**ORIGINAL ARTICLE**

**Coagulation factor VII gene polymorphisms are not associated with the occurrence or the survival of hepatocellular carcinoma: a report of 37 cases**

Chih-Che Lin¹, Chun-Hsien Wu¹,², Li-Yu Chen¹,², Ming-Chao Tsai³, Ahmed M. Elsarawy¹, Kuang-Tzu Huang¹,²

¹Liver Transplantation Center, Department of Surgery, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung 833, Taiwan, China; ²Institute for Translational Research in Biomedicine, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung 833, Taiwan, China; ³Division of Hepato-Gastroenterology, Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung 833, Taiwan, China

**ABSTRACT**

**Objective:** Coagulation factor VII (FVII) triggers the extrinsic pathway of blood coagulation. In our previous study, we showed that FVII plays an important role in tumorigenesis of hepatocellular carcinoma (HCC). However, the role of FVII polymorphism in HCC is still unknown. The present study aimed to investigate the relationship between HCC carcinogenesis and single nucleotide polymorphism of FVII.

**Methods:** Thirty-seven HCC patients and 30 healthy donors were recruited in this study. Four common FVII gene polymorphisms – a decanucleotide insertion at position −323 (−323ins10-bp), a G to T substitution at position −401 (−401G/T), a G to A substitution at position −402 (−402G/A), and a T to C substitution at position −122 (−122T/C) – were analyzed by sequencing or commercialized assays using genomic DNA isolated from blood samples. Clinicopathological parameters between control and HCC subjects were compared according to the specific genotypes.

**Results:** The most common nucleotide variation was −402G/A. However, no statistically significant difference was observed between healthy controls and HCC subjects for all four polymorphisms in terms of genotype distribution and allele frequencies, indicating that these polymorphisms may not affect HCC tumorigenesis. Furthermore, no association was found between −402G/A polymorphisms and tumor stage, recurrence, and overall survival.

**Conclusions:** Our results indicate that FVII polymorphisms may not be a key factor that clinically impact tumorigenesis and outcomes of HCC, although further investigations should be conducted to confirm our findings.

**KEYWORDS**
Factor VII; gene; polymorphism; liver; hepatocellular; cancer; survival

**Introduction**

It is a widely accepted idea that cancer patients exhibit elevated levels of circulating procoagulants that correlate with the risk of thrombosis, suggesting a potential link between coagulation and cancer development¹. In fact, extensive studies showed that coagulation plays an important role in tumor growth, invasion, and metastasis². Coagulation factor VII (FVII) is an approximately 50-kDa vitamin K-dependent precursor of a serine protease that triggers the extrinsic coagulation cascade³. This protease precursor is produced predominantly in the liver and released into the circulation. Upon injury, FVII interacts with tissue factor (TF), an integral membrane protein that functions as the cellular receptor for FVII, resulting in its conversion to the active form (FVIIa). The TF-FVIIa binary complex then initiates a downstream coagulation cascade, ultimately leading to formation of thrombin. Thrombin subsequently activates platelets for deposition of fibrin, another component of a hemostatic plug. Nevertheless, TF is also often expressed on the surface of cancer cells and tumor vasculature⁴. Binding of circulating FVIIa to TF also triggers pathways that do not cause blood coagulation but rather activate protease-activated receptor 2 (PAR2) and subsequent signaling events⁵. The activation of PAR2 is associated with numerous physiological and pathological mechanisms and also drives the production of pro-oncogenic and immunological factors. Additionally, shed membrane-derived vesicles (generally
referred to as microparticles) from tumor cells contain TF, which are major contributors of coagulopathy in cancer.

Hepatocellular carcinoma (HCC) is the fifth most frequent neoplasm worldwide and the third most common cause of tumor-related deaths. Invasion and metastasis are characteristic features of HCC and the major causes of treatment failure in some cases. Although several target therapies had been developed recently, the overall clinical outcomes remain unsatisfactory. Thus, there is an urgent need for further insights into the molecular mechanisms responsible for the biological behavior of HCC and development of new targets that supplement existing treatment protocols.

Our previous studies revealed that the coagulation pathway actively participates in the autophagic process. FVII, together with TF, negatively regulates autophagy and enhances the invasiveness of HCC through activation of downstream signaling driven by PAR2. We found that overexpression of FVII in HCC tumor carries high incidence of recurrence after curative hepatectomy. A mouse xenograft model also showed that FVII increases tumor microvessel density, suggesting that FVII may be involved in vascular invasion, a major prognostic factor of HCC.

