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# The *CHEK2* gene I157T mutation and other alterations in its proximity increase the risk of sporadic colorectal cancer in the Czech population

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ABSTRACT

Checkpoint kinase 2 (*CHEK2*) gene codes for an important mediator of DNA damage response pathway. Its mutations increase risk of several types of cancer. We analysed selected *CHEK2* mutations in 631 Czech colorectal cancer (CRC) patients.

The increased risk of CRC was associated with mutations in *CHEK2* gene region involving fork head-associated domain [39/631 (6.2%) cases versus 19/683 (2.8%) controls; odds ratio (OR) = 2.3; 95% confidence interval (CI) = 1.3–4.0; $p = 0.003$], and with the most frequent I157T mutation [30/631 (4.8%) cases versus 17/683 (2.5%) controls; OR = 2.0; 95% CI = 1.1–3.6; $p = 0.03$]. Prevalence of 1100delC mutation in CRC patients (4/631) did not differ from that in the control population (2/730; $p = 0.4$). The deletion of 5395 bp was not found in any of the successfully analysed CRC cases. We observed no association of analysed mutations with CRC family history. We conclude that the I157T and other alterations in its proximity predispose to sporadic but not to familial CRC in the Czech population.



## 1. Introduction

Colorectal cancer (CRC) is the most frequent cancer diagnosed in adult population in the Czech Republic ranking our country at the second place in the world incidence of CRC (incidence in 2005 = 77.9 per 100,000 persons).[1] The vast majority of CRC diagnoses arise in the form of sporadic disease; however, the hereditary predisposition to CRC could be found in about 5% of cases.[2] The causal role of mutations in APC gene (OMIM 175100) or mismatch repair genes (OMIM 120435) in CRC is now well established. In contrast, the role of mutations in low penetrance genes is not clear and is currently intensively studied. In comparison to the major predisposing genes, the low penetrance alleles display several distinct characteristics. Alongside the mild elevation of cancer risk (increase in RR ~ 2), the substantial regional differences in distribution

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and frequency (and hence clinical importance), and predisposition to wider spectrum of cancer diagnoses are frequently noted. Recently, mutations in checkpoint kinase 2 [*CHEK2*, Chk2, (OMIM 604373)] were shown to increase the susceptibility for CRC development.[3,4]

CHK2 is a nuclear phosphoprotein involved in genome integrity maintenance, and regulation of cell cycle, apoptosis and senescence (reviewed in [5]). Activation of CHK2 is initiated by its phosphorylation by ataxia-telangiectasia-mutated (ATM) kinase following DNA damage.[6] Three distinct structural/functional domains within CHK2 polypeptide were characterised. The N-terminal SQ/TQ domain (residues 20–75) contains the $Thr^{68}$ targeted by ATM kinase.[7] The conservative fork head-associated domain (FHA; residues 112–175) promotes homodimerisation of CHK2 following $Thr^{68}$ phosphorylation.[8] Autocatalytically activated kinase domains (residues 225–490) of CHK2 homodimer catalyse phosphorylation of CHK2-targeted downstream effectors.[9] The substrates of CHK2 kinase activity include several critical cell cycle and apoptosis regulators and DNA repair proteins (p53, PML, E2F1 and BRCA1).

Mutation analyses indicate that *CHEK2* acts as the multiorgan cancer susceptibility gene contributing to the development of numerous cancers, including breast, colorectal, prostate, ovarian, thyroid and kidney cancer.[10–13] The frameshifting 1100delC mutation leading to the translation of truncated protein product lacking kinase activity (fs381X) has been the most studied gene alteration in *CHEK2*, especially in patients with breast cancer. Its occurrence varies substantially among different populations being highly incident in Northern and Western Europe[14] and in Russia,[15] but rare in Southern Europe,[16] South America[17] or Asia.[18] Three other founder mutations in *CHEK2* were primarily shown to influence the development of breast cancer.[19] The c.470T>C (I157T) affects CHK2 FHA domain and reduces CHK2 activation in response to DNA damage.[7] The IVS2+1G>A (fs154X) leads to splicing aberration resulting in frame shift and synthesis of truncated protein [20] The large deletion of 5395 bp causes synthesis of protein with truncated kinase domain.[11]

