The Association of Adiponectin Gene Promoter Variations with Non-Small Cell Lung Cancer in a Han Chinese Population

Yingfu Li1*, Yueting Yao2*, Xu Qian3, Li Shi2, Jingxian Zhou2, Qianli Ma4*, Yufeng Yao2*

1 Department of Geriatrics, The No.1 Affiliated Hospital of Kunming Medical University, Kunming, 650032, China, 2 Institute of Medical Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, Yunnan Key Laboratory of Vaccine Research & Development on Severe Infectious Disease, Kunming, 650118, China, 3 Department of Cardiothoracic Surgery, Yan’an Hospital of Kunming, Kunming, 650051, China, 4 Department of Thoracic Surgery, The No.3 Affiliated Hospital of Kunming Medical University, Kunming, 650118, China

☯ These authors contributed equally to this work.
* maqianli78@126.com (QM); leoyyf@gmail.com (Yufeng Yao)

Abstract

Recently, in vitro studies have demonstrated that adiponectin has antiangiogenic and tumor growth-limiting properties. Additionally, serum adiponectin levels have been associated with the risk of several cancers; specifically, serum adiponectin was significantly lower in lung cancer patients with advanced-stage disease. In this study, we examined the association of adiponectin gene promoter variations associated with adiponectin gene expression and plasma levels in non-small cell lung cancer (NSCLC) in a Han Chinese population. A total of 319 patients with NSCLC and 489 healthy individuals were recruited to evaluate the association of four adiponectin gene promoter single-nucleotide polymorphisms (SNPs) (SNP-12140G>A, SNP-11426A>G, SNP-11391G>A and SNP-11377C>G) with NSCLC risk. Additionally, we constructed haplotypes of these four SNPs and evaluated the association of these haplotypes with NSCLC risk. Our results showed that among these four SNPs, only SNP-12140G>A was associated with NSCLC risk (P<0.05). The haplotype analysis showed that no haplotype was associated with NSCLC risk (P>0.05). Additionally, an association analysis of the four SNPs stratified into pathologic stages I+II and III+IV showed that these SNPs did not exhibit significant differences between pathologic stages I+II and III+IV. Moreover, we did not observe any differences in allele and genotype frequency for these SNPs between adenocarcinoma and squamous cell carcinoma. Our results indicated that the G allele of SNP-12140 may be a risk factor for NSCLC (OR = 1.516; 95% CI: 1.098–2.094) in this Han Chinese population.

Introduction

Lung cancer is one of the leading causes of cancer deaths worldwide and has a 5-year survival rate of approximately 15%[1], and non-small cell lung cancer (NSCLC) accounts for
approximately 80% of lung cancer cases [2]. In China, the incidence and mortality of lung cancer are estimated to be 0.7 and 0.6 million cases, respectively.

Adiponectin is an adipose tissue-secreted protein that acts as an endogenous insulin sensitizer by binding to insulin receptors [3]. Previous studies have shown that adiponectin is associated with obesity [4, 5], insulin resistance [5, 6] and type 2 diabetes [7, 8]. Recently, several studies have reported that adiponectin has antiproliferative and proapoptotic effects in breast cancer cell lines in vitro [9, 10]. Additionally, many studies have reported that lower adiponectin levels are associated with an increased risk of endometrial cancer [11–14], renal cancer [15], colon cancer [16] and breast cancer [10, 17–19]. The above results suggest that adiponectin may play an important role in tumor development and growth. In 2007, Petridou et al. found that serum adiponectin was not significantly different in patients with lung cancer compared with controls, though it was significantly lower in patients with advanced-stage disease, suggesting that adiponectin could be a potential marker for lung cancer progression [3]. Recently, Nigro et al. provided evidence for a direct effect of adiponectin on the proliferation and inflammation status of A549 cells, which supported the hypothesis that adiponectin plays a protective role in the lung and suggested that adiponectin could be a promising therapeutic target in lung diseases [20].

In 2006, Heid et al. reported that adiponectin promoter single-nucleotide polymorphisms (SNPs) are associated with adiponectin concentrations [21]. Laumen et al. later demonstrated that adiponectin promoter SNPs regulate adiponectin promoter activity [22]. Therefore, adiponectin promoter variations may have an impact on cancer risk.

We previously reported the association of two adiponectin gene promoter SNPs (SNP-12410G>A and SNP-11377C>G) with NSCLC risk in a Han Chinese population, and the results showed that SNP-12140G>A (rs266730) is associated with increased NSCLC risk [23]. To confirm these results, in this study, we increased the sample size (from 179 to 319 cases, from 242 to 489 controls) and added two other adiponectin gene promoter SNPs (SNP-11426A>G and SNP-11391G>A) to evaluate the association of these four adiponectin gene promoter SNPs, namely, SNP-12140G>A (rs266730), SNP-11426A>G (rs16861194), SNP-11391G>A (rs17200539) and SNP-11377C>G (rs266729), and their haplotypes with NSCLC risk in a Han Chinese population.

