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Single Nucleotide Polymorphisms within Interferon Signaling Pathway Genes Are Associated with Colorectal Cancer Susceptibility and Survival

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Abstract

Interferon (IFN) signaling has been suggested to play an important role in colorectal carcinogenesis. Our study aimed to examine potentially functional genetic variants in interferon regulatory factor 3 (IRF3), IRF5, IRF7, type I and type II IFN and their receptor genes with respect to colorectal cancer (CRC) risk and clinical outcome. Altogether 74 single nucleotide polymorphisms (SNPs) were covered by the 34 SNPs genotyped in a hospital-based case-control study of 1327 CRC cases and 758 healthy controls from the Czech Republic. We also analyzed these SNPs in relation to overall survival and event-free survival in a subgroup of 483 patients. Seven SNPs in IFNA1, IFNA13, IFNA21, IFNK, IFNAR1 and IFNGR1 were associated with CRC risk. After multiple testing correction, the associations with the SNPs rs2856968 (IFNAR1) and rs2234711 (IFNGR1) remained formally significant (P = 0.0015 and P<0.0001, respectively). Multivariable survival analyses showed that the SNP rs6475526 (IFNA7/IFNA14) was associated with overall survival of the patients (P = 0.041 and event-free survival among patients without distant metastasis at the time of diagnosis, P = 0.034). The hazard ratios (HRs) for rs6475526 remained statistically significant even after adjustment for age, gender, grade and stage (P = 0.029 and P = 0.036, respectively), suggesting that rs6475526 is an independent prognostic marker for CRC. Our data suggest that genetic variation in the IFN signaling pathway genes may play a role in the etiology and survival of CRC and further studies are warranted.

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Introduction

Colorectal cancer (CRC) is an important contributor to cancer incidence and death, with more than 1.3 million new cases resulting in about 694,000 deaths in 2012 worldwide [1]. Nutrition, lifestyle factors and environment [1], as well as genetic events have been implicated both in the causation of CRC and survival of patients after diagnosis of CRC [2,3]. So far, 50 single nucleotide polymorphisms (SNPs) located in 40 loci have been associated with the risk of CRC by genome-wide association studies (GWAS) [4–6]. Although molecular markers have been suggested for follow-up of treated CRC patients, their complete clinical application is under evaluation. Clinico-pathologic stage is still the main prognostic marker used in the clinical practice.

Inflammatory responses play a crucial role in colorectal carcinogenesis. Several studies, although not any GWAS, have reported SNPs in immune-related genes to be associated with CRC risk or prognosis [7–10]. Interferons (IFNs) are immunorelated proteins produced and released by host cells in response to the presence of pathogens. IFN-mediated signaling has a diverse range of functions, including antiviral and antimicrobial response, antiproliferation, immunomodulation and apoptosis [11,12]. There are two main classes of IFNs, type I and type II. The two type I IFNs, IFNA and IFNB, have been reported to have an effect on tumor suppression and antiviral immune defense through induction of p33 responses [13]. IFNG, the only type II IFN, has been suggested to play a vital role in the disruption of the intestinal epithelial barrier function [14,15]. It has also been identified as an important modulator of immune-related genes, such as toll-like receptor 3 (TLR3), the gene which showed association with CRC.
survival in our previous study [8]. Interferon regulatory factors (IRFs) regulate IFNs and some IFN-inducible oncoproteins by serving as transcription mediators of pathogens and IFN-induced signaling pathways. Interferon receptors are essential for IFNs to exert their biological effects [11,12]. All type I IFNs bind to a receptor composed of two subunits, IFNAR1 and IFNAR2, while the type II interferon IFNG binds to another dimeric receptor composed of IFNGR1 and IFNGR2.

So far, few studies have investigated the association between genetic variants in the IFN signaling pathway and CRC. A previous study examined genetic variation in IFNG, IFNGR1, IFNGR2 and IRF1-9 with the risk and survival of colon cancer [16]. In that study, tagSNP approach was applied; several SNPs in IRF3, IFNG and its receptors were found to be associated with CRC risk or survival [16]. To further explore the role of genetic variants in the IFN signaling pathway genes in CRC, we genotyped a set of potentially functional SNPs in the IFNGR1 and 2 and 5′ and 3′ untranslated regions (UTRs) and the promoter (up to approximately 1 kb from the transcription start site); pairwise linkage disequilibrium (LD, $r^2 \leq 0.80$) between the SNPs in Utah residents with Northern and Western European ancestry from the CEPH collection (CEU). SNPnexus (http://snp-nexus.org/) was used to predict functional consequences of the selected SNPs. For the SNPs rs2856968, rs2243711 and rs6475526 (including SNPs captured by these SNPs), which associated with CRC risk or survival, we also used additional web-based tools [HaploReg v2 (http://www.broadinstitute.org) and SNPInfo Web Server (http://snpinfo.niehs.nih.gov/cgi-bin/snpfunc.cgi)] to predict their effects on potential regulatory elements.