Previously, we have shown that 1100delC, IVS2+1G>A and I157T mutations are not significantly associated with breast cancer development in the Czech population; however, we characterised several rare alterations within or flanking to FHA-coding sequence of *CHEK2*.[21,22] Here, we summarise the results of analyses of *CHEK2* gene loci harbouring selected mutations including I157T, and other alterations in its neighbourhood, 1100delC, IVS2+1G>A and the 5395 bp deletion in CRC patients from the Czech Republic.

## 2. Patients and methods

### 2.1. Patients

The study involved 631 CRC patients and 683 unrelated non-cancer individuals. All CRC cases and controls were of Czech origin. CRC patients (367 males and 264 females) were recruited from six oncology departments throughout the Czech Republic since September 2004 to February 2006. This study was coordinated by the Department of Oncology, General Teaching Hospital and 1st Faculty of Medicine Charles University in Prague. Histologically, confirmed CRC diagnosis was the only inclusion criterion for group of cases. Data on personal and family history, clinical and histological characteristics of disease and its therapy were retrieved from medical records. A family history of cancer was available in 576 of 631 analysed cases. Positive family history (at least one cancer case in the first or second degree relative) was recorded in 279 patients (48.4%); history of CRC in at least one in the first or second degree relative was present in 100 patients (17.4%).

Control group consisting of two populations – 524 non-cancer controls and 159 blood donors – was described previously, including the results of mutation analysis of *CHEK2* fragment containing FHA domain-coding exons 2–3.[22] Briefly, the subgroup of non-cancer control population (250 males and 274 females), aged 59.0 ± 16.6 years (mean ± SD), consisted of randomly selected adult persons examined at the Department of Clinical Biochemistry and Laboratory Medicine, General Teaching Hospital in Prague between January 2003 and November 2005 excluding those with primary cancer diagnosis. Control blood donors subgroup comprised randomly chosen fully anonymised healthy individuals (69 females and 90 males) enrolled between April 2006 and August 2006 in the Department of Blood Transfusion of the Thomayer Faculty Hospital in Prague. The frequency of 1100delC mutation in control group was also assessed in our previous report.[21] This group (consisting of 730 non-cancer individuals) represented enlarged set of samples of the above-described control group of 524 non-cancer controls. All examined individuals were asked to read and sign the Informed Consent in agreement with the requirements of the Ethical Committee of the General Teaching Hospital.

### 2.2. DNA extraction

Genomic DNA was isolated from peripheral blood lymphocytes by the phenol/chloroform extraction method or using Wizard DNA extraction blood kit (Promega) according to the supplier's instruction. DNA samples were stored at –20 °C.

### 2.3. Genotyping

#### 2.3.1. Analysis of CHEK2 gene fragment containing coding sequence for FHA domain

The PCR-amplified *CHEK2* gene fragment (covering FHA-coding exons 2 and 3 with adjacent intronic sequences of introns 1 and 3, and whole sequence of intron 2) was analysed using denaturant high-performance liquid chromatography (DHPLC; WAVE 3500; Transgenomic) as described in details previously.[22] Both I157T and IVS2+1G>A alleles were screened in this analysis. Samples showing aberrant DHPLC chromatograms were bidirectionally sequenced from independently amplified samples using ABI 3130 sequencer (Applied Biosystems).

### 2.4. Analysis of 1100delC mutation

Mutation 1100delC was detected by DHPLC as we reported previously.[21] Analysis involved DNA amplification using nested PCR (to avoid random coincidence of numerous pseudogenes with high homology to *CHEK2* sequence) followed by DHPLC analysis. Presence of mutation was confirmed by DNA sequencing.

