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TLR-3 polymorphism is an independent prognostic marker for stage II colorectal cancer

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ABSTRACT

Background: Clinicopathologic stage is still the main parameter to evaluate the prognosis of newly diagnosed colorectal cancer (CRC) patients. Although molecular markers have been suggested for follow up of treated CRC patients, their complete clinical application is still under evaluation.

Materials and methods: To evaluate the association of immune-related genes with CRC prognosis and survival, a total of 19 single nucleotide polymorphisms (SNPs) were genotyped in 614 German patients within the Kiel cohort (POPGEN).

Results: A promoter variant (rs1800872) in the Interleukin-10 (*IL-10*) gene was associated with an increased lymph node metastasis involvement [odds ratio (OR) = 2.1, 95% confidence interval (CI) = 1.03–4.2, for carriers of the TT genotype]. More importantly, among 582 followed up patients the SNP rs3775291 in the toll-like receptor 3 (*TLR-3*) gene was associated with CRC specific survival (150 events). Patients carrying the TT genotype had a 93% increased risk of death compared with the CC carriers [hazard ratio (HR) = 1.93, 95% CI 1.14–3.28]. The observed effect of the *TLR-3* variant was restricted to stage II patients (HR = 4.14, 95% CI 1.24–13.84) and to patients who did not receive adjuvant therapy (HR = 3.2, 95% CI 1.4–7.7).

Conclusions: Our results may provide additional candidates for risk assessment in stage II CRC patients for treatment decision. Further validation of the presented findings is warranted.

1. Introduction

Colorectal cancer (CRC) is the third most common malignant tumour worldwide.[1] The main parameter to evaluate the prognosis of newly diagnosed patients is still clinicopathologic stage[2] and risk assessment for stage II CRC patients is a major challenge. Although patients diagnosed with stages II or III tumours have an approximately 75% overall 5-year

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survival after the surgery[3], 25% of the patients will have disease recurrence and die of their disease.[4,5] Contrary to stage III patients, it is still a dilemma whether to give postoperative adjuvant chemotherapy in stage II disease or not. Therefore, there is a need of new tools for risk assessment in stage II CRC.

Recently, the development of molecular markers has started to show promising results regarding CRC therapy (see review[2]). KRAS mutation status has been validated as a predictive marker of non-response to epidermal-growth-factor-receptor (EGFR)-target drugs in CRC patients and it is the only biological marker accepted for metastatic CRC and response to drug treatment. Microsatellite instability, p53 expression, loss of heterozygocity at chromosome 18q, thymidylate synthase, VEGF, IL-8 and MUC12 mRNA expression are other genetic markers that have been suggested. However, there is no final conclusion about the impact of those markers in the management and surveillance of CRC.

It is widely acknowledged that chronic inflammation plays an important role as a risk factor in CRC.[6,7] Furthermore, inherited genetic variation in immune-related genes, such as *UBD*[8], Interleukin-10 (*IL-10*)[9], *CARD15*[10] has been suggested to associate with susceptibility or prognosis to CRC. Whether similar inflammatory mechanisms can be useful markers for prognosis of CRC patients, remains open.

The aim of the present study was to improve our knowledge about the effect of genetic variation in immune/inflammation-related genes on different phenotypic features of CRC and survival. Understanding the mechanistic process of the observed variants may have important implications, especially in stage II CRC management, where there clearly is a need for new prognostic markers.

2. Patients and methods

2.1. Study population

A case study was conducted within the Kiel cohort (POPGEN), which has been previously described.[11] Briefly, patients with histologically proven CRC were identified via the regional cancer registry of Schleswig-Holstein, and from surgical departments in Northern Germany, namely at Kiel, Eckernförde, Rendsburg, Schleswig, Flensburg, Husum, Heide, Niebüll, Neumünster, Itzehoe, Rotenburg, Stade, Reinbek, Bad Oldesloh, Detmold, Neustadt, Hamburg-Harburg, Hamburg-Altona, Hamburg-Eilbek, Hamburg-Bergedorf and Lüneburg. The period for diagnosis from the records of the surgical departments and the regional cancer registry of Schleswig-Holstein was from 2002 to 2005, except for University Hospital Kiel, where it was from 1993 to 2005. The study was restricted to individuals of German ethnicity, i.e. only cases and controls with both parents born in Germany were included. Patients fulfilling either the clinical Amsterdam or Bethesda criteria for hereditary non-polyposis colorectal cancer (HNPCC) were excluded from the study, as were patients with a history of malignant disease or inflammatory bowel disease. All patients were contacted by mail and invited to participate in the study. Patients who did not respond were sent one written reminder. Individuals who agreed to participate were contacted by the POPGEN project team (http://www.popgen.de).

