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Original Article

Association analysis of genetic variants of adiponectin gene and risk of pancreatic cancer

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Abstract: Adiponectin is a cytokine exclusively secreted from adipocyte, and could perform direct or indirect effects on anti-inflammation and anti-tumor. Previous researches have studied the correlation between plasma adiponectin levels and the risk of pancreatic cancer. So we aimed at investigating the association of genetic variants of adiponectin gene and the risk of pancreatic cancer. In this study, we genotyped 6 SNPs of adiponectin gene in a case-control study of recruited 172 patients of pancreatic cancer and 181 healthy people in Chinese Han population. The results indicated that two of the SNPs had significant associations with pancreatic cancer. Of which, the SNP rs1501299C>A decreased the risk of PC (P=0.016, OR=0.662 95% CI 0.472-0.928), while rs1065358T>C increased the risk of PC (P=0.027, OR=1.421 95% CI 1.040-1.941). Furthermore, in the clinical correlation analysis, we found rs1501299 was correlated with tumor size (P=0.026), cigarette smoking (P=0.022) and alcohol consumption (P=0.001) and rs1063538 was correlated with alcohol consumption (P=0.026). In conclusion, we provided evidences that the variants in adiponectin gene might influence the development and progression of pancreatic cancer.

Keywords: Adiponectin gene, pancreatic cancer, single nucleotide polymorphism

Introduction

Pancreatic cancer (PC) leads the sixth cause of cancer related death in China, since it has the poorest prognosis among the solid malignant cancers [1, 2]. The 5-year overall survival rate of pancreatic cancer patients has been <5% over the last three decades [3]. Due to the studies of epidemiological characteristics, approximate 80% of patients with pancreatic cancer were diagnosed until advanced or metastasis stages in which there were extremely limited treatments toward tumor [4]. So pre-diagnosis and mechanism study should be taken in the early stage of pancreatic cancer in case of irreparable results. In the recent decade, pancreatic cancer has been one of the best focused neoplasms at the genetic level in both mechanism of tumorigenesis and therapy [5].

Adiponectin is a group of cytokine exclusively secreted from adipose tissue, and the main functions of adiponectin are Anti-atherosclerosis, anti-inflammation and insulin sensitization [6, 7]. Previous researches found that the expression levels of adiponectin on the circulation were adversely associated with cancers, such as breast cancer [8, 9], endometrial cancer [10], colorectal cancer [11] and gastric cancer [12]. However, the results could be controversial in pancreatic cancer proposed by several researches. Dalamaga [13] and colleagues found that adiponectin concentration had positive association with pancreatic cancer risk, while the opposite conclusion was proposed in a prospective study by Stolzenberg-Solomon [14]. But we found the majority of researches prevailed in the viewpoint that adiponectin had its protective effects in physiological and pathological conditions.

In addition, in the area of genetic association study, it has demonstrated the association of single nucleotide polymorphisms (SNPs) in adiponectin gene which expresses adiponectin with cancers, including breast cancer [15], colorectal cancer [16], prostate cancer [17] and esophageal cancer [18]. However, to our best

Table 1. The gene type frequency of adiponectin polymorphism in gastric cancer case-control study

				HWE		MAF			
SNP No.	rs#	alleles	position	controls	cases	controls	cases	*P*-value	OR (95% CI)
1	182052	G→A	186560782	0.527	0.276	0.484	0.450	0.377	0.871 (0.640-1.184)
2	16861205	G→A	186561634	0.801	0.762	0.193	0.146	0.109	0.716 (0.475-1.078)
3	822396	A→G	186566877	1.000	0.713	0.101	0.120	0.431	1.217 (0.746-1.987)
4	1501299	C→A	186571123	0.152	0.682	0.333	0.249	0.016	0.662 (0.472-0.928)
5	1063537	C→T	186574075	0.200	0.025	0.267	0.256	0.739	0.943 (0.666-1.335)
6	1063538	T→C	186574183	0.821	0.534	0.396	0.482	0.027	1.421 (1.040-1.941)

Table 2. The haplotype distribution in gastric cancer case-control study

	Frequency				
	Controls	Cases	Haplotype	OR (95% CI)	*P*-value
SNP 4,6	0.393	0.476	CC	1.209	0.033
	0.332	0.241	AT	0.955	0.010
	0.273	0.276	CT	1.163	0.940
SNP 4,5,6	0.396	0.477	CCC	1.207	0.036
	0.333	0.243	ACT	0.957	0.010
	0.276	0.254	CTT	1.118	0.688

knowledge, the association analysis on SNPs of ADIPOQ gene and pancreatic cancer is still lacking. Therefore, we initially did genetic association analysis of adiponectin gene and pancreatic cancer in a case-control study of Chinese Han population.