**Table 1 – Frequency of alterations in the CHEK2 gene region covering coding sequence of FHA domain.**

| Exon/intron | Genetic change | Protein change | CRC patients (*N* = 631) | Controls[a] (*N* = 683) | OR[b] | 95% CI[c] | *p* Value[d] |
|---|---|---|---|---|---|---|---|
| e2 | c.434G>A[f] | R145Q | 1 (0.2%) | 0 | –[e] | | |
| e3 | c.470T>C[g] | I157T | 30 (4.8%) | 17 (2.5%) | 2.0 | 1.1–3.6 | 0.03 |
| e3 | c.538C>T | R180C | 0 | 1 (0.2%) | –[e] | | |
| e3 | c.541C>T[g] | R181C | 2 (0.3%) | 0 | –[e] | | |
| i1 | IVS1-5T>A[g] | ? | 1 (0.2%) | 0 | –[e] | | |
| i2 | IVS2+1G>A | fs154X | 2 (0.3%) | 0 | –[e] | | |
| i2 | IVS2+24C>T[g] | ? | 3 (0.5%) | 1 (0.2%) | 3.4 | 0.4–32.4 | 0.4 |
| i2 | IVS2-55C>T[f] | ? | 1 (0.2%) | 0 | –[e] | | |
| All alterations within coding sequence | | | 33 (5.3%) | 18 (2.6%) | 2.1 | 1.2–3.7 | 0.02 |
| Alterations excluding I157T | | | 10 (1.6%) | 2 (0.3%) | 5.6 | 1.2–25.7 | 0.02 |
| All alterations | | | 39 (6.2%)[h] | 19 (2.8%) | 2.3 | 1.3–4.1 | 0.003 |

*Note*: Patients and controls were categorised as follows: (1) carriers of any alteration within coding sequence (R145Q, I157T, R180C, R181C); (2) carriers of any alteration excluding I157T and (3) carriers of any alteration.

a The frequency of all alterations within analysed fragment in the control subgroups of hospital-based controls and blood donors was 2.9% (15/524) and 2.5% (4/169), respectively ($p = 0.8$; ANOVA test for difference). The frequency of I157T mutation in the control subgroups of hospital-based controls and blood donors was 2.6% (14/524) and 1.9% (3/169), respectively ($p = 0.6$; ANOVA test for difference).

b Mantel–Haenszel common odds ratio (OR) estimate.

c 95% confidence interval (CI).

d Fisher's exact test, *p* (2-sided).

e Not performed due to the presence of 0 value in one group.

f Novel mutation.

g Alterations characterised in Czech breast cancer patients.[22]

h One patient carrier of both I157T and IVS2+24C>T was found.

### 2.5. *Analysis of large deletion of 5395 bp*

For the assessment of the large deletion (del5395), method previously published by Walsh and colleagues was used with minor modifications.[19] Briefly, two primers flanking the deletion (CHEK2delUSF primer located in intron 7 and CHEK2delUSR primer located in intron 9) were used for PCR identification of 1325 bp fragment indicating the large deletion in *CHEK2*. Separate PCR with primers CHEK2delUSF and CHEK2delUSR2 (annealed to the sequence in intron 7 lost in the case of deletion) amplified the wild-type *CHEK2* sequence, and served as a positive control of PCR (1195 bp fragment). Horizontal 1% agarose gel electrophoresis stained with ethidium bromide was used for visualisation of fragments. Samples with deletion were verified by DNA sequencing.

### 2.6. *Statistical analysis*

Crude odds ratios (ORs) were calculated from $2 \times 2$ tables by Mantel–Haenszel statistics (unconditional, df = 1). Two-sided Fisher's Exact Test was used for the evaluation of significance of results. The differences in clinical and histopathological characteristics between mutation carriers and non-carriers were calculated using Pearson's chi-square test and ANOVA. The *p* value lower than 0.05 was considered significant. Analyses were performed by Win SPSS v 13.0 program (SPSS Inc., Chicago, IL, USA).

