Functional genetic variants in complement component 7 confer susceptibility to gastric cancer

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Background Complement system plays an important role in innate immunity which involved in the changes tumor immune microenvironment by mediating the inflammatory response. This study aims to explore the relationship between complement component 7 (*C7*) polymorphisms and the risk of gastric cancer (GC). **Materials and methods** All selected SNPs of *C7* were genotyped in 471 patients and 471 controls using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Odds ratios (ORs) and 95% confidence intervals (*CI*s) were calculated by unconditional Logistic regression to analyze the relationship between each genotype and the genetic susceptibility to gastric cancer. The level of *C7* expression in GC was analyzed by Gene Expression Profiling Interactive Analysis (GEPIA) and detected by Enzyme Linked Immunosorbent Assay. Kaplan-Meier plotter were used to reveal *C7* of prognostic value in GC. We examined SNPs association with the expression of *C7* using the GTEx database. The effect of *C7* polymorphisms on the regulatory activity of *C7* was detected by luciferase reporter assay. **Results** Unconditional logistic regression showed that individuals with *C7* rs1376178 AA or CA genotype had a higher risk of GC with OR (95%*CI*) of 2.09 (1.43-3.03) and 1.88(1.35-2.63), respectively. For *C7* rs1061429 C>A polymorphism, AA genotype was associated with the elevated risk for developing gastric cancer (OR = 2.16, 95%*CI* = 1.37-3.38). In stratified analysis, *C7* rs1376178 AA genotype increased the risk of GC among males (OR = 2.88, 95%*CI* = 1.81-4.58), but not among females (OR = 1.06, 95%*CI* = 0.55-2.06). Individuals carrying rs1061429 AA significantly increased the risk of gastric cancer among youngers (OR = 2.84, 95%*CI* = 1.39-5.80) and non-smokers (OR = 2.79, 95%*CI* = 1.63-4.77). *C7* was overexpressed in gastric cancer tissues and serum of cancer patients and was significantly associated with the prognosis. *C7* rs1061429 C>A variant contributed to reduced protein level of *C7* (*P* = 0.029), but rs1376178 didn't. Luciferase reporter assay showed that rs1376178C-containing plasmid exhibited 2.86-fold higher

luciferase activity than rs1376178 A-containing plasmid ($P < 0.001$). We also found that rs1061429A allele contributed 1.34-fold increased luciferase activity than rs1061429C allele when co-transfected with miR-591 ($P = 0.0012$). **Conclusions** These findings highlight the role of *C7* in the development of gastric cancer.

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15 **ABSTRACT**

16 **Background** Complement system plays an important role in innate immunity which involved in

17 the changes tumor immune microenvironment by mediating the inflammatory response. This

18 study aims to explore the relationship between complement component 7 (*C7*) polymorphisms

19 and the risk of gastric cancer (GC). **Materials and methods** All selected SNPs of *C7* were

20 genotyped in 471 patients and 471 controls using the polymerase chain reaction-restriction

21 fragment length polymorphism (PCR-RFLP). Odds ratios (ORs) and 95% confidence intervals

22 (*CI*s) were calculated by unconditional Logistic regression to analyze the relationship between

23 each genotype and the genetic susceptibility to gastric cancer. The level of *C7* expression in GC

24 was analyzed by Gene Expression Profiling Interactive Analysis (GEPIA) and detected by

25 Enzyme Linked Immunosorbent Assay. Kaplan-Meier plotter were used to reveal *C7* of

26 prognostic value in GC. We examined SNPs association with the expression of *C7* using the

27 GTEx database. The effect of *C7* polymorphisms on the regulatory activity of *C7* was detected

28 by luciferase reporter assay. **Results** Unconditional logistic regression showed that individuals

29 with *C7* rs1376178 AA or CA genotype had a higher risk of GC with OR (95%*CI*) of 2.09 (1.43-

30 3.03) and 1.88(1.35-2.63), respectively. For *C7* rs1061429 C>A polymorphism, AA genotype

31 was associated with the elevated risk for developing gastric cancer (OR = 2.16, 95%*CI* = 1.37-

32 3.38). In stratified analysis, *C7* rs1376178 AA genotype increased the risk of GC among males

33 (OR = 2.88, 95%*CI* = 1.81-4.58), but not among females (OR = 1.06, 95%*CI* = 0.55-2.06).

34 Individuals carrying rs1061429 AA significantly increased the risk of gastric cancer among

35 youngers (OR=2.84, 95%CI=1.39-5.80) and non-smokers (OR=2.79, 95%CI=1.63-4.77). *C7* was

36 overexpressed in gastric cancer tissues and serum of cancer patients and was significantly

37 associated with the prognosis. *C7* rs1061429 C>A variant contributed to reduced protein level of

38   *C7* (*P* = 0.029), but rs1376178 didn't. Luciferase reporter assay showed that rs1376178C-

39   containing plasmid exhibited 2.86-fold higher luciferase activity than rs1376178 A-containing

40   plasmid (*P* < 0.001). We also found that rs1061429A allele contributed 1.34-fold increased

41   luciferase activity than rs1061429C allele when co-transfected with miR-591 (*P* = 0.0012).

42   **Conclusions** These findings highlight the role of *C7* in the development of gastric cancer.

44   **Key words** *C7*, Gastric cancer, Genetic variant, Single nucleotide polymorphism

INTRODUCTION

Gastric cancer is one of the common malignant cancer worldwide, especially in East Asia(Siegel

et al. 2021). The incidence of gastric cancer ranks the third and fourth among men and women in

China, respectively (Chen et al. 2016). For most gastric cancer patients, more symptoms are

usually considered to be related to an advanced stage and the surgical resection is still the main

therapeutic choice (Digklia & Wagner 2016; Song et al. 2017). Several Genome-wide

association studies (GWAS) datasets showed that genetic variants were significantly associated

with gastric cancer risk (Jin et al. 2020; Saeki et al. 2013).