Table 1 – Characteristics of the colorectal cancer (CRC) cases at the time of diagnosis.

Parameter	No. of patients[a]	(%)
Total patients	614	(100.0)
Gender[a]		
Male	325	(53.3)
Female	285	(46.7)
Age at diagnosis (year)		
<65	341	(55.5)
⩾65	273	(44.5)
Histopathologic grade (G)		
G1	20	(3.3)
G2	479	(78.0)
G3	98	(16.0)
G4	1	(0.2)
Gx	16	(2.6)
Pretherapeutic UICC TNM stage[b]		
Stage I	161	(26.2)
Stage II	157	(25.6)
Stage III	149	(24.3)
Stage IV	133	(21.7)
Other[c]	14	(2.3)
Primary tumour site (T)		
T0	6	(1.0)
T1	76	(12.3)
T2	138	(22.5)
T3	327	(53.3)
T4	58	(9.4)
Tis or Tx	9	(1.5)
Regional lymph node involvement (N)		
N–	355	(57.8)
N+	255	(41.5)
Nx	4	(0.6)
Distant metastatic spread (M)		
M–	479	(78.0)
M+	135	(21.9)
Localisation		
Rectum	328	(53.4)
Colon	286	(46.6)
Adjuvant therapy		
Any[d]	250	(40.4)
None	283	(45.8)
Unknown	81	(13.2)
Pretreatment		
Any[e]	68	(11.0)
None	517	(83.7)
Unknown	29	(4.7)

[a] Missing values for number of cases were only among the gender category ($n = 4$).

[b] – Stage I indicates T1–T2, no regional lymph node metastasis and no metastasis; stage II indicates T3–T4, no regional lymph node metastasis and no metastasis; stage III indicates any tumour, node 1–3, and no metastasis; and stage IV indicates any tumour, any node and distant metastasis.

[c] Not available.

[d] Comprises radiotherapy ($n = 13$), chemotherapy ($n = 86$), radio-chemotherapy ($n = 59$), immunotherapy ($n = 3$), chemotherapy plus 5FU/FA ($n = 68$), radio-chemotherapy plus 5FU/FA ($n = 18$) and other ($n = 3$).

[e] Comprises radiotherapy ($n = 5$) and radio-chemotherapy ($n = 63$).

They were interviewed by mail questionnaire and a venous EDTA blood sample was obtained either at the POPGEN office or by the patient's general practitioner. A total of 10,152 patients received an initial invitation letter, 2928 (29%) agreed to participate in the study, provided a blood sample and completed the questionnaire, 620 (6%) refused to participate, 1189 (12%) deceased before inclusion to the study, 2158 (21%) moved out and 3257 (32%) patients did not respond. From the total CRC cohort of 2928 individuals, the following clinical information for 614 patients were obtained by an on-site chart review and a contact to the patient and/or to the treating physician: (1) full clinical stage information as confirmed by intra-operative documentation and pathology scoring, (2) last contact to the patient no less than 1 year before November 2009, if still alive, (3) confirmed death by family doctor, (4) German ethnicity as defined by a consistent birthplace of both parents and self-reported ethnicity of the patient, (5) DNA availability at least 5 μg, (6) information about the type of surgery: only patients with elective surgery were included. In the present project, the 614 patients fulfilling these criteria as of November 2009 were included. The study protocols were approved by the institutional ethics committee and the local data protection officers. Written informed consent was obtained from all study participants. DNA was prepared from all samples using the FlexiGene chemistry (Qiagen, Hilden, Germany) according to the manufacturer's protocols. Characteristics of the patients at the time of diagnosis are shown in Table 1.

2.2. *Genotyping of markers*

Single nucleotide polymorphisms (SNPs) within a set of candidate genes (Table 2) were selected from The National Center for Biotechnology Information dbSNP database (http://www.ncbi.nlm.nih.gov/snp). Selection criteria for the included markers were: an established intragenic location, minor allele frequency (MAF) of >10% in the Caucasian population and a previously reported association with cancer susceptibility through a possible inflammatory mechanism.[9,12–14] Genotyping was performed by the previously developed SNP genotyping assay based on Luminex technology, as described.[15,16] A quality control process was undertaken to assess potential misclassification of genotyping results where 90 DNA samples from the Caucasian population cohort (CEPH, Utah residents with ancestry from northern and western Europe) of the International HapMap Project[17] were genotyped. No discrepancies were observed among the HAPMAP genotypes.

