RESEARCH ARTICLE

Identification of a Novel BRCA2 and CHEK2 A-C-G-C Haplotype in Turkish Patients Affected with Breast Cancer

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Abstract

Background: Many breast cancers are caused by certain rare and familial mutations in the high or moderate penetrance genes BRCA1, BRCA2 and CHEK2. The aim of this study was to examine the allele and genotype frequencies of seven mutations in BRCA1, BRCA2 and CHEK2 genes in breast cancer patients and to investigate their isolated and combined associations with breast cancer risk. Methods: We genotyped seven mutations in BRCA1, BRCA2 and CHEK2 genes and then analyzed single variations and haplotype associations in 106 breast cancer patients and 80 healthy controls. Results: We found significant associations in the analyses of CHEK2-1100delC (p=0.001) and BRCA1-5382insC (p=0.021) mutations in breast cancer patients compared to controls. The highest risk was observed among breast cancer patients carrying both CHEK2-1100delC and BRCA2-Met784Val mutations (OR=0.093; 95%CI 0.021-0.423; p=0.001). We identified one previously undescribed BRCA2 and a CHEK2 four-marker haplotype of A-C-G-C which was overrepresented (χ^2=7.655; p=0.0057) in the patient group compared to controls. Conclusion: In this study, we identified a previously undescribed BRCA2 and CHEK2 A-C-G-C haplotype in association with the breast cancer in our population. Our results further suggest that the CHEK2-1100delC mutation in combination with BRCA2-Met784Val may lead to an unexpected high risk which needs to be confirmed in larger cohorts in order to better understand their role in the development and prognosis of breast cancer.

Keywords: Breast cancer - BRCA1 - BRCA2 - CHEK2 - haplotype

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Introduction

Breast cancer is one of the main causes of cancer-related death among women worldwide (Desrichard et al., 2011). Breast cancer studies have provided the evidence for at least two major cancer susceptibility genes, BRCA1 and BRCA2 (Miki et al., 1994; Wooster et al., 1995). Besides, CHEK2, ATM, PALB2, NCOA3 and BRIP genes have also been reported to be low susceptibility genes associated with a nearly three fold increased risk of developing breast cancer and they are also attributed as predisposing factor in some hereditary breast cancer families (Gabrovska et al., 2011; Shuen and Foulkes, 2011).

BRCA1 (MIM 113705) located on chromosome 17q21 and has a central role in various cellular processes in response to DNA damage, including homologous repair, checkpoint control, spindle regulation and transcriptional regulation that maintain genomic integrity (Miki et al., 1994; Gudmundsdottir and Ashworth, 2006; Hansmann et al., 2012). On the other hand, BRCA2 (MIM 600185) located on chromosome 13q12 acts downstream of BRCA1 and is involved in homologous repair by regulating RAD51 filament formation (Wooster et al., 1995; Gudmundsdottir and Ashworth, 2006; Shuen and Foulkes, 2011). Both BRCA1 and BRCA2 function as tumor suppressor genes in response to DNA damage. In different population worldwide, the spectrum of BRCA1 and BRCA2 mutations has been investigated, these genes were demonstrated to contribute to hereditary cancer and sporadic origin between populations (Saxena et al., 2006). According to studies in hereditary breast cancer families, dominant mutations of 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 have been reported in Ashkenazi Jewish (Abeliovich et al., 1997; Levy-Lahad et al., 1997) and furthermore 5382insC in exon 20 of BRCA1 is determined to be a common mutation in Central and Eastern Europe (Backe et al., 1999; Gorski et al., 2000; Konstantopoulu et al., 2000; Machackova et al., 2000; Terschenko et al., 2002). The 185delAG was also reported in non-Jewish individuals from different ethnic backgrounds (Berman et al., 1996; Diez et al., 1998).