The human immune system is made up of two distinct parts, innate immune system and adaptive

immune system. As the body's first line of defense against germs and foreign substances, innate

immune system provides immediate and non-specific immune responses, which is different from

the way in which adaptive immune system specifically recognizes and eliminates pathogens

through specialized T and B lymphocytes (Berraondo et al. 2016; Saeki et al. 2013).

Complement system is a critical component of innate immunity, which can be activated by three

major pathways: the classical pathway, the alternative pathway, and the Mannose-binding Lectin

(MBL) pathway. Many studies have demonstrated that complement activation enhances innate

immunity against cancer through immune infiltrating or complement-dependent cytotoxicity

(Bao et al. 2020; Park et al. 2012). In addition, complement components *C5a* and *C3a* generated

by complement cascades facilitate cancer cell proliferation and regeneration (Markiewski et al.

2008; Ostrand-Rosenberg 2008).

Complement component 7 (*C7*) plays a central role in the activation of complement system as

66 the final product of the complement cascade (Würzner 2000), which acts as one of major rate-

67 limiting factors for the formation of membrane attack complex (MAC) (Walport 2001; Ying et

68 al. 2016). A study showed that *C7* had increased expressed in liver cancer stem cells and

69 enhanced the stemness of liver cancer cells by up-regulating Nanog, Oct4, Sox2, and C-myc

70 (Seol et al. 2016). *C7* is also identified as a potential tumor suppressor and may serve as a

71 prognostic biomarker for certain cancers (Chen et al. 2020; Ying et al. 2016).

72 Complement gene polymorphism is closely related to the occurrence of cancer. DAF (decay

73 accelerating factor), as one of key inhibitors of the complement system, inhibits complement

74 activation by preventing the formation of C3/C5 convertase from interfering with the formation

75 of MAC (Mikesch et al. 2006; Spendlove et al. 2006). Studies have indicated that DAF

76 rs2564978 T>C variant contributed to an increased risk of NSCLC (Zhang et al. 2017) and

77 rs10746463 G>A polymorphism was related to elevated risk of gastric cancer (Song et al. 2015).

78 Complement receptor 1 (CR1), acting as the receptor of C3b and C4b to inhibit the complement

79 activity (Liu & Niu 2009). The tag genetic variant rs9429942 in CR1 had great effect on the

80 susceptibility to gastric cancer (Zhao et al. 2015). However, the relationship between the

81 polymorphisms of *C7* and the susceptibility to gastric cancer still needs to be explored.

82 In this study, we conducted a case-control study in the Chinese population to verify the

83 hypothesis that the potential functional polymorphism of *C7* contributes to the susceptibility of

84 gastric cancer.

85

86 **MATERIALS AND METHODS**

Study population

This case-control study contains 471 gastric cancer patients and 471 healthy controls. All

patients were recruited from North China University of Science and Technology Affiliated

Tangshan Renmin Hospital and Affiliated Tangshan Gongren Hospital from January 2011 to

May 2015. Healthy individuals were from a large population underwent physical examinations in

same area during the same period. We also detected the *C7* expression level in serum of 70

healthy control and 70 gastric cancer patients. This study was approved by Institutional Review

Board of North China University of Science and Technology (2019021). All subjects signed an

informed consent form.

Potentially functional SNPs filtering

Based on NCBI dbSNP (https://www.ncbi.nlm.nih.gov/snp/) and Ensembl

(http://asia.ensembl.org/index.html) database, we screened out all SNPs in the promoter (2000bp

upstream of the transcription revelation site) and 3' untranslated region (3'UTR) of *C7* with

minor allele frequency (MAF) over than 0.05 in Chinese population. Alibaba 2.1

(http://generegulation.com/pub/programs/alibaba2) tool was then used to predict the binding

ability of transcription factors and SNP info Web server (https://manticore.niehs.nih.gov/) to

predict the miRNA binding changes of SNPs in 3'UTR.

Genotyping of Genetic Variants

Peripheral blood DNA was extracted by using TIANamp Blood DNA Kit (TIANGEN, Beijing,

China) according to manufacturer's instructions. PCR-restriction fragment length polymorphism

(PCR-RFLP) analysis were applied for genotyping. The target DNA fragment containing *C7*

108   rs1376178 or rs1061429 was amplified with primer pairs, rs1376178 PF(5'-

109   GCTAGAATCAATGCAAAGCTATGCG-3')/ PR (5'-TCAGATCACTGTGTTGGAAAGTT-

110   3') and rs1061429 PF (5'-GCTAGAATCAATGC AAAGCTATGCG-3')/ PR (5'-

111   AAGGAAAAGCTGTCCAGTGC-3'), respectively. PCR was performed in 6 μL PCR reaction

112   mixture with 1×Ftaq PCR Mix, 20 ng genomic DNA and 0.1 μM each primer. The thermal

113   cycling conditions for both *C7* rs1376178 and rs1061429 variants were 3 min at 94°C followed

114   by 30s at 94°C, 30s at 60°C, and 30s at 72°C for 30 cycles, and then a final extension 3 min at

115   72°C. PCR products for *C7* rs1376178 C>A (125 bp) and *C7* rs1061429 C>A (242 bp) were

116   digested by *Hha* I (NEB, Ipswich, MA, USA) and *Nco* I (NEB, Ipswich, MA, USA) and then

117   was separated on 3% agarose gel. For quality assurance, approximately 10% of the samples were

118   randomly selected for re-genotyping and all results were in 100% concordance.