2.3. *Statistical analysis*

SAS software version 9.1 (SAS Institute, Inc., Cary, NC) was used in all analyses. The observed genotype frequencies were tested for Hardy–Weinberg equilibrium. Unadjusted associations between the genetic markers and patient characteristics were evaluated by chi-square test and odds ratios (ORs) with the 95% confidence intervals (CIs) were estimated using unadjusted polytomous generalised logit regression models. Effects of the different genotypes on CRC survival were evaluated using Kaplan-Meier method and were compared using log-rank testing. Analysis of different parameters for prognostic significance was done by univariate and multivariate Cox proportional hazard models. *P*-values <0.05 were considered significant. Follow-up time was calculated from the date of CRC diagnosis to the CRC specific death or censored at the time of death by other causes or end of follow-up (date of last contact with the treating physician). The proportional hazard assumption of the Cox model was

Table 2 – Polymorphisms evaluated in this study.

Gene	rs number	Chr.	Nucleotide allele Major/minor	Variation	Minor allele frequency (MAF)
CCR-2	rs1799864	3	G/A	V64I	0.11
CTLA-4	rs231775	2	A/G	T17A	0.40
CTLA-4	rs3087243	2	G/A	nearGene-3	0.40
EVER2	rs7208422	17	T/A	N306I	0.50
IL-10	rs1800896	1	A/G	Promoter	0.48
IL-10	rs1800872	1	G/T	Promoter	0.24
IL-1B	rs1143627	2	T/C	nearGene-5	0.32
IL4-R	rs1805010	16	A/G	I75V	0.47
IL4-R	rs1805015	16	A/G	S503P	0.16
IL4-R	rs1801275	16	A/G	Q576R	0.20
IL-6	rs1554606	7	G/T	Intron 3	0.45
IL-6	rs1800797	7	G/A	Promoter	0.48
LTA	rs1041981	6	C/A	T60N	0.36
TIRAP	rs8177374	11	C/T	S180L	0.17
Toll-like receptor 3 (TLR-3)	rs5743305	4	A/T	Promoter	0.38
TLR-3	rs3775291	4	C/T	L412F	0.29
TLR-8	rs3761623	X	G/A	Promoter	0.49[a]
TLR-9	rs5743836	3	T/C	Promoter	0.14
TLR-9	rs352140	3	A/G	P545P	0.42

Chr, chromosome position.

[a] Only genotyping data for females were included.

examined by including time-dependent covariates in the model as interactions of the predictors with time. None of the interactions were statistically significant, suggesting that the models fulfilled the Cox regression model assumptions. Taking into account the possible biological effects of the selected variants in relation with inflammatory processes in cancer development, and a possible correlation between the studied variables, correction for multiple comparisons was excluded.

3. Results

3.1. Genetic markers and CRC progression

The SNP description and MAFs for each marker are shown in Table 2. All SNPs were successfully genotyped in 600 patients (97.7% of the samples) with a mean call rate of 99.4% and none of them exhibited a departure from HWE. Five of the 19 SNPs investigated were found to be statistically signifi-

Table 3 – Association between patient genotypes and CRC pretherapeutic UICC TNM stage, tumour stage (T), lymph nodes (N) and metastases (M).

