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Report

CHEK2 Is a Multiorgan Cancer Susceptibility Gene

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A single founder allele of the *CHEK2* gene has been associated with predisposition to breast and prostate cancer in North America and Europe. The CHEK2 protein participates in the DNA damage response in many cell types and is therefore a good candidate for a multisite cancer susceptibility gene. Three founder alleles are present in Poland. Two of these result in a truncated CHEK2 protein, and the other is a missense substitution of an isoleucine for a threonine. We ascertained the prevalence of each of these alleles in 4,008 cancer cases and 4,000 controls, all from Poland. The majority of the common cancer sites were represented. Positive associations with protein-truncating alleles were seen for cancers of the thyroid (odds ratio [OR] 4.9; $P = .0006$), breast (OR 2.2; $P = .02$), and prostate (OR 2.2; $P = .04$). The missense variant I157T was associated with an increased risk of breast cancer (OR 1.4; $P = .02$), colon cancer (OR 2.0; $P = .001$), kidney cancer (OR 2.1; $P = .0006$), prostate cancer (OR 1.7; $P = .002$), and thyroid cancer (OR 1.9; $P = .04$). The range of cancers associated with mutations of the *CHEK2* gene may be much greater than previously thought.

The *CHEK2* gene (MIM +604373) encodes the human analogue of the yeast checkpoint kinases Cds1 and Rad53 (Matsuoka et al. 1998). Activation of CHEK2 in response to DNA damage prevents the cell from entering into mitosis. The frequency of the 1100delC variant is 0.2%–1.4% in European populations (Meijers-Heijboer et al. 2002; CHEK2 Breast Cancer Case-Control Consortium 2004), but the allele appears to be infrequent in North America (Offit et al. 2003). This allele has been found to confer an elevated risk of breast cancer (Meijers-Heijboer et al. 2002; Offit et al. 2003; CHEK2 Breast Cancer Case-Control Consortium 2004) and prostate cancer (Dong et al. 2003; Seppala et al. 2003). It has also been reported in excess among Dutch families with aggregates of both breast and colon cancer (Meijers-Heijboer et al. 2003). In Poland, there are three polymorphic variants of *CHEK2*, which, in aggregate, are present in 5.5% of the population. Two of these (1100delC and IVS2+1G→A) are rare and result in premature protein truncation; the third is a common missense variant (I157T) that results in the substitution of an isoleucine for a threonine. We have recently reported that all three variants are associated with an increased risk of prostate cancer in Poland (Cybulski et al. 2004). The CHEK2 protein is expressed in a wide range of tissues, and the full range of cancers associated with inactivating *CHEK2* mutations has not yet been determined.

To establish the range of cancer types associated with *CHEK2* mutations, we genotyped 4,008 cases of cancer and 4,000 controls in Poland. We included 7 of the 10 most common types of cancer in Poland, and, together, these sites are representative of about two-thirds of all cancer cases in the country. Cases were collected from hospitals in Szczecin and surrounding counties. Study subjects were asked to participate at the time of diagnosis or during outpatient visits to surgical and medical oncology clinics. In general, patient participation rates exceeded 80% for each cancer site. Study subjects were unselected for age, sex, and family history.

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Three control groups were combined. The first group consisted of 2,000 newborn children from 10 hospitals throughout Poland (Szczecin, Bialystok, Gorzow, Katowice, Wroclaw, Poznan, Opole, Lodz, and Rzeszow) in 2003 and 2004. Samples of cord blood from unselected infants were forwarded to the study center in Szczecin. The second control group was taken from adult patient rolls of three family doctors practicing in the Szczecin region. One thousand controls were selected at random from the patient lists of these family doctors. The third control group consisted of adults from Szczecin who submitted blood for paternity testing. A sample of DNA was forwarded to the reference laboratory without identifying information. To ensure the comparability of the control groups, the allele frequencies of the three alleles were computed separately for the adult and neonatal control groups and were compared.