## 3. Results

### 3.1. *Analysis of CHEK2 gene fragment containing coding sequence for FHA domain*

Seven different *CHEK2* alterations (Table 1) were found in 39 of 631 CRC patients (6.2%) contrary to only two alterations found within the same gene fragment in 19 of 683 controls (2.8%) analysed previously using the same method.[22] The presence of any alteration elevated the risk of CRC in the group of patients more than twofold (OR = 2.3; 95% confidence interval (CI = 1.3–4.0; $p = 0.003$). Alongside the most frequent c.470T>C (I157T) mutation, and four alterations described previously (R181C, IVS1-5T>A, IVS2+1G>A and IVS2+24C>T) we characterised two novel gene variants – the missense variant c.434G>A (R145Q) and the intronic variant IVS2-55C>T (Fig. 1). The missense variant R180C was detected in one of 683 control samples only. The prevalence of I157T mutation was significantly higher in CRC patients – 30/631 (4.8%) than in controls [17/683 (2.5%); $p = 0.03$]. The inheritance of I157T mutation enhanced the risk of CRC twofold (OR = 2.0; 95% CI = 1.1–3.6; Table 1). The prevalence of other alterations detected in the *CHEK2* gene region containing FHA domain-coding sequence was also found to differ significantly between CRC patients and controls (10/631 versus 2/683; $p = 0.02$), and the risk of CRC associated with the inheritance of these allelic variants was enhanced accordingly (OR = 5.6; 95% CI = 1.2–25.7; Table 1). The inheritance of any *CHEK2* missense variant within FHA-coding sequence enhanced the risk of CRC more than twofold (OR = 2.1; 95% CI = 1.2–3.7; $p = 0.02$; Table 1). Both I157T and IVS2+24T>C variants were detected in one CRC patient.

### 3.2. *Analysis of c.1100delC mutation*

Truncating mutation 1100delC was found in four of 631 CRC patients (0.6%). Compared to previously analysed controls (2/730; 0.3%), the difference in frequency of 1100delC was not statistically significant (OR = 2.3; 95% CI = 0.4–12.8; $p = 0.4$).[21] The average age of CRC diagnosis in 1100delC carriers was 60.5 ± 8.5 years (mean ± SD). Positive familial history of cancer was scored in one of the four patients carrying