**Materials and Methods**

1.1 Ethics statement

This protocol was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Boards of the No.1 and No.3 Affiliated Hospitals of Kunming Medical University. All participants provided written informed consent.

1.2 Subjects

The case group included 319 patients (213 males and 106 females) who were diagnosed with NSCLC at the No.1 and No.3 Affiliated Hospitals of Kunming Medical University from July 2012 to May 2014. The histological type of lung cancer was identified according to the World Health Organization (WHO 2004) classifications. The pathologic stage was determined according to the International System for Staging Lung Cancer [24]. Based on the pathomorphological reports, the NSCLC cases included adenocarcinoma (AC), squamous cell carcinoma (SCC), and adenocarcinoma and squamous cell carcinoma (AC+SCC). NSCLC patients with a prior history of primary cancer other than lung cancer were excluded from the current study. Additionally, individuals with hypertension, coronary heart disease and diabetes were also excluded from this study to avoid any potential interference from overlapping genes. Clinical
characteristics and data, such as sex, age, family history of cancer, and histological type of cancer, were obtained. The healthy control group included 489 subjects (313 males and 176 females) who had no family history of NSCLC and were recruited from a population undergoing routine health checkups at the No.1 and No.3 Affiliated Hospitals of Kunming Medical University. All participants (NSCLC patients and healthy controls) self-reported as ethnic Hans and lived roughly within the same geographic region (Yunnan Province, China).

1.3 NP genotyping using TaqMan assay method

Genomic DNA was extracted from peripheral lymphocytes using a standard hydroxybenzene-chloroform method. Four adiponectin promoter SNPs, namely SNP-12140G>A (rs266730), SNP-11426A>G (rs16861194), SNP-11391G>A (rs17200539) and SNP-11377C>G (rs266729), were genotyped using PCR amplification with a TaqMan assay. Primers and probes were purchased from Applied Biosystems (Foster City, CA, USA). Selected PCR products were characterized by direct sequencing on a 3100 Genetic Analyzer (Applied Biosystems, Tokyo, Japan).

1.4 Statistical analysis

The allele and genotype frequencies of the four SNPs were calculated by the direct-counting method. Hardy-Weinberg equilibrium (HWE) was tested for the SNPs in both the NSCLC and control groups. The linkage disequilibrium (LD) and haplotype frequencies (deduced from the phenotype) were calculated based on the genotyping results by the expectation-maximization algorithm in SHEsis software[25, 26]. A χ² test was used to determine differences in allele, genotype and haplotype frequencies between the NSCLC and control groups, and the odds ratios (OR) with associated 95% confidence intervals (CIs) of allele-specific risks were calculated. Bonferroni correction was used for testing multiple comparisons. The association between each SNP and NSCLC was analyzed for the mode of inheritance using SNPStats software [27]. The Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used to determine the best-fit model for each SNP. The statistical power was calculated using PS Software[28]. The statistical analyses were performed using SPSS 13 (Chicago, IL). A P value less than 0.05 was considered statistically significant.

Results

1.1 Subject characteristics

Table 1 lists the characteristics of the enrolled subjects. There were no age or gender differences between the NSCLC and control groups (P>0.05). In the NSCLC individuals, 193 had AC, 121 had SCC, and 5 had AC and SCC. There were 47 patients in pathological stage I, 55 in stage II, 115 in stage III and 102 in stage IV.

1.2 Association of adiponectin gene promoter SNP-12140G>A, SNP-11426A>G, SNP-11391G>A, and SNP-11377C>G with NSCLC

The allele and genotype frequencies for SNP-12140G>A, SNP-11426A>G, SNP-11391G>A and SNP-11377C>G in the NSCLC and control groups are listed in Table 2. SNP-11391G>A was found to be monomorphic (G allele) in both the NSCLC and control groups. The genotype frequencies for SNP-12140G>A, SNP-11426A>G and SNP-11377C>G were in HWE for the NSCLC and control groups (P>0.05). However, the allele and genotype frequencies of SNP-12140G>A were significantly different in the NSCLC and control groups (P = 0.011 and 0.046, respectively). The G allele for SNP-12140G>A occurred at a significantly higher frequency in
the NSCLC group compared with the control group (OR = 1.516; 95% CI: 1.098–2.094). The allele and genotype frequencies for SNP-11426A>G and SNP-11377C>G were not significantly different in the NSCLC and control groups (P > 0.05).

