3**) revealed a marked correlation of rs143584207 and rs6713444 with lung cancer susceptibility in participants with BMI <24. Precisely, rs143584207 could significantly reduce

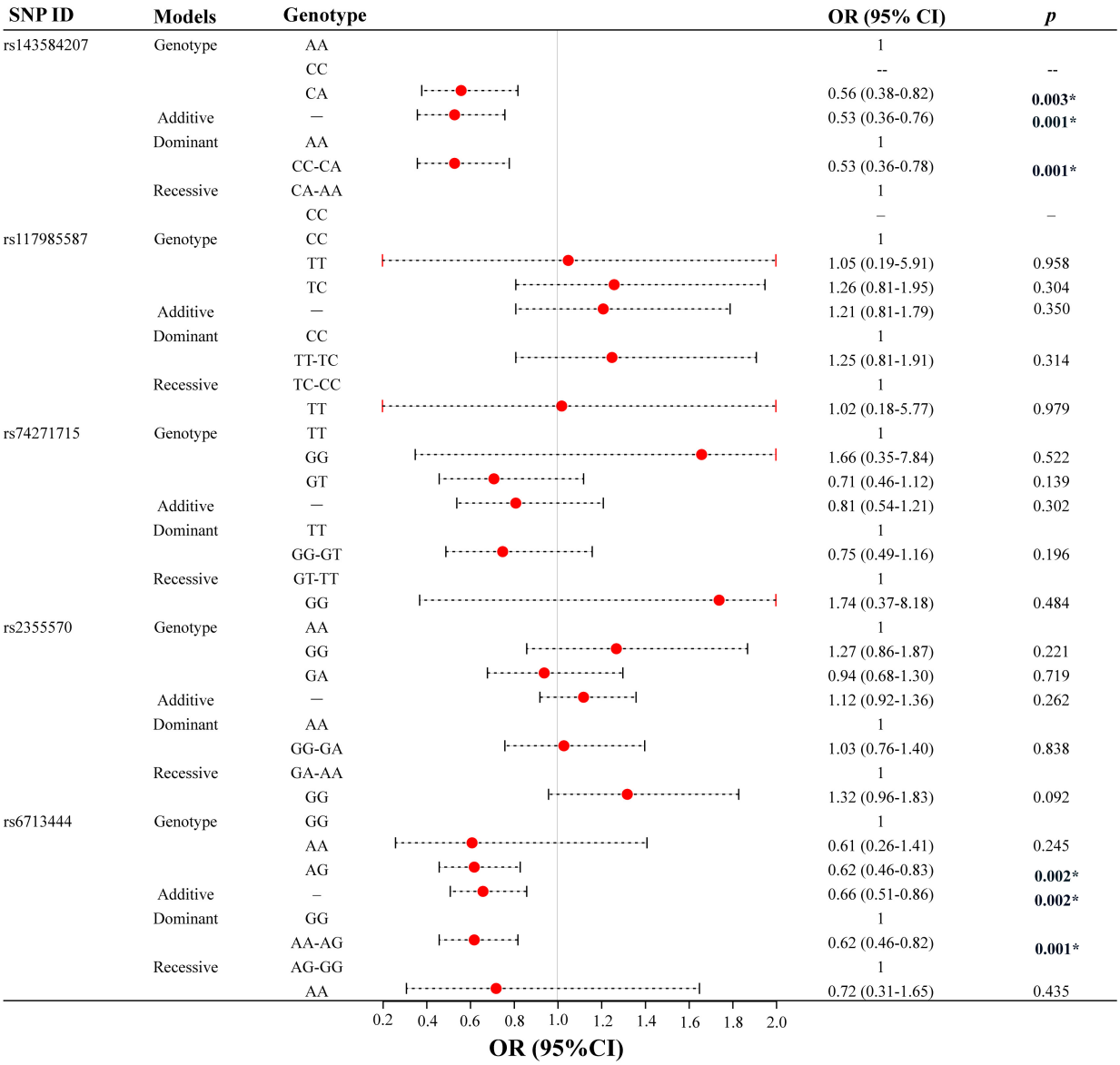

Fig. 3. Association between five candidate *GLS* SNPs and lung cancer susceptibility (Lung adenocarcinoma, N = 314). *$p < 0.05$ shows statistically significant. All OR and 95% CIs have been adjusted.

