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Significance of Gene Polymorphism and Gene Expression of BACE2 in Swedish Patients with Colorectal Cancer

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Keywords
BACE2 · Single nucleotide polymorphism · Colorectal cancer · Clinical parameters

Abstract
Introduction: β-site amyloid precursor protein (APP) cleaving enzyme 2 (BACE2) cleaves APP which is ubiquitously expressed in a variety of cell types including cancer cells. BACE2 can process APP in several ways and appears to be involved in the pathogenesis of cancer. Our purpose was to assess the association of mRNA expression and genetic polymorphism of BACE2 in colorectal cancer (CRC) susceptibility and its association to clinicopathological factors in Swedish patients with CRC. ***Methods:*** A total of 720 CRC patients and 470 healthy controls were genotyped for *BACE2* gene polymorphism rs2012050, using TaqMan single nucleotide polymorphism (SNP) assays based on polymerase chain reaction. Reverse transcription quantitative PCR was used to investigate the *BACE2* gene expression in 192 CRC tissue and 181 paired normal tissue. ***Results:*** Assessing clinicopathological factors, we noted that carrying of T allele in C/T and C/T+T/T was significantly associated with a protective role against disseminated cancer and higher lymph node status. Moreover, individuals carrying T/T genotype were significantly more likely to have poorly differentiated cancer. Follow-up data for patients in poorly differentiated cancer and the Kaplan-Meier analysis showed that the cancer-specific survival curves differed between C/C and C/T+T/T for the *BACE2* gene polymorphism and that the carriers of the genotype C/C were associated with more favorable prognosis. We found no significant differences in the genotypic frequencies between the patients and healthy controls. BACE2 mRNA level was significantly 2.2-fold upregulated in CRC tissue when compared to noncancerous tissue. A higher BACE2 mRNA level was observed in smaller tumors and in rectal cancer when compared to colon cancer. ***Conclusion:*** In patients with CRC, our results indicate BACE2 rs2012050 as a useful potential predictor of poor differentiation, disseminated cancer and lymph node status and that the BACE2 mRNA expression is associated to tumor size and cancer location.

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Introduction

Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality worldwide [1]. The etiology of CRC is not completely known but initiation and progression of CRC have been described by various genetic and epigenetic pathways and with inflammatory factors [2, 3]. Molecular biomarkers may be important to detect CRC and to determine prognosis as well as to meet the demand for a more personalized treatment following increased awareness that CRC is a heterogeneous disease [4, 5].

Amyloid precursor protein (APP) is a membrane-bound protein expressed in a variety of cell types including cancers cells [6]. However, the protein has received most attention as an important factor in the neurodegenerative disease Alzheimer's disease (AD) [7]. Research indicates that APP may exert effects in the progression, proliferation, and migration of cancer such as pancreatic, lung, and colon cancer [6]. A previous study provides evidence that APP is involved in growth of human colon carcinoma cells both in vitro and in vivo [8].

β-site APP cleaving enzyme 1 and 2 (BACE1 and BACE2) are integral membrane aspartic proteases localized on the cell surface membrane and on the membrane of intracellular vesicles [9]. BACE1 is particularly expressed with in the central nervous system. There is a moderate amount of its homology BACE2, and they both can cleave APP to generate the toxic unit Aβ [9, 10]. Outside the central nervous system, BACE2 is prominently found in peripheral tissues such as kidney, colon and pancreas with unclear functions [11]. However, in pancreas BACE2 has a characterized role in maintenance β cell mass and its function [12]. Unlike BACE1, BACE2 can cleave APP within the Aβ domain and consequently prevents the generation of Aβ which is considered as the toxic entity driving neurodegeneration in AD [13, 14].

Studies have shown that BACE2 is upregulated in a broad range of tumors such as glioblastoma, melanoma, pancreatic adenocarcinoma, and CRC [15, 16]. Genetic variations, such as single nucleotide polymorphisms (SNPs), have been shown to be associated with CRC and play a role in susceptibility and as well as survival of CRC patients [17]. The polymorphism rs2012050 in *BACE2* gene on chromosome 21q22.3 is located in the 3′-untranslated region (UTR) (www.ncbi.nlm.nih.gov/snp) and is associated with altered BACE2 gene expression [18].

