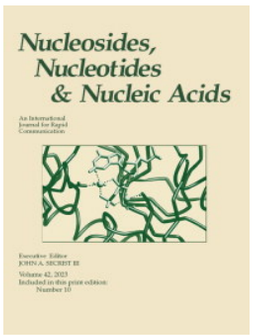

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# Clinicopathological and prognostic value of CD44 gene polymorphism (rs187115) in Swedish patients with colorectal cancer

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**ABSTRACT**

Cluster of differentiation (CD) 44 plays a crucial role in apoptosis, cell–cell interactions, angiogenesis, metastasis and proliferation. The aim of the present study was to examine the influence of CD44 gene polymorphism rs187115 on colorectal cancer (CRC) susceptibility and the association with various clinical features including long-term survival in Swedish patients with CRC. Genotypes were screened, using TaqMan single nucleotide polymorphism (SNP) assays based on polymerase chain reaction, in 612 CRC patients and 575 healthy controls.

The carriers of G allele, genotypes (AG + GG), were found to be associated with an increased risk of CRC with an odds ratio (OR) of 1.35 (95% confidence interval (CI) = 1.01–1.81; $p = 0.039$) and found to be more common in patients with mucinous cancer compared with non-mucinous cancer, OR = 1.69 (95% CI = 1.02–2.80; $p = 0.011$). By using Kaplan–Meier analysis, the patients with genotype GG showed shorter cancer-specific and recurrence free survival with a hazard ratio (HR) of 1.25 (95% CI = 1.02–1.54; $p = 0.036$) and 1.52 (95% CI = 1.12–2.06; $p = 0.007$), respectively, in comparison with the carriers of A allele (AG + AA). The present findings demonstrated that the variant G allele of CD44 gene polymorphism rs187115 was related to risk for CRC and associated to mucinous cancer and predict worse prognosis in Swedish patients with CRC.

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**KEYWORDS**
CD44; SNP; colorectal cancer; survival

## 1. Introduction

Colorectal cancer (CRC) is one of the most commonly occurring malignancies and a major cause of cancer death worldwide[1].

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The etiology of CRC is still unclear but it has been described that interactions between genetic factors and environmental factors contribute affecting CRC induction and progression[2,3]. Moreover, the connection between inflammation and CRC is well-established[4,5].

In general, CRC patients are identified based on pathological and clinical parameters with CRC staging based on the tumor-nodes-metastasis (TNM) system according to The American Joint Committee on Cancer (AJCC) classification system[6]. However, some of the parameters are considered weak prognostic markers[7,8]. Moreover, some studies suggest that CRC is characterized by interpatient and intratumor heterogeneity, which may affect response to therapy and prognosis[9]. Identifying new biomarkers that can guide the choice of treatment and predict prognosis would have great clinical relevance[10,11].

Cluster of differentiation (CD) 44 is a membrane glycoprotein with several isoforms, which is involved in several cellular functions including apoptosis, cell–cell interactions, cellular adhesion, angiogenesis, metastasis and proliferation[12]. Moreover, CD44 can induce chemotherapy resistance in various cancers[13]. Several of these functions are preceded by CD44 activating a number of signaling pathways including MAPK, Wnt and PI3K/Akt pathways[12].

CD44 is a membrane receptor for hyaluronic acid which has been reported to activate various biological behaviors in normal and in cancer cells. CD44 and its isoforms play a different role in cancer and is a common reported cancer stem cells marker in CRC[13–15].

The prognostic value of CD44 in CRC remains controversial. Various studies have shown that overexpression of CD44 and its variants was associated with poor prognosis or advanced stage in CRC[16,17]. On the other hand, studies concluded that loss of CD44 is a poor prognostic factor in patients with CRC and that CD44 expression is inversely associated with invasive characteristics of CRC[18,19].

Genetic variations in genes are suggested to play a role in CRC susceptibility and in the survival of CRC patients. Single nucleotide polymorphisms (SNPs) in CD44 gene, which is located on chromosome 11p13, have been explored in several human cancers including gastric, hepatocellular, oral, breast, lung and colorectal cancer[20,21]. Associations of specific SNPs to cancer risk, prognosis, recurrence and diverse clinical features of CRC have been observed but the findings remain controversial[21–24].

