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ARTICLE

Germline CHEK2 mutations and colorectal cancer risk: different effects of a missense and truncating mutations?

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Germline mutations in cell cycle checkpoint kinase 2 (*CHEK2*) have been associated with a range of cancer types, in particular of the breast and prostate. Protein-truncating mutations in *CHEK2* have been reported to confer higher risks of cancer of the breast and the prostate than the missense I157T variant. In order to estimate the risks of colorectal cancer associated with truncating and missense *CHEK2* mutations, we genotyped 1085 unselected colorectal cancer cases and 5496 controls for four *CHEK2* founder mutations present in Poland. We observed an increased risk of colorectal cancer in association with the missense I157T mutation (odds ratios (OR) = 1.5; 95% CI 1.2–2.0; $P = 0.002$) but not with truncating mutations (OR = 1.0; 95% CI 0.5–1.8; $P = 0.9$); however the difference in the two OR was not statistically significant ($P = 0.2$). We conclude that the I157T mutation increases the risk of colorectal cancer in the population, but that truncating mutations may confer a lower risk or no increase in risk. It is important that other studies of *CHEK2* mutation carriers be conducted to confirm this hypothesis.
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Introduction

Cell cycle checkpoint kinase 2 (*CHEK2*) is a key component of the DNA damage pathway. Activation of this protein in response to DNA mutation prevents cellular entry into mitosis.[1,2] Germline mutations in *CHEK2* have been associated with a range of cancer types, in particular of the breast and the prostate.[3–23] The majority of predisposing *CHEK2* alleles are protein-truncating, but a missense variant (I157T) has also been associated with cancer at various sites.[9]

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Four *CHEK2* mutations are founder alleles in Poland. Three of these are protein-truncating (del5395, IVS2 + 1G > A, 1100delC) and the other is a common missense variant I157T. Previously, we genotyped 300 Polish patients with colorectal cancer and 4000 controls for three common *CHEK2* mutations.[9] We have now extended our series to include 1085 incident cases of colon cancer and 5496 controls, and we have genotyped these for the four *CHEK2* founder mutations that are present in the population.

Materials and methods

Patients

We studied 1085 colorectal cancer cases diagnosed between 1998 and 2005 in three centres in North-Western Poland. 964 colon cancer cases were diagnosed in Szczecin between

1998 and 2005 and 121 cases were diagnosed in Koszalin and Kołobrzeg between 2003 and 2005. Patients were recruited from the six contributing hospitals in these cities and were unselected for age and family history. All men and women with colorectal cancer were invited to participate. The participation rate exceeded 80%. The mean age of diagnosis was 63.1 years (range 21–92 years). A family history of colorectal cancer in relatives was available from all subjects. One hundred and ten cases (10.1%) had a first-degree relatives diagnosed with colon cancer (familial cases). The study was approved by the Ethics Committee of Pomeranian Medical University in Szczecin.

Controls

In order to estimate the frequency of the Polish founder mutations in the general population, three control groups were combined. The first group consisted of 2183 newborn children from 9 cities throughout Poland (Szczecin, Białystok, Gorzów, Katowice, Wrocław, Poznań, Opole, Łódź and Rzeszów) between 2003 and 2006. Samples of cord blood from unselected infants were forwarded to the study centre in Szczecin. The second control group was taken from healthy adult patients (1079 women and 817 men) of three family doctors practicing in the Szczecin region. These individuals were selected randomly from the patient lists of family doctors. The third control group consisted of 1417 young adults (705 women and 712 men) from Szczecin who submitted blood for paternity testing.

Genotyping

Two primer pairs were used for genotyping of large deletion of exon 9 and 10 in multiplex-PCR reaction as previously described.[22] The first pair flanked breakpoint site in intron 8 and the second pair flanked breakpoint site in intron 10. In mutation-negative cases, only two PCR fragments of 379 and 522 bp were amplified from the wild-type allele. In deletion-positive cases, the forward primer of the first pair and the reverse primer of the second pair amplified additional PCR product of 450 bp.

All-deletion positive cases were confirmed by sequencing. The other three mutations in *CHEK2* (IVS2 + 1G > A, 1100delC and I157T) were genotyped as previously described.[9] These variants are detected by ASO– or RFLP–PCR analyses. In all reaction sets, positive and negative controls (without DNA) were used. All PCR reactions or enzymatic digestions were performed under a layer of mineral oil.