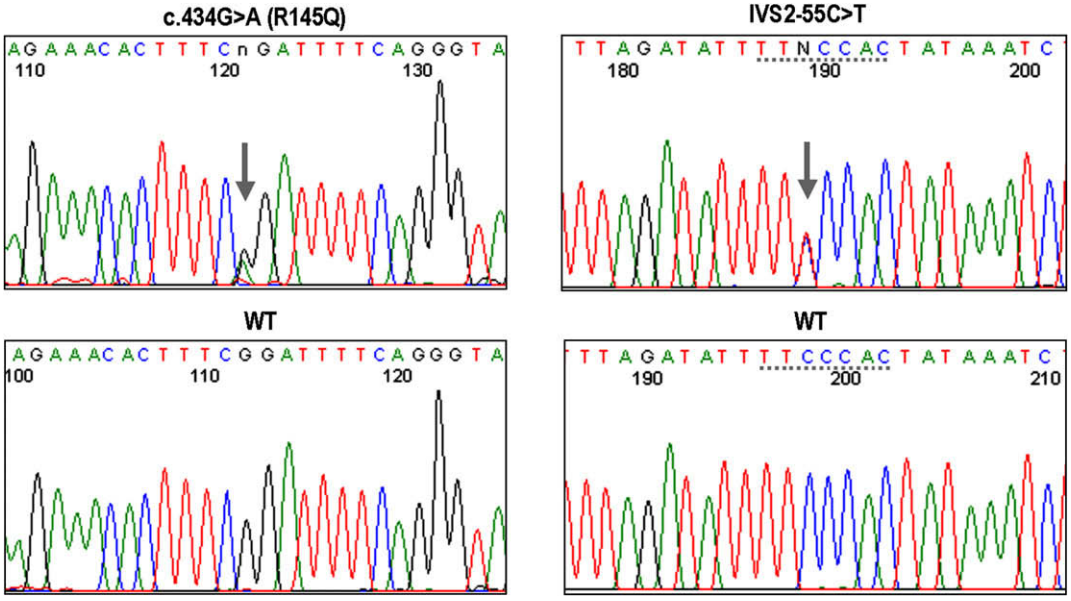


**Fig. 1 – Sequencing chromatograms show two novel alterations in *CHEK2* gene (R145Q and IVS2-55C>T, marked by arrow) and corresponding wild-type (WT) sequences. Position of predicted branch-site (5′-TTCCCAC; Supplementary Fig. 2) affected by IVS2-55C>T transition is underlined.**

1100delC (father with gastric cancer diagnosed at the age of 50).

### 3.3. *Analysis of 5395 bp deletion*

The large deletion of 5395 bp was successfully screened in 522 of 631 CRC patients and 565 of 683 controls. Analysis failed in 17% of the samples due to poor DNA quality or due to the lack of DNA sample. We have found no carrier of this mutation in the group of CRC patients. One heterozygote carrier was identified in the control group (1/565; 0.2%).[g]

### 3.4. *Association of CHEK2 gene I157T mutation and other alterations in its proximity with clinico-pathological characteristics of CRC patients*

To analyse the impact of various *CHEK2* allelic variants on clinical and histopathological characteristics of colorectal tumours, the group of CRC patients was categorised into subgroups containing (i) patients carrying any alteration within fragment containing coding sequence for FHA domain, (ii) carriers of I157T mutation and (iii) subjects with wild-type alleles (Table 2). Age at the diagnosis in 39 mutation carriers of any *CHEK2* mutation (59.4 ± 12.6 years; mean ± SD) and in 30 carriers of I157T mutation (60.1 ± 11.8) was similar to patients without mutation (61.0 ± 10.6; $p = 0.4$ compared to any mutation within analysed fragment). We did not note any relationship between the presence of *CHEK2* alteration and localisation of primary tumour or clinical stage (AJCC). However, statistically significant difference between mutation carriers and patients without mutation was associated with tumour grade ($p = 0.0495$ compared to any mutation carriers and wild-type patients; Table 2).

The frequency of positive family cancer history (defined as any cancer in the first or second degree relatives and index case) did not differ between CRC patients carrying any *CHEK2* alteration in analysed fragment containing FHA-coding sequence (18 of 39; 46.2%) and CRC patients with wild-type *CHEK2* alleles (261 of 576; 45.3%; Table 3). The most frequent cancer diagnoses in 18 families of *CHEK2* alteration carriers were colorectal and lung cancers (both in six families) and breast cancer (in three families). No association was observed between the presence of *CHEK2* alterations and hereditary CRC. The increased frequency of patients carrying *CHEK2* alterations was apparent only in the group of patients from colorectal and lung cancer families (defined as lung cancer in the first or second degree relatives and index case with CRC). Six CRC and lung cancer families were identified among 39 carriers of any *CHEK2* alteration and in 37 of 537 *CHEK2* wild-type CRC patients (15.4% and 6.9%, respectively; $p = 0.051$).

