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Published in:
GENES CHROMOSOMES & CANCER

DOI:
[10.1002/gcc.20195](https://doi.org/10.1002/gcc.20195)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2005

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

de Jong, MM., Nolte, IM., Meerman, GJT., van der Graaf, WTA., Mulder, MJ., van der Steege, G., Bruinenberg, M., Schaapveld, M., Niessen, RC., Berends, MJW., Sijmons, RH., Hofstra, RMW., de Vries, EGE., & Kleibeuker, JH. (2005). Colorectal cancer and the CHEK2 1100delC mutation. *GENES CHROMOSOMES & CANCER*, 43(4), 377-382. <https://doi.org/10.1002/gcc.20195>

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Colorectal Cancer and the *CHEK2* 1100delC Mutation

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The *CHEK2* 1100delC mutation was recently identified as a low-penetrance breast cancer susceptibility allele. The mutation occurred more frequently in families with clustering of breast and colorectal cancers (CRCs) than in families with clustering of breast cancer only. Hence, the 1100delC mutation could also be a low-penetrance CRC susceptibility allele. To test this hypothesis, we examined the mutation in 629 unselected CRC cases, 230 controls, and 105 selected CRCs diagnosed in patients before age 50. The mutation was observed in 1.6% of unselected patients and in 0.3% of controls (Not significant (NS)). After stratifying unselected patients according to defined genetic risk (on the basis of age at diagnosis and family history of colorectal and endometrial cancer), the highest frequency was observed in high-risk patients (12.5%), followed by moderate-risk patients (3.3%), and was lowest in low-risk patients (1.0%, P_{trend} 0.014). In selected patients, 1.6% carried the mutation (NS). Subgroup analyses for tumor localization, gender, and age at diagnosis did not reveal an association with the 1100delC genotype. In addition, a pooled analysis, combining data of one published study in unselected CRC cases and our study, also did not reveal an association. In conclusion, the frequency of the 1100delC genotype was neither significantly increased in unselected CRC patients nor in selected CRC patients diagnosed before age 50. However, after stratifying unselected CRC patients according to defined genetic risk, a significant trend of increasing frequency was observed. Together, the results are consistent with a low-penetrance effect (OR 1.5–2.0) of the *CHEK2* 1100delC on CRC risk. Large case–control studies are required to clarify the exact role of the *CHEK2* 1100delC mutation in CRC. © 2005 Wiley-Liss, Inc.

INTRODUCTION

Colorectal cancer (CRC) is one of the leading causes of cancer death in the Western countries. It is a multifactorial disease, with dietary and lifestyle habits, on the one hand, and hereditary predisposition, on the other, as contributing factors. First-degree relatives of sporadic CRC patients have a twofold increased cancer risk, which cannot be explained by the known CRC syndromes, that is, familial adenomatous polyposis, human homolog of bacterial MutY-polypsis, and hereditary nonpolyposis colorectal cancer (HNPCC). It is likely that low-penetrance alleles/mutations in several as-yet-unknown genes are involved. The *CHEK2* gene, recently identified as a breast cancer susceptibility gene (Meijers-Heijboer et al., 2002; Vahteristo et al., 2002; CHEK2 Breast Cancer Case-Control Consortium, 2004), could also be a candidate gene for CRC susceptibility.

The *CHEK2* gene encodes the human homologue of the Cds1 and RAD53 checkpoint kinases. In response to ionizing radiation–induced DNA damage, CHEK2 is activated by the ataxia telangiectasia mutated (*ATM*) protein and in turn stabilizes several substrates including TP53 and BRCA1

by phosphorylation, which leads to cell-cycle arrest, activation of DNA–repair, or apoptosis (reviewed by Bartek et al., 2001).

An increased frequency of the *CHEK2* 1100delC mutation was reported in familial breast cancer cases without *BRCA1/2* mutations (Meijers-Heijboer et al., 2002) and in unselected breast cancer cases (CHEK2 Breast Cancer Case–Control Consortium, 2004). The 1100delC mutation occurred even more frequently in families with clustering of both breast cancer and CRC compared to families with clustering of breast cancer only (Meijers-Heijboer et al., 2003).

To evaluate the effect of the 1100delC mutation on CRC risk, we examined the *CHEK2* 1100delC

Supported by: Dutch Cancer Society; Grant numbers: RUG-97-1544 and RUG-98-1665.