Genetic	Genotype	Cases[a]	Model	Stage (TMM)[b]				Model	Tumour stage (T)[c]			Lymph nodes (M)[d]			Metastases (M)[e]		
risk factor				TNM	OR	95% CI*			OR	95% CI*		OR	95% CI*		OR	95% CI*	
IL-10																	
RS1800872	TT	36	TT/GG	II	1.1	0.4	3.2	TT/GG	1.1	0.5	2.3	2.1	1.03	4.2	0.6	0.3	1.6
	GT	206	TT/GG	III	2.6	1.0	6.6	GT/GG	1.0	0.7	1.4	0.7	0.5	1.0	0.7	0.5	1.1
	GG	336	TT/GG	V	1.0	0.3	3.0										
			GT/GG	II	1.1	0.2	1.8										
			GT/GG	III	0.9	0.6	1.5										
			GT/GG	IV	0.5	0.5	1.3										
IL4-R																	
RS1805010	GG	135	GG/AA	II	1.0	0.5	1.8	GG/AA	1.0	0.6	1.5	1.2	0.7	1.9	0.5	0.3	1.0
	GA	271	GG/AA	III	1.4	0.8	2.6	GA/AA	1.6	1.1	2.4	1.1	0.8	1.7	1.0	0.6	1.5
	AA	164	GG/AA	IV	0.6	0.3	1.2										
			GA/AA	II	1.5	0.9	2.5										
			GA/AA	III	1.4	0.8	2.4										
			GA/AA	IV	1.2	0.7	2.1										
IL4-R																	
RS1805015	GG	21	GG/AA	II	0.4	0.1	1.3	GG/AA	0.6	0.2	1.4	0.5	0.2	1.3	0.1	0.0	1.1
	AG	147	GG/AA	III	0.5	0.2	1.6	AG/AA	0.9	0.6	1.4	0.9	0.6	1.3	0.8	0.5	1.3
	AA	414	GG/AA	IV	0.1	0.01	0.8										
			AG/AA	II	0.9	0.5	1.5										
			AG/AA	III	0.4	0.4	1.3										
			AG/AA	IV	0.2	0.4	1.2										
IL-6																	
RS1800797	AA	139	AA/GG	II	2.0	1.0	3.2	AA/GG	1.5	0.9	2.4	0.9	0.6	1.5	1.2	0.7	2.0
	GA	279	AA/GG	III	1.1	0.5	2.1	GA/GG	1.2	0.8	1.7	0.9	0.6	1.3	0.8	0.5	1.3
	GG	165	AA/GG	IV	1.3	0.0	2.5										
			GA/GG	II	1.6	0.9	2.8										
			GA/GG	III	1.0	0.6	1.7										
			GA/GG	IV	1.0	0.6	1.6										
TLR-9																	
RS5743836	CC	12	CC/TT	II	0.6	0.1	2.4	CC/TT	0.7	0.2	2.3	0.3	0.1	1.2	1.9	0.6	6.3
	TC	144	CC/TT	III	na	na	na	TC/TT	0.8	0.5	1.2	0.8	0.5	1.1	1.2	0.8	1.9
	TT	427	CC/TT	IV	0.9	0.2	3.5										
			TC/TT	II	0.0	0.4	1.1										
			TC/TT	III	0.5	0.3	0.9										
			TC/TT	IV	0.9	0.5	1.5										
TLR-9																	
RS5743836	CC + TC	156	CC + TC/TT	II	0.7	0.4	1.1	CC + TC/TT	0.8	0.5	1.1	0.7	0.5	1.0	1.2	0.8	1.9
	TT	427	CC + TC/TT	III	0.5	0.3	0.8										
			CC + TC/TT	IV	0.9	0.5	1.5										

OR, odds ratio and CI, confidence interval.

[a] The number of genotypes may differ because of missing data.

[b] Stage 1 as a reference category.

[c] Tumour stage T1 + T2 as a reference category; T0, Tx and Tis cases were excluded from the analysis.

[d] Absence of lymph nodes affected as a reference category, Nx cases were excluded from the analysis.

[e] Absence of metastasis as a reference category.

cantly associated with at least one of the pre-therapeutic UICC TNM stage (International Union against Cancer stage classification) when compared with the reference group of stage I patients (Table 3). However, after sub-analyses for each of the different components that form the UICC stage (T, N and M), only the association of the SNP rs1800872 (*IL-10* gene) with stage III (any T with lymph node metastasis) was confirmed (OR = 2.1, 95% CI = 1.03–4.2) for carriers of the TT genotype. There were no significant differences in the genotype distributions with the localisation of the tumour (data not shown). Since age of diagnosis and gender did not differ between the patients with tumours of different TNM, all analyses were unadjusted. Risk estimates for markers not associated with different progression stages of the disease are presented in the Supplementary Table 1.