We assessed the frequency of three *CHEK2* variants: IVS2+1G→A, 1100delC, and I157T. The IVS2+1G→A mutation was identified by RFLP-PCR by use of Hpy188 III (New England Biolabs) and primers Ch2/3f (5′-ATT TAT GAG CAA TTT TTA AAC G-3′) and Ch2/3r (5′-TCC AGT AAC CAT AAG ATA ATA ATA TTA C-3′). The PCR product was digested in samples in which a mutation was present. The 430T→C variant (Ile157Thr) was analyzed by RFLP-PCR by use of the Ch157f (5′-A CCC ATG TAT CTA GGA GAG CTG-3′) and Ch157r (5′-CCA CTG TGA TCT TCT ATG TCT GCA-3′) primers. The reverse primer introduced an artificial restriction site for the *Pst*I enzyme. The PCR products were digested in mutation-positive samples. The 1100delC variant was analyzed using an allele-specific PCR assay and primers Chk2ex10f (5′-TTA ATT TAA GCA AAA TTA AAT GTC-3′), Chk2ex10r (5′-GGC ATG GTG GTG TGC ATC-3′), and Chk2delC (5′-TGG AGT GCC CAA AAT CAT A-3′). All reactions were performed using Thermal Cycler 9600 (PerkinElmer). PCR products were separated in 2%–3% agarose gels and were visualized in UV light. For samples in which a mutation was detected in the RFLP-PCR and allele-specific oligonucleotides (ASO)–PCR analyses, a separate DNA sample was sequenced to confirm the presence of the mutation.

Exons 10–14 of CHEK2 share high homology with several pseudogenes located on chromosomes 2, 7, 10, 13, 15, 16, X, and Y (Sodha et al. 2000). To avoid amplification of pseudogene sequences, we designed a forward primer (Chk2ex10f) that is specific for the *CHEK2* gene on chromosome 22 and a reverse primer (Chk2delCr) that is specific for the 1100delC allele. In cases that were positive for 1100delC by ASO-PCR analysis, a separate DNA sample was sequenced with the Chk2ex10f and Chk2ex10r primers to confirm the presence of the mutation. Furthermore, to confirm the chromosomal location of the observed *CHEK2* mutations, we analyzed the polymorphic marker *D22S275,* which maps to intron 4 of *CHEK2*. We genotyped 36 patients with the I157T variant, all individuals with the 1100delC or the IVS2+1G→A mutation, and 50 individuals from the general population. All individuals with the 1100delC variant or the I157T variant carried the 165-bp allele of *D22S275,* which we estimate has a frequency of 15% in the Polish population. All individuals with the IVS2+1G→A mutation carried the 171-bp allele of *D22S275,* which we estimate has a frequency of 8% in the general population. These observations support the chromosome 22 assignment for the three variant alleles.

Statistical analysis included a comparison of the proportions of the prevalence of the allele in cases and controls. Odds ratios (ORs) were generated from two-by-two tables, and statistical significance was assessed using the Fisher's exact test.

The frequencies of the three *CHEK2* variants in cases and controls are presented in table 1. Because of their different effects on protein synthesis, the two truncating mutations (IVS2+1G→A or 1100delC) were considered separately from the missense mutation (I157T). A truncating *CHEK2* mutation was detected in 0.7% of Polish controls. For 6 of the 13 sites (breast, colon, melanoma, prostate, thyroid, and stomach), the prevalence of the mutant alleles was >1%. For three sites (breast, prostate, and thyroid), the excess was statistically significant. The prevalence of the truncating *CHEK2* alleles was particularly high (3.5%) in patients with thyroid cancer. The missense *CHEK2* I157T allele was found in 4.8% of controls. This variant was more common in cancer cases than in controls for nine sites, and for three sites (colon, kidney, and prostate) the association was significant at the $P \leq .01$ level.

Although any individual finding might be due to chance, our study, on the whole, suggests that mutations in *CHEK2* increase the risk of cancer in many different organs. A total of 52 comparisons were made. Of these, 13 were significant at the $P = .05$ level (2.6 expected by chance), and 5 were significant at the $P = .01$ level (0.5 expected). Furthermore, at all three sites for which a significant association was seen with the truncating mutation, a significant association was also seen with the missense mutation. This would be unlikely if the observations were due to chance.