Materials and Methods

Ethics statement

All participants gave a written informed consent to the use of their samples for research purpose. The study was approved by the ethical committees of the participating institutes, the Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague, Czech Republic and the Institute for Clinical and Experimental Medicine and Faculty Thomayer Hospital, Prague, Czech Republic.

Study population

The case group contained 1327 CRC patients recruited between the years 2004 and 2010 by several oncological departments in the Czech Republic [17]. Their mean age (± standard deviation) was 62.1 (±10.7) years, and 61.7% of them were men. The patients showed positive colonoscopic results for cancer or rectal carcinomas. Patients who met the Amsterdam criteria I or II for hereditary nonpolyposis colorectal cancer were not included in the study [18]. General information about gender and age at diagnosis was available for all patients. For 483 consecutively recruited, incident cases diagnosed between 2003 and 2010, clinical data at the time of diagnosis, including location of the tumor (colon/rectum), International Union against Cancer (UICC) TNM stage classification [size or direct extent of the primary tumor (T), degree of spread to regional lymph nodes (N), presence of metastasis (M)] and grade were available (Table 1). Information about distant metastasis, relapse and date of death was also collected, with a follow-up until August 31, 2011.

The control group contained 758 healthy individuals recruited by a blood-donor center in one hospital in Prague [19]. These disease-free individuals represent the general population of the Czech Republic, which has a genetically quite uniform population [20–22]. Their mean age (± standard deviation) was 45.6 (±8.3) years, and 56.2% of them were men.

SNP selection

20 candidate genes were selected from IFN signaling pathway based on their suggested functional role in CRC causation and survival, including IFNA (1, 2, 4, 5, 7, 8, 13, 16, 17, and 21), IFNB1, IFNK, IFNW1, IRF3, IRF5, IRF7, IFNAR1, IFNAR2, IFNGR1 and IFNGR2 genes in a case-control study of 1327 CRC patients and 758 healthy controls from the Czech Republic and evaluated their association with CRC susceptibility, progression, and prognosis.
adjusted for age, gender, T, N, M, TNM stage and grade separately, and in a final model for age, gender, tumor location, TNM stage and grade. Covariables were stratified for in the analysis if they did not meet the proportional hazards assumption.

**Results**

Altogether, 74 SNPs with MAF \( \geq 10\% \) in the CEU population were located within the regions of interest (promoter, 5’ and 3’UTR, non-synonymous SNPs) of the 20 genes \( IFNA (1, 2, 4, 5, 7, 8, 13, 16, 17, \) and 21), \( IFNB1, IFNK, IFNW1, IRF3, IRF5, IRF7, IFNAR1, IFNAR2, IFNGR1\) and \( IFNGR2\). From these, 34 SNPs were selected for genotyping based on LD \( (r^2 \leq 0.80) \) (Table 2). All \( IFNA \) genes as well as the \( IFNB1, IFNK \) and \( IFNW1 \) genes are located at the same locus at 9p21.3. Thus, although the SNPs were selected based on their potential functional effect on a specific gene, they may capture, and thus give information, about additional SNPs and other genes at the same locus, as shown in Table 2 and Figure S1. The genotype distribution of all 34 genotyped polymorphisms was consistent with HWE in the control group \( (P > 0.05) \). The MAFs in the control population were similar to the ones reported by the HapMap project for the CEU population (Table 2).

**Table 1.** Characteristics of the 483 newly diagnosed Czech colorectal cancer patients.

| Characteristics       | No. (%)               |
|-----------------------|-----------------------|
| Age at diagnosis, mean (range, SD) | 63.5 (27–89, 10.34) |
| <65                   | 243 (50.31)           |
| \( \geq 65 \)         | 240 (49.69)           |
| Gender                |                       |
| Female                | 180 (37.27)           |
| Male                  | 303 (62.73)           |
| Diagnosis             |                       |
| Colon                 | 298 (61.70)           |
| Rectum                | 185 (38.30)           |
| Grade                 |                       |
| 1, 2                  | 309 (63.98)           |
| 3, 4                  | 105 (21.74)           |
| Missing               | 69 (14.29)            |
| T                     |                       |
| T1, T2                | 88 (18.22)            |
| T3, T4                | 351 (72.67)           |
| Missing               | 44 (9.11)             |
| N                     |                       |
| N0                    | 216 (44.72)           |
| N1, N2                | 191 (39.54)           |
| Missing               | 76 (15.73)            |
| M                     |                       |
| M0                    | 325 (67.29)           |
| M1                    | 126 (26.09)           |
| Missing               | 32 (6.22)             |
| TNM stage             |                       |
| Stage I               | 55 (11.39)            |
| Stage II              | 128 (26.50)           |
| Stage III             | 123 (25.47)           |
| Stage IV              | 126 (26.09)           |
| Missing               | 51 (10.56)            |
| Relapse               |                       |
| Yes                   | 91 (18.84)            |
| No                    | 392 (81.16)           |
| Death                 |                       |
| Yes                   | 245 (50.72)           |
| No                    | 238 (49.28)           |