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In spite of the high incidence of breast cancer in Turkey which was reported to be 41.6 per 100.000 (Health Statistics Yearbook, 2010, The Ministry of Health of Turkey), no mutational hotspots have been found so far, in BRCA1 and BRCA2 analyses of a total of 385 Turkish breast cancer and ovarian cancer patients (Balci et al., 1999; Ozdag et al., 2000; Yazici et al., 2000; 2002, Manguoglu et al., 2003; 2011; Guran et al., 2005; Egeli et al., 2006).

CHEK2 (cell-cycle checkpoint kinase) gene is a strong candidate gene for a low risk breast cancer susceptibility on chromosome 22q12.1 (Bartek et al., 2001; Einarsdottir et al., 2006). The protein product of this gene functions as a serine/threonine protein kinase that phosphorylates various substrates such as BRCA1, p53, Cdc25 family proteins (Domagala et al., 2012) and also, it regulates the tumour suppressor functions of these proteins. In response to DNA double-strand breaks or replicative stress, CHEK2 activates cell-cycle checkpoints for promoting cell-cycle arrest and increases DNA repair efficiency. Several founder mutations in the CHEK2 gene (1100delC, IVS2+1G>A, I157T) have been reported. Especially deletion-mutation at position 1100 of CHEK2 gene is being associated with functional alteration of this gene involved in the DNA repair pathway (Meijers-Heijboer et al., 2002). This functional alteration of the 1100delC mutation causes protein truncation at codon 381 by deletion of a single cytosine at position 1100 (Walsh et al., 2006), which makes the mutant protein unstable and defects in kinase function (Sodha et al., 2006). Furthermore, an other protein-truncating variant IVS2+1G>A creates a 4-bp insertion attributable to abnormal splicing and leading to premature protein termination codon in exon 3 (Dong et al., 2003). Hence, these truncating mutations cause the disruption of protein expression. In contrast, another common variant I157T results in the substitution of an isoleucine to a threonine. The protein product of an I157T missense variant is stable, but defective in its ability to bind BRCA1, p53 and Cdc25A (Domagala et al., 2012).

The majority of the studies evaluating the frequency of CHEK2 mutations have been conducted in European populations. The highest prevalence of the 1100delC mutation was found among breast cancer cases from Finland (6.8%) (Vahteristo et al., 2002) followed by Netherlands (4.9%) (Meijers-Heijboer et al., 2002), the United Kingdom (1.2%), and Germany (0.8%) (CHEK2 Breast Cancer Case-Control Consortium. 2004). The missense I157T variant, which was identified in Germany (2.2%), confers lower penetrance than the truncating mutations, and women with this variant face a 1.5 fold increased risk of breast cancer (Cybulski et al., 2004). CHEK2-IVS2 +1G>A is reported to be rare and its contribution to the genetic risk at population level is limited (Einarsdottir et al., 2006). There are no published studies about the CHEK2 gene mutations in large Turkish breast cancer population and therefore spectrum of mutations for this gene is not known.

In the present study, we analyzed various mutations in BRCA1 (185delAG, 5382insC), BRCA2 (Met784Val, Met1915Thr) and CHEK2 (1100delC, IVS2+1G>A, I157T) genes in Turkish breast cancer patients. The aim of this study was to examine the allele and genotype frequencies of seven mutations in BRCA1, BRCA2 and CHEK2 genes in breast cancer patients and to investigate their isolated and combined associations with breast cancer risk.

Materials and Methods

Study population

BRCA1, BRCA2 and CHEK2 mutations were studied among 106 consecutive breast cancer patients (mean age; 55±8.33; age range; 32-65 years) who were admitted to Istanbul Training and Research Hospital, Department of General Surgery, and 80 age-matched female non-malignant healthy controls (mean age; 54.3±12.01; age range; 32-70 years). Subjects with a personal or family history of any cancer and chronic diseases such as cardiovascular or cerebrovascular disease, diabetes mellitus, hypertension, or renal disease were excluded from the study. Breast cancer patients were selected upon diagnosis of breast cancer further the mammography, ultrasonography, and finally pathological examination. Detailed questionnaire including family history, personal history and dietary habits were collected from both study groups. All patients and controls provide written informed consent. The blood samples were collected prior any chemotherapeutic or radiation therapy treatments. Controls were selected from surgery clinics among the healthy people who had no proven malignant disease or disease history. Control subjects were not taking any regular medication at time of the study. The specimens were taken after obtaining informed consent and the study was conducted prospectively. Local Ethical Committee approval was obtained for the study. The protocol followed was consistent with the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects).

