

## ELECTRONIC LETTER

**CHEK2 1100delC and colorectal cancer**

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Cell cycle checkpoint kinase 2 (CHEK2) is a tumour suppressor involved in the p53 pathway of DNA damage responses. 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 Bartek *et al*). A protein truncating mutation, 1100delC, which resides in exon 10 and abolishes the kinase function of CHEK2, has been shown to be significantly associated with a positive family history of breast cancer.<sup>2,3</sup> This allele is found with a 1.1–1.4% frequency in the normal population in the European countries studied so far but at a 4.9–5.9% frequency among familial *BRCA1/2* negative breast cancer patients.<sup>2,3</sup> The 1100delC allele appears to be a low penetrance susceptibility allele for breast cancer, with a twofold increased breast cancer risk for carriers.<sup>2</sup> Expression of the CHEK2 protein has been shown to be absent or grossly reduced in breast tumours of heterozygous 1100delC mutation carriers,<sup>3</sup> and loss of the wild-type allele has been reported in a breast tumour and a sarcoma of *CHEK2* mutation carriers in Li-Fraumeni syndrome (LFS).<sup>4</sup>

Very recently, the frequency of the 1100delC allele has been suggested to be higher among breast cancer families that also have colorectal cancer (CRC) than in those without CRC, identifying a hereditary breast and colorectal cancer phenotype (HBCC).<sup>5</sup> To evaluate the significance of the 1100delC allele for colorectal cancer we studied the frequency of the 1100delC in 662 colorectal cancer patients, including 149 familial CRC patients. We also studied the allelic imbalance at 1100delC in the colorectal tumours from patients with a germline 1100delC mutation.

**MATERIALS AND METHODS****Patients**

From a population based series of 1042 colorectal cancer cases described previously,<sup>6,7</sup> and a set of unselected CRC cases, 662 CRC patient DNA samples were selected. DNA was extracted either from blood or normal colon mucosa (separate site from tumour) according to standard procedure.<sup>8</sup> Informed consent was obtained from participating patients. All samples had been previously analysed for microsatellite instability (MSI) using polyA marker BAT26, a robust marker for high level MSI detection<sup>6,7</sup> (unpublished data). Of the 662 samples, 51 were MSI positive, and these samples were screened for germline mutations in the known familial CRC genes *MLH1* and *MSH2*. Of these 51 MSI positive cases, seven had mutations in one of these genes.<sup>6,7</sup> The series of 662 samples included 149 familial and 513 sporadic CRC cases. Familial CRC cases were defined as CRC probands with at least one first degree relative affected with CRC. In addition, the series included 14 CRC patients with breast cancer, and 99 CRC patients whose first degree relative had breast cancer. Four of these 113 pedigrees contained two breast cancer patients. The majority (70%) of the 662 CRC patient samples were collected and the patients originated from eastern Finland. We have previously defined a 1.4%

**Key points**

- *CHEK2* 1100delC is a low penetrance susceptibility allele identified in breast cancer families. Very recently, the allele was further suggested to associate with a hereditary breast and colorectal cancer phenotype.
- To assess the role of 1100delC in colorectal cancer, we studied the frequency of the 1100delC allele in 662 colorectal cancer patients, including 149 familial and 513 non-familial cases.
- We detected frequencies of 2.6% (17/662) for the whole patient cohort, 1.3% (2/149) for familial and 2.9% (15/513) for non-familial cases, which are not significantly higher than the geographically adjusted population frequency of 1.9% ( $p=0.266$ ,  $p=1.000$ ,  $p=0.134$ , respectively). A low frequency (3/17; 17.6%) of the loss of the wild-type allele was observed in the colorectal tumours of the carriers, and in one tumour the mutant allele was lost.
- Our findings suggest that the *CHEK2* 1100delC may not be a susceptibility allele for colorectal cancer; however, a very low penetrance effect on colorectal cancer could not be excluded.

population frequency of *CHEK2* 1100delC in Finland by screening 1885 healthy controls obtained from the Finnish Red Cross Blood Transfusion Service.<sup>3</sup> A slight variation in the population frequency is seen in eastern v other parts of Finland, and an adjusted frequency matching the geographical distribution of the CRC patient material was 1.9%.