## 4. Discussion

We studied the impact of four *CHEK2* founder mutations and other sequence variants on the development of CRC in Czech patients. The I157T mutation found in 30 of 631 CRC patients (4.8%) was the most prevalent *CHEK2* alteration. The occurrence of truncating mutations 1100delC and IVS2+1 G>A was higher in analysed CRC patients (0.6% and 0.3%) than in controls (0.3% and 0%); however, due to the low prevalence of these alterations in the Czech population their role in CRC

[g] Contrary to previously published description of 5395 bp deletion, we assume that the deletion of 5395 bp [c.909-2028_1095+330del5395; (Supplementary Fig. 1)] affects coding exons 8 and 9 (not 9 and 10) and causes synthesis of protein with truncated kinase domain (p.Met304Leufs15X).

**Table 2 – Selected clinico-pathological characteristics of colorectal tumours in patients analysed for the presence of mutations in the CHEK2 gene region covering coding sequence of FHA domain.**

| | Wild-type *N* (%) | Any CHEK2 alteration *N* (%) | *p* Value[a] | I157T *N* (%) | *p* Value[a] |
|---|---|---|---|---|---|
| Location of primary tumour | | | 0.6 | | 0.6 |
| Ascending colon | 76 (91.6%) | 7 (8.4%) | | 6 (7.2%) | |
| Transverse colon | 31 (100.0%) | 0 (0.0%) | | 0 (0.0%) | |
| Descending colon | 43 (93.5%) | 3 (6.5%) | | 2 (4.3%) | |
| Sigmoid rectum | 232 (93.2%) | 17 (6.8%) | | 14 (5.6%) | |
| Rectum | 177 (93.7%) | 12 (6.3%) | | 8 (4.2%) | |
| Staging (AJCC) | | | 0.4 | | 0.6 |
| Stage I | 17 (94.4%) | 1 (5.6%) | | 1 (5.6%) | |
| Stage IIA-B | 253 (95.1%) | 13 (4.9%) | | 10 (3.8%) | |
| Stage IIIA-C | 139 (90.8%) | 14 (9.2%) | | 10 (6.5%) | |
| Stage IV | 110 (92.4%) | 9 (7.6%) | | 7 (5.9%) | |
| Tumour grade[b] | | | 0.0495 | | 0.06 |
| G1 | 86 (90.5%) | 9 (9.5%) | | 7 (7.4%) | |
| G2 | 298 (95.5%) | 14 (4.5%) | | 10 (3.2%) | |
| G3 | 74 (89.4%) | 9 (10.6%) | | 7 (8.3%) | |

*Note*: Patients carrying any alteration within analysed *CHEK2* fragment covering exons 2 and 3, and patients carrying I157T were analysed separately against patients with wild-type sequence.

a Chi-square test.

b Two grade 4 tumours (wild-type in analysed sequence) were excluded from the statistics.

**Table 3 – Selected characteristics of colorectal cancer patients analysed for the presence of allelic variants in the CHEK2 gene region covering coding sequence of FHA domain.**

| | Wild-type *N* (%) | Any *CHEK2* alteration *N* (%) | *p* Value | I157T *N* (%) | *p* Value |
|---|---|---|---|---|---|
| CRC patients (*N* = 631) | 592 (93.8%) | 39 (6.2%) | – | 30 (4.8%) | – |
| Males; *N* (%) | 345 (94.0%) | 22 (6.0%) | – | 16 (4.4%) | – |
| Females; *N* (%) | 247 (93.6%) | 17 (6.4%) | – | 14 (5.3%) | – |
| Age at diagnosis (range) in years | 61.0 (23–86) | 59.4 (28–78) | – | 60.1 (28–76) | – |
| *Family cancer history (N = 576)*[c] | | | | | |
| Positive | 261 (93.5) | 18 (6.5%) | – | 14 (5.0%) | – |
| Age at diagnosis (range) in years | 60.3 (23–83) | 58.3 (28–78) | – | 57.9 (28–75) | – |
| Negative | 276 (92.9) | 21 (7.1%) | – | 16 (5.4%) | – |
| Age at diagnosis (range) in years | 61.3 (26–86) | 60.4 (28–76) | – | 62.1 (42–76) | – |
| HCC | 94 (94.0%) | 6 (6.0%) | – | 5 (5.0%) | – |
| Age at diagnosis (range) in years | 60.4 (23–83) | 56.5 (50–65) | 0.4[a] | 57.2 (50–65) | – |
| CC&LC | 37 (86.0%) | 6 (14.0%) | 0.051[b] | 4 (9.3%) | 0.2[b] |
| Age at diagnosis (range) in years | 59.7 (35–74) | 57.5 (41–78) | – | 56.5 (43–75) | – |