Up to now, there has been little information available about BACE2 in the clinical context of CRC. In the present study, we examined the gene expression of BACE2 and its gene polymorphism rs2012050 in Swedish patients with CRC to evaluate the significance on CRC susceptibility and the link with various clinical features and long-term survival.

Table 1. Clinicopathological characteristics of patients with CRC (n = 720)

Characteristic	Value
Age, median (range), years	73 (25–94)
Sex, *n* (%)	
Female	321 (45.6)
Male	399 (55.4)
Depth of tumor, *n* (%)	
T1+T2	152 (21.1)
T3+T4	568 (78.9)
Tumor differentiation, *n* (%)	
High/medium	564 (78.3)
Poor	156 (21.7)
TNM stage, *n* (%)	
I+II (localized cancer)	384 (53.3)
III+IV (disseminated cancer)	336 (46.7)
Tumor location, *n* (%)	
Colon	405 (56.2)
Rectum	315 (43.8)
Histologic type, *n* (%)	
Non-mucinous	627 (87.1)
Mucinous	93 (12.9)
Tumor size, *n* (%)[a]	
<4 cm	273 (42.1)
≥4 cm	376 (57.9)
Lymph vascular invasion, *n* (%)[a]	
No	412 (90.9)
Yes	41 (9.1)
Perineural invasion (PNI), *n* (%)[a]	
No	422 (81.2)
Yes	98 (18.8)
Lymph node status, *n* (%)[a]	
N0	92 (57.6)
N1+N2	288 (42.4)
Recurrence, *n* (%)[a]	
No	536 (78.8)
Yes	144 (21.2)

[a]Maximum available data.

Materials and Methods

Patients, Tissue Samples, and Healthy Controls

The study utilized blood samples from 720 patients with primary colorectal adenocarcinomas collected between 1996 and 2021 within the Department of Surgery, Ryhov County Hospital, Jönköping, Sweden. Blood samples were collected at the start of surgery. Tumor tissue from 192 patients and 181 paired normal tissue samples were collected between February 2014

Table 2. Clinicopathological characteristics of tumor specimens from 192 patients with CRC

Characteristic	Value
Age, median (range), years	74 (30–94)
Sex, *n* (%)	
Female	82 (42.7)
Male	110 (57.3)
Depth of tumor, *n* (%)	
T1+T2	40 (20.8)
T3+T4	152 (79.2)
Tumor differentiation, *n* (%)	
High/medium	156 (81.3)
Poor	36 (18.7)
TNM stage, *n* (%)	
I+II	108 (56.3)
III+IV	84 (43.7)
Tumor location, *n* (%)	
Colon	112 (58.3)
Rectum	80 (41.7)
Histologic type, *n* (%)	
Non-mucinous	169 (88.0)
Mucinous	23 (12.0)
Tumor size, *n* (%)	
<4 cm	93 (48.4)
≥4 cm	99 (51.6)
Perineural invasion (PNI), *n* (%)	
No	168 (87.5)
Yes	24 (12.5)
Lymph node status, *n* (%)	
N0	112 (58.3)
N1+N2	80 (41.7)

and November 2019. Tumor tissue and adjacent normal mucosa (about 5 cm from the tumor) were excised and immediately frozen at −78°C until analysis. All tissue samples intended for RNA analysis were stored in RNA protect tissue reagent (Qiagen) to maintain good RNA quality. Both blood and tissue samples have been collected according to availability in strict chronological order and the patient data have been prospectively recorded in a database. Follow-up for the estimation of cancer-specific survival ended on the date of death or in May 2023. Clinicopathological characteristics of the total patients are summarized in Table 1, and the tumors were classified according to the American Joint Committee on Cancer (AJCC) classification system [19]. Clinicopathological characteristics of the tumor specimens reserved for RNA analysis are shown in Table 2.

