The role of Vitamin D Receptor gene polymorphisms in colorectal cancer risk

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Abstract

Background: Vitamin D deficiency has been associated with increased colorectal cancer (CRC) incidence risk and mortality. Vitamin D mediates its action through binding of the vitamin D receptor (VDR) and polymorphisms of the VDR might explain these inverse associations. Methods: The aim of the study was the investigation of TaqI, ApaI, FokI and BsmI polymorphisms of the VDR gene for their relevance to colorectal carcinogenesis and progression. Peripheral blood was obtained from 397 patients with early operable stage II/III (n=202) and stage IV (n=195) CRC. Moreover, samples from 100 healthy donors and 40 patients with adenomatous polyps were also included as control groups. Genotyping in samples from patients and controls was performed using PCR-RFLP. Results: A significant association revealed between all four polymorphisms and cancer. Individuals with homozygous mutant (tt, aa, ff or bb) genotype are more susceptible to the disease ($p<0.001$). All mutant genotypes detected were also significantly associated with the stage IV disease ($p<0.001$) leading to significantly decreased survival ($p<0.001$). Moreover, all four polymorphisms were significantly associated with KRAS mutations and with TLR2, TLR4 and TLR9 genetic variants. In multivariate analysis, tt, aa and ff genotypes emerged as independent factors associated with decreased OS ($p=0.001$, $p<0.001$ and $p=0.001$, respectively). Conclusions: The detection of higher frequencies of the VDR polymorphisms in CRC patients highlights the role of these polymorphisms in cancer development and progression.

Background

Colorectal cancer (CRC) is the third leading cause of cancer both in men and women (1). CRC is of a major public health importance with increased mortality rates worldwide and accounts of 9% of all cancers (1). CRC is a multifactorial disease and involves the complex interactions between environmental and genetic factors (2, 3). However, understanding all the mechanisms and the associations between these factors is not an easy task. Earlier, it has been hypothesized that higher incidence rates of CRC in areas with low sunlight exposure might be attributable to lower levels of vitamin D (4). Indeed, it has been reported that vitamin D deficiency is a common phenomenon in Saudi Arabia, especially among women (5). Nowadays, a large number of candidate genes have been identified responsible for their potential role in tumorigenesis.

The importance of vitamin D for bone health is well established, but lately its role beyond skeletal system has gained scientific attention (6). Vitamin D regulates cellular differentiation and proliferation in normal and malignant tissues; regulates proliferation, apoptosis and cell adhesion in tumor cells; and modifies tumor angiogenesis, invasion and metastasis along with decreasing oxidative DNA damage (7). Vitamin D deficiency has been associated with various cancer types (8, 9); whereas, increased vitamin D serum levels play a role in decreased colorectal adenoma risk (9). Vitamin D mediates its action through binding to the vitamin D receptor (VDR), a member of the nuclear receptor superfamily and is expressed on various cell types, including colorectal epithelial cells, enabling transactivation of target genes (10, 11). Thus *VDR* gene has been implicated in CRC. Over 60 single nucleotide polymorphisms (SNPs) of the *VDR* gene located in the promoter region, in exons 2-9, in their proximity and in the 3'-UTR region have been

studied in relation to cancer occurrence and prognosis (12, 13). However, only few of them are potentially functional and affect expression of the *VDR* gene in relation to CRC risk. These include *Taq*I (rs731236) located in exon 9 (9, 14), *Apa*I (rs7975232) and *Bsm*I (rs1544410) located in the intron between exons 8 and 9 (14-16) and *Fok*I (rs10735810) located in the exon 2 (15, 17).

To this end, we aimed to investigate four single nucleotide polymorphisms (SNPs) (*Taq*I, *Apa*I, *Fok*I and *Bsm*I) of the *VDR* gene within patients with sporadic CRC, for the first time in the Greek population, and to evaluate this association with the risk of cancer development and progression. The selection of these SNPs was based on the common VDR SNPs sites examined in other populations in previous genetic epidemiological studies. In addition, the correlation of the expression of these molecules and previously genotyped Toll-like receptor (TLR) variants (TLR2: *196-to-174 del*; TLR4: *Asp299Gly* and *Thr399Ile*; TLR9: *T1237C* and *T1486C*) in the same patients' and controls' samples (18) was also investigated.

Methods

Patients' population

Three hundred and ninety seven patients with colon adenocarcinoma were enrolled in the study, between 2003 and 2013, from the Department of Medical Oncology, University Hospital of Heraklion.

Blood and tissue samples from control groups

In parallel to patients' samples, blood samples and formalin-fixed paraffin embedded (FFPE) tissues were also obtained from 100 healthy blood donors and from 40 patients with colon adenomas, in the absence of CRC disease, respectively, and were used as controls in the study.