Mutation analyses

Genomic DNA was exracted from peripheral blood containing EDTA according to the salt out technique. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) was used to detect the genotypes of each BRCA1, BRCA2 and CHEK2 mutations. BRCA1, 185delAG and 5382insC mutations, BRCA2, Met784Val and Met1915Thr mutations and CHEK2, 1100delC, IVS2+1G>A and I157T mutations are genotyped in Turkish breast cancer patients and controls (Figure 1). Thermal cycling conditions for BRCA1 variants (185del and 5382insC) were as follows: initial denaturation step at 95°C for 3 min, 40 cycles at 95°C for 45s, annealing step at 56°C for 30s and 72°C for 30s. The final extension step was performed at 72°C for 10 min. The 185del polymorphism was determined using the following primers: F 5'-AAAATGAAGTTGTCATTTTATAAACC-3' R 5'-CTGACTTACCAGATGGGACATT-3'. In the presence of the 185del mutation, the 176 bp product was cleaved by DdeI to fragments of 150 and 26bp, whereas the mutant product remained uncut. The 5382insC polymorphism was determined using the following

primers: F 5'-CCAAAGCGAGCAAGAGAATCAC-3 R 5'-GACGGGAATCCAAATTACACAG-3'. In the presence of the 5382insC mutation, the 273 bp product was cleaved by BstNI to fragments of 250 and 23 bp, whereas the wild-type product remained uncut (Rohlfs et al., 1997).

Thermal cycling conditions for BRCA2 variants (Met784Val and Thr1915Met) were as follows: initial denaturation step at 95°C for 5 min, 30 cycles at 95°C for 30s, annealing step at 56°C for 30s and 72°C for 30s. The final extension step was performed at 72°C for 5 min. The Met784Val polymorphism was determined using the following primers: F:5'-TGGAATACAGTGATACTGAC-3', R:5'-TTGGATTACTCTTAGATTTG-3'. In the presence of the Met784Val mutation, the 346bp product was cleaved by BspHI. The Met allele was digested into 296 and 50bp fragments, whereas the Val variant 235, 61 and 50 bp.The Thr1915Met polymorphism was determined using the following primers: F:5'-TTGCCAAACGAAAATTATGG-3', R:5'-AGATTTTCCACTTGCTGTGC-3'. The 304 bp product was digested overnight with restriction enzyme SphI. The Thr allele was digested into 95 bp and 209 bp fragments and the Met was intact (Krupa et al., 2009). Thermal cycling conditions for CHEK2 variant (1100delC, IVS2+1G>A, I157T) were as follows as BRCA1 condition. Two sets of PCR primers were used to screen for the CHEK2 1100delC mutation. Specific primers for the CHEK2 exon 10 on chromosome 22 were designed to avoid all other homologous sequences in the genome F:5'-TTAATTTAAGCAAAATTAAATGTC-3', R:5'-GGCATGGTGGTGTGCATC-3'. The PCR products were then re-amplified with nested primers, which were designed to amplify the region encompassing the site of CHEK2 1100delC. The forward primer contained one base substitution to generate a restriction site for restriction enzyme ScaI within the wild-type allele after PCR amplification. The forward primer sequence was F:5'-CCCTTTTGTACTGAATTTTAGAGTA-3' (a T to G substitution at position 1097). The reverse primer was 5'-ACAAGAACTTCAGGCGCCAAGTAG-3'. In the presence of the 1100delC mutation, the 116 bp product was cleaved by ScaI to fragments of 92 and 24 bp, whereas the mutant product remained uncut (Zhang et al., 2008). The I157T polymorphism was determined using the following primers F:5'-ACCCATGTATCTAGGAGAGCT-3', R: 5'-CCACTGTGATCTTCTATGTCTGCA-3'. In the presence of the I157T mutation, the 155 bp product was cleaved by PstI to fragments of 136 and 19 bp, whereas the wild-type product remained uncut. The following primers were used for the determination of IVS2 1G>A polymorphism. The PCR product was amplified with the use of the following primers, F: 5'-ATTTATGAGCAATTTTTAAACG-3' and R: 5'-TCCAGTAACCATAAGATAATAATATTAC-3'. In the presence of the IVS2 1G>A mutation, the 491bp product was cleaved by Hyp188III to fragments of 297 and 194bp, whereas the wild-type product remained uncut (Cybulski et al., 2004). All reactions were performed using Takara PCR Thermal Cycler Dice. PCR products were analyzed on 2% and the restriction fragments on 3% agarose gels and they were visualized under UV light.