Healthy blood donors (n = 470) at Ryhov County Hospital, with no known CRC history and from the same geographical region as the CRC patients were selected as the control population at the time of the blood donation. The cohort comprised 253 male and 217 female blood donors with a median age of 60 years (range 33–68). All blood samples were centrifuged to separate plasma and blood cells and then stored frozen at −70°C until analysis.

Genotyping of BACE2 *Gene Polymorphism*

Genomic DNA was isolated from all blood samples using QiaAmp DNA Blood Kit (Qiagen, Hilden, Germany). Genotyping was analyzed using the TaqMan SNP genotype assays BACE2 rs2012050 (ID C-9479137_10) (Applied Biosystems, Foster City, CA, USA). Ten ng DNA was mixed with TaqMan Genotyping Master Mix (Applied Biosystems) and was analyzed with the 7500 Fast Real-Time PCR System (Applied Biosystems). The PCR was performed using 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 manual calling option in the allelic discrimination application ABI PRISM 7500 SDS software version 1.3.1 (Applied Biosystems) was used to assign the genotypes.

RT-qPCR

CRC tissue (n = 192) and adjacent normal tissue samples (n = 181) were extracted for RNA using RNeasy Mini kit (Qiagen) in accordance with the manufacturer's instructions. Total RNA was reverse transcribed using Super Script III kit (#11752, Thermo Fisher Scientific) and the complementary DNA was amplified through RT-qPCR using probes for BACE2 (Hs00273238_ml) that was normalized against GAPDH (Hs02758991_g1) (Thermo Fisher Scientific) and semi-quantified from a relative standard curve.

Statistical Analysis

The differences in the frequencies of the *BACE2* gene polymorphism between patients and controls and the genotype associations according to clinicopathological characteristics within the CRC subgroups were analyzed using the χ^2 test. The strength of association was assessed by calculation of the odds ratio (OR) with 95% confidence interval using logistic regression. The ORs were adjusted for potential covariates in accordance with Table 1 by multiple logistic regression models. Survival analysis was performed by Kaplan-Meier analysis with log-rank test and Cox's regression. Statistical analysis was performed using Stata Statistical Software Release 15 (Stata

Table 3. Genotype numbers of *BACE2* gene polymorphism (rs2012050) in 720 patients with CRC and 470 healthy controls and CRC risk

Genotype	Controls, *n* (%)	CRC patients, *n* (%)	OR (95% CI)	*p* value
C/C	177 (37.7)	292 (40.6)	1.00 (reference)	
C/T	236 (50.2)	352 (48.9)	0.90 (0.70–1.16)	0.427
T/T	57 (12.1)	76 (10.5)	0.81 (0.54–1.19)	0.285
C/T+T/T	293 (62.3)	428 (59.4)	0.89 (0.70–1.12)	0.317

OR, odds ratio; CI, confidence interval.

Corp. College Station, TX, USA) and SPSS software for Windows, version 14.0 for (SPSS Inc., Chicago, IL, USA). The p value <0.05 was considered significant.

Results

BACE2 rs2012050 Polymorphism and the Risk of CRC and the Correlation with Clinicopathological Characteristics of CRC Patients

No significant differences in the genotype distributions were observed between the patients and the healthy control group (Table 3). This study explored the association of *BACE2* gene polymorphism to clinicopathological characteristics according to Table 1. A statistically significant association with the genotypic variants of BACE2 rs2012050 including TNM stage (Table 4), tumor differentiation (Table 5) and lymph node status (Table 6) was found. No association was found between BACE2 gene polymorphism and age, sex, depth of tumor location, tumor size, lympho-vascular invasion or perineural invasion and recurrence (data not shown). When the association between BACE2 gene polymorphism and clinicopathological characteristics was evaluated patients with C/T, T/T, or combined C/T+T/T genotypes were consistently compared with those carrying the C/C genotype. Carriers of T allele in C/T and C/T+T/T had a protective role against disseminated (stage III+IV) cancer (C/T: OR = 0.73, p = 0.048; C/T+T/T: OR = 0.72, p = 0.042) and higher node status (C/T: OR = 0.65, p = 0.012; C/T+T/T: OR = 0.66, p = 0.013) (Tables 4, 6, respectively). Moreover, individuals carrying T/T genotype were significantly more likely to have poorly differentiated cancer (OR = 1.37, p = 0.035) (Table 5).