No., number of patients; T, size or direct extent of the primary tumor; N, degree of spread to regional lymph nodes; M, presence of metastasis.

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Table 2. Polymorphisms evaluated in this study.

| Gene     | Genotyped SNP ID | Alleles (major/minor) | Chromosome Position | Location | MAF1 (NCBI) | MAF1 in controls | SNP captured with r^2=0.80^2 |
|----------|------------------|-----------------------|---------------------|----------|-------------|------------------|-----------------------------|
| IFNB1    | rs1424855        | C/G                   | 9p21.3              | 21078815 | 0.375       | 0.353            |                              |
| IFNB1    | rs10964859       | C/G                   | 9p21.3              | 21140672 | 0.394       | 0.343            |                              |
| IFNW1    | rs10757189       | G/A                   | 9p21.3              | 21142604 | 0.311       | 0.264            |                              |
| IFNA21   | rs2939           | T/C                   | 9p21.3              | 21166004 | 0.125       | 0.204            |                              |
| IFNA21   | rs12376071       | A/G                   | 9p21.3              | 21166902 | 0.317       | 0.305            |                              |
| IFNA21   | rs7047687        | A/C                   | 9p21.3              | 21167552 | 0.425       | 0.487            |                              |
| IFNA4    | rs2383183        | T/C                   | 9p21.3              | 21187700 | 0.1         | 0.122            |                              |
| IFNA16   | rs10964912       | A/C                   | 9p21.3              | 21218096 | 0.259       | 0.222            |                              |
| IFNA17   | rs7873404        | T/C                   | 9p21.3              | 21228497 | 0.183       | 0.233            |                              |
| IFNA7/IFN4 | rs6475525       | C/T                   | 9p21.3              | 21242162 | 0.376       | 0.352            |                              |
| IFNA5    | rs12156640       | G/A                   | 9p21.3              | 21306241 | 0.1         | 0.11             |                              |
| IFNA13   | rs641734         | A/T                   | 9p21.3              | 21368927 | 0.175       | 0.194            |                              |
| IFNA2    | rs10120977       | A/G                   | 9p21.3              | 21384363 | 0.248       | 0.209            |                              |
| IFNA8    | rs10738592       | C/T                   | 9p21.3              | 21408516 | 0.475       | 0.486            |                              |
| IFNA1    | rs10811536       | T/C                   | 9p21.3              | 21408693 | 0.233       | 0.2              |                              |
| IFNK     | rs700782         | G/A                   | 9p21.3              | 21752604 | 0.243       | 0.21             |                              |
| IRF3     | rs2304204        | A/G                   | 9q13                | 50169020 | 0.221       | 0.303            |                              |
| IRF5     | rs2070197        | T/C                   | 7q23                | 12858900 | 0.15        | 0.107            |                              |
| IRF5     | rs11770589       | G/A                   | 7q23                | 12858948 | 0.375       | 0.476            |                              |
### Table 2. Cont.

