Title

Genetic Susceptibility and Environmental Risk Factors of Liver Cancer, a Population-based Case-control Study in Jiangsu Province, China

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Publication Date

2015

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

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A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Epidemiology

by

Xing Liu

2015

ABSTRACT OF THE DISSERTATION

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Doctor of Philosophy in Epidemiology

University of California, Los Angeles, 2015

Professor Zuo-Feng Zhang, Chair

Background

Liver cancer is a major public health burden especially in China which accounts for 50% of new cases each year in the world. The main risk factors have been well established, including hepatitis virus infections, alcohol consumption, liver cirrhosis, tobacco smoking and intake of aflatoxin contaminated food. However, the facts that not all patients with chronic virus infection and liver cirrhosis develop liver cancer, and that family clustering of liver cancer has been frequently observed, indicate that genetic variation might also play a role. Genes from stem cell pathway and microRNA related genes have been associated with liver cancer in experimental studies. Also, GWAS reported that several SNPs were associated with the cancer. However, few well-designed large population-based studies in China have been conducted to evaluate the

impact of genetic susceptibility and environmental risk factors and their potential interaction on the development of liver cancer.

Methods

A population-based case-control study was conducted in Jiangsu from 2003 to 2010. A total of 2,011 cases with incident liver cancer and 7,933 healthy controls randomly selected from the community were consented and then interviewed by trained interviewers. A standard questionnaire was employed in collecting epidemiological data. Blood samples were drawn, DNAs were isolated, and 26 SNPs from stem cell pathway, 28 SNPs from microRNA related genes and 4 SNPs detected by GWAS were measured. Antibodies and/or antigens of hepatitis B and C viruses (HBV/HCV) were measured by ELISA procedure and serum HBV viral load was measured among those individuals who were with HBV infection. Unconditional logistic regression models were mainly used in determining the odds ratios of liver cancer and their 95% confidence intervals (CIs). Potential interactions between genetic susceptibility and environmental exposures were also examined.

Results

HBsAg positive was confirmed as a strong risk factor for liver cancer with an adjusted odds ratio (aOR) of 9.85 (95% CI: 8.28-11.72). Additionally, alcohol consumption (aOR: 1.91, 95% CI: 1.61-2.28), tobacco smoking (aOR: 1.48, 95% CI: 1.25-1.75), family history of liver cancer (aOR: 4.19, 95% CI: 3.17-5.53) and history of raw water drinking (aOR: 1.32, 95% CI: 1.13-1.55) were positively associated with liver cancer. Statistical interactions were evaluated among these five risk factors. Alcohol consumption, tobacco smoking and having family history of liver

cancer showed positive statistical interaction with HBsAg positive on liver cancer risk on both additive scale and multiplicative scale. Polymorphisms in microRNA related genes, rs896849 (TP53INP1 gene, C/C vs. T/T, aOR: 0.27, 95% CI: 0.08-0.87) and rs11614913 (miR-196a2 gene, C/C vs. T/T, aOR: 0.44, 95% CI: 0.27-0.71) showed inverse associations with liver cancer. Polymorphisms in genes from stem cell pathway, rs4730775 (WNT2 gene, C/T vs. C/C, aOR: 0.71, 95% CI: 0.50-0.99) and rs2241802 (FZD3 gene, A/A vs. G/G, aOR: 0.58, 95% CI: 0.37-0.91) showed inverse associations with liver cancer. Several SNPs were found to be associated with liver cancer in stratified analyses when stratified by HBV infection status, alcohol drinking or tobacco smoking. No obvious associations were found between SNPs identified from GWAS and liver cancer in this study population. Statistical interactions between rs896849 and HBV infection, drinking history and smoking history were observed. Rs11614813 was also found to interact with tobacco smoking. Among those 949 HBsAg positive participants, serum HBV viral load was measured and found to be positively associated with liver cancer and HBeAg positive. Rs12828 (WWOX gene) from microRNA related genes showed an inverse association with having higher level of HBV viral load (>10^5 IU/ml) (aOR=0.48, 95% CI: 0.27-0.84, G/A vs G/G; aOR=0.45, 95% CI: 0.22-0.93, A/A vs. G/G). Rs2740348 (Gemin4 gene) was associated with having higher HBV viral load (aOR=2.16, 95% CI: 1.16-4.03, G/C vs. G/G). Among SNPs from stem cell pathway, rs222851 (DVL2 gene) was associated with having higher HBV viral load (aOR=2.47, 95% CI: 1.42-4.28, A/G vs. A/A) and rs3734637 (HEY2 gene) was inversely associated with having higher HBV viral load (aOR=0.19, 95% CI: 0.05-0.70, C/C vs. A/A).

Conclusion

This study confirmed associations between HBV infection, alcohol consumption, tobacco smoking, unsafe water and food intake, family history of liver cancer and risk of liver cancer. Interactions were observed among these risk factors in this Chinese population. SNPs from microRNA related genes were inversely associated with liver cancer, and SNPs from stem cell pathway were associated with liver cancer in both directions based on overall analysis and stratified analyses. Some of the SNPs were further associated with serum HBV viral load among HBV infected participants. Polymorphisms in studied pathways and former mentioned environmental risk factors showed interaction on liver cancer development. Our results indicated that eliminating the infection of HBV is still the highest priority for liver cancer prevention by implementation of HBV vaccination in Chinese population, followed by interventions on high risk behaviors by reducing population prevalence of alcohol drinking, tobacco smoking and advocating safe water and foods. Our study also found several SNPs from microRNA related genes and stem cell pathway genes which were associated with liver cancer. These might serve as new markers for detecting carcinogenesis as well as therapy targets once the associations are further confirmed.

The dissertation of Xing Liu is approved.

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2015

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ACKNOWLEDGEMENTS

Above all, I would like to thank my parents for their support. Nothing compares to their love.

I would like to thank my PhD advisor, Professor Zuo-Feng Zhang for all his support, advice and encouragement during the past years. Professor Zhang's enthusiasm, wisdom, full of energy and sense of responsibility encourage me in every lecture, every group meeting and every conversation I have with him. He has not only provided scientific advice but built up a model of being an epidemiologist as well. I am also very grateful to the members of my PhD committee, Professors Detels, Afifi and Martínez-Maza for their nice help and suggestions in the progress. I would also like to express my deep appreciation to Dr. Jin-Kou Zhao, the Principal Investigator of Jiangsu study, who initiated the study and kindly allowed me to use the Jiangsu case-control study data for my dissertation research. Without his support, it would be impossible to conduct my PhD research.

I would like to thank all the participants in this study for providing their time, information and specimen. Thanks to all CDC staff members worked at each study site, did interviews, filled in questionnaires and collected blood samples. The study can never be done without their contribution.

I would like to thank Doctor Ming Wu, Associate Director of Jiangsu CDC and the current PI of the Jiangsu Study, and Doctors Jinyi Zhou, Jie Yang, Yu Qin, Renqiang Han, Yongqing Zhang and Qinglan Zhang from Department of Chronic Disease and Doctors Gengfeng Fu and Xiping

Huan from Department of HIV/AIDS Control and Prevention in Jiangsu Provincial CDC. They provided me the needed help in performing all the laboratory tests for this study. I would like to thank Mrs. Guifang Zhu for her strong support and help in the laboratory.

I am also grateful to all my graduate peers in the Cancer Epidemiology group. Thanks to Claire Kim and Fong Liu for supporting and helping me in preparing the exams together. Thanks to Dr. Shen-Chih Chang for all her nice and patient help. Thanks to Somee Jeong for her help in laboratory tests. I would also like to thank Drs. Weiming Zhu, Ying Yang, Po-yin Chang, Gina Wallar, Erin Peckham, Heather Tarleton, Fayth Miles, as well as Mr. Travis Meyers, Ms. Esther Chang, Ms. Aileen Baecker and Mr. Daniel Keebler for all the encouragement, insightful talks, idea-sharing and BBQ parties.

I would like to express my special appreciation to Professor Na He for his academic advice and support in the past seven years. Thanks to Doctors Haijiang Lin, Meiyang Gao, Sujuan Zhou and Yingying Ding for all their generous help.

Lastly, special thanks to Drs. Donglan Zhang, Chunqing Lin, Jelie Hsie, Kirral Xu, Mandy Zheng, Jie Wu, Xiaochen Lin and Ethan An. You are my family here in Los Angeles.

This project was supported by Jiangsu Provincial Health Department (RC 2003090, PI: Jin-Kou Zhao); partially supported by the National Cancer Institute, Fogarty International Center, National Institutes of Health, Department of Health and Human Services, Grants D43

TW000013-20S and 21S, CA0914; and the Alper Research Center for Environmental Genomics of the University of California, Los Angeles Jonsson Comprehensive Cancer Center.

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CHAPTER 1: BACKGROUND

1.1 Epidemiology of liver cancer

Liver cancer is the seventh most common neoplasm and the second most frequent cause of cancer death worldwide with 782,000 estimated new cases and 746,000 deaths in 2012[1]. The disease shows great global variations that the incidence is high in east Asia and sub-Saharan Africa and relatively low in North America and Europe, which might relates to distinctive distributions of risk factors or genetics among these populations[2]. China is the country most seriously affected by liver cancer, accounting for half of all newly diagnosed cases each year in the world[1]. Liver cancer is the third most common malignancy and the second leading cause of cancer death in this country, resulting in 394,770 incident cases and 383,203 deaths in 2012[1]. Liver cancer is a highly lethal cancer with an overall five-year survival of 16.1% estimated from 18 SEER geographic areas from 2003 to 2009, ranging from 3.0% (distant) to 29.1% (localized) depending on stage at diagnosis [3]. And the five-year survival for liver cancer in China is 10.1% (95% CI 9.5–10.7%) according to the report recently published [4]. Only a minority of liver cancer is detected at an early stage for curative therapies such as surgical resection or liver transplantation, which makes it important to explore risk and protective factors for primary prevention and to identify biological markers for precise prevention and control of the disease.

Most liver cancer cases (70-90%) develop the cancer with an established chronic liver disease (CLD) and risk factors vary among different regions [5,6].The established risk factors of liver cancer include hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, alcoholic cirrhosis, dietary aflatoxins and tobacco smoking. These factors have been classified as group

one carcinogens by the International Agency for Research on Cancer (IARC) [7-9]. Eventually not all patients infected with HBV or HCV would develop liver cancer, indicating other factors may play roles in the liver carcinogenesis. However, there still lack well-designed large scale population-based studies conducted to evaluate specific etiological factors and genetic susceptibility of liver cancer, especially the potential interactions between the HBV/HCV infections, genetic susceptibility and environmental exposures on liver cancer development.

1.2 HBV/HCV infection and liver cancer

It is estimated that HBV is responsible for 50-80% of liver cancer cases and HCV is responsible for 10-25%[2]. According to WHO, 2 billion people worldwide have been infected with HBV, more than 240 million are chronically infected, and about 600,000 people die each year due to hepatitis B[10]. Meanwhile, 3–4 million people are infected with the hepatitis C virus per year, about 150 million are chronically infected and more than 350,000 people die from hepatitis C-related liver diseases each year[11].

In 2006, the Ministry of Health of China estimated that among Chinese aged 1 to 59 years old as of 1992, the national prevalence of HBV infection (positive for HBsAg or any HBV marker) and HBV carriers (who have persistent virus and subvirus particles in their blood for more than 6 months, usually represented by HBsAg positive) was 57.6% and 9.8%, corresponding to 690 million infected persons and 120 million carriers, as well as 20 million people with chronic hepatitis [12]. And the nationwide prevalence of HCV Antibody was estimated as 0.43%, corresponding to 5.6 million infected persons in 2006 [13].

Studies show that most liver cancer cases in China were associated with HBV [12]. A meta-analysis including 32 case-control studies from 1966 to 2004 involving 3,201 cases and 4,005 controls in China reported the pooled odds ratio and 95% confidence interval (CI) for HBsAg positivity was 14.1 (95% CI: 10.6–18.8); for anti-HCV antibody/HCV RNA positivity was 4.6 (95% CI: 3.6–5.9) [14]. A large scale cohort in Jiangsu Province observing 12,351 people for 31 years showed that the incidence rate ratio was 11.70 (9.06-15.19) comparing people with HBsAg to those without HBsAg [15]. The risk of developing liver cancer among HBV carriers with CLD ranged from 10-fold to 100-fold greater compared with uninfected people depending on the markers and populations studied[16].

Only 5-10% people who acquire HBV infection in adulthood become carriers. But among the carriers, up to 30% would develop progressive CLD including hepatitis, fibrosis, cirrhosis and liver cancer[16]. HBV is the prototype member of the Hepadnaviridae (hepatotropic DNA virus) family [17]. Much has been studied about the underlying pathogenesis of liver cancer in HBV infection. The HBV replication cycle is not directly cytotoxic to cells, which is consistent with the observation that many HBV carriers do not have obvious symptoms and have minimal liver injury, despite the intrahepatic replication of the virus is going on and extensive. Instead, host immune responses to the virus displayed in infected liver cells are the major determinants of hepatocellular injury [17]. In the progress of liver cancer, the random integration of HBV DNA into the cell genome and the generation of the X protein are very important[18]. The integrated viral DNA might act as a mutagenic agent, leading to genomic instability and chromosomal rearrangement. The rearrangement might involve loss of tumor suppressor genes, or the amplification and over expression of growth factor genes. The protein product (HBx) of HBV X

gene might also act as a transcriptional transactivator of different genes involved in cellular growth and cell cycle control, may interfere with DNA repair and apoptosis [18].

For HCV, about 75- 85% newly infected persons develop chronic infection indicated by having persistent viremia [19]and 60-70% of chronically infected people develop chronic liver disease, 5-20% develop cirrhosis and 1-5% die from cirrhosis or liver cancer[11]. HCV is an RNA virus belongs to the family of flaviviruses [20]. HCV does not integrate into the cell genome and is not considered as a directly cytotoxic virus either. Hepatitis occurs as a result of the reaction of the host immune system against the virus infected cells[18].The acute phase of HCV infection is usually asymptomatic and is not frequently diagnosed[20]. In fact, although the number of HCV infected people is fewer than the number of those infected with HBV, the probability of developing chronic infection is much higher[18]. The viral replication of HCV is robust and lacks proofreading function, which results in a rapid evolution of viral subtypes, leading to great challenges to immune-mediated control and vaccine development [20].

In sum, the pathogenesis of HBV/HCV infections is basically immune mediated and multiple mechanisms are evolved in escaping immune elimination and in continuous replication. However, both innate and adoptive immune responses are not effective in virus clearance. Chronic infection results in persistent inflammation and oxidative stress. And the prolonged fibrotic response results in cirrhosis followed with localized hypoxia, change in tissue architecture and angiogenesis [16]. Virus encoded proteins may change host gene expression and cellular phenotypes. These changes promote growth factor-independent proliferation, resistance to

growth inhibition, tissue invasion and metastasis, angiogenesis, reprogramming of energy metabolism, and resistance to therapeutic intervention, which are hallmarks of cancer [16].

Moreover, for both HBV and HCV infection, plasma viral load are important predictors of viral replication and disease progression [21,22]. From the studies of natural history of HBV infection, levels of viremia in chronic infection are usually lower than in acute infection. High titers of HBV DNA in the blood are often observed along with the continuous presence of HBeAg [17]. And continuous high serum viral load and/or HBeAg expression are significantly associated with increased risk of liver cancer [22,23]. Thus, serum viral load may act as an important intermediate in the progress of carcinogenesis of liver cancer.

1.3 Alcohol consumption and liver cancer

Alcoholic cirrhosis is established group I carcinogen for liver cancer by IARC[8]. The relationship between alcohol abuse and development of cirrhosis, and the relationship between cirrhosis and liver cancer both have been well-studied [24,25]. Although the direct effect of alcohol intake on development of liver cancer is still under study, the mechanism of alcohol abuse induced liver injury has been well explored. First of all, liver cell is the place where ethanol is metabolized. The free radicals generated along with acetaldehyde by alcohol dehydrogenase (ADH) during the metabolism might combine to cell DNA as biochemical signs of oxidative damage which have been observed among alcoholic patients and experimental animals who were exposed to ethanol [26]. Second, the microsomal cytochrome P450 (CYP2E1) is also involved in metabolizing ethanol to acetaldehyde. CYP2E1 metabolism generates hydroxyethyl radical and leads to direct cellular damages [27,28], and alters distribution of

carcinogens, changes cell cycle duration and relates with nutritional deficiencies and abnormal immune responses as indirect effects[28,29]. Third, ethanol and its metabolism also shows impact on cell signaling pathways which regulate hepatocyte function, proliferation and apoptosis [28].

Apart from the effect of ethanol itself, there are other carcinogens that may exist in alcoholic beverages which have been summarized by IARC[8]. These substance include group I carcinogen aflatoxins from beer, arsenic, benzene, cadmium, etc. (Table 1.1).

Table 1.1 Summary of carcinogens that may be present in alcoholic beverages

Agent	Amount in alcoholic beverages[a]	*IARC Monographs* evaluation of carcinogenicity			*IARC Monographs* volume, year
		In animals	In humans	IARC group	
Acetaldehyde	Lower mg/L range	Sufficient	Inadequate	2B	**71**, 1999
Acrylamide	Beer; <10 µg/kg	Sufficient	Inadequate	2A	**60**, 1994
Aflatoxins	Beer (Table 1.22)	Sufficient	Sufficient	1	**56**, **82**, 2002
Arsenic	(Table 1.25)	Sufficient	Sufficient	1	**84**, 2004
Benzene	(no sufficient data)	Sufficient	Sufficient	1	**Suppl. 7**, 1987
Cadmium	(Table 1.24)	Sufficient	Sufficient	1	**58**, 1993
Deoxynivalenol	Beer (Table 1.19)	Inadequate	Inadequate	3	**56**, 1993
Ethanol	(2–80% vol)	Inadequate	Sufficient	1	**44**, **96**, 2010
Ethyl carbamate (urethane)	See monograph in this volume	Sufficient	Inadequate	2A	**7**, **96**, 2010
Furan	Beer; <20 µg/kg	Sufficient	Inadequate	2B	**63**, 1995
Lead	(Table 1.23)	Sufficient	Limited	2A	**87**, 2006
N-Nitrosodimethylamine	Beer: <0.5 µg/kg (Table 1.16)	Sufficient	Inadequate	2A	**Suppl. 7**, 1987
Nivalenol	Beer (Table 1.20)	Inadequate	Inadequate	3	**56**, 1993
Ochratoxin A	Wine, beer (Table 1.17)	Sufficient	Inadequate	2B	**56**, 1993
Organolead compounds	Wine; limited data	Inadequate	Inadequate	3	**87**, 2006
Patulin	Apple cider	Inadequate	Inadequate	3	**Suppl. 7**, 1987

[a] Most carcinogens are contained at very different concentration ranges depending on the origin and different production technologies, so that no average concentration can be derived.

A lot of human cancers have been reported to be associated with alcoholic beverage consumption in epidemiological studies including several large cohort studies, case-control studies and meta-analyses. Three early cohort studies conducted in western countries among alcoholics reported strong associations between alcoholism, cirrhosis and liver cancer [30-32]. However, two cohort studies performed in Chinese general population did not find statistically significant associations between alcohol consumption and increased risk of liver cancer [33,34]. Several case-control

studies mainly performed in western countries recruiting hospital-based controls reported increased risks especially among heavy drinkers, while lack of consistent dose-response relationship between drinking and liver cancer [8]. A meta-analysis reported relative risks of 1.17 (95% CI: 1.11-1.23), 1.36 (95% CI: 1.23-1.51) and 1.86 (95% CI: 1.53-2.27) for 25g, 50g and 100g alcohol intake per day [35]. And another meta-analysis reviewing Chinese case-control studies reported combined OR of 1.88 (95% CI: 1.53-2.32) comparing drinkers to never drinkers[36].

The interaction between virus infection and alcohol drinking is also of interest. Since cirrhosis is associated with hepatic regeneration once tissue damage occurred to liver, as two major causes of the injury, virus infection and alcohol may play a joint role in accelerating the cirrhosis [37]. The interaction between virus infection and alcohol consumption on development of liver cancer has not been thoroughly studied, mainly because the low prevalence of co-existence of these two risk factors in most areas [34,38-41]. Stratified analyses by HBV/HCV infection status were performed and positive association between alcohol consumption and liver cancer were reported within strata [38,39]. And the interaction between alcohol drinking and tobacco smoking was examined by three case-control studies, reporting relative risks from 5.9 to 7.2 comparing combined smoking and drinking exposure to non-smokers and non-drinkers[38-40].

1.4 Tobacco smoking and liver cancer

Carcinogens including different kinds of polynuclear aromatic hydrocarbons, heterocyclic hydrocarbons, N-Nitrosamines, aromatic amines, N-heterocyclic amines, aldehydes, phenolic compounds, bolatile hydrocarbons, miscellaneous organic compounds and metals and metal

compounds are found in cigarette smoke[9]. Associations between cigarette smoking and risk of liver cancer have been reported since 1980's. Most of the studies reported increased risk comparing current smokers to non-smokers after controlling for alcohol drinking[9]. A meta-analysis conducted overviewing 254 studies performed from 1961 to 2003 reported a pooled OR of 1.56 (95% CI: 1.29-1.87) for smoking on liver cancer [42]. A retrospective case control study comparing 36,000 adults died from liver cancer and 17,000 controls died from cirrhosis reported a risk ratio of 1.36 (95% CI: 1.29-1.43) in Chinese population[43].

The potential effect measure modification between smoking and other risk factors such as alcohol drinking and HBV/HCV infection was mostly examined by stratified analyses[9]. The magnitude of joint effect combined by two exposures has not been well studied.

1.5 Stem cell pathway in development of liver cancer

Stem cells are cells that have the potential to renew themselves and generate mature cells of tissues through ability of differentiation. Tumor cells share the same characteristics. Researchers noticed several associations between stem cells and cancer cells including the self-renewal regulation, the possibility of normal stem cells transform to cancer cells and the possibility of existence of cancer stem cells[44]. It has been hypothesized that many pathways classically found associated with cancer development may also play a role in regulating normal stem cell self-renewal mechanisms including Wnt, Shh and Notch pathways[45-52]. It has been suggested that Wnt signaling pathway plays key roles during embryogenesis, cell polarity generation, tissue regeneration, cell fate specification and carcinogenesis [53]. This signaling network is implicated in the maintenance of tissue homeostasis by regulating self-renewal of normal stem cells as well

as proliferation or differentiation of progenitor cells. Breakage of the stem cell signaling network may lead to carcinogenesis [54]. Epigenetic changes and loss-of-function mutation of negative regulators of the Wnt pathway have been observed in a variety of cancers [55-65].

It was firstly discovered in 1982 that the mouse Wnt1 gene (named as Int1 originally) was a integration site for the Mouse Mammary Tumor Virus (MMTV) which may induce breast cancer [66]. Later, researchers found that the human Wnt gene family consists of 19 members, encoding secreted glycoproteins with 22 or 24 Cys residues [67]. Many Wnt mutations have been found to be associated with special phenotypes when generated in mouse [55]. Moreover, mutations affecting the Wnt/β-catenin pathway appear to be the most frequent genetic event in human liver cancer [56].

It is believed that three different pathways are activated in Wnt pathway: the canonical Wnt/ β-catenin cascade, the non-canonical planar cell polarity (PCP) pathway and the Wnt/Ca^{2+} pathway [68,69]. In the canonical Wnt/ β-catenin pathway, when the activation is absent, the level of β-catenin is usually low in the hepatocyte because of a destruction complex comprised of APC, axin and GSK-3 binding it, phosphorylating it and degrading it [70]. Once the canonical Wnt/ β-catenin is initiated, the Wnt ligands bind to several receptors: Frizzled (FZD) family receptors, LDL-receptor-related transmembrane proteins (LRP5 and LRP6) as co-receptors and heparansulfate proteoglycans (HSPGs). Then the formation of the destruction complex is dissolved [67,70]. Unphosphorylated β-catenin then accumulates in the nucleus and relates to T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) family transcription factors, legless family docking proteins (BCL9 and BCL9L), and PYGO family co-activators (PYGO1 and

PYGO2) for activating target genes of the pathway, which include FGF20, JAG1, DKK1, MYC, CCND1 and AXIN2 [48,53,69]. These target genes up-regulates cell-proliferation, migration, invasion, cell cycle progression and metastasis [70]. It is reported that among liver cancer cases, accumulation of β-catenin has been observed and is closely associated with the clinic-pathological characteristics of the disease [71] and mutations [72].

Notch signaling pathway is another important pathway in the network. JAG1 gene is one of Notch ligand genes and it has been predicted as one of the targets of the canonical Wnt signaling pathway. And in the mammary carcinogenesis where Wnt signaling pathway gets activated, Notch4 is activated too. Notch and Wnt signaling pathways are both necessary for the self-renewal of hematopoietic stem cells. And they may have joint effect in inhibiting cell differentiation through the down-regulation of Atoh1/Hath1 bHLH transcription factor[54]. Together, these facts indicate that Notch and Wnt signaling pathways keep the homeostasis of stem cells and progenitor cells through the inhibition of terminal differentiation [53].

In sum, it is generally believed that the Wnt, FGF, Notch signaling network is important in the maintenance of tissue homeostasis by regulating stem cells in self-renewal and progenitor cells in proliferation and differentiation. Breakage of the network might lead to the transformation from normal stem cells to cancer stem cells. Acquisition of self-renewal potential in progenitor cells due to genetic or epigenetic change of stem cell signaling pathway related genes may also give rise to cancer stem cells[54]. However, very few epidemiologic studies have been conducted to assess the effect of SNPs in Wnt signaling genes and related signaling inhibitors on the development of liver cancer in human.

1.6 MicroRNA polymorphisms in development of liver cancer

MicroRNAs (miRNAs) are small, evolutionarily conserved, non-coding RNAs. They are of 18–25 nucleotides in length and have important function as key post-transcriptional regulators in gene regulation. MicroRNAs are believed to be involved in 30% of all protein-coding genes controlling and have been shown to participate in regulating almost all cellular process being investigated [73,74]. MiRNAs control gene regulation by regulating mRNA translation or stability in the cytoplasm [75,76]. MiRNAs mostly bind to mRNAs in the 3' untranslated regions (3'UTRs) and down-regulate the expression of genes [77]. They repress target genes and coordinate normal cell processes, including cellular proliferation, differentiation and apoptosis. And the aberrant miRNA function contributes to a number of human diseases including cancer [73]. Functional studies indicate that they involve in carcinogenesis as they can behave as oncogenes or tumor suppressor genes depending on the cellular function of their targets [78]. Many miRNA genes are located at cancer-related genomic regions or fragile sites. Germline mutations have been detected both in miRNA genes and in the binding sites of target mRNAs [78]. MiRNAs have been shown to be differentially expressed in cancer cells in which they are usually down-regulated or form distinct expression patterns. It is possible that the genetic instability changes these miRNAs' expression. Thus the expression might also be potentially important biomarker, cancer classifier and therapeutic target. As mentioned above, although the expression of some miRNAs is increased in cancer cells, the down regulation of miRNAs is more frequently observed in a wide range of studies. Furthermore, miRNAs are involved in many oncogenic networks. For example, MYC-driven reprogramming of miRNA expression has been suggested to be a factor in liver cancer because the reprogramming might be associated

with the aggressive phenotype of cancer[73]. In addition, epigenetic modifications in miRNA loci associated with altered transcription and metastatic ability of cancer cells have been observed [78].

Many reports have shown miRNA deregulation in human liver cancers. The miRNAs including miR-122, miR-192, miR-199 a/b-3p, miR-100 and miR-99a were found to have decreased expression to different extent and miR-21 was found to be up-regulated in observations [77,78]. Some studies found that miRNAs might regulate the hepatitis virus infection at the transcription level either by targeting at transcription factors required for HBV gene expression or by directly binding to HBV transcripts [79]. Some researchers reported that miRNA machinery is serving as a defense system in the HBV infection, and the expression of specific miRNAs is altered during the process. They found that miRNA-15a and miRNA-16-1 were down-regulated by HBx transcript [80]. MiR-141 was reported to repress HBV expression and replication in HepG2 cells [81]. Serum levels of miRNA-122 and miRNA-22 were tested and shown to be correlated, and both were elevated in chronic HBV infected patients. Levels of these two miRNAs were higher among those HBeAg positives than negatives. MiRNA-122 was found to be lower among those with advanced fibrosis [82]. MiRNA-501 was reported to be elevated in tissues with high HBV replication [83]. In sum, it has been widely observed that HBV infection might be associated with changing in miRNA expression and miRNA profiles were different at different stages of HBV associated disease [84].

A few epidemiologic studies have been performed exploring genetic variations in miRNA related genes and risk of hepatitis virus infection and development of liver cancer. One case-control

study examined three SNPs, rs1057035 in DICER1, rs3803012 in RAN and rs10773771 in PIWIL1 in a Chinese population and found that rs1057035 CT/CC variant genotypes was associated with decreased risk and rs3803012 AG/GG variant genotypes was associated with increased risk [85]. A meta-analysis was performed to assess whether several SNPs in the genes coding miRNAs are associated with liver cancer. miR-146a G>C (rs2910164) was found to be inversely associated with liver cancer and miR-196a-2 C>T (rs11614913) was positively associated with the risk [86].

1.7 Genome-wide association study (GWAS) of liver cancer

GWAS have been carried out for liver cancer in several studies and three have been conducted in Chinese populations [87-89]. These studies mainly focused on HBV related liver cancer since HCV is not a major risk factor for liver cancer in general Chinese population. However, the findings in these studies were not replicated in later studies [90-92]. One liver cancer GWAS study reported SNP rs9267673 lying in the MHC class II locus was significantly associated with liver cancer [92]. Another study reported SNP rs2596542 on HLA-S-MICA was associated with liver cancer [91]. And our study group detected two SNPs (rs9275572 and rs4678680) associated with liver cancer in the pilot Taixing study. Thus, in this analysis, these four SNPs detected from GWAS would be tested to explore their association with liver cancer.

1.8 The gap in the literature

The risk factors for liver cancer have been relatively well established. However, the interactions among hepatitis virus infection, alcohol consumption and smoking have not been sufficiently examined due to the low prevalence of co-existence of these risk factors. Furthermore, not all

people with these established risk factors would eventually develop liver cancer, implicating that genetic susceptibility might play a role in the underlying pathogenic mechanisms of the cancer. Very few studies have been conducted to assess the effect of polymorphisms in miRNA-related genes and stem cell pathway genes on the development of liver cancer, and their potential interaction with environmental risk factors in the liver carcinogenesis.

CHAPTER 2: STUDY OBJECTIVES AND METHODOLOGY

2.1 Research Objectives

To explore the effect of genetic susceptibility including polymorphisms in microRNA related genes, stem cell pathway genes and GWAS identified liver cancer related SNPs, and environmental risk factors such as hepatitis B virus infection, hepatitis C virus infection, alcohol consumption and tobacco smoking on risk of liver cancer and their interactions in a Chinese population.

2.2 Specific Aims and Hypotheses

Hypothesis 1

We hypothesize that HBV/HCV infection, alcohol consumption and tobacco smoking are risk factors and may interact with each other in the development of liver cancer in Chinese population.

Specific aim 1

To confirm and determine the strength of the association between HBV/HCV infection, alcohol consumption, tobacco smoking and other potential factors with liver cancer in a general population from Jiangsu, China.