BACE2 rs2012050 Polymorphism and Cancer-Specific Survival

Follow-up data available for 142 patients with poor differentiation and Kaplan-Meier analysis showed significant (p = 0.036) survival difference controlled by the genotypes for BACE2 gene polymorphism. The carriers of the genotype C/C were associated with more favorable prognosis (Fig. 1). Stratification analysis with regard to other clinical parameters showed no significant survival difference controlled by the genotypes for *BACE2 rs2012050* (data not shown).

BACE2 mRNA Expression in CRC and Normal Paired Tissue

There was a 2.2-fold (p < 0.001) higher expression of BACE2 mRNA with an upregulation of 81% of the cases in cancer tissue compared with normal paired tissue (Table 7) as determined by RT-qPCR. When the relationship between BACE2 mRNA level in cancer tissue and clinicopathological characteristics was analyzed there was a significant association to tumor size and tumor location. Small tumor size (<4 cm) and rectal cancer showed higher (p = 0.001) expression in comparison with ≥4 cm and colon cancer (Table 7). The level of BACE2 mRNA was not associated to any genotype of BACE2 rs2012050 (data not shown).

Discussion

Little information is available about BACE2 and its clinical implication on CRC. We performed our study on a large cohort of 720 patients with CRC, investigating the association of BACE2 gene polymorphism rs2012050 with various clinicopathological features and evaluating the significance on CRC susceptibility. In addition, in 192 of the patients we examined the mRNA expression of BACE2 in both cancer tissue and normal tissue.

We found that carrying T/T genotype was associated with poor differentiation in a multivariate model adjusting for covariates. Moreover, we noted that cancer-specific survival differed between genotypes for rs2012050 and that carriers of the genotype C/C had a more favorable prognosis among patients with poor

Table 4. Association of *BACE2* gene polymorphism (rs2012050) with localized and disseminated disease in 720 CRC patients

Genotype	Localized (N = 384), n (%)	Disseminated (N = 336), n (%)	OR (95% CI)	p value	AOR (95% CI)	p value
C/C	142 (37.0)	150 (44.6)	1.00 (reference)			
C/T	199 (51.8)	153 (45.6)	0.72 (0.53–0.98)	**0.038**	0.73 (0.53–0.98)	**0.048**
T/T	43 (11.2)	33 (9.8)	0.73 (0.44–1.21)	0.218		
C/T+T/T	242 (63.0)	186 (55.4)	0.72 (0.54–0.97)	**0.030**	0.72 (0.53–0.98)	**0.042**

OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio. Statistically significant p values are shown in bold.

Table 5. Association of *BACE2* gene polymorphism (rs2012050) and tumor differentiation in 720 CRC patients

Genotype	High/medium (N = 564), n (%)	Poor (N = 156), n (%)	OR (95% CI)	p value	AOR (95% CI)	p value
C/C	234 (41.5)	58 (37.2)	1.00 (reference)			
C/T	278 (49.3)	74 (47.4)	1.04 (0.85–1.26)	0.717		
T/T	52 (9.2)	24 (15.4)	1.37 (1.03–1.81)	**0.030**	1.37 (1.02–1.85)	**0.035**
C/T+T/T	330 (58.5)	98 (62.8)	1.10 (0.91–1.31)	0.332		

OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio. Statistically significant p values are shown in bold.

