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Genetic variants associated with colorectal brain metastases susceptibility and survival

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Abstract

Colorectal brain metastases (BM) are rare (1–2%) and a late-stage disease manifestation. Molecular mechanisms for BM development are not well understood. We tested whether variants within genes involved in overcoming the blood–brain barrier (BBB) are associated with BM susceptibility and survival in patients with BM. Germline single-nucleotide polymorphisms (SNPs, $n = 17$) in seven genes (*CXCR4, MMP9, ST6GALNAC5, ITGAV, ITGB1, ITGB3, KLF4*) were analyzed from germline DNA in patients with resected BM ($n = 70$) or no clinical evidence of BM after at least 24 months from diagnosis (control group, $n = 45$). SNPs were evaluated for association with BM susceptibility and overall survival (OS) from BM diagnosis. *ST6GALNAC5* rs17368584 and *ITGB3* rs3809865 were significantly associated with BM susceptibility. In multivariable analysis adjusted for patient characteristics, *KLF4* rs2236599, *ITGAV* rs10171481, *ST6GALNAC5* rs1883778, *CXCR4* rs2680880 and *ITGB3* rs5918 were significant for OS. This study shows for the first time that variants within genes involved in breaching the BBB are associated with BM susceptibility and survival. These findings warrant further validation to develop better screening guidelines and to identify novel therapy targets for patients with BM.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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INTRODUCTION

In colorectal cancer (CRC), brain metastases (BM) are rare, occurring in only 1–2% of the patients.[1] As median overall survival (OS) in metastatic CRC has reached ~ 30 months, the incidence of BM is expected to increase in the future, which will make them a relevant clinical factor.[2] BM usually occur after 24 months from diagnosis of CRC with survival times of <6 months after BM diagnosis.[3] Understanding the processes and pathways involved in the development of BM is critical to identify prognostic markers and to develop new treatment strategies to improve clinical outcome.

To migrate to the brain, cancer cells need to overcome the blood–brain barrier (BBB), which is an effective defense mechanism protecting the central nervous system.[4] This process requires the activation of cellular pathways that lead to migration, invasion, adhesion and breakdown of the BBB.[5] Moreover, cancer cells require the induction of angiogenesis to grow out and form tumors. Genes that are involved in these pathways are promising biomarkers and may also represent potential drug targets.[6]

We had the opportunity to study a unique cohort of 70 patients with metastatic CRC and resected BM. We investigated single-nucleotide polymorphisms (SNPs) in a comprehensive panel of genes involved in the breakdown of the BBB and the development of BM previously reported for other malignancies that are frequently associated with BM, such as melanoma, lung cancer and breast cancer, but have not been described for colorectal BM before. These genes have been reported to be involved in migration-, invasion- and adhesion-mediating (*ITGB1* (encoding for integrin β_1), *ITGB3* (integrin β_3), *ITGAV* (integrin α_v), *CXCR4* (chemokine (C-X-C motif) receptor 4), *MMP9* (matrix metallopeptidase 9), *ST6GALNAC5* (ST6 (Alpha-*N*-Acetyl-Neuraminyl-2,3-Beta-Galactosyl-1,3)-*N*-Acetylgalactosaminide Alpha-2,6-Sialyltransferase 5)) and cancer stem cell-promoting (*KLF4* (kruppel-like factor 4)) pathways.[7–13] To identify markers for BM susceptibility, we compared the genotypes of these SNPs between the 70 patients with BM and a control group of patients who had no clinical evidence of BM after at least 24 months of follow-up. Moreover, we tested whether these SNPs predict OS in the 70 patients with BM. The identification of clinically relevant polymorphisms may help to identify prognostic biomarkers and potential drug targets, and to guide brain-screening strategies in risk patients in the future.

MATERIALS AND METHODS

A total of 115 patients (Caucasian, median 61 years (range 37–76), 38 (54.3%) male) with metastatic CRC who underwent surgical resection for BM ($n = 70$) or who had no clinical evidence of BM after at least 24 months of follow-up (median 52.8 months (range 24.2–115.7); control group (CG); $n = 45$) were investigated. Patient characteristics are given in Table 1. The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional review board and all participants signed informed consent for the analysis of molecular correlates.