Genomic DNA extraction

Peripheral blood mononuclear cells (PBMC) from all individuals (patients and healthy donors) were obtained by Ficoll–Hypaque density gradient (d = 1,077 g/ml; Sigma-Aldrich, GmbH, Germany) as described previously (18). Representative FFPE specimens from the primary tumor were examined by an experienced pathologist and the appropriate area for microdissection was defined. Microdissection and malignant cells collection were performed using a piezoelectric microdissector (Eppendorf, Germany) as described previously (19).

DNA extraction both all samples was performed using the MasterPure™ Complete DNA and RNA Purification Kit (Epicenter, Madison, Wisconsin, USA) according to the manufacturer's instructions. NanoDrop ND-1000 v3.3 (ThermoFisher Scientific, Waltham, Massachusetts, USA) was used for DNA quantification.

VDR Genotyping

For genotyping of the SNPs at *TaqI*, *ApaI*, *FokI* and *BsmI* positions of the VDR gene, polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods were used. The sequences of the primers used for PCR amplification of the fragments are provided in Suppl Table S1. Allele types, SNP reference numbers and PCR conditions for all the analyzed polymorphisms are shown in Suppl Table S2.

*Taq*I (Minotech Biotechnology, IMBB-FORTH, Heraklion, Greece), *Apa*I (ThermoFisher Scientific, MA, USA), *Fok*I (ThermoFisher Scientific) and *Bsm*I (ThermoFisher Scientific) restriction enzymes were used to digest the amplified products of the VDR gene, according to manufacturer's instructions. Briefly, 10 μl of each related PCR product was mixed with 1 μl of each restriction enzyme and 2 μl of 10× buffers. DEPC treated water was added to a final volume of 20 μl (for *Taq*I) and 30 μl (for *Apa*I, *Fok*I and *Bsm*I). After incubation at 65°C for 15 min (*Taq*I) and at 37°C for 5 min (*Apa*I, *Fok*I and *Bsm*I), the restriction fragments were separated by electrophoresis on a 2% agarose gel, stained with Sybr Safe DNA Gel Stain (ThermoFisher Scientific), and visualized with the AlphaImager ultraviolet transilluminator (Alpha Innotech Corp., San Leandro, CA). The usual nomenclature for restriction fragment length polymorphism alleles was used in this study (20, 21). The lowercase (t, a, f, b) allele represents the presence of the restriction site and the uppercase allele (T, A, F, B) represents the absence of the restriction site.

Study design and statistics

The current study is a retrospective, single institution study aiming to investigate the VDR gene polymorphisms in CRC patients with before the initiation of any treatment. Disease-free survival (DFS), progression-free survival (PFS) and overall survival (OS) were calculated as previously described (18); from the date of surgery to the date of disease recurrence, diagnosis of new colorectal primary or death from any cause, from the date of diagnosis to documented disease progression or death from any cause and from the date of diagnosis to the date of death, from any cause, respectively. Laboratory analysis was carried out blindly to clinical data and statistical analysis was based on contingency tables, including calculations of hazard ratios (HR) and 95% CI, as previously described (18). Statistical significance was set at p=0.05.

Results

Patients' demographics and molecular characteristics

From 09/2003 to 11/2013, 397 patients were recruited in the study and presented newly diagnosed CRC and histologically documented disease. The patients' characteristics are listed in Table 1. Patients' median age was 65 years, 246 (62.0%) were males, 202 (50.9%) were of stage II/III, 372 (93.7%) had PS

(ECOG) 0-1, 205 (47.4%) had a high tumor grade and 279 (70.3%) had a colon/sigmoid tumor location. Moreover, 230 (58.7%) patients had early operable stage II/III disease, 64 (27.8%) of which relapsed, whereas 223 (56.2%) patients had stage IV disease, 197 (88.3%) of which relapsed (Tables 1 and Research Data).

VDR gene *Taq*I t allele (silent T→C transition in exon 9), *Apa*I a allele (T→G transition in intron 8), *Fok*I f allele (C→T transition at the junction of intron 1 and exon 2) and *Bsm*I b allele (G→A transition in intron 8) genotypes and allele frequencies were investigated in all 397 patients and are shown in Tables 1 and Research Data; whereas KRAS mutations were investigated in 245 patients due to no sample availability (Tables 1 and Research Data).