Hypothesis 2

We hypothesize that the genetic susceptibility including SNPs in stem cell pathway, SNPs in microRNA related genes and SNPs discovered by GWAS may be associated with liver cancer and that genetic susceptibility might interact with environmental factors such as HBV/HCV infection, tobacco smoking, alcohol consumption, etc. in the development of liver cancer in a Chinese population.

Specific aim 2

To explore the association between SNPs from microRNA related genes, stem cell pathway and SNPs detected by GWAS and risk of liver cancer, and the potential interaction between SNPs and other major risk factors such as HBV/HCV infection, alcohol consumption and tobacco smoking in this Chinese population.

Hypothesis 3

We hypothesize that the genetic and environmental risk factors associated with risk of liver cancer may also be related with serum viral load of HBV among those HBV infected participants. In other words, HBV viral load may play a role as an intermediate marker, which was affected by risk factors and then act as a predictor of the development of liver cancer in the disease progression.

Specific aim 3

To explore the association between risk factors of liver cancer observed in this study and serum HBV viral load levels in the HBV infected participants.

2.3 Study Design and Methods

Study overview

A population-based case-control study designed to systematically evaluate risk factors for four common cancers including esophagus, stomach, lung and liver cancer in Jiangsu, China was performed from 2003 to 2010. The cases and controls were individually matched by age (±5 years) and gender for each cancer site originally. Then the controls were pooled together in later analyses in order to increase the power of the study. For liver cancer study, a total of 2,011 liver cancer cases and 7,933 healthy controls were included. Epidemiological data including socio-demographic characteristics and exposures such as alcohol consumption and tobacco smoking were collected using a comprehensive questionnaire in face-to-face interviews. Blood samples were collected and laboratory tests were performed. Serum HBV infection markers (HBsAg, HBsAb, HBeAg, HBeAb, HBcAb) and HCV infection marker (anti-HCV) were measured among 1,216 cases and 6,529 controls with serum samples. Serum HBV viral load test was then performed among 915 HBsAg positive individuals who had sufficient serum sample. 58 SNPs from microRNA-related genes (28 SNPs), stem cell pathway genes (26 SNPs) and GWAS detected genes (4 SNPs) were measured among 419 cases and 2,193 controls. Statistical analyses were then performed to: 1) Confirm and determine the strength of association and interaction between known risk factors such as HBV/HCV infection, alcohol consumption and tobacco smoking on liver cancer in this Chinese population; 2) Explore the effect of genetic susceptibility representing by polymorphisms from above mentioned pathways on liver cancer and their interaction with environmental exposures; 3) Explore the association between risk factors of liver cancer and serum HBV viral load among HBV infected participants in this Chinese population.

2.4 Study population

Jiangsu is an eastern coastal province in China with heavy burden of cancer, reporting an annual cancer mortality of 209/100,000 in 2003-2005, which is higher than the national mortality rate (136/100,000). Cancer is the leading cause of death in this province since 1970s and liver cancer ranks the third among all cancers in 2010. In fact, Jiangsu Province is among the highest risk areas for liver cancer in this country, with an incidence of 32/100,000 and mortality of 30/100,000 in 2007. County-level cancer registries have been established and gradually improved in these counties since the late 1990s. This study was conducted in four counties: Dafeng, Ganyu, Chuzhou and Tongshan. All these four counties locate in northern Jiangsu with population sizes as the following: 0.71 million in Dafeng, 0.95 million in Ganyu, 0.98 million in Chuzhou and 1.14 million in Tongshan (6th national census data, 2010).

Cases

Eligible cases were patients with pathologically or clinically confirmed diagnosis of primary liver cancer from January 2003, to December 31, 2010, reported to the population-based cancer registries. The diagnostic methods of these incident liver cancer patients included pathologic or clinical diagnosis including computed tomography (CT), ultrasound, alpha-fetoprotein (AFP), cytology, surgery, magnetic resonance imaging (MRI), and others. During the study period, researchers attempted to interview all incident cases with primary liver cancer who consented to participate in the study with the following restrictions: patients had to be newly diagnosed, aged 20 years or older, and in stable medical condition as determined by their physicians, without previous history of cancer, and willing to sign a written informed consent. The study was restricted to people residing in four counties for 5 or more years. In the study period, a total of

2,011 patients with primary liver cancer diagnosed in four counties from a total of 4,541 newly diagnosed cases were included and interviewed during the study period with a response rate of 44.3%.

Controls

Eligible controls were randomly selected from the same county where cases arose using a county demographic database. Controls were included and interviewed during the study period based on the following eligibility criteria: aged 20 years or older, in stable medical condition, without previous history of cancer, and willing to sign a written informed consent form. The study was restricted to people residing in the four counties for at least 5 years. The control group was originally selected according to the frequency distribution of sex and age (5 year categories) of cancer cases interviewed from each village (or resident block in the city) where the cancer cases originated. For each village (or resident block), a list was generated of residents within the same sex and age group. Random numbers were used to select healthy controls according to the control-to-case ratio of 1:1. When the control did not fit the criteria or refused to be interviewed, their basic demographic data were recorded and the same selection process was used to choose another control. Approximately 5-8 ml of blood specimen was then collected from each control. In order to increase the power of the study, controls of all four cancer sites were combined as the population healthy control group for this proposed study. A total of 8,720 controls were interviewed and among these, 7,933 had questionnaire completed and 6,529 had blood samples drawn. The response rates exceeded 90% at all study sites.

2.5 Epidemiological data collection

Cases and controls were interviewed using a standard questionnaire by trained interviewers. Face-to-face interviews were monitored by professional staff in the Division of Chronic Disease Prevention of the county CDC and the quality control procedure was maintained by Jiangsu CDC staff. For cases, the interviews took place either in the hospital or at their homes. All healthy control subjects were interviewed at their homes. The collected information included: (1) Socio-demographic characteristics, including age, gender, residence, place of birth, education, annual income, blood type, and disease diagnostic information; (2) residence and water drinking history, including "raw water" intake history; (3) detailed dietary history using a pretested Chinese food frequency questionnaire, including 30 dietary indicators and 86 food items; (4) detailed tobacco smoking history; (5) alcohol drinking habits; (6) tea drinking habits; (7) detailed information on disease history; (8) occupation history and related exposures; (9) family history of liver cancer and other cancers; and (10) physical activities. For female cases and controls, reproductive factors were also collected.

Quality Control

Five percent of the participants were re-interviewed to ensure the quality of data. The epidemiological data collected were first reviewed by a research staff at the county level and then by an epidemiologist at Provincial CDC. Data were doubly entered into an epidemiology database designed using EpiData (Odense, Denmark) at each county CDC and then cleaned and managed at Jiangsu Provincial CDC.

2.6 Blood Sample collection and Laboratory assays

Blood Samples Collection

Five 5-8 ml peripheral blood sample into EDTA or heparin coated tube were collected for each consented participant after their interviews. Blood specimens were assigned an identification number, separated into serum, red blood cells, white blood cells, and then stored under -20℃ at local CDCs. Samples from all study sites were then sent to the Jiangsu CDC. The Jiangsu CDC was responsible for the storage of all samples under -70℃ for future examination. DNA samples were extracted in the molecular epidemiology lab for chronic disease in the Department of NCD of Jiangsu CDC.

HBV and HCV serum antigens and antibodies

HBV and HCV markers were measured at Jiangsu CDC. The presence of HBsAg, HBsAb, HBeAg, HBeAb, HBcAb and anti-HCV IgG antibody in serum were measured by the enzyme-linked immunosorbant assay (ELISA) using kits from Shanghai Kehua Biological Pharmacy (Shanghai, China), according to the manufacturer's instructions.

Measurement of HBsAg

In brief, 75 μL serum samples, one positive control and three negative controls provided by the manufacturer were pipetted into pre-coated micro-well plates and incubated at 37℃ for 30 minutes. After adding the conjugate, the plates were incubated at 37℃ for another 30 minutes, and then washed five times followed by dispensing the substrate solution. Finally, stop solution was added, and the plates were read under 450/630 nm dual wavelengths. Samples were

considered positive when OD ≥ (0.1+ ODNC). ODNC represents the average OD value of negative controls.

Measurement of HBsAb

In brief, 50 μL serum samples, two positive controls and two negative controls provided by the manufacturer were pipetted into pre-coated micro-well plates. After adding the 50 μL conjugate, the plates were incubated at 37℃ for 30 minutes, and then washed five times followed by dispensing the 100 μL substrate solution (50 μL substrate solution A and B each). They were incubated at 37℃ for another 15 minutes. Finally, 50 μL stop solution was added, and the plates were read under 450/630 nm dual wavelengths. Samples were considered positive when OD ≥ (2.1*ODNC). ODNC represents the average OD value of negative controls if ODNC was greater than 0.05 and less than or equal to 0.1, or use 0.05 if ODNC was less than 0.05.

Measurement of HBeAg

In brief, 50 μL serum samples, two positive controls and two negative controls provided by the manufacturer were pipetted into pre-coated micro-well plates. After adding the 50 μL conjugate, the plates were incubated at 37℃ for 30 minutes, and then washed five times followed by dispensing the 100 μL substrate solution (50 μL substrate solution A and B each). They were incubated at 37℃ for another 15 minutes. Finally, 50 μL stop solution was added, and the plates were read under 450/630 nm dual wavelengths. Samples were considered positive when OD ≥ (2.1*ODNC). ODNC represents the average OD value of negative controls if ODNC was greater than 0.05 and less than or equal to 0.1, or use 0.05 if ODNC was less than 0.05.

Measurement of HBeAb

In brief, 50 μL serum samples, two positive controls and two negative controls provided by the manufacturer were pipetted into precoated microwell plates. After adding the 50 μL conjugate, the plates were incubated at 37℃ for 30 minutes, and then washed five times followed by dispensing the 100 μL substrate solution (50 μL substrate solution A and B each). They were incubated at 37℃ for another 15 minutes. Finally, 50 μL stop solution was added, and the plates were read under 450/630 nm dual wavelengths. Samples were considered positive when OD < 0.5*(ODPC+ODNC). ODPC represents the average OD value of positive controls and ODNC represents the average OD value of negative controls.

Measurement of HBcAb

There are two methods for measuring HBcAb. One is use the serum sample directly without dilution, presenting results of epidemiologic meaning, the other is using the 30 times diluted sample, presenting results of clinical meaning. We chose the direct one. In brief, 50 μL serum samples, two positive controls and two negative controls provided by the manufacturer were pipetted into pre-coated micro-well plates. After adding the 50 μL conjugate, the plates were incubated at 37℃ for 30 minutes, and then washed five times followed by dispensing the 100 μL substrate solution (50 μL substrate solution A and B each). They were incubated at 37℃ for another 15 minutes. Finally, 50 μL stop solution was added, and the plates were read under 450/630 nm dual wavelengths. Samples were considered positive when OD < 0.3*ODNC. ODNC represents the average OD value of negative controls.

Measurement of Anti-HCV IgG antibody

In brief, serum samples, two positive controls and two negative controls provided by the manufacturer were diluted 1 to 10 with sample dilution and pipetted into microwell plates coated with HCV antigen. The plates were incubated at 37℃ for 60 minutes, followed by five washings. After adding the conjugate, the plates were incubated for an additional 30 minutes. Then, the plates were washed three times, followed by dispensing the substrate solution. Finally, stop solution was added and the plates were read under 450 nm single wavelength. Samples were considered positive when OD ≥ (0.1*ODPC+ODNC). ODPC represents the average OD value of positive controls and ODNC represents the average OD value of negative controls.

Serum HBV viral load

Serum HBV DNA was extracted and quantified by a real-time polymerase chain reaction (PCR) technique using commercially available kits for the quantification (Quantitative Diagnostic Kit for Hepatitis B Virus DNA, PCR-Fluorescent Probing, Qiagen Biotech Ltd., Shenzhen, China). The limit of detection was 500 IU ml-1 and the linear range of quantification was from 1.0×10^3 to 5.0×10^7 IU ml-1. In brief, 100 μL serum sample, one strong positive control, one critical positive control and one negative control sample provided by the company was added into a 0.5 ml centrifuge tube. After adding 100 μL DNA extraction solution 1, vibrate and mix the liquid. Then centrifuge for 10 minutes at 13,000 rpm. Dispose the upper level clear liquid. Add 25 μL DNA extraction solution 2, vibrate and mix until sediment get completely solved. Sit the tube in spoiling water for 10 minutes and then centrifuge for 10 minutes at 13,000 rpm. Keep the upper level clear liquid. The PCR reaction system contains 37.6 μL HBV PCR reaction solution, 0.4 μL Taq enzyme, 0.06 μL UNG and 2 μL products from the DNA extraction progress and standard

positive controls No.1 to No.4. ABI 7500 system for RT-PCR was used. The PCR program was as following: 37℃ for 5 mins, 94℃ for 1 min. Circles: 95℃ for 5 seconds, 60℃ for 30 seconds. 40 circles in all. The collection for the fluorescent signals was set at 60℃. The concentrations of the standard positive controls were (1-5)x10^7 IU/ml for No.1, (1-5)x10^6 IU/ml for No.2, (1-5)x10^5 IU/ml for No.3 and (1-5)x10^4 IU/ml for No.4. The standard curve was built using the concentrations of the four standard samples and their measured C_T value. The degree of fitting of the curve should be less than -0.980, otherwise the result was invalid. The concentration read by the standard curve for strong positive control should be located between 1x10^5-1x10^7 IU/ml, for critical positive control should be located between 1x10^3-9x10^4 IU/ml, for negative control should be 0 IU/ml, otherwise the result was invalid. The concentrations of HBV viral load of the serum samples were then read from the valid standard curve by their C_T values.

SNP Selection and Genotyping Assays

We selected 28 SNPs in microRNA pathway, 26 SNPs in stem cell pathway and 4 SNPs from GWAS into this analysis (Table 2.1). The minor allele frequencies of all these SNPs were at least 5% in Han Chinese population. Genotyping were performed at UCLA Genotype and Sequencing Core, with a customized Fluidigm Dynamic 96.96 Array™ Assay (Fluidigm, South San Francisco, CA). The assays were based on allele-specific PCR SNP detection chemistry with the reliability of Dynamic Array™ integrated fluidic circuits (IFCs). The SNPtype Assay employed tagged, allele specific PCR primers and a common reverse primer. A universal probe set was used in every reaction, producing uniform fluorescence and Fluidigm provided locus-specific primer sequences that allowed one to confirm target locations. After genotyping, SNPs did not follow Hardy-Weinberg Equilibrium (HWE) would be excluded from the analysis. And

we used Bonferroni-corrected p value, which was 0.05/96 based on our tests with 96 SNPs together as the cut-off of HWE.

Table 2.1. Gene, chromosome location and minor allele frequency in Chinese population of the 58 SNPs

Gene	Chromosome location	SNP	MAF in Chinese	Gene	Chromosome location	SNP	MAF in Chinese
MicroRNA pathway				**Stem cell pathway**			
CXCL12	10q11.1	rs1804429	0.09	Rex1	4q35.2	rs6815391	0.37
IL15	4q31	rs10519613	0.49	Oct4	6p21.31	rs13409	0.35
WWOX	16q23.3-q24.1	rs12828	0.37	Oct4	6p21.31	rs3130932	0.43
TP53INP1	8q22	rs896849	0.13	Ctbp2	10q26.13	rs3740535	0.20
TAB3	Xp21.2	rs3816757	0.19	GLI1	12q13.2	rs2228224	0.27
miR-196a2	12q13.13	rs11614913	0.48	EpCAM	2p21	rs1126497	0.17
pre-miR-146a	5q34	rs2910164	0.44	AXIN2	17q23-q24	rs2240308	0.38
miR-27	19p13.13	rs895819	0.31	WNT2	7q31.2	rs3729629	0.20
miR-26a1	3p21.3	rs7372209	0.32	WNT2	7q31.2	rs4730775	0.16
Dicer1	14q32.13	rs3742330	0.27	WNT8A	5q31	rs4835761	0.47
Ago2	8q24	rs4961280	0.11	FZD1	7q21	rs3750145	0.27
Ran	12q24.3	rs14035	0.19	FZD3	8p21	rs2241802	0.37
Gemin3	1p21.1-p13.2	rs197412	0.33	DVL2	17p13.1	rs222851	0.35
Gemin4	17p13	rs2740348	0.12	AXIN1	16p13.3	rs1981492	0.26
Gemin4	17p13	rs7813	0.29	TCF7L1	2p11.2	rs6754757	0.28
XPO5	6p21.2	rs11077	0.09	Notch4	6p21.3	rs915894	0.48
KRAS	12p12.1	rs9266	0.27	HEY1	8q21	rs1046472	0.22
IL6R	1q21	rs4072391	0.10	HEY2	6q21	rs3734637	0.16
RCHY1	4q21.1	rs2126852	0.24	HES2	1p36.31	rs11364	0.18
TP53INP1	8q22	rs7760	0.13	Notch4	6p21.3	rs520692	0.17
CDK6	7q21-q22	rs42031	0.05	JAG2	14q32	rs9972231	0.15
E2F2	1p36	rs2075993	0.38	JAG1	20p12.1-p11.23	rs8708	0.12
DOCK4	7q31.1	rs3801790	0.29	DLL1	6q27	rs1033583	0.31
Rbl2	16q12.2	rs3929	0.20	Dec1	9q32	rs2269700	0.23
THBS1	15q15	rs2292305	0.34	Notch1	19p13.2	rs3815188	0.39
Wnt2B	1p13	rs2273368	0.49	EpCAM	2p21	rs1421	0.15
CTNNB1	3p21	rs2953	0.24	**GWAS**			
PPARGC1A	4p15.1	rs3774923	0.17	CCR4-GLB1	3p22.3	rs4678680	0.06
				ZBTB12-C2	6p21.33	rs9267673	0.13
				HLA-DQB1-HLA-DQA2	6p21.32	rs9275572	0.29
				HLA-S-MICA	6p21.33	rs2596542	0.27

2.7 Statistical analysis

The major outcome in this analysis was the diagnosis of liver cancer. The second outcome was having higher serum HBV viral load defined as greater than 10^5 IU/mL. The distributions of outcomes within categorical variables were compared by Chi-square tests or Fisher's exact tests. The measurements of relative risks including crude odds ratios (OR) and adjusted odd ratios (aOR) with their 95% confidence intervals were calculated by unconditional logistic regression models to compare the risks of liver cancer among different levels of covariates.

The associations of liver cancer and 58 SNPs were explored in a subgroup of the study population. For each SNP, maximum likelihood estimates of allele frequencies were tested for departures from Hardy-Weinberg Equilibrium (HWE) using the chi-square goodness of fit tests among the controls. Under the assumption of a rare disease in a case-control study design, controls represent the general population. SNPs that are not in HWE among controls were excluded from subsequent analyses. Each SNP was analyzed individually for association with liver cancer using a co-dominant model first. Unconditional logistic regression analysis was used to test for the crude associations between liver cancer risk and genotype using additive, dominant, recessive and co-dominant genetic models. Co-dominant genetic models adjusting for age, gender, county and other potential confounding variables were used to determine the adjusted association.

HBV, HCV infection, SNPs and environmental exposures and their product terms were included into the final logistic regression model along with other covariates to further explore the potential

interaction on multiplicative scale on liver cancer, reporting the ratio of the odds ratio (ROR). Statistical interactions on additive scale were also explored, reporting the relative excess risk due to interaction (RERI), the attributable proportion (AP) and the synergy index(S).

Population attributable fraction (PAF) was calculated based on the adjusted relative risk (approximated by odds ratio) and the population prevalence of exposure (approximated by the prevalence of exposure among the controls). PAF was calculated by the formula: PAF=P*(RR-1)/[P*(RR-1)+1] [93].

Multiple Imputation for Missing data

Multiple imputation was used to impute missing values of covariates in the analysis of association between SNPs and liver cancer. We assume that missing depends on the observed data only. 5 rounds of imputation were run to generate the values for the missing observations. And the results of the 5 rounds imputation were combined as the final value. All the variables included in the logistic regression models for complete case analysis were included in the imputation. After all, the logistical regression models were re-run using the imputed data sets and results were combined to generate the final adjusted odds ratios.

Bayesian approach in relative risk estimates

The frequentist techniques including the statistical models used in the analyses perform well in the context of randomized trials. However, our case-control study was one of the observational studies which are faced with the questions of objectivity and realism of assumptions required for the statistical models. In observational studies, the mechanisms generating the samples and the

exposure status were not random, and hard to investigate or estimate [94]. Thus, Bayesian method was used for adjusting the estimates from our data with prior information from previous study results to generate the posterior estimates of the measures of association. Also, to achieve a shrinkage of the estimate when multiple comparisons were made or no informative prior exists, the null prior was used to generate a conservative estimate. Information-weighted averaging and data augmentation were used in carrying out the adjustment [94-96]. Information used as the weights in averaging is the inverse of the variance and the posterior estimate is the weighted average of prior information and study data, which are both assumed to be normally distributed. Or the prior is represented by a prior data base which was incorporated to the original study data base in generating posterior estimates as the method of data augmentation [95].

The prior information used for calculating the posterior estimate of association between the major environmental risk factors and liver cancer was from a meta-analysis published in 2005 based on a thorough search of literature both in Chinese and in English focusing on studies of liver cancer risk factors in Chinese population from 1966 to 2003 (Table 2.2)[36]. They included 55 studies in total, 26 studies for HBV infection, 15 for HCV infection, 25 for family history of liver cancer, 22 for alcohol drinking, 15 for tobacco smoking, 3 for intake of musty food and 15 for drinking water from pond, river or well. A null prior with variance of 0.5 (OR=1, 95% CI: 0.25-4) was used in shrinkage of main effects of those risk factors without informative priors and SNPs from above mentioned pathways [95]. Also, the same null prior was used to adjust all the product terms examining statistical interactions in regression models.

Statistical analyses were performed using SAS 9.3 statistical software (SAS Institute Inc.).

Table 2.2 Summary of the association between factors and PLC

Factors	Total case/control	Combined OR	95%CI	χ^2 (g = 1)	P
Liver diseases					
HBV infection	3 390/4 604	11.34	8.72-14.75	327.60	0.000[b]
HCV infection	1 737/2 534	4.28	3.30-5.56	119.93	0.000[b]
Liver cirrhosis	1 689/2 609	11.97	6.19-23.19	54.46	0.000[b]
History of hepatitis	3 625/4 903	5.71	4.11-7.92	108.12	0.000[b]
Family history					
Family history of PLC	3 681/4 932	3.49	2.68-4.53	87.24	0.000[b]
Psychological factors					
Negative living events	1 688/2 096	2.65	1.69-4.15	17.90	0.000[b]
Unstable emotion	1 502/2 086	2.20	1.74-2.77	44.48	0.000[b]
Depressed character	1 355/1 777	3.07	2.10-4.47	33.99	0.000[b]
Past history of exposure to poison					
Pesticide	755/969	1.55	0.82-2.93	1.84	0.175
Aflatoxin	327/327	1.80	1.44-2.25	26.90	0.000[b]
Living style					
Alcoholic	3 207/3 983	1.88	1.53-2.32	35.60	0.000[b]
Smoking	2 408/3 347	1.24	1.09-1.41	10.90	0.001[b]
Intake of musty food	623/723	1.87	1.42-2.47	19.74	0.000[b]
Intake of pickle	1 233/1 602	1.69	1.34-2.13	47.56	0.000[b]
Intake of bean products	814/1 158	0.74	0.29-1.90	0.13	0.718
Tea	656/870	0.69	0.31-1.51	0.88	0.348
Drinking water from pond	1 561/1 614	1.77	1.09-2.87	8.65	0.003[b]
Drinking water from river	379/437	1.41	0.38-5.19	0.27	0.603
Drinking water from well	636/856	0.79	0.45-1.36	7.99	0.392

The values of χ^2 shown are statistical values of significant test of combined OR. [b]P<0.01 *vs* significant difference.

CHAPTER 3: RESULTS

3.1 Specific Aim 1: Environmental risk factors for liver cancer

Socio-Demographic characteristics of the total study population

A total of 2,011 cases and 7,933 controls were included in the study. Among them, 73% were male and the mean age was 63 years old. Table 3.1 shows and compares the distribution of demographic and social-economic characteristics among cases and controls of the total study population. In the original four parallel studies, the cases and controls were individually matched by age and gender. After the combination of the four groups of controls, the cases tended to be younger and to have larger proportion of male ($p<0.001$) than the controls. The two groups were also different in study area (residency), marriage status, education levels and BMI ($p<0.001$). There was no significant difference in family income 10 years ago per capita. A total of 7,745 (78%) participants, 1,216 (61%) cases and 6,529 (82%) controls had blood samples stored. And there is a significant difference in the collection rates of blood samples among the study sites: 90% participants from Tongshan, 79% from Dafeng, 68% from Ganyu and 67% from Chuzhou have blood drawn. The proportion of having serum also differed by gender (male 77% and female 80%), age, family income per capita 10 years ago, marriage status, education level and BMI.

Table 3.1 Socio-demographic characteristics of subjects in Jiangsu liver cancer study 2003-2010 (N=9944), 2014

Characteristics	Total	%	Case	%	Control	%	p-value
Total	9944		2011		7933		
Study area							
Dafeng	3166	31.8	632	31.4	2534	31.9	<0.001
Ganyu	2400	24.1	390	19.4	2010	25.3	
Chuzhou	1435	14.4	301	15.0	1134	14.3	
Tongshan	2943	29.6	688	34.2	2255	28.4	
Gender							
Male	7239	72.8	1534	76.3	5705	71.9	<0.001
Female	2705	27.2	477	23.7	2228	28.1	
Age group							
<50	1343	13.5	471	23.4	872	11.0	<0.001
50-59	2376	23.9	603	30.0	1773	22.4	
60-69	3057	30.7	515	25.6	2542	32.0	
70-79	2490	25.0	322	16.0	2168	27.3	
80-	678	6.8	100	5.0	578	7.3	
In marriage							
Yes	8143	82.4	1717	86.0	6426	81.5	<0.001
No	1742	17.6	279	14.0	1463	18.5	
missing	59		15		44		
Education level							
Illiteracy	4560	46.0	764	38.2	3796	48.0	<0.001
Primary	3152	31.8	662	33.1	2490	31.5	
Middle	1759	17.7	461	23.1	1298	16.4	
High	393	4.0	101	5.1	292	3.7	
College	52	0.5	12	0.6	40	0.5	
missing	28		11		17		
Income 10 years ago per capita (RMB yuan/year)							
<1000	2048	21.1	392	20.0	1656	21.3	0.119
1000-	1917	19.7	405	20.6	1512	19.5	
1500-	2544	26.1	487	24.8	2057	26.5	
2500-	3222	33.1	680	34.6	2542	32.7	
missing	213		47		166		
BMI							
<18.5	663	6.7	211	10.7	452	5.7	<0.001
18.5-	6106	61.9	1315	66.4	4791	60.7	
24-	2577	26.1	379	19.1	2198	27.9	
28-	524	5.3	75	3.8	449	5.7	
missing	74		31		43		

An overview of risk factors for liver cancer

The associations between major risk factors including HBV infection, HCV infection, alcohol consumption, tobacco smoking, history of raw water drinking, history of possible consumption of mildew contaminated food and family history of liver cancer, etc. and liver cancer were examined in the study population and were shown in Table 3.2. HBsAg positive was confirmed to be a strong risk factor for liver cancer with an aOR of 9.85 (95% CI: 8.28-11.72). Anti-HCV positive showed an aOR of 1.40 with a wide confidence interval (95% CI: 0.62-3.14) because only 64 participants were tested positive in this study population. Alcohol consumption (aOR: 1.91, 95% CI: 1.61-2.28) and tobacco smoking (aOR: 1.48, 95% CI: 1.25-1.75) as two known risk factors were both positively associated with liver cancer. Family history of liver cancer was another strong indicator with an aOR of 4.19 (95% CI: 3.17-5.53). History of raw water drinking showed an aOR of 1.32 (95% CI: 1.13-1.55) for liver cancer.

After using the prior information from the meta-analysis in Chinese population or a null prior if no informative prior exists, we calculated the posterior ORs of the major risk factors on liver cancer and the 95% posterior limits. The SB-adjusted OR for HBV infection was 10.28 with 95% Posterior Interval (PI): 8.89-11.88. HCV infection is not a major risk factor for liver cancer in this population showing an aOR of 1.40 (95% CI: 0.62-3.14). We got a SB-adjusted OR for HCV infection of 3.85 (95% PI: 3.01-4.94) mainly because of the prior from the meta-analysis was an aOR of 4.28 (95% CI: 3.30-5.56). For alcohol consumption (SB-adjusted OR=1.90, 95% PI: 1.66-2.17), tobacco smoking (SB-adjusted OR=1.32, 95% PI: 1.20-1.47) and history of raw water drinking (SB-adjusted OR=1.31, 95% PI: 1.13-1.51), the SB-adjusted ORs were quite close to the results from our data. For possibility of mildew contaminated food consumption, the

posterior aOR was 1.51 (95% PI: 1.24-1.83), which was increased and statistically significant. After adjusting, family history of liver cancer showed aOR of 3.80 (95% PI: 3.14-4.60), which was slightly decreased from the study result.

Table 3.2. The association between risk factors and liver cancer in Jiangsu Study 2003-2010 (N=9944), 2014

Variables	Case	%	Control	%	Crude OR (95% CI)	Adjusted OR (95% CI)[a]	Bayesian adjusted OR (95% PI)[a]
Total	2011		7933				
HBsAg positive							
No	689	56.8	6074	93.5	1.00	1.00	1.00
Yes	524	43.2	425	6.5	**10.87 (9.35-12.63)**	**9.85 (8.28-11.72)**	**10.28 (8.89-11.88)**
missing	798		1434				
HCV-Ab positive							
No	1204	99.1	6446	99.2	1.00	1.00	1.00
Yes	11	0.9	53	0.8	1.11 (0.58-2.13)	1.40 (0.62-3.14)	**3.85 (3.01-4.94)**
missing	796		1434				
Ever drink							
No	885	44.0	4254	53.6	1.00	1.00	1.00
Yes	1126	56.0	3679	46.4	**1.47 (1.33-1.62)**	**1.91 (1.61-2.28)**	**1.90 (1.66-2.17)**
Ever smoke							
No	971	48.3	4241	53.5	1.00	1.00	1.00
Yes	1040	51.7	3692	46.5	**1.23 (1.12-1.36)**	**1.48 (1.25-1.75)**	**1.32 (1.20-1.47)**
Migrated from Qidong/Haimen							
No	1866	97.4	7625	97.8	1.00	1.00	1.00
Yes	50	2.6	173	2.2	1.18 (0.86-1.62)	1.05 (0.65-1.67)	1.04 (0.67-1.63)
Missing	95		135				
Ever drink raw water							
No	712	36.5	3528	45.6	1.00	1.00	1.00
Yes	1238	63.5	4216	54.4	**1.46 (1.31-1.61)**	**1.32 (1.13-1.55)**	**1.31 (1.13-1.51)**
missing	61		189				
Possibility of mildew contaminated food consumption							
No	1758	88.9	7206	91.8	1.00	1.00	1.00
Yes	219	11.1	646	8.2	**1.39 (1.18-1.63)**	1.23 (0.94-1.61)	**1.51 (1.24-1.83)**
missing	34		81				
Family history of liver cancer							
No	1735	86.3	7684	96.9	1.00	1.00	1.00
Yes	276	13.7	249	3.1	**4.91 (4.10-5.87)**	**4.19 (3.17-5.53)**	**3.80 (3.14-4.60)**

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0, except for variable of family history of liver cancer), pack-year of smoking (continuous, except for variable of tobacco smoking), ethanol consumption (gram/week, continuous, except for variable of alcohol drinking status), HBsAg status (1=positive, 0=negative, except for variable of HBsAg status) and study area (Dafeng=1, Ganyu=2, Chuzhou=3, Tongshan=4).