Table 6. Association of BACE2 gene polymorphism (rs2012050) and lymph node status in 680 CRC patients

Genotype	N0 (N = 392), n (%)	N1+N2 (N = 288), n (%)	OR (95% CI)	p value	AOR (95% CI)	p value
C/C	144 (36.7)	134 (46.5)	1.00 (reference)			
C/T	204 (52.0)	124 (43.1)	0.65 (0.47–0.90)	**0.010**	0.65 (0.47–0.91)	**0.012**
T/T	44 (11.3)	30 (10.4)	0.73 (0.44–1.23)	0.241		
C/T+T/T	248 (63.3)	154 (53.5)	0.67 (0.49–0.91)	**0.010**	0.66 (0.48–0.92)	**0.013**

OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio. Statistically significant p values are shown in bold.

differentiation. Little data are available on the role of BACE2 rs2012050 polymorphism, but one study reported that T/T genotype has a modulating effect regarding the gene expression of BACE2 in disease of AD [18]. In our study, we noted that an upregulation of BACE2 mRNA in cancer tissue was not associated to any genotype of BACE2 rs2012050. Furthermore, we found that carrying of T allele in C/T and C/T+T/T had a protective role against disseminated cancer and higher lymph node status. Understanding the mechanisms underlying the effects of SNPs resulting in cancer susceptibility is critical to understand the molecular pathogenesis of various cancers. As mentioned before, the polymorphism rs2012050 in *BACE2* gene is located in the 3′-UTR. SNPs located in the 3′-UTRs affect gene expression by different mechanisms including microRNA binding, mRNA degradation and translation efficiency [20]. It is plausible that BACE2 rs2012050 concomitant with other gene-gene or gene-environmental interactions controlling the colorectal carcinogenesis. Further experimental and functional studies are required to clarify the underlying mechanisms.

A previous study with 22 cancer tissue samples and 24 normal tissue samples from patients with CRC showed an upregulation of BACE2 mRNA in the cancer tissue [16]. Consistent with these data, we established that cancer tissue showed 2.2-fold higher level of BACE2 mRNA in comparison with paired normal tissue. In our study, we

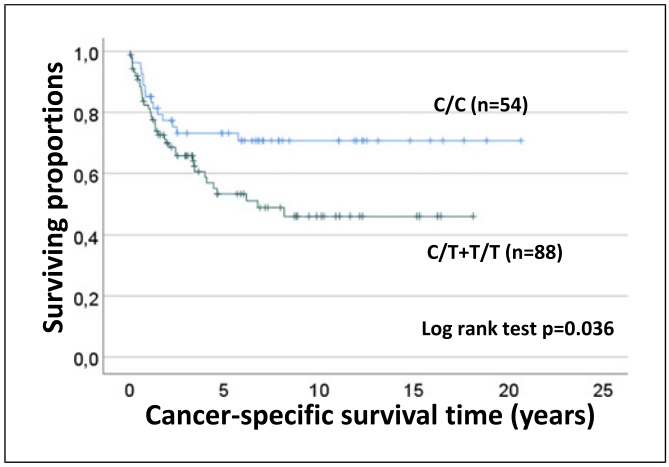

Fig. 1. Kaplan-Meier plot comparing cancer-specific survival among patients with poor differentiation CRC considering genotypes of BACE2 rs201050 polymorphism.

Table 7. Cancer and normal tissue level of BACE2 mRNA and the relation to characteristics in 192 patients with CRC

Variable	Cases, *n*	mRNA (AU)	*p* value
Paired			
Cancer tissue	181	74.5 (9.6–1,510)	
Normal tissue	181	34.6 (8.1–222.5)	<0.001
Tumor size			
<4 cm	93	84.4 (24.7–1,510)	
≥4 cm	99	62.3 (6.8–409.1)	0.001
Tumor location			
Colon	112	66.0 (6.8–360.2)	
Rectum	80	93.6 (9.6–1,510)	0.001

Data are shown as median (range). AU, arbitrary unit. *p* value <0.05 is statistically significant.

used cancer tissues and normal paired tissues from 181 patients with CRC. To evaluate associations between BACE2 mRNA expression in cancer tissue and various clinical features, we analyzed the expression of BACE2 in tumor tissue from 192 patients and identified that the expression was higher in tumors <4 cm and in rectal cancer in comparison with larger sized tumors and colon cancer, respectively. The detailed mechanism of BACE2 mRNA accumulation due to tumor size and location is expected to be elucidated in the future. It has been suggested that cancer in colon and rectum should be considered as two distinct entities with difference molecular carcinogenesis [21]. Through this, there could be an explanation for the difference in BACE2 mRNA expression.

