Association of WNT-1 Inducible Signaling Pathway Protein-1 (WISP1) Genetic Polymorphisms with the Risk of Gastric Cancer in Guangxi Chinese

Yanqiong Liu
Guangxi Medical University First Affiliated Hospital https://orcid.org/0000-0001-9159-4278
Weijuan Qin
the second Affilicated hospital of Guangxi Medical University
Fuyong Zhang
Guangxi Medical University First Affiliated Hospital
Wang Jian
Guangxi Medical University First Affiliated Hospital
Xi Li
Guangxi Medical University First Affiliated Hospital
Shan Li
Guangxi Medical University First Affiliated Hospital
Xue Qin
Indian Academy of Sciences
Yuefeng Lu (2233795319@qq.com)
Guangxi Medical University First Affiliated Hospital

Primary research

Keywords: WISP1, polymorphism, gastric cancer, risk

Posted Date: May 4th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-439482/v1

Version of Record: A version of this preprint was published at Cancer Cell International on July 30th, 2021. See the published version at https://doi.org/10.1186/s12935-021-02116-2.

Abstract

Background

To date, no study has investigated the association of the WNT1 inducible signaling pathway protein-1 (WISP1) polymorphisms with susceptibility to gastric cancer. Therefore, we conducted this study to explore their relation.

Methods

204 gastric cancer patients and 227 normal controls were enrolled. The WISP1 SNPs rs2929973, rs7843546 and rs10956697 were selected, and their genotypic distributions were determined through PCR-RFLP

Results

In overall, we could not identify a significant association between WISP1 SNP rs2929973, rs7843546 and rs10956697) and gastric cancer risk. However, subgroup analysis demonstrated that the presence of the rs7843546 T allele was associated with a significantly decreased risk of gastric cancer in Han Chinese ethnicity (CT vs. CC: OR= 0.33, 95%CI = 0.14-0.78; TT vs. CC: OR = 0.29, 95%CI = 0.11-0.76; dominant model CT+TT vs. CC: OR = 0.32, 95%CI = 0.14-0.74). In addition, patients with the rs7843546 TT genotype were around one-third as 0.34 likely (OR = 0.34; 95% CI: 0.14-0.84) than those with the CC genotype to develop stage I/II gastric cancer. Furthermore, individuals ≥50 years old who carried the rs10956697 AC genotype represented significantly decreased risk for gastric cancer (AC vs. CC: OR = 0.58, 95%CI = 0.35-0.98). Smokers with the rs10956697 AC and AC+AA genotype were around one-third likely to develop gastric cancer (OR = 0.28, 95% CI= 0.09-0.82 and OR = 0.32, 95% CI = 0.12-0.89, respectively)

Conclusions

WISP1 SNPs rs7843546 and rs10956697 polymorphisms were the first time discovered to reduce the susceptibility to gastric cancer in different subgroup in Guangxi Chinese.

Background

Globally, the burden of cancer incidence and mortality is rapidly growing worldwide. According to estimates from the World Health Organization (WHO) in 2020, gastric cancer remains a commonly cancer worldwide and is responsible for 1089,103 new cases and an estimated 768,793 deaths, ranking fifth for incidence and fourth for mortality globally [1]. China is a high incidence region of gastric cancer. According to the 2020 global cancer statistics, 478,508 new gastric cancer cases and 373,789 deaths were estimated to have occurred in China in 2020, accounting for 43.9% and 48.6% of the cases worldwide, respectively [2]. The high incidence and mortality in China highlights the importance and necessary to understand the risk factors related to gastric cancer development. While, gastric cancer is a multifactorial etiology in terms of risk factors, carcinogenesis, and epidemiologic patterns [3]. Chronic

Helicobacter pylori infection is considered the primary cause, with almost all cases attributed to this bacterium [4]. The prevalence of *Helicobacter pylori* infection is extremely high, infecting half of the world's population [5]. However, only about 1% people with *Helicobacter infections* will develop gastric cancer, indicating because of differences in host genetics, gender, age of infection acquisition, alcohol consumption, tobacco smoking and environmental factors [6]. More novel and powerful identification of the genetic predisposing factors is expected to provide new insights for the basic molecular pathways involved in tumorigenesis.

WNT1 inducible signaling pathway protein-1 (WISP1), also known as CCN4, is a cysteine-rich protein that belongs to the CCN protein family [7]. WISP1 is a target of the pathway Wnt1, which can modulate multiple processes that involve tumorigenesis and stem cell proliferation [8]. WISP1 aberrant expression is associated with the promotion of various pathologies including osteoarthritis, fibrosis and cancer [9]. In 2017, Jia et al. firstly presented that WISP1was up-regulated in gastric cancer and acted as an oncogene by promoting proliferation, migration, and invasion in gastric cancer cells [10]. Additionally, Zhang et al. demonstrated that significantly up-regulated WISP1 expression was associated with cancer progression, chemotherapy outcome, and poor prognosis in gastric cancer in 2019 [11]. The observation of WISP1 plays an important role in the progression of gastric cancer highlights the importance of identification the variants of this gene, because single nucleotide polymorphism (SNP) can change the encoded amino acids in a protein when arising in the related coding sequence, thus influencing gene function and phenotype [12].