HBV infection and liver cancer

HBsAg positive was confirmed to be a strong risk factor for liver cancer with an aOR of 9.85 (95% CI: 8.28-11.72). Besides HBsAg, other four serum markers of HBV infection and their associations with risk of liver cancer of and the combined patterns were also examined and results were shown in Table 3.3. The proportions of cases tested positive for the five markers were: 43.2% for HBsAg, 36.2% for HBsAb, 14.4% for HBeAg, 28.7% for HBeAb and 91.3% for HBcAb. The proportions of controls tested positive were 6.5% for HBsAg, 55.6% for HBsAb, 1.7% for HBeAg, 14.8% for HBeAb and 93.0% for HBcAb. The distributions of subjects in different patterns of HBV infection determined by these five markers were also shown. There are 9 common patterns and 16 relatively rare patterns. The crude and adjusted odds ratios of liver cancer comparing the 9 common patterns were listed. Compared to those tested all negatives for the five markers, people who were positive for HBsAg and HBcAb showed the highest risk (aOR: 12.31, 95% CI: 8.05-18.80), followed by those positive for HBsAg, HBeAg and HBcAb (aOR: 7.40, 95% CI: 4.79-11.41) and those positive for HBsAg, HBeAb and HBcAb (aOR: 3.36, 95% CI: 2.31-4.89). Meanwhile, compared to all negatives, people who were positive for HBcAb only (aOR: 0.41, 95% CI: 0.28-0.58), those positive for HBsAb, HBeAb and HBcAb (aOR: 0.59, 95% CI: 0.40-0.88) and those positive for HBsAb and HBcAb (aOR: 0.46, 95% CI: 0.33-0.65) showed decreased risks for liver cancer, after controlling for potential confounding variables.

Using the prevalence of HBsAg positive in the control group of 6.5% as the population prevalence of HBsAg positive, the population attributable risk due to HBsAg positive was 36.5%.

Table 3.3. The distribution of patterns of HBV markers and liver cancer in Jiangsu Study 2003-2010 (N=9944), 2014

	Status of HBV markers (0-negative, 1-positive)					n=7689	Case	Control	Crude OR	Adjusted OR
	HBsAg	HBsAb	HBeAg	HBeAb	HBcAb	(%)	(%)	(%)	(95% CI)	(95% CI)[a]
Pattern of HBV markers										
I	0	0	0	0	0	383 (5.0)	59 (4.9)	324 (5.0)	1.00	1.00
II	0	0	0	0	1	2189 (28.5)	172 (14.2)	2017 (31.1)	**0.47 (0.34-0.64)**	**0.41 (0.28-0.58)**
III	0	0	0	1	1	147 (1.9)	32 (2.6)	115 (1.8)	1.53 (0.95-2.47)	1.33 (0.77-2.29)
IV	0	1	0	0	0	125 (1.6)	23 (1.9)	102 (1.6)	1.24 (0.73-2.11)	0.98 (0.54-1.76)
V	0	1	0	1	1	653 (8.5)	86 (7.1)	567 (8.8)	0.83 (0.58-1.19)	**0.59 (0.40-0.88)**
VI	1	0	0	0	1	175 (2.3)	121 (10.0)	54 (0.8)	**12.31 (8.05-18.80)**	**8.48 (5.23-13.75)**
VII	0	1	0	0	1	3215 (41.8)	312 (25.8)	2903 (44.8)	**0.59 (0.44-0.80)**	**0.46 (0.33-0.65)**
VIII	1	0	0	1	1	469 (6.1)	208 (17.2)	261 (4.0)	**4.38 (3.14-6.10)**	**3.36 (2.31-4.89)**
IX	1	0	1	0	1	232 (3.0)	151 (12.5)	81 (1.3)	**10.24 (6.95-15.08)**	**7.40 (4.79-11.41)**
Other						101 (1.3)				
X	1	0	0	0	0	10 (0.1)	7 (0.6)	3 (0.1)		
XI	1	0	0	1	0	12 (0.2)	9 (0.7)	3 (0.1)		
XII	1	0	1	0	0	3 (0.0)	3 (0.3)	0		
XIII	1	0	1	1	1	9 (0.1)	8 (0.7)	1 (0.0)		
XIV	1	1	0	0	0	2 (0.0)	2 (0.2)	0		
XV	1	1	0	0	1	13 (0.2)	1 (0.1)	12 (0.2)		
XVI	1	1	0	1	0	0	0	0		
XVII	1	1	0	1	1	9 (0.1)	3 (0.3)	6 (0.1)		
XVIII	1	1	1	0	1	12 (0.2)	10 (0.8)	2 (0.0)		
XIX	0	0	1	0	0	7 (0.1)	1 (0.1)	6 (0.1)		
XX	0	0	1	0	1	4 (0.1)	1 (0.1)	3 (0.1)		
XXI	0	0	1	1	1	0	0	0		
XXII	0	1	0	1	0	2 (0.0)	1 (0.1)	1 (0.0)		
XXIII	0	1	1	0	0	2 (0.0)	0	2 (0.0)		
XXIV	0	1	1	0	1	13 (0.2)	1 (0.1)	12 (0.2)		
XXV	0	0	0	1	0	3 (0.0)	0	3 (0.1)		

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0, except for variable of family history of liver cancer), pack-year of smoking (continuous, except for variable of tobacco smoking), ethanol consumption (gram/week, continuous, except for variable of alcohol drinking status) and study area (Dafeng=1, Ganyu=2, Chuzhou=3, Tongshan=4).

Alcohol consumption and liver cancer

Among all the participants, 885 (44%) cases and 4254 (53.6%) never drank during their lifetime till the study performed. Ever drinking was defined as having any history of alcohol drinking habit, except for only drink on holidays. Occasionally drinking was defined as drank less than twice a week, and frequently drinking was defined as drank more than twice a week and less than six times a week. Among those ever drank, drinking at each level of amount was consistently associated with elevated odds of liver cancer. Comparing to those who never drank, people who drank occasionally (aOR=1.73, 95% CI: 1.40-2.14), who drank frequently (aOR=2.40, 95% CI: 1.89-3.04) and who drank every day (aOR=1.76, 95% CI: 1.37-2.26) all showed increased risks. In Table 3.4, the association between alcohol consumption and the risk of liver cancer was further analyzed in detail. First, most drinkers stared drinking at the age younger than 35 years old. And starting drinking at an earlier age was associated with higher risk. For participants who started alcohol consumption when they were aged between 25 and 34 years old, the adjusted OR of getting liver cancer was 1.64 (95% CI: 1.29-2.08); for those who started earlier, under 25 years old, the aOR was 2.31 (95% CI: 1.89-2.82), both comparing to those who never drank. Second, the duration of drinking was also positively associated with the risk. When looking into the weekly ethanol intake back in 1980s, those drank less than 500g per week in 1980s showed an aOR of 1.48 (95% CI: 1.17-1.89) comparing to those never drank; while those drank more than 500g per week showed an further elevated aOR of 1.89 (95% CI: 1.50-2.38). Similar patterns could be seen at the drinking volume in 1990s: those drank less than 500g per week in 1990s showed an aOR of 1.72 (95% CI: 1.41-2.10) comparing to those never drank; while those drank more than 500g per week showed a higher aOR of 2.19 (95% CI: 1.77-2.70). However, the

weekly ethanol intake one year ago was not statistically significantly associated with risk of liver cancer, and the point estimates of aOR were also close to 1 for both volume groups.

Using the prevalence of ever drinking in the control group of 46.4% as the population prevalence of ever alcohol drinking, the population attributable risk due to ever drinking was 29.7%.

Table 3.4. The association between alcohol drinking and liver cancer in Jiangsu Study 2003-2010 (N=9944), 2014

Variables	Case n=2011	%	Control n=7933	%	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
Age at start drinking (years)						***P* for trend<0.001**
Never drink	885	44.0	4254	53.6	1.00	1.00
45-	39	1.9	329	4.2	0.57 (0.41-0.80)	1.29 (0.81-2.05)
35-	72	3.6	368	4.6	0.94 (0.72-1.22)	1.44 (0.95-2.19)
25-	380	18.9	1332	16.8	**1.37 (1.20-1.57)**	**1.64 (1.29-2.08)**
<25	635	31.6	1650	20.8	**1.85 (1.65-2.08)**	**2.31 (1.89-2.82)**
Years of drinking						***P* for trend<0.001**
Never drink	885	45.1	4254	54.3	1.00	1.00
1-	39	2.0	123	1.6	**1.52 (1.06-2.20)**	**1.81 (1.06-3.07)**
10-	142	7.2	353	4.5	**1.93 (1.57-2.38)**	**2.14 (1.53-2.98)**
20-	284	14.5	775	9.9	**1.76 (1.51-2.06)**	**1.71 (1.31-2.23)**
30-	612	31.2	2329	29.7	**1.26 (1.13-1.42)**	**1.97 (1.61-2.42)**
Weekly ethanol intake in 1980s (g)						***P* for trend<0.001**
0	1228	62.5	5392	69.6	1.00	1.00
1-	322	16.4	1136	14.7	**1.25 (1.08-1.43)**	**1.48 (1.17-1.86)**
500-	414	21.1	1218	15.7	**1.49 (1.31-1.70)**	**1.89 (1.50-2.38)**
Weekly ethanol intake in 1990s (g)						***P* for trend<0.001**
0	885	44.0	4254	53.6	1.00	1.00
1-	490	24.4	1877	23.7	**1.26 (1.11-1.42)**	**1.72 (1.41-2.10)**
500-	636	31.6	1802	22.7	**1.70 (1.51-1.91)**	**2.19 (1.77-2.70)**
Weekly ethanol intake one year ago (g)						***P* for trend** =0.303
0	1314	66.8	5153	65.8	1.00	1.00
1-	306	15.6	1408	18.0	**0.85 (0.74-0.98)**	1.01 (0.81-1.26)
500-	346	17.6	1271	16.2	1.07 (0.93-1.22)	1.15 (0.90-1.46)

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0, except for variable of family history of liver cancer), pack-year of smoking (continuous), HBsAg status (1=positive, 0=negative) and study area (Dafeng=1, Ganyu=2, Chuzhou=3, Tongshan=4).

Tobacco smoking and liver cancer

Tobacco smoking was also positively associated with liver cancer (Table 3.5). Among all the participants, 971 (51%) cases and 4241 (56.4%) controls never smoked during their lifetime till the study time. Ever smoking was defined as had any history of tobacco smoking habit, and had smoked more than 100 cigarettes life time. Ages at start smoking reported by smokers were divided into four groups and at least 30% started smoking at the age younger than 25 year old among both cases and controls. Except for those who started smoking after 45 years old (aOR=1.36, 95% CI: 0.66-2.77), those who started smoking before 45 years old all showed at least 50% increased odds of liver cancer. Most smokers smoked 10 to 30 cigarettes a day, and had smoked for more than 20 years. Daily amount of cigarettes smoking, duration of smoking and pack-years of smoking were all positively associated with liver cancer. Although the p for trend tests gave significant results (all <0.05), there was no clear dose-response pattern observed. 229 (12%) cases and 671 (8.9%) controls reported themselves as former smokers who quitted from smoking. And former smokers (aOR=1.94, 95% CI: 1.49-2.53) showed higher risk increase than current smokers (aOR=1.47, 95% CI: 1.22-1.77), both comparing to never smokers.

Using the prevalence of ever smoking in the control group of 46.5% as the population prevalence of ever tobacco smoking, the population attributable risk due to ever smoking was 18.2%.

Table 3.5. The association between tobacco smoking and liver cancer in Jiangsu Study 2003-2010 (N=9944), 2014

Variables	Case n=2011	%	Control n=7933	%	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
Smoking status						
Never smoking	971	51.0	4241	56.4	1.00	1.00
Former smoking	229	12.0	671	8.9	**1.49 (1.26-1.76)**	**1.94 (1.49-2.53)**
Current smoking	703	36.9	2609	34.7	**1.18 (1.06-1.31)**	**1.47 (1.22-1.77)**
Age at start smoking (yrs)						$\boldsymbol{P}_{\textbf{for trend}}$**<0.001**
Never smoke	971	48.3	4241	53.5	1.00	1.00
45-	14	0.7	118	1.5	**0.52 (0.30-0.91)**	1.36 (0.66-2.77)
35-	51	2.5	211	2.7	1.06 (0.77-1.45)	**1.67 (1.03-2.71)**
25-	262	13.0	995	12.5	1.15 (0.99-1.34)	**1.49 (1.16-1.91)**
<25	713	35.5	2368	29.9	1.32 (1.18-1.47)	**1.47 (1.22-1.77)**
Daily amount of smoking (cig/day)						$\boldsymbol{P}_{\textbf{for trend}}$**=0.002**
Never smoke	971	52.4	4241	56.9	1.00	1.00
<10	104	5.6	439	5.9	1.04 (0.83-1.30)	**1.80 (1.31-2.47)**
10-	291	15.7	983	13.2	**1.29 (1.12-1.50)**	**1.65 (1.30-2.09)**
20-	338	18.2	1311	17.6	1.13 (0.98-1.29)	**1.27 (1.01-1.61)**
30-	149	8.0	483	6.5	**1.35 (1.11-1.64)**	**1.52 (1.09-2.12)**
Duration of smoking (years)						$\boldsymbol{P}_{\textbf{for trend}}$**<0.001**
Never smoke	971	50.9	4241	55.8	1.00	1.00
<20	118	6.2	309	4.1	**1.67 (1.33-2.09)**	**1.55 (1.10-2.19)**
20-	239	12.5	520	6.8	**2.01 (1.70-2.38)**	**1.75 (1.34-2.30)**
30-	275	14.4	940	12.4	**1.28 (1.10-1.49)**	**1.34 (1.05-1.71)**
40-	306	16.0	1587	20.9	**0.84 (0.73-0.97)**	**1.45 (1.14-1.84)**
Pack-years of smoking						$\boldsymbol{P}_{\textbf{for trend}}$**=0.0057**
Never smoke	971	52.4	4241	56.9	1.00	1.00
<10	106	5.7	354	4.8	**1.31 (1.04-1.64)**	**1.71 (1.22-2.39)**
10-	179	9.7	483	6.5	**1.62 (1.35-1.95)**	**1.75 (1.32-2.32)**
20-	184	9.9	581	7.8	**1.38 (1.16-1.66)**	**1.59 (1.19-2.11)**
30-	135	7.3	571	7.7	1.03 (0.85-1.26)	1.12 (0.81-1.56)
40-	277	15.0	1218	16.4	0.99 (0.86-1.15)	**1.42 (1.10-1.83)**

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0, except for variable of family history of liver cancer), ethanol consumption (gram/week), HBsAg status (1=positive, 0=negative) and study area (Dafeng=1, Ganyu=2, Chuzhou=3, Tongshan=4).

Interactions between major risk factors

The potential effect measure modification among major risk factors on liver cancer were explored.

First, the statistical interaction between risk factors and HBsAg positive as the marker of HBV infection was examined (Table 3.6). Ever drinking alcohol, ever smoking tobacco and having family history of liver cancer all showed significant statistical interaction with HBsAg positive on both additive scale and multiplicative scale. More specifically, among those never drinkers, being positive for HBsAg was associated with about 7 times of risk increase in development of liver cancer (aOR=8.34, 95% CI: 6.52-10.67); while among HBsAg negatives, ever drinking was positively associated with liver cancer showing an aOR of 1.73 (95% CI: 1.42-2.11). However, when the effects of alcohol drinking and HBsAg positive were combined together, a very strong aOR of 20.89 (95% CI: 15.97-27.31) was observed in this study. The RERI was 11.82 (95% CI: 6.54-17.09), AP was 0.57 (95% CI: 0.44-0.69) and the S was 2.46 (95% CI: 1.79-3.39) when the interaction was examined on additive scale. On the multiplicative scale, the ratio of the odds ratios was 1.44 (95% CI: 1.02-2.04) and remained significant after Semi-Bayesian adjustment using a null prior (SB-adjusted ROR: 1.41, 95% PI: 1.01-1.97). Similarly, smoking and being HBsAg positive showed a strong joint effect too. The aOR comparing HBsAg positive ever smokers to HBsAg negative and never smokers reached 18.03 (95% CI: 13.99-23.24), which was at the same time super-additive and super-multiplicative, given the aOR of HBsAg positive alone as 7.42 (95% CI: 5.89-9.35) and smoking alone as 1.42 (95% CI: 1.18-1.71). And the significant ROR remained existing after SB-adjustment (SB-adjusted ROR: 1.66, 95% PI: 1.20-2.30).

Besides these two behavioral factors, having family history of liver cancer was another strong risk factor interacting with HBsAg positive. Comparing having the family history to having no history among HBsAg negatives, the aOR was 3.23 (95% CI: 2.29-4.56). However, people in the group of HBsAg positive and having family history of liver cancer showed an aOR of 73.76 (95% CI: 47.71-130.44). This joint effect was also both super-additive (RERI: 62.40, 95% CI: 20.55-104.24; AP: 0.85, 95% CI: 0.76-0.94; S: 7.02, 95% CI: 3.89-12.67) and super-multiplicative (ROR: 2.50, 95% CI: 1.28-4.88). The ROR remained significant after SB-adjustment too (SB-adjusted ROR: 2.10, 95% PI: 1.15-3.84).

Second, the interaction between alcohol consumption and other major factors on risk of liver cancer was examined on both additive scale and multiplicative scale (Table 3.7). Overall, besides the interaction between alcohol and HBV infection as above described, statistically significant interaction on additive scale was detected between alcohol consumption and having family history of liver cancer. Comparing to those never drinkers who did not have family history of liver cancer, those drinkers who didn't have family history of the cancer showed aOR of 1.87 (95% CI: 1.56-2.25), those never drinkers who had family history showed aOR of 3.62 (95% CI: 2.35-5.57), while those drinkers had family history showed aOR of 8.66 (95% CI: 5.96-12.59). The combined effects were super-additive with RERI of 4.16 (95% CI: 0.81-7.52), AP of 0.48 (95% CI: 0.24-0.73) and S of 2.19 (95% CI: 1.24-3.89), but not statistically super-multiplicative with a ROR of 1.28 (95% CI: 0.74-2.21). There were no significant interactions detected between alcohol consumptions and other risk factors such as smoking, food contamination by mildew or raw water drinking.

Third, the potential interactions between tobacco smoking and raw water drinking, having family history of liver cancer were explored (Table 3.8). Although these three factors all have statistically significant main effects on liver cancer, only a significant interaction on additive scale between smoking and having family history of liver cancer was detected, reporting an AP of 0.34 (95% CI: 0.02-0.66).

Table 3.6. Interaction between risk factors and HBsAg positive on liver cancer in Jiangsu Study 2003-2010 (N=9944), 2014

Variables	HBsAg positive	Case/control	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
Ever alcohol drinking				
No	No	314/3276	1.00	1.00
No	Yes	204/238	**8.94 (7.18-11.14)**	**8.34 (6.52-10.67)**
Yes	No	375/2798	**1.40 (1.19-1.64)**	**1.73 (1.42-2.11)**
Yes	Yes	320/187	**17.85 (14.41-22.12)**	**20.89 (15.97-27.31)**
Additive	RERI: **11.82 (6.54-17.09)**	AP: **0.57 (0.44-0.69)**	S: **2.46 (1.79-3.39)**	
Multiplicative	ROR: **1.44 (1.02-2.04)**	SB-adjusted ROR: **1.41 (1.01-1.97)**		
Ever smoking				
No	No	368/3248	1.00	1.00
No	Yes	229/247	**8.18 (6.64-10.09)**	**7.42 (5.89-9.35)**
Yes	No	321/2826	1.00 (0.86-1.17)	**1.42 (1.18-1.71)**
Yes	Yes	295/178	**14.63 (11.80-18.14)**	**18.03 (13.99-23.24)**
Additive	RERI: **10.19 (5.84-14.55)**	AP: **0.57 (0.44-0.69)**	S: **2.49 (1.82-3.40)**	
Multiplicative	ROR: **1.71 (1.22-2.38)**	SB-adjusted ROR: **1.66 (1.20-2.30)**		
Ever drink raw water				
No	No	254/2671	1.00	1.00
No	Yes	176/191	**9.69 (7.61-12.34)**	**9.02 (6.87-11.84)**
Yes	No	415/3254	**1.34 (1.14-1.58)**	**1.27 (1.06-1.53)**
Yes	Yes	335/224	**15.73 (12.72-19.45)**	**13.41 (10.48-17.15)**
Additive	RERI: 4.12 (0.70-7.53)	AP: 0.31 (0.10-0.52)	S: **1.50 (1.07-2.08)**	
Multiplicative	ROR: 1.17 (0.82-1.66)	SB-adjusted ROR: 1.16 (0.82-1.63)		
Family history of liver cancer				
No	No	637/5881	1.00	1.00
No	Yes	412/407	**9.35 (7.97-10.96)**	**9.13 (7.61-10.95)**
Yes	No	52/193	**2.49 (1.81-3.42)**	**3.23 (2.29-4.56)**
Yes	Yes	112/18	**57.44 (34.69-95.11)**	**73.76 (41.71-130.44)**
Additive	RERI: **62.40 (20.55-104.24)**	AP: **0.85 (0.76-0.94)**	S: **7.02 (3.89-12.67)**	
Multiplicative	ROR: **2.50 (1.28-4.88)**	SB-adjusted ROR: **2.10 (1.15-3.84)**		

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0, except for variable of family history of liver cancer), pack-year of smoking (continuous, except for variable of tobacco smoking), ethanol consumption (gram/week, continuous, except for variable of alcohol drinking status), and study area (Dafeng=1, Ganyu=2, Chuzhou=3, Tongshan=4).

Table 3.7. Interaction between major risk factors and alcohol drinking on liver cancer in Jiangsu Study 2003-2010 (N=9944), 2014

Variables	Ever drink	case/control	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
Ever smoke				
No	No	651/3128	1.00	1.00
No	Yes	320/1113	**1.38 (1.19-1.61)**	**1.85 (1.47-2.33)**
Yes	No	234/1126	1.00 (0.85-1.18)	**1.44 (1.11-1.87)**
Yes	Yes	806/2566	**1.51 (1.34-1.69)**	**2.41 (1.96-2.95)**
Additive	RERI: 0.12 (-0.43-0.66)	AP: 0.05 (-0.18-0.27)	S: 1.09 (0.72-1.66)	
Multiplicative	ROR: 0.90 (0.65-1.26)	SB-adjusted ROR: 0.91 (0.66-1.25)		
Ever drink raw water				
No	No	346/2018	1.00	1.00
No	Yes	366/1510	**1.41 (1.20-1.66)**	**1.96 (1.51-2.54)**
Yes	No	504/2142	**1.37 (1.18-1.59)**	**1.29 (1.03-1.61)**
Yes	Yes	734/2074	**2.06 (1.79-2.38)**	**2.53 (2.00-3.19)**
Additive	RERI: 0.28 (-0.26-0.82)	AP: 0.11 (-0.10-0.32)	S: 1.23 (0.81-1.86)	
Multiplicative	ROR: 1.00 (0.73-1.37)	SB-adjusted ROR: 1.00 (0.74-1.36)		
Family history of liver cancer				
No	No	774/4141	1.00	1.00
No	Yes	961/3543	**1.45 (1.31-1.61)**	**1.87 (1.56-2.25)**
Yes	No	111/113	**5.26 (4.00-6.91)**	**3.62 (2.35-5.57)**
Yes	Yes	165/136	**6.49 (5.11-8.25)**	**8.66 (5.96-12.59)**
Additive	RERI: **4.16 (0.81-7.52)**	AP: **0.48 (0.24-0.73)**	**S: 2.19 (1.24-3.89)**	
Multiplicative	ROR: 1.28 (0.74-2.21)	SB-adjusted ROR: 1.24 (0.74-2.06)		

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0, except for variable of family history of liver cancer), pack-year of smoking (continuous, except for variable of tobacco smoking), HBsAg status (1=positive, 0=negative) and study area (Dafeng=1, Ganyu=2, Chuzhou=3, Tongshan=4).

Table 3.8. Interaction between major risk factors and tobacco smoking on liver cancer in Jiangsu Study 2003-2010 (N=9944), 2014

Variables	Ever smoke	case/control	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
Ever drink raw water				
No	No	370/1990	1.00	1.00
No	Yes	342/1538	**1.20 (1.02-1.41)**	**1.46 (1.13-1.88)**
Yes	No	570/2149	**1.43 (1.23-1.65)**	**1.37 (1.11-1.68)**
Yes	Yes	668/2067	**1.74 (1.51-2.00)**	**2.00 (1.59-2.51)**
Additive	RERI: 0.18 (-0.25-0.61)	AP: 0.09 (-0.12-0.30)	S:1.22 (0.73-2.05)	
Multiplicative	ROR: 1.01 (0.74-1.36)	SB-adjusted ROR: 1.01 (0.75-1.36)		
Family history of liver cancer				
No	No	849/4121	1.00	1.00
No	Yes	886/3563	**1.21 (1.09-1.34)**	**1.46 (1.23-1.74)**
Yes	No	122/120	**4.94 (3.80-6.42)**	**3.65 (2.42-5.52)**
Yes	Yes	154/129	**5.79 (4.53-7.41)**	**6.24 (4.31-9.04)**
Additive	RERI: 2.13 (-0.44-4.70)	AP: **0.34 (0.02-0.66)**	S: 1.68 (0.91-3.11)	
Multiplicative	ROR: 1.17 (0.68-2.00)	SB-adjusted ROR: 1.15 (0.69-1.89)		

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0, except for variable of family history of liver cancer), ethanol consumption (gram/week), HBsAg status (1=positive, 0=negative) and study area (Dafeng=1, Ganyu=2, Chuzhou=3, Tongshan=4).

3.2 Specific Aim 2: Polymorphisms and the risk of liver cancer

DNA samples were collected and genotyping was performed successfully from 2,612 participants' (419 cases and 2,193 controls) blood samples from Dafeng and Ganyu counties.

Hardy-Weinberg Equilibrium (HWE) Test

By using the customized Fluidigm Dynamic 96.96 Array Assay, there were totally 96 SNPs tested together for the DNA samples. Thus, after Bonferroni-correction, the cut-off value used in HWE was 0.05/96=0.000052 in this analysis. After testing HWE for each SNP among the controls which represent the source population, 10 SNPs (4 from microRNA related genes, 5 from stem cell pathway genes and 1 from GWAS) were excluded from the further analysis with HWE p-values less than the cut-off value.

Socio-demographic characteristics, and major risk factors for liver cancer in this Dafeng, Ganyu study population for polymorphisms

Table 3.9 showed the socio-demographic characteristics of participants from Dafeng and Ganyu with SNP data. Most of the participants were from Dafeng (86.9%) and were male (72.4%). liver cancer cases and controls differed in all these examined factors. Larger proportion of controls were from Dafeng (88.5% vs. 78.3%, $p<0.001$) and were female (28.6% vs. 22.4%, $p=0.01$). Cases tended to be younger than controls ($p<0.001$). More cases were in marriage (85.2% vs. 80.4%, $p=0.022$). The cases and controls also differed in education level, family income 10 years ago and BMI.

Table 3.10 showed the association between major risk factors and liver cancer in this population. The association between HBsAg positive and liver cancer was even stronger, with an aOR of 20.23 (95% CI: 14.92-27.44). Drinking (aOR=1.23, 95% CI: 0.89-1.69) and smoking (aOR=1.30, 95% CI: 0.95-1.79) showed positive but not significant association probably because of the insufficient sample size. Family history was still a strong risk factor in this population with an aOR of 4.42 (95% CI: 2.97-6.58).

Table 3.9 Socio-demographic characteristics of subjects in Dafeng and Ganyu from the Jiangsu liver cancer study 2003-2010 (n=2612), 2014

Characteristics	Total	%	Case	%	Control	%	p-value
Total	2612		419		2193		
Study area							
Dafeng	2269	86.9	328	78.3	1941	88.5	<0.001
Ganyu	343	13.1	91	21.7	252	11.5	
Gender							
Male	1892	72.4	325	77.6	1567	71.5	0.010
Female	720	27.6	94	22.4	626	28.6	
Age group							
<50	364	13.9	117	27.9	247	11.3	<0.001
50-59	579	22.2	114	27.2	465	21.2	
60-69	862	33.0	99	23.6	763	34.8	
70-79	668	25.6	75	17.9	593	27.0	
80-	139	5.3	14	3.3	125	5.7	
In marriage							
Yes	2119	81.2	356	85.2	1763	80.4	0.022
No	492	18.8	62	14.8	430	19.6	
Education level							
Illiteracy	1115	42.7	154	36.8	961	43.8	<0.001
Primary	926	35.5	140	33.4	786	35.8	
Middle school	427	16.4	93	22.2	334	15.2	
Highs school and above	144	5.5	32	7.6	112	5.1	
Income 10 years ago per capita (RMB yuan/year)							
<1000	363	14.0	57	13.7	306	14.1	0.018
1000-	509	19.6	105	25.2	404	18.6	
1500-	768	29.6	115	27.6	653	30.0	
2500-	952	36.7	139	33.4	813	37.4	
BMI							
<18.5	237	9.1	59	14.2	178	8.1	<0.001
18.5-	1622	62.2	264	63.3	1358	62.0	
24-	599	23.0	75	18.0	524	23.9	
28-	148	5.7	19	4.6	129	5.9	

Table 3.10. The association between risk factors and liver cancer among subjects in Dafeng and Ganyu from the Jiangsu Study 2003-2010 (n=2612), 2014

Variables	Case n=419	%	Control n=2193	%	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
HBsAg positive						
No	145	38.4	1952	93.6	1.00	1.00
Yes	233	61.6	134	6.4	**23.41 (17.85-30.70)**	**20.23 (14.92-27.44)**
Ever drink						
No	156	37.2	939	42.8	1.00	1.00
Yes	263	62.8	1254	57.2	**1.26 (1.02-1.57)**	1.23 (0.89-1.69)
Ever smoke						
No	145	34.6	876	40.0	1.00	1.00
Yes	274	65.4	1317	60.0	**1.26 (1.01-1.56)**	1.30 (0.95-1.79)
Ever drink raw water						
No	135	33.1	809	38.1	1.00	1.00
Yes	273	66.9	1313	61.9	**1.25 (1.00-1.56)**	0.96 (0.71-1.30)
Mildew contaminated food consumption						
No	391	94.2	2086	95.9	1.00	1.00
Yes	24	5.8	90	4.1	1.42 (0.90-2.26)	1.53 (0.83-2.83)
Family history of liver cancer						
No	321	76.6	2060	93.9	1.00	1.00
Yes	98	23.4	133	6.1	**4.73 (3.55-6.30)**	**4.42 (2.97-6.58)**

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0, except for variable of family history of liver cancer), pack-year of smoking (continuous, except for variable of tobacco smoking), ethanol consumption (gram/week, continuous, except for variable of alcohol drinking status), HBsAg status (1=positive, 0=negative, except for variable of HBsAg status) and study area (Dafeng=1, Ganyu=2).