Analysis of the VDR gene polymorphisms

The VDR gene *Taq*I, *Apa*I, *Fok*I and *Bsm*I polymorphisms amplification products were expected to be 740 bp, 740 bp, 265 and 825 bp, respectively. The PCR products were digested by *Taq*I, *Apa*I, *Fok*I and *Bsm*I enzymes, respectively. Following electrophoresis, 143 (36.0%), 129 (32.5%) and 125 (36.5%) patients presented the homozygous mutant (tt), the heterozygous (Tt) and the wild type (TT) genotype, respectively, for *Taq*I polymorphisms (Fig 1a, Tables 1 and Research Data). Similarly, 130 (32.7%), 122 (30.7%) and 145 (36.5%) presented the homozygous mutant (aa), the heterozygous (Aa) and the wild type (aa) genotype, respectively, for *Apa*I polymorphisms (Fig 1b. Tables 1 and Research Data). Moreover, 128 (32.2%), 147 (37.0%) and 122 (30.7%) presented the homozygous mutant (ff), the heterozygous (Ff) and the wild type (FF) genotype, respectively, for *Fok*I polymorphisms (Fig 1c, Tables 1 and Research Data). Finally, 131 (33.0 %), 191 (48.1%) and 75 (18.9%) presented the homozygous mutant (bb), the heterozygous (Bb) and the wild type (BB) genotype, respectively, for *Bsm*I polymorphisms (Fig 1d, Tables 1 and Research Data).

The results showed that both genotype and allelic frequencies in patients were significantly associated with the control groups, and this was the fact for all four polymorphisms. More specifically, it was shown that the healthy donors presented mainly the wild type and the heterozygous genotypes. Moreover, adenomatous polyps control patients presented also mainly the wild type and the heterozygous genotype polymorphisms, and this was observed in all four *Taq*I, *Apa*I, *Fok*I, *Bsm*I polymorphisms (Table 2).

Association of VDR variants and disease stage

Table 2 shows the association observed between VDR polymorphisms and disease stage of the patients. The tt genotype was more prevalent in stage IV patients whereas, the Tt and TT genotypes were

mostly seen in stage II/III patients (62.4% vs 12.0%, 28.6% vs 36.1% and 9.0% vs 51.9%, respectively; $p<0.001$) (Tables 2 and Research Data). The aa genotype was more prevalent in stage IV patients, whereas the Aa and AA genotypes were also mostly prevalent in stage II/III patients (61.4% vs 6.7%, 25.4% vs 35.6% and 13.2% vs 57.7%, respectively; $p<0.001$) (Tables 2 and Research Data). Moreover, the ff genotype was also more prevalent in stage IV patients, whereas the Ff and the FF genotypes were mostly met in stage II/III patients (56.6% vs 10.1%, 30.7% vs 42.8% and 12.7% vs 47.1%, respectively; $p<0.001$) (Tables 2 and Research Data). Similarly, the bb genotype was more prevalent in stage IV patients, whereas the Bb and BB genotype were mostly prevalent in stage II/III patients (49.7% vs 17.8%, 39.7% vs 55.8% and 10.6% vs 26.4%, respectively; $p<0.001$) (Tables 2 and Research Data).

Correlation of VDR and Toll-like receptor variants

The correlation between the different VDR and Toll-like receptor (TLR) variants was analyzed and is presented in Suppl Table S3. When analyzing the whole group of patients, a statistically significant coexistence was observed both between all different VDR combinations and between VDR and TLR genotype combinations (Tables S3 and Research Data). When patients were analyzed according to their disease stage, it was observed that stage II/III patients had a significant coexistence of only *Taq*I-*Fok*I, $p=0.005$; *Taq*I-*Bsm*I, $p<0.001$; *Apa*I-*Bsm*I, $p=0.014$; *Fok*I-*Bsm*I, $p<0.001$; *Fok*I- *Asp299Gly*, $p=0.035$ and *Fok*I-*Thr399Ile* , $p=0.035$ (Tables S3 and Research Data). Similarly, in stage IV patients a significant coexistence was observed in *Taq*I-*Apa*I, $p<0.001$; *Taq*I-*Bsm*I, $p<0.001$; *Apa*I-*Bsm*I, $p<0.001$; *Fok*I- *Apa*I, $p=0.001$; *Fok*I-*Bsm*I, $p<0.001$; *Taq*I-T1237C, $p=0.042$; *Taq*I-T1486C, $p=0.042$; *Fok*I- *Asp299Gly*, $p=0.037$ and *Fok*I-*Thr399Ile*, $p=0.037$ (Tables S3 and Research Data).

