ELECTRONIC LETTER

CHEK2 I157T associates with familial and sporadic colorectal cancer

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Background: Recently, a functionally defective *CHEK2* variant I157T has been proposed to associate with an increased risk of several types of cancer. We investigated the *CHEK2* I157T variant for colorectal cancer (CRC) predisposition in a large population based study including a significant number of familial CRC cases.

Methods: We screened the *CHEK2* I157T variant in a population based series of 1042 Finnish CRC patients using restriction fragment length polymorphism. Mutation status was studied for correlation with clinical characteristics and family history of CRC and other cancers.

Results: The frequency of *CHEK2* I157T was significantly higher in CRC patients (7.8%, 76/972) than in healthy population controls (5.3%, 100/1885) (OR=1.5, 95% CI 1.1 to 2.1, $p=0.008$). The significant association of *CHEK2* I157T with CRC was observed among patients with (10.4%, 14/135) and without (7.4%, 62/837) a family history of CRC (OR=2.1, 95% CI 1.1 to 3.7, $p=0.01$; OR=1.4, 95% CI 1.0 to 2.0, $p=0.03$; respectively). A trend towards higher variant frequency was also noted among patients with multiple primary tumours and a family history of any cancer.

Conclusions: *CHEK2* I157T associates with an increased risk of CRC: the association was observed both among familial and sporadic CRC patients. Furthermore, the higher frequency of I157T among patients with multiple primary tumours as well as those with a family history of any cancer supports a role for *CHEK2* I157T as a susceptibility allele for multiple cancer types.

High penetrance mutations and low penetrance variants contribute to genetic predisposition to colorectal cancer (CRC). Rare cancer syndromes, such as familial adenomatous polyposis and Lynch syndrome (also known as HNPCC) affecting *APC* and mismatch repair (MMR) genes, respectively, only account for approximately 5% of CRC cases. A large proportion of genetic predisposition to CRC may be due to low penetrance variants in genes encoding proteins involved, for example, in carcinogen metabolism, methylation, DNA repair, or cell cycle control.

Cell cycle checkpoint kinase 2 (*CHEK2*) is a serine/threonine kinase which takes part in various cellular functions regulating cell cycle progression, apoptosis, and DNA damage repair (reviewed in Bartek and Lukas¹). *CHEK2* protein consists of three functional domains: SQTQ cluster domain (SCD), forkhead associated (FHA) domain, and kinase domain. The SCD is a target for (auto)phosphorylation and is thus important for activation and regulation of *CHEK2* functions. The FHA domain is responsible for substrate specificity of *CHEK2* through phosphorylation dependent protein-protein interactions and is also involved in the activation process of *CHEK2*. The *CHEK2* variant I157T, first

associated with an increased risk of breast cancer² and subsequently also with prostate cancer³ and a number of other cancers,⁴ resides in the FHA domain of *CHEK2*. Functional studies indicate that *CHEK2* I157T protein is stable and becomes normally activated,⁵ but also that binding to its substrates p53,⁶ BRCA1,⁷ and Cdc25A⁸ is adversely affected. The *CHEK2* I157T protein is capable of forming dimers in vivo with other *CHEK2* I157T molecules, as well as with the wild type *CHEK2* protein,² although impaired *CHEK2* oligomerisation and reduced autophosphorylation in I157T *CHEK2* proteins has also been reported.⁹ Taken together, these impaired properties of *CHEK2* I157T could result in a grossly reduced number of functional *CHEK2* units in the cell, suggesting a molecular mechanism behind the cancer susceptibility associated with the I157T allele. The I100delC allele of *CHEK2*, residing in the kinase domain and leading to a truncated protein product, has also been found as a low penetrance breast cancer predisposition allele, with a twofold increased breast cancer risk and significant contribution to familial breast cancer.^{10–12}