119   **Bioinformatic analysis of *C7* expression**

120   To analyze the levels of the *C7* mRNA expression in GC, the GEPIA databases were analyzed.

121   GEPIA (http://gepia.cancer-pku.cn/) is a comprehensive and interactive web resource for

122   analyzing cancer data, which includes 9736 tumors and 8587 normal samples from Genotype-

123   Tissue Expression (GTEx) and The Cancer Genome Atlas (TCGA) (Tang et al. 2017).

124   Additionally, we also extracted eQTL data from the GTEx database, where differences in gene

125   levels under different SNPs were examined. *P*-value with <0.05 was considered as statistical

126   significance.

127   **Enzyme Linked Immunosorbent Assay (ELISA)**

128   The serum samples from 70 normal individuals and 70 gastric cancer patients were used to detect

129   the level of *C7* protein by using enzyme-linked immunosorbent assay (ELISA). Human

130   Complement (*C7*) kit were purchased from Cusabio company (Wuhan, China). We conducted

131   the *C7* expression analysis in serum according to manufactory's instructor.

132   **Cell culture**

133   Gastric cancer cell line (BGC823) was purchased from Cell Bank of Type Culture Collection of

134   the Chinese Academy of Sciences Shanghai Institute of Biochemistry and Cell Biology. BGC823

135   cells were cultured in RPMI-1640 (Thermo Fisher Scientific, Waltham, USA) supplemented

136   with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham, USA) and 1%

137   antibiotics (100U/ml penicillin and 100μg/mL streptomycin) in an atmosphere of 5% CO_2 at

138   37°C.

139   **Plasmid constructure and luciferase reporter gene assay**

140   A 1615bp DNA fragment containing rs1376178 site in the promoter of *C7* were inserted into

141   pGL3-basic plasmid (Promega, Madison, USA) to conduct luciferase reporter gene assay. The

142   PCR primers with *Kpn* I and *Xho* I (NEB, Ipswich, USA) cutting site adaptor were 5'-

143   GGGTACCCTTTCCCACTTCCAGTGGTGC-3') and 5'-CCGCTCGAG

144   CTGAGATTTAGCTCCTACCCC-3'. The final plasmids with rs1376178 C or A allele were

145   designed as pGL3$_{rs1376178C}$ and pGL3$_{rs1376178A}$, respectively. A 1268bp fragment with rs1061429

146   site in the 3' untranslated region (UTR) was cloned into psiCHECK2 plasmid (Promega,

147   Madison, USA). The PCR primer pairs with *Xho* I and *Not* I (NEB, Ipswich, USA) sites were 5'-

148   CCGCTCGAGTGCGAGGA AGAAGGGTTT-3' and 5'-GAATGCGGCCG

149   CTGGGACTGTATCCACAGAA-3'. The constructors with rs1061429C or A allele were named

150   as psiCHECK2$_{rs1061429C}$ and psiCHECK2$_{rs1061429A}$, respectively.

151   BGC823 gastric cancer cells were seeded in 24-well plates at a density of $2×10^5$ cells/well. As

152   the cells reached 60-70% confluent, 300ng of each pGL3-containing plasmids and 5ng pRL-

153   SV40 (Promega, Madison, USA) were co-transfected into the cells using Lipofectamine 2000

154   (Invitrogen, Carlsbad, USA). For psiCHECK2-containing plasmids, 20ng of plasmids was

155   transfected into gastric cancer cells with or without 30pmol of miR-591 mimic (GenePharma,

156   Shanghai, China). Gastric cancer cells were then collected 24 h after transfection. The activity of

157   luciferase and renilla reporter gene activity were measured using GloMax 20/20 Luminometer

158   (Promega, Madison, USA).

159   **Statistical analysis**

160   The statistical analyses in our study were conducted by SPSS 23.0 (SPSS Inc., Chicago, USA).

161   The Hardy-Weinberg equilibrium (HWE) of *C7* polymorphisms in controls were assessed by *χ2*

162   test. Differences of basic characteristics between cases and control subjects were evaluated by *χ2*

163   test. The association of *C7* genotypes with the susceptibility to gastric cancer was evaluated by

164   unconditional logistic regression with OR (95% *CI*) after adjusted by age, sex, smoking status

165   and drinking status. The interaction between gene and environment was analyzed by epiR

166   program in R platform (version 3.6.1). We defined the smokers as they smoked more than 100

167   cigarettes in their lifetime. Drinkers were categorized as the individuals took at least 12 drinks on

168   one occasion during the previous year according to international guide for monitoring alcohol

169   consumption and harm of WHO. *P* value < 0.05 was regarded as statistical significance. To

170 explore the associations of *C7* expression level and the prognosis of gastric cancer, we

171 performed survival analysis by Kaplan-Meier online program (www.kmpolt.com).

172

173 **RESULTS**

174 **Subject Characteristics.**

175 The distribution of select characteristics of all subjects were shown in Table 1. The study

176 involved in 471 gastric cancer patients and 471 healthy controls. There was no statistically

177 significant difference in distribution of age and gender between cases and controls (age: $P =$

178 1.00; gender $P = 0.44$). Regard to the distribution of smoking and drinking status, there was no

179 significant difference between cases and controls (smoking: $P = 0.32$; drinking $P = 0.25$).