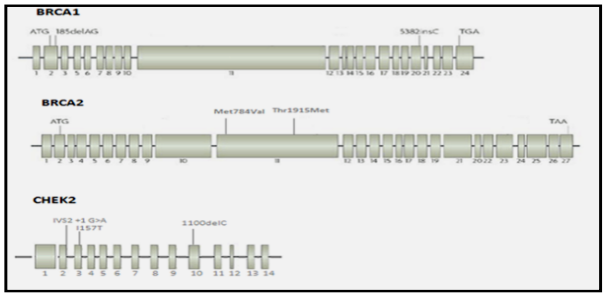

Figure 1. Position of Several Mutations within BRCA1 (top) and BRCA2 (middle) and CHEK2 (bottom). BRCA1 gene (MIM 113705), BRCA2 (MIM 600185) and CHEK2 (MIM 604373) genes are located on chromosome 17q21, 13q12, and 22q12.1, respectively. To date many different mutations have been detected in patients with different ethnic backgrounds. Gray boxes indicate the exons with the exon number provided below each box. Shown in the figure are the mutations in various exons of the related genes that are analyzed in this paper

Statistical analysis

Statistical analyses were performed using the SPSS software package (version 11.5 SPSS Inc., Chicago, IL, U.S.A.). Data are expressed as means±SD. Distributions of genotypes and haplotypes were compared using the Chi-square test, respectively. The distribution of genotypes in all groups was tested for deviation from Hardy-Weinberg equilibrium (HWE) using the goodness-of-fit test. Relative risk at 95% confidence intervals (CI) was calculated as the odds ratio (OR). Linkage disequilibrium between variations was assessed using D0 and r2 values obtained through the Haploview program (http://www.broad.mit.edu/mpg/haploview/ documentation.php). P-values less than 0.05 were considered statistically significant.

Results

The mean age of the 106 breast cancer patients was 55±8.33 (age range 32-65 years), while the mean age of 80 healthy controls was 54.3±12.01 (age range 32-70 years).

We investigated the presence of seven mutations in BRCA1, BRCA2 and CHEK2 genes in 106 breast cancer patients with a personal and/or family history of breast cancer. 80% of the patients were diagnosed with stage I and II disease and the rest with stage III and IV disease. The pathological assesment of the tumors revealed that, the majority of the tumors were poorly (71%), or intermediately (23%) differentiated invasive ductal carcinomas. 90% of the patients were receiving adjuvant chemotherapy following surgery.