| Gene | Genotyped SNP ID | Alleles (major/minor) | Chromosome | Position | Location | MAF1 (NCBI) | MAF1 in controls | SNP captured with $r^2 \geq 0.80$ |
|------|-----------------|-----------------------|------------|----------|----------|-------------|----------------|------------------|
| IRF5 | rs1874327$^1$   | T/A                   | 7q23       | 128945322| intron   | 0.400       | 0.358          | IRF5 IRF5 IRF5    |
| IRF7 | rs1061502       | A/G                   | 11p15.5    | 614318   | missense | 0.283       | 0.231          | IRF7 IRF7 IRF7 IRF7 |
|      |                  |                       |            |          |          |             |                | rs7943546 rs1061505 rs1055582 rs12805435 rs1131665 |
|      |                  |                       |            |          |          |             |                | 612148 613297 612382 612355 613208    |
|      |                  |                       |            |          |          |             |                | 3' UTR 3' UTR 5' near gene |
| IFNAR2 | rs1131668     | G/A                   | 21         | 33262573 | missense | 0.332       | 0.326          | IFNAR2           |
| IFNAR1 | rs2856968$^2$ | A/G                   | 21         | 33325676 | intron   | 0.424       | 0.345          | IFNAR1 IFNAR1 IFNAR1 |
|      |                  |                       |            |          |          |             |                | rs17875752 rs17875753 rs2843710 |
|      |                  |                       |            |          |          |             |                | 33324192 33324196 33324402          |
|      |                  |                       |            |          |          |             |                | 5' near gene 5' near gene 5' near gene |
| IFNAR1 | rs2850015     | C/T                   | 21         | 34697264 | 5' UTR   | 0.308       | 0.296          |                   |
| IFNAR1 | rs2257167     | G/C                   | 21         | 34715699 | missense | 0.127       | 0.14           |                   |
| IFNAR1 | rs2834202     | A/G                   | 21         | 34730954 | 3' UTR   | 0.261       | 0.218          |                   |
| IFNGR1 | rs2234711     | T/C                   | 6          | 13750520 | 5' UTR   | 0.353       | 0.375          |                   |
| IFNGR1 | rs17181457    | C/T                   | 6          | 137540536| 5' UTR   | 0.117       | 0.078          |                   |
| IFNGR1 | rs327474      | G/A                   | 6          | 137541075| 5' near gene | 0.398   | 0.447          |                   |
| IFNGR2 | rs17882748    | T/C                   | 21         | 34775721 | 5' UTR   | 0.413       | 0.49           |                   |
| IFNGR2 | rs9808753     | A/G                   | 21         | 34787312 | missense | 0.142       | 0.12           |                   |
| IFNGR2 | rs1059293     | T/C                   | 21         | 34809693 | 3' UTR   | 0.478       | 0.445          |                   |

1Minor allele frequency (MAF) based on Utah residents with Northern and Western European ancestry from the CEPH collection in the HapMap project.

2Pairwise linkage disequilibrium ($r^2$) was calculated for the SNPs with MAF$\geq 10\%$ within the regions of interest based on Utah residents with Northern and Western European ancestry from the CEPH collection in the HapMap project.

3Because no assays were available for the potentially functionally SNPs, the SNPs rs6473526, rs1874327 and rs2856968, respectively, were genotyped instead.

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Seven SNPs were associated with CRC susceptibility

Minor allele carriers of the IFNA13 promoter SNP rs641734, and IFNA21 3’UTR SNP rs2939, had a decreased risk of CRC, while the minor allele carriers of the IFNA1 missense SNP rs33965070, IFNK 3’UTR SNP rs700782, IFNARI 3’UTR SNP rs2834202, IFNARI SNP rs2856968, which was genotyped instead of the promoter SNPs in the same gene, and IFNGR1 5’UTR SNP rs2234711 had an increased risk of CRC (Table S3). These associations did not differ by tumor location at colon or rectum (data not shown). To correct for multiple testing, we used the SNPSpD approach. The study-wise effective number of independent markers Meff was calculated to be 27, which gave the significance threshold of 0.0019. Thus, the associations with the SNPs rs2856968 (IFNARI) and rs2234711 (IFNGR1) remained formally significant ($P = 0.0015$ and $P < 0.0001$, respectively). The other genotyped SNPs were not associated with CRC risk (Table S1).

Two SNPs were associated with CRC survival

In the univariable analysis, the following parameters were associated with overall survival rate: gender, size or direct extent of the primary tumor (T), degree of spread to regional lymph nodes (N), presence of metastasis (M), TNM stage and tumor grade (Table S2). Interestingly, the SNP rs6475526, located about 2.2 kb 5’ of IFNA14 and genotyped instead of the IFNA7 promoter SNPs, and the IFNA21 promoter SNP rs7047687, showed an association with overall survival among the 483 patients with follow-up data ($HR = 1.33, 95\% CI 1.01–1.74$ and $HR = 0.77, 95\% CI 0.59–0.99$, respectively) (Table 4, Table S3) and SNP rs6475526 also with event-free survival among patients without distant metastasis at the time of diagnosis ($HR = 1.51, 95\% CI 1.03–2.21$) (Table 4). Moreover, compared to the GG genotype carries, the AA carries of IRF5 SNP rs11770859 had a better overall survival ($HR = 0.67, 95\% CI 0.47–0.96$). The Kaplan-Meier survival curves representing the overall and event-free survival rates of the patients according to their rs6475526 genotypes and the overall survival rates of the patients according to their rs7047687 genotypes are presented in Figure 1. The survival differences between the carriers of the different genotypes were statistically significant with log-rank $p$-values of 0.04, 0.03 and 0.04, respectively. The associations were strongest among stage 1