HCC – hereditary CRC (defined as CRC in the first or second degree relatives and index case); HBCC – hereditary breast cancer and CRC (defined as breast cancer in the first or second degree relatives and index case); CC&LC – CRC and lung cancer (defined as lung cancer in the first or second degree relatives and index case).

*Note*: patients carrying any alteration within analysed *CHEK2* fragment covering exons 2 and 3, and patients carrying I157T were analysed separately against patients with wild-type sequence.

a ANOVA test.

b Chi-square test.

c Cases, where family history of cancer was available.

development is of limited clinical importance. The lack of the 5395 bp deletion in analysed CRC patients suggests that the effect of this mutation may be limited to an increase in breast cancer risk only. Moreover, recent studies showed limited relevance of *CHEK2* truncating mutations to CRC development.[4,23]

According to our data, the I157T mutation associates with an increased risk of CRC in the Czech population (OR = 2.0). The frequency of I157T mutation in both CRC and control groups of Czech origin (4.8% and 2.5%, respectively; OR = 2.0) was lower compared to that reported by Kilpivaara and colleagues in Finland (7.8% and 5.3%, respectively; OR = 1.5) and Cybulski and colleagues in Poland (7.1% and 4.8%, respectively; OR = 1.5).[24,4] The frequency of I157T mutation in control population similar to our observation was reported by Brennan and colleagues in different control groups of Czech origin (2.5%; 16/636) contributing to analysis of I157T prevalence in patients with tobacco-related cancers.[11] Contrary to the above-mentioned studies from Finland and Poland, we did not find association of I157T with family history of CRC.

In its place we observed increased frequency of lung cancer in relatives of I157T carriers with CRC [4/30 (13.3%) cases with I157T versus 37/537 (6.9%) wild-type cases; $p = 0.2$]. This trend turned even stronger when all alterations detected in the gene fragment containing exons 2 and 3 were included [6/39 (14.0%) cases with *CHEK2* alteration; $p = 0.051$]. However, we are aware that interpretation of this association is limited by the small sample size but it remains interesting, as the I157T mutation was recently demonstrated to associate negatively with sporadic lung cancer development.[11,25] Our results indicate that I157T moderately increases the risk of CRC, but the alteration is not linked to familial CRC development in the Czech Republic. Several genetic aspects can contribute to this effect: (i) Genetic origin of CRC in patients not carrying disease-causing mutations in high-penetrant genes is multifactorial. Recently, Cybulski and colleagues reported the cooperative increase of breast cancer and CRC risk in patients carrying both c.326T>G (V109G) allele in p27 and one of I157T, IVS2+1G>A, 1100delC or del5395 mutations in *CHEK2*.[26] (ii) The penetrance of these (so far poorly characterised or undisclosed) multifactorial genetic loci varies in broad scale below the threshold, in which it turns into the *sine qua non* condition for cancerogenesis initiation. Because of usually low penetrance of contributing alleles (maximally ~OR 2.0), their frequencies could be quite high in population. However, they may vary substantially among diverse populations (e.g. the occurrence of 1100delC allele has been shown to decrease in European countries in North-to-South direction). (iii) Current evidences have shown that carriage of low penetrant alleles influences the risk of particular cancer type. The I157T could serve as an example, increasing the risk of CRC but protecting against tobacco-related lung cancer.[11] (iv) The individual genetic cancer risk in cancer patients is probably driven by the mutual interplay of risk factors. The multifactorial interplay of numerous 'low penetrant' or 'modifying' alleles with diverse population frequencies could explain the association to sporadic but not to hereditary CRC. CRC develops in a subset of CRC patients that inherited 'cancer-promoting collection' of alleles (e.g. including I157T) from their parents. This 'collection' is assembled from two allele pools that were alone incapable to evoke cancer in their parents (the cumulative OR for CRC in each parent is lower than the cumulative OR of combined genotype in their CRC-affected child). It should also be considered that siblings of such CRC patient might be at an increased risk. However, it is probable that the composition of 'cancer-promoting collection' will be diluted in descent of patient. We hypothesise that numerous (however limited) such 'cancer-promoting collections' may exist, and at least some of them may have a population-specific character. We speculate that the I157T mutation (and possibly other alterations within FHA domain-coding sequence) participates as one of several genetic contributors to CRC development in our population.