Materials and methods

Study population

Totally 172 patients with pancreatic cancer and 181 cancer-free people were recruited from Chinese PLA General Hospital between 2009 and 2012. The patients with mean age of 61.6±9.5 (age from 34 to 82), comprised of 134 males and 38 females, had consistent diagnosis by two pathologists. All the patients had complete clinical pathological data. We drew blood samples prior to surgical operation, chemotherapy or radiotherapy. Unrelated volunteers were comprised of 136 males and 45 females with mean age of 63.1±8.4 (age from 29 to 83). Patients and healthy people were all Chinese Han popolations. Individuals who participated in the study were all given informed consent, and this study was approved by the Clinic Research Ethics Committee of Chinese PLA General Hospital.

SNP selection and genotyping

Six SNPs of adiponectin gene were selected according to results from Haploview software. Genotyping of SNPs in this study was performed by Sequenom MassARRAY system (Sequenom, San Diego, California, USA). Locus-specific PCR and detection were designed using MassARRAY Design 3.0 software. The DNA samples were then amplified by multiplex PCR reaction, and the product used in the locus-specific single-base extension reaction. The final products were desalted and transferred to a 384-element SpectroCHIP array. Allele detection was performed using MALDI-TOF MS. The mass spectrograms were analyzed using the MassARRAY Typer software (Sequenom).

Statistical analysis

We used a logistic regression model to analyze the association of the SNPs with the risk of gastric cancer. The Hardy-Weinberg equilibrium for each SNP was determined according to the control samples (P>0.01). The association between variants and gastric cancer risk was analyzed by calculating the odds ratios (OR) and 95% confidence intervals (95% CI) of the minor allele frequency. The statistical software Plink version 1.07 The *P* values reported in the study were based on a two-sided probability test with a significance level of (http://pngu.mgh.harvard.edu/purcell/plink/) and Stata/SE version 10 were used for statistical analyses (StataCorp LP, College Station, TX, USA).

Results

Genotype and haplotype frequency

We genotyped six SNPs of adiponectin gene all of which were accord with assumption of Hardy-

Table 3. The correlation between clinical parameters and adiponectin polymorphisms

	tumor size				Cigarette smoking				alcohol consumption			
	<2 cm	≥2 cm	*P*-value	OR (95% CI)	Yes	Never	*P*-value	OR (95% CI)	Yes	Never	*P*-value	OR (95% CI)
rs1501299												
CC	29	66	0.026	1	48	46	0.022	1	47	47	0.001	1
CA	9	58	0.014	2.832 (1.239-6.474)	28	39	0.246	1.453 (0.773-2.745)	20	47	0.037	2.230 (1.051-4.733)
AA	8	1	0.246	3.515 (0.420-29.409)	8	1	0.059	0.130 (0.016-1.084)	8	1	0.126	0.183 (0.021-1.165)
rs1065358												
TT	7	35	0.616	1	25	17	0.276	1	25	17	0.026	1
TC	20	67	0.410	0.670 (0.258-1.737)	39	48	0.120	1.810 (0.857-3.821)	31	56	0.170	1.776 (0.782-4.036)
CC	9	27	0.366	0.600 (0.198-1.817)	19	16	0.644	1.238 (0.500-3.066)	18	17	0.415	1.513 (0.559-4.096)

Weinberg equilibrium (P>0.01), and two of these SNPs showed significant associations with pancreatic cancer risk. Of which rs1501299C>A decreased the risk of PC (P=0.016, OR=0.662 95% CI 0.472-0.928), while rs1065358T>C increased the risk of PC (P=0.027, OR=1.421 95% CI 1.040-1.941). We did not find any associations of the other four SNPs with PC risk (**Table 1**).

Then we further performed haplotype analysis using Haploview Software. The six SNPs were distributed in two blocks. Of which, the SNP rs182052 and rs16861205 (SNP 1 and 2) were in a block (LD plot 1), while the SNP rs1501299, rs1063537 and rs1063538 (SNP 4, 5 and 6) were in another block (LD plot 2). We found the haplotypes of adiponectin gene affected susceptibility with PC. The result was consistent with the results of genotype. We did not find any association between the SNPs in LD plot 1 and the risk of pancreatic cancer (Data not shown). The haplotype CC (P=0.033) with major allele of rs1501299 and minor allele of rs1065358 increased the risk of PC, and the haplotype AT (0.010) with minor allele of rs1501299 and major allele of rs1065358 decreased the risk of PC. In combination of alleles of rs1501299 and rs1065358 with major allele of rs1063537 which the three SNPs were in a strong LD plot, the result was still constant (**Table 2**).