Polymorphism and liver cancer

Polymorphism in microRNA related genes and liver cancer

Table 3.11 showed the crude associations between SNPs from microRNA related genes and liver cancer evaluated by additive, dominant, recessive and co-dominant genetic models and the adjusted associations using co-dominant models. Rs896849 (TP53INP1 gene) T/T, T/C and C/C showed inverse association in additive model with OR of 0.77 (95% CI: 0.61-0.98) and C/C showed OR of 0.36 (95% CI: 0.13-1.00) in the co-dominant model comparing to T/T type. Rs11614913 (miR-196a2 gene) T/T, T/C and C/C also showed inverse association with liver cancer in all the four models with OR of 0.75 (95% CI: 0.64-0.89) in additive model, 0.72 (95% CI: 0.56-0.92) in dominant model and 0.63 (95% CI: 0.46-0.87) in recessive model. Comparing to T/T type, C/C showed an inverse association with an OR of 0.55 (95% CI: 0.39-0.79) on liver cancer.

After controlling for age, gender, study site, education level, marriage status, family income per capita 10 years ago, BMI, HBsAg status, family history of liver cancer, pack-year of smoking, and weekly ethanol intake in 1990s, rs896849 (C/C vs. T/T) still showed an inverse association with aOR of 0.27 (95% CI: 0.08-0.87). Also, rs11614913 (C/C vs. T/T) still showed an inverse association with aOR of 0.44 (95% CI: 0.27-0.71). And this association remained significant after Semi-Bayesian adjustment using a null prior, reporting an aOR of 0.48, 95% PI: 0.31-0.76 (Table 3.14).

Table 3.11 The association between SNPs from microRNA related genes and liver cancer in Jiangsu study 2003-2010 (n=2612), 2014

SNP	Case 419	%	Control 2193	%	Crude OR additive (95% CI)	Crude OR dominant (95% CI)	Crude OR recessive (95% CI)	Crude OR co-dominant (95% CI)	Adjusted OR co-dominant (95% CI)[a]
rs1804429	347		2060						298/1893
T:T	311	89.6	1795	87.1	1.00	1.00	1.00	1.00	1.00
T:G	35	10.1	247	12.0	0.77 (0.55-1.09)	0.78 (0.54-1.13)		0.82 (0.56-1.19)	0.98 (0.61-1.59)
G:G	1	0.3	18	0.9			0.33 (0.04-2.46)	0.32 (0.04-2.41)	0.61 (0.05-7.01)
rs10519613	341		2066						296/1896
C:C	122	35.8	732	35.4	1.00	1.00	1.00	1.00	1.00
C:A	142	41.6	936	45.3	1.06 (0.90-1.24)	0.98 (0.77-1.25)		0.91 (0.70-1.18)	0.96 (0.67-1.36)
A:A	77	22.6	398	19.3			1.22 (0.93-1.61)	1.16 (0.85-1.58)	1.05 (0.68-1.62)
rs12828	336		2022						290/1853
G:G	148	44.1	847	41.9	1.00	1.00	1.00	1.00	1.00
G:A	129	38.4	883	43.7	1.02 (0.87-1.20)	0.92 (0.73-1.16)		0.84 (0.65-1.08)	0.88 (0.63-1.24)
A:A	59	17.6	292	14.4			1.26 (0.93-1.72)	1.16 (0.83-1.61)	0.96 (0.61-1.52)
rs896849	343		2008						299/1911
T:T	262	76.4	1495	74.5	1.00	1.00	1.00	1.00	1.00
T:C	77	22.4	450	22.4	**0.77 (0.61-0.98)**	0.79 (0.61-1.03)		0.84 (0.64-1.11)	0.85 (0.59-1.22)
C:C	4	1.2	63	3.1			0.38 (0.14-1.05)	**0.36 (0.13-1.00)**	**0.27 (0.08-0.87)**
rs11614913	325		2022						281/1855
T:T	126	38.8	632	31.3	1.00	1.00	1.00	1.00	1.00
T:C	151	46.5	955	47.2	**0.75 (0.64-0.89)**	**0.72 (0.56-0.92)**		0.79 (0.61-1.03)	0.76 (0.54-1.07)
C:C	48	14.8	435	21.5			**0.63 (0.46-0.87)**	**0.55 (0.39-0.79)**	**0.44 (0.27-0.71)**
rs2910164	342		2072						293/1901
C:C	115	33.6	754	36.4	1.00	1.00	1.00	1.00	1.00
C:G	173	50.6	957	46.2	1.02 (0.87-1.20)	1.13 (0.89-1.44)		1.19 (0.92-1.53)	0.97 (0.70-1.36)
G:G	54	15.8	361	17.4			0.89 (0.65-1.21)	0.98 (0.69-1.39)	0.65 (0.41-1.04)

Table 3.11 cont. The association between SNPs from microRNA related genes and liver cancer in Jiangsu study 2003-2010 (n=2612), 2014

SNP	Case 419	%	Control 2193	%	Crude OR additive (95% CI)	Crude OR dominant (95% CI)	Crude OR recessive (95% CI)	Crude OR co-dominant (95% CI)	Adjusted OR co-dominant (95% CI)[a]
rs895819	345		2038						299/1910
T:T	205	59.4	1145	56.2	1.00	1.00	1.00	1.00	1.00
T:C	114	33.0	732	35.9	0.89 (0.74-1.07)	0.84 (0.66-1.05)		0.82 (0.64-1.05)	0.73 (0.53-1.02)
C:C	26	7.5	161	7.9			0.97 (0.63-1.50)	0.90 (0.58-1.40)	0.86 (0.48-1.55)
rs7372209	340		2073						290/1907
C:C	182	53.5	1038	50.1	1.00	1.00	1.00	1.00	1.00
C:T	121	35.6	828	39.9	0.94 (0.79-1.12)	0.87 (0.69-1.10)		0.83 (0.65-1.07)	0.89 (0.64-1.25)
T:T	37	10.9	207	10.0			1.10 (0.76-1.59)	1.02 (0.70-1.50)	0.97 (0.58-1.63)
rs3742330	348		2097						301/1925
A:A	158	45.4	903	43.1	1.00	1.00	1.00	1.00	1.00
A:G	153	44.0	909	43.3	0.89 (0.76-1.06)	0.91 (0.72-1.14)		0.96 (0.76-1.22)	0.88 (0.64-1.22)
G:G	37	10.6	285	13.6			0.76 (0.53-1.09)	0.74 (0.51-1.09)	0.61 (0.36-1.02)
rs4961280	335		2024						291/1858
C:C	261	77.9	1557	76.9	1.00	1.00	1.00	1.00	1.00
C:A	68	20.3	422	20.8	0.94 (0.74-1.20)	0.95 (0.72-1.25)		0.96 (0.72-1.28)	0.91 (0.62-1.33)
A:A	6	1.8	45	2.2			0.80 (0.34-1.90)	0.80 (0.34-1.88)	0.71 (0.25-2.02)
rs14035	317		1966						276/1822
C:C	219	69.1	1300	66.1	1.00	1.00	1.00	1.00	1.00
C:T	86	27.1	568	28.9	0.88 (0.71-1.09)	0.87 (0.68-1.13)		0.90 (0.69-1.18)	1.00 (0.70-1.43)
T:T	12	3.8	98	5.0			0.75 (0.41-1.38)	0.73 (0.39-1.35)	1.17 (0.57-2.43)
rs197412	347		2024						300/1853
T:T	162	46.7	914	45.2	1.00	1.00	1.00	1.00	1.00
T:C	144	41.5	889	43.9	0.99 (0.83-1.17)	0.94 (0.75-1.18)		0.91 (0.72-1.17)	0.92 (0.66-1.27)
C:C	41	11.8	221	10.9			1.09 (0.77-1.56)	1.05 (0.72-1.52)	1.27 (0.77-2.11)

Table 3.11 cont. The association between SNPs from microRNA related genes and liver cancer in Jiangsu study 2003-2010 (n=2612), 2014

SNP	Case 419	%	Control 2193	%	Crude OR additive (95% CI)	Crude OR dominant (95% CI)	Crude OR recessive (95% CI)	Crude OR co-dominant (95% CI)	Adjusted OR co-dominant (95% CI)[a]
rs2740348	324		2012						279/1843
G:G	253	78.1	1612	80.1	1.00	1.00	1.00	1.00	1.00
G:C	68	21.0	363	18.0	1.06 (0.82-1.36)	1.13 (0.85-1.50)		1.19 (0.89-1.60)	1.17 (0.78-1.74)
C:C	3	0.9	37	1.8			0.50 (0.15-1.63)	0.52 (0.16-1.69)	0.40 (0.10-1.62)
rs7813	337		2008						291/1844
T:T	170	50.5	1026	51.1	1.00	1.00	1.00	1.00	1.00
T:C	142	42.1	774	38.5	0.95 (0.80-1.13)	1.03 (0.82-1.29)		1.11 (0.87-1.41)	1.10 (0.80-1.53)
C:C	25	7.4	208	10.4			0.69 (0.45-1.07)	0.73 (0.47-1.13)	0.61 (0.33-1.11)
rs11077	341		2061						292/1893
A:A	284	83.3	1771	85.9	1.00	1.00	1.00	1.00	1.00
A:C	54	15.8	270	13.1	1.18 (0.89-1.56)	1.23 (0.90-1.67)		1.25 (0.91-1.71)	1.01 (0.65-1.55)
C:C	3	0.9	20	1.0			0.91 (0.27-3.07)	0.94 (0.28-3.17)	0.85 (0.16-4.56)
rs9266	350		2014						300/1908
C:C	220	62.9	1253	62.9	1.00	1.00	1.00	1.00	1.00
C:T	116	33.1	653	32.8	0.90 (0.74-1.09)	0.90 (0.71-1.14)		0.92 (0.72-1.18)	0.91 (0.65-1.27)
T:T	14	4.0	108	5.4			0.76 (0.43-1.34)	0.74 (0.42-1.31)	0.48 (0.21-1.08)
rs4072391	345		2077						297/1907
C:C	278	80.6	1701	81.9	1.00	1.00	1.00	1.00	1.00
C:T	60	17.4	341	16.4	1.09 (0.85-1.40)	1.09 (0.82-1.46)		1.08 (0.80-1.46)	0.99 (0.64-1.51)
T:T	7	2.0	35	1.7			1.21 (0.53-2.74)	1.22 (0.54-2.78)	0.60 (0.19-1.85)
rs2126852	339		2013						293/1850
A:A	186	54.9	1118	55.5	1.00	1.00	1.00	1.00	1.00
A:G	122	36.0	721	35.8	1.03 (0.86-1.23)	1.03 (0.82-1.30)		1.02 (0.80-1.30)	0.82 (0.59-1.15)
G:G	31	9.1	174	8.6			1.06 (0.71-1.59)	1.07 (0.71-1.62)	0.95 (0.55-1.63)

Table 3.11 cont. The association between SNPs from microRNA related genes and liver cancer in Jiangsu study 2003-2010 (n=2612), 2014

SNP	Case 419	%	Control 2193	%	Crude OR additive (95% CI)	Crude OR dominant (95% CI)	Crude OR recessive (95% CI)	Crude OR co-dominant (95% CI)	Adjusted OR co-dominant (95% CI)[a]
rs7760	333		2065						286/1898
T:T	257	77.2	1574	76.2	1.00	1.00	1.00	1.00	1.00
T:G	72	21.6	442	21.4	0.91 (0.71-1.17)	0.95 (0.72-1.25)		1.00 (0.75-1.32)	1.08 (0.73-1.58)
G:G	4	1.2	49	2.4			0.50 (0.18-1.40)	0.50 (0.18-1.40)	0.39 (0.11-1.42)
rs2075993	340		2014						295/1848
G:G	112	32.9	762	37.8	1.00	1.00	1.00	1.00	1.00
G:A	165	48.5	915	45.4	1.14 (0.97-1.34)	1.24 (0.97-1.58)		1.23 (0.95-1.59)	1.37 (0.97-1.95)
A:A	63	18.5	337	16.7			1.13 (0.84-1.52)	1.27 (0.91-1.78)	1.24 (0.78-1.95)
rs3801790	349		2050						298/1883
A:A	140	40.1	772	37.7	1.00	1.00	1.00	1.00	1.00
A:G	157	45.0	928	45.3	0.91 (0.78-1.07)	0.90 (0.72-1.14)		0.93 (0.73-1.19)	0.99 (0.70-1.38)
G:G	52	14.9	350	17.1			0.85 (0.62-1.17)	0.82 (0.58-1.15)	0.65 (0.41-1.05)
rs3929	355		2110						305/1936
G:G	232	65.4	1415	67.1	1.00	1.00	1.00	1.00	1.00
G:C	108	30.4	610	28.9	1.06 (0.87-1.29)	1.08 (0.85-1.37)		1.08 (0.84-1.38)	0.94 (0.67-1.31)
C:C	15	4.2	85	4.0			1.05 (0.60-1.84)	1.08 (0.61-1.90)	1.20 (0.56-2.58)
rs2292305	333		2032						289/1867
T:T	148	44.4	916	45.1	1.00	1.00	1.00	1.00	1.00
T:C	152	45.7	861	42.4	0.96 (0.81-1.14)	1.03 (0.81-1.30)		1.09 (0.86-1.40)	1.03 (0.74-1.44)
C:C	33	9.9	255	12.5			0.77 (0.52-1.12)	0.80 (0.54-1.20)	0.77 (0.45-1.32)
rs2953	346		2088						298/1919
T:T	199	57.5	1155	55.3	1.00	1.00	1.00	1.00	1.00
T:G	125	36.1	783	37.5	0.93 (0.77-1.11)	0.91 (0.73-1.15)		0.93 (0.73-1.18)	0.86 (0.62-1.19)
G:G	22	6.4	150	7.2			0.88 (0.55-1.39)	0.85 (0.53-1.37)	1.03 (0.56-1.89)

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0), pack-year of smoking (continuous), ethanol consumption (gram/week, continuous), HBsAg status (1=positive, 0=negative) and study area (Dafeng=1, Ganyu=2).

Polymorphism in stem cell pathway genes and liver cancer

Table 3.12 showed the crude associations between SNPs from stem cell pathway and liver cancer evaluated by additive, dominant, recessive and co-dominant genetic models and adjusted associations by co-dominant model. Rs3729629 (WNT2 gene, G/G, G/C and C/C) showed inverse associations in additive model with OR of 0.82 (95% CI: 0.69-0.98) and OR of 0.79 (95% CI: 0.63-0.99) in dominant model comparing to T/T. Rs4730775 (WNT2 gene, C/C, C/T and T/T) also showed inverse associations with liver cancer with OR of 0.82 (95% CI: 0.67-0.99) in additive model, 0.77 (95% CI: 0.61-0.97) in dominant model and 0.77 (95% CI: 0.60-0.99) in co-dominant model when comparing C/T to T/T type. Rs3734637 (HEY2 gene, A/A, A/C, and C/C) showed inverse associations in additive model with OR of 0.78 (95% CI: 0.64-0.95), in dominant model with OR of 0.70 (95% CI: 0.55-0.88) and in co-dominant model with OR of 0.67 (95% CI: 0.52-0.87) when comparing A/C to A/A type.

After controlling for age, gender, study site, education level, marriage status, family income per capita 10 years ago, BMI, HBsAg status, family history of liver cancer, pack-year of smoking, and weekly ethanol intake in 1990s, rs3729629 and rs3734637 were no longer statistically significantly associated with liver cancer. Rs4730775 (WNT2 gene, C/T vs. C/C) remained being inversely associated with liver cancer, showing an aOR of 0.71 (95% CI: 0.50-0.99). And comparing the T/T type to C/C type, the inverse association still existed with an aOR of 0.56, while the confidence interval was wide (95% CI: 0.29-1.07). Moreover, rs2241802 (FZD3 gene, G/G, G/A and A/A) showed an inverse association when comparing A/A to G/G with an aOR of 0.58 (95% CI: 0.37-0.91). And this association remained significant after the Bayesian adjustment, showing an adjusted ROR of 0.60, 95% PI: 0.40-0.92 (Table 3.15).

Table 3.12 The association between SNPs from stem cell pathway with risk of liver cancer in Jiangsu study 2003-2010 (n=2612), 2014

SNP	Case 419	%	Control 2193	%	Crude OR additive (95% CI)	Crude OR dominant (95% CI)	Crude OR recessive (95% CI)	Crude OR co-dominant (95% CI)	Adjusted OR co-dominant (95% CI)[a]
rs6815391	342		2032						293/1867
T:T	149	43.6	893	43.9	1.00	1.00	1.00	1.00	1.00
T:C	150	43.9	862	42.4	0.99 (0.84-1.16)	1.02 (0.81-1.28)		1.04 (0.82-1.33)	1.21 (0.87-1.69)
C:C	43	12.6	277	13.6			0.91 (0.65-1.29)	0.93 (0.65-1.34)	1.02 (0.61-1.68)
rs13409	327		2072						283/1904
C:C	120	25.3	770	37.2	1.00	1.00	1.00	1.00	1.00
C:T	138	29.1	915	44.2	1.06 (0.90-1.24)	1.02 (0.80-1.30)		0.97 (0.74-1.26)	1.02 (0.72-1.45)
T:T	69	14.5	387	18.7			1.16 (0.87-1.55)	1.14 (0.83-1.58)	1.21 (0.79-1.86)
rs3130932	351		2073						300/1906
T:T	177	50.4	962	46.4	1.00	1.00	1.00	1.00	1.00
G:T	133	37.9	875	42.2	0.92 (0.78-1.09)	0.85 (0.68-1.07)		0.83 (0.65-1.05)	0.85 (0.62-1.18)
G:G	41	11.7	236	11.4			1.03 (0.72-1.47)	0.94 (0.65-1.37)	0.91 (0.55-1.48)
rs3740535	354		2075						302/1905
G:G	194	54.8	1144	55.1	1.00	1.00	1.00	1.00	1.00
G:A	128	36.2	767	37.0	1.04 (0.87-1.23)	1.01 (0.81-1.27)		0.98 (0.77-1.25)	1.07 (0.78-1.48)
A:G	32	9.0	164	7.9			1.16 (0.78-1.72)	1.15 (0.77-1.73)	0.87 (0.49-1.56)
rs2228224	342		2038						295/1871
G:G	189	55.3	1135	55.7	1.00	1.00	1.00	1.00	1.00
G:A	134	39.2	735	36.1	0.95 (0.79-1.13)	1.02 (0.81-1.28)		1.10 (0.86-1.39)	1.17 (0.85-1.62)
A:G	19	5.6	168	8.2			0.66 (0.40-1.07)	0.68 (0.41-1.12)	0.57 (0.29-1.09)
rs3729629	362		2076						309/1907
G:G	185	51.1	938	45.2	1.00	1.00	1.00	1.00	1.00
G:C	146	40.3	908	43.7	**0.82 (0.69-0.98)**	**0.79 (0.63-0.99)**		0.82 (0.64-1.03)	0.95 (0.69-1.30)
C:C	31	8.6	230	11.1			0.75 (0.51-1.11)	0.68 (0.46-1.03)	0.73 (0.42-1.28)

Table 3.12 cont. The association between SNPs from stem cell pathway with risk of liver cancer in Jiangsu study 2003-2010, 2014

SNP	Case 419	%	Control 2193	%	Crude OR additive (95% CI)	Crude OR dominant (95% CI)	Crude OR recessive (95% CI)	Crude OR co-dominant (95% CI)	Adjusted OR co-dominant (95% CI)[a]
rs4730775	347		2051						296/1883
C:C	221	63.7	1177	57.4	1.00	1.00	1.00	1.00	1.00
C:T	106	30.6	730	35.6	**0.82 (0.67-0.99)**	**0.77 (0.61-0.97)**		**0.77 (0.60-0.99)**	**0.71 (0.50-0.99)**
T:T	20	5.8	144	7.0			0.81 (0.50-1.31)	0.74 (0.45-1.21)	0.56 (0.29-1.07)
rs4835761	347		2021						302/1862
A:A	118	34.0	698	34.5	1.00	1.00	1.00	1.00	1.00
A:G	167	48.1	943	46.7	0.99 (0.85-1.16)	1.02 (0.81-1.30)		1.05 (0.81-1.35)	0.97 (0.68-1.36)
G:G	62	17.9	380	18.8			0.94 (0.70-1.26)	0.97 (0.69-1.35)	0.97 (0.62-1.52)
rs3750145	297		1917						259/1763
A:A	179	60.3	1204	62.8	1.00	1.00	1.00	1.00	1.00
A:G	96	32.3	614	32.0	1.14 (0.93-1.39)	1.11 (0.87-1.43)		1.05 (0.81-1.37)	1.21 (0.85-1.72)
G:G	22	7.4	99	5.2			1.47 (0.91-2.37)	1.50 (0.92-2.44)	1.49 (0.79-2.82)
rs2241802	347		2023						296/1858
G:G	131	37.8	682	33.7	1.00	1.00	1.00	1.00	1.00
G:A	153	44.1	929	45.9	0.89 (0.76-1.04)	0.84 (0.66-1.06)		0.86 (0.67-1.11)	0.90 (0.64-1.26)
A:A	63	18.2	412	20.4			0.87 (0.65-1.16)	0.80 (0.58-1.10)	**0.58 (0.37-0.91)**
rs222851	332		2031						287/1864
A:A	118	35.5	805	39.6	1.00	1.00	1.00	1.00	1.00
A:G	162	48.8	910	44.8	1.09 (0.92-1.28)	1.19 (0.94-1.52)		1.21 (0.94-1.57)	1.17 (0.83-1.65)
G:G	52	15.7	316	15.6			1.01 (0.73-1.39)	1.12 (0.79-1.60)	1.19 (0.75-1.91)
rs1981492	333		2048						289/1882
G:G	162	48.7	1057	51.6	1.00	1.00	1.00	1.00	1.00
G:A	141	42.3	787	38.4	1.05 (0.88-1.25)	1.13 (0.89-1.42)		1.17 (0.92-1.49)	1.10 (0.80-1.53)
A:A	30	9.0	204	10.0			0.90 (0.60-1.34)	0.96 (0.63-1.46)	0.75 (0.44-1.28)

Table 3.12 cont. The association between SNPs from stem cell pathway with risk of liver cancer in Jiangsu study 2003-2010 (n=2612), 2014

SNP	Case 419	%	Control 2193	%	Crude OR additive (95% CI)	Crude OR dominant (95% CI)	Crude OR recessive (95% CI)	Crude OR co-dominant (95% CI)	Adjusted OR co-dominant (95% CI)[a]
rs1046472	338		2074						292/1904
C:C	222	65.7	1310	63.2	1.00	1.00	1.00	1.00	1.00
C:A	100	29.6	661	31.9	0.92 (0.75-1.13)	0.90 (0.70-1.14)		0.89 (0.69-1.15)	0.94 (0.67-1.32)
A:A	16	4.7	103	5.0			0.95 (0.56-1.63)	0.92 (0.53-1.58)	1.06 (0.50-2.24)
rs3734637	354		2071						301/1902
A:A	241	68.1	1237	59.7	1.00	1.00	1.00	1.00	1.00
A:C	92	26.0	704	34.0	**0.78 (0.64-0.95)**	**0.70 (0.55-0.88)**		**0.67 (0.52-0.87)**	0.75 (0.53-1.06)
C:C	21	5.9	130	6.3			0.94 (0.59-1.52)	0.83 (0.51-1.34)	0.67 (0.34-1.31)
rs11364	322		1824						277/1671
G:G	227	70.5	1296	71.1	1.00	1.00	1.00	1.00	1.00
G:A	84	26.1	452	24.8	0.99 (0.80-1.23)	1.03 (0.79-1.33)		1.06 (0.81-1.39)	1.21 (0.83-1.75)
A:A	11	3.4	76	4.2			0.81 (0.43-1.55)	0.83 (0.43-1.58)	0.92 (0.41-2.07)
rs520692	337		2076						295/1907
A:A	261	77.5	1571	75.7	1.00	1.00	1.00	1.00	1.00
A:G	72	21.4	459	22.1	0.88 (0.69-1.13)	0.91 (0.69-1.19)		0.94 (0.71-1.25)	0.99 (0.68-1.43)
G:G	4	1.2	46	2.2			0.53 (0.19-1.48)	0.52 (0.19-1.47)	0.82 (0.23-2.99)
rs8708	341		2042						291/1875
A:A	241	70.7	1403	68.7	1.00	1.00	1.00	1.00	1.00
A:G	84	24.6	552	27.0	0.95 (0.77-1.17)	0.91 (0.71-1.17)		0.89 (0.68-1.16)	1.04 (0.73-1.47)
G:G	16	4.7	87	4.3			1.11 (0.64-1.91)	1.07 (0.62-1.86)	1.17 (0.53-2.60)
rs1033583	319		1880						275/1724
A:A	180	56.4	1065	56.6	1.00	1.00	1.00	1.00	1.00
A:C	107	33.5	684	36.4	1.09 (0.90-1.31)	1.01 (0.79-1.28)		0.93 (0.72-1.20)	0.98 (0.69-1.39)
C:C	32	10.0	131	7.0			1.49 (0.99-2.23)	1.45 (0.95-2.19)	1.37 (0.76-2.47)

Table 3.12 cont. The association between SNPs from stem cell pathway with risk of liver cancer in Jiangsu study 2003-2010 (n=2612), 2014

SNP	Case 419	%	Control 2193	%	Crude OR additive (95% CI)	Crude OR dominant (95% CI)	Crude OR recessive (95% CI)	Crude OR co-dominant (95% CI)	Adjusted OR co-dominant (95% CI)[a]
rs2269700	355		2084						301/1914
T:T	237	66.8	1400	67.2	1.00	1.00	1.00	1.00	1.00
T:C	103	29.0	604	29.0	1.03 (0.84-1.25)	1.02 (0.80-1.29)		1.01 (0.78-1.29)	0.93 (0.66-1.31)
C:C	15	4.2	80	3.8			1.11 (0.63-1.94)	1.11 (0.63-1.96)	0.81 (0.37-1.79)
rs915894	336		2024						292/1870
C:C	111	33.0	601	29.7	1.00	1.00	1.00	1.00	1.00
C:A	163	48.5	970	47.9	0.87 (0.74-1.02)	0.85 (0.66-1.08)		0.95 (0.73-1.24)	0.91 (0.64-1.29)
A:A	62	18.5	453	22.4			0.79 (0.59-1.06)	0.74 (0.53-1.04)	0.98 (0.63-1.51)
rs9972231	287		1778						246/1695
C:C	204	71.1	1224	68.8	1.00	1.00	1.00	1.00	1.00
C:T	71	24.7	482	27.1	0.86 (0.69-1.09)	0.81 (0.61-1.06)		0.78 (0.58-1.04)	0.85 (0.59-1.25)
T:T	12	4.2	72	4.0			1.07 (0.58-2.00)	1.00 (0.53-1.88)	0.76 (0.27-2.15)

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0), pack-year of smoking (continuous), ethanol consumption (gram/week, continuous), HBsAg status (1=positive, 0=negative) and study area (Dafeng=1, Ganyu=2).

Polymorphism from GWAS and liver cancer

Table 3.13 showed the crude associations between SNPs from GWAS and liver cancer evaluated by additive, dominant, recessive and co-dominant genetic models and adjusted associations by co-dominant model. Rs4678680 (CCR4 -GLB1 gene, T/T, T/G and G/G) showed positive association in recessive model with OR of 2.45 (95% CI: 1.01-5.94) and in co-dominant model (G/G vs. T/T) with OR of 2.41 (95% CI: 0.99-5.85). And rs9275572 (HLA-DQB1- HLA-DQA2 gene, G/G, G/A and A/A) showed inverse association with OR of 0.80 (95% CI: 0.63-1.00) in dominant model.

After controlling for age, gender, study site, education level, marriage status, family income per capita 10 years ago, BMI, HBsAg status, family history of liver cancer, pack-year of smoking, and weekly ethanol intake in 1990s, no SNPs remain significantly associated with liver cancer in this analysis and in the further stratified analysis (Table 3.20).

Multiple Imputation

After five times imputation and combined the imputed results together, the logistic regression model was run for each SNP using the imputed data (Table 2.6, 2.7, 2.8). From microRNA SNPs, rs9266 (KRAS gene, T/T vs. C/C) showed an aOR of 0.45 (95% CI: 0.21-0.95) as an inverse association with liver cancer. Rs2075993 (E2F2 gene, G/A vs. G/G) showed an aOR of 1.36 (95% CI: 1.00-1.85) as a positive association with liver cancer (Table 3.14-3.16).

Table 3.13 The association between candidate SNPs from GWAS with risk of liver cancer in Jiangsu study 2003-2010 (n=2612), 2014

SNP	Case 419	%	Control 2193	%	Crude OR additive (95% CI)	Crude OR dominant (95% CI)	Crude OR recessive (95% CI)	Crude OR co-dominant (95% CI)	Adjusted OR co-dominant (95% CI)[a]
rs4678680	360		2113						309/1943
T:T	319	88.6	1864	88.2	1.00	1.00	1.00	1.00	1.00
T:G	34	9.4	232	11.0	1.06 (0.78-1.43)	0.96 (0.68-1.37)		0.86 (0.59-1.25)	1.01 (0.61-1.68)
G:G	7	1.9	17	0.8			**2.45 (1.01-5.94)**	**2.41 (0.99-5.85)**	0.86 (0.23-3.17)
rs9267673	358		2016						304/1903
C:C	257	71.8	1426	70.7	1.00	1.00	1.00	1.00	1.00
C:T	91	25.4	524	26.0	0.88 (0.71-1.10)	0.87 (0.68-1.11)		0.87 (0.67-1.12)	0.83 (0.59-1.18)
T:T	10	2.8	66	3.3			0.87 (0.45-1.72)	0.84 (0.43-1.66)	1.43 (0.63-3.28)
rs9275572	345		2052						297/1894
G:G	201	58.3	1085	52.9	1.00	1.00	1.00	1.00	1.00
G:A	117	33.9	774	37.7	0.84 (0.70-1.01)	**0.80 (0.63-1.00)**		0.81 (0.63-1.03)	1.07 (0.77-1.50)
A:A	27	7.8	193	9.4			0.82 (0.54-1.25)	0.76 (0.49-1.16)	1.32 (0.77-2.28)

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0), pack-year of smoking (continuous), ethanol consumption (gram/week, continuous), HBsAg status (1=positive, 0=negative) and study area (Dafeng=1, Ganyu=2).