lung cancer susceptibility under the additive (OR = 0.66, 95% CI 0.44–0.97, p = 0.033) and dominant (CC-CA *vs*. AA: OR = 0.66, 95% CI 0.44–0.99, p = 0.042) models. Rs6713444 could significantly reduce lung cancer susceptibility under the heterozygote (AG *vs*. GG: OR = 0.64, 95% CI 0.45–0.91, p = 0.014) and dominant (AA-AG *vs*. GG: OR = 0.67, 95% CI 0.48–0.94, p = 0.022) models. Rs2355570 and rs6713444 were significantly correlated with lung cancer susceptibility in participants with BMI ≥24. Among them, rs6713444 could significantly reduce lung cancer susceptibility under the homozygous (AA *vs*. GG: OR = 0.38, 95% CI 0.15–0.96, p = 0.040) and recessive (AA *vs*. AG-GG: OR = 0.40, 95% CI 0.16–0.99, p = 0.048) models. However, rs2355570 could significantly increase lung cancer susceptibility under the recessive model (GG *vs*. GA-AA: OR = 1.52, 95% CI 1.09–2.11, p = 0.014).

Smoking-stratified analysis (**Supplementary Table 3**) showed that in non-smoking participants, only rs143584207 could significantly reduce lung cancer susceptibility under the additive (OR = 0.59, 95% CI 0.38–0.92, p = 0.020) and dominant (CC-CA *vs*. AA: OR = 0.61, 95% CI 0.39–0.96, p = 0.031) models. The other four genetic loci (rs117985587, rs74271715, rs2355570, and rs6713444) had no marked correlation with lung cancer susceptibility in non-smoking participants.

The clinical information about patients with different tumor types was analyzed, suggesting that (Fig. 3 and **Supplementary Table 4**) in patients with lung adenocarcinoma, those carrying the CA heterozygote of rs143584207 had a lower risk of lung adenocarcinoma than wild-type homozygous AA carriers (CA *vs*. AA: OR = 0.56, 95% CI 0.38–0.82, p = 0.003). And under the additive (OR =

Table 3. SNP-SNP interactions in lung cancer risk based on MDR analysis.

Models	Training balanced accuracy	Testing balanced accuracy	OR (95% CI)	*p*	CVC
rs6713444	0.5307	0.5175	1.29 (1.04–1.61)	0.0216	8/10
rs143584207, rs6713444	0.5475	0.5395	1.46 (1.18–1.81)	0.0004	9/10
rs143584207, rs117985587, rs6713444	0.5530	0.5329	1.53 (1.23–1.89)	<0.0001	8/10
rs143584207, rs117985587, rs74271715, rs6713444	0.5566	0.5336	1.56 (1.26–1.94)	<0.0001	7/10
rs143584207, rs117985587, rs74271715, rs2355570, rs6713444	0.5608	0.5044	1.62 (1.31–2.01)	<0.0001	10/10

Abbreviation: CVC, cross-validation consistency.

0.53, 95% CI 0.36–0.76, p = 0.001) and dominant (CC-CA *vs*. AA: OR = 0.53, 95% CI 0.36–0.78, p = 0.001) models, rs143584207 could significantly reduce lung adenocarcinoma susceptibility. And rs6713444 could also significantly reduce lung adenocarcinoma susceptibility under the additive (OR = 0.66, 95% CI 0.51–0.86, p = 0.002) and dominant (AA-AG *vs*. GG: OR = 0.62, 95% CI 0.46–0.82, p = 0.001) models. However, in patients with squamous-cell lung cancer, we found that these five candidate SNPs of *GLS* had no significant correlation with squamous-cell lung cancer susceptibility.

According to the four clinical stages of lung cancer, we took patients with stage I–II lung cancer as the control group. And the association between *GLS* SNPs and lung cancer susceptibility in patients with stage III-IV lung cancer was analyzed, as shown in **Supplementary Table 5**. Among patients with late-stage lung cancer, rs117985587 could significantly reduce lung cancer susceptibility under the additive (OR = 0.60, 95% CI 0.38– 0.95, p = 0.029) and dominant (TT-TC *vs*. CC: OR = 0.59, 95% CI 0.36–0.96, p = 0.035) models. However, rs6713444 was associated with a significantly increased susceptibility to lung cancer under the heterozygote (AG *vs*. GG: OR = 1.70, 95% CI 1.20–2.39, p = 0.003), additive (OR = 1.48, 95% CI 1.10–2.00, p = 0.011), and dominant (AA-AG *vs*. GG: OR = 1.64, 95% CI 1.18–2.28, p = 0.004) models.