The CD44 gene polymorphism rs187115 is located in the first intron of CD44. Intronic SNPs may have an important role in gene transcription and splicing and a previous study showed an altered transcriptional regulation for rs187115 by in silico analys is[25–27]. From what we have found, there are so far no available results that reflect a connection between CD44 rs187115 and a Swedish population regarding CRC.

The aim of the present study was to examine the influence of CD44 gene polymorphism rs187115 on CRC susceptibility and the association with various clinical features including long-term survival in Swedish patients with CRC.

## 2. Materials and methods

### 2.1. Patients and controls

This study comprised blood samples from 612 consecutively collected patients who underwent surgical resection for primary colorectal adenocarcinoma at the Department of Surgery, Ryhov County Hospital, Jönköping, Sweden between 1996 and 2019. The samples were collected using the same standardized protocol during the period. Ensured by the same principle investigator responsible for this. The DNA was extracted using the same kit and protocol.

Clinicopathological characteristics of the patients are shown in Table 1. The tumors were classified according to AJCC.

Blood samples were collected at the start of surgery and patient data has been prospectively recorded in a database. Follow-up for the estimation of cancer specific survival ended on the date of death or on February 23, 2021.

Healthy blood donors ($n = 575$) at Ryhov County Hospital were collected as the control population at the time of the blood donation. The control

**Table 1.** Clinicopathological characteristics of 612 patients with CRC.

| Characteristic | Value |
|---|---|
| Age (years) | |
| Mean ± SD | 71 ± 11.1 |
| Gender, *n* (%) | |
| Female | 269 (44.0) |
| Male | 343 (56.0) |
| T stage, *n* (%) | |
| T1 + T2 | 131 (21.4) |
| T3 | 420 (68.6) |
| T4 | 61 (10.0) |
| Tumor differentiation, *n* (%) | |
| High/medium | 480 (78.4) |
| Poor | 132 (21.6) |
| TNM stage, *n* (%) | |
| I | 105 (17.2) |
| II | 229 (37.4) |
| III | 197 (32.2) |
| IV | 81 (13.2) |
| Localization, *n* (%) | |
| Colon | 335 (54.7) |
| Rectum | 277 (45.3) |
| Histologic type, *n* (%) | |
| Non-mucinous | 530 (86.6) |
| Mucinous | 82 (13.4) |
| Recurrence, *n* (%) | |
| No-recurrence | 486 (79.4) |
| Recurrence | 126 (20.6) |

population with no known CRC history was from the same geographical region (Healthcare region Southeast Sweden) as the CRC patients and comprised 310 males and 265 females, with a mean age of 58 years (SD = ±7.2). All blood samples were centrifuged to separate plasma and blood cells and then stored frozen at −70 °C until analysis.

The investigation was approved by the Regional Ethical Review Board in Linköping, Linköping, Sweden (Dnr. 2013/271-31) and informed consent was obtained from each of the participants.

### 2.2. Genotyping of CD44 gene polymorphism

In the present study genomic DNA was isolated from all blood samples using QiaAmp DNA Blood Kit (Qiagen, Hilden, Germany). The TaqMan SNP genotype assays were used for analysis of the CD44 rs187115 genotypes (Applied Biosystems, Foster City, CA, USA). Ten nanograms DNA were mixed with TaqMan Genotyping Master Mix (Applied Biosystems) and was analyzed with the 7500 Fast Real-Time Polymerase Chain Reaction (PCR) System (Applied Biosystems). The PCR conditions were an initial cycle at 50 °C for 2 min followed by one cycle at 95 °C for 10 min and finally 40 cycles at 95 °C for 15 s and at 60 °C for 1 min. The allelic discrimination application ABI PRISM 7500 SDS software version 1.3.1 (Applied Biosystems) was used to characterize the genotypes.