Alongside the I157T and IVS2+1G>A mutations, the analysis of *CHEK2* gene fragment containing the FHA-coding exons 2 and 3 with adjacent intronic sequences revealed the presence of five another alterations in eight CRC patients. The novel c.434G>A transition (R145Q) found in one CRC patient leads to the replacement of highly conservative Arg to Glu. Other missense variants affecting Arg145 [c.433C>T (R145W) and c.434G>C (R145P)] were described elsewhere in patients with Li-Fraumeni syndrome, breast and prostate cancer and in CRC cell line HCT15.[20,27–29] The R145W variant has been shown to cause reduced ATM-dependent CHK2 phosphorylation and CHK2 kinase activity, and thus affecting the association of CHK2 with other cellular proteins in response to DNA damage.[7,8,30] Therefore, it is possible that R145Q may also alter CHK2 activation. The c.541C>T transition (R181C) detected in two CRC patients affects non-conservative amino acid residue located in proximity to C-end of FHA domain. This alteration was earlier described by Dong and colleagues in one of 178 prostate cancer tumour samples. It was not found in any of 298 men with familial prostate cancer, 400 men with sporadic prostate cancer or 423 unaffected men.[20] We identified recently R181C in one breast cancer patient from the Czech Republic.[22] The occurrence of intronic variants IVS1-5T>A (identified in one CRC patient) and IVS2+24C>T (found in three CRC patients and one control sample) was described in previously analysed population of breast cancer patients. Based on computer prediction, we deduced that both variants might interfere with the binding of splicing factors.[22] In this study, we characterised another intronic variant IVS2-55C>T in one CRC patient. The IVS2-55C>T transition alters the most probable branching-site (based on software prediction in ESE finder algorithm; Supplementary Fig. 2) in intron 2, and hence may lead to aberrant mRNA splicing. However, this assumption needs to be confirmed by analysis at mRNA level.

Clinical and histopathological characteristics in CRC patients with *CHEK2* alterations and wild-type alleles were similar, except for tumour grading in carriers of I157T. However, instead of the clear trend showing increased mutation frequency with higher grading, we detected uneven distribution of grading with increased mutation frequencies in both grade 1 and 3 tumours. Thus, this observation may be a result of limited size of analysed groups or due to multiple comparisons.

In conclusion, the analysis of a gene fragment containing coding sequence of *CHEK2* FHA domain in CRC population supports our previous observation in breast cancer patients that exons 2 and 3 and flanking intronic sequences are subject to numerous population-specific genetic alterations.[22] Alterations in this region enhanced the effect of I157T and together contributed to an increased risk of sporadic CRC development (OR = 2.3) in the Czech population. Prevalence of truncating mutation 1100delC is low in CRC patients, and play clinically less important role in CRC tumourigenesis.

## Conflict of interest statement

None declared.

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Hospital, and Drahomira Springer from the Department of Clinical Biochemistry and Laboratory Medicine, and their patients, and volunteers for their collaboration.

## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2008.09.022.

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