SNPs in genes previously reported to be associated with breakdown of the BBB and development of BM were selected when functionally relevant according to the literature or if they were Tag SNPs.[7–10,12–14] Functional relevance was assessed according to Queen's University F-SNP and National Institute of Environmental Health Sciences SNP Function Prediction tools.[15,16] SNPs were selected if the minor allele frequency was ⩾10% in Caucasians according to the Ensembl database.[17]

Germline DNA was extracted from the formalin-fixed paraffin-embedded specimen from resected BM or primary tumors (control group) using the QIAamp DNAeasy Kit (Qiagen, Hilden, Germany). Germline SNPs were analyzed using polymerase chain reaction (primers given in Table 2) and direct Sanger sequencing. ABI Sequencing Scanner v1.0 (Applied Biosystems, Foster City, CA, USA) was used for sequence analyses. Investigators performing sequence analyses were blinded to patients' clinical data.

Statistical analysis

For allele distribution of each polymorphism, deviation from the Hardy–Weinberg equilibrium was assessed using a goodness-of-fit χ^2-test with 1 degree of freedom. The co-dominant, dominant or recessive inheritance model was considered whenever appropriate. A dominant model was used when the patient number in the homozygous variant group was < 10% (seven patients).

The primary outcome was OS measured from diagnosis of BM to death, or last follow-up if patients were still alive. Association between genetic variants and OS were examined using Kaplan–Meier curves and log-rank test in the univariable analysis, and Cox proportional hazards regression model adjusting patient baseline characteristics (stepwise selection) in the multivariable analysis. Recursive partitioning was performed to identify the patterns of genetic variants associated with OS.

To compare the differences in the allele distribution between patients with and without BM, Fisher's exact test was used. With 70 cases of BM and 45 controls, we would have ~ 80% power to detect a genetic variant with increased relative risk of 3.00–3.33 using 0.05 level, two-sided tests for the allele frequency of 0.1–0.35. The power analysis was conducted using Quanto (Version 1.2.4, May 2009).

Analyses were carried out with the statistical software package SAS version 9.4 (SAS Institute, Cary, NC, USA) or rpart function in R (the R foundation for Statistical Computing).

All *P*-values were two-sided. *P*-values were adjusted for multiple testing using the false discovery rate (the Benjamini and Hochberg method). Both raw *P*-values and false discovery rate-adjusted *P*-values were provided. The false discovery rate-adjusted *P*-values <0.15 were considered as significant.

RESULTS

Variants and Hardy–Weinberg equilibrium

In BM patients, 9 out of 17 SNPs were not in Hardy–Weinberg equilibrium (*ST6GALNAC5* rs1146671 G>A, *ST6GALNAC5* rs1883778 G>A, *ST6GALNAC5* rs17368584 T>C, *KLF4* rs2236599 G>A, *ITGAV* rs1839123 G>A, *ITGAV* rs10171481 A>G, *ITGAV* rs11902171 G>C, *ITGB3* rs4642 A>G, *ITGB3* rs5918 T>C).

Variants and BM susceptibility

ST6GALNAC5 rs17368584 T>C and *ITGB3* rs3809865 A>T were significantly associated with BM susceptibility ((BM T/T 40%, T/C 35%, C/C 25% vs CG T/T 43%, T/C 50%, C/C 7%; $P = 0.045$) and (BM A/A 58%, A/T 23%, T/T 19% vs CG A/A 40%, A/T 49%, T/T 11%; $P = 0.017$), respectively).