When stratified by lymph node metastasis, we evaluated the association between *GLS* SNPs and lung cancer susceptibility, taking lung cancer patients without lymph node metastasis as the control group. No marked correlation between *GLS* SNPs and lung cancer susceptibility was observed in patients with lymph node metastasis (**Supplementary Table 5**).

3.5 FPRP Analysis

The FPRP analysis of positive results of the overall and subgroup analyses was further carried out, suggesting that (**Supplementary Table 6**) the association between rs143584207 and lung cancer susceptibility should not be concerned in non-smokers and participants with BMI <24. The correlation between rs6713444 and lung cancer susceptibility should not be concerned in participants with BMI ≥24. The correlation between rs117985587 and lung cancer susceptibility should not be concerned in patients with clinical stage III–IV. The FPRP values of other positive results were less than 0.2, indicating that the observed significant association between *GLS* polymorphisms and lung cancer susceptibility was notable, which was worth further considering.

3.6 Analysis of SNP-SNP Interactions

The interactions among five candidate SNPs of *GLS* were analyzed by the MDR method. As shown in Fig. 4, the blue lines indicate redundant interactions among the three candidate SNPs (rs6713444, rs74271715, and rs2355570). Additionally, MDR analysis (Table 3) showed that the two-locus model (the combination of rs143584207 and rs6713444) was considered as the best one (testing balanced accuracy = 0.5395, CVC = 9/10) to predict lung cancer susceptibility.

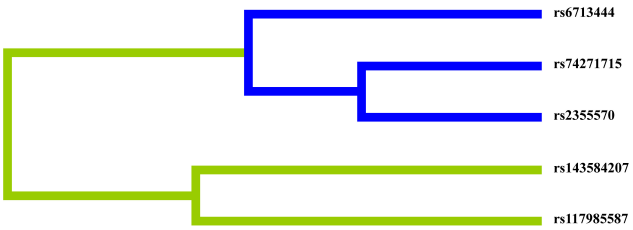

Fig. 4. Dendrogram of SNP-SNP interactions. Blue lines indicate redundancy between SNPs. Green lines indicate synergy between SNPs.

4. Discussion

We analyzed the association between five candidate *GLS* SNPs and lung cancer susceptibility among 1368 participants. The results indicated that *GLS* rs143584207 and rs6713444 were significantly associated with lung cancer susceptibility, while the other three SNPs (rs117985587, rs74271715, and rs2355570) showed little correlation with lung cancer susceptibility. The overall analysis revealed that *GLS* rs143584207 and rs6713444 might be protective factors against lung cancer. The subgroup and clinical information analyses further revealed that *GLS* rs143584207 and rs6713444 could significantly reduce lung cancer susceptibility in different subgroups (age >60,

females, BMI <24, and lung adenocarcinoma). Furthermore, rs143584207 could markedly reduce lung cancer susceptibility in non-smokers, and rs6713444 could obviously reduce lung cancer susceptibility in participants with advanced lung cancer.

Previous studies have found that SNPs in coding or non-coding areas may regulate gene function, gene expression level and enzyme activity by influencing the binding affinity of transcription factors and changing RNA splicing, thus significantly affecting tumor susceptibility [29]. Hu *et al*. [25] have found that the heterozygotes of rs1045411 can reduce the expression levels of the *HMGB1* gene and thus decrease lung cancer susceptibility. Gemignani *et al*. [5] have detected that some gene polymorphisms encoding xenobiotic metabolizing enzymes (XMEs) are closely related to lung cancer susceptibility, and they may influence the levels of related metabolites and enzyme activities, thereby affecting lung cancer susceptibility. In this study, we preliminarily confirmed that *GLS* gene polymorphisms (rs143584207 and rs6713444) were markedly related to the reduction of lung cancer susceptibility. And we further speculated that rs143584207 and rs6713444 could reduce the expression of *GLS*, inhibit the hydrolysis of glutamine, affect the growth of tumor cells, and ultimately decrease lung cancer susceptibility. But the specific mechanism of *GLS* SNPs in lung cancer development needs to be further demonstrated by follow-up study.