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Received 8 October 2004; Accepted 1 March 2005

DOI 10.1002/gcc.20195

Published online 25 April 2005 in Wiley InterScience (www.interscience.wiley.com).

mutation in a large sample of unselected CRC cases, in selected CRC cases diagnosed before the age of 50, and in controls.

PATIENTS AND METHODS

Colorectal Cancer Patients and Controls

Unselected CRC cases ($n = 629$), selected CRC cases ($n = 105$), and controls ($n = 230$) were examined.

The unselected CRC patients and controls participated in the SCOPE study, which aimed to detect CRC susceptibility genes. The controls were the spouses of the unselected CRC patients. All participants were Caucasian and living in the northern part of the Netherlands. CRC patients were identified through the cancer registry of the Comprehensive Cancer Center Northern Netherlands and were subsequently invited to participate in the study by their physicians, either in the outpatient clinic or by letter via mail. Cases were diagnosed after 1989 and were included between December 1998 and December 2002, regardless of family history or age at diagnosis. About 60% of the invited subjects consented to participate. Patients completed a questionnaire that included questions on family history of colorectal and endometrial cancers. A positive family history (first- or second-degree relative) for CRC was reported by 30% of participants. No attempts were made to confirm cancer diagnoses in relatives.

All DNA samples and data in this study were handled anonymously, and patients were aware that they would not be informed about individual test results. For the SCOPE study, the unselected CRC patients were not screened for mutations in the mismatch-repair (MMR) genes.

To study the 1100delC mutation in different genetic risk groups, the unselected CRC cases were assigned to high-, moderate-, or low-genetic-risk groups on the basis of age at diagnosis and family history of colorectal and endometrial cancer. Families fulfilling the modified Amsterdam criteria were labeled high risk (Vasen and Wijnen, 1999). Patients with an age at diagnosis before 50 years or at least 2 first- or second-degree relatives with CRC (on the same side of the family) and not fulfilling the high-risk criteria were labeled moderate risk (Bradshaw et al., 2003; Grady, 2003; Rose et al., 2004). The remaining patients were considered low risk. Subgroup analyses were performed for tumor localization (proximal, distal, or rectal), gender, and age at diagnosis (before or after 50 years of age).

In addition, a pooled analysis was performed for the *CHEK2* 1100delC mutation and CRC by combining the data of the one published study that genotyped the mutation in unselected CRC patients (Kilpivaara et al., 2003) and data of the unselected CRC patients in the present study. In the pooled analysis, subgroup analysis was performed for sporadic and familial CRC cases, with the latter defined by the earlier study as patients with at least one first-degree relative affected by CRC (Kilpivaara et al., 2003).

A second group of CRC patients (the selected patients) was included in this study. These patients had participated in another study on the role of MMR gene mutations in individuals suspected of having HNPCC (Wu et al., 2001). One of the inclusion criteria was CRC diagnosed before the age of 50 years. The selected patients were screened for *MLH1*, *MSH2*, and *MSH6* germ-line mutations, and patients having no observed pathogenic mutation in one of the MMR genes were included in the present study.

The medical ethical committees of the participating hospitals approved both studies. All included subjects gave written informed consent.

Genotyping

DNA was extracted from 20 ml of EDTA-blood following standard procedures and stored at -80°C . Polymerase chain reaction (PCR) primers to amplify the 1100C/1100delC site were based on the published gene sequence and chosen with Primer3 software (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). Primer sequences were checked for specificity using BLAST (<http://www.ncbi.nlm.nih.gov/>). The forward primer was ATCACCTCC TACCAGTCTGTGC, and the reverse primer was GCAAGTTCAACATTATTCCTTT.