We have previously found the *CHEK2* I157T variant to be associated with a 1.4-fold increased breast cancer risk among unselected Finnish breast cancer patients.² In another study on a combined German and Belorussian breast cancer series, an even stronger association with breast cancer risk was found (OR = 4.1, 95% CI 1.8 to 9.2, $p<0.001$).¹³ Cybulski *et al* further reported an elevated frequency of *CHEK2* I157T in breast, colon, kidney, prostate, and thyroid cancer patients as well as in non-Hodgkin lymphoma patients.⁴ Their results on *CHEK2* I157T and both breast and prostate cancers are similar to findings previously reported in Finland,^{2,3} and the association of *CHEK2* I157T and hereditary prostate cancer is strong in both populations.^{3,14} The population frequencies in Finland and Poland are also very similar: 5.3%² and 4.8%,⁴ respectively. The I157T variant is also present in German and Belorussian populations at comparable frequencies,^{13–15} while in other studied populations *CHEK2* I157T is very rare or non-existent.¹⁶

We have here elucidated the role of *CHEK2* I157T in CRC predisposition in Finland by analysing the frequency of the I157T variant in a population based series of 1042 CRC patients, including a significant number of familial CRC cases. Association of the I157T variant with familial CRC has not been assessed previously.

METHODS

Patients

CHEK2 I157T was screened in a Finnish population based series of 1042 CRC patients. The data were collected prospectively at nine Finnish central hospitals between

Abbreviations: *CHEK2*, cell cycle checkpoint kinase 2; CRC, colorectal cancer; FHA, forkhead associated; MMR, mismatch repair; RER, replication error; RFLP, restriction fragment length polymorphism; SCD, SQTQ cluster domain

1994 and 1998 and have previously been described in detail.^{17,18} The patient series includes 135 familial CRC cases, defined as two or more first degree relative CRC cases in the same family, and 290 patients with a family history of any cancer (three or more cancer cases in first degree relatives including the index case). The clinical data collected for the patients include age at diagnosis, family history of cancer, pathology reports, and tumour grade. A complete data set was available for every CRC patient. Patient information and samples were obtained with full informed consent. The study was approved by the appropriate ethics review committee (Helsinki University Central Hospital Ethics Committee E8). The healthy Finnish population control subjects (n = 1885) were previously screened for *CHEK2* I157T as described in Kilpivaara *et al.*² The Finnish population frequency for I157T was 5.3%, with little or no geographical variation.

Detection of *CHEK2* I157T

The *CHEK2* I157T mutation was studied using restriction fragment length polymorphism (RFLP) analysis. The *CHEK2* exons 2 and 3 were amplified from genomic DNA using *CHEK2* specific primers 2–3F (5'-GGCTATTTCTACAATTA GC) and 2–3R (5'-CATATTCTGTAAGGACAGGAC) on genomic DNA. The PCR product was then digested with *BtsI* enzyme (New England Biolabs, Beverly, MA, USA) and resulting fragments were separated using 2% agarose gel electrophoresis. The *BtsI* enzyme digests the PCR product with a wild type allele once and a PCR product with the T470C (I157T) allele twice. All I157T positive findings were confirmed by independent analysis.

Statistical methods

The frequencies of the *CHEK2* I157T variant between CRC patients and population controls were compared using χ^2 test, and when required, Fisher's exact test. The ANOVA test was used for comparison of age at diagnosis between groups. A p value of <0.05 was considered statistically significant. SPSS version 12.0 for Windows (SPSS, Chicago, IL, USA) was used for the statistical analyses.

RESULTS

A total of 972 CRC patient samples were successfully analysed for the *CHEK2* I157T mutation, which is 93.3% of the whole patient cohort (972/1042). No results were obtained for 70 samples because of missing DNA or unsuccessful RFLP analysis due to poor DNA quality. The mutation frequency among unselected CRC patients was 7.8% (76/972), which is significantly higher than the Finnish population frequency of 5.3% (OR = 1.5, 95% CI 1.1 to 2.1, p = 0.008). In order to analyse the association between familial aggregation of CRC and *CHEK2*, the patients were

grouped into familial cases, defined as two or more first degree CRC cases in the same family, and non-familial cases. The frequencies of *CHEK2* I157T separately for familial and non-familial CRC cases were both significantly higher than the population frequency, with a carrier frequency of 10.4% (14/135, OR = 2.1, 95% CI 1.1 to 3.7, p = 0.01) among familial cases and 7.4% (62/837, OR = 1.4, 95% CI 1.0 to 2.0, p = 0.03) among non-familial cases. Two patients were homozygous for *CHEK2* I157T. These patients had no specific phenotypic features.