3.2. *Immune-related SNPs and CRC specific survival*

Based on the data from our cohort with up to 13 years of follow-up, we examined the impact of the analysed SNPs on the specific long term survival. The median patient follow-up time was 46.9 months among 582 patients who had complete genotype and follow-up information. The 5-year survival rate was 73% and the 10-year survival rate was 65%. In the univariate analysis, the following parameters were found to be associated with patient survival rate: age at diagnosis, UICC TMN stage, tumour differentiation, lymph node metastasis and distant metastasis (Table 4). Interestingly, one of the analysed genetic markers, the SNP rs3775291 in the toll-like receptor 3 (TLR-3) gene, was statistically significantly associated with CRC survival. Patients carrying the TT genotype had a 93% increased risk of death compared with the CC carriers (HR = 1.93, 95% CI 1.14–3.28) (Table 4). The Kaplan-Meier survival curves representing the CRC-specific survival rates of the patients and TLR-3 genotypes are presented in Fig. 1a. Among all patients, those who had the TT genotype, had a significantly shorter survival compared with patients with the CC or the CT genotype (log-rank test, $P = 0.03$). Kaplan-Meier survival estimates by the TLR-3 genotypes were calculated for all different TNM stages; the effect of the TLR-3 variant was restricted to those patients with diagnosis of stage II CRC (Fig. 1b, log-rank test, $P = 0.03$, HR = 4.14, 95% CI 1.24–13.84). There was no significant association between the TLR-3 variant and the CRC-survival in patients who were diagnosed for stages I, III and IV ($P = 0.73$, $P = 0.61$, and $P = 0.87$, for log-rank test, respectively). Restricted analyses of CRC survival by TLR-3 genotypes in those patients who did not have any adjuvant therapy, showed a stronger effect on CRC survival (HR = 3.2, 95% CI 1.4–7.7, for the homozygote TT genotype) by the TLR-3 variant than observed in the whole group of patients (HR = 1.93, 95% CI 1.14–3.28) as shown in Fig. 1c (log-rank test, $P = 0.03$).

Subsequently, we examined whether inclusion of other associated variables with CRC survival affected the parameter estimate for TLR-3 polymorphism (Table 5). The risk of death for carriers of the TT genotype remained significantly increased after adjustment for age at diagnosis, pathological tumour stage and lymph node metastasis. No other SNPs were associated with survival (Supplementary Table 2).

Table 4 – Univariate analysis of CRC survival and known prognostic factors.

Parameter	No. at risk[a]	Deaths	HR	95% CI	
Age at diagnosis					
⩾65	256	77	1.53	1.11	2.11
<65	327	73	1.00	–	–
Gender					
Male	308	79	1.02	0.74	1.42
Female	271	67	1.00	–	–
TNM stage[b]					
Stage IV	125	92	27.98	14.40	54.37
Stage III	140	27	3.39	1.64	7.00
Stage II	149	19	1.87	0.87	4.03
Stage I	154	10	1.00	–	–
Pathological tumour stage (T)					
T4 + T3	362	125	3.60	2.31	5.62
T1 + T2	205	23	1.00	–	–
Pathological lymph nodes (N)					
N+	239	103	4.37	3.07	6.21
N–	339	45	1.00	–	–
Pathological metastases (M)					
M+	126	92	13.73	9.66	19.51
M–	452	56	1.00	–	–
Localisation					
Colon	275	72	0.98	0.73	1.38
Rectum	307	78	1.00	–	–
TLR-3 rs3775291					
TT	39	17	1.93	1.14	3.28
CT	246	62	1.01	0.72	1.43
CC	280	69	1.00	–	–

[a] Number of cases may differ due to missing data.
[b] Pretherapeutic UICC TNM stage (TNM); HR, hazard ratio and CI, confidence interval.

4. Discussion

There is a clear need to discover new prognostic markers in CRC, especially to improve the situation of newly diagnosed stage II CRC patients, since a considerable proportion of those patients will have a recurrence (approximately 25%).[18] This study sought to understand the possible role of 19 selected variants in immune/inflammation-related genes in the progression of the disease and the prognosis of CRC patients. Based on a relatively large and homogeneous German population we demonstrated that a missense polymorphism (Leu412Phe, rs3775291) in the TLR-3 gene is an independent prognostic marker for disease specific survival in patients with stage II CRC. The prominent effect of this SNP in CRC survival was confirmed among patients who did not get any adjuvant therapy. In these two groups, patients carrying the TT genotype of the SNP rs3775291 in the TLR-3 gene had a decreased CRC survival rate compared to patients carrying the common genotypes.