180 **Association of *C7* gene polymorphisms with gastric cancer risk**

181 After predicting potential regulatory functional SNPs, we found that *C7* rs1376178 C>A variant

182 enhanced the binding capability to transcription factor STAT1 and *C7* rs1061429 C>A allele

183 created a binding site with has-miR-591 in 3'UTR. Genotype distributions of rs1376178 and

184 rs1061429 polymorphisms in controls were conformed to Hardy-Weinberg equilibrium (HWE)

185 (Table2).

186 The relationship between each genetic variant and the susceptibility to gastric cancer was shown

187 in Table 3. After adjusted by gender, age, drinking and smoking status, non-conditional logistic

188 regression analysis showed that the distribution of *C7* rs1376178 AA and CA genotypes was

189 statistically different between cases and controls ($P < 0.001$). The individuals with *C7* rs1376178

190 AA or CA genotype had a higher risk of GC with OR (95%*CI*) of 2.09 (1.43-3.03) and

191   1.88(1.35-2.63), respectively. For *C7* rs1061429 C>A polymorphism, AA genotype was

192   associated with the elevated risk for developing gastric cancer (OR = 2.16, 95%*CI* = 1.37-3.38).

193   **Stratification analysis of *C7* variants with GC risk**

194   To further analyze the effect of age, gender, smoking and drinking on the association of *C7*

195   variants (rs1376178 and rs1061429) with the risk of GC, we performed stratification analysis

196   (Table 4). Gender stratification analysis showed that *C7* rs1376178 AA genotype was associated

197   with the elevated risk of gastric cancer among males (OR = 2.88, 95%*CI* = 1.81-4.58, *P* <

198   0.001), but not among females (OR = 1.06, 95%*CI* = 0.55-2.06, *P* > 0.05). When stratified by

199   age, individuals with *C7* rs1376178 AA had an increased risk of gastric cancer in both groups

200   with OR (95%*CI*) of 2.19 (1.26-3.78) for the youngers and 2.07 (1.23-3.48) for the elders. Our

201   data also showed that the risk of gastric cancer was associated with the rs1376178 AA regardless

202   of smoking and drinking status (OR = 2.05, 95%*CI* = 1.31-3.21, *P* = 0.002 for nonsmokers; OR

203   = 2.40, 95%*CI* = 1.19-4.87, *P* = 0.015 for smokers; OR = 1.94, 95%*CI* = 1.28-2.93, *P* = 0.002

204   for nondrinkers; OR = 3.55, 95%*CI* = 1.37-9.17, *P* = 0.009 for drinkers).

205   Stratification analysis of *C7* rs1061429 polymorphism was showed in Table5. Our data

206   suggested that individuals with rs1061429 AA had an increased risk of gastric cancer among

207   youngers (OR = 2.84, 95%*CI* =1.39-5.80, *P* = 0.004) and non-smokers (OR = 2.79, 95%*CI* =

208   1.63-4.77, *P* < 0.001), but not among elders (OR = 1.81, 95%*CI* = 1.00-3.28, *P* = 0.050) and

209   smokers (OR =1.03, 95%*CI* = 0.43-2.5, *P* = 0.947). The stratification analysis by gender or

210   drinking status showed that rs1061429 AA genotype was contributed to the risk of gastric cancer

211   regardless of gender and drinking status (OR = 1.94, 95%*CI* = 1.13-3.33, *P* = 0.017 for males;

212 OR = 2.87, 95%CI = 1.26-6.54, P = 0.012 for females; OR = 1.92, 95%CI = 1.18-3.13, P =

213 0.008 for nondrinkers; OR = 5.23, 95%CI = 1.34-20.40, P = 0.017 for drinkers). Use R package

214 epiR to build Logistic regression gene-environment interaction mode, we found that rs1061429

215 had no additive and multiplicative interaction with drinking or gender to affect the risk of gastric

216 cancer (P > 0.05).

217 **The expression of *C7* in gastric cancer and its effect on prognosis and clinical-pathological**

218 **characteristics of gastric cancer**

219 Furthermore, we explore the potential function of gene *C7* in gastric cancer, based on GEPIA

220 data, we analyzed the differential expression of *C7* in gastric cancer tissues and adjacent normal

221 tissues and found that the level of *C7* mRNA in gastric cancer tissues (n=408) was significantly

222 depressed when compared with that in adjacent normal tissues (n=211) (Fig. 1A). To verify the

223 result from GEPIA data, we further compared the expression of *C7* in serum between normal

224 individuals and gastric cancer patients and found a significant differential expression of *C7* (P =

225 0.043) (Fig. 1B).

226 We also analyzed the impact of *C7* expression on the prognosis of gastric cancer using Kaplan-

227 Meier online program and demonstrated that higher expression of *C7* was related with poor

228 overall survival time (OS) and post-progression survival time (PPS) of gastric patients with HR

229 (95%CI) of 1.29 (1.09-1.53) and 1.51 (1.19-1.91), respectively (Fig.1C and 1D). We also

230 evaluated the association of *C7* expression level with TNM stage. Our data didn't show any

231 correlation between *C7* expression and TNM stage (Supplementary Figure 1).

232 ***C7* genotypes and clinical-pathological characteristics**

233   We used Kendall's rank correlation tests to estimate the correlation between these two SNPs and

234   clinicopathological characteristics. Our results showed that rs1061029 C>A variant has a positive

235   correlation with lymph node metastasis, but the correlation strength was low ($P = 0.022$,

236   Kendall's Tau-b= 0.104). We didn't find any correlation between rs1376178 C>A variant and

237   TNM stage, tumor size, lymph node metastasis, distant metastasis (Table 6).

238   **The effect of *C7* polymorphisms on the regulatory activity of *C7***

239   To substantiate the association between the identified SNPs and GC risk, using the genotype and

240   gene expression data of 324 normal gastric tissues in the GTEx to analyze, the eQTL data results

241   showed that the mRNA expression of *C7* was significantly related to the rs1061429 genotype (P

242   < 0.001) (Fig. 2A). However, there is no eQTL data related to rs1376178 in GEPIA database.