A slightly higher frequency of *CHEK2* I157T was also observed among cases with a family history of any type of cancer, defined as three or more cancer cases in first degree relatives (including the index case), in comparison with cases having no family history of cancer (29/290, 10.0% v 47/679, 6.9%, p = 0.10). The I157T variant frequency among index CRC cases with a family history of other types of cancer (excluding familial CRC) was 8.9% (17/192). In addition, the *CHEK2* I157T variant was more common among patients with multiple primary tumours than among those with only one primary tumour (16/140, 11.4% v 60/832, 7.2%, p = 0.09). It is noteworthy that multiple colorectal tumours do not explain the higher frequency among patients with multiple tumours since only one of those 16 *CHEK2* I157T carriers belonged to that group because of another colorectal tumour. The frequencies of *CHEK2* I157T in various subgroups are given in table 1.

No statistically significant association was observed between *CHEK2* I157T status and tumour grade, tumour stage (Dukes), or replication error (RER) status (table 2). The ages at onset of CRC were very similar (67 years) for both *CHEK2* I157T carriers and non-carriers (data not shown), as also reported previously.⁴ In addition, there was no difference in frequencies between patients with tumours located distally (left) or proximally (right) to the splenic flexure.

DISCUSSION

We have here analysed the incidence of *CHEK2* I157T in Finnish CRC patients and found a significant association of *CHEK2* I157T with CRC risk. Our results are in agreement with the suggested association between *CHEK2* I157T and colon cancer among unselected Polish patients⁴ and indicate that *CHEK2* I157T may be a risk allele for CRC in the Finnish population.

Association of I157T with familial CRC has not previously been assessed. We found here that *CHEK2* I157T also significantly associates with the familial form of CRC. The I157T variant was also more frequent among CRC patients with a family history of any cancer (CRC or other cancers) as well as among those with multiple primary tumours, although these findings did not reach statistical significance.

Table 1 Frequency of *CHEK2* I157T in colorectal cancer cases and controls

	Total	Wild type	I157T	%	p*	OR*	95% CI
Controls	1885	1785	100	5.3		1.0	
All CRC cases	972	896	76	7.8	0.008	1.5	1.1 to 2.1
Familial CRC cases	135	121	14	10.4	0.01	2.1	1.1 to 3.7
Non-familial CRC cases	837	775	62	7.4	0.03	1.4	1.0 to 2.0
					p†	OR†	
Female	483	449	34	7.0			
Male	489	447	42	8.6	0.37	1.2	0.8 to 2.0
Cases with one colorectal tumour	832	772	60	7.2			
Cases with multiple tumours	140	124	16‡	11.4	0.09	1.7	0.9 to 3.0
No family history of cancer	679	632	47	6.9			
Family history of cancer	290	261	29	10.0	0.10	1.5	0.9 to 2.4

*Versus population controls; †versus the other subgroup; ‡only one patient belonged to this group because of another colorectal tumour.

Family history of cancer is defined as three or more cancer cases in first degree family members including the index case. Family history of CRC is defined as two or more CRC cases in first degree family members including the index case.

Table 2 Characteristics of colorectal tumours from 972 unselected CRC patients analysed for *CHEK2* I157T