Clinical susceptibility analysis of SNPs in ADIPOQ gene with pancreatic cancer

We performed the stratified association analysis with regards to the six SNPs in different clinical datum which we got from the epidemiological survey (including age, gender, TNM stage, differentiation, tumor location, tumor size, diabetes, pancreatitis, cigarette smoking, alcohol consumption and family history). The results were summarized in **Table 3** and indicated that the SNP rs1501299 was significantly correlated with tumor size (P=0.026), smoking (P=0.022) and alcohol drinking (P=0.001). Meanwhile, the SNP rs1065358 had significant correlation with alcohol drinking (P=0.026). The other four SNPs, however, had no correlations with any clinical parameters (data not shown).

Discussion

In this study, a hospital based genetic association analysis of adiponectin gene was performed with pancreatic cancer in Chinese Han populations. Although previous researches have demonstrated the correlations between plasma adiponectin levels and pancreatic cancer, this could be the initial research on genetic variants analysis. Our results indicated that the two SNPs rs1501299C>A and rs1063538T>C in adiponectin gene played a reverse role in the risk of pancreatic cancer.

We demonstrated that the SNP rs1501299 with C to A change was significantly associated with decreased risk of PC, while the SNP rs1063538 with T to C change had strong association with increased the risk of PC. The result from haplotype analysis would provide us the same trend. That is, when the haplotype of rs1501299 and rs1063538 was CC, it increased the risk of pancreatic cancer, while the haplotype of these two SNPs was AT, there was a decreased risk between the SNPs and pancreatic cancer. The SNP rs1063537 which was also in a block with rs1501299 and rs1063538 might have not distinct effects on the pancreatic cancer risk.

It has been reported that the rs1063538 was located in the 3-untranslational region (3'-UTR), which the change of this variant might influence adiponectin expression levels and subsequent biological effects directly [19]. For another, although the SNP rs1501299 was located in the intron region of adiponectin gene, it has been reported previously that rs1501299 variant was associated with plasma adiponectin levels [20]. However, there were some controversies about the role of adiponectin on carcinogenesis. Bao et al. [21] conducted a prospective research on five cohorts in American populations and found the plasma adiponectin concentration inversely associated with the risk of pancreatic cancer. But some case-control studies proposed by Dalagama and Chang have drawn the inconsistent conclusion in the relative small-scale cohorts in retrospective studies, and found that up-regulation of plasma adiponectin concentration increased the risk of pancreatic cancer [13, 22]. Furthermore, another prospective study proposed by Grote found that there were no association between plasma adiponectin concentration and the risk of PC in smokers, while lateral study reported an inverse association among never smokers [23]. It was reported previously that cigarette smoking represented the only-established environment risk

factor in development of PC [24]. And cigarette smoking increased the risk of PC by 1.5 to 5 folds, while the risk would be low in the quit-smoking cohorts [25]. We inferred that the controversial conclusions might be modified by environment effects such as smoking, alcohol consumption or diet [26]. In addition, the plasma adiponectin concentration in retrospective studies might be influenced by complicated factors due to severe pathological states of PC [27, 28]. So we were inclined to approve the viewpoint of Bao's research. Appealingly, our result indicated that the SNP rs1501299 was correlated to the cigarette smoking.

We also found the SNP rs1501299 had significant correlation with tumor size. As we know, tumor growth depends quite a lot on the formation of new blood vessels. Adiponectin preferentially influenced the tumor angiogenesis in pathological states, indicating the adiponectin might be related to the formation rate of new blood vessels [29]. White [30] and colleagues found the rapid tumor growth inversely correlated with plasma adiponectin concentration in animal models. Saxena [31] and colleagues demonstrated that adiponectin expression levels were inversely correlated with tumor size of hepatocellular cancer in a tissue microarray study. Meanwhile, similar result was found by research of Jeong and colleagues in the study of breast cancer [32]. We might infer that the SNP rs1501299 would function in the development of pancreatic cancers.

Furthermore, we observed both of SNPs in ADIPOG gene, rs1501299 and rs1063538, were significantly correlated with alcohol consumption. Alcohol has been not admitted as an independent risk factor of pancreatic cancer, although the risk of chronic pancreatitis was undoubtedly increased and commonly caused by alcohol consumption [33]. Wed infer that in the pathogenesis of pancreatic cancers, alcohol might promote other environmental effects which could play a part in development of PC, such as smoking [34].

Limitation of our research should be taken into account. Our research was restricted in the genetic variants analysis in a relatively small-scale Chinese Han population, so further validation in large-scale panels of patients and distinct population samples should be taken to enhance the credibility of results.

In conclusion, we initially investigated the associations between six SNPs in adiponectin gene and pancreatic cancer risk, and two SNPs were significantly associated with the risk of pancreatic cancer. Of which, the SNP rs1501299 decreased the risk of PC, while the SNP rs1063538 increased the risk of PC. Our study might provide a novel insight into understanding of genetic based development of pancreatic cancer. However, further functional analysis of ADIPOQ gene would be conducted to discuss the mechanisms of adiponectin in development of pancreatic cancer.

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Disclosure of conflict of interest

None.

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