Journal of BUON **15:** 314-317, 2010

ORIGINAL ARTICLE

CHEK2 I157T and colorectal cancer in Bulgaria

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Summary

Purpose: *Germline variants of the CHEK2 gene have been shown to act as low-penetrance cancer susceptibility alleles for a wide range of human malignancies. CHEK2 I157T has particularly been linked to colorectal cancer (CRC) risk. We aimed at establishing the population frequency and contribution of this variant to colorectal carcinogenesis in Bulgaria.*

Methods: *We have genotyped 802 population controls and 343 CRC patients from Bulgaria for the CHEK2 I157T variant.*

Results: *Heterozygous were 9 of 343 patients (2.62%, odds ratio/OR=1.0, 95% confidence interval/CI = 0.42 - 2.33, p=0.99) and 21 of 802 controls (2.62%). Higher frequencies were found among patients with multiple polyposis (2/40, 5%, p=0.28) and the rarer mucinous histology (1/11, 9.09%, p= 0.26).*

Conclusion: *We conclude that CHEK2 I157T is not relevant for CRC risk in Bulgaria, but studies on a larger scale might help evaluate its possible significance in respect to disease characteristics.*

Key words: CHEK2, colorectal cancer, I157T, gene

Introduction

The CHEK2 gene encodes a cell cycle checkpoint kinase which plays a crucial role for mammalian DNA-damage-signaling pathway and cell cycle regulation. Following double-strand DNA breaks, the CHEK2 protein is activated in an ataxia-telangiectasia mutated (ATM) - dependent fashion to phosphorylate its substrates among which are TP53, BRCA1, Cdc25C and Cdc25A, thus regulating their function and coordinating subsequent DNA repair or apoptosis [1].

Germline variants in CHEK2 have been demonstrated to act as low-penetrance cancer predisposing alleles - the protein truncating 1100delC mutation contributes to a substantial fraction of BRCA-mutation negative familial breast cancer [2], and the single nucleotide polymorphism I157T, resulting in the substitution of an isoleucine for a threonine within the functional forkhead associated (FHA) domain of the protein, has been linked to colon cancer [3-6]. CHEK2 I157T protein has been shown to be unable to bind and phosphorylate its substrates but capable of dimerizing with wild-type CHEK2 [7], thus supposedly exerting a negative effect on the pool of wild-type CHEK2 protein.

Previous relative studies have shown a low-penetrance effect of CHEK2 I157T in CRC predisposition [3-5]. To estimate the significance of this variant for CRC predisposition in Bulgaria, we have genotyped in a case-control study a cohort of 343 CRC patients and 802 population controls.

Methods

Patient and control cohorts

CRC patients were referred by the University Hospital "Queen Giovanna", Sofia, and the University Hospital "St. Marina", Varna. The former acquires patients

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Received 08-05-2009; Accepted 06-08-2009

mainly from the western part of the country, while the latter from the eastern part. A written informed consent was obtained along with information on personal and family history of cancer at hospital departments where blood was drawn. All cases were histologically confirmed.

Cases where at least 2 first-degree relatives had cancer or where at least 1 first-degree relative had a hereditary nonpolyposis colon cancer (HNPCC) reminiscent cancer as defined by Lynch [8], one being diagnosed before the age of 50, were classified as familial.

A total of 612 controls were recruited from adult individuals who had previously submitted blood for paternity testing. Another 190 controls were selected from adult individuals who underwent routine yearly testing in a clinical laboratory. All control samples have been anonymised before use.

The study was approved by the Ethics Committee of the Medical University of Sofia.

Mutation analysis

DNA was extracted from peripheral blood mononuclear cells and stored in tris-EDTA (TE) buffer. The Ile157Thr variant was analyzed by using PCR-RFLP as described in [4]. PCR products were digested, run on an acrylamide gel along with a heterozygous control sample and visualized by silver staining.

Samples in which a mutation was detected in the PCR-RFLP were sequenced with primers: F: 5′ - CGT TTG ATA CAT GAA ATT CA - 3′ and R: 5′ - CCA GTA ACC ATA AGA TAA TA - 3′.

Statistical methods

The distribution of genotype frequencies among groups were compared using x^2 test, and when required, Fisher's exact test. OR and 95% CI were calculated using unconditional logistic regression.

A t-test was used for comparison of mean ages at disease onset between carriers and noncarriers.

Results

I157T variant was identified in 30 subjects and only in heterozygous cytosine/thymine (C/T) state.

The prevalence of C/T heterozygotes among patient and control cohorts did not differ (OR 1.0; 95% CI 0.42 - 2.33; Table1).