Association of TLR2, TLR4 and TLR9 variants and KRAS status

VDR variants and *KRAS* status of CRC patients presented a significant association (Table 3). The tt genotype was more prevalent in patients with a mutant *KRAS* status whereas the Tt and the TT alleles were mostly seen in *KRAS* wild type (53.8% vs 39.0%, 24.0% vs 34.8% and 22.1% vs 26.2%, respectively, $p=0.041$). The aa genotype was again more frequent in *KRAS* mutants, whereas Aa and AA genotypes were more frequent in patients with *KRAS* wild type (55.8% vs 36.9%, 19.2% vs 34.8% and 25.0% vs 28.4%, respectively; $p=0.007$). Similarly, ff genotype was more prevalent in *KRAS* mutants, whereas the Ff and FF genotypes were more frequent in *KRAS* wild type patients (59.6% vs 27.7%, 25.0% vs 42.6% and 15.4% vs 29.8%, respectively; $p<0.001$). Finally, the bb genotype was also more frequent in *KRAS* mutants, whereas Bb and BB genotypes were more frequent in patients with *KRAS* wild type (65.4% vs 27.7%, 24.0% vs 55.3% and 10.6% vs 17.0%, respectively; $p<0.001$) (Tables 3 and Research Data).

Association of VDR variants and clinical outcome

Sixty four (27.8%) adjuvant and 197 (49.7%) metastatic patients presented a disease progression following their adjuvant and first-line treatment, respectively (Research Data Table). The median DFS was 19 months (95% CI: 15.5-22.5) and median OS was 155 months (95% CI: 59.1-250.9), respectively for stage II/III patients. According to the presence of different VDR genotypes, no significant differences were observed, whereas only a significant shorter OS in patients with the aa genotype (p<0.001) was observed (Fig 2a).

For the case of stage IV patients, the median PFS was 8 months (95% CI: 7.1-8.9) and median OS was 31 months (95% CI: 25.2-36.8), respectively (Research Data Table). Again, there was no difference in PFS according to the presence of different VDR genotypes, whereas only a significant decreased OS in patients with *Taq*I homozygous mutant or heterozygous allele (p=0.037) was observed (Fig 2b). Analysis of all patients presented a median OS of 75 months (95% CI: 56.8-93.2) prevailing a significantly decreased OS in patients with *Taq*I, *Apa*I, *Fok*I and/or *Bsm*I homozygous mutant allele (p<0.001, p<0.001, p<0.001 and p<0.001, respectively) (Figs 2c-f and Research Data Table).

Univariate and multivariate analysis

Univariate analysis revealed that PS (ECOG) was significantly associated with a shorter PFS and PS (ECOG), tumor grade and all VDR polymorphisms were significantly associated with shorter OS (Table 4). In multivariate analysis, adjusting for these factors, tumor grade and *Taq*I, *Apa*I and *Fok*I variants emerged as independent factors associated with decreased OS (HR: 2.6, 95%CI: 1.0–3.4, p = 0.045; HR: 1.5, 95%CI: 1.2–1.9, p = 0.001; HR: 1.9, 95%CI: 1.5–2.3, p < 0.001; HR: 1.5, 95%CI: 1.2–1.9, p = 0.001) (Table 4).

Discussion

Undoubtedly, the risk of sporadic CRC has been linked to environmental and genetic factors (2, 22) and more recently to gut microbiota. The microbiota influences both human health and disease, by affecting the development of the host immune system development and by maintaining homeostasis to influencing diseases and allergies that cannot simply be parsed into strict pathogenesis and commensalism (23, 24). Chronic infection and inflammation are the most important epigenetic factors contributing to tumorigenesis and tumor progression (25). Moreover, vitamin D deficiency has been implicated, among other diseases and metabolic syndromes, in human malignancies (26, 27). Since vitamin D mediates its action by binding to *VDR* gene, suboptimal responsiveness of the *VDR* can be manifested as vitamin D deficiency. Furthermore, the *VDR* gene as part of the innate immunity is responsible for the prevention and elimination of infection and determination of gut microbiome (28-30). Thus, polymorphisms in the human *VDR* gene may play important role in the structure of the gut microbiome. Interestingly, it has been reported previously that VDR conditional knockout ($vdr^{\Delta IEC}$) in the intestinal epithelial or low intestinal VDR protein level may lead to dysbiosis (28) and to reduced

autophagy, accompanied by a reduction of ATG16L1, an inflammatory bowel disease risk gene (30); whereas, absence of intestinal VDR leads to susceptibility to colon cancer via reducing JAK/STAT signaling, a pathway with critical role in intestinal and microbial homeostasis, and dampening inflammatory responses (30). Therefore, the vitamin D/VDR pathway may significantly influence homeostasis, signaling between the microbiota and host in intestinal inflammation and tumorigenesis (30). The aim of the current study, was to evaluate the detection of VDR (*Taq*I, *Apa*I, *Fok*I and *Bsm*I) polymorphisms in adjuvant and metastatic CRC patients.