Variants and association with OS after BM diagnosis

Patients with a C/C genotype in *ITGB3* rs5918 T>C had a significantly shorter OS compared with those with T/T or T/C in univariable analysis (4.0 versus 8.0 months; hazard ratio (HR) 2.66 (95% confidence interval (CI) 1.13−6.25), $P = 0.010$), which remained significant in multivariable analysis (HR 2.72 (95% CI 1.14−6.48), $P = 0.024$) (Supplementary Figure 1). Patients with a G/G genotype of *MMP9* rs17577 G>A had a significantly longer OS compared with those with a variant allele (7.4 versus 5.1 months; HR 1.83 (95% CI 0.95−3.53), $P = 0.044$), however this difference did not remain significant in multivariable analysis ($P = 0.20$) (Supplementary Figure 2). Patients with a variant G/G genotype of *ITGB3* rs4642 A>G had significantly shorter OS compared with those with A/A or A/G (4.3 versus 8.0 months; HR 2.31 (95% CI 1.08−4.93), $P = 0.014$) (Supplementary Figure 3). This difference also did not remain significant in multivariable analysis ($P = 0.16$). Four SNPs were not significant in univariable analysis (*KLF4* rs2236599 G>A G/G 7.4 months, G/A or A/A 4.8 months; HR 1.44 (95% CI 0.83−2.49), $P = 0.16$; *ITGAV* rs10171481 A>G A/A or A/G 5.3 months, G/G 15.5 months; HR 0.58 (95% CI 0.29−1.15), $P = 0.10$; *ST6GALNAC5* rs1883778 G>A G/G 4.6 months, G/A or A/A 9.4 months; HR 0.68 (95% CI 0.40−1.16), $P = 0.14$; *CXCR4* rs2680880 A>T A/A or A/T 7.1 months, T/T 4.6 months; HR 1.09 (95% CI 0.60−2.00), $P = 0.76$, respectively), however these differences became significant in multivariable analysis (*KLF4* rs2236599 G>A HR 2.12 (95% CI 1.01−4.45), $P = 0.048$; *ITGAV* rs10171481 A>G HR 0.39 (95% CI 0.18−0.85), $P = 0.018$; *ST6GALNAC5* rs1883778 G>A HR 0.53 (95% CI 0.30−0.93), $P = 0.028$; *CXCR4* rs2680880 A>T HR 2.30 (95% CI 1.19−4.44), $P = 0.013$, respectively) (Supplementary Figures 4–7). After adjusting for multiple testing, *ITGAV* rs10171481 A>G, *ST6GALNAC5* rs1883778 G>A, *ITGB3* rs5918 T>C and *CXCR4* rs2680880 A>T remained significant ($P<0.15$). Data on OS are given in Table 3.

Recursive partitioning and OS after BM diagnosis

ITGB3 rs4642 A>G was the dominant OS-predicting SNP. *MMP9* rs17577 G>A, *ITGB1* rs11009151 T>A and *ITGAV* rs11902171 G>C predicted OS in subgroups. Median OS was 15.4 months (95% CI 4.6−30.9) for node 1, 6.9 months (95% CI 4.0−13.2) for nodes 2+3

and 4.4 months (95% CI 2.0−8.0) for nodes 4+5 (log-rank test, $P = 0.003$) (Figure 1). In the multivariable Cox regression model, the tree nodes were significantly associated with OS after adjusting for age, Karnofsky performance status at diagnosis of BM, primary tumor site and localization of BM (P for trend = 0.043).

DISCUSSION

This study shows for the first time that variants of genes involved in cancer cell migration, invasion and adhesion, and breakdown of the BBB predict BM susceptibility and clinical outcome in patients with resected colorectal BM. Intriguingly, variants within *ITGB3* and *ST6GALNAC5* were associated with both risk of developing BM and OS, and are therefore promising biomarkers and drug targets.

The results of this study strongly suggest that integrins, which are cell surface receptors involved in cell adhesion, migration and interaction with the microenvironment, are involved in both developing BM and determining the prognosis. Integrins form heterodimers with α- and β-subunits and are expressed on various cell types including cancer cells.[18] Binding of integrins on these cells to extracellular matrix components, such as collagen, laminin, vitronectin, fibronectin and fibrinogen, leads to activation of downstream signaling, cross-talk with growth factor signaling, stimulating cell proliferation, and to increased cell motility, stimulating adhesion, migration and angiogenesis.[19] In the present study, three SNPs in *ITGB3* and *ITGAV* were associated with OS, suggesting that various types of integrins are clinically relevant in this setting. The expression of the heterodimer integrin $\alpha_v\beta_3$ is frequently upregulated in endothelial cells upon induction of angiogenic signaling in CRC and in cancers prone to metastasize to the brain, and is associated with poor clinical outcome.[20–22] A previous study has demonstrated the critical relevance of the heterodimer integrin $\alpha_v\beta_3$ in the development of BM under normoxic conditions in a vascular endothelial growth factor-dependent manner.[9] A more recent study investigating SNPs in integrins has found that *ITGB3* rs4642 A>G was associated with time to recurrence in stage II and III CRC patients receiving fluoropyrimidine-based adjuvant chemotherapy.[23] In line with our study, the G allele was associated with inferior outcome. Inhibition of integrins is a promising approach in cancer treatment, which may be directly facilitated by inhibition of growth of cancer cells or indirectly by inhibition of angiogenesis. Recent data suggested that integrin inhibitors should be considered, for example, in breast cancer treatment.[24] However, the clinical meaning of this approach in CRC with or without BM remains to be elucidated.[25]