Multiorgan cancer predisposition is characteristic of other genes in the DNA damage–signaling pathway, including *BRCA1* (MIM 113705), *BRCA2* (MIM 600185), and *NBS1* (MIM 602667). We expected that the effects of truncating mutations and of missense mutations might differ. Prostate, breast, and thyroid cancer were associated with mutations of both types. Interestingly, kidney cancer and colon cancer were associated with the *CHEK2* missense variant but not with truncating mutations. However, the number of truncating mutations in this study was

Table 1

Association between CHEK2 Variants and Selected Types of Cancer

Subject Group and Type of Cancer	No. of Subjects Tested	No. of Subjects with Mutation (Frequency in %)				Odds Ratio				P			
		IVS2+1G→A	1100delC	Any Truncating Mutation	I157T	IVS2+1G→A	1100delC	Any Truncating Mutation	I157T	IVS2+1G→A	1100delC	Any Truncating Mutation	I157T
Cases:													
Bladder	172	1 (.6)	0	1 (.6)	12 (7.0)	1.2	0	.8	1.5	.7	0	.8	.3
Breast	1,017	11 (1.1)	5 (.5)	16 (1.6)	68 (6.7)	2.3	2.0	2.2	1.4	.04	.3	.02	.02
Colon	300	1 (.3)	2 (.7)	3 (1)	28 (9.3)	.7	2.7	1.4	2.0	.9	.4	.8	.001
Kidney	264	0	2 (.8)	2 (.8)	26 (9.8)	0	2.7	1.0	2.1	0	.5	.8	.0006
Larynx	245	0	0	0	10 (4.1)	0	0	0	.8	0	0	0	.7
Lung	272	0	0	0	7 (2.6)	0	0	0	.5	0	0	0	.1
Melanoma	129	2 (1.5)	1 (.8)	3 (2.3)	6 (4.6)	3.3	3.1	3.2	1.0	.3	.8	.1	.9
Non-Hodgkin lymphoma	120	1 (.8)	0	1 (.8)	11 (9.2)	1.8	0	1.1	2.0	.9	0	.7	.05
Ovary	292	0	0	0	14 (4.8)	0	0	0	1.0	0	0	0	.9
Pancreas	93	0	0	0	6 (6.4)	0	0	0	1.4	0	0	0	.6
Prostate	690	8 (1.2)	3 (.4)	11 (1.6)	54 (7.8)	2.5	1.7	2.2	1.7	.05	.2	.04	.002
Stomach	241	4 (1.7)	0	4 (2.1)	13 (5.4)	3.5	0	2.3	1.1	.05	0	.2	.8
Thyroid	173	5 (2.9)	1 (.6)	6 (3.5)	15 (8.7)	6.2	2.3	4.9	1.9	.0003	.9	.0006	.04
Controls	4,000	19 (.48)	10 (.25)	29 (.73)	193 (4.8)	…	…	…	…	…	…	…	…

Table 2

Frequencies of CHEK2 Variant Alleles in Adult and Newborn Controls

Control Group	No. of Subjects with Variant (Frequency in %)		
	IVS2+1G→A	1100delC	I157T
Newborns (n = 2,000)	9 (.45)	5 (.25)	92 (4.6)
Adults (n = 2,000)	10 (.50)	5 (.25)	101 (5.0)

relatively small, and some associations may have been missed as a result of limited study power.

There were no clear associations between age at onset and the presence of a *CHEK2* mutation for any of the 13 cancer sites studied. However, for all sites except colon and thyroid, the average age at onset was younger for carriers of truncating mutations than for noncarriers, but the difference was not statistically significant for any of the comparisons. The ages at onset of cancer for patients with and without the missense mutation were very similar (data not shown).