PCR reactions were performed in a volume of 10 μl , which included ~ 25 ng of DNA. For each PCR, 0.5 units *Taq* DNA polymerase (Amersham Pharmacia Biotech, Uppsala, Sweden) were used to amplify the fragments. Reaction mixtures contained 0.2 mM dNTP (Roche Diagnostics, Mannheim, Germany), 2.5 mM MgCl_2 , 10 mM Tris-HCl (pH 9.0), 50 mM KCl (Amersham Pharmacia Biotech), and 0.25 μM of each primer, with the 5' primer labeled with fluorochrome 6-FAM (Sigma, Malden, the Netherlands). Cycling was performed on a PTC-225 thermal cycler (MJ Research, Waltham, MA) and a PrimusHT (MWG Biotech, Ebersberg, Germany). A standard protocol was used for amplification. A 2.3- μl sample of the PCR product was mixed with 2.5 μl of MilliQ and 0.2 μl of ET-400R size standard (Amersham

TABLE 1. *CHEK2* 1100delC Variant in Colorectal Cancer Cases and Controls

	Individuals positive for the 1100delC variant/total number of individuals (%)	OR	95% CI
Controls	1/230 ^{a,b} (0.4%)	1	
Unselected colorectal cancer cases	10/629 (1.6%)	3.70	0.30–45.6
Low risk	5/498 ^a (1.0%)	2.32	0.17–32.0
Moderate risk	4/123 ^a (3.3%)	7.70	0.53–112
High risk	1/8 ^a (13%)	32.7	0.99–1082
Selected colorectal cases (<50), MMR gene mutation–negative	2/105 ^b (1.9%)	4.45	0.24–84.0

Abbreviations: OR, odds ratio; CI, confidence interval.

^aP value for trend 0.014 (controls, low risk, moderate risk, and high risk, corrected for 3 tests using a Bonferroni correction).

^bP value versus controls 0.55 (corrected for 3 tests using a Bonferroni correction).

Pharmacia Biotech) and separated on a MegaBACE 1000 capillary sequencer (Amersham Pharmacia Biotech) according to the manufacturer's protocol. Results were analyzed using Genetic Profiler, version 1.1 (Amersham Pharmacia Biotech). Scoring of the alleles was performed blinded for status of whether affected.

Statistical Methods

Present study

For the genotype association analysis, the frequencies of the genotypes were compared between patients and controls using the chi-square test or, when one or more of the expected numbers were smaller than 5, Fisher's exact test. A chi-square trend analysis on the different genetic risk groups was used to test whether the frequency of heterozygotes increased with genetic risk.

An ANOVA test was performed using age at diagnosis as dependent variable with the *CHEK2* 1100delC genotype a factor.

For the subgroup analyses, each subgroup was compared with the entire control group, assuming random mating and no stratification in the population.

A multiple testing correction was performed for three tests in the unselected patients, in the selected patients, and in the trend analysis and for six tests in the subgroup analyses, using a Bonferroni correction. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated, without adjustment for external variables, also corrected for three or six tests.

Pooled analysis

We tested the population studied by Kilpivaara et al. (2003) and our own group for homogeneity of the *CHEK2* 1100delC genotype frequency in order to validate pooling of the samples by the chi-square test with one degree of freedom. Both the case and control samples were sufficiently homoge-

TABLE 2. Unselected Colorectal Cancer Cases and Subgroup Characteristics

	Individuals positive for the 1100delC variant/total number of individuals (%)	P value ^a
Controls	1/230 (0.4%)	
Patients	10/629 (1.6%)	0.89
Tumor localization		
Proximal	3/227 (1.3%)	0.94
Distal	2/201 (1.0%)	1.00
Rectal	4/190 (2.1%)	0.70
Gender		
Male	2/331 (0.6%)	1.00
Female	8/295 (2.7%)	0.41
Age at diagnosis		
<50	3/68 (4.4%)	0.95
>50	7/560 (1.3%)	0.97

^aP values corrected for 6 tests using Bonferroni correction.

nous for the *CHEK2* 1100delC genotype frequency ($P = 0.22$ and 0.23 , respectively) for pooling to be valid. Therefore, the raw data from both studies on frequency of the *CHEK2* 1100delC genotype were combined. In addition, subgroup analysis was executed for a family history of CRC, as defined by Kilpivaara et al. (2003). A multiple testing correction was performed using a Bonferroni correction, implying that the reported P values and CIs were corrected for three independent tests. The ORs and 95% CIs were calculated, without adjustment for external variables (de Jong et al., 2002b).

RESULTS

A total of 629 unselected CRC patients, 105 selected CRC patients, and 230 controls were studied. The mean age at diagnosis was 64.5 years (range 25–87 years) in the unselected patients and 42.4 years (range 20–49 years) in the selected patients.

In the unselected CRC patients, the 1100delC mutation was detected 10 times (1.6%, Table 1).