Recent studies have provided evidence that gene polymorphisms are associated with the protein level. Given the implications that FVII levels are linked to malignant progression of HCC, one would expect that FVII polymorphisms may also be a critical participating factor. Three common polymorphisms (–401G/T, –402G/A, –323ins10-bp) in the promoter region of the FVII gene locus have been reported to be associated with circulating levels of FVII. These variants result in differences in promoter activity. Additionally, the relationship between FVII polymorphisms and tumorigenesis has been observed in breast cancer. However, whether these gene variants affect the development and progression of HCC has not yet been determined. Therefore, in this study, we investigated the contribution of common functional polymorphisms in the promoter region of FVII gene and discussed their relevance in the development and outcomes of HCC.

Materials and methods

Patient samples

Peripheral blood was collected from HCC patients who underwent curative resection for HCC (n=37) at Kaohsiung Chang Gung Memorial Hospital. The clinicopathological features are listed in Table 1. Healthy donors (n=30) were recruited as controls. All controls were free from any medical comorbidities. Written informed consent was obtained from each patient and healthy subject. This study was approved by the Institutional Review Board of Chang Gung Medical Foundation (100-3002A3, 101-1377A3, and 102-5206B). Genomic DNA was extracted using the Genomic DNA Extraction Mini Kit (Favorgen Biotech Corp.; Pingtung, Taiwan).

Genotyping of FVII polymorphisms

A 705-bp DNA fragment was amplified by polymerase chain reaction (PCR) for DNA sequencing using the following specific primers: forward primer 5'-GGCTC ACCTA AGAAA CCAGC-3' and reverse primer 5'-AAGAA ATTGA ACAGG AGCCG-3'. Four common functional polymorphisms in the promoter region of the FVII gene – a decanucleotide insertion at position –323 (–323ins10-bp), a G to T substitution at position –401 (–401G/T), a G to A substitution at position –402 (–402G/A), and a T to C substitution at position –122 (–122T/C) – were analyzed in this study. The PCR reactions started with 2 min at 94 °C for initial denaturation, followed by 30 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min. A final extension was carried out at 72 °C for 10 min. DNA sequencing was performed by outsourcing services from Genomics BioSci &

| Table 1 Clinico-pathological features of 37 patients with HCC undergoing hepatectomy |
|---------------------------------|-----------------|
| Item                            | Value           |
| Patient demographics            |                 |
| Age (years) (median; range)     | 55 (37–75)      |
| Sex (male : female)             | 29 : 8          |
| AFP (ng/mL) (median; range)     | 24.9 (1.99–303145) |
| Multiple tumor (+) (%)          | 9 (24.32%)      |
| Tumor size (cm) (median; range) | 3.5 (1–19.3)    |
| Liver cirrhosis (+) (%)         | 18 (48.65%)     |
| Hepatitis (B : C : B+C : none)  | 23 : 6 : 0 : 8  |
| TNM stage (I : II : III)b       | 7 : 19 : 9      |
| Hypertension (+) (%)            | 9 (24.3%)       |
| Diabetes mellitus (+) (%)       | 9 (24.3%)       |
| Ischemic heart disease (+) (%)  | 0 (0%)          |
| Pathological features           |                 |
| Capsule (yes : no)              | 6 : 28          |
| Satellite nodule (yes : no)     | 5 : 30          |
| Microvascular invasion (yes : no)| 25 : 10        |
| Histological grade (I : II : III)| 5 : 27 : 1    |

*a Measured by the diameter of the largest tumor nodule. b TNM stage classification according to AJCC staging 7th Edition.*
Tech, Ltd. (New Taipei City, Taiwan), and distribution of FVII polymorphisms was determined after DNA alignment using BioEdit Sequence Alignment Editor (Ibis Biosciences; Carlsbad, CA, USA).

Analysis of –402G/A polymorphism of the FVII gene

Determination of –402G/A in the FVII promoter was conducted using the TaqMan SNP Genotyping Assays (Thermo Fisher; Waltham, MA, USA). Allele-specific TaqMan fluorescent probes targeting –402G/A and a PCR primer pair were mixed with test samples and analyzed on an ABI 7500 fast PCR system (Applied Biosystems; Grand Island, NY, USA).