### 2.3. Statistical analysis

The differences in genotypes of the CD44 gene polymorphism between CRC patients and the control subjects and in subgroups of CRC patients according to clinical parameters were analyzed using the Chi-squared test. The Hardy–Weinberg equilibrium was tested for the genotypes. Survival analysis was analyzed by Kaplan–Meier method with log-rank test. Statistical analysis was performed using Stata Statistical Software Release 15 (Stata Corp. College Station, TX, USA) and SPSS software for Windows, version 14.0 for (SPSS Inc., Chicago, IL, USA). Results were considered to indicate statistical significance at $p < 0.05$.

## 3. Results

### 3.1. Genotype frequencies of the CD44 gene polymorphisms in CRC patients and control individuals

Statistically significant differences in the genotype distributions were observed between the patients and the healthy control group for CD44 rs187115 (Table 2). Moreover, the prevalence of AA genotype was 39.5% (242/612) and that of AG + GG was 60.5% (370/612) among patients, whilst

**Table 2.** Genotype numbers of *CD44* gene polymorphism (rs187115) in patients with CRC and healthy controls.

| Variables | Cases (*n*) | Genotypes (%) | | | *p* value |
|---|---|---|---|---|---|
| | | AA | AG | GG | |
| Patients | 612 | 242 (39.5) | 287 (46.9) | 83 (13.6) | |
| Controls | 575 | 269 (46.8) | 234 (40.7) | 72 (12.5) | 0.039 |
| Patients/histologic type Mucinous | | | | | |
| No | 530 | 218 (41.1) | 236 (44.6) | 76 (14.3) | |
| Yes | 82 | 24 (29.3) | 51 (62.2) | 7 (8.5) | 0.011 |

**Table 3.** Association of CD44 gene polymorphism (rs187115) and CRC risk.

| Genotype | CRC patients (*N* = 612) *n* (%) | Controls (*N* = 575) *n* (%) | OR (95% CI) | *p* value | AOR (95% CI) | *p* value* |
|---|---|---|---|---|---|---|
| AA | 242 (39.5) | 269 (46.8) | 1.00 (reference) | | | |
| AG | 287 (46.9) | 234 (40.7) | 1.36 (1.07–1.74) | 0.013 | 1.54 (0.93–1.81) | 0.120 |
| GG | 83 (13.6) | 72 (12.5) | 1.28 (0.89–1.84) | 0.177 | 1.21 (0.71–1.95) | 0.440 |
| AG + GG | 370 (60.5) | 306 (53.2) | 1.34 (1.07–1.69) | 0.012 | 1.35 (1.01–1.81) | 0.039 |

Odds ratio (OR); Adjusted odds ratio (AOR) and *p* value* after controlling for age and gender; 95% confidence interval (95% CI).

being 46.8% (269/575) and 53.2% (306/575) respectively, in the healthy control group. The carriers of G allele (AG + GG) were found to be associated with an increased risk of CRC with an odds ratio (OR) of 1.34 (95% confidence interval (CI) = 1.07–1.69; $p = 0.012$) and after adjusting for age and gender we obtained (OR) of 1.35 (95% confidence interval (CI) = 1.01–1.81; $p = 0.039$) (Table 3). The patients and the control group were in agreement with Hardy–Weinberg equilibrium.

### 3.2. Clinicopathological features in relation to CD44 gene polymorphism

Stratification analysis of association between CD44 gene polymorphism and clinicopathological features such as age, gender, tumor size, tumor differentiation, stage, localization and recurrence showed no statistically significant differences (data not shown). However, statistically significant differences in the genotype distributions were observed between the patients with mucinous and non-mucinous cancer (Table 2). Moreover, the result shows that the carriers of G allele (AG + GG) in patients with mucinous cancer was 70.7% (58/82) and was significantly different from those with non-mucinous cancer with the rate 58.9% (312/530) which corresponds an OR = 1.69 (95% CI = 1.02–2.80; $p = 0.011$).