WISP1 gene is constituted of 5 exons and 4 introns, located on chromosome 8q24.1 to 8q24.3 and has been shown to be highly polymorphic [13]. Several clinical studies have indicated a significant association between WISP1 polymorphisms and various cancers, such as breast cancer [14], urothelial cell carcinoma [15], hepatocellular carcinoma [16], oral squamous cell carcinoma [17], lung cancer [18], uterine cervical cancer [19]. Up until now, to our knowledge, no study has established a connection between WISP1 genetic polymorphisms and gastric cancer. Therefore, we conducted this study to explore the association between WISP1 SNPs of rs2929973, rs7843546 and rs10956697 and the susceptibility of gastric cancer in a southwest Chinese population.

Methods

Study subjects

We enrolled 204 gastric cancer patients in this study. All cases were clinically and pathologically confirmed to be primary gastric cancer admitted to the First Affiliated Hospital of Guangxi Medical University, Guangxi, China, which have been described in our period study [20]. Patients were excluded if they had any of the following: a) concomitant malignant neoplasias, b) acquired immunodeficiency syndrome, c) acute or chronic inflammatory diseases, d) antibody of *Helicobacter pylori* were positive.

For the control group, we selected 227 healthy individuals recruited from the general health check-up centers at the same hospital during the same period of the study. The individuals in the control group had no previous genetic history of the tumor and were matched with the case group in terms of gender and age. Clinical and pathological characteristics of all subjects were collected based on electronic medical record system.

Selection of WISP1 polymorphisms

In this study, SNPs were selected based on data from the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/), dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/) and the finding of previous studies reported the effect of WISP1 genetic polymorphisms on cancer susceptibility [14–19, 21]. All SNPs had minor allele frequencies (MAF) of > 5% to prevent false negative results. Based on the aforementioned criteria, three SNPs were selected: rs2929973, rs7843546 and rs10956697.

DNA extraction and WISP1 genotyping

Genomic DNA was isolated from from EDTA anticoagulated venous blood using the phenol-chloroform protocol, as described in detail in our previous studies [20, 22]. The concentration and purity of the DNA were determined spectrophotometrically. The obtained DNA was stored at -20℃ and prepared for genotyping using the polymerase chain reaction (PCR). Genotyping was conducted by restriction fragment length polymorphism (RFLP) assay, as described previously [20, 22].

DNA sequencing

To determine the accuracy of the PCR-RFLP method, a random selection of > 5% of all samples was genotyped by the direct sequencing method with an ABI Prism 3100 (Applied Biosystems, Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., China). The resultant genotypes showed no differences.

Statistical analysis

Student's *T*-test or Mann–Whitney *U* test was applied to analyze the continuous variables. The χ^2 test or Fisher's exact test was applied to analyze the categorical variables. Adjusted odds ratios (AORs) and 95% confidence intervals (CIs) were estimated using logistic regression models. AORs and 95%CIs were used to assess association between genotype frequencies with gastric cancer risk and clinical and pathological characteristics. To evaluate the joint effects of the three SNPs in the WISP1 gene, SHEsis software (http://analysis.bio-x.cn/myAnalysis.php) [23] was employed to construct haplotypes between the patients and controls. SPSS version 16.0 for Windows (SPSS Inc., IL, USA) software was used for all the statistical analyses. A two-sided P value of < 0.05 was accepted as statistically significant.

Results

Characteristics of the study subjects

Table 1 shows the demographic and clinical characteristics of all the subjects in the study. In total, 204 gastric cancer patients and 227 controls were enrolled in the study. The age and sex were well matched (P = 0.057 and P = 0.954, respectively). There were no differences between the two groups in terms of smoke, alcohol drinking and ethnicity. The patient groups had a significantly lower BMI compared to those of the healthy controls. Most patients (70.6%) had stage III/IV gastric cancer; 29.4% had stage I/II disease (Table 1). Most tumors (82.4%) were classified as undifferentiated and poor differentiated (Table 1).