TLR-3 is a member of the toll-like receptor molecules, which are involved in the recognition of different pathogen-associated molecular patterns, leading to the activation of the innate immune response and determining the

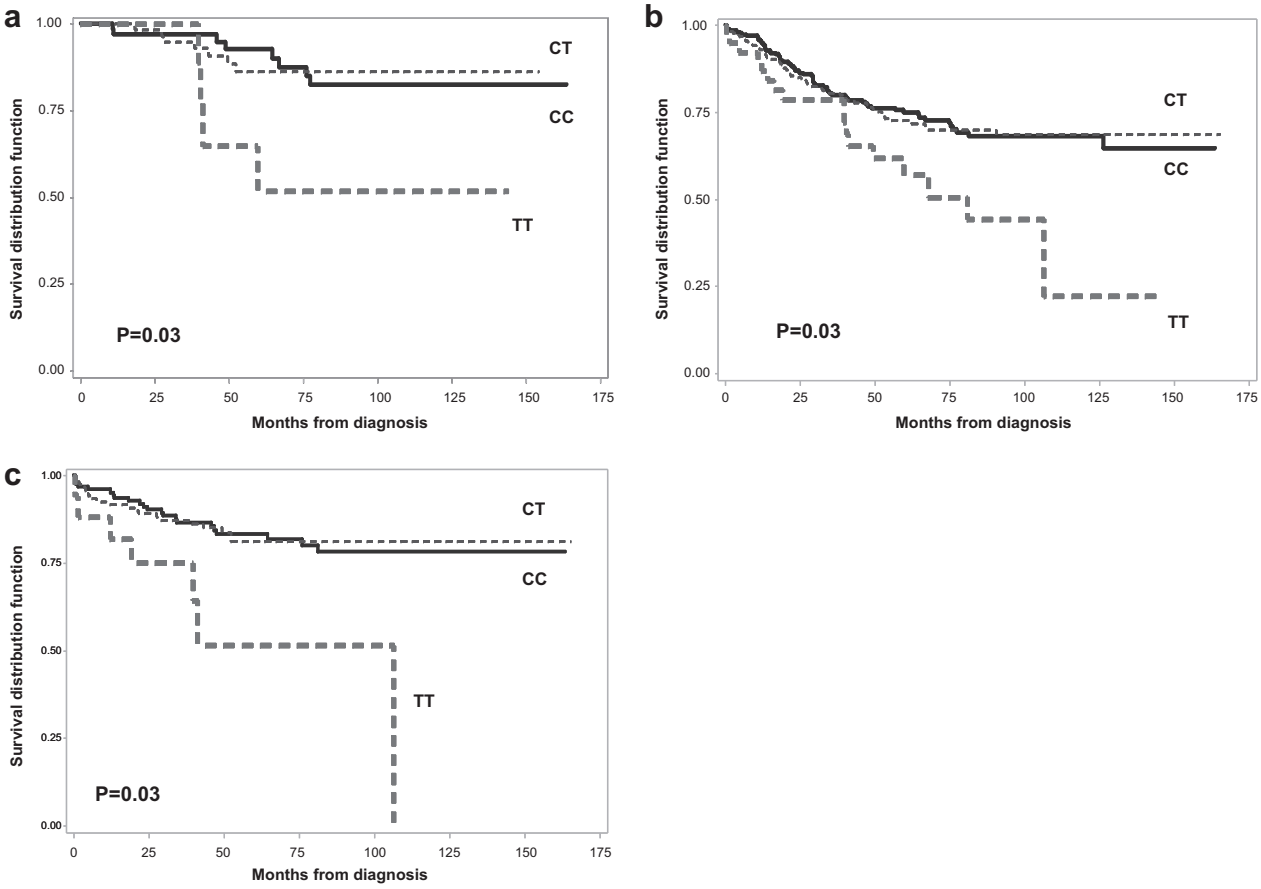

Fig. 1 – Kaplan-Meier estimates of colorectal cancer (CRC)-specific survival according to toll-like receptor 3 (TLR-3) genotypes. (a) Among all colorectal cancer patients (*n* = 582), (b) among only those patients that were diagnosed for stage II (*n* = 157). (c) Among patients without adjuvant therapy (*n* = 283). *P*-values are for the log-rank test.