### 3.3. Survival analysis according to CD44 gene polymorphism

Follow-up data were available for 611 patients and Kaplan–Meier analysis of the effect of CD44 rs187115 polymorphism on cancer-specific survival ($p = 0.104$) is described in Figure 1. Moreover, the cancer-specific and recurrence-free survival curves were different between GG and AG + AA

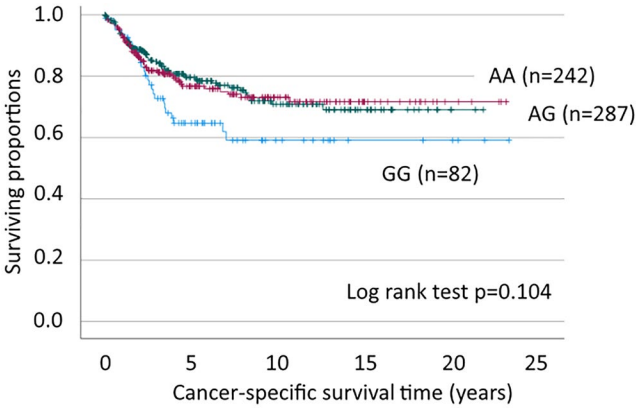


**Figure 1.** Kaplan–Meier plot describing cancer-specific survival estimates among CRC patients according to genotypes of rs187115 SNP of CD44 gene. Log rank test, $p = 0.104$.

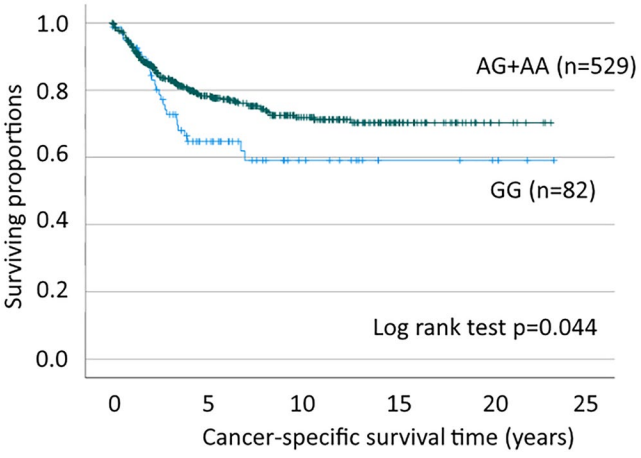


**Figure 2.** Kaplan–Meier plot comparing cancer-specific survival among CRC patients considering genotypes of rs187115 SNP of CD44 gene. Log rank test, $p = 0.044$.

for SNP CD44 with $p = 0.044$ and $p = 0.006$, respectively (Figures 2 and 3). The patients with genotype GG showed shorter cancer-specific and recurrence free survival with a hazard ratio (HR) of 1.25 (95% CI = 1.02–1.54; $p = 0.036$) and 1.52 (95% CI = 1.12–2.06; $p = 0.007$), respectively.

## 4. Discussion

Several studies have shown that CD44 polymorphisms are significantly associated with susceptibility and prognosis in different types of cancer[21]. In clinical investigations it has been found that the presence of rs187115 polymorphism in the gene of CD44 can be a risk factor, is associated with clinical features of CRC and affects its prognosis but the findings have been inconsistent[23,24,28]. To the best of our knowledge, the present study

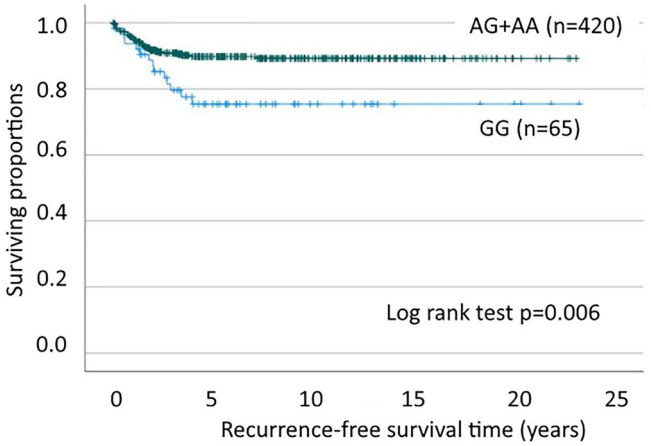


**Figure 3.** Kaplan–Meier plot comparing recurrence free survival among CRC patients considering genotypes of rs187115 SNP of CD44 gene. Log rank test, $p = 0.006$.

assesses the potential association of the CD44 rs187115 polymorphism with clinicopathological parameters, the effect on susceptibility and prognosis related to Swedish patients with CRC.