Table 3.14 The multiple imputed and semi-Bayesian adjusted association between polymorphisms in microRNA related genes and liver cancer in Jiangsu study, n=2612 2003-2010, 2014

miRNA SNP	Case	Control	Adjusted OR co-dominant (95% CI)[a]	Multiple Imputation Adjusted OR co-dominant (95% CI)[a]	Semi-Bayesian Adjusted OR co-dominant (95% PI)[a]
Total	419	2193	(case/control)		
rs1804429	347	2060	298/1893		
T:T	311	1795	1.00	1.00	1.00
T:G	35	247	0.98 (0.61-1.59)	0.96 (0.56-1.64)	0.98 (0.62-1.55)
G:G	1	18	0.61 (0.05-7.01)	0.49 (0.04-5.62)	0.86 (0.27-2.79)
rs10519613	341	2066	296/1896		
C:C	122	732	1.00	1.00	1.00
C:A	142	936	0.96 (0.67-1.36)	0.99 (0.70-1.39)	0.95 (0.68-1.32)
A:A	77	398	1.05 (0.68-1.62)	1.14 (0.74-1.76)	1.04 (0.69-1.57)
rs12828	336	2022	290/1853		
G:G	148	847	1.00	1.00	1.00
G:A	129	883	0.88 (0.63-1.24)	0.84 (0.60-1.17)	0.88 (0.64-1.21)
A:A	59	292	0.96 (0.61-1.52)	1.00 (0.64-1.56)	0.96 (0.62-1.48)
rs896849	343	2008	299/1911		
T:T	262	1495	1.00	1.00	1.00
T:C	77	450	0.85 (0.59-1.22)	0.88 (0.63-1.24)	0.86 (0.61-1.23)
C:C	4	63	**0.27 (0.08-0.87)**	**0.20 (0.06-0.64)**	0.45 (0.19-1.04)
rs11614913	325	2022	281/1855		
T:T	126	632	1.00	1.00	1.00
T:C	151	955	0.76 (0.54-1.07)	0.83 (0.59-1.16)	0.79 (0.57-1.10)
C:C	48	435	**0.44 (0.27-0.71)**	**0.53 (0.33-0.84)**	**0.48 (0.31-0.76)**
rs2910164	342	2072	293/1901		
C:C	115	754	1.00	1.00	1.00
C:G	173	957	0.97 (0.70-1.36)	1.04 (0.76-1.42)	0.97 (0.70-1.34)
G:G	54	361	0.65 (0.41-1.04)	0.72 (0.45-1.15)	0.67 (0.43-1.04)
rs895819	345	2038	299/1910		
T:T	205	1145	1.00	1.00	1.00
T:C	114	732	0.73 (0.53-1.02)	0.79 (0.59-1.06)	0.74 (0.54-1.02)
C:C	26	161	0.86 (0.48-1.55)	0.86 (0.51-1.44)	0.89 (0.52-1.51)
rs7372209	340	2073	290/1907		
C:C	182	1038	1.00	1.00	1.00
C:T	121	828	0.89 (0.64-1.25)	0.86 (0.64-1.16)	0.88 (0.64-1.21)
T:T	37	207	0.97 (0.58-1.63)	0.82 (0.51-1.33)	0.95 (0.59-1.53)
rs3742330	348	2097	301/1925		
A:A	158	903	1.00	1.00	1.00
A:G	153	909	0.88 (0.64-1.22)	1.01 (0.72-1.43)	0.88 (0.65-1.20)
G:G	37	285	0.61 (0.36-1.02)	0.73 (0.46-1.14)	0.64 (0.40-1.03)

Table 3.14 cont. The multiple imputed and semi-Bayesian adjusted association between polymorphisms in microRNA related genes and liver cancer in Jiangsu study, 2003-2010, 2014

miRNA SNP	Case	Control	Adjusted OR co-dominant (95% CI)[a]	Multiple Imputation Adjusted OR co-dominant (95% CI)[a]	Semi-Bayesian Adjusted OR co-dominant (95% PI)[a]
rs4961280	335	2024	291/1858		
C:C	261	1557	1.00	1.00	1.00
C:A	68	422	0.91 (0.62-1.33)	0.95 (0.61-1.49)	0.91 (0.63-1.31)
A:A	6	45	0.71 (0.25-2.02)	0.57 (0.21-1.57)	0.81 (0.36-1.84)
rs14035	317	1966	276/1822		
C:C	219	1300	1.00	1.00	1.00
C:T	86	568	1.00 (0.70-1.43)	0.95 (0.66-1.38)	0.98 (0.70-1.39)
T:T	12	98	1.17 (0.57-2.43)	0.96 (0.44-2.10)	1.10 (0.58-2.11)
rs197412	347	2024	300/1853		
T:T	162	914	1.00	1.00	1.00
T:C	144	889	0.92 (0.66-1.27)	0.97 (0.63-1.48)	0.90 (0.65-1.23)
C:C	41	221	1.27 (0.77-2.11)	1.23 (0.76-1.99)	1.23 (0.76-1.97)
rs2740348	324	2012	279/1843		
G:G	253	1612	1.00	1.00	1.00
G:C	68	363	1.17 (0.78-1.74)	1.12 (0.73-1.72)	1.15 (0.78-1.69)
C:C	3	37	0.40 (0.10-1.62)	0.32 (0.08-1.26)	0.63 (0.25-1.60)
rs7813	337	2008	291/1844		
T:T	170	1026	1.00	1.00	1.00
T:C	142	774	1.10 (0.80-1.53)	1.20 (0.85-1.69)	1.10 (0.80-1.50)
C:C	25	208	0.61 (0.33-1.11)	0.66 (0.36-1.21)	0.65 (0.38-1.12)
rs11077	341	2061	292/1893		
A:A	284	1771	1.00	1.00	1.00
A:C	54	270	1.01 (0.65-1.55)	1.19 (0.81-1.76)	0.99 (0.66-1.51)
C:C	3	20	0.85 (0.16-4.56)	0.70 (0.14-3.46)	0.92 (0.32-2.66)
rs9266	350	2014	300/1908		
C:C	220	1253	1.00	1.00	1.00
C:T	116	653	0.91 (0.65-1.27)	0.87 (0.64-1.17)	0.91 (0.66-1.25)
T:T	14	108	0.48 (0.21-1.08)	**0.45 (0.21-0.95)**	0.57 (0.29-1.13)
rs4072391	345	2077	297/1907		
C:C	278	1701	1.00	1.00	1.00
C:T	60	341	0.99 (0.64-1.51)	1.11 (0.77-1.59)	0.99 (0.66-1.48)
T:T	7	35	0.60 (0.19-1.85)	0.52 (0.17-1.54)	0.73 (0.31-1.73)
rs2126852	339	2013	293/1850		
A:A	186	1118	1.00	1.00	1.00
A:G	122	721	0.82 (0.59-1.15)	0.85 (0.62-1.16)	0.83 (0.60-1.14)
G:G	31	174	0.95 (0.55-1.63)	0.82 (0.46-1.48)	0.96 (0.58-1.59)

Table 3.14 cont. The multiple imputed and semi-Bayesian adjusted association between polymorphisms in microRNA related genes and liver cancer in Jiangsu study, 2003-2010, 2014

miRNA SNP	Case	Control	Adjusted OR co-dominant (95% CI)[a]	Multiple Imputation Adjusted OR co-dominant (95% CI)[a]	Semi-Bayesian Adjusted OR co-dominant (95% PI)[a]
rs7760	333	2065	286/1898		
T:T	257	1574	1.00	1.00	1.00
T:G	72	442	1.08 (0.73-1.58)	1.13 (0.81-1.58)	1.08 (0.74-1.56)
G:G	4	49	0.39 (0.11-1.42)	0.29 (0.08-1.02)	0.59 (0.24-1.45)
rs2075993	340	2014	295/1848		
G:G	112	762	1.00	1.00	1.00
G:A	165	915	1.37 (0.97-1.95)	**1.36 (1.00-1.85)**	1.31 (0.94-1.83)
A:A	63	337	1.24 (0.78-1.95)	1.26 (0.83-1.93)	1.18 (0.77-1.82)
rs3801790	349	2050	298/1883		
A:A	140	772	1.00	1.00	1.00
A:G	157	928	0.99 (0.70-1.38)	1.06 (0.78-1.43)	0.98 (0.71-1.36)
G:G	52	350	0.65 (0.41-1.05)	0.74 (0.47-1.18)	0.67 (0.43-1.04)
rs3929	355	2110	305/1936		
G:G	232	1415	1.00	1.00	1.00
G:C	108	610	0.94 (0.67-1.31)	1.02 (0.73-1.42)	0.93 (0.67-1.29)
C:C	15	85	1.20 (0.56-2.58)	1.19 (0.59-2.41)	1.14 (0.58-2.23)
rs2292305	333	2032	289/1867		
T:T	148	916	1.00	1.00	1.00
T:C	152	861	1.03 (0.74-1.44)	1.23 (0.89-1.71)	1.01 (0.74-1.39)
C:C	33	255	0.77 (0.45-1.32)	0.88 (0.54-1.44)	0.77 (0.47-1.27)
rs2953	346	2088	298/1919		
T:T	199	1155	1.00	1.00	1.00
T:G	125	783	0.86 (0.62-1.19)	0.88 (0.63-1.23)	0.85 (0.62-1.16)
G:G	22	150	1.03 (0.56-1.89)	0.90 (0.50-1.64)	0.99 (0.57-1.73)

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0), pack-year of smoking (continuous), ethanol consumption (gram/week, continuous), HBsAg status (1=positive, 0=negative) and study area (Dafeng=1, Ganyu=2).

Table 3.15 The multiple imputed and semi-Bayesian adjusted association between polymorphisms in stem cell pathway genes and liver cancer in Jiangsu study, n=2612 2003-2010, 2014

Stem cell SNP	Case	Control	Adjusted OR co-dominant (95% CI)[a]	Multiple Imputation Adjusted OR co-dominant (95% CI)[a]	Semi-Bayesian Adjusted OR co-dominant (95% PI)[a]
Total	419	2193	(case/control)		
rs6815391	342	2032	293/1867		
T:T	149	893	1.00	1.00	1.00
T:C	150	862	1.21 (0.87-1.69)	1.16 (0.78-1.74)	1.16 (0.84-1.60)
C:C	43	277	1.02 (0.61-1.68)	1.07 (0.61-1.86)	0.98 (0.61-1.57)
rs13409	327	2072	283/1904		
C:C	120	770	1.00	1.00	1.00
C:T	138	915	1.02 (0.72-1.45)	0.92 (0.68-1.25)	1.00 (0.71-1.39)
T:T	69	387	1.21 (0.79-1.86)	1.23 (0.78-1.94)	1.16 (0.78-1.75)
rs3130932	351	2073	300/1906		
T:T	177	962	1.00	1.00	1.00
G:T	133	875	0.85 (0.62-1.18)	0.81 (0.59-1.13)	0.85 (0.62-1.16)
G:G	41	236	0.91 (0.55-1.48)	0.88 (0.55-1.42)	0.91 (0.57-1.44)
rs3740535	354	2075	302/1905		
G:G	194	1144	1.00	1.00	1.00
G:A	128	767	1.07 (0.78-1.48)	1.01 (0.72-1.42)	1.06 (0.78-1.45)
A:G	32	164	0.87 (0.49-1.56)	0.94 (0.56-1.57)	0.88 (0.51-1.51)
rs2228224	342	2038	295/1871		
G:G	189	1135	1.00	1.00	1.00
G:A	134	735	1.17 (0.85-1.62)	1.12 (0.81-1.54)	1.16 (0.85-1.56)
A:G	19	168	0.57 (0.29-1.09)	0.58 (0.31-1.08)	0.61 (0.34-1.10)
rs3729629	362	2076	309/1907		
G:G	185	938	1.00	1.00	1.00
G:C	146	908	0.95 (0.69-1.30)	0.94 (0.69-1.26)	0.94 (0.69-1.28)
C:C	31	230	0.73 (0.42-1.28)	0.71 (0.44-1.15)	0.75 (0.45-1.25)
rs4730775	347	2051	296/1883		
C:C	221	1177	1.00	1.00	1.00
C:T	106	730	**0.71 (0.50-0.99)**	0.82 (0.61-1.12)	0.72 (0.52-1.00)
T:T	20	144	0.56 (0.29-1.07)	0.67 (0.37-1.21)	0.61 (0.34-1.09)
rs4835761	347	2021	302/1862		
A:A	118	698	1.00	1.00	1.00
A:G	167	943	0.97 (0.68-1.36)	0.90 (0.62-1.30)	0.96 (0.69-1.33)
G:G	62	380	0.97 (0.62-1.52)	0.98 (0.59-1.62)	0.97 (0.63-1.47)
rs3750145	297	1917	259/1763		
A:A	179	1204	1.00	1.00	1.00
A:G	96	614	1.21 (0.85-1.72)	1.24 (0.90-1.70)	1.18 (0.84-1.65)
G:G	22	99	1.49 (0.79-2.82)	1.24 (0.63-2.44)	1.39 (0.78-2.48)

Table 3.15 cont. The multiple imputed and semi-Bayesian adjusted association between polymorphisms in stem cell pathway genes and liver cancer in Jiangsu study, 2003-2010, 2014

Stem cell SNP	Case	Control	Adjusted OR co-dominant (95% CI)[a]	Multiple Imputation Adjusted OR co-dominant (95% CI)[a]	Semi-Bayesian Adjusted OR co-dominant (95% PI)[a]
rs2241802	347	2023	296/1858		
G:G	131	682	1.00	1.00	1.00
G:A	153	929	0.90 (0.64-1.26)	0.90 (0.63-1.29)	0.89 (0.64-1.24)
A:A	63	412	**0.58 (0.37-0.91)**	**0.63 (0.43-0.93)**	**0.60 (0.40-0.92)**
rs222851	332	2031	287/1864		
A:A	118	805	1.00	1.00	1.00
A:G	162	910	1.17 (0.83-1.65)	1.25 (0.89-1.74)	1.14 (0.82-1.58)
G:G	52	316	1.19 (0.75-1.91)	1.30 (0.80-2.12)	1.15 (0.74-1.79)
rs1981492	333	2048	289/1882		
G:G	162	1057	1.00	1.00	1.00
G:A	141	787	1.10 (0.80-1.53)	1.09 (0.77-1.54)	1.09 (0.79-1.49)
A:A	30	204	0.75 (0.44-1.28)	0.65 (0.38-1.10)	0.77 (0.47-1.26)
rs1046472	338	2074	292/1904		
C:C	222	1310	1.00	1.00	1.00
C:A	100	661	0.94 (0.67-1.32)	0.95 (0.65-1.39)	0.93 (0.67-1.30)
A:A	16	103	1.06 (0.50-2.24)	1.11 (0.55-2.24)	1.05 (0.54-2.02)
rs3734637	354	2071	301/1902		
A:A	241	1237	1.00	1.00	1.00
A:C	92	704	0.75 (0.53-1.06)	0.73 (0.51-1.05)	0.77 (0.55-1.07)
C:C	21	130	0.67 (0.34-1.31)	0.78 (0.43-1.39)	0.72 (0.40-1.31)
rs11364	322	1824	277/1671		
G:G	227	1296	1.00	1.00	1.00
G:A	84	452	1.21 (0.83-1.75)	1.21 (0.77-1.90)	1.17 (0.82-1.67)
A:A	11	76	0.92 (0.41-2.07)	0.81 (0.32-2.02)	0.93 (0.46-1.86)
rs520692	337	2076	295/1907		
A:A	261	1571	1.00	1.00	1.00
A:G	72	459	0.99 (0.68-1.43)	1.01 (0.72-1.40)	0.98 (0.69-1.40)
G:G	4	46	0.82 (0.23-2.99)	0.85 (0.27-2.66)	0.89 (0.35-2.26)
rs8708	341	2042	291/1875		
A:A	241	1403	1.00	1.00	1.00
A:G	84	552	1.04 (0.73-1.47)	0.93 (0.67-1.29)	1.02 (0.72-1.43)
G:G	16	87	1.17 (0.53-2.60)	1.00 (0.48-2.06)	1.12 (0.56-2.24)
rs1033583	319	1880	275/1724		
A:A	180	1065	1.00	1.00	1.00
A:C	107	684	0.98 (0.69-1.39)	1.01 (0.72-1.43)	0.97 (0.69-1.35)
C:C	32	131	1.37 (0.76-2.47)	1.22 (0.67-2.24)	1.31 (0.76-2.25)

Table 3.15 cont. The multiple imputed and semi-Bayesian adjusted association between polymorphisms in stem cell pathway genes and liver cancer in Jiangsu study, 2003-2010, 2014

Stem cell SNP	Case	Control	Adjusted OR co-dominant (95% CI)[a]	Multiple Imputation Adjusted OR co-dominant (95% CI)[a]	Semi-Bayesian Adjusted OR co-dominant (95% PI)[a]
rs2269700	355	2084	301/1914		
T:T	237	1400	1.00	1.00	1.00
T:C	103	604	0.93 (0.66-1.31)	0.96 (0.70-1.33)	0.93 (0.67-1.29)
C:C	15	80	0.81 (0.37-1.79)	0.89 (0.43-1.83)	0.83 (0.42-1.65)
rs915894	336	2024	292/1870		
C:C	111	601	1.00	1.00	1.00
C:A	163	970	0.91 (0.64-1.29)	0.95 (0.68-1.32)	0.90 (0.64-1.26)
A:A	62	453	0.98 (0.63-1.51)	0.98 (0.66-1.45)	0.97 (0.64-1.46)
rs9972231	287	1778	246/1695		
C:C	204	1224	1.00	1.00	1.00
C:T	71	482	0.85 (0.59-1.25)	0.80 (0.49-1.30)	0.87 (0.60-1.25)
T:T	12	72	0.76 (0.27-2.15)	1.14 (0.50-2.60)	0.84 (0.37-1.90)

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0), pack-year of smoking (continuous), ethanol consumption (gram/week, continuous), HBsAg status (1=positive, 0=negative) and study area (Dafeng=1, Ganyu=2).

Table 3.16 The multiple imputed and semi-Bayesian adjusted association between polymorphisms in GWAS genes and liver cancer in Jiangsu study, n=2612 2003-2010, 2014

GWAS SNP	Case	Control	Adjusted OR co-dominant (95% CI)[a]	Multiple Imputation Adjusted OR co-dominant (95% CI)[a]	Semi-Bayesian Adjusted OR co-dominant (95% PI)[a]
Total	419	2193	(case/control)		
rs4678680	360	2113	309/1943		
T:T	319	1864	1.00	1.00	1.00
T:G	34	232	1.01 (0.61-1.68)	0.96 (0.61-1.51)	1.00 (0.62-1.60)
G:G	7	17	0.86 (0.23-3.17)	0.97 (0.29-3.24)	0.93 (0.36-2.39)
rs9267673	358	2016	304/1903		
C:C	257	1426	1.00	1.00	1.00
C:T	91	524	0.83 (0.59-1.18)	0.83 (0.57-1.21)	0.83 (0.59-1.17)
T:T	10	66	1.43 (0.63-3.28)	1.08 (0.47-2.49)	1.32 (0.64-2.72)
rs9275572	345	2052	297/1894		
G:G	201	1085	1.00	1.00	1.00
G:A	117	774	1.07 (0.77-1.50)	1.07 (0.77-1.47)	1.05 (0.76-1.44)
A:A	27	193	1.32 (0.77-2.28)	1.13 (0.62-2.06)	1.25 (0.75-2.07)

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0), pack-year of smoking (continuous), ethanol consumption (gram/week, continuous), HBsAg status (1=positive, 0=negative) and study area (Dafeng=1, Ganyu=2).

Interaction between polymorphism and environmental factors on liver cancer

SNP and SNP interaction

There were four SNPs showing inverse associations with the liver cancer after controlling for potential confounding variables: rs896849, rs11614913 from microRNA related genes and rs4730775, rs2241803 from stem cell pathway genes. The potential interaction between these SNPs on liver cancer were examined and shown in Table 3.17. Since the measurements of interaction on additive scales ask for both of the factors to be coded as risk factors and the group with lowest risk to be used as the reference group, these SNPs were recoded to show positive associations with liver cancer. After adjusting for confounders, statistical interaction between rs896849 and rs4730775 in the regression model was detected. Comparing to rs896849 T/C+C/C and rs4730775 C/C type, rs896849 (T/C+C/C) and rs4730775 (C/T+T/T) type showed an aOR of 1.63 (95% CI: 0.86-3.09), rs896849 (T/T) and rs4730775 (C/C) type showed an aOR of 1.99 (95% CI: 1.22-3.22), while rs896849 (T/T) and rs4730775 (C/T+T/T) showed an aOR of 1.02 (95% CI: 0.60-1.74). The RERI was -1.60 (95% CI: -3.07-(-0.13)) and the AP was -1.56 (95% CI: -2.81-(-0.32)), both showed inverse interactions on additive scale. And the ratio of odds ratio was 0.31 (95% CI: 0.15-0.66), showing an inverse interaction on multiplicative scale. Moreover, this product term remained existing after the Semi-Bayesian shrinkage (aOR=0.40, 95% PI: 0.21-0.77).

Table 3.17 Interactions between SNPs on liver cancer in Jiangsu study, n=2612 2003-2010, 2014

SNP	SNP	Cases	Controls	aOR (95% CI)[a]	Interaction (95% CI)	SB-adjusted ROR (95% PI)
Between microRNA SNPs						
rs896849	rs11614913					
T:C+C:C	T:C+C:C	47	391	1.00	ROR: 1.20 (0.55-2.61)	1.15 (0.58-2.26)
T:C+C:C	T:T	27	169	1.33 (0.67-2.61)	RERI: 0.40 (-0.59-1.39)	
T:T	T:C+C:C	151	984	1.21 (0.76-1.94)	AP: 0.21 (-0.30-0.71)	
T:T	T:T	95	446	**1.93 (1.16-3.21)**	S: 1.74 (0.26-11.60)	
Between stem cell SNPs						
rs4730775	rs2241802					
T:C+C:C	G:A+A:A	74	560	1.00	ROR: 0.95 (0.49-1.86)	0.96 (0.53-1.75)
T:C+C:C	G:G	50	289	1.31 (0.77-2.23)	RERI: 0.05 (-0.88-0.99)	
T:T	G:A+A:A	134	756	1.47 (0.97-2.22)	AP: 0.03 (-0.48-0.54)	
T:T	G:G	78	371	1.83 (1.15-2.91)	S: 1.07 (0.32-3.51)	
Between microRNA and stem cell SNPs						
rs11614913	rs4730775					
T:C+C:C	C:T+T:T	73	599	1.00	ROR: 1.35 (0.67-2.72)	1.27 (0.68-2.37)
T:C+C:C	C:C	123	772	1.33 (0.88-2.02)	RERI: 0.68 (-0.32-1.67)	
T:T	C:T+T:T	41	253	1.26 (0.72-2.21)	AP: 0.30 (-0.10-0.70)	
T:T	C:C	77	347	2.26 (1.42-3.59)	S: 2.15 (0.46-10.03)	
rs11614913	rs2241802					
T:C+C:C	G:A+A:A	126	901	1.00	ROR: 1.55 (0.78-3.09)	1.42 (0.77-2.63)
T:C+C:C	G:G	69	449	1.05 (0.68-1.61)	RERI: 0.74 (-0.29-1.76)	
T:T	G:A+A:A	68	400	1.25 (0.82-1.92)	AP: 0.36 (-0.04-0.77)	
T:T	G:G	51	199	**2.04 (1.26-3.32)**	S: 3.42 (0.29-40.76)	

Table 3.17 cont. Interactions between SNPs on liver cancer in Jiangsu study, n=2612 2003-2010, 2014

SNP	SNP	Cases	Controls	aOR (95% CI)[a]	Interaction (95% CI)	SB-adjusted ROR (95% PI)
rs896849	rs4730775					
T:C+C:C	C:C	42	333	1.00	**ROR: 0.31 (0.15-0.66)**	**0.40 (0.21-0.77)**
T:C+C:C	C:T+T:T	36	236	1.63 (0.86-3.09)	**RERI: -1.60 (-3.07-(-0.13))**	
T:T	C:C	170	826	**1.99 (1.22-3.22)**	**AP: -1.56 (-2.81-(-0.32))**	
T:T	C:T+T:T	83	627	1.02 (0.60-1.74)	S: 0.01 (0.00-+++)	
rs896849	rs2241802					
T:C+C:C	G:G	23	196	1.00	ROR: 0.63 (0.29-1.36)	0.70 (0.36-1.38)
T:C+C:C	G:A+A:A	57	368	1.15 (0.58-2.26)	RERI: -0.63 (-1.80-0.54)	
T:T	G:G	104	474	1.74 (0.92-3.27)	AP: -0.50 (-1.28-0.27)	
T:T	G:A+A:A	148	954	1.26 (0.68-2.30)	S: 0.29 (0.06-1.49)	

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0), pack-year of smoking (continuous), ethanol consumption (gram/week, continuous), HBsAg status (1=positive, 0=negative) and study area (Dafeng=1, Ganyu=2).

Interaction between SNPs and environmental risk factors

Stratified analysis by major risk factors

Polymorphism in microRNA related genes in stratified analysis

Stratified analyses of associations between liver cancer and polymorphism in microRNA related genes were further performed. Models were fitted stratifying by HBsAg status, drinking status and smoking status which were major risk factors (Table 3.18).

First, the inverse association between rs896849 (C/C vs. T/T) mainly existed among those HBsAg positive participants with an aOR of 0.17 (95% CI: 0.03-0.91) and didn't show such association among HBsAg negatives. While the inverse association between rs11614913 (C/C vs. T/T) remained significant when stratified by HBsAg status: looking into those HBsAg negative participants, rs11614913 (C/C vs. T/T) showed an aOR of 0.50 (0.28-0.91); while looking into HBsAg positives, rs11614913 (C/C vs. T/T) showed an aOR of 0.41 (95% CI: 0.18-0.94). Moreover, rs12828 (WWOX gene), rs2910164 (pre-miR-146a gene) and rs4072391 (IL6R gene) which did not show significant association in the overall analyses showed associations among HBsAg positives in the stratified one. Rs12828 (G/A vs. G/G) showed an inverse association with aOR of 0.52 (95% CI: 0.28-0.99), and rs12828 (A/A vs G/G) also showed a borderline association with aOR of 0.47 (95% CI: 0.20-1.09). Rs2910164 (G/G vs C/C) showed an inverse association with aOR of 0.42 (94% CI: 0.18-0.98) and rs4072391 (T/T vs. C/C) showed an inverse association with aOR of 0.22 (95% CI: 0.05-0.95). Meanwhile, rs895819 (miR-27 gene, T/C vs. T/T) showed an inverse association with aOR of 0.64 (95% CI: 0.42-1.00) among HBsAg negatives.

Second, when stratified by drinking status, the inverse association between rs896849 (C/C vs. T/T, aOR=0.06, 95% CI= 0.01-0.52), rs11614913 (C/C vs. T/T, aOR=0.39, 95% CI=0.21-0.74) and liver cancer remained significant among those ever drinkers. Also, rs7813 (Gemin4 gene, C/C vs. T/T) showed an inverse association with an aOR of 0.26 (95% CI: 0.08-0.88) among those never drinkers.

Third, when stratified by smoking status, the inverse association between rs896849 (T/C vs. T/T) appeared with an aOR of 0.52 (95% CI: 0.32-0.85) among ever smokers. Also, rs11614913 (T/C vs. T/T) appeared to be inversely associated with the risk reporting an aOR of 0.62 (95% CI: 0.40-0.96), while the association between (C/C vs. T/T) remained significant with an aOR of 0.33 (95% CI: 0.18-0.60) among ever smokers. Moreover, rs2910164 (G/G vs. C/C) showed inverse association with an aOR of 0.52 (95% CI: 0.28-0.97) among ever smokers and rs2126852 (RCHY1 gene, A/G vs. A/A) showed an inverse association with an aOR of 0.50 (95% CI: 0.28-0.89) among those never smokers.

Stem cell pathway polymorphism in stratified analysis

Stratified analyses of associations between liver cancer and stem cell polymorphism were also further performed. Models were fitted stratifying by HBsAg status, drinking status and smoking status which were major risk factors (Table 3.19).

First, the inverse associations between rs4730775 (T/T vs. C/C), rs2241802 (A/A vs. G/G) and liver cancer risk no longer existed after stratified by HBsAg status. However, inverse associations between rs3130932 (Oct4 gene, G/G vs. T/T, aOR=0.42, 95% CI: 0.18-0.99),

rs2228224 (GLI1 gene, A/A vs G/G, aOR=0.13, 95% CI: 0.04-0.40) and rs3734637 (HEY2 gene, A/C vs. A/A, aOR=0.28, 95% CI: 0.09-0.85) were observed with liver cancer among HBsAg positive participants. Meanwhile, the inverse association between rs3734637 with liver cancer also existed among HBsAg negatives, showing an aOR of 0.56, 95% CI: 0.35-0.88 comparing A/C to A/A types. Apart from the inverse associations, rs222851 (DVL2 gene, A/G vs. A/A, aOR=1.99, 95% CI: 1.07-3.71) and rs8708 (HES2 gene, G/G vs. A/A, aOR=6.31, 95% CI: 1.10-36.34) were both observed to be positively associated with liver cancer among HBsAg positive participants.

Second, when stratified by drinking status, inverse association between rs3130932 (G/G vs. T/T, aOR=0.42, 95% CI= 0.18-0.98), rs3734637 (A/C vs. A/A, aOR=0.57, 95% CI=0.33-0.98) and liver cancer were observed among those never drinkers. Also, rs1981492 (AXIN1 gene, G/A vs. G/G) showed a positive association with an aOR of 1.71 (95% CI: 1.01-2.88) among those never drinkers. No significant association was observed among drinkers in this analysis.

Third, when stratified by smoking status, inverse association between rs2228224 (A/A vs. G/G, aOR= 0.29 (95% CI: 0.11-0.75), rs2241802 (FZD3 gene, A/A vs. G/G, aOR=0.45, 95% CI: 0.25-0.81) and rs3734637 (A/C vs. A/A, aOR=0.64, 95% CI: 0.41-0.99) and liver cancer were observed among ever smokers. Also, rs222851 (DVL2 gene, A/A, A/G and G/G) showed positive associations among never smokers. Comparing to A/A type, A/G showed an aOR of 1.77 (95% CI: 1.01-3.11) and G/G showed an aOR of 2.28 (95% CI: 1.10-4.71).