Table 3. Associations between candidate SNPs and colorectal cancer susceptibility.

| Gene      | SNP rs#  | Genotype | Case No. (%)$^1$ | Control No. (%)$^1$ | OR (95% CI)       | $P$ value$^2$ |
|-----------|----------|----------|------------------|---------------------|------------------|--------------|
| IFNA1     | rs33965070 | CC       | 1060 (82.23)     | 640 (86.37)         | 1                |              |
|           |          | GG       | 0                | 0                   |                  |              |
|           |          | CG+GG    | 229 (17.77)      | 101 (13.63)         | 1.37 (1.06–1.75) | 0.015        |
| IFNA13    | rs641734 | CC       | 900 (69.39)      | 473 (64.35)         | 1                |              |
|           |          | CT       | 358 (27.60)      | 239 (32.52)         | 0.79 (0.65–0.96) | 0.018        |
|           |          | TT       | 39 (3.01)        | 23 (3.13)           | 0.89 (0.53–1.52) | 0.668        |
|           |          | TC+CC    | 397 (30.61)      | 262 (35.65)         | 0.80 (0.66–0.97) | 0.024        |
| IFNA21    | rs2939   | TT       | 882 (67.90)      | 466 (62.38)         | 1                |              |
|           |          | CT       | 383 (29.48)      | 257 (34.40)         | 0.79 (0.65–0.95) | 0.016        |
|           |          | CC       | 34 (2.62)        | 24 (3.21)           | 0.75 (0.44–1.28) | 0.288        |
|           |          | CT+CC    | 417 (32.10)      | 281 (37.62)         | 0.79 (0.65–0.95) | 0.012        |
| IFNK      | rs700782 | GG       | 743 (57.15)      | 467 (62.18)         | 1                |              |
|           |          | AG       | 484 (37.23)      | 252 (33.56)         | 1.20 (1.00–1.47) | 0.054        |
|           |          | AA       | 73 (5.62)        | 32 (4.26)           | 1.43 (0.93–2.22) | 0.102        |
|           |          | AG+AA    | 557 (42.85)      | 284 (37.82)         | 1.23 (1.03–1.49) | 0.023        |
| IFNAR1    | rs2834202| AA       | 715 (56.08)      | 448 (62.22)         | 1                |              |
|           |          | AG       | 481 (37.73)      | 230 (31.94)         | 1.32 (1.08–1.59) | 0.007        |
|           |          | GG       | 79 (6.20)        | 42 (5.83)           | 1.18 (0.79–1.75) | 0.412        |
|           |          | AG+GG    | 560 (43.92)      | 272 (37.78)         | 1.30 (1.08–1.56) | 0.007        |
| IFNAR1    | rs2856968| AA       | 469 (37.05)      | 321 (44.15)         | 1                |              |
|           |          | AG       | 583 (46.05)      | 311 (42.78)         | 1.28 (1.05–1.56) | 0.014        |
|           |          | GG       | 214 (16.90)      | 95 (13.07)          | 1.54 (1.16–2.04) | 0.003        |
|           |          | AG+GG    | 797 (62.95)      | 406 (55.85)         | 1.35 (1.12–1.61) | 0.0015       |
| IFNGR1    | rs2234711| TT       | 395 (30.91)      | 266 (40.00)         | 1                |              |
|           |          | CT       | 673 (52.66)      | 299 (44–96)         | 1.52 (1.23–1.85) | <0.0001      |
|           |          | CC       | 210 (16.43)      | 100 (15.04)         | 1.41 (1.06–1.89) | 0.017        |
|           |          | CT+CC    | 883 (69.09)      | 399 (60.00)         | 1.49 (1.22–1.82) | <0.0001      |

$^1$Number of cases may differ due to missing data.

$^2$Two-sided $X^2$ test for genotype distribution between the cases and controls, adjusted for age and gender.

No., number of subjects; OR, odds ratio; CI, confidence interval. Bold numbers indicate a statistical significance at 5% level.

Bold numbers in Italics indicate a statistical significance at 5% level after adjustment for multiple comparisons.