Characteristic	Germline <i>CHEK2</i>		p
	Wild type	I157T	
Tumour site			
Ascending colon	101 (93.5%)	7 (6.5%)	ND
Caecum	113 (91.9%)	10 (8.1%)	
Descending colon	36 (94.7%)	2 (5.3%)	
Flexura hepatic	19 (90.5%)	2 (9.5%)	
Flexura lienalis	17 (89.5%)	2 (10.5%)	
Rectum	353 (90.1%)	39 (9.9%)	
Rectosigma	20 (95.2%)	1 (4.8%)	
Sigma	178 (94.7%)	10 (5.3%)	
Transversum	53 (94.6%)	3 (5.4%)	0.84
Tumour grade, n = 953*			
1	179 (93.2%)	13 (6.8%)	
2	624 (92.0%)	54 (8.0%)	
3	76 (91.6%)	7 (6.4%)	0.95
Tumour stage (Dukes), n = 967			
A	180 (91.8%)	16 (8.2%)	
B	354 (92.7%)	28 (7.3%)	
C	244 (92.1%)	21 (7.9%)	
D	113 (91.1%)	11 (8.9%)	0.60
RER status, n = 972			
Negative	783 (92.0%)	68 (8.0%)	
Positive	113 (93.4%)	8 (6.6%)	

*Two grade 4 tumours (wild type for the *CHEK2* I157T) were not included in the statistics.

ND, not determined; RER, replication error.

due to the small number of cases. Similarly, among our series of 627 unselected breast cancer cases previously analysed for the I157T variant,² a higher frequency of I157T was also seen among breast cancer patients with multiple cancers (other than breast) than among those with only one primary tumour (8/68, 11.8% *v* 36/518, 6.5%). The I157T variant has so far been found significantly associated with breast, prostate, kidney, colon, and thyroid cancers and non-Hodgkin lymphoma.^{2-4 13} An elevated frequency has also been reported among bladder and pancreatic cancer patients as compared to population controls.⁴ Our findings of an increased risk for multiple cancers among *CHEK2* I157T carriers as well as with a family history of any cancer support the suggested role of I157T as a risk allele for many different cancer types.⁴

These data on the increased cancer risk of the I157T variant are from northern or central/eastern European populations including Finns, Poles, Germans, and Belorussians, among whom *CHEK2* I157T is present at fairly similar population frequencies, while it is very rare or absent in other studied populations. The absence or rarity of both the *CHEK2* I157T and 1100delC variants in many studied populations limits the possibility of evaluating the cancer risks associated with these variants. The evidence for association of the 1100delC allele with breast cancer is strong and there is evidence for an increased risk of prostate cancer as well,^{3 10-12} but the role of *CHEK2* 1100delC in other cancer types remains so far unestablished. *CHEK2* 1100delC has also been suggested to associate with CRC, as the incidence of *CHEK2* 1100delC was observed to be higher in breast cancer families with than without CRC.¹⁹ However, recent results suggest that *CHEK2* 1100delC is not associated with increased risk of either familial or sporadic CRC or that the possible effect on risk is very low.²⁰ Cybulski *et al* observed very low frequencies of *CHEK2* 1100delC among Polish colon cancer patients and controls (0.7% and 0.25%, respectively) and concluded that the 1100delC allele does not increase colon cancer risk.⁴ However, a low risk effect can not be ruled out based on these results. The 1100delC allele was not found associated with colorectal disease either.²¹ Altogether, the evidence for CRC

risk conferred by 1100delC suggests a lower CRC risk than that conferred by I157T, or no elevated risk at all.^{20 22 23}

While both *CHEK2* 1100delC and I157T confer increased risk for breast and prostate cancer, differences in tissue specificity or magnitude of cancer risk may also exist.

The *CHEK2* I157T variant is likely to harm cells through defective binding of CHEK2 protein to its substrates⁶⁻⁸ in the process of mediating DNA damage induced cell cycle checkpoints, DNA repair, and apoptosis. Brown *et al* have reported that CHEK2 associates with MSH2,²⁴ an MMR complex member, and a CRC susceptibility gene. Furthermore, they suggest that CHEK2 activation upon ionising radiation would be challenged in cells deficient in MMR. Whether interaction with MSH2 protein is also affected in the *CHEK2* I157T cells remains to be established.

In conclusion, our results suggest that *CHEK2* I157T is a risk allele for both familial and sporadic CRC. A higher frequency of I157T among patients with multiple primary tumours as well as those with a family history of any cancer supports a role for *CHEK2* I157T as a susceptibility allele for multiple cancer types.

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