1.3 Mode of inheritance analysis of adiponectin promoter SNP-12140G>A, SNP-11426A>G and SNP-11377C>G

Tables 3–5 present the results of the mode of inheritance analysis for the four SNPs. To compare each inheritance model (codominant, dominant, recessive, overdominant and log-additive) to the most general model (codominant), the AIC and BIC were calculated to identify the inheritance model that best fit the data[27]. The model with the smallest AIC and BIC value corresponds to the minimal expected entropy[27]. The best fit inheritance model with the lowest AIC and BIC for SNP-12140G>A and SNP-11426A>G was the log-additive model. The best fit inheritance model with the lowest AIC and BIC for SNP-11377C>G was the dominant and log-additive model. The analysis of different genetic models revealed that the GG genotype of SNP-12140G>A conferred more NSCLC risk in the log-additive model. No significant differences for SNP-11426A>G and SNP-11377C>G were found between the NSCLC and control groups in the different genetic models.

### Table 1. Clinical characteristics of the subjects enrolled in the present study (Data are mean±SD).

|                          | NSCLC       | Control    | P value |
|--------------------------|-------------|------------|---------|
| N                        | 319         | 489        |         |
| Ages (years)             | 55.55±10.69 | 54.68±10.38| 0.25    |
| Sex (M/F)                | 213/106     | 313/176    | 0.42    |
| Adenocarcinoma (AC)      | 193(60.5%)  |            |         |
| Squamous cell carcinoma (SSC) | 121(37.9%) |            |         |
| Adenocarcinoma and Squamous cell carcinoma (AC+SSC) | 5(1.6%) |            |         |
| Clinical stage           |             |            |         |
| I                        | 47(14.7%)   |            |         |
| II                       | 55(17.2%)   |            |         |
| III                      | 115(36.1%)  |            |         |
| IV                       | 102(32.0%)  |            |         |

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### Table 2. Comparison of genotypic and allelic distribution of SNP-12140G>A, SNP-11426A>G and SNP-11377C>G between NSCLC and control groups.

| SNPs             | Genotypes[n(%)] | P value | Alleles[n(%)] | P value | Odds Ratio[95 CI] |
|------------------|-----------------|---------|---------------|---------|------------------|
| SNP-12140G>A     |                 |         |               |         |                  |
| NSCLC            | A/A(freq) 4(13.0%) | 0.046  | A(freq) 60(9.4%) | 0.011  | 1.516[1.098–2.094] |
| Control          | 12(2.5%) | 109(22.3%) | 368(75.3%) | 845(86.4%) |                  |
| SNP-11426A>G     |                 |         |               |         |                  |
| NSCLC            | 223(69.9%) | 86(27.0%) | 10(3.1%) | 0.335  | 0.825[0.626–1.086] |
| Control          | 360(73.6%) | 120(24.5%) | 9(1.8%) | 138(14.1%) |                  |
| SNP-11377C>G     |                 |         |               |         |                  |
| NSCLC            | 166(52.0%) | 129(40.4%) | 24(7.5%) | 259(26.5%) |                  |
| Control          | 264(54.0%) | 191(39.1%) | 34(7.0%) |                  |                  |

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1.4 Linkage disequilibrium (LD) and haplotype analysis of adiponectin promoter SNP-12140G>A, SNP-11426A>G and SNP-11377C>G

Significant LD (D') values between the SNP-12140G>A, SNP-11426A>G and SNP-11377C>G loci were found in all individuals. The D' value was 0.899 between SNP-12140G>A and SNP-11426A>G, 0.857 between SNP-12140G>A and SNP-11377C>G and 0.864 between SNP-11426A>G and SNP-11377C>G. Based on the LD result, we calculated the haplotypes of these three SNPs, and Table 6 shows the estimated frequencies of SNP-12140G>A-SNP-11426A>G-SNP-11377C>G haplotypes with frequencies of more than 3%. None of the haplotypes were significantly different in the NSCLC and control groups after Bonferroni correction (P>0.05).

1.5 Association analysis of adiponectin promoter SNP-12140G>A, SNP-11426A>G, and SNP-11377C>G with different pathologic stages

There were no differences in the allele and genotype frequencies for SNP-12140G>A, SNP-11426A>G and SNP-11377C>G between pathologic stages I+II and III+IV (Table 7).

| Table 3. Different inheritance models analysis of the SNP-12140G>A in adiponectin gene promoter between NSCLC and control groups. |
| --- | --- | --- | --- | --- |
| Model | Genotype | Control[n(%)] | NSCLC[n(%)] | Before adjusted by Age and Sex | After adjusted by Age and Sex |
| | | OR (95% CI) | P value | OR (95% CI) | P value |
| Codominant | G/G | 368 (75.3%) | 263 (82.5%) | 1.00 | 0.042 |
| | G/A | 109 (22.3%) | 52 (16.3%) | 0.67 (0.46–0.96) | 0.67 (0.46–0.96) |
| | A/A | 12 (2.5%) | 4 (1.2%) | 0.47 (0.15–1.46) | 0.45 (0.14–1.42) |
| Dominant | G/G | 368 (75.3%) | 263 (82.5%) | 1.00 | 0.015 |
| | G/A-A/A | 121 (24.7%) | 56 (17.6%) | 0.65 (0.45–0.92) | 0.64 (0.45–0.92) |
| Recessive | G/G-G/A | 477 (97.5%) | 315 (98.8%) | 1.00 | 0.220 |
| | G/A | 109 (22.3%) | 52 (16.3%) | 0.67 (0.46–0.96) | 0.67 (0.46–0.96) |
| | A/A | 12 (2.5%) | 4 (1.2%) | 0.47 (0.15–1.46) | 0.45 (0.14–1.42) |
| Overdominant | G/G-A/A | 380 (77.7%) | 267 (83.7%) | 1.00 | 0.035 |
| | G/A | 109 (22.3%) | 52 (16.3%) | 0.67 (0.49–0.92) | 0.67 (0.49–0.92) |
| Log-additive | --- | --- | --- | --- | --- |