Another gene that was associated with both risk of developing BM and OS in patients with BM was *ST6GALNAC5*. ST6GALNAC5 is a sialyltransferase that promotes the addition of sialic acid to glycoproteins that facilitates cell-cell interaction. In breast cancer cells, expression of *ST6GALNAC5* was shown to be associated with penetration and overcoming of the BBB, whereas knockdown decreased this activity.[7] *ST6GALNAC5* rs1883778 G>A is an intronic SNP that is associated with transcriptional regulation suggesting functional relevance. To our knowledge, ST6GALNAC5 has not been studied for its therapeutic potential so far, but appears to be a promising drug target.

Other SNPs that were associated with OS were *CXCR4* rs2680880 A>T, *KLF4* rs2236599 G>A and *MMP9* rs17577 G>A. *CXCR4* rs2680880 A>T is an intronic SNP associated with transcriptional regulation. The chemokine receptor CXCR4 is expressed by various cancer types and binds to its ligand CXCL12.[26] Beside its physiological function in immune cell attraction (chemotaxis) to inflammatory sites, the CXCR4/CXCL12 axis has been shown to be involved in metastasis development.[27] Cells expressing CXCR4 follow a concentration gradient of CXCL12 to the metastatic site. This biological mechanism has also been demonstrated to be relevant in the formation of BM.[10] Although CXCR4 inhibition is currently being investigated in the treatment of hematological malignancies, CXCR4 is also a promising target to inhibit angiogenesis, tumor growth and metastases in solid tumors (NCT02179970, NCT01391130 and NCT01439568).[28]

KLF4 rs2236599 G>A was also found to be clinically relevant in this study. Although KLF4, which belongs to a family of regulatory transcription factors, has previously been regarded as a tumor suppressor, recent studies have shown that KLF4 exerts an oncogenic effect by maintaining cancer stem cells.[13,29] Cancer stem cells represent a small subpopulation of cancer cells that are the main drivers of tumor growth and cancer recurrence. In a study investigating breast cancer stem cells, high KLF4 expression was significantly associated with the development of BM *in vivo*.[13] In hematological malignancies, a pharmacological inducer of KLF4 expression (APTO-253 HCl) that is thought to intensify the tumor suppressive effect of KLF4 is currently being investigated (NCT02267863). However, in a context where KLF4 exerts an oncogenic effect, as suggested for BM, inhibition of KLF4 appears to be a treatment approach worth pursuing.

Finally, the non-synonymous SNP *MMP9* rs17577 G>A was associated with OS, however this SNP did not remain significant in multivariable analysis. Expression of MMP9 is upregulated in CRC and associated with poor prognosis.[30] MMP9 is involved in the degradation of the basal membrane, the BBB and the extracellular matrix in cerebral malignant processes, such as metastasis.[11] Early studies on MMP9 inhibitors (for example, GS-5745) in solid tumors are ongoing (NCT01803282), which may be also relevant for patients with BM.

Recursive partitioning analysis provided insights into the clinical relevance of the investigated SNPs in an unbiased hierarchical manner. These findings may help to develop genetic profiling panels for a clinical characterization of patients with colorectal BM. The observation that other SNPs were relevant in recursive partitioning analysis than in univariable and multivariable analyses for OS can be explained by the fact that recursive partitioning analysis showed hidden interactions between SNPs and OS. The effect of a SNP could be indirectly associated with OS through another SNP.