A number of studies on VDR polymorphisms have been performed in patients with sporadic CRC. Some studies reported contradictory results between various VDR genetic variants and CRC (31, 32) or even no association (13, 33) probably due to limitations such as the recruitment of patients with different characteristics, such as ethnicity, among these studies and the small sample size. However, many of these studies presented significant associations (31, 34). Indeed, in a meta-analysis conducted by Serrano *et al*., (35) the authors presented a significant increased risk for CRC in the presence of tt and aa genotypes, with strong frequency variations be present among different ethnic groups. Moreover, Gandini *et al*., (27) reviewed 79 studies with more than 52,000 cancer cases, including CRC patients, and 62,000 controls; and demonstrated that *Bsm*I, *Fok*I and *Taq*I polymorphisms are associated with CRC. Additional studies reported that the bb genotype of the *Bsm*I polymorphism, are more susceptible to CRC whereas, BB, Bb, TtFf and TTFf genotypes are significantly associated with a decreased risk of CRC (14, 31, 36, 37). Our results are in accordance with these findings. In fact, we demonstrate a higher frequency of the tt, aa, ff and bb genotypes in CRC patient compared to the control groups, thus highlighting the role of these polymorphisms in colorectal carcinogenesis. Moreover, higher frequencies of the tt, aa, ff and bb genotypes were detected in metastatic CRC patients compared to stage II/III patients, emphasizing the role of these polymorphisms in CRC progression and in patients' overall survival.

The Ras/MAPK pathway and its continuous activation, due to mutations presented in codon 12 of the *KRAS* gene, plays an important role in treatment resistance in patients with various carcinomas, including CRC (38). To this end, we also aimed to associate the frequency of VDR polymorphisms in patients with different *KRAS* status. Despite about 40% of the enrolled patients were not evaluated for their *KRAS* status, a significant association was demonstrated between tt, aa, ff and bb genotypes with the *KRAS* mutant patients.

The TLR pathway, increases the risk of colitis-associated CRC due to commensal gut microbiota (39, 40). TLRs play an important role in immunity and are expressed in various cell types, including tumor cells (41, 42). Since TLR polymorphisms have been associated with changes in susceptibility to many diseases, including cancers (43) and TLRs promote survival of cancer cells (44), we also correlated the coexistence of previously genotyped TLR (TLR2, TLR4 and TLR9) polymorphisms (18) with the VDR polymorphisms. It has been reported previously that the human TLRs are considered as regulators in vitamin D/VDR signaling. In human, when a pathogen is detected by TLRs, gene expressions of VDR and Cyp27B1 are induced (45, 46). In accordance to these reports, we demonstrated that both *TLR* (18) and VDR polymorphisms are associated with increased CRC risk of development and progression with an

impact on patients survival; and also demonstrated a significant correlation between *TLR* and *VDR* gene polymorphisms.

Conclusions

In conclusion, the results of the present study highlight the significant role of VDR polymorphisms in carcinogenesis, disease progression and patients' survival. Our data also showed a correlation between TLR and VDR expression. Based on the present results, therapies targeting the activity of VDRs, including modulation of the TLR-VDR pathways, might provide new approaches to the management of CRC.

Abbreviations

CRC: Colorectal cancer

VDR: Vitamin D receptor

SNPs: Single nucleotide polymorphisms

TLR: Toll-like receptor

FFPE: Formalin-fixed paraffin embedded

PCR-RLFP: Polymerase chain reaction - Restriction fragment polymorphism

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee/Institutional review board of the University Hospital of Heraklion (1265/29-11-17) and signed informed consent has been obtained from all enrolled patients. All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article as “Research Data Table” [and its supplementary information files].

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Competing Interest

Conflict of interest relevant to this article was not reported.

Authors' contributions

Study Concept and Design: I.M, J.S

Data Acquisition: I.M, A.K, M.Sf, N.G, E.A, I.T, E.X, D.M, M.T, J.S

Quality Control of Data and Algorithms: I.M, M.Sf, J.S

Data Analysis and Interpretation: I.M, M.Sf

Statistical Analysis: I.M, J.S

Manuscript Preparation: all authors

Manuscript review: all authors

All authors have read and approved the manuscript, and ensure that this is the case.