All mutations were within the distribution expected according to Hardy-Weinberg equilibrium in both groups. The genotype and allele frequencies of the individual mutation are summarized in Table 1. The genotype and the allele frequencies of rs11571653 (BRCA2; g.26226A>G, c.2350A>G, p.Met784Val), rs4987117 (BRCA2; g.29620C>T, c.5744C>T, p.Thr1915Met), rs17879961 (CHEK2; g.21736T>C, c.599T>C, p.Ile157Thr) and

Table 1. Association Results of the Five Selected BRCA1 and CHEK Variations with Breast Cancer

Mutations	Allele 1	Allele 2	Patients (n=106) 11	12	22	Controls (n=80) 11	12	22	Genotype P value	Allele P value
"BRCA2- Met784Val rs11571653"	A	G	5	75	0	1	71	0	0.124	0.671
"BRCA2-Thr1915Met rs4987117"	C	T	97	1	0	74	4	0	0.103	0.177
"CHEK2-IVS2+1G>A rs113994006"	A	G	84	0	0	77	0	0	-	1.000
"CHEK2-I157T rs17879961"	C	T	97	2	1	76	5	0	0.547	0.512
"BRCA1-5382insC rs76171189"	-	C	79	1	4	75	1	0	0.072	**0.021**

*Patients refer to patients with breast cancer, and controls refer to control subjects. The P-values given are corrected by means of Bonferroni correction. Association reaching nominal significance (P<0.05) is shown in bold

CHEK2-IVS2+1G>A (c.444+1G>A) were no different between the groups. Besides, the allele frequency of rs76171189 (BRCA1; g.160921delC, c.5382insC, p.Gln1709Argfs) was significantly different between the patient and control groups (p=0.021) (Table 1).

The BRCA1-185delAG carriers were significantly different among the groups (p=0.00072). Interestingly, in control group heterozygous mutants were significantly higher compared to patient group. The frequency of 185delAG carriers was 0.195 among the patients as opposed to 0.375 in controls (OR=0.086; 95%CI 0.038-0.191; p=0.001) indicating a protective effect to breast cancer risk in our population. In our patient group, BRCA1-5382insC mutation was present in 5 of our patients. There was a statistically significant difference between the patients and controls (OR=0.117; 95%CI 0.015-0.935; p=0.021). Carriers of the BRCA2-Thr1915Met allele alone were higher in controls, but no protective effect was determined (OR=0.195; 95%CI 0.022-1.761; p=0.177). And, BRCA2-Met784Val carriers in the patient group alone were more than the controls, but no risk effect was identified (OR=0.907; 95%CI 0.578-1.424; p=0.671). In CHEK2 gene, IVS2+1G>A, Ile157Thr and 1100delC mutations were analyzed. And, CHEK2-IVS2+1G>A mutation was not found in our patient or control groups. CHEK2-Ile157Thr mutation was found higher in controls than patients, though there was no protective effect (OR=0.641; 95%CI 0.169-2.426; p=0.521). We have further studied the 1100delC mutation in CHEK2 gene, this truncating mutation is known to double the risk of unselected women, but gives rise to much higher risk for women who have a family history of breast cancer. In our patient group, we found that 1100delC carriers were nearly two fold higher compared to non-carriers and there was a significant difference between the patients and controls (p=0.001). In patient group and controls, the frequency of 1100delC carriers was determined 0.309 and 0.08, respectively (p=0.001; χ^2=41.57). The deletion was more common in the cases than in the controls, and the corresponding odds ratio for deletion carriers versus noncarriers was 12.6 (95% CI 3.67-43.9) (Table 2).

Table 2. Overall Association of the CHEK2 1100delC Mutation with Breast Cancer Risk

1100 delC Genotype	Number of Patients/Controls	OR (95%CI)
C/C	81/75	1 (Reference)
C/-	28/03	12.6 (3.67-43.9)
-/-	00/00	-
C/- and -/-	28/03	12.6 (3.67-43.9)

*Patients refer to patients with breast cancer, and controls refer to control subjects. OD; Odds Ratio, CI; Confidence Interval. "-" refers to deletion

In order to understand the combined effect of BRCA1, BRCA2 and CHEK2 genes on breast cancer risk, we have further analyzed the breast cancer patients carrying no mutations in BRCA1-CHEK2 and BRCA2-CHEK2 genes and carrying a mutation in both genes. As described above, there is a significant increase in the risk of breast cancer associated with a CHEK2-1100delC mutation alone. However, when compared the breast cancer patients carrying no mutations in BRCA1-CHEK2 and BRCA2-CHEK2 genes and carrying a mutation in both genes, we found that the breast cancer patients carrying CHEK2-1100delC and BRCA2-Met784Val (23.1%) were at significantly higher risk in comparison to non mutation carriers (2.8%) (OR=0.093; 95% CI 0.021-0.423; p=0.001).