243   We then measured the *C7* protein level in serum of 70 individuals. For rs1061429 variant, the

244   expression of *C7* was significantly lower in individuals with AA genotype when compared with

245   those with CC genotype ($P = 0.029$) (Fig. 2B). For rs1376178 polymorphism, our data didn't

246   show any effect on *C7* expression (Fig. 2C).

247   Our luciferase reporter assay showed that pGL3$_{rs1376178C}$ exhibited 2.86-fold higher luciferase

248   activity than pGL3$_{rs1376178A}$ ($P < 0.001$) (Fig. 3A). We also found that psiCHECK2$_{rs1061429A}$ had

249   1.34-fold increased luciferase activity in comparison to psiCHECK2$_{rs1061429C}$ when co-transfected

250   with miR-591 ($P = 0.0012$) (Fig. 3B). There was no effect of rs1061429 polymorphism on the

251   reporter gene activity without additional miR-591.

252

253   **DISCUSSION**

254   The development of gastric cancer is a long-term multistage process with complex etiology.

255   Genetic epidemiological studies have shown that gastric cancer is the result of long-term effects

256   of environmental and individual genetic factors (Jin et al. 2020). Many studies provided strong

257   evidence for the effect of complement activation on tumor development (Kwak et al. 2018;

258   Markiewski et al. 2008; Roumenina et al. 2019). *C7* is a terminal component of complement

259   activation which plays essential roles within innate immunity (Fujita et al. 2004; Walport 2001).

260   Membrane-associated *C7* was acted a regulator of the excessive proinflammatory reaction (Bossi

261   et al. 2009). As an essential part of the membrane attack complex (MAC), *C7* participated in

262   various microbial defense responses and immune injury responses. The lack of *C7* may affect the

263   function of MAC and further increase the susceptibility to infection (Barroso et al. 2010; Sarma

264   & Ward 2011). Tumor infection promoted cancer aggression, and prevented inflammation

265   reduces metastasis and improved anti-tumor treatment (Maller et al. 2021).*C7* played an

266   important role in the occurrence of various cancers. It was demonstrated that *C7* affected the

267   progression of liver cancer via affecting the transcription of stemness factors (Seol et al. 2016).

268   Similarly, *C7* was verified to be related to the prognosis of prostate cancer patients (Chen et al.

269   2020). Consistent with our finding which showed a significantly down-regulated *C7* in gastric

270   cancer tissue, researchers also found that the *C7* was down expressed in ovarian, it was also

271   demonstrated that the decreased expression of *C7* was related to poor differentiation in patients

272   with NSCLC (Ying et al. 2016). These studies indicated its significance in the occurrence and

273   development of tumors.

274   Single Nucleotide Polymorphism (SNP) was widely present in human genome and is the most

275 common type of genetic variation, which can affect gene regulation by changing gene structure

276 or expression (Zhang et al. 2014). There are few reports on the relationship between *C7* genetic

277 variants and cancer risk. In this study, we discovered that *C7* rs1376178 C>A increased the

278 susceptibility to gastric cancer. This was consistent with the *C7* expression analysis and dual-

279 luciferase reporter gene results which showed that the rs1376178 A allele significantly reduced

280 the promoter activity and the expression level of *C7*. For rs1061429 polymorphism, we found

281 that *C7* rs1061429 C>A increased the binding ability of has-miR-591 to reduce the activity of

282 reporter gene. Literature reported dysregulation of miR-591 confer paclitaxel resistance to

283 ovarian cancer (Huh et al. 2013), and circ_0091581 could promote the progression of

284 hepatocellular carcinoma through miR-591/FOSL2 Axis (Ji et al. 2020). Therefore, binding of

285 hsa-mir-591 may inhibit translation of *C7* and attenuate its expression further participate in the

286 development of gastric cancer.

287 Gastric cancer is a complex disease, in which the interaction of genetic variants with several

288 compounding factors, such as age, sex and environment, has been demonstrated to modulate the

289 risk phenotypes (Favé et al. 2018; Jiang et al. 2021). For gastric cancer, the environmental risk

290 factors involved in *Helicobacter pylori* (*H. pylori*) infection, smoking and drinking (Brenner et

291 al. 2009; Terry et al. 2002). Due to the missing data of *H. pylori* infection, we only analyzed the

292 impact of these compounding factors, including age, sex, smoking status and drinking status on

293 genetic risk of disease.

294 Smoking was one of established and important risk factors contributing to the risk of gastric

295 cancer (Butt et al. 2019). Thus, we analyzed the effects of *C7* variants on the risk of GC when

296   stratified by smoking status. Our data presented that rs1061429 variant had an effected on gastric

297   cancer risk among non-smokers, but not among smokers. Cigarette smoke contains multiple

298   known human carcinogens. Exposure to nicotine-derived nitrosamine ketone, a key carcinogenic

299   ingredient of cigarette smoke, had been proved to result in mitochondrial dysfunction (Wu et al.

300   2019), to promote immune dysfunction, and further to influence tumor immune

301   microenvironment (de la Iglesia et al. 2020; Lee et al. 2012). It has been reported that cigarette

302   smoke can induce oxidative injury and dose-dependently stimulated gastric cancer cell

303   proliferation (Bhattacharyya et al. 2014; Shin et al. 2004). Smoking status could affect the

304   association of *C7* rs1061429 variant with the gastric cancer risk which was consistent with the

305   report on gene-environment interaction between smoking and SNP in decay-accelerating factor

306   (DAF) gene (Song et al. 2015). This could be supported by which tobacco enhanced the

307   activation of the classical pathway of the complement system (Yin et al. 2008).