All four *CHEK2* alleles present in Poland are in the functional copy of *CHEK2* gene at chromosome 22 (not in pseudogenes located elsewhere).[9,20,22]

Statistical analysis

The prevalence of each of the three *CHEK2* alleles in cases and in population controls was compared. ORs were generated from two-by-two tables and statistical significance was assessed using the χ^2 test. The ORs were used as estimates of relative risk.

Results

One of four founder *CHEK2* mutations was identified in 87 of 1085 subjects with colorectal cancer (8.0%), including I157T (77 times), 1100delC (five times), del5395 deletion (four times) and IVS2 + 1G > A (two times). The OR for colorectal cancer, given a *CHEK2* missense mutation (I157T) was 1.5 (95% CI1.2–2.0; $P = 0.002$) (Table 1). The I157T variant was seen in 10% of familial cases (OR = 2.2, 95% CI 1.2–4.1; $P = 0.01$). In contrast, we saw no association between *CHEK2* truncating alleles and colorectal cancer risk (OR = 1.0; 95% CI 0.5–1.8; $P = 0.9$). A test for homogeneity of OR was conducted, but we were unable to reject the null hypothesis that both ORs are from the same underlying distribution ($P = 0.2$).

The mean age of diagnosis of colon cancer in mutation non-carriers was 63.3. The mean age of diagnosis of carriers of a *CHEK2* missense mutation was 61.8 years ($P = 0.3$ for difference).

Discussion

We studied four *CHEK2* alleles in relation to colon cancer risk in Poland. We saw a modest but significantly increased risk of colon cancer associated with the I157T variant (OR = 1.5, $P = 0.002$). The association was particularly strong in the subgroup of patients with familial colon cancer (OR 2.2, $P = 0.01$) but this subgroup was small (110 cases). A predisposing I157T mutation in *CHEK2* was present in 7% of Polish colon cancer patients. On average, individuals who carry this mutation have a 1.5-fold increased risk of colon cancer risk. We estimate that this particular *CHEK2* allele is responsible for approximately 3% of all colon cancer cases in the country.

There was no association between the allele frequencies and either age or sex in our control population. The frequency of a *CHEK2* mutation was 6.0% in 1529 adult men (5.0% for the I157T and 1.0% for a truncating variant) and 5.8% in 1784 adult women (4.9% for the I157T and 0.9% for a truncating variant) The frequency was 5.8% in 2183 newborns (4.6% for the I157T and 1.2% for a truncating variant), 5.6% in 1417 young adults who submitted blood for paternity testing (4.8% for the I157T and 0.8% for a truncating variant), and 6.0% in 1896 adult patients from family doctors (5.0% for the I157T and 1.0% for a truncating variant) (Table 2).

Previously, we genotyped 300 individuals with colorectal cancer and 4000 controls.[9] We have extended our series to include an additional 785 cases and 1496 controls. In the initial sample, the I157T variant was seen in 9.3% of cases

Table 1 Prevalence of CHEK2 mutations in cases and controls with corresponding odds ratios

Mutation subjects	*No. of carriers/total (frequency)*	*OR*	*95% CI*	P-*value*
A truncating mutation[a]				
Controls	58/5496 (1.1%)	1.0		
Unselected cases	11/1085 (1.0%)	1.0	0.5–1.8	0.90
Familial cases	2/110 (1.8%)	1.7	0.4–7.2	0.44
I157T				
Controls	264/5496 (4.8%)	1.0		
Unselected cases	77/1085 (7.1%)	1.5	1.2–2.0	0.002
Familial cases	11/110 (10%)	2.2	1.2–4.1	0.01

One case and one control had both truncating and missense mutations.
[a]A truncating mutation – del5395 or IVS2+1G>A or 1100delC.

Table 2 Frequencies of CHEK2 variant alleles in adult and newborn controls

	Variant			
	del5395	*IVS2+1G>A*	*1100delC*	*I157T*
Newborns ($n=2183$)	11 (0.50%)	10 (0.46%)	5 (0.23%)	101 (4.6%)
Adults ($n=3313$)	13 (0.39%)	12 (0.36%)	7 (0.21%)	163 (4.9%)

versus 4.8% of controls (OR = 2.0; $P=0.001$), and a truncating mutation was seen in 1.3% of cases *versus* 1.1% of controls (OR = 1.2; $P=0.7$). In the additional series, the I157T was present in 6.2% of cases *versus* 4.7% of controls (OR = 1.3; $P=0.1$), and a truncating mutation was present in 0.9% of cases and controls, respectively, (OR = 1.0; $P=0.9$). Although the association in the second sample was less strong than that seen in the original report, in the combined sample the statistical significance was maintained ($P=0.002$).