Table 3.18 The stratified association between microRNA polymorphisms and liver cancer in Jiangsu study, n=2612 2003-2010, 2014

	Adjusted ORs in co-dominant models (95% CI)					
SNP	HBsAg negative	HBsAg positive	Never drink	Ever drink	Never smoke	Ever smoke
rs1804429	114/1779	184/114	114/814	184/1079	103/769	209/1171
T:T	1.00	1.00	1.00	1.00	1.00	1.00
T:G	1.30 (0.76-2.24)	0.56 (0.22-1.42)	0.65 (0.29-1.46)	1.42 (0.77-2.62)	0.72 (0.34-1.52)	1.31 (0.71-2.42)
G:G	na	na	na	1.17 (0.09-16.00)	na	0.66 (0.05-9.46)
rs10519613	109/1782	187/114	113/813	183/1083	101/774	207/1170
C:C	1.00	1.00	1.00	1.00	1.00	1.00
C:A	1.12 (0.70-1.77)	0.72 (0.39-1.35)	0.83 (0.48-1.42)	1.06 (0.66-1.70)	0.79 (0.46-1.37)	1.12 (0.71-1.76)
A:A	1.29 (0.74-2.24)	0.68 (0.32-1.43)	0.96 (0.48-1.90)	1.18 (0.67-2.09)	0.88 (0.44-1.78)	1.25 (0.72-2.16)
rs12828	112/1738	178/115	109/798	181/1055	99/766	204/1138
G:G	1.00	1.00	1.00	1.00	1.00	1.00
G:A	0.99 (0.65-1.52)	**0.52 (0.28-0.99)**	0.77 (0.46-1.29)	0.96 (0.62-1.51)	0.91 (0.52-1.58)	0.83 (0.54-1.26)
A:A	1.08 (0.61-1.94)	0.47 (0.20-1.09)	0.76 (0.35-1.67)	1.10 (0.62-1.98)	1.40 (0.70-2.78)	0.69 (0.38-1.28)
rs896849	115/1794	184/117	113/826	186/1085	104/776	206/1185
T:T	1.00	1.00	1.00	1.00	1.00	1.00
T:C	0.95 (0.60-1.49)	0.75 (0.39-1.45)	1.23 (0.71-2.15)	0.63 (0.38-1.05)	1.63 (0.94-2.85)	**0.52 (0.32-0.85)**
C:C	0.36 (0.05-2.66)	**0.17 (0.03-0.91)**	1.52 (0.34-6.79)	**0.06 (0.01-0.52)**	**na**	0.34 (0.09-1.34)
rs11614913	106/1744	175/111	109/798	172/1057	97/758	195/1145
T:T	1.00	1.00	1.00	1.00	1.00	1.00
T:C	0.68 (0.44-1.06)	0.93 (0.49-1.76)	0.85 (0.48-1.48)	0.75 (0.48-1.19)	1.12 (0.63-1.98)	**0.62 (0.40-0.96)**
C:C	**0.50 (0.28-0.91)**	**0.41 (0.18-0.94)**	0.60 (0.29-1.27)	**0.39 (0.21-0.74)**	0.71 (0.32-1.55)	**0.33 (0.18-0.60)**
rs2910164	116/1785	177/116	111/826	182/1075	103/781	204/1169
C:C	1.00	1.00	1.00	1.00	1.00	1.00
C:G	0.98 (0.65-1.49)	0.77 (0.40-1.47)	0.93 (0.55-1.57)	1.01 (0.65-1.57)	0.77 (0.45-1.31)	1.03 (0.67-1.58)
G:G	0.80 (0.44-1.44)	**0.42 (0.18-0.98)**	0.67 (0.33-1.38)	0.67 (0.35-1.26)	0.94 (0.48-1.85)	**0.52 (0.28-0.97)**
rs895819	116/1795	183/115	114/828	185/1082	103/779	208/1181
T:T	1.00	1.00	1.00	1.00	1.00	1.00
T:C	**0.64 (0.42-1.00)**	0.87 (0.48-1.59)	0.74 (0.44-1.24)	0.72 (0.46-1.12)	0.79 (0.46-1.34)	0.75 (0.49-1.14)
C:C	1.16 (0.60-2.24)	0.40 (0.13-1.20)	0.78 (0.33-1.86)	0.97 (0.43-2.16)	0.59 (0.23-1.49)	1.15 (0.55-2.43)

Table 3.18 cont. The stratified association between microRNA polymorphisms and liver cancer in Jiangsu study, n=2612 2003-2010, 2014

	Adjusted ORs in co-dominant models (95% CI)					
SNP	HBsAg negative	HBsAg positive	Never drink	Ever drink	Never smoke	Ever smoke
rs7372209	109/1791	181/116	112/827	178/1080	102/782	202/1173
C:C	1.00	1.00	1.00	1.00	1.00	1.00
C:T	0.79 (0.52-1.21)	1.13 (0.62-2.08)	0.68 (0.40-1.14)	0.99 (0.63-1.55)	0.87 (0.51-1.47)	0.87 (0.57-1.34)
T:T	0.87 (0.43-1.75)	1.18 (0.49-2.85)	0.77 (0.33-1.80)	1.05 (0.53-2.08)	1.22 (0.56-2.69)	0.69 (0.35-1.38)
rs3742330	117/1805	184/120	117/829	184/1096	108/788	207/1187
A:A	1.00	1.00	1.00	1.00	1.00	1.00
A:G	0.90 (0.60-1.35)	0.88 (0.48-1.62)	0.91 (0.55-1.51)	0.82 (0.54-1.25)	0.80 (0.48-1.31)	1.05 (0.69-1.58)
G:G	0.63 (0.32-1.24)	0.57 (0.24-1.33)	0.69 (0.32-1.51)	0.54 (0.26-1.11)	0.66 (0.29-1.52)	0.64 (0.33-1.23)
rs4961280	110/1747	181/111	113/797	178/1061	102/753	203/1153
C:C	1.00	1.00	1.00	1.00	1.00	1.00
C:A	0.87 (0.53-1.43)	1.00 (0.51-1.97)	0.71 (0.37-1.36)	0.99 (0.61-1.61)	1.19 (0.64-2.21)	0.74 (0.46-1.21)
A:A	0.42 (0.06-3.21)	1.10 (0.25-4.79)	0.54 (0.09-3.13)	0.83 (0.21-3.21)	0.53 (0.09-2.96)	0.73 (0.19-2.84)
rs14035	108/1712	168/110	110/786	166/1036	97/742	190/1122
C:C	1.00	1.00	1.00	1.00	1.00	1.00
C:T	1.12 (0.71-1.74)	0.80 (0.41-1.57)	1.16 (0.67-2.02)	0.95 (0.58-1.53)	1.19 (0.66-2.13)	0.87 (0.56-1.36)
T:T	1.66 (0.75-3.67)	0.44 (0.09-2.19)	0.81 (0.22-2.99)	1.46 (0.60-3.57)	1.21 (0.39-3.78)	1.11 (0.43-2.90)
rs197412	112/1738	188/115	114/797	186/1056	107/756	205/1147
T:T	1.00	1.00	1.00	1.00	1.00	1.00
T:C	0.91 (0.60-1.41)	0.92 (0.51-1.67)	1.00 (0.59-1.68)	0.83 (0.54-1.28)	0.92 (0.55-1.54)	0.90 (0.59-1.38)
C:C	1.75 (0.99-3.09)	0.66 (0.26-1.68)	0.85 (0.39-1.87)	1.77 (0.92-3.42)	0.75 (0.32-1.75)	1.83 (0.98-3.41)
rs2740348	109/1734	170/109	108/796	171/1047	97/749	194/1142
G:G	1.00	1.00	1.00	1.00	1.00	1.00
G:C	1.12 (0.67-1.87)	1.41 (0.67-2.96)	1.33 (0.72-2.47)	1.06 (0.62-1.82)	0.99 (0.50-1.96)	1.18 (0.72-1.93)
C:C	na	0.50 (0.07-3.68)	0.35 (0.04-3.32)	0.46 (0.08-2.79)	0.27 (0.03-2.35)	0.50 (0.08-3.40)
rs7813	113/1737	178/107	114/794	177/1050	104/749	200/1142
T:T	1.00	1.00	1.00	1.00	1.00	1.00
T:C	1.15 (0.77-1.73)	1.10 (0.60-2.02)	1.30 (0.78-2.16)	0.97 (0.63-1.51)	0.90 (0.53-1.52)	1.31 (0.87-1.98)
C:C	0.68 (0.31-1.47)	0.46 (0.15-1.37)	**0.26 (0.08-0.88)**	0.83 (0.40-1.71)	0.55 (0.20-1.48)	0.67 (0.32-1.41)

Table 3.18 cont. The stratified association between microRNA polymorphisms and liver cancer in Jiangsu study, n=2612 2003-2010, 2014

	Adjusted ORs in co-dominant models (95% CI)					
SNP	HBsAg negative	HBsAg positive	Never drink	Ever drink	Never smoke	Ever smoke
rs11077	109/1776	183/117	113/819	179/1074	101/771	205/1171
A:A	1.00	1.00	1.00	1.00	1.00	1.00
A:C	1.25 (0.74-2.13)	0.62 (0.29-1.34)	0.79 (0.39-1.58)	1.20 (0.68-2.11)	1.77 (0.91-3.46)	0.72 (0.41-1.27)
C:C	0.97 (0.12-7.69)	1.15 (0.06-24.01)	0.33 (0.03-4.43)	3.45 (0.45-26.72)	1.74 (0.16-18.80)	0.45 (0.04-5.03)
rs9266	112/1794	188/114	115/827	185/1081	104/782	210 /1173
C:C	1.00	1.00	1.00	1.00	1.00	1.00
C:T	1.11 (0.74-1.67)	0.59 (0.32-1.09)	1.11 (0.67-1.85)	0.78 (0.50-1.22)	1.05 (0.63-1.77)	0.88 (0.58-1.34)
T:T	0.32 (0.08-1.34)	0.47 (0.13-1.73)	0.37 (0.10-1.39)	0.63 (0.22-1.80)	0.32 (0.06-1.78)	0.54 (0.21-1.37)
rs4072391	111/1791	186/116	113/821	184/1086	104/784	207/1172
C:C	1.00	1.00	1.00	1.00	1.00	1.00
C:T	0.87 (0.50-1.51)	1.44 (0.67-3.10)	0.94 (0.49-1.80)	1.00 (0.57-1.78)	1.03 (0.53-2.01)	1.07 (0.63-1.81)
T:T	1.37 (0.31-6.11)	**0.22 (0.05-0.95)**	0.20 (0.02-2.39)	0.91 (0.24-3.50)	0.47 (0.09-2.47)	0.89 (0.21-3.86)
rs2126852	113/1738	180/112	113/799	180/1051	100/755	206/1141
A:A	1.00	1.00	1.00	1.00	1.00	1.00
A:G	0.93 (0.61-1.41)	0.68 (0.37-1.25)	1.00 (0.59-1.70)	0.77 (0.49-1.21)	**0.50 (0.28-0.89)**	1.22 (0.81-1.84)
G:G	0.81 (0.39-1.68)	0.87 (0.34-2.21)	1.09 (0.47-2.52)	0.92 (0.46-1.84)	0.49 (0.19-1.25)	1.32 (0.67-2.62)
rs7760	107/1780	179/118	112/821	174/1251	102/774	197/1172
T:T	1.00	1.00	1.00	1.00	1.00	1.00
T:G	1.24 (0.78-1.98)	0.92 (0.45-1.84)	1.42 (0.80-2.51)	0.85 (0.50-1.44)	1.74 (0.96-3.14)	0.71 (0.43-1.17)
G:G	0.47 (0.06-3.54)	0.28 (0.04-1.88)	0.50 (0.04-6.39)	0.31 (0.06-1.54)	0.64 (0.11-3.60)	0.19 (0.03-1.35)
rs2075993	108/1735	187/113	114/794	181/1054	105/745	202/1150
G:G	1.00	1.00	1.00	1.00	1.00	1.00
G:A	1.38 (0.88-2.14)	1.24 (0.66-2.32)	1.17 (0.68-2.03)	1.51 (0.95-2.41)	1.51 (0.87-2.64)	138 (0.88-2.17)
A:A	0.96 (0.51-1.80)	1.80 (0.80-4.06)	1.12 (0.57-2.19)	1.29 (0.69-2.43)	1.04 (0.51-2.12)	1.56 (0.87-2.81)
rs3801790	113/1769	185/114	114/809	184/1074	102/765	211/1165
A:A	1.00	1.00	1.00	1.00	1.00	1.00
A:G	0.93 (0.61-1.41)	1.11 (0.59-2.08)	0.93 (0.56-1.56)	1.02 (0.65-1.60)	1.16 (0.68-1.98)	0.86 (0.56-1.32)
G:G	0.71 (0.38-1.32)	0.53 (0.24-1.15)	0.49 (0.23-1.04)	0.82 (0.44-1.52)	0.55 (0.26-1.18)	0.79 (0.43-1.42)

Table 3.18 cont. The stratified association between microRNA polymorphisms and liver cancer in Jiangsu study, n=2612 2003-2010, 2014

	Adjusted ORs in co-dominant models (95% CI)					
SNP	HBsAg negative	HBsAg positive	Never drink	Ever drink	Never smoke	Ever smoke
rs3929	116/1818	189/118	117/833	188/1103	104/793	215/1196
G:G	1.00	1.00	1.00	1.00	1.00	1.00
G:C	0.97 (0.64-1.48)	0.81 (0.46-1.46)	1.04 (0.62-1.75)	0.88 (0.56-1.37)	1.54 (0.92-2.58)	0.75 (0.49-1.14)
C:C	0.81 (0.28-2.31)	5.34 (0.56-50.89)	1.31 (0.39-4.40)	1.16 (0.44-3.11)	1.62 (0.46-5.78)	1.22 (0.48-3.07)
rs2292305	111/1754	178/113	113/804	176/1063	101/759	199/1155
T:T	1.00	1.00	1.00	1.00	1.00	1.00
T:C	1.26 (0.83-1.90)	0.65 (0.36-1.19)	0.87 (0.51-1.46)	1.14 (0.74-1.77)	0.68 (0.40-1.18)	1.32 (0.87-2.00)
C:C	0.71 (0.35-1.45)	0.75 (0.28-1.98)	1.04 (0.47-2.28)	0.64 (0.31-1.33)	1.08 (0.49-2.38)	0.61 (0.30-1.27)
rs2953	113/1799	185/120	115/829	183/1090	106/787	205/1181
T:T	1.00	1.00	1.00	1.00	1.00	1.00
T:G	0.93 (0.61-1.41)	0.86 (0.49-1.51)	1.00 (0.61-1.65)	0.73 (0.47-1.14)	0.88 (0.53-1.45)	0.75 (0.49-1.15)
G:G	1.36 (0.67-2.75)	0.73 (0.24-2.22)	1.33 (0.54-3.27)	0.88 (0.37-2.08)	0.66 (0.22-1.92)	1.35 (0.64-2.82)

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0), pack-year of smoking (continuous, except for when stratified by tobacco smoking), ethanol consumption (gram/week, continuous, except for when stratified by alcohol drinking status), HBsAg status (1=positive, 0=negative, except for when stratified by HBsAg status) and study area (Dafeng=1, Ganyu=2).

Table 3.19 The stratified association between polymorphisms in stem cell pathway and liver cancer in Jiangsu study, n=2612 2003-2010, 2014

	Adjusted ORs in co-dominant models (95% CI)					
SNP	HBsAg negative	HBsAg positive	Never drink	Ever drink	Never smoke	Ever smoke
rs6815391	110/1757	183/110	110/805	183/1062	101/753	205/1161
T:T	1.00	1.00	1.00	1.00	1.00	1.00
T:C	1.28 (0.84-1.97)	1.37 (0.75-2.51)	1.36 (0.79-2.33)	1.13 (0.73-1.75)	1.43 (0.83-2.46)	1.07 (0.70-1.63)
C:C	1.31 (0.70-2.44)	0.86 (0.35-2.11)	1.54 (0.77-3.08)	0.68 (0.31-1.49)	1.39 (0.65-2.97)	0.82 (0.42-1.58)
rs13409	109/1793	174/111	113/827	170/1077	100/770	194/1184
C:C	1.00	1.00	1.00	1.00	1.00	1.00
C:T	0.82 (0.52-1.28)	1.64 (0.87-3.09)	1.75 (1.00-3.08)	0.68 (0.43-1.08)	1.39 (0.79-2.46)	0.85 (0.55-1.32)
T:T	1.17 (0.70-1.96)	1.10 (0.49-2.43)	1.94 (0.99-3.82)	0.86 (0.48-1.52)	1.40 (0.69-2.82)	1.12 (0.65-1.93)
rs3130932	118/1788	182/118	116/823	184/1083	104/780	210/1173
T:T	1.00	1.00	1.00	1.00	1.00	1.00
G:T	0.71 (0.46-1.08)	1.11 (0.61-2.03)	0.77 (0.47-1.28)	0.88 (0.57-1.36)	0.90 (0.53-1.51)	0.75 (0.50-1.13)
G:G	1.35 (0.77-2.37)	**0.42 (0.18-0.99)**	**0.42 (0.18-0.98)**	1.67 (0.90-3.11)	0.63 (0.28-1.44)	0.96 (0.51-1.79)
rs3740535	116/1789	186/116	113/820	189/1085	104/783	212/1171
G:G	1.00	1.00	1.00	1.00	1.00	1.00
G:A	1.35 (0.91-2.00)	0.72 (0.40-1.28)	1.11 (0.68-1.84)	1.00 (0.65-1.54)	1.40 (0.84-2.32)	0.90 (0.60-1.36)
A:G	0.58 (0.22-1.49)	0.90 (0.31-2.55)	0.80 (0.29-2.23)	0.92 (0.45-1.89)	1.68 (0.67-4.23)	0.76 (0.38-1.53)
rs2228224	113/1755	182/116	114/806	181/1065	104/766	205/1153
G:G	1.00	1.00	1.00	1.00	1.00	1.00
G:A	1.45 (0.97-2.18)	0.85 (0.46-1.56)	1.44 (0.86-2.40)	1.00 (0.65-1.54)	1.51 (0.91-2.52)	0.95 (0.63-1.44)
A:A	1.35 (0.66-2.76)	**0.13 (0.04-0.40)**	1.55 (0.65-3.74)	0.21 (0.07-0.61)	1.06 (0.43-2.65)	**0.29 (0.11-0.75)**
rs3729629	119/1790	190/117	116/826	193/1081	107/778	216/1178
G:G	1.00	1.00	1.00	1.00	1.00	1.00
G:C	1.00 (0.67-1.49)	0.91 (0.51-1.60)	0.83 (0.50-1.36)	1.05 (0.69-1.59)	0.64 (0.38-1.07)	1.13 (0.76-1.69)
C:C	0.85 (0.43-1.68)	0.50 (0.18-1.43)	0.64 (0.25-1.66)	0.85 (0.42-1.69)	0.62 (0.26-1.48)	0.77 (0.39-1.53)
rs4730775	114/1768	182/115	113/813	183/1070	102/763	208/1169
C:C	1.00	1.00	1.00	1.00	1.00	1.00
C:T	0.75 (0.50-1.15)	0.61 (0.32-1.17)	0.65 (0.37-1.13)	0.73 (0.47-1.14)	0.58 (0.33-1.02)	0.89 (0.59-1.35)
T:T	0.46 (0.16-1.30)	0.56 (0.19-1.62)	0.53 (0.19-1.48)	0.54 (0.22-1.31)	0.49 (0.18-1.35)	0.68 (0.30-1.53)
rs4835761	112/1750	190/112	115/802	187/1060	108/759	207/1149
A:A	1.00	1.00	1.00	1.00	1.00	1.00
A:G	0.98 (0.63-1.53)	0.97 (0.52-180)	1.14 (0.68-1.93)	0.84 (0.52-1.34)	0.87 (0.51-1.49)	0.94 (0.60-1.46)
G:G	1.16 (0.67-2.00)	0.81 (0.37-1.78)	0.72 (0.35-1.51)	1.20 (0.67-2.12)	0.82 (0.41-1.65)	1.10 (0.63-1.91)

Table 3.19 cont. The stratified association between polymorphisms in stem cell pathway and liver cancer in Jiangsu study, 2003-2010, 2014

	Adjusted ORs in co-dominant models (95% CI)					
SNP	HBsAg negative	HBsAg positive	Never drink	Ever drink	Never smoke	Ever smoke
rs3750145	102/1658	157/105	105/756	154/1007	93/710	177/1096
A:A	1.00	1.00	1.00	1.00	1.00	1.00
A:G	1.10 (0.71-1.69)	1.28 (0.67-2.47)	1.13 (0.65-1.95)	1.37 (0.86-2.18)	1.65 (0.95-2.84)	0.98 (0.62-1.55)
G:G	1.08 (0.44-2.63)	2.04 (0.65-6.44)	1.45 (0.50-4.26)	1.67 (0.75-3.74)	0.98 (0.32-3.02)	1.94 (0.89-4.22)
rs2241802	114/1747	182/111	112/808	184/1050	104/764	206/1141
G:G	1.00	1.00	1.00	1.00	1.00	1.00
G:A	0.90 (0.59-1.37)	0.91 (0.48-1.74)	0.87 (0.50-1.52)	0.87 (0.56-1.35)	0.71 (0.41-1.25)	1.07 (0.69-1.64)
A:A	0.55 (0.29-1.02)	0.59 (0.29-1.24)	0.56 (0.28-1.12)	0.57 (0.31-1.04)	0.65 (0.32-1.33)	**0.45 (0.25-0.81)**
rs222851	110/1750	177/114	113/811	174/1053	101/759	198/1153
A:A	1.00	1.00	1.00	1.00	1.00	1.00
A:G	0.93 (0.60-1.43)	**1.99 (1.07-3.71)**	1.60 (0.93-2.74)	0.92 (0.59-1.45)	**1.77 (1.01-3.11)**	0.97 (0.63-1.49)
G:G	1.12 (0.63-1.98)	1.65 (0.69-3.91)	1.64 (0.79-3.38)	0.87 (0.46-1.66)	**2.28 (1.10-4.71)**	0.78 (0.42-1.46)
rs1981492	111/1765	178/117	110/816	179/1066	100/765	200/1164
G:G	1.00	1.00	1.00	1.00	1.00	1.00
G:A	1.15 (0.76-1.74)	0.96 (0.53-1.75)	**1.71 (1.01-2.88)**	0.78 (0.51-1.20)	1.01 (0.59-1.72)	1.11 (0.74-1.68)
A:A	0.78 (0.38-1.60)	0.55 (0.22-1.35)	1.24 (0.57-2.70)	0.50 (0.23-1.09)	0.80 (0.36-1.80)	0.69 (0.33-1.41)
rs1046472	110/1786	182/118	112/823	180/1081	103/778	201/1176
C:C	1.00	1.00	1.00	1.00	1.00	1.00
C:A	1.02 (0.66-1.57)	0.75 (0.41-1.36)	0.96 (0.58-1.60)	0.92 (0.58-1.46)	0.90 (0.52-1.55)	0.89 (0.58-1.37)
A:A	1.21 (0.52-2.76)	1.05 (0.21-5.34)	0.68 (0.18-2.59)	1.31 (0.51-3.36)	1.38 (0.44-4.34)	1.08 (0.43-2.69)
rs3734637	113/1788	188/114	115/816	186/1086	107/778	208/1173
A:A	1.00	1.00	1.00	1.00	1.00	1.00
A:C	**0.56 (0.35-0.88)**	1.00 (0.53-1.88)	**0.57 (0.33-0.98)**	0.87 (0.55-1.36)	0.76 (0.44-1.31)	**0.64 (0.41-0.99)**
C:C	0.87 (0.39-1.96)	**0.28 (0.09-0.85)**	0.93 (0.34-2.53)	0.48 (0.19-1.23)	0.94 (0.36-2.49)	0.49 (0.20-1.21)
rs11364	104/1578	173/93	107/731	170/940	97/681	192/1035
G:G	1.00	1.00	1.00	1.00	1.00	1.00
G:A	1.21 (0.77-1.91)	1.43 (0.68-2.98)	1.50 (0.85-2.65)	0.95 (0.57-1.58)	1.31 (0.72-2.41)	1.13 (0.71-1.80)
A:A	0.78 (0.24-2.58)	1.45 (0.40-5.28)	1.04 (0.33-3.28)	0.85 (0.26-2.79)	1.50 (0.52-4.27)	0.49 (0.13-1.85)
rs520692	112/1791	183/116	114/822	181/1085	105/775	201/1180
A:A	1.00	1.00	1.00	1.00	1.00	1.00
A:G	0.92 (0.58-1.48)	1.15 (0.58-2.28)	1.08 (0.59-1.97)	0.89 (0.55-1.44)	0.73 (0.39-1.38)	1.29 (0.81-2.03)
G:G	0.77 (0.18-3.27)	0.78 (0.04-14.38)	1.55 (0.28-8.48)	0.38 (0.04-3.22)	0.43 (0.05-3.74)	1.31 (0.24-7.06)

Table 3.19 cont. The stratified association between polymorphisms in stem cell pathway and liver cancer in Jiangsu study, 2003-2010, 2014

	Adjusted ORs in co-dominant models (95% CI)					
SNP	HBsAg negative	HBsAg positive	Never drink	Ever drink	Never smoke	Ever smoke
rs8708	111/1762	180/113	111/819	180/1056	97/762	208/1161
A:A	1.00	1.00	1.00	1.00	1.00	1.00
A:G	0.86 (0.54-1.36)	1.55 (0.80-3.00)	1.19 (0.69-2.06)	0.93 (0.58-1.50)	1.18 (0.66-2.11)	0.83 (0.53-1.31)
G:G	0.65 (0.20-2.14)	**6.31 (1.10-36.34)**	2.24 (0.82-6.12)	0.55 (0.14-2.21)	1.69 (0.58-4.92)	0.86 (0.28-2.62)
rs1033583	102/1621	173/103	107/747	168/977	96/707	190/1061
A:A	1.00	1.00	1.00	1.00	1.00	1.00
A:C	1.01 (0.65-1.57)	0.94 (0.50-1.74)	1.39 (0.81-2.40)	0.73 (0.46-1.15)	1.25 (0.72-2.19)	0.80 (0.51-1.24)
C:C	1.71 (0.85-3.45)	0.72 (0.26-2.05)	1.29 (0.47-3.53)	1.36 (0.64-2.89)	0.93 (0.32-2.73)	1.44 (0.71-2.92)
rs2269700	116/1794	185/120	115/828	186/1086	105/785	210/1178
T:T	1.00	1.00	1.00	1.00	1.00	1.00
T:C	0.77 (0.49-1.21)	1.51 (0.82-2.80)	0.84 (0.49-1.44)	1.01 (0.64-1.58)	0.94 (0.54-1.64)	0.93 (0.61-1.42)
C:C	1.45 (0.56-3.78)	0.37 (0.10-1.32)	0.46 (0.06-3.66)	0.89 (0.36-2.18)	0.69 (0.19-2.42)	0.97 (0.35-2.67)
rs915894	111/1758	181/112	112/803	180/1067	102/761	201/1158
C:C	1.00	1.00	1.00	1.00	1.00	1.00
C:A	0.81 (0.52-1.27)	1.29 (0.68-2.45)	0.86 (0.49-1.51)	0.92 (0.58-1.47)	0.72 (0.41-1.25)	0.99 (0.63-1.56)
A:A	1.01 (0.59-1.71)	1.02 (0.44-2.36)	1.09 (0.55-2.15)	0.88 (0.49-1.58)	0.85 (0.43-1.68)	0.97 (0.55-1.70)
rs9972231	94/1590	152/105	98/727	148/968	81/684	177/1053
C:C	1.00	1.00	1.00	1.00	1.00	1.00
C:T	0.92 (0.57-1.50)	0.77 (0.39-1.53)	0.57 (0.30-1.06)	1.06 (0.65-1.71)	0.64 (0.33-1.24)	0.94 (0.59-1.50)
T:T	0.79 (0.23-2.65)	1.07 (0.14-8.08)	1.24 (0.37-4.12)	0.29 (0.04-2.34)	1.96 (0.60-6.46)	0.26 (0.05-1.41)

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0), pack-year of smoking (continuous, except for when stratified by tobacco smoking), ethanol consumption (gram/week, continuous, except for when stratified by alcohol drinking status), HBsAg status (1=positive, 0=negative, except for when stratified by HBsAg status) and study area (Dafeng=1, Ganyu=2).

Table 3.20 The adjusted and stratified association between polymorphisms in GWAS SNPs and liver cancer in Jiangsu study, n=2612 2003-2010, 2014

	Adjusted ORs in co-dominant models (95% CI)					
SNP	HBsAg negative	HBsAg positive	Never drink	Ever drink	Never smoke	Ever smoke
rs4678680	117/1825	192/118	120/835	189/1108	109/796	214/1195
T:T	1.00	1.00	1.00	1.00	1.00	1.00
T:G	1.07 (0.58-1.96)	0.93 (0.35-2.42)	1.26 (0.60-2.66)	0.78 (0.38-1.60)	0.76 (0.31-1.88)	1.05 (0.57-1.92)
G:G	1.05 (0.13-8.63)	0.86 (0.17-4.49)	4.89 (0.75-31.93)	0.15 (0.01-1.67)	2.21 (0.42-11.65)	0.33 (0.05-2.11)
rs9267673	116/1786	188/117	115/818	189/1085	107/774	211/1179
C:C	1.00	1.00	1.00	1.00	1.00	1.00
C:T	0.85 (0.54-1.34)	0.64 (0.34-1.20)	0.82 (0.47-1.42)	0.78 (0.49-1.25)	0.95 (0.55-1.65)	0.84 (0.54-1.32)
T:T	1.26 (0.48-3.29)	4.71 (0.28-79.32)	2.80 (0.87-8.99)	0.99 (0.31-3.13)	1.31 (0.33-5.13)	1.63 (0.56-4.69)
rs9275572	113/1777	184/117	114/823	183/1071	102/776	209/1165
G:G	1.00	1.00	1.00	1.00	1.00	1.00
G:A	1.40 (0.92-2.12)	0.65 (0.35-1.19)	0.93 (0.56-1.56)	1.20 (0.77-1.88)	1.06 (0.62-1.80)	1.13 (0.74-1.71)
A:A	1.51 (0.79-2.90)	1.24 (0.40-3.87)	1.03 (0.43-2.48)	1.54 (0.76-3.13)	1.93 (0.82-4.54)	0.99 (0.49-2.01)

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0), pack-year of smoking (continuous, except for when stratified by tobacco smoking), ethanol consumption (gram/week, continuous, except for when stratified by alcohol drinking status), HBsAg status (1=positive, 0=negative, except for when stratified by HBsAg status) and study area (Dafeng=1, Ganyu=2).

SNP and HBV interaction

Statistical interaction on additive scale was observed between rs896849 and HBsAg status on liver cancer risk (Table 3.21). After recoding, comparing to rs896849 (T/C+C/C) type and HBsAg negatives, rs896849 (T/C+C/C) type and HBsAg positives showed an aOR of 15.99 (95% CI: 8.62-29.65), rs896849 (T/T) type and HBsAg negatives showed an aOR of 1.10 (95% CI: 0.71-1.72), while rs896849 (T/T) type and HBsAg positives showed an aOR of 34.12 (95% CI: 20.30-57.35). The AP was 0.41 (95% CI: 0.08-0.74), indicating a positive interaction on additive scale.

SNP and drinking interaction

Participants' drinking status used in examining the interaction was never/ever drinkers. Rs896849 and drinking showed statistically significant interaction on both additive scale and multiplicative scale (Table 3.22). Comparing to those with rs896849 (T/C+C/C) type ever drinkers, never drinkers with same genetic type showed an aOR of 1.55 (95% CI: 0.81-2.95); rs896849 (T/T) type and ever drinkers showed an aOR of 1.89 (95% CI: 1.17-3.06); while rs896849 (T/T) type and never drinkers showed an aOR of 1.23 (95% CI: 0.73-2.10). The ratio of the odds ratio was 0.42 (95% CI: 0.20-0.87) and the AP was -0.98 (95% CI: -1.92-(-0.03)), both showing negative interactions. Moreover, the product term remained significant after the Semi-Bayesian shrinkage (aROR=0.51, 95% PI: 0.27-0.97).

SNP and smoking interaction

Participants' smoking status was also examined as never/ever smoking (Table 3.23). Rs896849 again showed interaction with ever smoke on liver cancer. Comparing to those with rs896849

(T/C+C/C) type ever smokers, never smoker with same genetic type showed an aOR of 1.56 (95% CI: 0.82-2.95); rs896849 (T/T) type ever smokers showed an aOR of 1.93 (95% CI: 1.22-3.05); while rs896849 (T/T) never smokers showed an aOR of 1.12 (95% CI: 0.67-1.88). On the multiplicative scale, the ratio of the odds ratio was 0.37 (95% CI: 0.18-0.77) and on the additive scale the RERI was -1.37 (95% CI: -2.69-(-0.05)) and AP was -1.22 (95% CI: -2.29-(-0.15)). Moreover, the product term remained significant after the Semi-Bayesian shrinkage (aOR=0.46, 95% PI: 0.24-0.87). Rs896849 negatively interacted with smoking on liver cancer.

