The CHEK2 1100delC Variant in Swedish Colorectal Cancer

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Abstract. Background: The cell cycle checkpoint kinase 2 (CHEK2) 1100delC variant has recently been identified at high frequency in families with both breast and colorectal cancer, suggesting the possible role of this variant in colorectal cancer predisposition. Patients and Methods: To evaluate the role of CHEK2 1100delC among Swedish colorectal cancer patients, the variant frequency was determined in 174 selected familial cases, 644 unselected cases and 760 controls, as well as in 18 families used in the genome-wide linkage analysis, where weak linkage was seen for the region harboring the CHEK2 gene. Results: CHEK2 1100delC was found in 1.15% of familial and in 0.93% of unselected cases, compared to 0.66% of controls, showing no significant difference between groups. One out of 45 familial cases with a family history of breast cancer was shown to be a carrier. The variant was not identified in the 18 families included in the linkage analysis. Conclusion: The CHEK2 1100delC was not significantly increased in Swedish colorectal cancer patients, however, in order to determine the role of the variant in colorectal cancer families with the history of breast cancer a larger sample size is needed.

The genetic contribution in colorectal cancer has been estimated to be ~35% (1). However, to date, high-penetrance mutations in any of the known colorectal cancer predisposing genes have been found in 5% or less of families with an inherited predisposition to colorectal cancer (2). Despite intensive efforts there has been little success in identifying additional high-penetrance colorectal cancer predisposing genes, which may suggest the role of low to moderate penetrance alleles in as yet unknown genes that could account for a substantial fraction of familial colorectal cancers.

Cell cycle checkpoint kinase 2 (CHEK2) is a human homologue of the yeast Cds1 and Rad53 kinases. In the presence of DNA damage induced by ionizing radiation CHEK2 is activated by ATM and, through phosphorylation of substrates like TP53, BRCA1, CDC25A and CDC25C, is involved in regulation of cell cycle arrest and DNA repair (3). The protein truncating mutation 1100delC that abolishes kinase function of CHEK2 has been suggested to act as a low penetrance breast cancer susceptibility allele in families without BRCA1/2 mutations (4, 5). This variant has been found among healthy individuals with a frequency of

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about 1%, while the frequency among non-BRCA1/BRCA2 familial patients was ~5% (4, 5). The frequency was, however, suggested to be higher among families with hereditary breast and colorectal cancer (18%), identifying the hereditary breast and colorectal cancer phenotype (HBCC) (6). It was also suggested that the variant is not the major predisposing factor for HBCC phenotype, but instead acts with another, as yet unidentified predisposing gene (4).

In a recently performed genome-wide linkage analysis in 18 non-HNPPC/non-FAP colorectal cancer families from Sweden, no single predisposing locus was identified (7). Three chromosomes were, however, suggested to be of interest when the analysis was performed under the assumption of locus heterogeneity. One of the suggested chromosomes was chromosome 22 where the heterogeneity LOD (HLOD) score of 1.25 for α=0.60, where α value represents a proportion of families, was observed for the region around marker D22S315, where the CHEK2 gene resides.

To determine the effect of CHEK2 1100delC on colorectal cancer risk the frequency of the variant among Swedish familial and non-familial colorectal cancer cases and in controls was studied.

**Patients and Methods**

**Patient material.** In total 1,047 patients and 760 controls were tested for the CHEK2 1100delC variant. Three hundred eighty-two patients were from families where two or more family members were affected with colorectal cancer. These families were recruited from the Cancer Family Clinic at Karolinska University Hospital, Sweden, where they underwent counseling due to the suggested inheritance of the disease. A family history including clinicopathological details was obtained by interview and confirmed by medical records, pathological reports or death certificates. None of these families had classical or attenuated polyposis. HNPCC was separated on 2% agarose gels stained with ethidium bromide.

**Statistical analysis.** Comparison of the CHEK2 1100delC variant frequency between cases and controls was done using Fisher’s exact test.

**Results and Discussion**

Genotyping of the CHEK2 1100delC variant was successful in 94.2% of patients and 100% of controls (Table I). Of the 192 selected familial cases 174 were genotyped; 45 out of 47 cases with a family history of breast cancer and 129 out of 145 cases without reported family history of breast cancer. A family history including clinicopathological details was obtained by interview and confirmed by medical records, pathological reports or death certificates. None of these families had classical or attenuated polyposis. HNPCC was separated on 2% agarose gels stained with ethidium bromide.

**Statistical analysis.** Comparison of the CHEK2 1100delC variant frequency between cases and controls was done using Fisher’s exact test.
Eighteen families included in the linkage analysis were tested for the prevalence of CHEK2 1100delC variant in this study. Analysis was not restricted to index cases only but to all available family members (190 individuals) who had been genotyped for the linkage analysis. None of the tested individuals was found to be a carrier of CHEK2 1100delC. Although the effect of this variant on colorectal cancer was excluded in these 18 families, the region on chromosome 22q is still of interest.

Eight out of 818 successfully genotyped cases were shown to be carriers of CHEK2 1100delC (Table I). The frequency in selected familial cases and unselected cases was 1.15% and 0.95%, respectively, showing no over-representation in either of the two groups tested (Table I). In the familial group with a family history of breast cancer one out of 45 successfully genotyped cases (2.22%) carried CHEK2 1100delC, whereas a variant frequency of 0.78% was detected in selected familial cases without a family history of breast cancer. The slightly increased variant frequency seen in relation to breast cancer was not significant. However, due to the small sample size, the comparison was underpowered. The only CHEK2 variant carrier detected in the familial group with breast cancer was a patient diagnosed with colorectal cancer at the age of 48, bilateral breast cancer at 57 and 69 years of age and melanoma at the age of 55. There were two additional cases of breast cancer and one case of colorectal cancer in her family. None of the 160 unselected cases with breast cancer in the family carried the CHEK2 1100delC variant.

Meijers-Heijboer et al. have reported a CHEK2 1100delC frequency of 18% among families with hereditary breast and colorectal cancer (HBCC families). In the same study a variant frequency of 4.5% was detected in HNPPC and HNPCC-like families with HBCC-like colorectal cancer, suggesting that the variant also confers a colorectal cancer risk, although lower than in the case of breast cancer (6). The CHEK2 1100delC variant frequency analysis of females diagnosed with at least one breast and one colorectal cancer from the south of Sweden did not detect an increase in the frequency among cases (11). Consistent with our result, studies of this variant and colorectal cancer cases from the Netherlands and Finland failed to show a significant difference in the frequency between cases and controls, although these results may still be compatible with the slightly increased risk of 1.5-2.0 (12, 13). In addition, it has been shown that the variant was unlikely to be of importance in patients with multiple colorectal adenomas (14).

In conclusion, the CHEK2 1100delC variant was detected at a low frequency in colorectal cancer cases from Sweden. No over-representation of the variant was detected in either selected familial cases or in unselected cases. One out of 45 familial cases with a family history of breast cancer was determined to be a carrier of the variant. Due to the low frequency of the variant in our population we were not able to exclude a very low penetrance effect among Swedish colorectal cancer cases. A larger sample set is necessary to evaluate the role of CHEK2 1100delC in colorectal cancer families with HBCC-like phenotype.

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