308   Drinking was another important risk factor for gastric cancer (Ma et al. 2017; Na & Lee 2017).

309   In current study, individuals with *C7* rs1376178AA or rs1061429AA genotype were contributed

310   to the risk of gastric cancer regardless of drinking status. The result was consistent with some

311   previous studies that drinking significantly effect on association between polymorphisms and

312   gastric cancer risk (Li et al. 2021; Qiu et al. 2015).

313   Besides smoking and drinking, age also contributed to the occurrence and development of a

314   variety of cancers (Hansen et al. 2019). In this study, when stratified by age, our data showed

315   that individuals carrying *C7* rs1061429 AA genotype had an increased risk of gastric cancer

316   among youngers, but not among elders. The pathogenesis of gastric cancer in the elders was

317   mostly induced by environmental factors (Forman & Burley 2006); however, young patients are

318   more effected by genetic factors (Machlowska et al. 2020). Several previous studies from our

319   laboratory also provided the evidence that the effect of genetic variants in TNFSF15 and XAB2

320   on the susceptibility to gastric cancer could be modified by age (Gao et al. 2019; Pei et al. 2015).

321   These findings further verified the importance of interaction of age with the genetic

322   polymorphism on the development of gastric cancer.

323   Gender was also an important factor affecting the development of cancers (Li et al. 2019; Lou et

324   al. 2020). When stratified by gender, our data shows that individuals with the *C7* rs1061429 AA

325   genotype have an increased risk of gastric cancer in both men and women, but the risk in women

326   is significantly higher than that in men. This might be related to different lifestyle between men

327   and women (Song et al. 2008) and other risk factors, such as diet, microbial virulence, and Hp

328   infection (Cover & Peek 2013; Xia et al. 2016). Similarly, researchers found that *C7* rs1063499

329   GG genotype increased the risk of liver cancer among men, but not among women (de Lima et

330   al. 2018). Our previous study demonstrated that TNFSF15 -638 GG genotype was associated

331   with an increased risk of SCLC among males compared with the AA genotype, but not among

332   females (Gao et al. 2019). These findings further indicated the contribution of gender to cancer

333   development.

334   There are still some limitations. The sample size is relatively small after stratification. In

335   addition, as one of the best-established environmental factors of gastric cancer, *H. Pylori*

336   infection should be considered in this study (Wang et al. 2014). In our future research, we should

337   pay more attention to the exploration of reducing the impact of more environmental

338   compounding factors.

339

340   **CONCLUSIONS**

341   In summary, our study demonstrated that *C7* genetic variants were related to gastric cancer's

342   susceptibility which implying the critical role of the complement genes in the development of

343   gastric cancer.

344

345   **LIST OF ABBREVIATIONS**

346   *C7*: Complement component 7

347   GC: Gastric cancer

348   GEO: Gene Expression Omnibus

349   PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism technique

350   MAC: Membrane attack complex

351   HWE: Hardy-Weinberg equilibrium

352

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364

Competing Interests

The authors declare there are no competing interests.

367

Author Contributions

Siyue Wang performed the experiments, prepared figures and tables, authored or reviewed drafts of the paper, and approved the final draft.

Wenqian Hu performed the experiments and approved the final draft.

Hongjiao Wu and Zhi Zhang performed the clinical diagnosis and sample collection, prepared figures and tables, and approved the final draft.

Yuning Xie and Zhenxian Jia analyzed the data and approved the final draft.

Xuemei Zhang performed the conceived and designed the experiments, reviewed drafts of the paper, provided financial support that are vital for the project, and approved the final draft.

377

Data Availability

380   The raw measurements are available in Tables 1-5. Anonymized patient information is

381   available in Supplemental Files.

382

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569

570   Fig. 1 *C7* expression in gastric cancer and its effect on the prognosis of gastric cancer patients

571   (A) *C7* expression in gastric cancer and adjacent normal tissues in GEPIA database. (*$P < 0.05$).

572   (B) *C7* expression in serum of normal individuals and gastric cancer patient. (C) Overall survival

573   and (D) post-progression survival analysis of gastric cancer patients based on *C7* expression.

574

575   Fig. 2 Association of *C7* expression in gastric cancer patients with *C7* genetic polymorphisms

576   (A) rs1061429 genotypes from GTEx database. (B) rs1061429 genotypes and (C) rs1376178

577   genotypes in serum.

578

579   Fig 3. The effect of *C7* polymorphisms on transcription activity. (A) Different allele of

580   rs1376178 had different regulatory effects in BGC823 cells. (B) Different allele of rs1061429

581   had different 3'UTR activity in BGC823 cells. ***$P < 0.001$; **$P < 0.01$.

Frequency distribution of the study population.

1   Table 1 Frequency distribution of the study population.

Variables	Cases(n=471)		Controls(n=471)		P value [a]
	NO	%	NO	%	
Gender					
Male	332	70.5	321	68.2	0.44
Female	139	29.5	150	31.8	
Age					
<60	209	44.4	209	44.4	1.00
≥60	262	55.6	262	55.6	
Smoking status					
Non-smoker	323	68.6	337	71.5	0.32
smoker	148	31.4	134	28.5	
Drinking status					
No	374	79.4	388	82.4	0.25
Yes	97	20.6	83	17.6	

2   [a] Two-sided $\chi 2$ test.

Single nucleotide polymorphism information and Hardy-Weinberg test.