The tumor size has been reported to be prognostic in CRC with negative relationship between tumor size and survival but the prognostic value of tumor size remains controversial [22, 23]. How BACE2 mRNA expression is linked to tumor size require detailed analyses. It has become clear that APP has effects in the progression, proliferation and migration of cancer such as pancreatic, lung, and colon cancer [6, 8]. BACE2 can cleave APP generating the unit Aβ but can also cleaves the APP within the Aβ domain and thereby prevents the formation of the unit Aβ [13, 14]. BACE2-driven mechanisms and CRC progression are not known but one may speculate that Aβ peptide is involved. In a previous study using normal human cerebral endothelial cells, and in vivo, using the chick embryo was shown that the Aß peptide may contribute to angiogenesis [24]. In this study, we observed that BACE2 mRNA expression was higher in tumor size <4 cm compared with tumor size ≥4 cm. The reduced tumor size could potentially be a result of less angiogenesis through higher activity of BACE2, which prevents the formation of Aβ due to the higher BACE2 expression in smaller tumors.

Previously it has been shown that BACE2 is highly expressed in glioma and positively modulates nuclear factor-kappa B (NF-κB) signaling to promote tumor growth and invasiveness [25]. The NF-κB signaling pathway is a regulator of cell proliferation, apoptosis, angiogenesis, inflammation, and metastasis in CRC. An over-activation of the NF-κB pathway is a feature of CRC [26]. Furthermore, dysfunctional calcium homeostasis contributes to colon cancer cell proliferation and migration [27]. In a study on ocular melanoma, it has been noted that high BACE2 activity modulates intracellular Ca^{2+} pathways to support cancer growth [28]. Moreover, to explore underlying mechanisms the research group found that BACE2 could regulate the transcriptional factor CTNNB1 (β-catenin) which is relevant to colorectal carcinogenesis by Wnt signaling during epithelial-mesenchymal transition leading to activate central pro-oncogenic cell-cycle controller genes such as c-Myc and cyclin D1 [2]. Further studies may confirm whether the referred signaling pathways can be translated to CRC.

This study is exploratory and factors influencing carcinogenesis such as environmental and lifestyle factors were not considered. Moreover, other data of oncogenes, such as RAS, BRAF, and HER2, and microsatellite instability were not available, which is a limitation of the study.

A further verification study with a larger number of patients and controls from another cohort is needed to confirm our results. An additional prospective study would be of interest in the future to confirm the results.

Conclusions

In conclusion, the current study is, to our knowledge, the first study in which the association between BACE2 rs2012050 and a wide range of clinicopathological factors has been described in patients with CRC. We observed that a SNP in BACE2 (rs2012050) is a useful predictor of differentiation, clinical stage and lymph node status and a useful indicator for clinical prognosis for patients with poorly differentiated cancer. Moreover, the gene expression of BACE2 reflects both tumor size and cancer location. Larger cohorts of patients would be a step further in determining usefulness of BACE2 as a diagnostic and prognostic indicator for CRC.

Statement of Ethics

This study was approved by the Regional Ethical Review Board in Linköping, Sweden, Approval No. 2013/271-31, and written informed consent was obtained from each of the participants. All research was performed in accordance with relevant guidelines/regulations and in accordance with the Declaration of Helsinki.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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Author Contributions

Research design and prepared the main manuscript and analyzed data and statistical analysis: J.D. and D.W. L.S. prepared clinical samples. Performed the laboratory work: L.S. and K.G. Responsible for patient data and follow-up: K.L. and L.S. Review and revision: J.D., D.W., L.S., K.G., and K.L.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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