Statistical analysis

The continuous variables were presented as means ± SD, and statistics were performed using Student’s *t*-test. The correlations between gene polymorphisms and recurrence-free/overall survival were examined using the Kaplan-Meier method with log rank tests for comparison between both study groups. Other discrete variables were analyzed using the Chi-square test. Differences were considered statistically significant when *P*<0.05. Statistical analysis was performed using the SPSS software (SPSS Inc.; Chicago, IL, USA).

Results

Comparison of demographics and blood test levels

The mean age at diagnosis of all patients was 55 years (range 37–75 years); 29 patients were male, and 8 were female. The majority of underlying disease was hepatitis B/C. The tumor characteristics are described in Table 1. In comparing the basic blood test status between healthy donors and HCC patients, we found that the international normalized ratio, hemoglobin, and hematocrit showed no significant difference. HCC patients were found to have lower platelet counts than healthy donors due to underlying liver cirrhosis and portal hypertension with subsequent thrombocytopenia as expected (Table 2).

402G/A was the most frequent FVII polymorphism in both HCC patients and healthy donors

To assess the frequencies and distribution of four common polymorphisms (–402G/A, –401G/T, –323ins10-bp, and –122T/C) in the FVII gene among HCC patients and healthy controls, DNA sequencing was conducted. Our results revealed that three of the common variants (–401G/T, –323ins10-bp, and –122T/C) were very conserved, showing 100% and 93.75% wild-type homozygous genotypes in healthy donors and HCC patients, respectively (Table 3). Interestingly, the –402G/A polymorphism occurred most frequently among studied subjects, with G and A alleles having almost equal frequencies (50%/50% in healthy donors, 55.41%/44.59% in HCC patients) (Table 4).

FVII polymorphisms were not associated with the occurrence of HCC

Given that –402G/A was the most frequent FVII polymorphism in HCC patients, we next compared whether there was a prevalence of major polymorphism at this particular site in healthy donors and HCC patients. Our results showed that there was no significant difference in terms of (GG vs. GA vs. AA) or (GG vs. non-GG) between HCC and healthy individuals (Table 4), indicating that this FVII polymorphism may not be associated with the development of HCC.

FVII polymorphisms were not associated with the clinical features and outcomes of HCC

We then examined whether there was a correlation between
the –402G/A polymorphism and the clinicopathological features of HCC patients. Comparisons between GG and non-GG alleles are shown in Table 5. Once again, there was no significant difference among these clinical parameters, indicating that FVII polymorphisms may not play a critical role in regulating pathological features of HCC.

We further assessed the effects of –402G/A polymorphism
on HCC outcomes. As shown in Figure 1 (left), there was no significant difference in recurrence-free survival among HCC patients with −402GG, GA, or AA alleles. No association was found when the patients were categorized into −402GG and non-GG groups (Figure 1, right). Similarly, no significant effects of −402G/A polymorphisms on 3-year overall survival were observed in HCC patients (Figure 2).

**Discussion**

In the current investigation, we aimed to study the correlations between common FVII polymorphisms and incidence/outcomes of HCC. We have previously shown that FVII is highly associated with autophagy and migration in HCC cells and tumor stage, vascular invasion, and recurrence in clinical HCC cases. Although several other studies found TF as the major deciding factor in the TF-FVII-PAR2 signaling in other cancers, our previous findings suggested that changes in FVII levels were the main contributor to aberrant PAR2 signaling in HCC, which may partially result from the fact that FVII is principally synthesized in the liver and in close proximity with cell surface-anchored TF and PAR2. However, the polymorphisms examined did not seem to correlate well with the clinical parameters of HCC.

Clinical studies have shown correlations between FVII levels and various human disorders, including cardiovascular diseases and cancer. We have previously demonstrated

![Figure 1](image1.png) **Figure 1** Kaplan-Meier curves showing cumulative recurrence-free survival of HCC patients who underwent curative resection categorized according to −402GG, GA and AA (left) or −402GG and non-GG (right) genotypes in the FVII promoter region. HCC patients (n=37) were followed up after tumor resection. P value was calculated using a log-rank test.