1   Table 2 Single nucleotide polymorphism information and Hardy-Weinberg test.

Gene	Position	SNP	Region	Allele gene	MAF	Functional change	P value
C7	chr5:40908799	rs1376178	promoter	C/A	0.45	STAT1[a]	0.393
C7	chr5:40981587	rs1061429	3'UTR	C/A	0.39	has-miR-591[b]	0.710

2   [a] Typical transcription factor changes of promoter.

3   [b] Typical miRNA binding changes of 3'UTR.

Genotype frequencies of *C7* and their association with gastric carcinoma .

1 Table 3 Genotype frequencies of *C7* and their association with gastric carcinoma.

Genotypes	Controls(n=471)		Cases(n=471)		OR (95% CI) [a]	P value
	N	(%)	N	(%)		
C7 rs1376178						
CC	130	27.6	77	16.4	1.00ref	
CA	226	48.0	252	53.5	1.88(1.35-2.63)	<0.001
AA	115	24.4	142	30.1	2.09(1.43-3.03)	<0.001
C7 rs1061429						
CC	246	52.2	225	47.8	1.00ref	
CA	191	40.6	179	38.0	1.03(0.78-1.35)	0.861
AA	34	7.2	67	14.2	2.16(1.37-3.38)	0.001

2 [a]Adjusted for age, gender smoking status and drinking status.

Association of *C7* rs1376178 C > A polymorphism with GC risk stratified by selected variables.

1 Table 4 Association of *C7* rs1376178 C > A polymorphism with GC risk stratified by selected
2 variables.

Variables	Genotypes (Controls/Cases)			AA/CC model	*P* value
	CC	CA	AA	OR (95% CI) [a]	
Gender					
Male	91/48	158/176	72/108	2.88(1.81-4.58)	<0.001
Female	39/29	68/76	43/34	1.06(0.55-2.06)	0.862
Age					
<60	66/40	97/108	46/61	2.19(1.26-3.78)	0.005
≥60	64/37	129/144	69/81	2.07(1.23-3.48)	0.006
Smoking status					
No	92/51	156/172	89/100	2.05(1.31-3.21)	0.002
Yes	38/26	70/80	26/42	2.40(1.19-4.87)	0.015
Drinking status					
No	105/62	181/196	102/116	1.94(1.28-2.93)	0.002
Yes	25/15	45/56	13/26	3.55(1.37-9.17)	0.009

3 [a] Data were calculated by unconditional logistic regression and adjusted for age, gender, smoking
4 and drinking status, where they were appropriate.

Association of *C7* rs1061429C > A polymorphism with GC risk stratified by selected variables.

1 Table 5 Association of *C7* rs1061429C > A polymorphism with GC risk stratified by selected
2 variables.

Variables	Genotypes (Controls/Cases)			AA/CC model	*P* value
	CC	CA	AA	OR (95% CI) [a]	
Gender					
Male	172/165	125/123	24/44	1.94(1.13-3.33)	0.017
Female	74/60	66/56	10/23	2.87(1.26-6.54)	0.012
Age					
<60	110/106	87/70	12/33	2.84(1.39-5.80)	0.004
≥60	136/119	104/109	22/34	1.81(1.00-3.28)	0.050
Smoking status					
Non-smoker	172/149	142/119	23/55	2.79(1.63-4.77)	<0.001
smoker	74/76	49/60	11/12	1.03(0.43-2.5)	0.947
Drinking status					
No	203/180	154/141	31/53	1.92(1.18-3.13)	0.008
Yes	43/45	37/38	3/14	5.23(1.34-20.40)	0.017

3 [a] Data were calculated by unconditional logistic regression and adjusted for age, gender, smoking
4 and drinking status, where they were appropriate.

Table 6 Correlation between *C7* polymorphisms and clinical characteristics

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Clinical pathological characteristics	rs1061429 C>A		rs1376178 C>A	
	Tau-b	*P* value	Tau-b	*P* value
TNM stage	0.074	0.089	0.021	0.631
Tumor size	0.071	0.123	-0.004	0.935
Lymph node metastasis	0.104	0.022	0.065	0.156
Distant metastasis	0.086	0.075	0.027	0.569

Figure 1

Fig. 1 C7 expression in gastric cancer and its effect on the prognosis of gastric cancer patients

(A) C7 expression in gastric cancer and adjacent normal tissues in GEPIA database. (*$P <$ 0.05). (B) *C7* expression in serum of normal individuals and gastric cancer patient. (C) Overall survival and (D) post-progression survival analysis of gastric cancer patients based on C7 expression.