TABLE 3. ANOVA Test of Age at Diagnosis

	Wild-type <i>CHEK2</i>		1100delC genotype		<i>P</i> value ^a
	Patients	Mean age at diagnosis	Patients	Mean age at diagnosis	
All colorectal cancer cases	720	61.4	12	57.5	0.36
Unselected colorectal cancer cases	617 ^b	64.5	10	59.6	0.19
Selected colorectal cases (<50) ^c	103	42.3	2	47.0	0.33

^a*P* values corrected for 6 tests using a Bonferroni correction.^bFor 2 cases, age at diagnosis was not known.^cMMR gene mutation negative.TABLE 4. Pooled Analysis of *CHEK2* 1100delC Mutation and Unselected Colorectal Cancer Patients

	Individuals positive for the 1100delC mutation/total number of individuals (%)			<i>P</i> value ^{a,b}	OR	95% CI ^b
	Present study	Kilpivaara, 2003	Pooled Analysis			
Controls	1/230 (0.4%)	26/1,885 (1.4%)	27/2,115 (1.3%)			
Unselected colorectal cancer cases	10/629 (1.6%)	17/662 (2.6%)	27/1,291 (2.1%)	0.18	1.65	0.86–3.18
Familial colorectal cancer cases	4/126 (3.2%)	2/149 (1.3%)	6/275 (2.2%)	0.54	1.72	0.52–5.72

Abbreviations: OR, odds ratio; CI, confidence interval.

^a*P* values were calculated by either a chi-square test or, when one or more of the expected values was smaller than 5, a Fisher's exact test.^bCorrected for 3 tests using a Bonferroni correction.

This frequency was not significantly different from that in controls (0.4%). However, when the patients were stratified according to the assumed genetic risk group (low, moderate, or high), the highest frequency was observed in the high-risk group (1 of 8, 12.5%), followed by that in the moderate-risk group (4 of 123, 3.3%), with the lowest frequency in the low-risk group (5 of 498, 1.0%). This trend of increasing frequency was significant ($P_{\text{trend}} = 0.014$). When we excluded patients who fulfilled the criteria for the high-risk group, that is, the Amsterdam criteria, because of a first-degree relative with endometrial cancer, the trend remained significant ($P_{\text{trend}} = 0.039$).

The subgroup analyses for tumor localization, gender, and age at diagnosis did not reveal any specific association of CRC with the 1100delC genotype (Table 2). Also, no difference in mean age at diagnosis was observed between patients with and without the 1100delC mutation in both the unselected and selected CRC cases (Table 3).

In the 68 unselected patients diagnosed before the age of 50, the mutation was detected three times (4.4%, P versus controls = 0.95). In 105 selected CRC cases (all diagnosed before the age 50), two (1.9%) carried the 1100delC mutation, a frequency that again was not significantly different from that in the controls ($P = 0.55$). Combining all patients diagnosed before the age of 50 (i.e., 5 of 173, 2.9%) also did not reveal an association with the 1100delC mutation ($P = 0.38$).

The pooled analysis (Table 4) did not show a difference in the genotype frequency of the 1100delC mutation either between unselected CRC patients and controls or between familial CRC cases and controls.

DISCUSSION

In the present study, the unselected CRC patients showed no increased frequency of the *CHEK2* 1100delC genotype compared to controls. However, we observed a trend of increasing frequency of the *CHEK2* 1100delC mutation controls via low- and moderate-risk to high-genetic-risk individuals.

There was no association of the 1100delC genotype with tumor localization, gender, or age at diagnosis. The selected CRC patients (diagnosed before the age of 50 years) revealed no increased frequency of the 1100delC genotype. There also was no difference in mean age at diagnosis between patients with and without the 1100delC mutation in both the unselected and the selected CRC cases.