| Table 4. Different inheritance models analysis of the SNP-11426A>G in adiponectin gene promoter between NSCLC and control groups. |
| --- | --- | --- | --- | --- |
| Model | Genotype | Control[n(%)] | NSCLC[n(%)] | Before adjusted by Age and Sex | After adjusted by Age and Sex |
| | | OR (95% CI) | P value | OR (95% CI) | P value |
| Codominant | A/A | 360 (73.6%) | 223 (69.9%) | 1.00 | 0.340 |
| | G/A | 120 (24.5%) | 86 (27%) | 1.16 (0.84–1.60) | 1.16 (0.84–1.61) |
| | G/G | 9 (1.8%) | 10 (3.1%) | 1.79 (0.72–4.48) | 1.78 (0.71–4.45) |
| Dominant | A/A | 360 (73.6%) | 223 (69.9%) | 1.00 | 0.250 |
| | G/A-G/G | 129 (26.4%) | 96 (30.1%) | 1.20 (0.88–1.64) | 1.21 (0.88–1.65) |
| Recessive | A/A-G/A | 480 (98.2%) | 309 (96.9%) | 1.00 | 0.240 |
| | G/G | 9 (1.8%) | 10 (3.1%) | 1.73 (0.69–4.30) | 1.71 (0.69–4.26) |
| Overdominant | A/A-G/A | 369 (75.5%) | 233 (73%) | 1.00 | 0.440 |
| | G/A | 120 (24.5%) | 86 (27%) | 1.13 (0.82–1.57) | 1.14 (0.83–1.57) |
| Log-additive | --- | --- | --- | --- | --- |
1.6 Association analysis of *adiponectin* promoter SNP-12140G>A, SNP-11426A>G, and SNP-11377C>G with adenocarcinoma (AC) and squamous cell carcinoma (SCC)

Our results revealed no differences in the allele and genotype frequencies for SNP-12140G>A, SNP-11426A>G and SNP-11377C>G between AC and SCC (Table 8).

### Discussion

Recent evidence has demonstrated that adiponectin has antiproliferative and proapoptotic effects in breast cancer cell lines in vitro[9, 10]. In 2007, Petridou *et al.* found that serum adiponectin was not significantly different in patients with lung cancer compared with controls but that it was significantly lower in patients at advanced disease stages, suggesting that adiponectin could be a potential marker for lung cancer progression[3]. Pei *et al.* then undertook a meta-analysis to exploit the causal relevance of circulating adiponectin with cancer; their findings demonstrated that genetically higher circulating adiponectin conferred a protective effect against lung cancer but a risk for colorectal cancer[29].

In 2011, Cui *et al.* investigated the association of SNPs in the adiponectin gene (rs266729, rs822395, rs822396 and rs2241766) with NSCLC[30], and the SNPs they chose were reported to be related to circulating adiponectin levels[30]. Although Cui *et al.* failed to observe an association for SNP-11377C>G (rs266729), which is located in the *adiponectin* promoter, with

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### Table 5. Different inheritance models analysis of the SNP-11377C>G in adiponectin gene promoter between NSCLC and control groups.

| Model       | Genotype | Control[n(%)] | NSCLC[n(%)] | Before adjusted by Age and Sex | After adjusted by Age and Sex |
|-------------|----------|---------------|-------------|--------------------------------|-------------------------------|
|             |          |               |             | OR (95% CI)                     | P value                       | OR (95% CI)                     | P value |
| Codominant  | C/C      | 264 (54%)     | 166 (52%)   | 1.00                           | 0.85                          | 1.00                           | 0.840   |
|             | G/C      | 191 (39.1%)   | 129 (40.4%) | 1.07 (0.80–1.44)               | 1.08 (0.80–1.45)              |                                |         |
|             | G/G      | 34 (7%)       | 24 (7.5%)   | 1.12 (0.64–1.96)               | 1.13 (0.65–1.98)              |                                |         |
| Dominant    | C/C      | 264 (54%)     | 166 (52%)   | 1.00                           | 0.59                          | 1.00                           | 0.570   |
|             | G/C-G/G  | 225 (46%)     | 153 (48%)   | 1.08 (0.82–1.43)               | 1.09 (0.82–1.44)              |                                |         |
| Recessive   | C/C-G/C  | 455 (93%)     | 295 (92.5%) | 1.00                           | 0.76                          | 1.00                           | 0.740   |
|             | G/G      | 34 (7%)       | 24 (7.5%)   | 1.09 (0.63–1.87)               | 1.10 (0.64–1.89)              |                                |         |
| Overdominant| C/C-G/G  | 298 (60.9%)   | 190 (59.6%) | 1.00                           | 0.70                          | 1.00                           | 0.680   |
|             | G/G      | 191 (39.1%)   | 129 (40.4%) | 1.06 (0.79–1.41)               | 1.06 (0.80–1.42)              |                                |         |
| Log-additive| —        | —             | —           | 1.07 (0.85–1.33)               | 0.58                          | 1.07 (0.85–1.34)               | 0.560   |