Haplotype association analysis

Pair-wise linkage disequilibrium (LD) was calculated between all pairs of variations by the use of the Haploview program and the results are shown in Table 3. Four markers of CHEK2 and BRCA2 genes; CHEK2-IVS2+1G>A, CHEK2-Ile157Thr, BRCA2-Met784Val and BRCA2-Thr1915Met were part of one haplotype block –A-C-G-C (Table 3). The analysis of sliding windows of two and three-marker haplotypes did not reveal any significant difference in haplotype distribution between patients and controls (data not shown). The four-marker haplotypes showed significant differences in the distributions of two haplotypes A-C-A-C and A-C-G-C between populations.

Table 3. Four-SNP haplotype Frequencies and Results of the Association Analysis between Patients and Controls

Haplotype Number	Haplotype Associations CHEK2-IVS2+1G>A/ CHEK2-Ile157Thr/BRCA2-Met784Val/ BRCA2-Thr1915Met	Haplotype Frequency Overall	Patients	Controls	χ^2	P value
1	ACAC	0.436	0.346	0.494	5.234	**0.0222**
2	ACGC	**0.029**	0.065	0.005	7.655	**0.0057**
3	ACGT	**0.021**	0.012	0.027	0.643	0.4226

*Patients refers to patients with breast cancer, and control refers to control subjects. Haplotypes listed are only those with a frequency ≥1%. The P-values given are based on 10000 permutations using the Haploview program. Associations reaching nominal significance (P<0.05) are shown in bold

The A-C-A-C haplotype was slightly underrepresented in patients as opposed to A-C-G-C haplotype which is overrepresented in patients (p=0.0022; p=0.0057, respectively), suggesting the latter one to be a high risk haplotype.

Discussion

In this study, we have identified a previously undescribed BRCA2 and CHEK2 A-C-G-C haplotype in association with the breast cancer in our population. Our results further suggest that CHEK2-1100delC mutation in combination BRCA2-Met784Val mutation may lead to an unexpected high risk in breast cancer.