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Tables

Table 1. Patients’ characteristics

	Frequency (N=397)	%
Age (range)	65 (18-88)	
<70	260	65,5
>=70	137	34,5
Gender		
Male	246	62
Female	151	38
Stage		
IIA-IIIC	202	50,9
IV	195	49,1
Location		
Colon/Sigmoid	279	70,3
Rectum	118	29,7
PS (ECOG)		
0-1	372	93,7
>=2	25	6,3
Surgery		
Yes	347	87,8
No	50	12,2
Radiotherapy		
Yes	80	20,4
No	313	79,6
Adjuvant Treatment		
Yes	230	58,7
No	162	41,4
First Line Treatment		
Yes	223	56,2
No	174	46,8
Grade		
High	205	47,4
Low	228	52,6
TaqI (T to C)		
Wt (TT)	125	31,5
Hetero (Tt)	129	32,5
Homo (tt)	143	36
ApaI (T to G)		
Wt (AA)	145	36,5
Hetero (Aa)	122	30,7
Homo (aa)	130	32,7
FokI (C to T)		
Wt (FF)	122	30,7
Hetero (Ff)	147	37
Homo (ff)	128	32,2
BsmI (T to C)		
Wt (BB)	75	18,9
Hetero (Bb)	191	48,1
Homo (bb)	131	33
KRAS		
Mutant	104	42,4
Wild type	141	57,6

	ND	152

Table 2. Association of VDR polymorphisms between patients and control groups and between different patients' stages

SNP	Genotype	Patient No (%)	Healthy blood donors No (%)	Adenomatous polyps No (%)	*p* value	Stage II/III	Stage IV	*p*-value
TaqI	**Wt (TT)**	125 (31,5)	71 (71)	24 (60,0)	<0.001	108 (51,9%)	17 (9,0%)	<0,001
	Hetero (Tt)	129 (32,5)	26 (26,0)	11 (27,5)		75 (36,1%)	54 (28,6%)	
	Homo (tt)	143 (36,0)	3 (3,0)	5 (12,5)		25 (12,0%)	118 (62,4%)	
ApaI	**Wt (AA)**	145 (36,5)	56 (56,0)	22 (55,0)	<0.001	120 (57,7%)	25 (13,2%)	<0,001
	Hetero (Aa)	122 (30,7)	40 (40,0)	17 (42,5)		74 (35,6%)	48 (25,4%)	
	Homo (aa)	130 (32,7)	4 (4,0)	1 (2,5)		14 (6,7%)	116 (61,4%)	
FokI	**Wt (FF)**	122 (30,7)	55 (55,0)	21 (52,5)	<0.001	98 (47,1%)	24 (12,7%)	<0,001
	Hetero (Ff)	147 (37,0)	40 (40,0)	16 (40,0)		89 (42,8%)	58 (30,7%)	
	Homo (ff)	128 (32,2)	5 (5,0)	3 (7,5)		21 (10,1%)	107 (56,6%)	
BsmI	**Wt (BB)**	75 (18,9)	55 (55,0)	13 (32,5)	<0.001	55 (26,4%)	20 (10,6%)	<0,001
	Hetero (Bb)	191 (48,1)	43 (43,0)	24 (60,0)		116 (55,8%)	75 (39,7%)	
	Homo (bb)	131 (33,0)	2 (2,0)	3 (7,5)		37 (17,8%)	94 (49,7%)	

Table 3. Association of VDR polymorphisms and KRAS status

		KRAS		
SNP	Genotype	Wt (%)	Mutant (%)	*p* value
TaqI	Wt (TT)	37 (26,2)	23 (22,1)	0,041
	Hetero (Tt)	49 (34,8)	25 (24,0)	
	Homo (tt)	55 (39,0)	56 (53,8)	
ApaI	Wt (AA)	40 (28,4)	26 (25,0)	0,007
	Hetero (Aa)	49 (34,8)	20 (19,2)	
	Homo (aa)	52 (36,9)	58 (55,8)	
FokI	Wt (FF)	42 (29,8)	16 (15,4)	<0,001
	Hetero (Ff)	60 (42,6)	26 (25,0)	
	Homo (ff)	39 (27,7)	62 (59,6)	
BsmI	Wt (BB)	24 (17,0)	11 (10,6)	<0,001
	Hetero (Bb)	78 (55,3)	25 (24,0)	
	Homo (bb)	39 (27,7)	68 (65,4)	