| Model       | Genotype | Control[n(%)] | NSCLC[n(%)] | P value | Fisher's Bonferroni correction | OR [%95 CI] |
|-------------|----------|---------------|-------------|---------|-------------------------------|-------------|
| SNP-12140G>A| A        | A             | C           | 0.025   | >0.05                         | 0.691       |
| SNP-11426A>G| A        | A             | G           | 0.03(0.0%) | 5.45(0.6%)               | -           |
| SNP-11377C>G| A        | G             | C           | 0.00(0.0%) | 0.35(0.0%)               | -           |
| SNP-12140G>A| G        | A             | C           | 298.53(46.8%) | 456.69(46.7%) | 0.980 | >0.05 | 0.997 | [0.816–1.219] |
| SNP-11426A>G| G        | A             | G           | 173.47(27.2%) | 250.67(25.6%) | 0.511 | >0.05 | 1.079 | [0.860–1.353] |
| SNP-11377C>G| G        | G             | C           | 102.5(16.1%) | 134.7(18.3%)   | 0.214 | >0.05 | 1.193 | [0.903–1.577] |
| SNP-12140G>A| G        | G             | G           | 3.5(0.5%) | 2.89(0.3%)               | -           |

**Note:** All P values were calculated using the Fisher's exact test, and the 95% confidence intervals (CI) were calculated using the Bonferroni method.
NSCLC[30], their results revealed that the rs2241766 TT genotype was significantly associated with NSCLC susceptibility. In 2006, Heid et al. reported that rs2241766 was associated with adiponectin levels, with the GG genotype correlating with increased adiponectin levels compared with the TT genotype [21]. In addition, Pei et al. also observed that the rs2241766 GG genotype or G allele was associated with significantly higher circulating adiponectin levels than the TT genotype, without heterogeneity[29]. Moreover, a meta-analysis of cancer risk and adiponectin gene polymorphisms by Xu et al. and Yang et al. indicated that the G allele of rs2241766 was a potential protection factor for cancer risk[31, 32]. Therefore, rs2241766TT is associated with lower adiponectin levels, and the rs2241766TT genotype may be significantly associated with NSCLC susceptibility.

In 2009, Laumen et al. found that SNP-11426A>G, SNP-11391G>A and SNP-11377C>G within the adiponectin promoter were regulatory SNPs for adiponectin promoter activity[22]. Consequently, we choose four adiponectin promoter SNPs to evaluate the association of these SNPs and their haplotypes with NSCLC in a Han Chinese population. Our results confirmed our previous finding that the G allele of the adiponectin promoter SNP-12140G>A is a risk factor for NSCLC[23] and that individuals with the SNP-12140G>A GG genotype have increased risk for NSCLC under log-additive models. The other three adiponectin gene promoter SNPs, including SNP-11377C>G, were not correlated with NSCLC in the current study. In 2008, Kaklamani et al. reported that adiponectin gene promoter SNP-11377C>G is associated with colorectal cancer risk[33]. However, Carvajal-Carmona et al. failed to observe an association between SNP-11377C>G and colorectal cancer risk in patients from the UK[34], stating that

| SNPs         | Genotypes[n(%)] | P value | Alleles[n(%)] | P value | Odds Ratio[95 CI] |
|--------------|-----------------|---------|---------------|---------|------------------|
| SNP-12140 G>A | A/A(freq)       | A/G(freq) | G/G(freq) | 0.850  | 37(9.6%) 490(90.4%) 0.702 1.116[0.636-1.956] |
| Stage I+II   | 3(1.6%)         | 31(16.1%)  | 159(82.4%) |         |                  |
| Stage III+IV | 10(8.8%)        | 19(15.7%)  | 101(83.5%) |         |                  |
| SNP-11426 A>G | A/A(freq)       | A/G(freq) | G/G(freq) | 0.591  | 325(84.2%) 61(15.8%) 0.363 1.217[0.797-1.860] |
| Stage I+II   | 138(71.5%)      | 49(25.4%)  | 6(3.1%)   |         |                  |
| Stage III+IV | 80(66.1%)       | 37(30.6%)  | 4(3.3%)   |         |                  |
| SNP-11377 C>G | C/C(freq)       | C/G(freq) | G/G(freq) |         |                  |
| Stage I+II   | 101(52.3%)      | 80(41.5%)  | 12(6.2%)  | 0.617  | 282(73.1%) 104(26.9%) 0.668 1.081[0.756-1.548] |
| Stage III+IV | 63(52.1%)       | 47(38.8%)  | 11(9.1%)  |         |                  |