A

B

C

OS

D

PPS
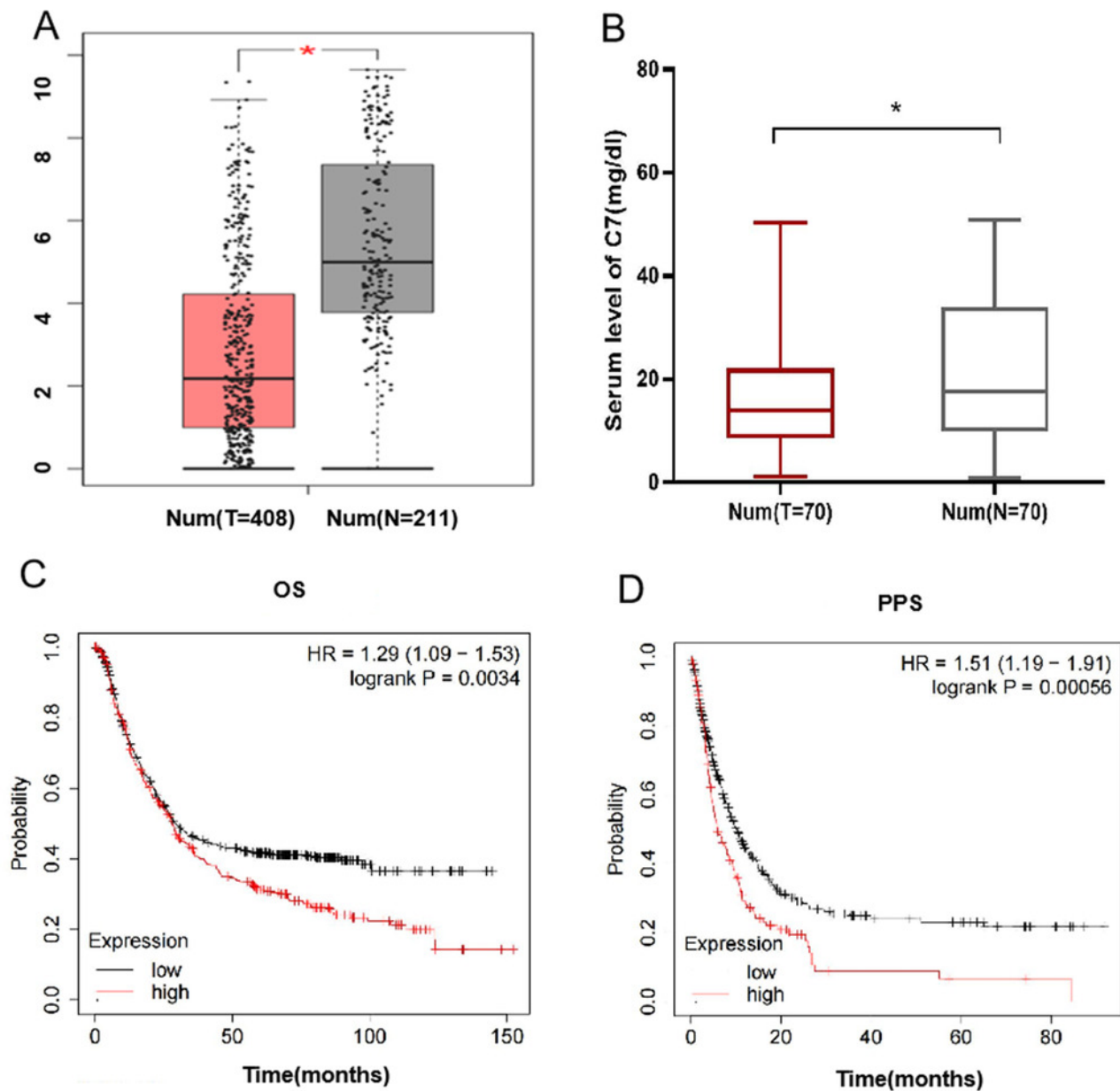
Figure 2

Fig. 2 Association of C7 expression in gastric cancer patients with C7 genetic polymorphisms

(A) rs1061429 genotypes from GTEx database. (B) rs1061429 genotypes and (C) rs1376178 genotypes in serum.
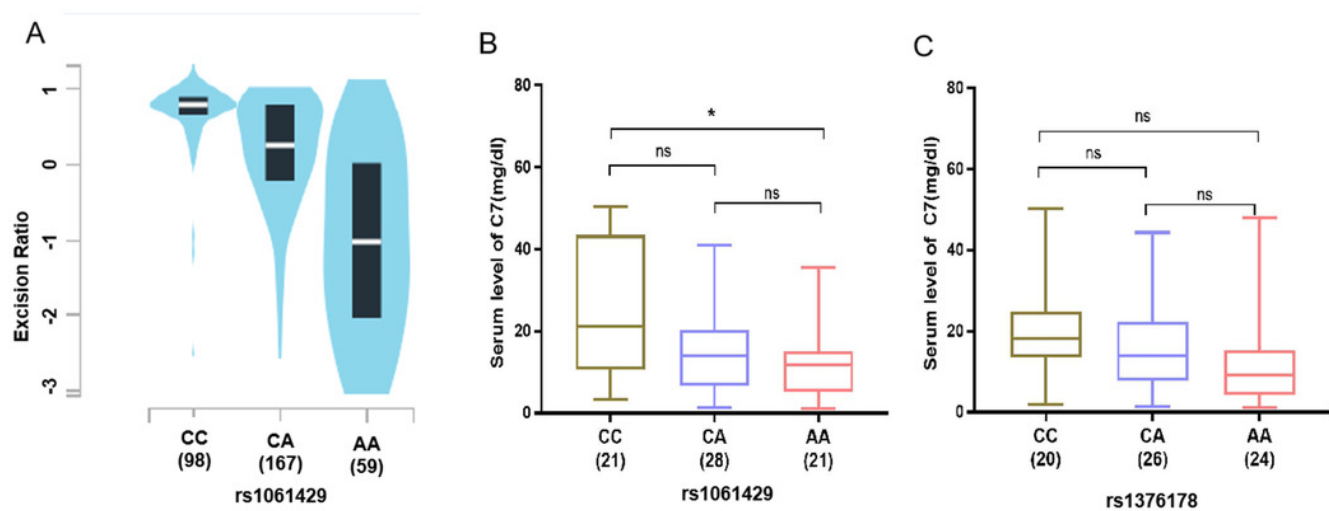
Figure 3

Fig 3. The effect of C7 polymorphisms on transcription activity

(A) Different allele of rs1376178 had different regulatory effects in BGC823 cells. (B) Different allele of rs1061429 had different 3'UTR activity in BGC823 cells. ***$P < 0.001$; **$P < 0.01$.