Table 1
The distributions of demographical characteristics in 227 controls and 204 patients with gastric cancer

Characteristics	Patients (N = 204)	Controls (N = 227)	*P* value
Ages(mean ± SD, years)	54.31 ± 12.00	52.58 ± 5.13	0.057
BMI (mean ± SD, kg/m^2)	20.59 ± 3.11	22.47 ± 3.47	**< 0.001***
Gender			
Male	134 (65.7%)	138 (60.8%)	0.954
Female	70 (34.0%)	89 (39.2%)	
Ethnicity			
Han	99 (48.5%)	112 (49.3%)	0.975
Zhuang	92 (45.1%)	100 (44.1%)	
Other	13 (6.4%)	15 (6.6%)	
Smoke			
Yes	58 (28.4%)	71 (31.3%)	0.519
No	146 (71.6%)	156 (68.7%)	
Alcohol			
Yes	52 (25.5%)	67 (29.5%)	0.351
No	152 (74.5%)	160 (70.5%)	
Cell differentiation			
Moderate and poor	168 (82.4%)		
Well	36 (17.6%)		
Clinical state			
I/II	60 (29.4%)		
III/IV	144 (70.6%)		
SD standard deviation			
* p value < 0.05 as statistically significant.			

WISP1 polymorphisms and gastric cancer risk

Genotypic distributions of WISP1 SNPs rs2929973, rs7843546 and rs10956697 in the gastric cancer group and normal control group were all in accordance with the Hardy–Weinberg equilibrium (P > 0.05).

The frequency distribution and logistic regression analysis of the polymorphism of WISP1 gene in gastric cancer and control group are shown in Table 2. After logistic regression adjustment analysis based on gender, age, BMI, ethnicity, smoking and alcohol drinking, no significant differences were observed between gastric patients and control group in the rs2929973, rs7843546 and rs10956697 polymorphisms of the WISP1 gene.

Table 2
The frequency distribution and logistic regression analysis of the polymorphism of WISP1 gene in gastric cancer and control group.

Variables		Gastric cancer (N = 204) n (%)	Controls (N = 227) n (%)	AOR (95% CI)	*P*
rs2929973					
Alleles	T	285 (69.9)	295 (65.0)	1.00[ref]	
	G	123 (30.1)	159 (35.0)	0.83 (0.61–1.13)	0.230
Co-dominant	TT	100 (49.0)	102 (44.9)	1.00[ref]	
	TG	85 (41.7)	91 (40.1)	0.99 (0.64–1.54)	0.967
	GG	19 (9.3)	34 (15.0)	0.63 (0.32–1.21)	0.164
Dominant	TT	100 (49.0)	102 (44.9)	1.00[ref]	
	TG + GG	104 (51.0)	125 (55.1)	0.88 (0.58–1.32)	0.536
Recessive	TT + TG	185 (90.7)	193 (85.0)	1.00[ref]	
	GG	19 (9.3)	34 (15.0)	0.62 (0.33–1.17)	0.141
rs7843546					
Allele	C	191 (46.8)	191 (42.1)	1.00[ref]	
	T	217 (53.2)	263 (57.9)	0.84 (0.63–1.12)	0.224
Co-dominant	CC	41 (20.1)	36 (15.9)	1.00[ref]	
	CT	109 (53.4)	119 (52.4)	0.86 (0.49–1.48)	0.577
	TT	54 (26.5)	72 (31.7)	0.68 (0.37–1.24)	0.204

ref: reference

AOR : Adjusted odds ratio; 95% CI, 95% confidence interval; adjusted for age, BMI, ethnicity, smoke and alcohol drinking.

Variables		Gastric cancer (N = 204) n (%)	Controls (N = 227) n (%)	AOR (95% CI)	*P*
Dominant	CC	41 (20.1)	36 (15.9)	1.00[ref]	
	CT + TT	163 (79.9)	191 (84.1)	0.79 (0.47–1.33)	0.376
Recessive	CT + CC	150 (73.5)	155 (68.3)	1.00[ref]	
	TT	54 (26.5)	72 (31.7)	0.78 (0.50–1.21)	0.262
rs10956697					
Allele	C	278 (68.1)	286 (63.0)	1.00[ref]	
	A	130 (31.9)	168 (37.0)	0.88 (0.65–1.20)	0.421
Co-dominant	CC	95 (46.6)	86 (37.9)	1.00[ref]	
	AC	88 (43.1)	114 (50.2)	0.78 (0.50–1.19)	0.248
	AA	21 (10.3)	27 (11.9)	0.90 (0.45–1.79)	0.771
Dominant	CC	95 (46.6)	86 (37.9)	1.00[ref]	
	AC + AA	109 (53.4)	141 (62.1)	0.79 (0.52–1.19)	0.254
Recessive	CC + AC	183 (89.7)	200 (88.1)	1.00[ref]	
	AA	21 (10.3)	27 (11.9)	1.02 (0.53–1.96)	0.947
ref: reference					
AOR : Adjusted odds ratio; 95% CI, 95% confidence interval; adjusted for age, BMI, ethnicity, smoke and alcohol drinking.					