Table 8. Association analysis of the adiponectin promoter SNP-12140G>A, SNP-11426A>G, SNP-11377C>G between pathologic stages I+II and III+IV.
the discrepancy between their results and those of Kaklamani et al. may be due to differences between ethnic groups, differential environmental effects or false-positive results[34]. Pei et al. reported an interesting finding that genetically elevated circulating adiponectin may confer a protective effect against lung cancer but a risk for colorectal cancer[29]. Thus, the type of cancer could be a key factor in the relationship between cancer and the adiponectin gene. Our results, together with those of Cui et al. [30], indicated that SNP-11377C>G is not associated with NSCLC risk and suggested that the role of adiponectin gene SNP-11377C>G may be specific to the cancer type or ethnicity. Additionally, our results indicated that the role of adiponectin gene promoter SNPs may be specific to the SNP site, e.g., SNP-12140G>A maybe more important than SNP-11377C>G in the Chinese population. Based on these results, we hypothesized that SNP-12140G>A may influence adiponectin promoter activity, gene expression, plasma adiponectin level and, ultimately, NSCLC risk. Unfortunately, to date, the role of SNP-12140G>A in adiponectin promoter activity and gene expression has not been tested. Therefore, the function of this SNP in adiponectin promoter activity and gene expression should be investigated in the future. It was an interesting finding that only SNP-12140G>A was associated with NSCLC in our study. Our data showed that SNP-12140G>A just was partly in linkage disequilibrium with SNP-11426A>G and SNP-11377C>G. Therefore, we assumed this could be possible one of causes of the divergence that only SNP-12140G>A associated with NSCLC in this Han Chinese population, but not SNP-11426A>G and SNP-11377C>G.

In this study, we also observed that there were no differences in allele and genotype frequency for SNP-12140G>A, SNP-11426A>G and SNP-11377C>G between AC and SCC (Table 8). In addition, there were no differences in allele and genotype frequency for SNP-12140G>A, SNP-11426A>G and SNP-11377C>G between pathologic stages I+II and III+IV (Table 7). However, in 2007, Petridou et al. found that adiponectin level was not significantly different between lung cancer individuals compared with controls, though it was significantly lower in patients with advanced disease stage[3]. These author spostulated that the reason that a significantly lower adiponectin level was observed in advanced disease-stage patients may be due to the decrease in overall fat mass in advanced lung cancer, leading to the decreased production of adiponectin by subcutaneous adipose tissue [3]. Thus, SNP-12140G>A may be merely a susceptibility factor for NSCLC in the Han Chinese population and not associated with lung cancer stage. The association of lower adiponectin with advanced disease stage could be the due to the decreased production of adiponectin induced by the decrease in fat mass in advanced lung cancer stages. Because we did not measure adiponectin levels in the NSCLC and control groups, which is a limitation of our study, we did not observe an association between genetic data, adiponectin levels and NSCLC risk and NSCLC pathologic stage.

There were other several limitations in the present study. A relatively small sample size may limit the statistical power of our study. The statistical power for the effect of SNP-12140G>A was calculated using PS software [28], and we found that our sample reached 74.1% of statistical efficacy. Thus, a larger population should be investigated to further verify our results. The other limitation of this study was that we did not ascertain the smoking status of the control individuals, making it difficult to perform future analyses of such exposure variables and to perform a gene-smoking interaction analysis. This limitation may neutralize the effect of smoking and expose the effects of genetic variants in our study.

Conclusions

In this study, we performed an association study for adiponectin promoter SNP-12140G>A, SNP-11426A>G and SNP-11377C>G and NSCLC in a Han Chinese population and found that the G allele of SNP-12140G>A is a risk factor for NSCLC; individuals with the
SNP-12140G>A GG genotype showed increased risk for NSCLC under log-additive models. In the future, larger scale studies are needed to better clarify and examine the association of adiponectin gene promoter variations with NSCLC susceptibility. Moreover, the function of SNP-12140 in adiponectin promoter activity and gene expression should be investigated.