**Mutation detection**

Specific PCR primers (described in detail in reports by Vahteristo *et al*<sup>3,9</sup>) for the *CHEK2* exon 10 on chromosome 22 were designed to avoid amplification of the homologous sequences on other chromosomes. The 1100delC variant was screened using minisequencing (primer extension),<sup>10</sup> and for this purpose an internal pair of primers was used in addition to the original exon 10 primers (nested PCR). All positive minisequencing results were confirmed by reamplification from the original genomic DNA sample using another independent detection method, either direct sequencing (ABI Big Dye Cycle Sequencing Kit version 3.0, ABI Prism 310 Genetic Analyzer; Applied Biosystems, Foster City, CA, USA) or conformation sensitive gel electrophoresis.<sup>11</sup>

**Abbreviations:** CHEK2, cell cycle checkpoint kinase 2; CRC, colorectal cancer; HBCC, hereditary breast and colorectal cancer phenotype; HNPCC, hereditary nonpolyposis colorectal cancer; LFS, Li-Fraumeni syndrome; MSI, microsatellite instability

### Analysis of allelic imbalance

Allelic imbalance at the 1100delC was studied in the colorectal tumours from all 17 patients with a germline 1100delC mutation. Tumour DNA was extracted from fresh frozen specimens. A pathologist evaluated the proportion of tumour tissue histologically before DNA extraction. All samples displayed greater than 55% carcinoma tissue, typically 60–80%. *CHEK2* exon 10 was PCR amplified and sequenced for analysing allelic imbalance.

In statistical analyses, differences in dichotomous variables were tested by Pearson  $\chi^2$  test or by Fisher's exact test using SPSS version 11.0 for Windows (SPSS Inc., Chicago, IL, USA). All *p* values are two sided.

### RESULTS AND DISCUSSION

The frequency of the *CHEK2* 1100delC among colorectal cancer patients was 2.6% (17/662). The proportions were 1.3% (2/149) and 2.9% (15/513) in familial and non-familial cases, respectively. These frequencies are not significantly higher (odds ratio (OR) 1.393, 95% confidence interval (CI) 0.775 to 2.504, *p* = 0.266, for all cases; OR 0.720, 95% CI 0.172 to 3.020, *p* = 1.000 for familial cases and OR 1.592, 95% CI 0.863 to 2.939, *p* = 0.134 for non-familial cases) than in the normal population, compared with the geographically adjusted population frequency of 1.9%. These results suggest that the 1100delC variant is not significantly associated with familial colorectal cancer or with colorectal cancer risk in the population; however, larger studies would be needed to detect or exclude any slight increase with a high confidence. In the group of non-familial colorectal cancer cases, there was no difference in allele frequency among those patients with a personal or family history of breast cancer (3/104, 2.9%) and those without a breast cancer history (12/409, 2.9%). Among the familial CRC cases, 1/9 with breast cancer in the family carried the 1100delC allele while only 1/140 (0.7%) of those without a breast cancer family history did so. The *CHEK2* mutation carrier belonged to a family with two members affected with breast cancer. Altogether, four CRC patients had two breast cancer cases in the family and one (25%) was found to carry the 1100delC variant, which is consistent with the association of the 1100delC allele with familial breast cancer. Two colorectal cancers in the 17 1100delC carriers had previously been found positive for MIS, but neither patient has a germline mutation in the *MLH1* or *MSH2* gene.