![Figure 2](image2.png) **Figure 2** Cumulative overall survival of patients with resected HCC categorized according to −402GG, GA and AA (left) or −402GG and non-GG (right) genotypes in the FVII promoter region. HCC patients (n=37) were followed up for the analysis. P value was calculated using a log-rank test.
that FVII levels are associated with malignant behavior and recurrence in HCC. However, the molecular mechanisms of how hepatic FVII expression is regulated and its reflection on circulating levels are still unknown. Recent studies have provided evidence linking polymorphic markers of the FVII gene with FVII transcriptional activity and plasma protein levels. The −323ins10-bp polymorphism is shown to reduce promoter activity and related to low plasma levels of FVII\textsuperscript{13}. The −401G/T polymorphism is also found to be associated with lower plasma levels, but −402A is related to higher FVII levels compared with the common G allele\textsuperscript{14}. FVII polymorphisms may determine up to about one third of the differences in FVII levels\textsuperscript{20}. However, the influence of these genetic variants on disease risks can be inconsistent. For example, the FVII-lowering −323ins10-bp polymorphism has presented a positive association with total and arterial thrombosis in patients with myeloproliferative neoplasms, while several studies have shown a protective effect on thrombotic events\textsuperscript{17,21,22}. In addition, a number of studies indicate that certain polymorphisms in the FVII gene may contribute to a more hypercoagulable state. However, other studies have failed to find any associations\textsuperscript{23,24}. The discrepancies have not been resolved although cohort selection may have affected the outcome of analysis. In addition, the link between observed polymorphisms and disease mechanisms also remains to be elucidated.

A possible contribution of altered −402G/A genotype and allele frequency in the FVII gene has been implicated in breast cancer over control cases\textsuperscript{16}. In our study, there was no significant difference in the distribution of −402G/A or any other genotype among HCC cases versus control subjects. The lack of association between FVII polymorphisms and clinical parameters and prognosis of HCC may be due to the relatively small size of the cohort. However, several interesting findings can be concluded based on our results. First, the incidence of the “rare” −402A allele was nearly equal to that of −402G, which is distinct from other studies. Furthermore, almost only wild-type genotypes (but one HCC case) were observed for −401G/T, −122T/C, and −323ins10-bp in both control and HCC subjects. The underlying cause of these observations is currently unknown. Further studies with a larger series need to be conducted before reaching more precise conclusions. It is also important to associate the observed polymorphisms with plasma FVII levels and TMN stages of enrolled HCC patients. Additional clinical parameters should also be included besides recurrence and survival rate in future studies.

Conclusions

In conclusion, our data indicate that the four common polymorphisms (−122T/C, −323ins10-bp, −401G/T, and −402G/A) in the promoter region of the FVII gene are not contributing factors to incidence, recurrence, and survival in HCC patients. In addition, −402G/A is the most common variant of FVII in both control and HCC subjects, while others present almost no variations. As HCC is a consequence of multifactorial events, apart from the classic biological factors that are often discussed in the literature\textsuperscript{25}, our study is the first attempt to demonstrate the association of FVII polymorphisms with the presence of HCC. From our results, there may be other or even unidentified polymorphic variants in the FVII gene that are related to HCC, although further studies are needed to confirm this.

Conflict of interest statement

No potential conflicts of interest are disclosed.