Supporting Information

S1 Table. (XLSX)
S2 Table. (XLSX)

Author Contributions

Conceived and designed the experiments: QM Yufeng Yao. Performed the experiments: YL Yueting Yao. Analyzed the data: LS JZ. Contributed reagents/materials/analysis tools: XQ. Wrote the paper: LS Yufeng Yao.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005; 55(2):74–108. PubMed PMID:15761078.
2. Johnson JL, Pillai S, Chellappan SP. Genetic and biochemical alterations in non-small cell lung cancer. Biochim Biophys Acta. 2012; 940405. doi:10.1152/2012/940405 PubMed PMID:22928112; PubMed Central PMCID: PMC3426175.
3. Petridou ET, Mitsiades N, Gialamas S, Angelopoulos M, Skalkidou A, Desypris N, et al. Circulating adiponectin levels and expression of adiponectin receptors in relation to lung cancer: two case-control studies. Oncology. 2007; 73(3–4):261–9. doi:10.1159/000127424 PubMed PMID:18424891.
4. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun. 1999; 257(1):79–83. PubMed PMID:10092513.
5. Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, et al. Plasma adiponectin levels in overweight and obese Asians. Obes Res. 2002; 10(11):1104–10. doi:10.1038/oby.2002.150 PubMed PMID:12429873.
6. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab. 2001; 86(5):1930–5. doi:10.1210/jcem.86.5.7463 PubMed PMID:11344187.
7. Li S, Shin HJ, Ding EL, van Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA. 2009; 302(2):179–88. doi:10.1001/jama.2009.976 PubMed PMID:19564347.
8. Li Y, Yang Y, Shi L, Li X, Zhang Y, Yao Y. The association studies of ADIPOQ with type 2 diabetes mellitus in Chinese populations. Diabetes Metab Res Rev. 2012; 28(7):551–9. Epub 2012/04/28. doi:10.1002/dmr.2309 PubMed PMID:22539443.
9. Dieudonne MN, Bussiere M, Dos Santos E, Leneuve MC, Giudicelli Y, Pecqueury R. Adiponectin mediates antiproliferative and apoptotic responses in human MCF7 breast cancer cells. Biochem Biophys Res Commun. 2006; 345(1):271–9. doi:10.1016/j.bbrc.2006.04.076 PubMed PMID:16678125.
10. Korner A, Pazzitou-Panayiotou K, Kelesidis T, Kelesidis I, Williams CJ, Kaprara A, et al. Total and high-molecular-weight adiponectin in breast cancer: in vitro and in vivo studies. J Clin Endocrinol Metab. 2007; 92(3):1041–8. doi:10.1210/jc.2006-1858 PubMed PMID:17192291.
11. Dal Maso L, Augustin LS, Karalis A, Talamini R, Franceschi S, Trichopoulos D, et al. Circulating adiponectin and endometrial cancer risk. J Clin Endocrinol Metab. 2004; 89(3):1160–3. doi:10.1210/jc.2003-031716 PubMed PMID:15001602.
12. Petridou E, Mantzoros C, Desypris N, Koukoulomatis P, Addy C, Voulgaris Z, et al. Plasma adiponectin concentrations in relation to endometrial cancer: a case-control study in Greece. J Clin Endocrinol Metab. 2003; 88(3):993–7. doi:10.1210/jc.2002-021209 PubMed PMID:12629074.
13. Soliman PT, Wu D, Tortolero-Luna G, Schmeler KM, Slomovitz BM, Bray MS, et al. Association between adiponectin, insulin resistance, and endometrial cancer. Cancer. 2006; 106(11):2376–81. doi: 10.1002/cncr.21866 PubMed PMID: 16639730.

14. Cust AE, Kaaks R, Friedenreich C, Bonnet F, Laville M, Lukanova A, et al. Plasma adiponectin levels and endometrial cancer risk in pre- and postmenopausal women. J Clin Endocrinol Metab. 2007; 92(1):255–63. doi: 10.1210/jc.2006-1371 PubMed PMID: 17062789.

15. Spyridopoulos TN, Petridou ET, Skalkidou A, Dessypris N, Chrousos GP, Mantzoros CS, et al. Low adiponectin levels are associated with renal cell carcinoma: a case-control study. Int J Cancer. 2007; 120(7):1573–8. doi: 10.1002/jic.22526 PubMed PMID: 17205522.

16. Wei EK, Giovannucci E, Fuchs CS, Willett WC, Mantzoros CS. Low plasma adiponectin levels and risk of colorectal cancer in men: a prospective study. J Natl Cancer Inst. 1999; 92(22):1688–94. doi: 10.1093/jnci/dji376 PubMed PMID: 10373014.

17. Mantzoros C, Petridou E, Dessypris N, Chavelas C, Dalamaga M, Alexe DM, et al. Adiponectin and breast cancer risk. J Clin Endocrinol Metab. 2004; 89(3):1102–7. doi: 10.1210/jc.2003-031804 PubMed PMID: 15001594.

18. Miyoshi Y, Funahashi T, Kihara S, Taguchi T, Tamaki Y, Matsuzawa Y, et al. Association of serum adiponectin with breast cancer risk. J Clin Endocrinol Metab. 2003; 89(3):308–13. doi: 10.1210/jc.2003-031804 PubMed PMID: 12854273.