Several previous observations suggest that the I157T missense mutation is pathogenic. It is localized in a functionally important domain of *CHEK2* (the FHA domain), and the protein with this mutation has been shown to be defective in its ability to bind and phosphorylate Cdc25A and to bind p53 and BRCA1 (Falck et al. 2001*a*, 2001*b*; Li et al. 2002). The I157T protein may also have a dominant negative effect by forming heterodimers with wild-type CHEK2 (Kilpivaara et al. 2004). A recent paper from Finland (Kilpivaara et al. 2004) also reported that the *CHEK2* I157T mutation was associated with an increase in the risk of breast cancer of a magnitude similar to that reported here (OR 1.4; $P = .02$). Other groups have only found an association between breast cancer risk and the 1100delC variant (Schutte et al. 2003; Dufault et al. 2004). The CHEK2 Breast Cancer Case-Control Consortium (2004) reported an OR of 2.3 in association with the 1100delC variant but did not report on other variants.

It was also reported by Kilpivaara et al. (2003) that the 1100delC variant is not associated with colon cancer (Kilpivaara et al. 2003). This is consistent with our observation of no increased colon cancer risk with either truncating mutation, but we did see a highly significant association with the missense variant (OR 2.0; $P = .001$). An association of similar magnitude was seen with kidney cancer (OR 2.0; $P = .0006$) (to our knowledge, no previous group has studied the relationship between *CHEK2* mutations and kidney cancer). All of the kidney cancer cases were clear cell renal carcinomas. Our strongest observed association was with thyroid cancer (OR 4.9; $P = .0006$), but this estimate was based on only six truncating mutations and needs to be confirmed. These six cancers were all of papillary histology.

In summary, these observations support the hypothesis that *CHEK2* is a multisite cancer gene that increases the risk for several cancers other than breast and prostate cancer. This is perhaps not surprising, since *CHEK2* was first reported to be a gene for Li-Fraumeni type families (MIM 151623) (Bell et al. 1999), and these families suffer from a wide range of cancer types. In the original studies of the CHEK2 1100delC mutation and Li-Fraumeni syndrome, cancers of the breast, colon, stomach, ovary, endometrium, kidney, and prostate were reported (Bell et al. 1999; Vahteristo et al. 2001).

Most of the patients in our case group were recruited from the Szczecin region, which is populated by ethnic Poles who immigrated to the region from throughout Poland after the Second World War, as ethnic German residents were relocated elsewhere. Our control group was drawn both from the adult population of Szczecin and from newborns in 10 cities throughout Poland. The frequency of the *CHEK2* alleles was similar in the newborn and adult controls (table 2). Also, there was no statistical difference between the *CHEK2* allele frequencies of the newborns recruited from the Szczecin metropolitan region and those of newborns from other Polish cities (data not shown).

After *BRCA1* and *BRCA2* were discovered in 1994 and 1995, it was hoped that the identification of a third rare high-risk breast cancer gene would follow—or at least that common variants of these genes, conferring modest lifetime cancer risks, would be detected. To date, these genes remain undiscovered. Arguably, the most important discovery of a breast cancer gene in the past 8 years has been *CHEK2,* which is typical of a third category of genes; mutations in genes in this category are rare and are associated with modest penetrance. It is difficult to study these genes, because very large sample sizes are needed to identify significant relative risks. Not all populations harbor carriers at the same frequency, and different mutations may be associated with different cancer risks. Large, well-controlled studies are needed to establish the full range of risks associated with *CHEK2* founder alleles in different populations and to estimate the corresponding risks associated with various mutations for various cancer sites.

Electronic-Database Information

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (*CHEK2, BRCA1, BRCA2, NBS1,* and Li-Fraumeni syndrome)

References

Bell DW, Varley JM, Szydlo TE, Kang DH, Wahrer DC, Shannon KE, Lubratovich M, Verselis SJ, Isselbacher KJ, Fraumeni JF, Birch JM, Li FP, Garber JE, Haber DA (1999)

Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. Science 286:2528–2531

CHEK2 Breast Cancer Case-Control Consortium (2004) CHEK2*1100delC and susceptibility to breast cancer: a collaborative analysis involving 10,860 breast cancer cases and 9,065 controls from 10 studies. Am J Hum Genet 74:1175–1182

Cybulski C, Huzarski T, Górski B, Masojć B, Mierzejewski M, Dębniak T, Gliniewicz B, Matyjasik J, Złowocka E, Kurzawski G, Sikorski A, Posmyk M, Szwiec M, Czajka R, Narod SA, Lubiński J (2004) A novel founder CHEK2 mutation is associated with increased prostate cancer risk. Cancer Res 64:2677–2679