Also, rs11614913 showed interaction with smoking on additive scale. Comparing to those with rs11614913 (T/C+C/C) type never smokers, ever smoker with same genetic type showed an aOR of 1.12 (95% CI: 0.72-1.73); rs11614913 (T/T) type never smokers showed an aOR of 1.03 (95% CI: 0.60-1.79); while rs11614913 (T/T) ever smokers showed an aOR of 2.13 (95% CI: 1.32-3.42). On the additive scale, the RERI was 0.98 (95% CI: 0.11-1.85), AP was 0.46 (95% CI: 0.12-0.80) and S was 7.62 (95% CI: 0.03-1954.83), indicating positive interactions.

Table 3.21 Interactions between SNPs and HBsAg status on liver cancer

SNP	HBsAg	cases	controls	aOR (95% CI)[a]	Interaction (95% CI)	SB-adjusted ROR (95% PI)
rs896849						
T:C+C:C	negative	28	514	1.00	ROR: 1.54 (0.75-3.14)	1.41 (0.74-2.66)
T:C+C:C	positive	44	39	**15.99 (8.62-29.65)**	RERI: 11.03 (-0.73-22.79)	
T:T	negative	90	1343	1.10 (0.71-1.72)	**AP: 0.41 (0.08-0.74)**	
T:T	positive	152	83	**27.12 (16.55-44.45)**	S: 1.73 (0.96-3.13)	
rs11614913						
T:C+C:C	negative	61	1235	1.00	ROR: 0.87 (0.44-1.71)	0.89 (0.49-1.64)
T:C+C:C	positive	117	80	**24.38 (15.97-37.24)**	RERI: 9.14 (-7.83-26.10)	
T:T	negative	47	569	**1.61 (1.07-2.41)**	AP: 0.27 (-0.13-0.66)	
T:T	positive	70	35	**34.12 (20.30-57.35)**	S: 1.38 (0.79-2.40)	
rs4730775						
C:T+T:T	negative	43	781	1.00	ROR: 1.02 (0.53-1.96)	1.02 (0.56-1.84)
C:T+T:T	positive	70	47	**21.29 (12.48-36.34)**	RERI: 10.00 (-3.69-23.70)	
C:C	negative	75	1048	1.47 (0.98-2.20)	AP: 0.32 (-0.04-0.67)	
C:C	positive	128	73	**31.76 (20.01-50.43)**	S: 1.48 (0.87-2.53)	
rs2241802						
G:A+A:A	negative	71	1203	1.00	ROR: 1.07 (0.55-2.08)	1.06 (0.58-1.92)
G:A+A:A	positive	124	80	**21.83 (14.51-32.86)**	RERI: 6.87 (-7.47-21.21)	
G:G	negative	47	603	1.24 (0.84-1.85)	AP: 0.24 (-0.17-0.64)	
G:G	positive	73	36	**28.95 (17.48-47.93)**	S: 1.33 (0.76-2.31)	

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0), pack-year of smoking (continuous), HBsAg status (1=positive, 0=negative) and study area (Dafeng=1, Ganyu=2).

Table 3.22 Interactions between SNPs and drinking status on liver cancer

SNP	Ever drink	cases	controls	aOR (95% CI)[a]	Interaction (95% CI)	SB-adjusted ROR (95% PI)
rs896849						
T:C+C:C	Yes	45	332	1.00	**ROR: 0.42 (0.20-0.87)**	**0.51 (0.27-0.97)**
T:C+C:C	No	36	253	1.55 (0.81-2.95)	RERI: -1.20 (-2.51-0.10)	
T:T	Yes	172	854	**1.89 (1.17-3.06)**	**AP: -0.98 (-1.92-(-0.03))**	
T:T	No	90	641	1.23 (0.73-2.10)	S: 0.16 (0.02-1.34)	
rs11614913						
T:C+C:C	No	82	600	1.00	ROR: 1.18 (0.61-2.30)	1.14 (0.63-2.08)
T:C+C:C	Yes	117	790	1.09 (0.71-1.68)	RERI: 0.31 (-0.60-1.21)	
T:T	No	40	267	1.38 (0.82-2.31)	AP: 0.17 (-0.31-0.66)	
T:T	Yes	86	365	**1.78 (1.11-2.86)**	S: 1.66 (0.27-10.06)	
rs4730775						
C:T+T:T	No	41	381	1.00	ROR: 0.83 (0.43-1.60)	0.86 (0.47-1.56)
C:T+T:T	Yes	85	493	1.41 (0.81-2.45)	RERI: -0.13 (-1.09-0.84)	
C:C	No	87	499	1.66 (0.99-2.78)	AP: -0.07 (-0.56-0.43)	
C:C	Yes	134	678	**1.95 (1.16-3.25)**	S: 0.88 (0.36-2.16)	
rs2241802						
G:A+A:A	No	85	594	1.00	ROR: 0.94 (0.49-1.80)	0.95 (0.53-1.71)
G:A+A:A	Yes	131	747	1.28 (0.83-1.98)	RERI: -0.02 (-0.88-0.85)	
G:G	No	42	282	1.32 (0.79-2.19)	AP: -0.01 (-0.56-0.54)	
G:G	Yes	89	400	1.59 (0.99-2.54)	S: 0.97 (0.23-4.10)	

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0), pack-year of smoking (continuous), HBsAg status (1=positive, 0=negative) and study area (Dafeng=1, Ganyu=2).

Table 3.23 Interactions between SNPs and smoking status on liver cancer

SNP	Ever smoke	cases	controls	aOR (95% CI)[a]	Interaction (95% CI)	SB-adjusted ROR (95% PI)
rs896849						
T:C+C:C	Yes	50	350	1.00	**ROR: 0.37 (0.18-0.77)**	**0.46 (0.24-0.87)**
T:C+C:C	No	31	235	1.56 (0.82-2.95)	**RERI: -1.37 (-2.69-(-0.05))**	
T:T	Yes	179	909	**1.93 (1.22-3.05)**	**AP: -1.22 (-2.29-(-0.15))**	
T:T	No	83	586	1.12 (0.67-1.88)	S: 0.08 (0.00-5.03)	
rs11614913						
T:C+C:C	No	73	529	1.00	ROR: 1.85 (0.94-3.64)	1.64 (0.89-3.02)
T:C+C:C	Yes	126	861	1.12 (0.72-1.73)	**RERI: 0.98 (0.11-1.85)**	
T:T	No	34	273	1.03 (0.60-1.79)	**AP: 0.46 (0.12-0.80)**	
T:T	Yes	92	359	**2.13 (1.32-3.42)**	**S: 7.62 (0.03-1954.83)**	
rs4730775						
C:T+T:T	No	36	355	1.00	ROR: 0.63 (0.32-1.22)	0.69 (0.38-1.26)
C:T+T:T	Yes	90	519	**1.89 (1.09-3.28)**	RERI: -0.55 (-1.73-0.64)	
C:C	No	77	454	**1.85 (1.08-3.19)**	AP: -0.25 (-0.77-0.27)	
C:C	Yes	144	723	**2.20 (1.30-3.71)**	S: 0.69 (0.35-1.33)	
rs2241802						
G:A+A:A	No	79	552	1.00	ROR: 0.84 (0.43-1.62)	0.87 (0.48-1.58)
G:A+A:A	Yes	137	789	1.42 (0.93-2.17)	RERI: -0.15 (-1.09-0.79)	
G:G	No	36	257	1.43 (0.84-2.42)	AP: -0.09 (-0.65-0.47)	
G:G	Yes	95	425	**1.70 (1.08-2.69)**	S: 0.83 (0.26-2.59)	

[a] Adjusted for age (continuous), gender (male=1, female=0), education level (illiteracy=0, primary=1, middle=2, high and college=3), in marriage (1=yes, 0=no) income (Yuan/year) 10 years ago (continuous), BMI (continuous), family history of liver cancer (yes=1, no=0), ethanol consumption (gram/week, continuous), HBsAg status (1=positive, 0=negative) and study area (Dafeng=1, Ganyu=2).

3.3 Specific Aim 3: Risk factors of higher serum HBV viral load

A total of 949 participants in this study were HBsAg positive. The serum HBV DNA viral load tests were performed among 915 (95.4%), 495 cases and 420 controls those who had sufficient serum sample left after ELISA. Overall, 156 (17.1%) showed undetectable HBV DNA in serum, 105 (11.5%) had the viral load under 10^3 IU/ml, 276 (30.2%) were between 10^3 and 10^5 IU/ml, 283 (30.9%) were between 10^5-10^7 IU/ml and 95 (10.4%) were over 10^7 IU/ml. Liver cancer cases tended to show higher HBV viral loads than controls ($p<0.001$) (Table 3.24). HBV viral load was closely related with HBV serum markers. Table 3.25 showed the distribution of viral load in different HBV serum marker patterns. People with positive HBeAg were showing higher proportion in the higher viral load group.

The distribution of HBV viral load in different characteristics were shown in Table 3.26 and 3.27 overall and by liver cancer cases and controls separately. Among all these socio-demographic characteristics such as gender, age, education level, family income 10 years ago and BMI, gender and age were different in distribution of HBV viral loads among all subjects. But when cases and controls were examined separately, HBV viral load was only different between men and women among liver cancer cases ($p=0.030$).

The associations between major risk factors of liver cancer and the distribution of HBV viral load were shown in Table 3.28 and 3.29 altogether and by liver cancer cases and controls separately. We found that among all these risk factors, HBV viral load was different between those who ever drank and never drank ($p=0.020$), who had or had not family history of liver

cancer (p=0.001). When looking into cases and controls separately, HBV viral load only differed between those had or had not family history of liver cancer among controls (p=0.038).

Table 3.24. The distribution of HBV viral load and liver cancer among HBsAg positives in Jiangsu Study 2003-2010 (n=915), 2014

HBV viral load (IU/mL)		Liver cancer		
		Case	Control	Total
Undetectable		35	121	156
	Row %	22.4	77. 6	
	Column %	7.1	28.8	17.1
500-10^3		38	67	105
	Row %	36.2	63.8	
	Column %	7.7	16.0	11.5
10^3-10^5		154	122	276
	Row %	55.8	44.2	
	Column %	31.1	29.1	30.2
10^5-10^7		221	62	283
	Row %	78.1	21.9	
	Column %	44.7	14.8	30.9
10^7-		47	48	95
	Row %	49.5	50.5	
	Column %	9.5	11.4	10.4
Total		495	420	915
	Row %	54.1	45.9	

Table 3.25. The distribution of HBV viral load in different patterns of HBV markers among those HBsAg positives in Jiangsu Study 2003-2010 (N=909), 2014

HBV Pattern (HBsAg, HBsAb, HBeAg, HBeAb, HBcAb) (0-negative, 1-positive)	Total	Number of patients in each level of HBV viral load (IU/mL) (%)				
		Undetectable	500-10^3	10^3-10^5	10^5-10^7	10^7-
Total	909	153 (16.8)	103 (11.3)	275 (30.3)	283 (31.1)	95 (10.5)
VI (1,0,0,0,1)	171	28 (16.4)	16 (9.4)	63 (36.8)	59 (34.5)	5 (2.9)
VIII (1,0,0,1,1)	464	113 (24.4)	84 (18.1)	177 (38.2)	82 (17.7)	8 (1.7)
IX (1,0,1,0,1)	231	0	3 (1.3)	23 (10.0)	125 (54.1)	80 (34.6)
X (1,0,0,0,0)	2	1 (50.0)	0	0	1 (50.0)	0
XI (1,0,0,1,0)	2	0	0	2 (100)	0	0
XII (1,0,1,0,0)	1	0	0	0	0	1 (100)
XIII (1,0,1,1,1)	9	2 (22.2)	0	1 (11.1)	6 (66.7)	0
XIV (1,1,0,0,0)	0	0	0	0	0	0
XV (1,1,0,0,1)	11	4 (36.4)	0	5 (45.5)	2 (18.2)	0
XVII (1,1,0,1,1)	8	4 (50.0)	0	2 (25.0)	2 (25.0)	0
XVIII (1,1,1,0,1)	10	1 (10.0)	0	2 (20.0)	6 (60.0)	1 (10.0)

Table 3.26 Socio-demographic characteristics and the distribution of HBV viral load in Jiangsu study(n=915), 2003-2010, 2014

Characteristics	HBV Viral load (IU/ml)						
	Und.-10^3	%	10^3-10^5	%	>10^5	%	p
Gender							
Male	114	15.9	290	40.5	313	43.7	0.017
Female	42	21.2	91	46.0	65	32.8	
Age							
<50	22	10.6	86	41.4	100	48.1	0.001
50-59	37	13.5	123	44.7	115	41.8	
60-69	57	21.9	101	38.9	102	39.2	
70-79	35	25.4	60	43.5	43	31.2	
80-	5	14.7	11	32.4	18	52.9	
Education level							
Illiteracy	67	19.9	132	39.3	137	40.8	0.153
Primary	52	16.7	139	44.7	120	38.6	
Middle	27	12.6	85	39.5	103	47.9	
High school& above	10	19.2	24	46.2	18	34.6	
Income 10 years ago per capita (RMB yuan/year)							
<1000	31	16.4	81	42.9	77	40.7	0.981
1000-	27	16.0	73	43.2	69	40.8	
1500-	50	18.7	106	39.6	112	41.8	
2500-	46	17.2	109	40.7	113	42.2	
BMI							
<18.5	13	17.6	28	37.8	33	44.6	0.055
18.5-	89	15.0	242	40.9	261	44.1	
24-	47	23.6	86	43.2	66	33.2	
28-	7	16.3	21	48.8	15	34.9	

Table 3.27 Socio-demographic characteristics and the distribution of HBV viral load by case and control in Jiangsu study, 2003-2010, 2014

	HBV Viral load (IU/ml)													
	liver cancer cases							controls						
Characteristics	$<10^3$	%	10^3-10^5	%	$>10^5$	%	p	$<10^3$	%	10^3-10^5	%	$>10^5$	%	p
Gender							**0.030**							0.146
Male	23	5.7	158	38.9	225	55.4		91	29.3	132	42.4	88	28.3	
Female	12	13.5	34	38.2	43	48.3		30	27.5	57	52.3	22	20.2	
Age							0.297							0.705
<50	13	7.7	64	37.9	92	54.4		9	23.1	22	56.4	8	20.5	
50-59	9	5.6	68	42.2	84	52.2		28	24.6	55	48.3	31	27.2	
60-69	12	10.5	39	34.2	63	55.3		45	30.8	62	42.5	39	26.7	
70-79	1	2.7	18	48.7	18	48.7		34	33.7	42	41.6	25	24.8	
80-	0	0	3	21.4	11	78.6		5	25.0	8	40.0	7	35.0	
Education level							0.153							0.606
Illiteracy	11	7.6	55	37.9	79	54.5		56	29.3	77	40.3	58	30.4	
Primary	12	7.3	67	40.6	86	52.1		40	27.4	72	49.3	34	23.3	
Middle	6	4.2	50	35.0	87	60.8		21	29.2	35	48.6	16	22.2	
High school and above	6	14.6	19	46.3	16	39.0		4	36.4	5	45.5	2	18.2	
Income 10 years ago per capita (RMB yuan/year)							0.998							0.946
<1000	7	6.7	42	40.0	56	53.3		24	28.6	39	46.4	21	25.0	
1000-	7	7.2	39	40.2	51	52.6		20	27.8	34	47.2	18	25.0	
1500-	10	6.9	53	36.6	82	56.6		40	32.5	53	43.1	30	24.4	
2500-	10	7.2	54	38.9	75	54.0		36	27.9	55	42.6	38	29.5	
BMI							0.369							0.638
<18.5	3	6.1	17	34.7	29	59.2		10	40.0	11	44.0	4	16.0	
18.5-	21	6.1	130	38.0	191	55.9		68	27.2	112	44.8	70	28.0	
24-	10	12.7	34	43.0	35	44.3		37	30.8	52	43.3	31	25.8	
28-	1	0.2	8	40.0	11	55.0		6.0	26.1	13	56.5	4.0	17.4	

Table 3.28 Major risk factors on liver cancer and the distribution of HBV viral load in Jiangsu study(n=915), 2003-2010, 2014

Risk factor	HBV Viral load (IU/ml)						
	$<10^3$	%	10^3-10^5	%	$>10^5$	%	p
Ever drink							
No	88	20.3	182	42.0	163	37.6	**0.020**
Yes	68	14.1	199	41.3	215	44.6	
Ever smoke							
No	86	18.7	193	42.0	181	39.4	0.308
Yes	70	15.4	188	41.3	197	43.3	
Ever drink raw water							
No	69	19.6	142	40.2	142	40.2	0.298
Yes	84	15.6	225	41.7	231	42.8	
Mildew contaminated food consumption							
No	140	17.0	338	41.1	345	41.9	0.588
Yes	13	16.5	37	46.8	29	36.7	
Family history of liver cancer							
No	142	18.0	340	43.0	308	39.0	**0.001**
Yes	14	11.2	41	32.8	70	56.0	

Table 3.29 Major risk factors on liver cancer and the distribution of HBV viral load by outcome in Jiangsu study, 2003-2010, 2014

	HBV Viral load (IU/ml)													
	liver cancer cases							controls						
Risk factor	$<10^3$	%	10^3-10^5	%	$>10^5$	%	p	$<10^3$	%	10^3-10^5	%	$>10^5$	%	p
Ever drink														
No	16	8.3	73	38.0	103	53.7	0.681	72	29.9	109	45.2	60	24.9	0.744
Yes	19	6.3	119	39.3	165	54.5		49	27.4	80	44.7	50	27.9	
Ever smoke														
No	22	10.1	82	37.6	114	52.3	0.067	64	26.5	111	45.9	67	27.7	0.430
Yes	13	4.7	110	39.7	154	55.6		57	32.0	78	43.8	43	24.2	
Ever drink raw water														
No	10	6.1	66	40.0	89	53.9	0.725	59	31.4	76	40.4	53	28.2	0.343
Yes	25	7.8	120	37.6	174	54.6		59	26.7	105	47.5	57	25.8	
Mildew contaminated food consumption														
No	28	6.3	174	39.1	243	54.6	0.080	112	29.6	164	43.4	102	27.0	0.151
Yes	7	15.2	17	37.0	22	47.8		6	18.2	20	60.6	7	21.2	
Family history of liver cancer														
No	31	8.0	156	40.2	201	51.8	0.087	111	27.6	184	45.8	107	26.6	**0.038**
Yes	4	3.7	36	33.6	67	62.6		10	55.6	5	27.8	3	16.7	

Associations between SNPs from microRNA related genes, stem cell pathway and GWAS and HBV viral load among HBsAg positive participants.

Among 2,612 participants from Dafeng and Ganyu who had successful DNA extraction and SNPs testing, 367 were HBsAg positive. Among them, 347 (94.6%) had sufficient serum sample for HBV viral load test.

Overall, 62 (17.9%) out of the 347 samples had undetectable HBV viral load, 34 (9.8%) had the viral load of less than 10^3 IU/mL, 109 (31.4%) were within 10^3-10^5 IU/mL, 113 (32.6%) were within 10^5-10^7 IU /mL and 29 (8.4%) were greater than 10^7 IU/mL.

Table 3.30, 3.31 and 3.32 showed the distribution of participants with HBV viral load divided into three groups: $<10^3$ IU/mL, 10^3-10^5 IU/mL and $>10^5$ IU/mL in three types of each SNP from microRNA related genes, stem cell pathway and GWAS. Polymorphism in Rs11614913 (p=0.008), rs2740348 (p=0.003) from microRNA related genes, rs222851 (p=0.007), rs3734637 (p=0.043) and rs915894 (p=0.004) from stem cell pathway showed to have different distributions of HBV viral load levels.

HBV viral load greater than 10^5 IU/mL was considered as a high viral load level, representing highly active viral replication. There were 142 (40.9%) subjects having HBV viral load greater than 10^5 IU/mL. We further analyzed the association between SNPs and HBV viral load greater than 10^5 IU/mL as outcome using unconditional logistic regression, adjusting for gender, age, level of education, pack-year of smoking, ethanol consumption and family history of liver cancer.

Among microRNA related SNPs, rs12828 (aOR=0.48, 95% CI: 0.27-0.84, G/A vs G/G; aOR=0.45, 95% CI: 0.22-0.93, A/A vs. G/G) showed inverse association with having higher level of HBV viral load. And rs12828 previously showed inverse association with liver cancer among HBsAg positives with an aOR of 0.52, (95% CI: 0.28-0.99) when comparing G/A to G/G type (Table 2.8). Rs2740348 (aOR=2.16, 95% CI: 1.16-4.03, G/C vs. G/G) showed positive association with having higher HBV viral load (Table 3.30). Among SNPs from stem cell pathway, rs222851 (aOR=2.47, 95% CI: 1.42-4.28, A/G vs. A/A) showed increased risk of having higher HBV viral load. Also, rs222851 previously showed increased odds of liver cancer among HBsAg positives with an aOR of 1.99 (95% CI: 1.07-3.71) when comparing A/G to A/A (Table 2.9). And rs3734637 (aOR=0.19, 95% CI: 0.05-0.70, C/C vs. A/A) showed decreased risk of having higher HBV viral load (Table 3.31). Rs3734637 showed inverse associations with liver cancer in previous stratified analyses (Table 2.31). No other SNPs including SNPs detected by former GWAS showed significant association from logistic regression models after confounding control (Table 3.32).

Table 3.30 The association between SNPs from microRNA related genes and HBV vial load

SNP	HBV viral load (IU/mL) <10^3	%	10^3-10^5	%	>10^5	%	P	aOR (95% CI)[a] (>10^5 as outcome)
rs1804429								
T:T	49	17.3	116	41.0	118	41.7	0.684	1.00
T:G	7	24.1	11	37.9	11	37.9		0.86 (0.37-1.99)
G:G	0	0.0	1	100.0	0	0.0		na
rs10519613								
C:C	22	18.8	45	38.5	50	42.7	0.998	1.00
C:A	23	18.0	51	39.8	54	42.2		0.97 (0.56-1.66)
A:A	12	17.7	28	41.2	28	41.2		0.94 (0.49-1.79)
rs12828								
G:G	23	17.2	47	35.1	64	47.8	0.144	1.00
G:A	24	19.8	55	45.5	42	34.7		**0.48 (0.27-0.84)**
A:A	7	13.2	27	50.9	19	35.9		**0.45 (0.22-0.93)**
rs896849								
T:T	38	16.5	94	40.9	98	42.6	0.345	1.00
T:C	17	23.9	28	39.4	26	36.6		0.69 (0.38-1.27)
C:C	3	27.3	6	54.6	2	18.2		0.32 (0.06-1.59)
rs11614913								
T:T	12	12.0	44	44.0	44	44.0	**0.008**	1.00
T:C	22	15.6	61	43.3	58	41.1		0.91 (0.52-1.58)
C:C	19	34.6	16	29.1	20	36.4		0.70 (0.33-1.46)
rs2910164								
C:C	18	18.6	46	47.4	33	34.0	0.552	1.00
C:G	27	17.4	59	38.1	69	44.5		1.39 (0.79-2.44)
G:G	11	19.6	23	41.1	22	39.3		1.28 (0.62-2.63)
rs895819								
T:T	29	17.0	78	45.6	64	37.4	0.369	1.00
T:C	23	20.0	39	33.9	53	46.1		1.30 (0.77-2.19)
C:C	3	13.0	10	43.5	10	43.5		1.01 (0.39-2.62)
rs7372209								
C:C	34	20.9	60	36.8	69	42.3	0.248	1.00
C:T	17	14.9	49	43.0	48	42.1		1.16 (0.69-1.94)
T:T	5	13.9	20	55.6	11	30.6		0.68 (0.30-1.53)
rs3742330								
A:A	25	17.5	63	44.1	55	38.5	0.729	1.00
A:G	27	20.2	49	36.6	58	43.3		1.31 (0.78-2.18)
G:G	6	16.2	17	46.0	14	37.8		0.81 (0.35-1.84)

Table 3.30 cont. The association between SNPs from microRNA related genes and HBV vial load

SNP	HBV viral load (IU/mL) <10^3	%	10^3-10^5	%	>10^5	%	P	aOR (95% CI)[a] (>10^5 as outcome)
rs4961280								
C:C	42	18.1	99	42.7	91	39.2	0.646	1.00
C:A	10	14.9	25	37.3	32	47.8		1.66 (0.92-3.00)
A:A	2	28.6	2	28.6	3	42.9		1.42 (0.30-6.67)
rs14035								
C:C	41	20.2	79	38.9	83	40.9	0.309	1.00
C:T	12	15.4	32	41.0	34	43.6		1.25 (0.70-2.24)
T:T	3	33.3	5	55.6	1	11.1		0.15 (0.02-1.33)
rs197412								
T:T	23	15.9	58	40.0	64	44.1	0.708	1.00
T:C	28	20.7	57	42.2	50	37.0		0.67 (0.40-1.13)
C:C	7	21.2	14	42.4	12	36.4		0.70 (0.30-1.65)
rs2740348								
G:G	48	21.5	93	41.7	82	36.8	**0.003**	1.00
G:C	6	9.5	23	36.5	34	54.0		**2.16 (1.16-4.03)**
C:C	0	0.0	6	100.0	0	0.0		na
rs7813								
T:T	27	17.7	69	45.1	57	37.3	0.483	1.00
T:C	24	20.2	41	34.5	54	45.4		1.41 (0.84-2.37)
C:C	4	14.8	11	40.7	12	44.4		1.24 (0.51-3.04)
rs11077								
A:A	48	18.0	110	41.2	109	40.8	0.582	1.00
A:C	9	20.5	19	43.2	16	36.4		0.84 (0.42-1.67)
C:C	1	33.3	0	0.0	2	66.7		3.33 (0.28-39.60)
rs9266								
C:C	31	16.6	76	40.6	80	42.8	0.363	1.00
C:T	23	21.3	41	38.0	44	40.7		0.78 (0.47-1.32)
T:T	2	12.5	10	62.5	4	25.0		0.38 (0.11-1.29)
rs4072391								
C:C	45	17.9	102	40.6	104	41.4	0.555	1.00
C:T	10	18.5	20	37.0	24	44.4		1.19 (0.62-2.27)
T:T	3	27.3	6	54.6	2	18.2		0.30 (0.06-1.47)
rs2126852								
A:A	28	18.2	67	43.5	59	38.3	0.226	1.00
A:G	21	17.5	43	35.8	56	46.7		1.51 (0.89-2.56)
G:G	8	25.0	16	50.0	8	25.0		0.49 (0.20-1.20)

Table 3.30 cont. The association between SNPs from microRNA related genes and HBV vial load

SNP	HBV viral load (IU/mL)							aOR (95% CI)[a]
	$<10^3$	%	10^3-10^5	%	$>10^5$	%	P	($>10^5$ as outcome)
rs7760								
T:T	44	18.3	97	40.3	100	41.5	0.953	1.00
T:G	13	20.6	26	41.3	24	38.1		0.77 (0.42-1.44)
G:G	2	25.0	3	37.5	3	37.5		0.79 (0.16-3.81)
rs2075993								
G:G	22	20.6	43	40.2	42	39.3	0.870	1.00
G:A	24	16.1	60	40.3	65	43.6		1.16 (0.67-2.01)
A:A	10	17.5	25	43.9	22	38.6		0.91 (0.44-1.87)
rs3801790								
A:A	20	17.1	44	37.6	53	45.3	0.851	1.00
A:G	27	18.6	61	42.1	57	39.3		0.91 (0.53-1.56)
G:G	8	16.3	22	44.9	19	38.8		0.91 (0.44-1.87)
rs3929								
G:G	46	21.9	77	36.7	87	41.4	0.106	1.00
G:C	13	12.8	50	49.0	39	38.2		0.77 (0.46-1.31)
C:C	0	0.0	4	57.1	3	42.9		0.80 (0.16-3.97)
rs2292305								
T:T	32	22.1	59	40.7	54	37.2	0.573	1.00
T:C	21	16.2	54	41.5	55	42.3		1.10 (0.66-1.86)
C:C	4	14.3	10	35.7	14	50.0		1.47 (0.63-3.42)
rs2953								
T:T	27	14.9	78	43.1	76	42.0	0.422	1.00
T:G	28	23.7	44	37.3	46	39.0		0.96 (0.58-1.58)
G:G	4	21.1	8	42.1	7	36.8		0.80 (0.28-2.28)

[a]Adjusted for gender, age, level of education, pack-year of smoking, ethanol consumption and family history of liver cancer

Table 3.31 The association between SNPs from stem cell pathway and HBV vial load

SNP	HBV viral load (IU/mL) <10^3	%	10^3-10^5	%	>10^5	%	P	aOR (95% CI)[a] (>10^5 as outcome)
rs6815391								
T:T	31	21.8	53	37.3	58	40.9	0.277	1.00
T:C	17	13.6	54	43.2	54	43.2		1.12 (0.67-1.89)
C:C	5	12.8	20	51.3	14	35.9		0.87 (0.40-1.89)
rs13409								
C:C	21	19.3	44	40.4	44	40.4	0.762	1.00
C:T	22	16.3	53	39.3	60	44.4		1.28 (0.74-2.23)
T:T	12	21.8	24	43.6	19	34.6		0.74 (0.36-1.51)
rs3130932								
T:T	32	20.9	54	35.3	67	43.8	0.117	1.00
G:T	16	13.1	59	48.4	47	38.5		0.76 (0.45-1.27)
G:G	10	26.3	15	39.5	13	34.2		0.61 (0.27-1.36)
rs3740535								
G:G	31	18.8	69	41.8	65	39.4	0.401	1.00
G:A	25	20.5	46	37.7	51	41.8		1.17 (0.70-1.95)
A:G	2	6.5	16	51.6	13	41.9		0.96 (0.42-2.23)
rs2228224								
G:G	27	15.7	72	41.9	73	42.4	0.224	1.00
G:A	21	18.0	49	41.9	47	40.2		0.96 (0.57-1.61)
A:G	8	36.4	7	31.8	7	31.8		0.72 (0.27-1.94)
rs3729629								
G:G	31	19.5	62	39.0	66	41.5	0.646	1.00
G:C	25	18.8	55	41.4	53	39.9		0.96 (0.58-1.58)
C:C	2	7.4	12	44.4	13	48.2		1.36 (0.54-3.41)
rs4730775								
C:C	31	15.7	80	40.4	87	43.9	0.512	1.00
C:T	20	22.0	39	42.9	32	35.2		0.59 (0.34-1.04)
T:T	6	23.1	9	34.6	11	42.3		0.78 (0.31-1.94)
rs4835761								
A:A	21	20.8	37	36.6	43	42.6	0.418	1.00
A:G	28	17.6	62	39.0	69	43.4		1.18 (0.69-2.01)
G:G	8	15.4	27	51.9	17	32.7		0.66 (0.31-1.42)
rs3750145								
A:A	36	20.5	66	37.5	74	42.1	0.524	1.00
A:G	14	17.7	33	41.8	32	40.5		0.86 (0.48-1.53)
G:G	3	14.3	12	57.1	6	28.6		0.51 (0.18-1.46)

Table 3.31 cont. The association between SNPs from stem cell pathway and HBV vial load