To clarify the role of the WISP1 genetic polymorphisms in gastric cancer demographic and clinical variables, the respective SNPs were analyzed for their correlations with clinical parameters. Table 3 and Additional files 1–6 present the results of the subgroup analyses by clinical stage, cell differentiation, gender, age, ethnicity, smoking and alcohol drinking status. In an evaluation of clinical stage and rs7843546 WISP1 genotypes, patients with the TT genotype were around one-third as 0.34 likely (OR = 0.34; 95% CI: 0.14–0.84; P = 0.020) (Table 3) than those with the CC genotype to develop stage I/II gastric cancer after adjustment for gender, age, BMI, ethnicity, smoke and alcohol drinking. In addition, after adjustment for the above-mentioned variables, subjects carrying at least one copy of the T allele for the rs7843546 SNP were around half as likely (OR = 0.46, 95% CI = 0.22–0.96, P = 0.038) (Table 3) than those

with the CC genotype (dominant model: CT + TT vs. CC) to develop stage I/II gastric cancer. However, the other SNPs genotypes did not have significant difference.

Table 3
Distribution frequency of WISP1polymorphisms in controls and gastric cancer patients stratified by clinical stage

Variables	Clinical stage I/II				Clinical stage Ⅲ/Ⅳ		
	Controls (N = 227)	Cancer (N = 60)	*OR (95% CI)	*P*	Cancer (N = 144)	AOR (95% CI)	*P*
rs2929973							
Co-dominant TT	102 (44.9)	26 (43.3)	1.00[ref]		74 (51.4)	1.00[ref]	
TG	91 (40.1)	28 (46.7)	1.30 (0.46–3.70)	0.624	57 (39.6)	0.88 (0.54–1.42)	0.591
GG	34 (15.0)	6 (10.0)	1.71 (0.60–4.85)	0.315	13 (9.0)	0.58 (0.28–1.21)	0.144
Dominant TT	102 (44.9)	26 (43.3)	1.00[ref]		74 (51.4)	1.00[ref]	
TG + GG	125 (55.1)	34 (56.7)	1.17 (0.62–2.20)	0.636	70 (48.6)	0.78 (0.50–1.23)	0.286
Recessive TT + TG	193 (85.0)	54 (90.0)	1.00[ref]		131 (91.0)	1.00[ref]	
GG	34 (15.0)	6 (10.0)	0.67 (0.25–1.81)	0.433	13 (9.0)	0.61 (0.30–1.23)	0.163
rs7843546							
Co-dominant CC	36 (15.9)	17 (28.3)	1.00[ref]		24 (16.7)	1.00[ref]	
CT	119 (52.4)	30 (50.0)	0.54 (0.25–1.17)	0.116	79 (54.9)	1.08 (0.58–2.02)	0.808
TT	72 (31.7)	13 (21.7)	**0.34 (0.14–0.84)**	**0.020***	41 (28.5)	0.90 (0.46–1.77)	0.756

ref: reference

AOR : Adjusted odds ratio; 95% CI, 95% confidence interval; adjusted for age, BMI, ethnicity, smoke and alcohol drinking.

*P < 0.05 as statically significant.

Variables	Clinical stage I/II				Clinical stage Ⅲ/Ⅳ		
	Controls (N = 227)	Cancer (N = 60)	*OR (95% CI)	*P*	Cancer (N = 144)	AOR (95% CI)	*P*
Dominant CC	36 (15.9)	17 (28.3)	1.00[ref]		24 (16.7)	1.00[ref]	
CT + TT	191 (84.1)	43 (71.7)	**0.46 (0.22–0.96)**	**0.038***	120 (83.3)	1.02 (0.56–1.85)	0.953
Recessive CT + CC	155 (68.3)	47 (78.3)	1.00[ref]		103 (71.5)	1.00[ref]	
TT	72 (31.7)	13 (21.7)	0.52 (0.25–1.10)	0.087	41 (28.5)	0.87 (0.54–1.41)	0.569
rs10956697							
Co-dominant CC	86 (37.9)	26 (43.3)	1.00[ref]		69 (47.9)	1.00[ref]	
AC	114 (50.2)	27 (45.0)	0.84 (0.43–1.63)	0.609	61 (42.4)	0.72 (0.45–1.16)	0.176
AA	27 (11.9)	7 (11.7)	1.08 (0.38–3.03)	0.888	14 (9.7)	0.83 (0.39–1.79)	0.636
Dominant CC	86 (37.9)	26 (43.3)	1.00[ref]		69 (47.9)	1.00[ref]	
AC + AA	141 (62.1)	34 (56.7)	0.88 (0.47–1.66)	0.696	75 (52.1)	0.73 (0.46–1.14)	0.166
Recessive CC + AC	200(88.1)	53 (88.3)	1.00[ref]		130 (90.3)	1.00[ref]	
AA	27 (11.9)	7 (11.7)	1.18 (0.45–3.13)	0.739	14 (9.7)	0.97 (0.47–2.01)	0.943

ref: reference

AOR : Adjusted odds ratio; 95% CI, 95% confidence interval; adjusted for age, BMI, ethnicity, smoke and alcohol drinking.