Our results are in agreement with those of a recent study from Finland,[19] wherein the frequency of I157T variant was higher in colorectal cancer patients (7.8%, 76/972) than in population controls (5.3%, 100/1885) (OR = 1.5, 95% CI = 1.1–2.1, $P=0.008$). In that study, the I157T was also found to be associated more strongly with familial cases (OR = 2.1, $P=0.01$). The frequencies of the I157T mutation are similar in Poland and Finland (4.8 and 5.3%) and the corresponding ORs are almost identical. Combining the data from the Finnish and Polish studies yields an overall OR of 1.5 (95% CI: 1.3–1.9, $P< 0.0001$), thus confirming that *CHEK2* is pathogenic for colorectal cancer.

It was initially suggested that the *CHEK2* 1100delC mutation was associated with colorectal cancer; the prevalence of *CHEK2* 1100delC was observed to be higher in breast cancer families with colorectal cancer than in families without colorectal cancer.[14] To date, this result has not been confirmed.[24] Two recent studies investigated the frequency of *CHEK2* 1100delC in unselected colorectal cancer cases and controls.[25,26] In Finland, the 1100delC mutation was not associated with increased colorectal cancer risk – the 1100delC allele was detected in 2.6% (17/662) of cases and 1.9% of 1885 controls.[24] A study from the Netherlands reported a trend towards a higher frequency of the 1100delC mutation in 629 unselected colorectal cancer cases than in 230 controls (1.6 *versus* 0.4%, OR = 3.7; $P=0.3$),[25] but in that study there was only one carrier of the 1100delC in the control group. In the UK, the CHEK2 1100delC allele was not seen in excess in 149 patients with multiple colorectal adenomas (some of whom developed colorectal cancer).[26]

We did not see an association between any *CHEK2* truncating mutation and colorectal cancer risk in Poland (OR = 1.0; $P=0.95$). A truncating variant was seen in 1.0% of the colon cancer patients. This contrasts strongly with our recent observations that a truncating variant was present in 2.4% of prostate cancer patients[22] and in 2.5% of unselected breast cancer patients.[23] Based on a study of 1085 cases and 5496 controls, and a frequency in controls of truncating mutations of 1.1%, we had a power of 76% to detect a relative risk of 2.0 associated with truncating mutations, and a power of 36% to detect a relative risk of 1.5 (at the $P=0.05$ level). Also, our study had limited power to provide evidence for heterogeneity between the missense mutation and the truncating mutations – however, together with the previous reports the aggregate data support the hypothesis that the I157T mutation confer increased colorectal cancer risk, and that truncating *CHEK2* mutations may confer a lower risk or no elevated risk.

CHEK2 is involved in the p53 pathway of DNA damage responses. CHEK2 interacts with many different proteins. Upon ionizing radiation-induced DNA damage, CHEK2 is

activated by ataxia telangiectasia mutated (ATM) and is in turn capable of phosphorylating several substrates including Cdc25A, Cdc25C, p53, and BRCA1, leading to cell cycle arrest, apoptosis and DNA repair (reviewed in[27]).

The protein with I157T variant is stable. The I157T missense variant is localized in a functionally important domain of CHEK2 (the FHA domain) and protein with this mutation has been shown to be defective in its ability to bind and to phosphorylate Cdc25A and to bind p53 and BRCA1.[28–30] The I157T protein may also have a dominant-negative effect by forming heterodimers with wild-type CHEK2.[10] A dominant negative-effect was shown to influence clinical presentation. For example, tumours that develop in heterozygous carriers of an *ATM* mutation gene (the gene in the same pathway as *CHEK2*) appear to be the consequence of a dominant-negative effect of the ATM protein, whereas heterozygous carriers of *ATM* truncating mutations do not seem to be at an increased cancer risk.[31,32] Similarly, only specific missense mutations, which cause local protein defects, confer high-risk of pheochromocytoma in VHL diesease, whereas typically VHL patients with truncating mutations do not develop pheochromocytoma.[33,34] Therefore, in theory, if a dominant negative-effect of CHEK2 protein is present the effects of *CHEK2* truncating and missense mutations might be different.

In conclusion, there are four variant founder alleles of the *CHEK2* gene in Poland. Of these, only the I157T was seen in significant excess in Polish patients with colorectal cancer. It is possible that there is a variation in the risks of cancers associated with *CHEK2* mutations, depending on the class of mutation studied. If present, such genotype–phenotype correlation might be due to a possible dominant-negative effect of the I157T variant. It is important that other studies of *CHEK2* mutation carriers be conducted to verify this hypothesis.

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