SNP	HBV viral load (IU/mL) <10^3	%	10^3-10^5	%	>10^5	%	P	aOR (95% CI)[a] (>10^5 as outcome)
rs2241802								
G:G	14	13.0	49	45.4	45	41.7	0.444	1.00
G:A	26	20.3	48	37.5	54	42.2		1.01 (0.58-1.77)
A:A	16	21.9	30	41.1	27	37.0		0.91 (0.48-1.75)
rs222851								
A:A	28	23.3	56	46.7	36	30.0	**0.007**	1.00
A:G	20	13.4	53	35.6	76	51.0		**2.47 (1.42-4.28)**
G:G	9	24.3	16	43.2	12	32.4		1.16 (0.51-2.64)
rs1981492								
G:G	25	17.0	64	43.5	58	39.5	0.389	1.00
G:A	23	18.7	45	36.6	55	44.7		1.09 (0.65-1.85)
A:A	7	19.4	19	52.8	10	27.8		0.49 (0.21-1.18)
rs1046472								
C:C	38	18.5	84	41.0	83	40.5	0.831	1.00
C:A	16	16.7	40	41.7	40	41.7		1.10 (0.65-1.86)
A:A	2	22.2	2	22.2	5	55.6		2.39 (0.58-9.84)
rs3734637								
A:A	33	16.1	75	36.6	97	47.3	**0.043**	1.00
A:C	18	19.2	43	45.7	33	35.1		0.61 (0.35-1.04)
C:C	6	33.3	9	50.0	3	16.7		**0.19 (0.05-0.70)**
rs11364								
G:G	32	16.3	85	43.4	79	40.3	0.619	1.00
G:A	12	17.7	23	33.8	33	48.5		1.21 (0.67-2.20)
A:A	3	23.1	4	30.8	6	46.2		1.41 (0.44-4.54)
rs520692								
A:A	43	17.4	106	42.9	98	39.7	0.498	1.00
A:G	12	19.7	22	36.1	27	44.3		1.18 (0.64-2.16)
G:G	1	50.0	1	50.0	0	0.0		na
rs8708								
A:A	45	20.6	82	37.6	91	41.7	0.599	1.00
A:G	12	15.6	34	44.2	31	40.3		1.01 (0.58-1.77)
G:G	1	7.1	6	42.9	7	50.0		1.29 (0.39-4.23)
rs1033583								
A:A	29	17.3	70	41.7	69	41.1	0.914	1.00
A:C	18	19.6	37	40.2	37	40.2		0.89 (0.52-1.54)
C:C	3	11.5	11	42.3	12	46.2		1.16 (0.48-2.82)

Table 3.31 cont. The association between SNPs from stem cell pathway and HBV vial load

SNP	HBV viral load (IU/mL) <10^3	%	10^3-10^5	%	>10^5	%	P	aOR (95% CI)[a] (>10^5 as outcome)
rs2269700								
T:T	39	18.7	85	40.7	85	40.7	0.796	1.00
T:C	19	19.8	42	43.8	35	36.5		0.82 (0.48-1.41)
C:C	2	13.3	5	33.3	8	53.3		2.13 (0.71-6.44)
rs915894								
C:C	8	7.9	55	54.5	38	37.6	**0.004**	1.00
C:A	30	19.7	54	35.5	68	44.7		1.44 (0.83-2.48)
A:A	14	26.9	21	40.4	17	32.7		0.78 (0.36-1.68)
rs9972231								
C:C	31	16.5	78	41.5	79	42.0	0.315	1.00
C:T	19	25.0	30	39.5	27	35.5		0.78 (0.43-1.43)
T:T	2	33.3	1	16.7	3	50.0		1.26 (0.20-8.10)

[a] Adjusted for gender, age, level of education, pack-year of smoking, ethanol consumption and family history of liver cancer

Table 3.32 The association between GWAS SNPs and HBV vial load

SNP	HBV viral load (IU/mL)							aOR (95% CI) ($>10^5$ as outcome)
	$<10^3$	%	10^3-10^5	%	$>10^5$	%	P	
rs4678680								
T:T	53	18.4	121	42.0	114	39.6	0.601	1.00
T:G	6	21.4	8	28.6	14	50.0		1.51 (0.66-3.43)
G:G	1	11.1	5	55.6	3	33.3		0.78 (0.19-3.29)
rs9267673								
C:C	41	18.4	90	40.4	92	41.3	0.837	1.00
C:T	16	17.6	41	45.1	34	37.4		0.79 (0.46-1.38)
T:T	0	0	2	40.0	3	60.0		2.07 (0.31-13.75)
rs9275572								
G:G	40	20.1	75	37.7	84	42.2	0.653	1.00
G:A	17	17.5	45	46.4	35	36.1		0.78 (0.45-1.34)
A:A	3	15.8	7	36.8	9	47.4		1.21 (0.45-3.23)

[a] Adjusted for gender, age, level of education, pack-year of smoking, ethanol consumption and family history of liver cancer.

CHAPTER 4: DISCUSSION

This is a population-based case-control study examined the associations and interactions among genetic susceptibility including polymorphisms in microRNA related genes, stem cell pathway and GWAS detected genes, environmental exposures including HBV/HCV infection, alcohol consumption and tobacco smoking with liver cancer.

Hepatitis virus infection

The results confirmed HBsAg positive as a strong risk factor for liver cancer with an aOR of 9.85 (95% CI: 8.28-11.72). While examining study sites of Dafeng and Ganyu alone with genetic information, the risk increase was even higher. Other studies performed before in Chinese populations also indicated more than ten times risk increase [12]. A meta-analysis based on 32 Chinese case-control studies from 1966 to 2004 involving 3,201 cases and 4,005 controls reported a pooled OR of 14.1 (95% CI: 10.6–18.8) [14]. A large cohort study observing 12,351 people for 31 years in Jiangsu Province showed that the incidence rate ratio was 11.70 (9.06-15.19) for HBsAg positive [15]. Actually, the risk of developing liver cancer among chronically HBV infected participants ranges from 10-fold to 100-fold greater compared to uninfected people depending on the markers used and populations studied[16].

This study also had sufficient sample size to further examine the associations between patterns of serologic markers of HBV infection and liver cancer. In this study population, the most common pattern is HBsAb and HBcAb positive, followed by HBcAb positive only. These two patterns accounted for almost 70% of all the participants. HBsAb positive usually represents recovery or immunity from HBV infection, while HBcAb positive indicates history of infection and may

persist for lifetime. The high prevalence of these two markers suggested a high level of past or present HBV infection in this population. We also noticed that the prevalence of HBcAb positive is higher in this study than others' reports in Jiangsu Province[97,98], probably because the method(dilution times) we used was different from clinical use. The diluted method is more of clinical meaning, while the undiluted one is more of epidemiological meaning and is better revealing the epidemic of the infection in populations. Thus the one we chose provided better sensitivity and reported higher positive rates[99]. People under these two patterns showed an inverse association with the risk of liver cancer comparing with those who are negative for all the five markers. This finding might indicate that people recovered from HBV virus infection with virus clearance may be less vulnerable in getting liver cancer.

Based on our results of an adjusted OR of 9.85 comparing HBsAg positive to negative, we estimated a risk fraction of 90% attributable to HBV infection and a population attributable risk of 36.5% in this Chinese population. In 2013, Fan et al. used the prevalence data around 1990 and relative risk estimate data from meta analyses and large-scale observational studies to estimate that the national population attributable fraction of liver cancer due to HBV infection is 65.9% in men and 58.4% in women in China in 2005[100]. Comparing to our result, the derived relative risk they employed as 18.1 were higher than the 9.85 level in our study, which might lead to the difference.

The prevalence of HCV antibody was less than 1% in both cases and controls, and did not show a statistically significant increase in liver cancer risk (aOR: 1.40, 95% CI: 0.62-3.14). This indicated that HCV infection was not a major cause of liver cancer in this study population. The

former mentioned meta-analysis including 32 case-control studies in China reported that the pooled odds ratio and 95% confidence interval (CI) for anti-HCV/HCV RNA positivity was 4.6 (95% CI: 3.6–5.9), which was a higher point estimate than ours [14]. By examining the individual studies from the meta-analysis, we found that 7 of them were conducted in Jiangsu from 1990 to 2004, with 4 using community based controls and 3 using hospital based controls. However, 3 studies failed to calculate an OR relating HCV infection to risk of liver cancer, and 4 reported ORs with wide confidence intervals, due to the small numbers of HCV infected participants. These information as well as our study result probably indicate that the HCV prevalence is relatively low in general population of Jiangsu.

Alcohol consumption

The results showed that alcohol consumption, at any frequency level, at earlier age of starting and having higher cumulative consumption were positively associated with liver cancer. This confirmed that alcohol consumption is a risk factor for liver cancer in Chinese general population. One cohort study performed in Shanghai (a city close to Jiangsu) in 1980s did not report a significant relationship between moderate alcohol consumption (their major exposure) and liver cancer. However, given the fact that the total number of liver cancer deaths was 13 with 122 300 person-years follow up and important confounders such as HBV infection had not been controlled in this early study, we do not take this prospective study a strong evidence[33]. Another cohort study in Chinese population was carried out in Taiwan among males with HBV infection status controlled [34]. They reported a marginally insignificant risk ratio of 1.46 (95% CI: 0.97-2.21) comparing alcohol drinkers to non-drinkers with 115 liver cancer cases by 91,885 person-years. Our results with more number of cases and better controlled confounding showed a

slightly higher but still moderate risk increase by alcohol consumption on liver cancer risk than the Taiwan study, and had similar conclusion with other case-control studies performed in western countries [8].

Furthermore, this study observed a strong effect measure modification between alcohol consumption and HBV infection on the risk of liver cancer with a sufficient sample size. With the aOR of 1.73 for alcohol consumption alone and 8.34 for being HBV infected alone, when the two exposures combined together, an odds ratio of 20.89 (95% CI: 15.97-27.31) was detected. Meanwhile, both measures of statistical interaction on additive scale (RERI, AP, S) and multiplicative scale (ROR) showed significant results, indicating a synergistic effect on the development of liver cancer by these two exposures. This finding was similar to the results from the study performed among Taiwan males [34]. Some other studies from western countries also explored the interaction between alcohol and HBV infection mainly by stratified analyses. Kuper et al. reported different effect of alcohol consumption on liver cancer when stratified by HBV/HCV infection status in Greece [38]; Donato et al. reported a synergy index for the interaction between HBV infection and alcohol drinking >60g/day as 1.7 in an Italian study [101]; Yuan et al. reported a synergy index for HBV/HCV infection and heavy drinking as 5.5 (95% CI: 3.9-7.0) and a ROR of 2.3 (95% CI: 0.5-12.1) in a US study [39]. Along with these findings, our results added evidence to the conclusion that there is synergetic effects on development of liver cancer for people who are infected with HBV and at the same time are alcohol drinkers.

Tobacco smoking

Our study confirmed that tobacco smoking was also moderately positively associated with liver cancer. The prevalence of cigarette smoking among adults in this Chinese population was about 50%. Almost 1/3 smokers began smoking before 25 years old, and most smokers smoked 10 to 30 cigarettes a day, and had smoked for more than 20 years. These factors indicated that tobacco smoking was still a prevalent behavior among Chinese adults. The overall aOR comparing ever smokers to non-smokers was 1.48 (95% CI: 1.25-1.75), and aORs from strata of different smoking characteristics ranged from 1.27-1.94 for point estimates which was close to most of the reported relative risks[9,42,43,102,103]. However, although in our results, daily amount of cigarettes smoking, duration of smoking and pack-years of smoking all showed significant results in trend tests ($p<0.05$), we did not see an obvious dose-response pattern between smoking and liver cancer in these three characteristics, different from others' reports[9]. There were some possible explanations. First of all, all the associations between groups of pack year of smoking and liver cancer risk were weak to moderate (ranging from 1.12 to 1.71). It was possible that the random variation played a role. Second, the pack year of smoking was divided into six groups which probably was not necessary and thus increased the random variation. Third, it was possible that there did exist the reverse dose-response pattern. One of the explanations would be that there existed some competing risks for death such as lung cancer and some heavier smokers have died from them and the participants of this study were strong survivors.

Similar to alcohol consumption, tobacco smoking also showed effect measure modification with HBV infection on liver cancer. The joint effect reached 18.03 (95% CI: 13.99-23.24). The statistical interaction measured were both super-additive with RERI of 11.82 (95% CI: 6.54-17.09), AP of 0.57 (95% CI: 0.44-0.69) and S of 2.46 (95% CI: 1.79-3.39) and super-

multiplicative with ROR of 1.44 (95% CI: 1.02-2.04). A meta-analysis summarizing 16 studies about interaction between smoking and HBV/HCV infection published in 2010 reported joint effect of HBV infection and tobacco smoking as 21.6 (95% CI: 15.2-30.5), with an S of 1.44 (95% CI: 1.00-2.06) and an ROR (V in their paper) of 1.60 (95% CI: 1.16-2.20) after the pooled analysis[104]. Our results showed an even stronger super-additivity and a similar super-multiplicativity as the pooled analysis.

Potential contamination of aflatoxin

Aflatoxin is another major risk factor of liver cancer. One study reported that the fraction of liver cancer deaths attributable to aflatoxin was 25.0% in China [100].We were not able to measure the level of aflatoxin in serum or tissues in this study. However, we used several questions to investigate the possibility of aflatoxin contamination. In our results, having history of raw water drinking showed an aOR of 1.32 (95% CI: 1.13-1.55) and self-reported possibility of mildew contaminated food consumption showed an aOR of 1.23 (95% CI: 0.94-1.61) with liver cancer, which both indicated the potential role played by aflatoxin on development of liver cancer in this population. Former studies performed in China reported that drinking unboiled water from pond was associated with liver cancer with about 70% of risk increase [36]. These evidences indicated that unsafe water drinking and mildew food consumption could be suggested to be used as a proxy of aflatoxin contamination.

Family history of liver cancer

Family history of liver cancer has been frequently reported to be positively associated with liver cancer. The meta-analysis showed that after adjusting for chronic hepatitis B/C viruses infection,

family history of liver cancer was still associated with liver cancer with an OR of 2.38, 95% CI, 1.01-5.58 [105]. The Shanghai Women's and Men's Health Study followed up 133,014 participants and reported 299 liver cancer cases through 2010. Family history of liver cancer was associated with liver cancer reporting an HR of 2.60, 95% CI: 1.77–3.80 [106]. Our study reported an aOR of 4.19 (95% CI: 3.17-5.53) after adjusting for important confounding factors including hepatitis B virus infection, alcohol consumption, tobacco smoking. This might suggest two potential risk factors. First, the family clustering of liver cancer might indicate common environmental exposures such as food contamination from aflatoxin. Second, this might indicate genetic characteristics in developing liver cancer.

Polymorphism in microRNA related genes

Rs896849 locates in tumor protein p53 inducible nuclear protein 1 (TP53INP1). TP53INP1 is one of the p53 target genes and is involved in cell death, cell cycle arrest and cell migration [107]. It was reported that TP53INP1 was also a target of miRNA-182, and associated with the drug resistance in cisplatin-treated liver cancer [108]. Our study found that rs896849 was associated with decreased odds of liver cancer, and the association existed among sub-populations as HBsAg positive participants, ever drinkers and ever smokers. In the further analysis, rs896849 continued to show significant statistical interaction with rs4730775, HBV infection, alcohol drinking and tobacco smoking. These might indicate the potential joint effect between TP53INP1 gene and other factors in tumor development.

Rs11614913 locates in miR-196a2 gene. The association between its polymorphism and risk of cancer has been relatively better studied. One study published in 2010 reported miRNA-196a2

was associated with cirrhosis-related liver cancer in Chinese population, with rs11614913 genotype associating with tumor size [109]. Another study found that rs11614913 was associated with liver cancer among male [110]. A third study reported no main effects, but potential interaction between rs11614913 and rs4938723 [111]. However, two meta-analyses looking into rs11614916 and liver cancer reported different conclusions [112,113]. Our study found that rs11614913 C/C type was associated with decreased odds of liver cancer comparing to T/T type (aOR=0.44, 95% CI: 0.27-0.71), while T/C type also showed decreased risk while not statistically significant (aOR=0.76, 95% CI: 0.54-1.07). The association was still significant in both HBsAg negative and positive participants, as well as among ever drinkers and ever smokers.

Our study also found that rs2910164 (pre-miR-146a gene) was inversely associated with liver cancer among HBsAg positives (aOR=0.42, 95% CI: 0.18-0.98, comparing G/G to C/C), and among ever smokers (aOR=0.52, 95% CI: 0.28-0.97, comparing G/G to C/C). MiR-146 is another miRNA that has been better studied while the conclusion remains controversial. Some found that G/G type was associated with increased odds of liver cancer [114,115] , or decreased odds [116], while others found no association [112,113,117-119].

Rs4072391 (IL6R gene) was found to be inversely associated with liver cancer among HBsAg positive participants with an aOR of 0.22 (95% CI: 0.05-0.95) when comparing T/T type to C/C type in this study. Interleukin 6 receptor (IL6R) is also known as CD126 and is a cytokine receptor which plays an important role in immune response. IL6R was identified among several proteins that were significantly different comparing liver cancer patients and hepatitis patients [120]. Also it was found to be involved in a microRNA-inflammatory feedback loop circuit

which initiating hepatocellular transformation [121]. One case-control study conducted in Chinese population exploring rs6684439 which was another SNP of IL6R gene reported inverse association with liver cancer [122].

Rs895819 (MiR-27 gene) is a relatively widely studied microRNA in its polymorphism and risk of cancers. It was found to be inversely associated with liver cancer among HBsAg negative participants with an aOR of 0.64 (95%CI: 0.42-1.00) in our study. It was suggested that miR-27 regulates the transcription factor Runx1 and thus have impact on the differentiation of myeloblasts into granulocytes [123]. Studies exploring miR-27 and risk of breast cancer were most commonly conducted, while the results remain controversial [124-129]. One meta-analysis concluded no significant association overall but decrease risk among Europeans [130]. Studies reported associations between miR-27 and risk of different sites of cancer including renal cell carcinoma [131,132], ovarian cancer [124], gastric cancer [133-137], colorectal cancer [138-141], lung cancer [142-144], esophageal cancer [145,146], and cervical cancer[147] . Two meta-analyses gave the association between rs896849 and risk of cancers as not significant overall, but significant among Caucasians [148,149]. Our study adds to the knowledge as the first one reporting a decreased odds of liver cancer.

Rs7813 (Gemin4 gene) was found to be inversely associated with liver cancer among never drinkers with an aOR of 0.26 (95% CI: 0.08-0.88) in this study. A variant of Gemin4 is liver cancer-associated protein, HCAP1 [150]. And its variant was suggested to have reduced ability of inhibition on hepatocarcinoma cell growth and impaired DNA repair [151]. One study in

China reported polymorphisms in Gemin4 was associated with liver cancer, however they tested different SNPs from ours [152].

Rs12828 (WWOX gene) was found to be inversely associated with liver cancer among HBsAg positives with an aOR of 0.52, 95% CI: 0.28-0.99 when comparing G/A to G/G type, and an aOR of 0.47, 95% CI: 0.20-1.09. WWOX gene locates at chromosome 16q23, which was reported having association between homozygous deletion and aflatoxin B1-exposed liver cancer [153]. As a tumor suppressor gene, WWOX was found to have lower expression in human liver cancer [154,155], and was also associated with prognosis [156,157]. Some studies suggested that WWOX probably was associated with Wnt pathway in cancer development [158,159].

Rs2126852 was found to be inversely associated with liver cancer among never smokers in our study with an aOR of 0.50 (95% CI: 0.28-0.89). RCHY1 gene encodes RING finger and CHY zinc finger domain-containing protein1. The protein binds with p53 and promotes its degradation and acts as an oncogene. Currently no epidemiological study has reported its polymorphism with liver cancer and the association was firstly explored by our study.

Polymorphism in stem cell pathway

In the analyses, mutations in rs4730775 (WNT2 gene) was inversely associated with liver cancer, showing an aOR of 0.71 (95% CI: 0.50-0.99) comparing C/T to C/C type, and an aOR of 0.56, while the confidence interval was wide (95% CI: 0.29-1.07) comparing the T/T to C/C type. Wnt 2 is one of the genes activating the canonical wnt pathway. It was shown to be up-regulated in gastric and colorectal cancers [55,160,161]. There were no studies reporting the gene expression

of wnt2 with regard to liver cancer currently, thus the underlying mechanism of our finding remained unknown.

Rs2241802 (FZD3 gene, G/G, G/A and A/A) also showed an inverse association when comparing A/A to G/G type with an aOR of 0.58 (95% CI: 0.37-0.91). And this association differed by smoking status for it only existed among ever smokers. FZD3, along with FZD4 and FZD6 acts as G-protein coupled receptors and stimulates inositol signaling and protein kinase C (PKC) [162]. Studies reported up-regulated Frizzled homolog 3 receptor in some of the cancers [163-165]. One study examined the frizzled homolog 3 expression among colorectal cancer patients' specimens, reporting high expression in cancer specimens and association with colorectal cancer progression. Moreover, they reported that FZD3 was expressed in lower percentage of metastatic hepatocellular carcinoma [166]. No epidemiologic study has yet been available reporting the association between FZD3 genes with liver cancer, our results might provide some pilot clue in this perspective.

In stratified analyses, several SNPs in stem cell pathway which did not show significant association with liver cancer overall however showed the association in certain sub-populations.

Rs3130932 (Oct4 gene) showed inverse associations with liver cancer among HBsAg positive participants (aOR=0.42, 95% CI: 0.18-0.99, G/G vs. T/T) and those who never drank (aOR=0.42, 95% CI: 0.18-0.98, G/G vs. T/T). The Oct4, also called Oct3, was a POU family transcription factor which expressed in embryonic stem cells [167]. The Oct4 gene was reported to be expressed in human cancer cells but not in normal tissues [168]. And it was hypothesized that

cells with Oct4 gene expression might be pluripotent stem cells and could serve as the targets for initiating carcinogenesis [169]. Several studies also suggested that Oct4 might be associated with poor clinical outcome among liver cancer patients [170], mediate the drug resistance in liver cancer cells [171], promote liver cancer progression [172] and is associated with tumor recurrence [173]. Results from these studies might suggest the underlying mechanisms of our findings.

Rs3734637 (HEY2 gene) was found to be inversely associated with liver cancer in stratified analyses. The HEY family it belongs to is related with Notch signaling pathway which maintains stem cells through the HEY family's transcriptional activation to repress tissue-specific transcription factors [174].

Rs2228224 (Gli1 gene) was found to be associated with liver cancer among those ever smokers (aOR=0.29, 95% CI: 0.11-0.75, A/A vs. G/G) and HBV positive participants (aOR=0.13, 95% CI: 0.04-0.40, A/A vs. G/G) in the stratified analyses. The glioma-associated oncogene-1 (Gli1) gene belongs to the hedgehog (Hh) pathway which has been shown to be associated with patterning, growth, and survival of many tissues, and is associated with several cancers [175]. It is correlated with caveolin-1 (Cav-1) which is an over-expressed marker in liver cancer which promotes liver cancer cell motility and invasion via inducing epithelial-mesenchymal transition (EMT) in tumor [176]. Moreover, HBV X protein was found to induce Gli-directed gene transaction by increasing the protein stability of Gli proteins [177]. This not only suggested the mechanism of association between rs2228224 and liver cancer, but the potential interaction between this SNP and HBV infection as well.

Rs1981492 (AXIN1 gene) was found to be positively associated with liver cancer among never drinkers in this study. Comparing to G/G type, G/A type showed an aOR of 1.71 (95% CI: 1.01-2.88). Axis inhibition protein 1 (AXIN1) encodes an important factor for Wnt signaling, and the mutation in AXIN1 has been found in liver cancer cases [178,179]. More recently, it was reported that the alterations in AXIN2 was associated with HBV-related liver cancer [180].

Semi-Bayesian approach in relative risk estimate

After using the prior information from the meta-analysis in Chinese population[36] or a null prior if no informative prior exists, we calculated the posterior ORs of the major risk factors on liver cancer and the 95% posterior limits. The SB-adjusted OR for HBV infection was 10.28 with 95% Posterior Interval (PI): 8.89-11.88, which was greater than the aOR of 9.85 (95% CI: 8.28-11.72) from the data since the prior OR used showed a greater risk increase (OR=11.34, 95% CI: 8.72-14.75). HCV infection was not a major risk factor for liver cancer in this population showing an aOR of 1.40 (95% CI: 0.62-3.14). We got a SB-adjusted OR for HCV infection of 3.85 (95% PI: 3.01-4.94) mainly because of the prior from the meta-analysis was an aOR of 4.28 (95% CI: 3.30-5.56). For alcohol consumption (data aOR= 1.91, 95% CI: 1.61-2.28; SB-adjusted OR=1.90, 95% PI: 1.66-2.17), tobacco smoking (data aOR= 1.48, 95% CI: 1.25-1.75; SB-adjusted OR=1.32, 95% PI: 1.20-1.47) and history of raw water drinking (data aOR= 1.32, 95% CI: 1.13-1.55; SB-adjusted OR=1.31, 95% PI: 1.13-1.51), the SB-adjusted ORs were quite close to the results from our data. These results may suggest that our study adds to the evidence that alcohol consumption was associated with doubling of liver cancer risk and tobacco smoking and raw water drinking were associated with about 30% risk increase. In our analysis,

possibility of mildew contaminated food consumption was measured as a proxy of exposure to aflatoxin. And it showed an aOR of 1.23 (95% CI: 0.94-1.61). After adjustment using the prior of aOR=1.87 (95% CI: 1.42-2.47) from the meta-analysis, the posterior aOR was 1.51 (95% PI: 1.24-1.83), which was increased and significant. After adjustment, family history of liver cancer showed aOR of 3.80 (95% PI: 3.14-4.60), which was slightly decreased from the data result (aOR=4.19, 95% CI: 3.17-5.53). Moreover, several product terms examining statistical interactions between the risk factors remained significant after Semi-Bayesian adjustment. In sum, for the main effects of risk factors for liver cancer, by using the prior from a meta-analysis performed in Chinese population, the estimated relative risks were adjusted and showed the magnitude of association based on both our data and former performed studies.

Strengths

Although liver cancer is a very serious public health problem in China, few well-designed large population-based studies have been conducted to systematically evaluate specific etiological factors and genetic susceptibility and potential interactions among these risk factors of liver cancer in China. This study might fill this gap with relatively sufficient sample size and well collected comprehensive epidemiological information. In fact, for some of the SNPs, this was the first epidemiologic study performed examining their association with liver cancer in population.

Limitations

There are several limitations in this study. First, the registry-based recruitment had relatively low response rate among cases. Because of the fast progress of liver cancer, some patients died before being reached by our researchers and some were not able to participate in the study because of the severe disease condition. This might lead to selection bias because the patients who were recruited in the study may be at an earlier stage of the disease, or under better condition, or were strong survivors comparing to those who did not participate.

Information bias may exist in this study too. First, most of the diagnoses were based on clinical diagnoses, which might lead to misclassification of non-cases to cases (it would be rare for misclassification from cases to non-cases because of very severe clinical prognosis), which will lead to an under-estimation of the true association and result in a conservative estimate. Second, as a case-control study, all the information of exposures were collected after the disease diagnose.

The recall bias may exist when reporting personal habits especially for alcohol drinking, tobacco smoking or even food storage as known risk factors for cancers especially for liver cancer.

Measurements of biomarkers of some environmental exposures, as well as additional functional SNPs are not possible in this study. For example, we were not able to measure the real exposure to aflatoxin. This might have certain impact on the association estimate as well as confounding control.

CHAPTER 5: CONCLUSIONS AND PUBLIC HEALTH IMPLICATIONS

Conclusions

Our study concluded that HBV infection, alcohol consumption, tobacco smoking, history of raw water drinking, history of possible consumption of mildew contaminated food and family history of liver cancer were the major risk factors of liver cancer in this Chinese population. All these associations remained similar after the semi-Bayesian adjustment. HCV infection was not a major risk factor in this population. Using the prevalence of the risk factors in the control group as the population prevalence, we conclude that the population attributable risk due to HBsAg positive was 36.5%, due to ever drinking alcohol was 29.7%, and due to ever smoking was 18.2%. We also concluded that HBV infection along with alcohol drinking, tobacco smoking and having family history of liver cancer showed synergic effects on liver cancer. Positive interaction was also observed between ever drinking, ever smoking with family history of liver cancer.

For SNPs tested from microRNA related genes, stem cell pathways and GWAS, rs896849 (TP53INP1 gene) and rs11614913 (miR-196a2 gene) from microRNA genes showed inverse association with liver cancer. Rs4730775 (WNT2 gene) and rs2241802 (FZD3 gene) from stem cell pathway showed inverse associations with liver cancer. After semi-Bayesian shrinkage adjustment using a null prior, the posterior estimate of rs11614913 and rs2241802 remained statistically significant with a similar effect magnitude. No GWAS SNPs showed obvious association with liver cancer in this population. From the stratified analyses stratified by major risk factors as HBsAg positive, ever drinking and ever smoking, several additional SNPs were found to be associated with the cancer risk including microRNA related genes rs12828 (WWOX

gene), rs2910164 (pre-miR-146a gene), rs4072391 (IL6R gene), rs7813 (Gemin4 gene), and rs2126852 (RCHY1 gene), and genes in stem cell pathways as rs3130932 (Oct4 gene), rs2228224 (GLI1 gene), 3734637 (HEY2 gene), rs222851 (DVL2 gene), rs8708 (HES2 gene), rs1981492 (AXIN1 gene) and rs2241802 (FZD3 gene).

Statistical interactions were observed between rs896849 and rs4730775, HBsAg positive, alcohol drinking and tobacco smoking on liver cancer. Rs11614913 also interacted with smoking on the cancer risk. We concluded that gene and gene interaction and gene and environment interaction existed in this study population in the development of liver cancer.

We concluded that among those HBsAg positive participants, serum HBV viral load was positively associated with the liver cancer risk. Liver cancer cases showed larger proportion of having higher serum HBV viral loads. Higher HBV vial load was also observed among those HBeAg positives. Meanwhile, among microRNA genes, rs12828 (WWOX gene) which previously showed inverse association with liver cancer among HBsAg positives also showed inverse association with having higher level of HBV viral load. Rs2740348 (Gemin4 gene) showed positive association with having higher HBV viral load. Among stem cell SNPs, rs222851 (DVL2 gene) which previously showed positive association with liver cancer among HBsAg positives showed positive association with having higher HBV viral load. And rs3734637 (HEY2 gene) which showed inverse association with liver cancer in previous stratified analysis showed inverse association with having higher HBV viral load. Thus we conclude that some SNPs showed associations with liver cancer also were associated with the

levels of serum HBV viral load. And HBV viral load might act as an intermediate factor in the progress of the disease.

Public Health Implications

Our results indicate that eliminating the infection of HBV is still the priority in liver cancer prevention in Chinese population, followed by interventions on high risk behaviors. With the implementation of HBV vaccination in the country from 1990's, it is important to reduce population prevalence of alcohol consumption, tobacco smoking and to advocate safe water and food, given the fact that we observed interaction among these risk factors. And the intervention should be emphasized among those with family history of liver cancer. Our study also found several SNPs from microRNA related genes and stem cell pathway genes which were associated with liver cancer, providing population evidence for the findings in the experimental studies. These genetic characteristics might serve as new markers for detecting carcinogenesis as well as therapy targets once the associations are further confirmed.

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