subsequent adaptive immune response. It has been suggested that it promotes not only an antiviral IFN response[19,20], but it may also trigger the pro-inflammatory pathway by activating NF-κβ and MAPK and influence apoptosis through caspase 8.[12,21] Cumulative evidence has been published about a possible role of intestinal TLRs in the development of sporadic colon cancer.[22,23] However, only one study has shown that polymorphisms of TLR2 and TLR4 genes may be associated with CRC.[24] Interestingly, evidence of an important role of TLR-3 in other cancers has been suggested. In breast cancer, women with TLR-3 positive breast cancer had a prolonged survival after receiving a TLR3 agonist, polyadenylic–polyuridylic acid (poly(A:U)), as adjuvant treatment in clinical trials.[25] TLR-3 may also have an important role in oesophageous cancer.[26] In CRC, one might speculate that the poor survival observed in patients carrying the TT genotype of *TLR-3* may be explained by changes in the properties of the TLR-3 protein or on its expression pattern. Such a variation could influence its role as anticancer immune stimulator but also alter the apoptotic process, thus resulting in a better tumour cell survival. A structural analysis of the TLR-3 molecule has shown that a glycosylation site (Asn^{413}) within the ligand-binding surface for dsRNA is required for receptor activation.[27,28] Asn^{413} is located adjacent to the Leu412Phe polymorphism, which in our study was associated with survival. Thus, alteration in the ligand binding or in the dimerisation of TLR due to the variant may be possible. The observed effect in the prognosis of CRC by TLR-3 may have important clinical relevance in CRC, since TLR3 agonists have been implemented as adjuvant therapy in clinical trials for different types of cancer.[29,30]

Association of the studied variants with different CRC stages of progression was also tested. The rs1800872 variant, located in the promoter of the *IL-10* gene (also described as *IL10* –592) was significantly associated with lymph node involvement in the CRC patients. The IL-10 promoter haplotype, CGG (from the variants at positions –1082, –819, –592), has been associated with high IL-10 production, the TAT haplotype with low IL-10.[31,32] We speculate that the observed association with stage III may be due to a decreased production of anti-inflammatory IL-10 in those individuals carrying the TT genotype of the SNP rs1800872. This finding is in the line with our previous observation in a Swedish population, showing that patients carrying the C allele of another promoter SNP (*IL-10* –1082, rs1800896) had significantly more tumours with Dukes stage A + B than with stages C + D.[9] Since nodal stage is the most important indicator of the need for adjuvant therapy, the presence of *IL-10* polymorphisms may

Table 5 – Multivariate analysis of CRC survival and TLR-3.

Parameter	No. at risk[a]	HR	95% CI	
Unadjusted model				
TLR-3 rs3775291				
TT	39	1.93	1.14	3.28
CT	246	1.01	0.72	1.43
CC	280	1.00	–	–
Adjusted model				
Age at diagnosis (>65)				
TT	39	1.91	1.12	3.25
CT	246	1.05	0.74	1.48
CC	281	1.00	–	–
TNM stage[b]				
TT	39	1.48	0.87	2.53
CT	241	1.11	0.79	1.56
CC	271	1.00	–	–
Pathological tumour stage (T)				
TT	39	1.83	1.08	3.12
CT	242	1.02	0.72	1.43
CC	270	1.00	–	–
Pathological lymph nodes (N)				
TT	39	1.99	1.17	3.39
CT	246	1.12	0.79	1.58
CC	277	1.00	–	–
Pathological metastases (M)				
TT	39	1.34	0.78	2.28
CT	246	0.77	1.52	0.66
CC	280	1.00	–	–
Final model[c]				
TT	39	1.94	1.13	3.30
CT	239	1.15	0.81	1.63
CC	270	1.00	–	–

HR, hazard ratio and CI, confidence interval.
[a] Number of cases at risk for each of the models.
[b] Pretherapeutic UICC TNM stage (TNM).
[c] Include age at diagnosis, T and N.

be a parameter to take into account for risk assessment related to nodal staging.

The strengths of our study include patients who were recruited from a rather homogeneous Caucasian population of German ancestry as determined by the birth place of both parents. Although the participation rate was only 29% of the initially identified patients and only patients with elective surgery were included in the study, we do not think that our study was biased due to preferential survival[33,34], because the UICC stage distribution was similar to the ones in other Western countries.[35,36] The total number of patients ($n = 582$) included into our study of CRC survival was relatively large, and detailed clinical data were available of the study participants, however, in the subgroup analyses the power of our study was limited.

Our findings suggest an important role of the immune/inflammatory mechanisms in the progression and survival of CRC. Since this is the first time that TLR-3 is found to play a role in the prognosis of CRC patients and to be useful for risk assessment of stage II patients, further independent studies are needed to evaluate the significance of our finding in the clinic for a correct risk assessment and treatment decision for patients with stage II of CRC.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2010.12.011.

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