Table 4. Univariate and multivariate Cox Regression analysis

	Univariate Analysis				Multivariate Analysis			
	PFS		OS		PFS		OS	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Gender (Male vs Female)	1,0 (0,8-1,4)	0,986	0,9 (0,7-1,2)	0,411	-	-	-	-
Histology (Colon/Sigmoid vs Rectum)	1,2 (0,9-1,6)	0,267	1,2 (0,9-1,7)	0,172	-	-	-	-
PS ECOG (≥2 vs 0-1)	2,6 (1,6-4,2)	<0,001	4,4 (2,8-6,9)	<0,001	-	-	-	-
Age (≥70 vs <70)	1,1 (0,8-1,4)	0,652	1,3 (1,0-1,8)	0,06	-	-	-	-
Grade (High vs Low)	1,4 (0,6-3,4)	0,493	4,4 (1,8-11,0)	0,001	-	-	2,6 (1,0-3,4)	0,045
TaqI (tt vs Tt/TT)	1,2 (0,9-1,2)	0,783	2,2 (1,8-2,7)	<0,001	-	-	1,5 (1,2-1,9)	0,001
ApaI (aa vs Aa/AA)	1,1 (0,9-1,3)	0,46	2,6 (2,1-3,1)	<0,001	-	-	1,9 (1,5-2,3)	<0,001
FokI (ff vs Ff/FF)	1,1 (0,9-1,2)	0,768	2,2 (1,9-2,6)	<0,001	-	-	1,5 (1,2-1,9)	0,001
BsmI (bb vs Bb/BB)	0,1 (0,9-1,3)	0,616	1,7 1,4-2,2)	<0,001	-	-	1,1 (0,7-1,7)	0,712

Figures

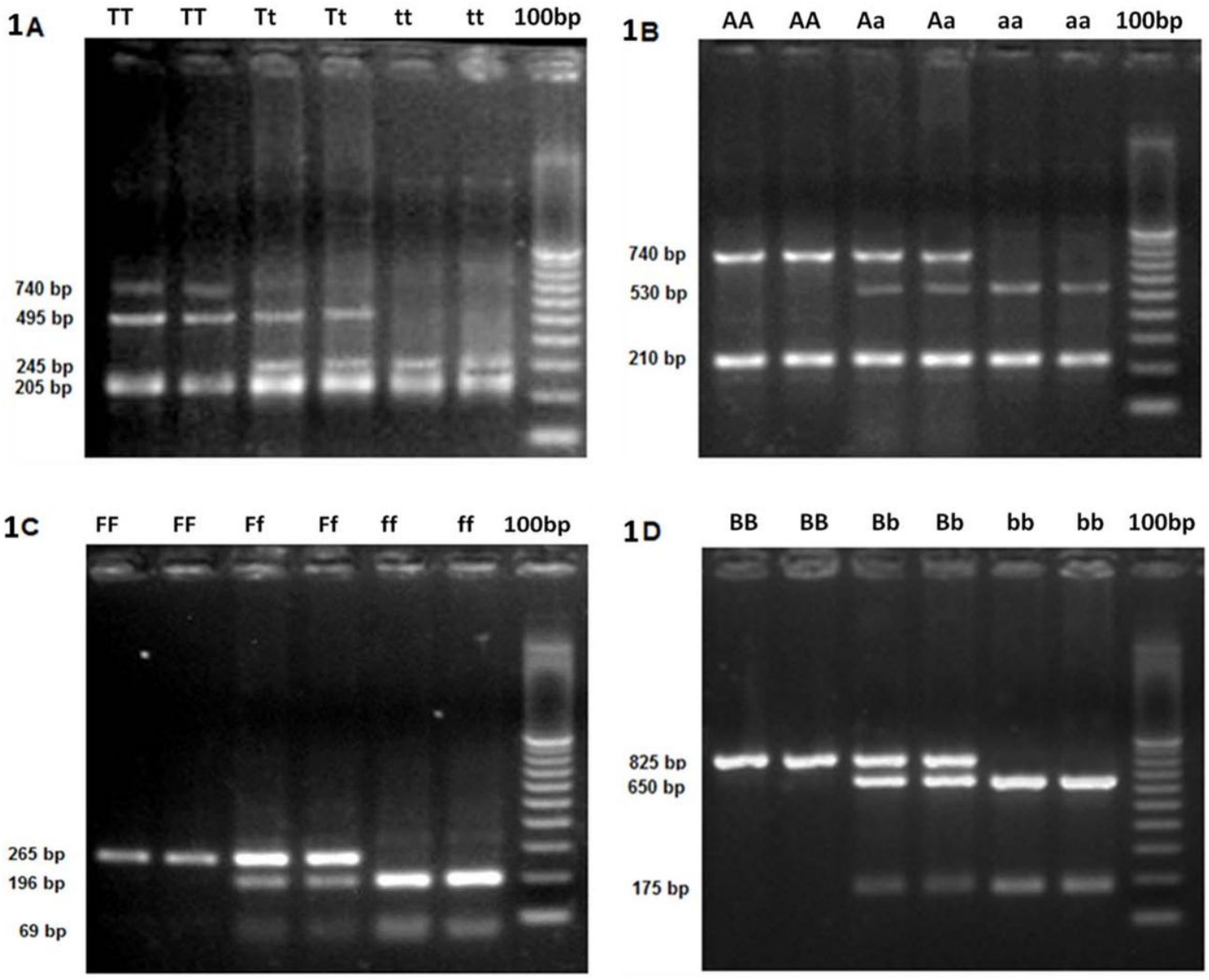

Figure 1

PCR-RFLP representative agarose gels of different SNPs of the a) TaqI, b) ApaI, c) FokI and d) BsmI respectively