*P < 0.05 as statically significant.

When the subjects were further divided into subgroups according to the age, significant differences were found in the genotypic distributions of WISP1 SNP rs10956697 in subjects ≥ 50 years old carrying the AC

genotype compared with those carrying the CC genotype (AC vs. CC: OR = 0.58, 95%CI = 0.35–0.98, P = 0.043) (Additional files 3). No difference was observed in subjects < 50 years old.

As regards ethnicity, in Han Chinese ethnicity, the presence of the rs7843546 T allele was associated with a significantly decreased risk of gastric cancer (CT vs. CC: OR = 0.33, 95%CI = 0.14–0.78, P = 0.012; TT vs. CC: OR = 0.29, 95%CI = 0.11–0.76, P = 0.012; dominant model CT + TT vs. CC: OR = 0.32, 95%CI = 0.14–0.74, P = 0.007), whereas the association was not statistically significant among Zhuang population cohort (Additional files 4).

In smoking status cohort, compared with patients carrying the CC genotype of SNP rs10956697, those with the AC and AC + AA genotype were around one-third likely to develop gastric cancer (OR = 0.28, 95% CI = 0.09–0.82,P = 0.021 and OR = 0.32, 95% CI = 0.12–0.89, P = 0.030, respectively) (Additional files 5). No difference was observed in non-smokers' cohort. No effect of cell differentiation, gender and drinking status on the association between the WISP1 polymorphism and susceptibility to gastric cancer was observed. No significant association was observed between the WISP1 rs2929973 polymorphism and risk of gastric cancer in all genetic models.

Discussion

WISP1 polymorphisms have been identified in various cancers, including breast cancer [13], urothelial cell carcinoma [14], hepatocellular carcinoma [15], oral squamous cell carcinoma [16], lung cancer [17], uterine cervical cancer [18], but data are scant as to the involvement of WISP1 polymorphisms in gastric cancer. As far as we are aware, our study is the first to investigate the distributions of the rs2929973, rs7843546 and rs10956697 SNPs and their associations with the development gastric cancer in Guangxi Chinese population. Our results revealed the correlations between WISP1 SNPs (rs7843546 and rs10956697) and gastric cancer susceptibility in different subgroups. In detailed, SNP rs7843546 TT and CT + TT genotype reduced the susceptibility to stage I/II gastric cancer with CC as a reference. The presence of the rs7843546 TT genotype was also associated with a significantly reduced risk of gastric cancer in Han population. In addition, we found that smokers or subjects ≥ 50 years old carrying the AC or AC + AA genotype of the WISP1 rs10956697 polymorphism were less likely than those with CC homozygotes to develop gastric cancer. Both of two SNPs were discovered for the first time to be associated with the gastric cancer.

Most previous researches about the association of WISP1 polymorphisms and cancer were focused on the polymorphisms of WISP1 SNPs rs2929970, rs2929973 and rs2977530. SNPs rs2929970 and rs2929973 are located in the 3'UTR of the WISP1 gene, and rs2977530 is located in introns. In the first second, in 2010, Frank *et al.* investigated the association between WISP1 SNP rs2929970 and colorectal cancer risk but found no evidence for the said risk [24]. Then, in 2015, Chen *et al.* found that WISP1 SNP rs16893344, rs2977530, rs2977537, and rs62514004 polymorphisms were related to susceptibility of lung cancer, but found no significant association in SNPs rs2929970 or rs2929973 [21]. By contrast, Lau *et al.* demonstrated that WISP1 SNP rs2929970 polymorphism carriers with at least one G allele were

susceptible to oral squamous cell carcinoma in 2017 [17]. Moreover, Chen *et al.* revealed that the WISP1 SNP rs2977530 (AG + GG) was associated with hepatocellular carcinoma development and WISP1 SNPs rs62514004 (AG + GG) and rs16893344 (CT + TT) were correlated with lower risks of greater tumor size and reaching a later clinical stage of hepatocellular carcinoma in 2018 [16]. Furthermore, Lin *et al.* demonstrated genotypes AG + GG in WISP1 SNP rs2977530 reduced the susceptibility of Taiwanese women to invasive cervical cancer, whereas genotype AA in rs2977537 increases the said risk [19]. In addition, Lee *et al.* indicated that patients with urothelial cell carcinoma carrying rs2977530 genetic variants (AG + GG) had a higher risk of developing a more invasive tumor stage and a large tumor [15]. Wang *et al.* found that breast cancer patients with the WISP1 rs2929973 GG + TT genotype were likely to develop estrogen receptor (ER)- and progesterone receptor(PR)-positive tumors status [14].

