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Missense mutations (p.H371Y, p.D438Y) in gene CHEK2 are associated with breast cancer risk in women of Balochistan origin

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Abstract CHEK2 encodes a serine/threonine-protein kinase which plays a critical role in DNA damage signaling pathways. CHEK2 directly phosphorylates and regulates the functions of p53 and BRCA1. Most women with breast and/or ovarian cancer are not carriers of mutant BRCA1 or BRCA2. Multiple studies have shown that a CHEK2*1100delC confers about a two-fold increased risk of breast cancer in unselected females and a tenfold increase in males. Moreover, studies have shown that first-degree relatives of bilateral breast cancer cases who carried the CHEK2*1100delC allele had an eight-fold increased risk of breast cancer. It has been suggested that CHEK2 functions as a low-penetrance susceptibility gene for cancers and multiplies the risks associated with other gene(s) to increase cancer risk. The main goal of this study was to evaluate and to compare the role of truncating mutations, splice junction mutations and rare missense substitutions in breast cancer susceptibility gene CHEK2. Present study was performed on 140 individuals including 70 breast cancer patients both with and without family history and 70 normal individuals. Written consent was obtained and 3 ml intravenous blood was drawn from all the subjects. DNA was extracted from all the samples through inorganic method published already. Primers were synthesized for all the 14 exons of CHEK2 gene. Coding and adjacent intronic sequences of CHEK2 gene were amplified and sequenced. Two genetic variants (p.H371Y, p.D438Y) were found in exon 10 and exon 11 of gene CHEK2 which were not found in any of the 70 control individuals from same geographical area and ethnic group. The genetic variant c.1312G>T (p.D438Y) identified in a patient with a family history of breast cancer. To our knowledge, this is first mutation scanning study of gene CHEK2 from Balochistan population.

Keywords CHEK2 · Breast cancer · Balochistan · Intermediate breast cancer gene · Mutational analysis

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Introduction

CHEK2 is the official symbol for the human gene CHK2 checkpoint homolog [1–3]. The human CHEK2 gene consists of 14 exons spans about 50 kb of genomic DNA [4]. This gene is located on the long (q) arm of

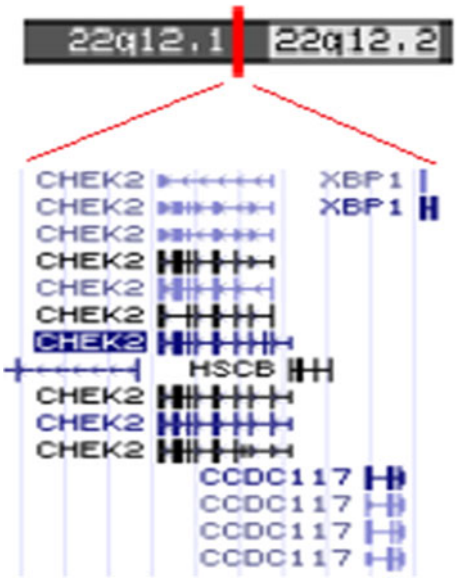

Fig. 1 Showing location of gene CHEK2 on chromosome 22q12.1 (29, 083, 730 bp–29, 137, 821 bp)

chromosome 22 (Fig. 1). A protein named as CHK2 protein kinase is encoded by CHEK2 gene, activated in response to DNA damage and involved in cell cycle arrest [3]. There are three characteristics domains in the structure of the CHEK2 protein: N-terminal SQ/TQ cluster domain, fork head associated (FHA) domain and a serine/threonine kinase domain [2, 5]. Mutations in CHEK2 gene contributes to molecular pathogenesis of different types of human cancers both hereditary and sporadic [6]. CHEK2 gene is an intermediate-risk gene for breast cancers. Loss of 22q, where the CHEK2 locus resides, is a common event in breast cancer [4, 7]. The link between CHEK2 and breast cancer was first proposed in 2002, when a faulty version of the gene was found to be present in some cases with a strong family history of breast cancer [6].

CHEK2*1100delC is an important breast cancer-predisposing variant, which increases the risk three- to five-fold. Because the cumulative risk of breast cancer at age 70 years among familial patient cases for CHEK2*1100delC heterozygotes is almost as high as that for BRCA1 and BRCA2 mutation heterozygotes, genotyping for CHEK2*1100delC should be considered together with BRCA1 and BRCA2 mutation screening in women with a family history of breast cancer [8]. Beside the 1100delC, another common mutation c.1111C4T (p.H371Y) also plays active role in the development of the breast cancer [9].

Present study was performed on 140 individuals including 70 breast cancer patients both with and without family history and 70 normal individuals. Two genetic variants (p.H371Y, p.D438Y) were found in exon 10 and exon 11 respectively. Both genetic variants in CHEK2 were found in two separate individuals, which were not found in any of the control individual from same geographical area. To our knowledge, this is first mutation scanning study of gene CHEK2 from Balochistan population.

Materials and methods

This study was carried out on 140 individuals of Balochistan origin, including 70 breast cancer patients and 70 normal individuals. An inform consent was taken from all the subjects. Breast cancer history was taken from the patients by providing them a questionnaire. 3 ml intravenous blood was drawn from all the subjects and the samples were stored at −20 °C (at least for 24 h) before further processing. DNA was extracted from all the samples using standard inorganic method [10]. Already reported primers sequences (Table 1) were synthesized for all exons of gene CHEK2 [11]. Coding exons and adjacent intronic sequences of the CHEK2 gene were amplified and sequenced. PCR products were amplified using 100 ng of genomic DNA in a 25 µl reaction mixture containing 10 pmol of forward and reverse primers, 0.2 mM dNTP, 10 mM Tris–HCl, 50 mM KCl, 1.5 Mm $MgCl_2$, and 0.5 U of *Taq* polymerase (Invitrogen Corp., Carlsbad, CA). After initial denaturation at 95 °C for 4 min, 30 cycles were performed, which consisted of 95 °C for 1 min, 55–62 °C (depending on the fragment) for 1 min, and 72 °C for 1 min, with a final extension step of 72 °C for 10 min for all exons. PCR products were digested with exonuclease I and shrimp alkaline phosphatase (Fermentas Life Sciences, Glen Burnie, MD) and sequenced bi-directionally using BigDye Terminator v.3.1 kit (Applied Biosystems, Darmstadt, Germany).