Tumour DNA from all 17 CRC patients positive for the 1100delC (two familial and 15 non-familial) was analysed for allelic imbalance at *CHEK2* exon 10. In three cases, the wild-type allele appeared to be lost (one familial, two non-familial); however, in one sporadic tumour sample the mutated allele was lost. The other 13 samples showed no *CHEK2* exon 10 allelic imbalance in tumour DNA. We have previously shown a gross reduction or loss of the *CHEK2* protein expression in familial breast tumours of 1100delC carriers.<sup>3</sup> Loss of the wild type allele has been reported in a sarcoma and a breast tumour of 1100delC or R145W *CHEK2* mutation carriers in Li-Fraumeni families.<sup>4</sup> In another study on familial breast cancer, however, one breast tumour did not show allelic imbalance of the 1100delC allele, while contradictory results were obtained for the other case studied.<sup>12</sup> Thus, alternative inactivation mechanisms may also be possible. However, the low frequency of the allelic imbalance observed in the CRC cases supports the view that the *CHEK2* 1100delC allele may not be a susceptibility allele for CRC.

Very recently, it has been proposed that the 1100delC allele identifies families with an hereditary breast and colorectal cancer phenotype (HBCC), with a significantly higher frequency among the HBCC families (10/55, 18.2%) than in breast cancer families without CRC (15/380, 4.0%).<sup>5</sup> An HBCC

family was defined as including at least two first or second degree relatives affected with breast cancer, of which at least one had been diagnosed before 60 years of age, and either: (a) at least one breast cancer case with CRC, or (b) a first/second degree relative to a breast cancer case diagnosed with CRC before 50 years of age, or (c) two or more CRC cases of which at least one was a first/second degree relative to a breast cancer case. Among our cohort of 507 familial breast cancer cases previously analysed for the 1100delC allele,<sup>3</sup> 80 families (15.8%) also include CRC cases, and 19 of these (3.7% of all) fulfil the definition of HBCC. However, among this cohort, the frequency of the 1100delC allele is not higher in the families with CRC (4/80 (5.0%) among all breast cancer families with CRC and 1/19, 5.3% among HBCC families) than among those without CRC (24/427 (5.6%)). Among the Dutch families, the 1100delC allele was suggested to act in concert with another putative allele predisposing to the HBCC phenotype, and possibly population specific differences in the prevalence of such alleles could underlie the difference seen between the Dutch and Finnish families with the HBCC phenotype. However, among the Dutch HNPCC (hereditary nonpolyposis colorectal cancer) or HNPCC-like CRC families, the association of the 1100delC allele with the HBCC-like phenotype was not unequivocal and any CRC risk associated with 1100delC was suggested to be low.<sup>5</sup> As the number of HBCC families in either study is quite small, larger studies will be needed to evaluate the suggested association between 1100delC and the HBCC phenotype, as well as possible very low penetrance effects of the 1100delC on colorectal cancer predisposition.

The *CHEK2* gene was first suggested as a susceptibility gene for Li-Fraumeni syndrome (LFS) upon identification of the 1100delC and another variant I157T in LFS families.<sup>13</sup> While neither appear to be a high penetrance LFS susceptibility allele,<sup>9, 14</sup> an additive or modifying effect on putative other susceptibility alleles in LFS or variant LFS families may be possible. In individual variant LFS families, an R145W mutation with deleterious effect on *CHEK2* function, as well as a missense mutation, R3W, of unknown function have been identified,<sup>9</sup> while in another report, no *CHEK2* germline variants were found in variant LFS families.<sup>15</sup> Recently, several novel germline variants in the *CHEK2* gene have been reported in prostate cancer patients, with a significant association of the overall variant frequency with sporadic but not with familial prostate cancer.<sup>15</sup> The 1100delC was found in germline DNA in 2/698 cases (0.3%); however, larger studies would be needed to evaluate whether the 1100delC is a risk factor for prostate cancer.<sup>16</sup> Rare *CHEK2* germline variants have been identified in some other cancer patients also. In Iceland, a T59K variant of unknown functional significance found in *CHEK2* mutation analysis of 120 breast tumours, was also identified in 2/119 colorectal, 4/685 breast, 1/37 stomach, and 1/43 ovarian cancer patients but not in 452 normal controls, and was suggested to be a low penetrance variant.<sup>17</sup> Another rare variant, R117G, has been recently reported in two familial breast cancer cases as well.<sup>18</sup>