Dong X, Wang L, Taniguchi K, Wang X, Cunningham JM, McDonnell SK, Qian C, Marks AF, Slager SL, Peterson BJ, Smith DI, Cheville JC, Blute ML, Jacobsen SJ, Schaid DJ, Tindall DJ, Thibodeau SN, Liu W (2003) Mutations in CHEK2 associated with prostate cancer risk. Am J Hum Genet 72:270–280

Dufault MR, Betz B, Wappenschmidt B, Hofmann W, Bandick K, Golla A, Pietschmann A, Nestle-Kramling C, Rhiem K, Huttner C, von Lindern C, Dall P, Kiechle M, Untch M, Jonat W, Meindl A, Scherneck S, Niederacher D, Schmutzler RK, Arnold N (2004) Limited relevance of the CHEK2 gene in hereditary breast cancer. Int J Cancer 110:320–325

Falck J, Lukas C, Protopopova M, Lukas J, Selinanova G, Bartek J (2001*a*) Functional impact on concomitant versus alternative defects in the Chk2-p53 tumour suppressor pathway. Oncogene 20:5503–5510

Falck J, Mailand N, Syljuåsen RG, Bartek J, Lukas J (2001*b*) The ATM-Chk2-Cdc25A checkpoint pathway guards against radioresistant DNA synthesis. Nature 410:842–847

Kilpivaara O, Laiho P, Aaltonen LA, Nevanlinna H (2003) CHEK2 1100delC and colorectal cancer. J Med Genet 40: e110

Kilpivaara O, Vahteristo P, Falck J, Syrjakoski K, Eerola H, Easton D, Bartkova J, Lukas J, Heikkila P, Aittomaki K, Holli K, Blomqvist C, Kallioniemi OP, Bartek J, Nevanlinna H (2004) CHEK2 variant I157T may be associated with increased breast cancer risk. Int J Cancer 111:543–547

Li J, Williams BL, Haire LF, Goldberg M, Wilker E, Durocher D, Yaffe MB, Jackson SP, Smerdon SJ (2002) Structural and functional versatility of the FHA domain in DNA-damage signaling by the tumor suppressor kinase Chk2. Mol Cell 9:1045–1054

Matsuoka S, Huang M, Elledge SJ (1998) Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. Science 282: 1893–1897

Meijers-Heijboer H, van den Ouweland A, Klijn J, Wasielewski M, de Snoo A, Oldenburg R, Hollestelle A, et al (2002) Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations. Nat Genet 31:55–59

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 (2003) The CHEK2 1100delC mutation identifies families with a hereditary breast and colorectal cancer phenotype. Am J Hum Genet 72:1308–1314

Offit K, Pierce H, Kirchhoff T, Kolachana P, Rapaport B, Gregersen P, Johnson S, Yossepowitch O, Huang H, Satagopan J, Robson M, Scheuer L, Nafa K, Ellis N (2003) Frequency of CHEK2*1100delC in New York breast cancer cases and controls. BMC Med Genet 4:1

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 (2003) Variants in CHEK2 other than 1100delC do not make a major contribution to breast cancer susceptibility. Am J Hum Genet 72:1023–1028

Seppala EH, Ikonen T, Mononen N, Autio V, Rokman A, Matikainen MP, Tammela TL, Schleutker J (2003) CHEK2 variants associate with hereditary prostate cancer. Br J Cancer 89:1966–1970

Sodha N, Williams R, Mangion J, Bullock SL, Yuille MR, Eeles RA (2000) Screening hCHK2 for mutations. Science 289: 359

Vahteristo P, Tamminen A, Karvinen P, Eerola H, Eklund C, Aaltonen LA, Blomqvist C, Aittomaki K, Nevanlinna H (2001) p53, CHK2, and CHK1 genes in Finnish families with Li-Fraumeni syndrome: further evidence of CHK2 in inherited cancer predisposition. Cancer Res 61:5718–5722