Limitations of this study are the patient number and the retrospective study design. However, considering the rare occurrence of BM in CRC, this cohort is, to the best of our knowledge, the largest described so far. Another limitation is the lack of a validation cohort, which can also be explained by the rarity of BM. The fact that 9 of 17 SNPs were not in Hardy–Weinberg equilibrium, verified by multiple sequencing runs, may be explained by this highly selected patient population. Another limitation is that patients were assigned to the control

group if they did not show any clinical symptoms of BM but did not undergo systematic brain imaging. Moreover, there were differences between the groups with respect to baseline characteristics.

In conclusion, this study demonstrates for the first time that variants in genes associated with adhesion, migration and breakdown of the BBB predict BM susceptibility and clinical outcome in patients who underwent resection for colorectal BM. The results of this study may contribute to the effort to identify predictive and/or prognostic biomarkers and potential drug targets in patients with BM and new brain-screening strategies in high-risk patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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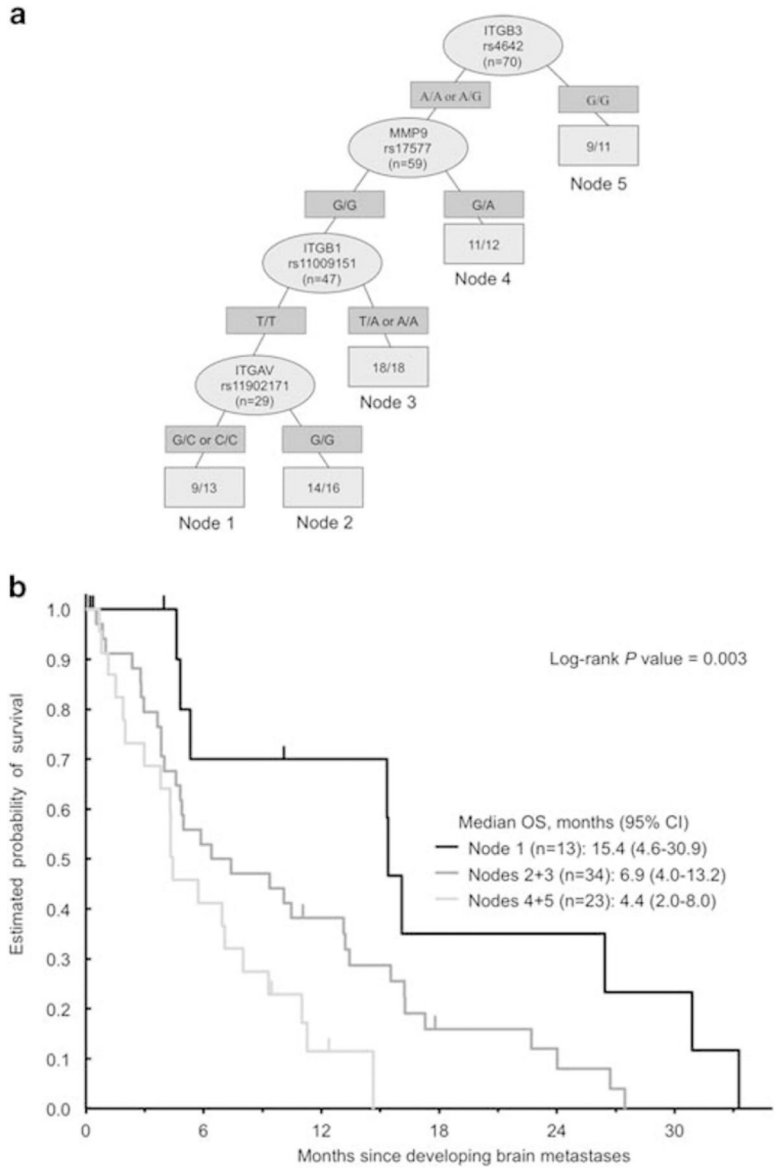

Figure 1.

(**a**) Recursive partitioning tree for OS after diagnosis of BM. (**b**) Kaplan–Meier curves for terminal nodes of recursive partitioning tree.

Table 1.