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Table 4. Association of rs7047687, rs6745526 and rs11770589 with overall and event-free survival of newly diagnosed colorectal cancer patients.

|                  | No.² | No.² died (%) | HR (95% CI) | P value | No.² | No.² died (%) | HR (95% CI) | P value |
|------------------|------|---------------|-------------|---------|------|---------------|-------------|---------|
| Overall survival¹ |      |               |             |         |      |               |             |         |
| rs7047687        |      |               |             |         |      |               |             |         |
| A/A              | 181  | 101 (55.80)   | 1           |         | 159  | 88 (55.35)    | 1           |         |
| A/C              | 163  | 80 (49.08)    | 0.84 (0.62–1.12) | 0.235  | 148  | 74 (50.00)    | 0.94 (0.69–1.28) | 0.689  |
| C/C              | 120  | 56 (46.67)    | 0.69 (0.50–0.95) | 0.025  | 108  | 48 (44.44)    | 0.65 (0.45–0.92) | 0.016  |
| dom              | 283  | 136 (48.06)   | 0.77 (0.59–1.00) | 0.045  | 256  | 122 (47.66)   | 0.79 (0.60–1.05) | 0.101  |
| rs6475526        |      |               |             |         |      |               |             |         |
| C/C              | 176  | 80 (45.45)    | 1           |         | 159  | 71 (44.65)    | 1           |         |
| C/T              | 224  | 125 (55.80)   | 1.41 (1.07–1.87) | 0.017  | 203  | 112 (55.17)   | 1.43 (1.06–1.93) | 0.021  |
| T/T              | 65   | 28 (43.08)    | 1.05 (0.68–1.61) | 0.834  | 55   | 25 (45.45)    | 1.26 (0.80–2.00) | 0.324  |
| dom              | 289  | 153 (52.94)   | 1.33 (1.01–1.74) | 0.041  | 258  | 137           | 1.38 (1.04–1.84) | 0.029  |
| rs11770589       |      |               |             |         |      |               |             |         |
| G/G              | 142  | 79 (55.63)    | 1           |         | 128  | 69 (53.91)    | 1           |         |
| A/G              | 210  | 106 (50.48)   | 0.83 (0.62–1.11) | 0.214  | 187  | 94 (50.27)    | 0.89 (0.65–1.21) | 0.455  |
| A/A              | 109  | 49 (44.95)    | 0.67 (0.47–0.96) | 0.028  | 96   | 44 (45.83)    | 0.76 (0.52–1.11) | 0.152  |
| dom              | 319  | 155 (48.59)   | 0.77 (0.59–1.01) | 0.062  | 283  | 138 (48.76)   | 0.85 (0.64–1.14) | 0.269  |
| Event-free survival (M=0)² |      |               |             |         |      |               |             |         |
| rs6475526        |      |               |             |         |      |               |             |         |
| C/C              | 118  | 53 (44.92)    | 1           |         | 111  | 49 (44.14)    | 1           |         |
| C/T              | 112  | 44 (39.29)    | 0.82 (0.55–1.22) | 0.323  | 106  | 42 (19.62)    | 0.82 (0.54–1.25) | 0.354  |
| T/T              | 83   | 33 (39.76)    | 0.77 (0.50–1.20) | 0.250  | 77   | 28 (36.36)    | 0.72 (0.50–1.50) | 0.168  |
| dom              | 195  | 77 (39.49)    | 0.80 (0.56–1.13) | 0.207  | 183  | 70 (38.25)    | 0.77 (0.54–1.12) | 0.172  |
| rs11770589       |      |               |             |         |      |               |             |         |
| G/G              | 115  | 39 (33.91)    | 1           |         | 107  | 34 (31.78)    | 1           |         |
| A/A              | 149  | 68 (45.64)    | 1.59 (1.07–2.36) | 0.021  | 143  | 64 (44.76)    | 1.61 (1.06–2.46) | 0.027  |
| A/G              | 86   | 36 (42.35)    | 1           |         | 85   | 36           | 1           |         |
| A/A              | 81   | 32 (39.51)    | 0.86 (0.54–1.37) | 0.52   | 76   | 30 (39.47)    | 0.95 (0.54–1.55) | 0.845  |
| A/G              | 140  | 58 (41.43)    | 0.93 (0.62–1.40) | 0.73   | 131  | 53 (40.46)    | 0.96 (0.63–1.48) | 0.855  |
patients; the HR for overall survival was 4.04 (95% CI 1.13–14.53) for SNP rs6475526 and 0.29 (95% CI 0.10–0.83) for IFNA21 SNP rs7047687; the HR for event-free survival was 3.78 (95% CI 1.27–11.67) for SNP rs6475526 (Table S4). However, these results should be taken with caution due to small number of patients who died (11/29 and 6/34 variant allele carriers of the stage 1 patients, respectively), and because the HRs among stage 2, 3 and 4 patients were similar to the ones for all 483 patients (Table S4). Moreover, no differences in overall survival between patients with grade 1+2 and grade 3+4 tumors or between patients without (M0) and with distant metastasis (M1) were observed. Stratified analysis according to tumor location showed that the worse overall survival of SNP rs6475526 was restricted to patients with rectal cancer (HR 2.10, 95% CI 1.31–3.36; colon cancer HR 1.01, 95% CI 0.72–1.42), the same tendency was observed also for event-free survival (rectal cancer HR 1.90, 95% CI 1.06–3.40; colon cancer HR 1.27, 95% CI 0.77–2.10) (Table S4).