We found no prevalence of C/T heterozygosity within the familial patient group. Table 2 displays the

Table 1. Prevalence of CHEK2 I157T carriers among patient and control populations

Tested	*Tested n*	*I157T carriers n*	*%*	*p-value*
CRC	343	9	2.62	0.99
female	160	2	1.25	0.18*
male	183	7	3.82	
Control subjects	802	21	2.62	
female	449	11	2.45	0.74*
male	353	10	2.83	

*Compared to subjects without that characteristic
CRC: colorectal cancer

Table 2. Distribution of CHEK2 I157T carriers in respect to patient and tumor characteristics

Characteristics	*Total no. tested*	*I157T carriers, n*	*%*	*p-value*
Familial cases	118	3	2.54	1.00*
Sporadic cases	219	6	2.73	
Personal history of cancer†	23	0		
Multiple polyps present	40	2	5.00	0.28*
Dukes' stage				0.51*
A	26	1	3.85	
B	133	5	3.76	
C	122	2	1.64	
D	62	1	1.61	
Grade				0.37*
I	62	1	1.61	
II	233	6	2.57	
III	48	2	4.17	
AdenoCa	332	8	2.41	0.26*
Mucinous adenoCa	11	1	9.09	

†For 6 cases no sufficient information existed and were regarded as unknown
*Compared to patients without that characteristic

distribution of carriers depending on patient characteristics. We found no association with tumor Dukes' stage or grade, but noticed a higher variant frequency among patients with multiple polyposis (5.00%) and those with mucinous histology (9.09%).

Patients who were heterozygous had a mean age of 56 years (SD±14) at diagnosis, while for noncarriers the mean age was 58.7 years (SD±15.4), the difference being non significant (p=0.62).

Discussion

CHEK2 is a serine / threonine protein kinase which is pivotal to cell cycle regulation and DNA damage response. The variant I157T (c.470T>C) is located in a functionally important phosphopeptide recognition domain (FHA domain), which normally enables the protein to form homodimers or bind to its substrates.

Mutations in the FHA domain of the protein have been shown to abrogate its ability to form complexes with some of its key substrates - p53 and Cdc25A. As its ability to form dimers remains intact, CHEK2 I157T has also been suggested to reduce the pool of wild-type CHEK2 protein through a dominant-negative interaction [7].

The CHEK2 gene is evolutionarily conserved and not so many germline variants have been described. CHEK2 I157T, as well as 1100delC, have initially been identified in families with Li-Fraumeni syndrome and proposed to be able to cause the disease. Further studies have demonstrated that these variants probably act as a low-penetrant rather than high-penetrant multiorgan tumor-susceptibility alleles [9].

CHEK2 I157T distribution among populations throughout Europe seems to be quite unequal and is considered to be the result of a founder effect and migration. Its frequency drops from 7.6% for Russia [10] and 5.3% for Finland [3] to 2.5% for the Czech Republic [10,11]. In Italy this variant has not been discovered in any one of 365 unrelated males [12]. Exceptions from this gradient seem to exist as in Germany it was discovered in only 0.6% of controls and in Byelorussia in 1.3% [13]. Despite the variations in allele frequency, the variant has consistently shown an association to CRC risk in previous analyses [3-6]. To our knowledge this study is the first to report a population in which CHEK2 I157T does not increase CRC risk, although a very low penetrance effect could not be excluded.

CHEK2 I157T has also been studied in relation to breast cancer and has been shown to increase breast cancer risk in some populations [14], but not in others [11,15].

An important consideration, besides cancer risk, is carrier clinical and tumor characteristics. CHEK2 variants, mainly 1100delC, have been extensively studied in this aspect among breast cancer patients. Association with higher tumor grade [16], larger tumor size and ductal histology [17], estrogen receptor positive status and risk of contralateral breast cancer [18] were found. The I157T variant, on the other hand, was strongly associated with lobular breast cancer histology [19].

One study related CHEK2 I157T to tumor characteristics of colon cancer [3], but prevalence in any of the stage or grade subgroups was not found. In our study we found no such prevalence too. Instead, we found that it might be related to 2 other tumor characteristics - histological type (p=0.26) and presence of multiple polyps (p=0.28), but the corresponding patient groups were small and no statistically significant differences could be observed.

We conclude that CHEK2 I157T is not relevant for CRC risk estimation in Bulgaria but studies on a larger scale may be able to further evaluate its significance in respect to CRC development.

Acknowledgements

We express our gratitude to all participants in this study. Financial support for this study was provided by research grant D8/2007 of the Medical University, Sofia, Bulgaria

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