Baseline characteristics

	Patients with BM (n = 70)		Control group (n = 45)	
Age at diagnosis of primary tumor				
Median years (range)	61	(37–76)	54	(28–74)
Gender				
Male	38	54.3%	17	37.8%
Female	32	45.7%	28	62.2%
Primary tumor site				
Right colon	15	21.4%	9[a]	20.0%
Left colon	16	22.9%	21	46.7%
Rectal	33	47.1%	15	33.3%
Unknown	6	8.6%		
Positive family history for colorectal cancer (1st degree relative)				
No	58	82.9%	N/A	N/A
Yes	4	5.7%		
Unknown	8	11.4%		
Stage IV at first diagnosis of primary tumor				
No	50	71.4%	18	40.0%
Yes	19	27.1%	27	60.0%
Unknown	1	1.4%		
Number of BM				
1	51	72.9%	N/A	N/A
2–4	18	25.7%		
Unknown	1	1.4%		
GPA classification				
I	4	5.7%	N/A	N/A
II	5	7.1%		
III	52	74.3%		
IV	8	11.4%		
Unknown	1	1.4%		
Time from diagnosis of CRC to BM				
Median months (range)	25	(1–103)	N/A	N/A
Unknown	6			
Neurological symptoms				
No	6	8.6%	N/A	N/A
Yes	62	88.6%		
Unknown	2	2.9%		
Localization of BM				
Supratentorial	34	48.6%	N/A	N/A
Infratentorial	24	34.3%		
Both	12	17.1%		

	Patients with BM (n = 70)		Control group (n = 45)	
Karnofsky performance status at diagnosis of BM				
< 80	19	27.1%	N/A	N/A
80	11	15.7%		
90–100	40	57.1%		
Synchronous BM+extracranial new metastatic site				
No	55	78.6%	N/A	N/A
Yes	15	21.4%		
Chemotherapy before diagnosis of BM				
No	26	37.1%	N/A	N/A
Yes	40	57.1%		
Unknown	4	5.7%		
First line treatment of BM				
Surgery	66	94.3%	N/A	N/A
Gamma knife	2	2.9%		
WBRT	1	1.4%		
Unknown	1	1.4%		

Abbreviations: BM, brain metastases; CRC, colorectal cancer; GPA, graded prognostic assessment; N/A, not applicable; WBRT, whole-brain radiotherapy.

[a]One patient had both right and left CRC.

Table 2.

Genes and SNPs involved in the breakdown of the blood–brain barrier and the development of brain metastases

rs number	MAF[a]	Base change	Function	Primer sequence
MMP9				
rs17577	18%	G > A	NA	F: GTGAAGGCGCAGATGGTG R: GCTTTTTCTTCCTCGCTCAG
rs17576	39%	A > G	Protein coding, splicing regulation, post translation	F: ACACTTGGGGGTTATAATGTGC R: GTGCAGGCGGAGTAGGATT
ST6GALNAC5				
rs1146671	39%	G > A	Transcriptional regulation	F: ATGGTGTGGTGAAGGCAGA R: AGCAGCCAGTCCTCCTTTCT
rs1883778	48%	G > A	Transcriptional regulation	F: TTTTTCCTTTTCAACCAAAGTTTC R: ATGCCTCCTTTGTGCAGATT
rs17368584	47%	T > C	Transcriptional regulation	F: AGCAGTTTCGTGAATAACCTGT R: GTTTTGCTCCTTTCCTTCCA
ITGAV				
rs1839123	32%	G > A	Transcriptional regulation	F: CCCAGGCCTTGAGGAACT R: AACCTTTCTGAAGTTCGTTAGTATAAA
rs1448424	32%	A > G	Tag	F: TATTTTAGCCCTTTGTAAAATCATTG R: AAGAAAGTTTTCTCATTACTGTGATCT
rs10171481	27%	A > G	Transcriptional regulation	F: GGAATACAGCCACACCCATT R: ATTTAGATTAGGGTTCAGCAAACTT
rs11902171	23%	G > C	Transcriptional regulation	F: AGCAATAGCATGATGTTACAGGAA R: CTAACGAACTTCAGAAAGGTTTAAAAG
ITGB1				
rs2298141	16%	G > A	Protein coding, splicing regulation	F: AAAATCACACTTAAAATTCACACACA R: TTGTTGGAAAACAGCGCATA
rs1187071	13%	T > C	Tag	F: CATGGAGAAGAATAATCTATTGCTAA R: TTTCAGTGGAGTGATGAGGAAA
rs11009151	16%	T > A	Tag	F: AATTAAATTGCAAAACATCAAATCA R: ACTTGGGTCAGTTCTGGGAAA
ITGB3				
rs5918	13%	T > C	Protein coding, splicing regulation, post translation	F: GCCTGCAGGAGGTAGAGAGT R: ACTCACTGGGAACTCGATGG
rs4642	30%	A > G	Protein coding, splicing regulation, post translation	F: GGACCTTTGAGTGTGGGGTA R: GTGGCAGACACATTGACCAC
rs3809865	29%	A > T	Protein coding, transcriptional regulation	F: TCCTCAAAGGGAGAGAGTGC R: ACAAGGCAGCCAAGAGGTAG