Our study, however, revealed that the WISP1 rs7843546 and rs10956697 was associated with gastric cancer susceptibility in different subgroups, whereas no significant associations were observed in SNPs rs2929973. These results demonstrated the variety of WISP1 polymorphisms in different cancers. There are two reasons for these inconsistencies we may consider. One is that the WISP1 expression was varied in different cancer. Recent researches revealed that the roles of WISP1 in cancer occurrence and progression were diverse in different kinds of cancer. For example, WISP1 was found to negatively regulate the progress of cell motility and invasion by the inhibition of Rac function through integrins in lung cancer [25]. On the contrary, WISP-1 was up-regulated in gastric cancer tissues compared with their adjacent noncancerous tissues, suggesting that WISP-1 acted as an oncogene in gastric cancer. Similar results were found in the previous studies in liver cancer [26] breast cancer [27], and endometrial adenocarcinoma [28]. The other one reason is that different ethnicities of patients included in the aforementioned studies. Study of Frank [24] studied in Caucasians, but studies of Wang [14], Lin [19], Lee[15], Lau [17], Chen [16, 18, 21] studied in Asian. Our samples are south Chinese population that is East Asian. In particular, our results revealed that SNP rs7843546 TT genotype was associated with a significantly reduced risk of gastric cancer in Han population but no Zhuang population. This further indicated that WISP1 genotype distributions were different in different ethnicities.

Helicobacter pylori infection, aging, gender, smoking and alcohol consumption are the main risk factor for the development of gastric cancer [29]. In order to rule out the influence of confounding factor: *Helicobacter pylori* infection, we did not include the *Helicobacter pylori* infection patients in our study. We further analyzed the correlations of WISP1 SNP polymorphisms with other confounding factors of gastric cancer patients. After stratifying individuals into smokers and nonsmokers, participants with AC genotype in WISP1 SNP rs10956697 displayed a 0.28-fold lower risk (95% CI = 0.09–0.82) of gastric cancer among the 132 smokers. Smoking is a well-known carcinogen for tumorigenesis, such as in gastric cancer, and nicotine exposure is suggested to promote cancer progression by activating the Wnt/β-catenin and Wnt/PCP signaling pathways [30]. Aging is also a significant risk factor for gastric cancer. We stratified our included subjects according a person's age. We found that subjects ≥ 50 years old carrying the WISP1 rs10956697 AC genotype were a 0.58-fold (95% CI = 0.35–0.98) lower risk than those with CC homozygotes to develop gastric cancer. Aging is the process of loss and degeneration of the body from constitutive substances and tissue structures to physiological functions [31]. The time-dependent

accumulation of cellular damage is widely considered the general cause of aging [32]. Concomitantly, cellular damage may occasionally provide aberrant advantages to certain cells, which can eventually produce cancer [31].

The current findings must be interpreted in light of several potential limitations. Firstly, the evidence for different effects of aging, smoking, and ethnicity on gastric cancer risk was suggestive but not conclusive. Sample size for the study was not large enough and the sample size in each subgroup was even too small, thus, the results lack statistical power and robustness. Larger independent cohort study is required to confirm the result we discovered. Secondly, the study was limited to eligible participations in Guangxi (Southwest China), which might not be representative of the entire Chinese population. Thirdly, this research was based on data from individual participants and only 3 SNPs of the WISP1 gene were selected, which restricted interpretations about gene-to-gene interactions. These limitations restrict the interpretation and extrapolation of the current findings.

Conclusions

In overall, we could not identify a significant association between WISP1 SNP rs2929973, rs7843546 and rs10956697) and gastric cancer risk. However, our results suggest that a subset of subjects may be affected, including: patients with ≥ 50 years old carrying AC genotype of rs10956697; smoking patients carrying AC or AC + AA genotype of rs10956697; Han people carrying CT or TT genotype of rs7843546; stage I/II gastric patients carrying TT and CT + TT genotype of rs7843546. All these polymorphisms were the first time discovered to represent a significantly reduced risk for gastric cancer. Replication in further epidemiologic studies and functional analyses are warranted to confirm these findings.

Abbreviations

BMI, body mass index; CI, confidence interval; OR; odds ratio; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; SNP, single-nucleotide polymorphism; WISP1, WNT1-inducible signaling pathway protein-1

Declarations

- Ethics approval and consent to participate: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

- Consent for publication: This manuscript is approved by all authors for publication

- Availability of data and materials: The datasets supporting the conclusions of this article (are) included within the article and its additional files.

- Competing interests: The authors declare that they have no conflict of interest.