19. Cust AE, Kaaks R, Friedenreich C, Bonnet F, Laville M, Lukanova A, et al. Plasma adiponectin levels and endometrial cancer risk in pre- and postmenopausal women. J Clin Endocrinol Metab. 2007; 92(1):255–63. doi: 10.1210/jc.2006-1371 PubMed PMID: 17062789.

20. Miyoshi Y, Funahashi T, Kihara S, Taguchi T, Tamaki Y, Matsuzawa Y, et al. Association of serum adiponectin with breast cancer risk. J Clin Endocrinol Metab. 2003; 89(3):308–13. doi: 10.1210/jc.2003-031804 PubMed PMID: 12854273.

21. Heid IM, Wagner SA, Gohlke H, Iglseder B, Mueller JC, Cip P, et al. Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1,727 healthy Caucasians. Diabetes. 2006; 55(2):375–84. PubMed PMID: 16443770.

22. Laumen H, Saningong AD, Heid IM, Hess J, Herder C, Claussnitzer M, et al. Functional characterization of promoter variants of the adiponectin gene complemented by epidemiological data. Diabetes. 2009; 58(4):984–91. Epub 2009/03/20. PubMed PMID: 19290020.

23. Li Y, Qian X, Zhou J, Chen N, Hu Y, Ma Q. ADIPO genetic polymorphisms are associated with susceptibility of non-small cell lung cancer. Journal of Kunming Medical University. 2014; 35(9):52–5.

24. Groome PA, Bolejack V, Crowley JJ, Kennedy C, Krasnik M, Sobin LH, et al. The IASLC Lung Cancer Staging Project: validation of the proposals for revision of the T, N, and M descriptors and consequent stage groupings in the forthcoming (seventh) edition of the TNM classification of malignant tumours. J Thorac Oncol. 2007; 2(8):694–705. doi: 10.1097/JTO.0b013e31812d05d5 PubMed PMID: 17762335.

25. Li Z, Zhang Z, He Z, Tang W, Li T, Zeng Z, et al. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn). Cell Res. 2009; 19(4):519–33. doi: 10.1038/cr.2009.33 PubMed PMID: 19074962; PubMed Central PMCID: PMC2661577.

26. Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res. 2009; 19(4):519–33. doi: 10.1038/cr.2009.33 PubMed PMID: 19290020.

27. Sole X, Guino E, Valls J, Iniesta R, Moreno V. SNStats: a web tool for the analysis of association studies. Bioinformatics. 2006; 22(15):1928–9. doi: 10.1093/bioinformatics/btl268 PubMed PMID: 16720584.

28. Dupont WD, Plummer WD Jr. Power and sample size calculations for studies involving linear regression. Control Clin Trials. 1998; 19(6):589–601. PubMed PMID: 9875838.

29. Pei Y, Xu Y, Niu W. Causal relevance of circulating adiponectin with cancer: a meta-analysis implementing Mendelian randomization. Tumour Biol. 2014. doi: 10.1007/s13277-014-2654-x PubMed PMID: 25273172.

30. Cui E, Deng A, Wang X, Wang B, Mao W, Feng X, et al. The role of adiponectin (ADIPOQ) gene polymorphisms in the susceptibility and prognosis of non-small cell lung cancer. Biochem Cell Biol. 2011; 89(3):308–13. doi: 10.1139/O11-005 PubMed PMID: 21619462.

31. Xu Y, He B, Pan Y, Gu L, Nie Z, Chen L, et al. The roles of ADIPOQ genetic variations in cancer risk: evidence from published studies. Mol Biol Rep. 2013; 40(2):1135–44. doi: 10.1007/s10077-012-2154-2 PubMed PMID: 23065236.
32. Yang Y, Zhang F, Ding R, Skrip L, Wang Y, Lei H, et al. ADIPOQ gene polymorphisms and cancer risk: a meta-analysis. Cytokine. 2013; 61(2):565–71. doi:10.1016/j.cyto.2012.10.030 PubMed PMID: 23200411.

33. Kaklamani VG, Wisinski KB, Sadim M, Gulden C, Do A, Offit K, et al. Variants of the adiponectin (ADIPOQ) and adiponectin receptor 1 (ADIPOR1) genes and colorectal cancer risk. JAMA. 2008; 300(13):1523–31. doi:10.1001/jama.300.13.1523 PubMed PMID: 18827209; PubMed Central PMCID: PMC2628475.

34. Carvajal-Camona LG, Spain S, Consortium C, Kerr D, Houlston R, Cazier JB, et al. Common variation at the adiponectin locus is not associated with colorectal cancer risk in the UK. Hum Mol Genet. 2009; 18(10):1889–92. doi:10.1093/hmg/ddp109 PubMed PMID: 19264763.