In the multivariable analysis, the HRs for rs6475526 remained statistically significant after adjustment for age, gender, tumor location and TNM stage (overall survival HR 1.38, 95% CI 1.04–1.84; event-free survival HR 1.55, 95% CI 1.03–2.32) (Table 4).

Discussion

IFN-signaling system may play a critical role in carcinogenesis of CRC by regulating immune responses during inflammation and it may additionally affect survival of CRC patients [8,13–16,20,25]. In this genetic association study, we investigated the associations between 34 SNPs capturing 74 potentially functional SNPs in the IFN-signaling system genes and CRC risk and clinical outcome. Two SNPs located in the IFNAR1 and IFNGR1 genes exhibited an association with CRC risk. In the multivariable survival analysis, the SNP rs6475526, located about 2.2 kb of IFNA14 and capturing two promoter SNPs in IFNA7, was associated with overall survival and also with event-free survival of non-metastatic CRC patients. These SNPs together with other common variants identified by the GWASs and the candidate gene studies may affect CRC risk and clinical outcome.

IFNAR1 has recently been proposed as a novel candidate CRC tumor suppressor gene [21]. IFNAR1 has also been reported to play an important role in the development of early-onset CRC, suggesting a role in genetic predisposition [23]. Polymorphisms in IFNAR1 have also been reported to be associated with susceptibility of multiple sclerosis, hepatocellular carcinoma and outcome of hepatitis B virus infection [32,33]. In our study, the minor allele of rs2856968 in the intron of IFNAR1 was associated with an increased risk of CRC. This polymorphism captured three promoter SNPs rs2843710, rs17875753 and rs17875752 with high LD (D’ = 1, r² = 1). Rs2843710 is located in the binding site of protein polymerase (DNA directed), epsilon, catalytic subunit (POLE), which has been reported to be associated with colorectal carcinogenesis [26,34]. Combined with the previously reported associations of IFNAR1 with early-onset CRC and effects of IFNAR1 on apoptosis and p53 signaling pathway in CRC cells [13,21,23], our data support the role of IFNAR1 in CRC causation.

The IFNGR1 protein is a prerequisite to the initiation of IFN signaling [35]. Reduced expression of IFNGR1 has been reported to be associated with clinicopathologic characteristics of esophageal cancer [36] and prognosis of ovarian cancer [37]. Published studies have reported that polymorphisms in IFNGR1 are significantly associated with susceptibility of chronic hepatitis B virus infection, early gastric carcinoma, and rectal cancer [16,38,39]. In this study, we found the minor allele of rs2234711
A  Overall survival

Log-rank \( *P = 0.04 \)

B  Event-free survival \((m=0)\)

Log-rank \( *P = 0.03 \)

C  Overall survival

Log-rank \( *P = 0.04 \)
in the promoter of IFNGRI to be associated with an increased risk of CRC. Rs2234711 has also been reported to be associated with the susceptibility of early gastric carcinoma, chronic hepatitis B virus infection and cerebral malaria [30–40]. A previous study indicated that rs2234711 may have functional effects on stimulating B cell lines, and C allele was associated with decreased IFNGRI gene activity, however, in a context-dependent manner [41]. Rs2234711 is located near an activating protein (AP)-2/AP-4 consensus binding site [42] and overexpression of AP-2α has been shown to reduce the expression of IFNGRI and to inhibit IFNG signaling [35]. Moreover, rs2234711 is located in the binding site of POLB, which has been associated with CRC [43,44]. Together with evidences above, our finding suggested that the functional variant rs2234711 might have an effect on CRC causation through regulating the expression or function of IFNGRI.