rs number	MAF[a]	Base change	Function	Primer sequence
KLF4				
rs2236599	20%	G > A	Protein coding	F: CAGTCCCGGGGATTTGTAG R: GTCTTCCCCTCTTTGGCTTG
CXCR4				
rs2680880	42%	A > T	Transcriptional regulation	F: TGTATATCTGCAAAAGAGGCAAA R: TTGTGCCCTTAGCCCACTAC

Abbreviations: A, adenine; C, cytosine; F, forward; G, guanine; HR, hazard ratio; MAF, minor allele frequency; m, months; NA, not available; R, reverse; rs, reference SNP number; T, thymine.

[a]According to Ensembl database for Caucasians

Table 3.

SNPs and overall survival

	N	*Median*, m *(95% CI)*	*HR (95% CI)*[a]	P-*value*[a]	*HR (95% CI)*[b]	P-*value*[b]
MMP9 rs17577				0.044		0.20 (0.40)
G/G	54	7.4 (4.8–13.2)	1 (reference)		1 (reference)	
G/A	14	5.1 (1.9–11.0)	1.83 (0.95–3.53)		1.59 (0.79–3.22)	
MMP9 rs17576				0.32		0.21 (0.40)
A/A	32	7.4 (4.6–15.5)	1 (reference)		1 (reference)	
A/G	25	5.1 (4.3–10.1)	1.51 (0.85–2.68)		1.45 (0.81–2.62)	
G/G	12	7.0 (0.8–11.0)	1.25 (0.60–2.62)		2.06 (0.84–5.06)	
ST6GALNAC5 rs1146671				0.68		0.52 (0.80)
G/G	27	5.8 (3.0–13.2)	1 (reference)		1 (reference)	
G/A	24	7.1 (4.3–16.1)	0.90 (0.50–1.63)		0.91 (0.48–1.73)	
A/A	18	7.2 (2.8–14.7)	1.21 (0.62–2.37)		1.38 (0.68–2.78)	
ST6GALNAC5 rs1883778				0.14		0.028 (0.12)
G/G	27	4.6 (3.0–9.3)	1 (reference)		1 (reference)	
G/A	22					
A/A	21					
G/A or A/A	43	9.4 (5.0–13.4)	0.68 (0.40–1.16)		0.53 (0.30–0.93)	
ST6GALNAC5 rs17368584				0.97		0.28 (0.48)
T/T	26	7.4 (3.8–14.7)	1 (reference)		1 (reference)	
T/C	23	5.0 (4.3–13.1)	1.03 (0.56–1.92)		0.69 (0.36–1.33)	
C/C	16	6.1 (2.0–13.4)	0.96 (0.49–1.88)		0.56 (0.27–1.18)	
ITGAV rs1839123				0.90		0.73 (0.83)
G/G	38	7.1 (4.6–11.0)	1 (reference)		1 (reference)	
G/A	21	6.9 (3.0–16.2)	0.89 (0.50–1.60)		1.02 (0.54–1.92)	
A/A	9	14.7 (0.5–17.3)	0.89 (0.41–1.95)		0.73 (0.31–1.71)	
ITGAV rs1448424				0.98		0.95 (0.95)
A/A	39	7.1 (4.8–11.0)	1 (reference)		1 (reference)	
A/G	22	6.9 (3.0–15.3)	0.96 (0.54–1.71)		0.90 (0.47–1.71)	
G/G	8	9.8 (0.5–17.3)	1.04 (0.47–2.27)		0.97 (0.42–2.27)	