This study demonstrated that CD44 gene polymorphism rs187115 is associated with susceptibility to develop CRC. Specifically, the carriers of G allele in patients were found to be associated with an increased risk of CRC. This finding is consistent with data from Chinese patients with CRC but inconsistent with another Chinese study where no significant differences in the genotype frequencies were observed between the cancer patients and controls for CD44 gene polymorphism rs187115[24, 28].

Studies suggested that different CD44 isoforms induce or promote apoptosis, anti-apoptotic effects and chemotherapy resistance in cancer[13,21]. A study has suggested a possible role of CD44 rs187115 functional variants with cellular stress responses and chemoresistance in a p53-dependent manner[29]. Moreover, Muys et al. observed that expression of oncogenic CD44 splice variants is controlled by p53 in human CRC[30]. Mucinous cancer is an aggressive subtype of CRC and is frequently associated with resistance to apoptosis[31]. The factors involved in the development of mucinous CRC are not yet known[31,32] but a different molecular signature has been suggested[33]. In a study by Zhu et al. the expression of the secretory mucin MUC5AC was found to be significantly elevated in colon cancer tissues when compared with the paired para-cancerous tissues in the clinical setting[34]. Interestingly, this secretory mucin has been shown to interact with CD44 and results in enhanced tumorigenesis and confers chemoresistance via CD44/β-catenin/p53/p21 signaling in human CRC[35]. In this study, stratification analysis of association between CD44 gene polymorphism and the clinicopathological features investigated showed no difference excepted the patients with mucinous and non-mucinous cancer.

Moreover, the result shows that the carriers of G allele were more common in patients with mucinous cancer and was different from those with non-mucinous cancer. However, there are limited data about the functional activity of this SNP. Whether our findings reflect a modulating role of CD44 rs187115 in a p53 signaling pattern aimed at controlling colorectal carcinogenesis during mucinous secretion will require further studies to determine.

We noted that both cancer-specific and recurrence-free survival differed between genotypes for rs187115 and that carriers of the genotype GG were associated with the worst prognosis for CRC. In a previous study Wen et al. reported that this genotype was associated with less favorable prognosis, in terms of overall survival, compared with other genotype in Chinese patients with CRC[24]. It appears likely that the referred genetic component GG has a modulating role regarding the clinical outcome and that CD44 rs187115 SNP could be a potentially useful prognostic biomarker. Different types of CD44 isoforms are expressed in cancer[13]. There is considerable evidence that the expression of the CD44 isoforms v6 and v9 has impact on tumor progression, metastasis and prognosis in CRC[13,36]. However, functional research is required to clarify the genetic regulation from CD44 rs187115 to influence the expression of CD44 isoforms v6 and v9.

Some limitations in this study are worth noting. It is important to note that this study is of exploratory nature. Factors influencing carcinogenesis such as environmental and lifestyle factors were not considered. Moreover, it cannot be excluded that the investigated CD44 rs187115 may be linked with other polymorphisms that affect susceptibility to CRC and the prognosis of patients. The patients and controls were selected from one hospital and may not represent the general populations. Therefore, additional studies in larger and broader groups of patients and controls to validate our finding are needed.

## 5. Conclusion

The present findings demonstrated that the SNP rs187115 of the CD44 gene was associated with risk, mucinous cancer and prognosis in Swedish patients with CRC. Further functional studies are required to confirm the genetic contribution of the CD44 rs187115 polymorphism and to evaluate its suitability as a biomarker for CRC in a broader population.

## Disclosure statement

The authors declare no conflicts of interest.

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