- Funding: This study was supported by Self-financing Scientific Research Subject of Guangxi Health Department (Z20200085)

- Authors' contributions: Y.Liu performed the experiments and wrote the manuscript. W.Qin performed the experiments. F.Zhang participated in the statistical analysis. J.Wang and X.Li participated in the design of the study. Y.Lu and X.Qin conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

- Acknowledgements: Not applicable

References

1. Sung H, Ferlay J, Siegel RL: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. 2021.doi:10.3322/caac.21660.
2. International Agency for Research on Cancer. The Global Cancer Observatory. Cancer Today. Available online: http://gco.iarc.fr/.
3. Klingelhöfer D, Braun M, Schöffel N, Brüggmann D, Groneberg DA: Gastric Cancer: Bibliometric Analysis of Epidemiological, Geographical and Socio-Economic Parameters of the Global Research Landscape. Int J Health Policy Manag 2020.doi:10.34172/ijhpm.2020.29.
4. Plummer M, Franceschi S, Vignat J, Forman D, de Martel C: Global burden of gastric cancer attributable to Helicobacter pylori. Int J Cancer 2015, 136(2):487-490.doi:10.1002/ijc.28999.
5. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, Malfertheiner P, Graham DY, Wong VWS, Wu JCY *et al*: Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. Gastroenterology 2017, 153(2):420-429.doi:10.1053/j.gastro.2017.04.022.
6. Kidd M, Lastovica AJ, Atherton JC, Louw JA: Heterogeneity in the Helicobacter pylori vacA and cagA genes: association with gastroduodenal disease in South Africa? Gut 1999, 45(4):499-502.doi:10.1136/gut.45.4.499.
7. Maiese K: WISP1: Clinical insights for a proliferative and restorative member of the CCN family. Curr Neurovasc Res 2014, 11(4):378-389.doi:10.2174/1567202611666140912115107.
8. Berwick DC, Harvey K: The regulation and deregulation of Wnt signaling by PARK genes in health and disease. J Mol Cell Biol 2014, 6(1):3-12.doi:10.1093/jmcb/mjt037.
9. Gurbuz I, Chiquet-Ehrismann R: CCN4/WISP1 (WNT1 inducible signaling pathway protein 1): a focus on its role in cancer. Int J Biochem Cell Biol 2015, 62:142-146.doi:10.1016/j.biocel.2015.03.007.
10. Jia S, Qu T, Feng M, Ji K, Li Z, Jiang W, Ji J: Association of Wnt1-inducible signaling pathway protein-1 with the proliferation, migration and invasion in gastric cancer cells. Tumour Biol 2017, 39(6):1010428317699755.doi:10.1177/1010428317699755.

11. Zhang LH, Wang Y, Fan QQ, Liu YK, Li LH, Qi XW, Mao Y, Hua D: Up-regulated Wnt1-inducible signaling pathway protein 1 correlates with poor prognosis and drug resistance by reducing DNA repair in gastric cancer. World J Gastroenterol 2019, 25(38):5814-5825.doi:10.3748/wjg.v25.i38.5814.
12. Shastry BS: SNPs: impact on gene function and phenotype. Methods Mol Biol 2009, 578:3-22.doi:10.1007/978-1-60327-411-1_1.
13. Davies SR, Watkins G, Mansel RE, Jiang WG: Differential expression and prognostic implications of the CCN family members WISP-1, WISP-2, and WISP-3 in human breast cancer. Ann Surg Oncol 2007, 14(6):1909-1918.doi:10.1245/s10434-007-9376-x.
14. Wang Y, Yang SH, Hsu PW, Chien SY, Wang CQ, Su CM, Dong XF, Zhao YM, Tang CH: Impact of WNT1-inducible signaling pathway protein-1 (WISP-1) genetic polymorphisms and clinical aspects of breast cancer. Medicine (Baltimore) 2019, 98(44):e17854.doi:10.1097/md.0000000000017854.
15. Lee HL, Chiou HL, Wang SS, Hung SC, Chou MC, Yang SF, Hsieh MJ, Chou YE: WISP1 genetic variants as predictors of tumor development with urothelial cell carcinoma. Urol Oncol 2018, 36(4):160.e115-160.e121.doi:10.1016/j.urolonc.2017.11.023.
16. Chen CT, Lee HL, Chiou HL, Chou CH, Wang PH, Yang SF: Impacts of WNT1-inducible signaling pathway protein 1 polymorphism on hepatocellular carcinoma development. PloS one 2018, 13(6):e0198967.doi:10.1371/journal.pone.0198967.
17. Lau HK, Wu ER, Chen MK, Hsieh MJ, Yang SF, Wang LY, Chou YE: Effect of genetic variation in microRNA binding site in WNT1-inducible signaling pathway protein 1 gene on oral squamous cell carcinoma susceptibility. PLoS One 2017, 12(4):e0176246.doi:10.1371/journal.pone.0176246.
18. Chen J, Yin J, Li X, Wang Y, Zheng Y, Qian C, Xiao L, Zou T, Wang Z, Liu J *et al*: WISP1 polymorphisms contribute to platinum-based chemotherapy toxicity in lung cancer patients. Int J Mol Sci 2014, 15(11):21011-21027.doi:10.3390/ijms151121011.
19. Lin YH, Hsiao YH, Yang SF, Liu YF, Hsu CF, Wang PH: Association Between Genetic Polymorphisms of WNT1 Inducible Signaling Pathway Protein 1 and Uterine Cervical Cancer. Reprod Sci 2018, 25(11):1549-1556.doi:10.1177/1933719118756749.
20. Li T, Qin W, Liu Y, Li S, Qin X, Liu Z: Effect of RAGE gene polymorphisms and circulating sRAGE levels on susceptibility to gastric cancer: a case-control study. Cancer Cell Int 2017, 17:19.doi:10.1186/s12935-017-0391-0.
21. Chen J, Yin JY, Li XP, Wang Y, Zheng Y, Qian CY, He H, Fang C, Wang Z, Zhang Y *et al*: Association of Wnt-inducible signaling pathway protein 1 genetic polymorphisms with lung cancer susceptibility and platinum-based chemotherapy response. Clin Lung Cancer 2015, 16(4):298-304.e291-292.doi:10.1016/j.cllc.2014.12.008.
22. Liu Y, Xie L, Zhao J, Huang X, Song L, Luo J, Ma L, Li S, Qin X: Association between catalase gene polymorphisms and risk of chronic hepatitis B, hepatitis B virus-related liver cirrhosis and hepatocellular carcinoma in Guangxi population: a case-control study. Medicine (Baltimore) 2015, 94(13):e702.doi:10.1097/md.0000000000000702.