The most common Ashkenazi Jewish mutations in BRCA1 gene, BRCA1-5382insC and BRCA1 185delC mutations were previously studied in Turkish breast cancer patients. The presence of BRCA1-5382insC mutation was found in three different studies from Turkey. Yazici et al. identified this mutation twice in her study group, while Manguoglu et al. in a patient with bilateral breast cancer having relatives diagnosed with cancer at different sites and very recently five patients with BRCA1-5382insC from the same family were reported by Purcu et al. (2010) (Yazici et al., 2000; Manguoglu et al., 2010; Purcu et al., 2010). In our study, we have identified BRCA1-5382insC mutation in 5.9% of our patients (5 of 84 patients) and the corresponding odds ratio was 8.54 (95% CI; 1.07-68.27). Our results supporting the previous Turkish studies enable us to suggest to screen BRCA1-5382insC mutation in high-risk Turkish individuals before the whole-gene screening analysis. Furthermore, we observed a significant difference between breast cancer patients and controls in regard to the frequency of CHEK2-1100delC. The deletion-mutation at position 1100 of the CHEK2 gene is associated with an increased risk of breast cancer as stated in the meta-analyses of 16 studies, including a total of 26,488 patient cases and 27,402 controls. The meta-analyses emphasizes that CHEK2-1100delC heterozygosity increases the risk of breast cancer three- to five-fold, which supports previous individual studies conducted in patients with unselected, early-onset, and familial breast cancer (Weischer et al., 2008). In women of Northern and Eastern European descent CHEK2-1100delC carrier status confers a nearly 2 fold risk of breast cancer. whereas the frequency was reported to be much lower in North America (Iniesta et al., 2010; Adank et al., 2011). In women of Northern and Eastern European descent, women with a familial history of breast cancer have a 4.8 fold risk of breast cancer equal to a lifetime risk of breast cancer of 37% (Meijers-Heijboer et al., 2002). BRCA2 mutations; BRCA2-Met784Val and BRCA2-Thr1915Met previously reported being independent markers of breast cancer (Ishitobi et al., 2003; Krupa et al., 2009) were also studied. In contrast to Polish and Japanese studies (Ishitobi et al., 2003; Krupa et al., 2009), we could not determine any significant association between BRCA2-Met784Val and BRCA2-Thr1915Met mutations and breast cancer in our population (p=0.671 and p=0.177, respectively). However, we demonstrated that the combined effect of CHEK2-1100delC and BRCA2-Met784Val mutations in breast cancer patients causes significantly higher risk in comparison to non mutation carriers (2.8%) (OR=0.093; 95% CI 0.021-0.423; p=0.001). Our results suggest that in isolation, the BRCA2-Met784Val mutation does not appear to be influential in cancer risk, but when combined with CHEK2-1100delC, the cancer risk seems to be increased in breast cancer patients. Moreover, this is the third study reporting the important impact of CHEK2 gene in gene-gene interaction analysis in cancer. Previously, the joint effect of CHEK2 and CDKN1B has been reported in prostate and colon cancer risk. (Cybulski et al., 2007). And, interestingly, a significant interaction effect was reported between CHEK2 mutations and BRCA2-Thr1915Met resulting in an unexpectedly high risk of beast cancer (Serrano-Fernandez et al., 2009).

To our knowledge, this is the first study reporting the presence of the high frequency of CHEK2 1100delC in Turkish population. The only study from Turkey reported no 1100del variant in CHEK2 gene in 16 familial, 29 early onset, 3 male breast cancer, and 2 bilateral breast/ovarian cancer high risk Turkish index cases (Manguoglu et al., 2011). The difference might be due to the small size of their study group. The high population frequency of 1100delC in the Turkish population is in line with some data reported from Europe, although different than Northern European countries and North America, and our result showed that the corresponding odds ratio for 1100delC carriers versus non-carriers was 12.6 (95% CI, 3.67-43.91). Interestingly, the prevalence of CHEK2 1100delC was reported to be increased with increasing numbers of breast cancer cases in the families and also, in families that were more severely affected with breast cancer, this genotype only partially segregated with the breast cancer phenotype. In a Dutch study, it was shown that nearly half of Dutch CHEK2 1100delC breast cancer families also included colorectal cancer cases (Wasielewski et al., 2008). Taken together the Dutch study and our results, we might suggest that, the high frequency of CHEK2 1100delC in our population might be due to the lack of early detection of the breast cancer cases and also, they need to be monitoring carefully for other cancers.

In conclusion, this is the first study to report a haplotype in Turkish population. The BRCA2 and CHEK2 A-C-G-C haplotype is related to breast cancer in our population. Our results might suggest a polygenic breast cancer susceptibility model in Turkish breast cancer patients, and the haplotype formed by CHEK2 1100delC and BRCA2 might well be acting together and/or with other yet- not identified breast cancer susceptibility alleles. And second major finding of our study is the presence of the high frequency of CHEK2-1100delC in Turkish breast cancer patients which might be associated with breast cancer in our population. Moreover, in combination with BRCA2-Met784Val mutation, the cancer risk seems to be increased in breast cancer patients. Further analyses in larger cohorts and in other cancer types need to be performed in order to elucidate the role of CHEK2-1100delC polymorphism in the development and prognosis of breast cancer. And, our results enable us to suggest to screen CHEK2-1100delC along with BRCA2-Met784Val and BRCA1-5382insC mutations with in high-risk Turkish individuals before

the whole-gene screening analysis.

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