So far, three studies have examined the *CHEK2* 1100delC mutation in relation to colorectal cancer (Kilpivaara et al., 2003; Lipton et al., 2003; Meijers-Heijboer et al., 2003). In the first study, by Meijer-Heijboer et al. (2003), the 1100delC mutation was analyzed in HNPCC and HNPCC-like (two first-degree relatives with colorectal cancer, at least one of which was diagnosed before the age of 50 years) patients. The frequency of the 1100delC

mutation was somewhat higher in MMR gene mutation carriers (2.4%, 3 of 127) and in patients without MMR gene mutations (2.6%, 3 of 107) compared with the controls (1.1%, 18 of 1,620), but this difference was not statistically significant ($P = 0.07$). In the same study, the 1100delC mutation was not observed in 95 familial adenomatous polyposis families (Meijers-Heijboer et al., 2003). Kilpivaara et al. (2003) analyzed 662 colorectal cancer patients. The frequency of the 1100delC mutation was 1.3% (2 of 149) in CRC patients whose disease was considered familial (defined as having at least one first-degree relative with CRC) and 2.9% (15 of 513) in sporadic CRC patients, which were not higher than the geographically adjusted population frequency of 1.9% (Kilpivaara et al., 2003). In the third study, by Lipton et al. (2003), the frequency of the 1100delC mutation was 2% (3 of 149) in multiple (5–100) colorectal adenoma patients, compared to 1.1% (18 of 1,620) in controls, again not a statistically significant difference. In the latter study, all patients were screened for mutations of the *APC* and *MYH* genes, and no pathogenic mutations were detected in those genes.

One other study (Kilpivaara et al., 2003) analyzed the association of CRC with the 1100delC mutation in unselected patients. Kilpivaara et al. (2003) also did not find a significant difference between patients and controls in the frequency of the mutation (reported $P = 0.27$). Pooling the raw data from the study by Kilpivaara et al. (2003) with those of our own study again did not reveal a difference between unselected CRC patients and controls.

With an OR of ~ 1.65 and a low genotype frequency (1%), a sample size of more than 3,000 cases is needed to detect an association with a power of 90% (de Jong et al., 2002a). Thus, the pooled sample size of the unselected CRC cases from these studies was still too small to detect an association. With the present pooled sample size ($\sim 1,300$), increased risk with an OR above 2.1 has been excluded.

Kilpivaara et al. (2003) performed subgroup analysis of familial CRC patients (defined as those having at least one first-degree relative with CRC). The frequency of the 1100delC mutation was 1.3% (2 of 149) in familial CRC patients and 2.9% (15 of 513) in sporadic CRC patients, which was not higher than the geographically adjusted population frequency of 1.9%. In our study, 126 unselected CRC patients had at least one first-degree relative with CRC, thereby fulfilling the definition of Kilpivaara et al. (2003) for familial CRC. Four of these 126 carried the 1100delC mutation. When

both studies were combined, no difference was observed between familial CRC and controls in genotype frequency of the mutation. Nevertheless, we observed that the higher the genetic risk of CRC, the more frequent was the 1100delC mutation, suggesting that this mutation has some kind of a role as a promoter or modifier of colorectal carcinogenesis. To some extent, this hypothesis is supported by the finding of Meijers-Heijboer et al. (2003) that the 1100delC mutation occurred somewhat more frequently in HNPCC and HNPCC-like patients (2.6%, 6 of 234) than in controls (1.1%, 18 of 1,620; $P = 0.07$).

We did not collect information from our patients about the prevalence of breast cancer in their families, so we cannot comment on the association of the combined familial occurrence of breast and colorectal cancer and the 1100delC mutation.

In conclusion, the frequency of the 1100delC genotype was not significantly increased either in unselected CRC patients or in CRC patients selected for age at diagnosis before 50 years. The pooled analysis showed that increased risk with an OR above 2.1 could be excluded. However, after stratifying the unselected CRC patients according to defined genetic risk, a significant trend of increasing frequency was observed. Together, the results are consistent with a low-penetrance effect (OR 1.5–2.0) of the *CHEK2* 1100delC on CRC risk. Large case-control studies are required to clarify the exact role of the *CHEK2* 1100delC mutation in CRC.

ACKNOWLEDGMENTS

We thank all families who donated their DNA to this project. We also thank coworkers of the Departments of Surgery of the University of Groningen Medical Center, the Medical Center Leeuwarden and Harlingen, Hospital Ny Smellinthe Drachten, Hospital Talma Sionsberg Dokkum, the Anthonius Hospital Sneek, and the Martini Hospital Groningen and coworkers of the Departments of Gastroenterology of the University of Groningen Medical Center and the Martini Hospital Groningen. Furthermore, we thank Giny Bokma and Gerry Sieling and the blood bank Sanguin Noord-Oost (Groningen, The Netherlands) for their work in enrolling patients and collecting blood, respectively.

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