In conclusion, while rare *CHEK2* variants have been identified in several cancer types, the 1100delC allele has been found to be significantly associated with familial breast cancer. In this study, no significant association between germline *CHEK2* 1100delC and familial or sporadic colorectal cancer was found. A very low penetrance effect on CRC could not be excluded.

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#### REFERENCES

- 1 **Bartek J**, Falck J, Lukas J. CHK2 Kinase—a busy messenger. *Nature Rev* 2001;**2**:877–86.
- 2 **Meijers-Heijboer H**, van den Ouweland A, Klijn J, Wasielewski M, de Snoo A, Oldenburg R, Hollestelle A, Houben M, Crepin E, van Veghel-Plandsoen M, Elstrodt F, van Duijn C, Bartels C, Meijers C, Schutte M, McGuffog L, Thompson D, Easton D, Sodha N, Seal S, Barfoot R, Mangion J, Chang-Claude J, Eccles D, Eeles R, Evans DG, Houlston R, Murday V, Narod S, Peretz T, Peto J, Phelan C, Zhang HX, Szabo C, Devilee P, Goldgar D, Futreal PA, Nathanson KL, Weber B, Rahman N, Stratton MR, the CHEK2-Breast Cancer Consortium. Low-penetrance susceptibility to breast cancer due to CHEK2(\*1100delC) in noncarriers of BRCA1 or BRCA2 mutations. *Nat Genet* 2002;**31**:55–9.
- 3 **Vahteristo P**, Bartkova J, Eerola H, Syrjakoski K, Ojala S, Kilpivaara O, Tamminen A, Kononen J, Aittomaki K, Heikkila P, Holli K, Blomqvist C, Bartek J, Kallioniemi OP, Nevanlinna H. A CHEK2 genetic variant contributing to a substantial fraction of familial breast cancer. *Am J Hum Genet* 2002;**71**:432–8.
- 4 **Lee SB**, Kim SH, Bell DW, Wahrer DC, Schiripo TA, Jorczak MM, Sgroi DC, Garber JE, Li FP, Nichols KE, Varley JM, Godwin AK, Shannon KM, Harlow E, Haber DA. Destabilization of Chk2 by a missense mutation associated with Li-Fraumeni syndrome. *Cancer Res* 2001;**61**:8062–7.
- 5 **Meijers-Heijboer H**, Wijnen J, Vasen H, Wasielewski M, Wagner A, Hollestelle A, Elstrodt F, van den Bos R, de Snoo A, Fat GT, Brekelmans C, Jagmohan S, Franken P, Verkuijlen P, van den Ouweland A, Chapman P, Tops C, Moslein G, Burn J, Lynch H, Klijn J, Fodde R, Schutte M. The CHEK2 1100delC mutation identifies families with an hereditary breast and colorectal cancer phenotype. *Am J Hum Genet*. 2003;**72**: (electronically published April 10, 2003).
- 6 **Aaltonen LA**, Salovaara R, Kristo P, Canzian F, Hemminki A, Peltomaki P, Chadwick RB, Kaariainen H, Eskelinen M, Jarvinen H, Mecklin JP, de la Chapelle A. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. *N Engl J Med* 1998;**338**:1481–7.
- 7 **Salovaara R**, Roth S, Loukola A, Launonen V, Sistonen P, Avizienyte E, Kristo P, Jarvinen H, Souhelnytskyi S, Sarlomo-Rikala M, Aaltonen LA. Population-based molecular detection of hereditary nonpolyposis colorectal cancer. *J Clin Oncol* 2000;**18**:2193–200.
- 8 **Lahiri DK**, Nurnberger J Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 1991;**19**:5444.