Our study suggested an association of IFNA SNPs with clinical outcome of CRC. Based on our data, the SNP rs6475526 may be associated with overall and event-free survival of CRC patients. The associations remained statistically significant after adjustment for known prognostic markers, suggesting that rs6475526 is an independent prognostic marker. Rs6475526 (about 2.2 kb 5’ of IFNA14) is capturing two IFNA7 promoter SNPs rs7045980 and rs7046208 with high LD (D’=0.97, r²=0.85). According to SNPinfo Web Server, all these SNPs are located nearby several transcription factor binding sites and may thus affect transcriptional activity. Previous studies have reported that IFNA-expressing tumor cells enhance generation and promote survival of tumor-specific cytotoxic T lymphocytes [45] and that IFNA improves the anti-proliferative effect of EGFR inhibitors in CRC cell lines [27,28]. Our finding gives novel evidence of the role of IFNA in CRC progression and survival.

Compared to the previous study, which investigated the association of tagSNPs in IFNG, IFNGRI, IFNGR2 and IRF1-9 with the risk and survival of colon and rectal cancer [16], our study focused on potentially functional SNPs and covered in addition to the IRFs, IFNG and its receptors also other IFNs and their receptors. Six genes, IFNG, IFNGRI, IFNGR2, IRF3, IRF5 and IRF7, were common in these two studies. In contrast to the previous study, which reported many associations both with colon and rectal cancer risk and survival, we observed only two associations with CRC risk and one association with overall and event-free survival. The only gene showing association in both studies was IFNGRI, however, the SNP rs2234711 which was associated with CRC risk in our study, was not covered by any tagSNP in the previous study. For the risk analysis, both studies were large [Slattery et. al. case/control, 1555/1956 (colon cancer), 754/959 (rectal cancer); we 1327/758 (CRC)]. There may be small differences in the origin of the study participants, with our study coming from a genetically quite uniform Czech population [22], while the recruitment area of the study by Slattery et. al. was Northern California and Utah, including also some 10–20% of Hispanic, Black and Asian participants. For the survival analysis, the studies had comparable follow-up time, but while Slattery et. al. had follow-up for all patients, we had it only for 483 patients, which decreased our power to detect small associations. However, this ensured that only newly diagnosed CRC cases (within one year of diagnosis before enrollment for this study) were included in our study, excluding a survival bias. For this subgroup, nearly complete clinical data were available, allowing evaluation of the SNPs as independent prognostic markers.

GWASs mainly describe only the most robust associations, which may be the reason that they have not reported any associations between CRC and interferon pathway genes. The tagSNP approach, used in the GWAS, is thought as a method with maximum SNP prediction accuracy, however, it does not cover all SNPs in the regulatory regions. A total of 74 SNPs in the regulatory and coding regions of the genes were covered by our study. However, due to sample size restrictions, we concentrated on SNPs with MAF ≥10% in Europeans and on SNPs located in the basic regulatory regions. It is possible that SNPs with a lower MAF or SNPs in still unknown regulatory regions of the studied genes, such as the enhancer and the silencer regions, might also modulate CRC susceptibility or survival.

In summary, our results, together with the previous study by Slattery et. al. suggest that genetic variation in the IFN signaling pathway genes plays a role in the etiology and survival of CRC. The strongest findings of our study included the associations of SNPs in IFNAR1 and IFNGRI with susceptibility to CRC, and of SNPs in IFNA7/IFNA14 with the survival of CRC patients. Validation of our findings and investigation of novel genetic variants in large, independent populations are encouraged.

Supporting Information

Figure S1 Haploview linkage disequilibrium (LD) pattern of the interferon alpha region on chromosome 9p21.3 showing pairwise LD values r² between the SNPs. Only SNPs with the minor allele frequency >10% in the Utah residents with Northern and Western European ancestry (CEU) from the CEPH collection in the 1000 genomes project are shown. Intensity of the gray color from white (r² = 0) to black (r² = 1) indicates the extent of LD. The two IFNA7 promoter SNPs rs7045980 and rs7046208 captured by rs6475526 (2.2 kb of IFNA14) are surrounded by a red line. (PNG)

Table S1 Association of all evaluated SNPs with colorectal cancer susceptibility in the whole study population of 1327 cases and 758 controls. (DOC)

Table S2 Univariable analysis of colorectal cancer survival and known prognostic factors. (DOC)

Table S3 Association of all evaluated SNPs with colorectal cancer overall survival for all patients and event-free survival among patients without distant metastasis at the time of diagnosis. (DOC)

Table S4 Stratified analysis of rs6475526, rs7047687 and rs11770589 for overall survival and rs6475526 for event-free survival among patients without distant metastasis at the time of diagnosis. (DOC)
Author Contributions
Conceived and designed the experiments: SL AF. Performed the experiments: SL. Analyzed the data: SI BC AF. Contributed reagents/... materials/analysis tools: BP AN SH VV LV TB KH PV. Wrote the paper: SL AF. Revised manuscript and contributed to the discussion of the results: All authors. Read and approved final manuscript: All authors.

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