23. Shi YY, He L: SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 2005, 15(2):97-98.doi:10.1038/sj.cr.7290272.
24. Frank B, Hoffmeister M, Klopp N, Illig T, Chang-Claude J, Brenner H: Single nucleotide polymorphisms in Wnt signaling and cell death pathway genes and susceptibility to colorectal cancer. Carcinogenesis 2010, 31(8):1381-1386.doi:10.1093/carcin/bgq082.
25. Su F, Overholtzer M, Besser D, Levine AJ: WISP-1 attenuates p53-mediated apoptosis in response to DNA damage through activation of the Akt kinase. Genes Dev 2002, 16(1):46-57.doi:10.1101/gad.942902.
26. Calvisi DF, Conner EA, Ladu S, Lemmer ER, Factor VM, Thorgeirsson SS: Activation of the canonical Wnt/beta-catenin pathway confers growth advantages in c-Myc/E2F1 transgenic mouse model of liver cancer. J Hepatol 2005, 42(6):842-849.doi:10.1016/j.jhep.2005.01.029.
27. Chiang KC, Yeh CN, Chung LC, Feng TH, Sun CC, Chen MF, Jan YY, Yeh TS, Chen SC, Juang HH: WNT-1 inducible signaling pathway protein-1 enhances growth and tumorigenesis in human breast cancer. Sci Rep 2015, 5:8686.doi:10.1038/srep08686.
28. Tang Q, Jiang X, Li H, Lin Z, Zhou X, Luo X, Liu L, Chen G: Expression and prognostic value of WISP-1 in patients with endometrial endometrioid adenocarcinoma. J Obstet Gynaecol Res 2011, 37(6):606-612.doi:10.1111/j.1447-0756.2011.01631.x.
29. Machlowska J, Baj J, Sitarz M, Maciejewski R, Sitarz R: Gastric Cancer: Epidemiology, Risk Factors, Classification, Genomic Characteristics and Treatment Strategies. Int J Mol Sci 2020, 21(11):4012.doi:10.3390/ijms21114012.
30. Chung TT, Pan MS, Kuo CL, Wong RH, Lin CW, Chen MK, Yang SF: Impact of RECK gene polymorphisms and environmental factors on oral cancer susceptibility and clinicopathologic characteristics in Taiwan. Carcinogenesis 2011, 32(7):1063-1068.doi:10.1093/carcin/bgr083.
31. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G: The hallmarks of aging. Cell 2013, 153(6):1194-1217.doi:10.1016/j.cell.2013.05.039.
32. Vijg J, Campisi J: Puzzles, promises and a cure for ageing. Nature 2008, 454(7208):1065-1071.doi:10.1038/nature07216.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfiles1.docx
- Additionalfiles2.docx
- Additionalfiles3.docx
- Additionalfiles4.docx
- Additionalfiles5.docx

- PersonalCover.doc