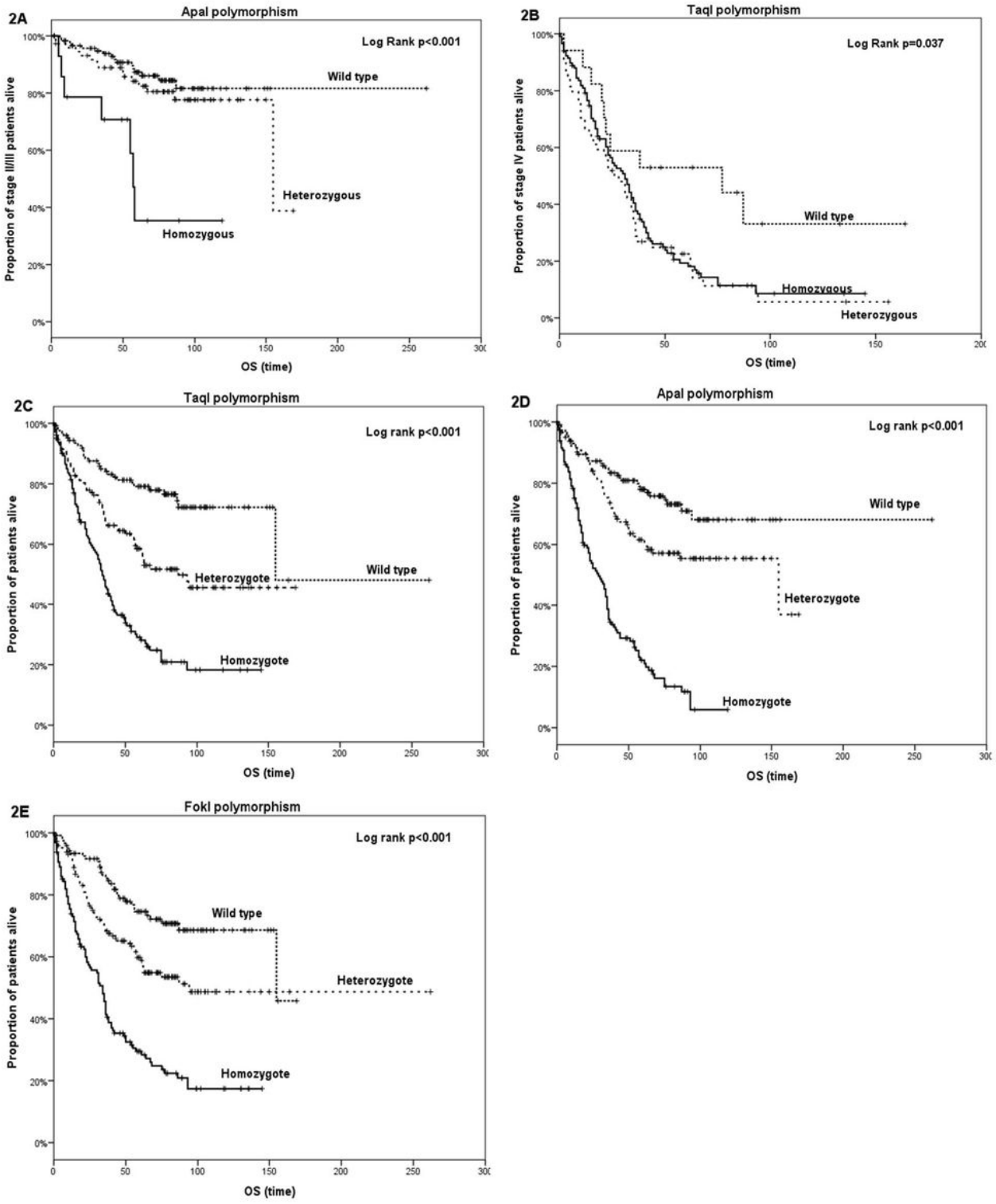

Figure 2

Overall survival according to the detection of a) ApaI polymorphisms and b) TaqI polymorphisms in stage II/III patients; and c) TaqI polymorphisms, d) ApaI polymorphisms, e) FokI polymorphisms and f) BsmI polymorphisms in stage IV patients

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTableS1.docx
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- SupplementaryTableS3.docx