- 9 **Vahteristo P**, Tamminen A, Karvinen P, Eerola H, Eklund C, Aaltonen LA, Blomqvist C, Aittomaki K, Nevanlinna H. p53, Chk2, and Chk1 genes in Finnish families with Li-Fraumeni syndrome: Further evidence of Chk2 in inherited cancer predisposition. *Cancer Res* 2001;**61**:5718–22.
- 10 **Syvanen AC**, Sajantila A, Lukka M. Identification of individuals by analysis of biallelic DNA markers, using PCR and solid-phase minisequencing. *Am J Hum Genet* 1993;**52**:46–59.
- 11 **Ganguly A**, Rock MJ, Prockop DJ. Conformation-sensitive gel electrophoresis for rapid detection of single-base differences in double-stranded PCR products and DNA fragments: evidence for solvent-induced bends in DNA heteroduplexes. *Proc Natl Acad Sci USA* 1993;**90**:10325–9.
- 12 **Sodha N**, Bullock S, Taylor R, Mitchell G, Guertl-Lackner B, Williams RD, Bevan S, Bishop K, McGuire S, Houlston RS, Eeles RA. CHEK2 variants in susceptibility to breast cancer and evidence of retention of the wild type allele in tumours. *Br J Cancer* 2002;**87**:1445–8.
- 13 **Bell DW**, Varley JM, Szydlow TE, Kang DH, Wahrer DC, Shannon KE, Lubratovich M, Verselis SJ, Isselbacher KJ, Fraumeni JF, Birch JM, Li FP, Garber JE, Haber DA. Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. *Science* 1999;**286**:2528–31.
- 14 **Sodha N**, Bullock S, Taylor R, Mitchell G, Guertl-Lackner B, Williams RD, Bevan S, Bishop K, McGuire S, Houlston RS, Eeles RA. Increasing evidence that germline mutations in CHEK2 do not cause Li-Fraumeni syndrome. *Hum Mutat* 2002;**20**:460–2.
- 15 **Bougeard G**, Limacher J-M, Martin C, Charbonnier F, Killian A, Delattre O, Longy M, Jonveaux P, Fricker J-P, Stoppa-Lyonnet D, Flaman J-M, Fréburg T. Detection of 11 germline inactivating TP53 mutations and absence of TP63 and HCHK2 mutations in French families with Li-Fraumeni or Li-Fraumeni-like syndrome. *J Med Genet* 2001;**38**:253–7.
- 16 **Dong X**, Wang L, Taniguchi K, Wang X, Cunningham JM, McDonnell SK, Qian C, Marks AF, Slager SL, Peterson BJ, Smith DI, Chevillet JC, Blute ML, Jacobsen SJ, Schaid DJ, Tindall DJ, Thibodeau SN, Liu W. Mutations in CHEK2 associated with prostate cancer risk. *Am J Hum Genet* 2003;**72**:270–80.
- 17 **Ingvarsson S**, Sigbjornsdottir BI, Huiping C, Hafsteinsdottir SH, Ragnarsson G, Barkardottir RB, Arason A, Egilsson V, Bergthorsson JT. Mutation analysis of the CHEK2 gene in breast carcinoma and other cancers. *Breast Cancer Res* 2002;**4**:R4.
- 18 **Schutte M**, Seal S, Barfoot R, Meijers-Heijboer H, Wasielewski M, Evans DG, Eccles D, Meijers C, Lohman F, Klijn J, van den Ouweland A, Futreal PA, Nathanson KL, Weber BL, Easton DF, Stratton MR, Rahman N, Breast Cancer Linkage Consortium. Variants in CHEK2 other than 1100delC do not make a major contribution to breast cancer susceptibility. *Am J Hum Genet* 2003;**72**:1023–8.