	N	*Median*, m (95% CI)	*HR (95% CI)*[a]	P-value[a]	*HR (95% CI)*[b]	P-value[b]
ITGAV rs10171481				0.10		0.018 (0.12)
A/A, A/G	58	5.3 (4.3–10.1)	1 (reference)		1 (reference)	
A/A	41					
A/G	17					
G/G	11	15.5 (2.8–22.7)	0.58 (0.29–1.15)		0.39 (0.18–0.85)	
ITGAV rs11902171				0.12		0.65 (0.83)
G/G	40	5.8 (3.8–9.3)	1 (reference)		1 (reference)	
G/C	20	15.3 (4.8–16.3)	0.60 (0.32–1.11)		0.73 (0.36–1.49)	
C/C	10	5.9 (1.1–13.1)	1.14 (0.53–2.44)		1.07 (0.49–2.33)	
ITGB1 rs2298141				0.49		0.62 (0.83)
G/G	44	7.1 (4.3–14.7)	1 (reference)		1 (reference)	
G/A	21					
A/A	4					
G/A or A/A	25	6.9 (4.8–11.3)	1.20 (0.71–2.02)		1.16 (0.65–2.06)	
ITGB1 rs1187071				0.57		0.21 (0.40)
T/T	55	7.4 (4.6–11.3)	1 (reference)		1 (reference)	
T/C	15	5.3 (3.8–14.7)	1.20 (0.63–2.28)		1.56 (0.78–3.15)	
ITGB1 rs11009151				0.65		0.88 (0.93)
T/T	45	5.8 (4.3–14.7)	1 (reference)		1 (reference)	
T/A	22					
A/A	2					
T/A or A/A	24	8.1 (4.8–13.1)	1.12 (0.66–1.91)		1.05 (0.59–1.86)	
ITGB3 rs5918				0.010		0.024 (0.12)
T/T or T/C	61	8.0 (4.9–13.1)	1 (reference)		1 (reference)	
T/T	47					
T/C	14					
C/C	8	4.0 (1.1–6.9)	2.66 (1.13–6.25)		2.72 (1.14–6.48)	
ITGB3 rs4642				0.014		0.16 (0.40)
A/A or A/G	59	8.0 (4.9–13.1)	1 (reference)		1 (reference)	
A/A	40					
A/G	19					

	N	*Median*, m *(95% CI)*	*HR (95% CI)*[a]	P-*value*[a]	*HR (95% CI)*[b]	P-*value*[b]
G/G	11	4.3 (1.1–6.9)	2.31 (1.08–4.93)		1.80 (0.79–4.08)	
ITGB3 rs3809865				0.82		0.72 (0.83)
A/A	40	7.4 (4.3–11.3)	1 (reference)		1 (reference)	
A/T	16	6.4 (2.0–16.1)	1.16 (0.61–2.23)		1.16 (0.58–2.32)	
T/T	13	6.9 (3.8–15.5)	0.92 (0.48–1.76)		1.34 (0.64–2.82)	
KLF4 rs2236599				0.16		0.048 (0.16)
G/G	45	7.4 (4.8–13.2)	1 (reference)		1 (reference)	
G/A	16					
A/A	9					
G/A or A/A	25	4.8 (3.8–10.1)	1.44 (0.83–2.49)		2.12 (1.01–4.45)	
CXCR4 rs2680880				0.76		0.013 (0.12)
A/A or A/T	55	7.1 (4.8–13.1)	1 (reference)		1 (reference)	
A/A	25					
A/T	30					
T/T	15	4.6 (2.8–11.0)	1.09 (0.60–2.00)		2.30 (1.19–4.44)	

Abbreviations: A, adenine; C, cytosine; CI, confidence interval; G, guanine; HR, hazard ratio; m, months; rs, reference SNP number; T, thymine.

[a]Based on the log-rank test in the univariable analysis.

[b]Based on Wald test in the multivariable analysis within Cox regression model adjusted for age at diagnosis of CRC (< 57 vs 57+ years), primary tumor site (colon vs rectal), BM location (supratentoriell vs infratentoriell or both) and Karnosky performance status (< 90, 90–100). FDR-adjusted *P*-values are shown in parentheses.