References

1. Blom JW, Doggen CJM, Osanto S, Rosendaal FR. Malignancies, prothrombotic mutations, and the risk of venous thrombosis. JAMA. 2005; 293: 715-22.
2. Horowitz N, Brenner B. Thrombophilia and cancer. Pathophysiol Haemos Thromb. 2008; 36: 131-6.
3. Furie B, Furie BC. The molecular basis of blood coagulation. Cell. 1988; 53: 505-18.
4. Versteeg HH, Peppelenbosch MP, Spek CA. The pleiotropic effects of tissue factor: a possible role for factor VIIa-induced intracellular signalling? Thromb Haemost. 2001; 86: 1353-9.
5. Versteeg HH, Ruf W. Emerging insights in tissue factor-dependent signaling events. Semin Thromb Hemost. 2006; 32: 24-32.
6. Rak J. Microparticles in cancer. Semin Thromb Hemost. 2010; 36: 888-906.
7. Waly Raphael S, Zhang YD, Chen YX. Hepatocellular carcinoma: focus on different aspects of management. ISRN Oncol. 2012; 2012: 421673
8. Best J, Schotten G, Theysohn JM, Wetter A, Muller S, Radunz S, et al. Novel implications in the treatment of hepatocellular carcinoma. Ann Gastroenterol. 2017; 30: 23-32.
9. Omata M, Lesmana LA, Tateishi R, Chen PJ, Lin SM, Yoshida H, et al. Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. Hepatol Int. 2010; 4: 439-74.
10. Chen KD, Huang KT, Tsai MC, Wu CH, Kuo IY, Chen LY, et al. Coagulation factor VII and malignant progression of hepatocellular carcinoma. Cell Death Dis. 2016; 7: e2110
11. Chen KD, Wang CC, Tsai MC, Wu CH, Yang HJ, Chen LY, et al. Interconnections between autophagy and the coagulation cascade in hepatocellular carcinoma. Cell Death Dis. 2014; 5: e1244
12. Tsai MC, Chen KD, Wang CC, Huang KT, Wu CH, Kuo IY, et al. Factor VII promotes hepatocellular carcinoma progression through ERK-TSC signaling. Cell Death Discov. 2015; 1: 15051
13. Pollak ES, Hung HL, Godin W, Overton GC, High KA. Functional characterization of the human factor VII 5'-flanking region. J Biol Chem. 1996; 271: 1738-47.
14. van’t Hooft FM, Silveira A, Tornvall P, Iliadou A, Ehrenborg E, Eriksson P, et al. Two common functional polymorphisms in the promoter region of the coagulation factor VII gene determining plasma factor VII activity and mass concentration. Blood. 1999; 93: 3432-41.
15. Sabater-Lleal M, Chillón M, Howard TE, Gil E, Almasy L, Blangero J, et al. Functional analysis of the genetic variability in the F7 gene promoter. Atherosclerosis. 2007; 195: 262-8.
16. Ergülu A, Öztürk A, Akar N. Association between the -402GA, -401GT, and -323ins10-bp polymorphisms of factor VII gene and breast cancer. Breast Cancer. 2011; 18: 282-3.
17. Buxhofer-Ausch V, Olcaydu D, Gisslinger B, Schalling M, Frantal S, Thiele J, et al. Decanucleotide insertion polymorphism of F7 significantly influences the risk of thrombosis in patients with essential thrombocythemia. Eur J Haematol. 2014; 93: 103-11.
18. de Maat MP, Green F, de Knijff P, Jespersen J, Kluf W. Factor VII polymorphisms in populations with different risks of cardiovascular disease. Arterioscler, Thromb, Vasc Biol. 1997; 17: 1918-23.
19. Meade TW, Brozovic M, Chakrabarti RR, Haines AP, Imeson JD, Mellows S, et al. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. Lancet. 1986; 328: 533-7.
20. Bernardi F, Marchetti G, Pinotti M, Arcieri P, Baroncini C, Papacchini M, et al. Factor VII gene polymorphisms contribute about one third of the factor VII level variation in plasma. Arterioscler, Thromb, Vasc Biol. 1996; 16: 72-6.
21. Iacoviello L, di Castelnuovo A, de Knijff P, D’Orazio A, Amore C, Arboretti R, et al. Polymorphisms in the coagulation factor VII gene and the risk of myocardial infarction. N Engl J Med. 1998; 338: 79-85.
22. Lopaciuk S, Windyg J, Watala CW, Bykowska K, Pietrucha T, Kwiecinski H, et al. Polymorphisms in the factor VII gene and ischemic stroke in young adults. Blood Coagul Fibrinolysis. 2010; 21: 442-7.
23. Doggen CJM, Cats MV, Bertina RM, Reitsma PH, Vandenbroucke JP, Rosendaal FR. A genetic propensity to high factor VII is not associated with the risk of myocardial infarction in men. Thromb Haemost. 1998; 80: 281-5.
24. Ken-Dror G, Drenos F, Humphries SE, Talmud PJ, Hingorani AD, Kivimäki M, Kumari M, et al. Haplotype and genotype effects of the F7 gene on circulating factor VII, coagulation activation markers and incident coronary heart disease in UK men. J Thromb Haemost. 2010; 8: 2394-403.
25. Li C, Li R, Zhang W. Progress in non-invasive detection of liver fibrosis. Cancer Biol Med. 2018; 15: 124-36.

Cite this article as: Lin C, Wu C, Chen L, Tsai M, Elsarawy AM, Huang K. Coagulation factor VII gene polymorphisms are not associated with the occurrence or the survival of hepatocellular carcinoma: a report of 37 cases. Cancer Biol Med. 2018; 15: 275-81. doi: 10.20892/j.issn.2095-3941.2017.0144