In addition, more and more studies have shown that smokers and non-smokers are both susceptible to lung cancer, nevertheless, their various clinicopathologic features may suggest that the etiologies of lung cancer in smokers and non-smokers are different [30]. Noticeably, some gene polymorphisms may have a marked impact on lung cancer susceptibility [31,32]. Li *et al*. [9] have found that genetic variants on chromosome 13q31.3 can reduce the expression of *GPC5* and are related to an increased lung cancer susceptibility in non-smokers. Jou *et al*. [33] have discovered that one SNP (8227G) located in the intron of *EGFR* has a major impact on increased lung cancer susceptibility, especially in non-smoking female patients with lung adenocarcinoma. Hence, we thought that SNPs in *GPC5* and *EGFR* might be risk factors for lung cancer in non-smokers. In contrast, Zhang *et al*. [32] have noticed that *AGBL1* rs4513061 can reduce lung cancer susceptibility in non-smoking females. Our study also found that *GLS* rs143584207 could significantly reduce lung cancer susceptibility in non-smoking females. Therefore, we considered that *AGBL1* rs4513061 and *GLS* rs143584207 might be protective factors against lung cancer in non-smoking females. These findings can give an important direction for the timely treatment of lung cancer among non-smoking females.

What's more, our research also showed that the association between *GLS* SNPs and lung cancer susceptibility was affected by age, sex, and pathological types. *GLS* rs143584207 and rs6713444 could significantly reduce the susceptibility of lung cancer in participants aged >60 years, females, and patients with lung adenocarcinoma. Previous studies have shown that age, sex, and pathological types of lung cancer play a key role in the occurrence and development of lung cancer. Aareleid *et al*. [34] have pointed out that there are differences in the incidence of lung cancer among people of different ages and genders in Estonia, and with the improvement of public awareness and stricter tobacco control, the overall incidence of lung cancer has declined in men, while not in women. He *et al*. [35] have also found that the occurrence of NSCLC is related to age, sex, and pathological types of lung cancer. Our research was consistent with these results, which proved the credibility of our results, and also provided an important reference for reducing the susceptibility of lung cancer in women and the elderly in the future.

It is undeniable that there are some shortcomings in this research. The sample size was relatively small, and all subjects were recruited from the same hospital, which cannot fully represent the Chinese Han population. We will further expand the sample size in follow-up studies so as to obtain more accurate and convincing results.

5. Conclusions

Our study found that *GLS* rs143584207 and rs6713444 could significantly reduce lung cancer susceptibility in the Chinese Han population, which will give a new direction for the timely treatment of lung cancer in high-risk populations.

Abbreviations

SNPs, single nucleotide polymorphisms; *GLS*, glutaminase; ORs, odds ratios; 95% CIs, 95% confidence intervals; FPRP, false-positive report probability; MDR, multi-factor dimensionality reduction; BMI, body mass index; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; *VEGF*, vascular endothelial growth factor; *IL-32*, interleukin-32; *PARK2*, parkin RBR E3 ubiquitin protein ligase; KGA or *GLS1*, kidney-type glutaminase; LGA or *GLS2*, liver-type glutaminase; TNM, tumor, node, metastasis; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; XMEs, xenobiotic metabolizing enzymes.

Availability of Data and Materials

The datasets generated and/or analyzed during this study are available from the corresponding author on reasonable request.

Author Contributions

YW and MingyC designed the study; YW, FY, and JX performed the research; CL, ZZ, and PW analyzed the data; YW, TJ, and MingwC wrote, reviewed, and edited the manuscript. All authors have participated sufficiently

in the work and agreed to be responsible for all aspects of this work. All authors have contributed to editorial changes in the manuscript and have read and approved the final manuscript.

Ethics Approval and Consent to Participate

This study was conducted under the standards approved by the Biomedical Ethics Committee of Xizang Minzu University (No. 20200-11), and conformed to the ethical principles of the World Medical Association Declaration of Helsinki for medical research involving humans. All participants signed informed consent forms before participating in this study.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2805095.

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