Results

In this study, 140 individuals including 70 breast cancer patients and 70 normal subjects were investigated. Pashtoon ethnic group was the most common among affected with the total of 20 patients followed by 18 Afghan patients and 16 Baloch patients (Fig. 2). Other patients belong to ethnic group, Punjabi (8 cases) and Hazara (8 cases). The common age group was 41–50 years with a total of 42 patients, followed by 31–40 age groups with a total of 20 patients and 8 patients belong to age group 51-60.

Invasive ductal carcinoma (IDC) was the most common type of breast cancer in this study with a total of 67 patients followed by breast cancer type Invasive lobular carcinoma (ILC) in 3 patients. Breast cancer with grade III was

Table 1 CHEK2 Primers sequences and conditions used

Exons	Size	Range	Forward primer	Reverse primer	Ann temp
1	565	1–106	GAACTATAGGTCTGGGCTGTTAGG	TCCACCTGGTAATACAACTTTCTG	57
2–3	582	107–197	TGCCTTCTTAGGCTATTTTCCTAC	AACCATATTCTGTAAGGACAGGAC	56
4	354	198–228	CTCAAGGGCTTTACAATATG	GAAATGAGAAACCACCAATC	54
5	499	229–264	GAATTTCACAATCCAGGGCTAC	CTCACAAATTCATCCATCTAAGCAG	56
6	632	265–282	TAGAGCTGGGTTTGGAACTCAG	AGCTAGGCATGTGTGTGAATG	68
7	434	283–304	AAGAAGACTGGGAAGAGACCTAGC	GCAAGCCTACATTAGATTCTTTGG	56
8	365	305–336	CATCTCATTCCTTAGTTTCCAACTG	TCTGCCTAATTCAGGGAGTAATTC	56
9	331	337–365	CTGTGAGATGTGTGTGTTGGTAAC	TCTGGATAAGAGCAGTATCACCTG	58
10	546	366–420	TTAATTTAAGCAAAATTAAATGTCC	GGCATGGTGGTGTGCATC	54
11	353	421–458	GCTGGGATTACAAGCCTAAGG	GAAGAAACTCCCACCACAGC	69
12	541	459–487	GGCCTGTTAATTCTGGCATACTC	AAAGGTTGTAGCCTGGCCAG	67
13	488	488–514	CCTCTGGGAAGGTAGAGGC	CAATCCCTAGCTGTGCTTATCG	66
14	585	515–543	CCCCCACTTTACTGGAAGC	GCAAAACCCTGTCTCTACAAAAT	64

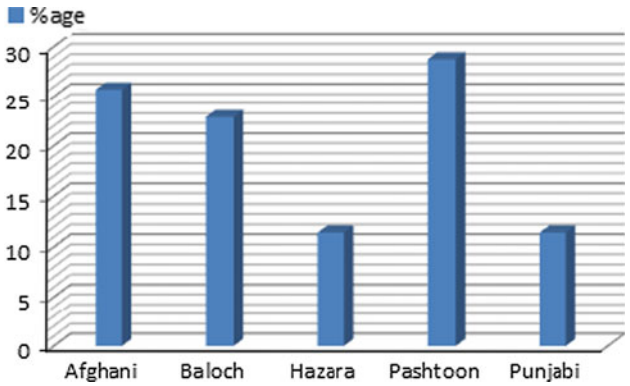

Fig. 2 Graph showing percentage of patients belonging to different ethnic groups

Table 2 Genetic variants observed in gene CHEK2 in breast cancer patients of Balochistan

Subject ID	Variant	Type	Effect on protein	Cases	Controls
BC109	c.96C>G	silent	p.S32S	1	2
BC125	c.1111C>T	missense	p.H371Y	1	0
BC167	c.1312G>T	missense	p.D438Y	1	0

common with a total of 45 patients followed by 18 patients with grade II and 7 patients with grade I. The normal subjects included in this study were between 25 and 65 years age group and the mean age was 46 years. Out of 70 breast cancer patients, five patients had a positive family history of breast cancer.

As a result of sequencing CHEK2 gene, two genetic variants (p.H371Y, p.D438Y) were found in two different individuals in exon 10 and exon 11 respectively (Table 2). Both individuals were breast cancer positive with CHEK2 gene mutation including one patient with a family history of breast cancer. In addition to two missense variants, a silent variant p.S32S was also observed.

Discussion

Breast cancer is the common malignancy and second leading cause of death among women worldwide [12]. We aimed to study the role of CHEK2 gene mutation in Balochistan (Pakistan) population affected with breast cancer. In present study, we investigated 70 breast cancer patients. Pashtoon was the most common ethnic group with a total of 20 patients followed by 18 Afghan and 16 Baloch patients (Fig. 2). Beside the multiple risk factors of the breast cancer, the genetic make-up (ethnicity) is an important risk factor for woman developing breast cancer. Family history, socioeconomic status, lifestyle, lack of education (awareness) and lack of health facilities make a woman to prone to breast cancer [13]. In our study, the most affected individuals with breast cancer were between 41 and 50 years of age. The age is also predisposing risk factor of breast cancer. In previous studies carried out in Pakistani populations had also the same results regarding the age group and shown that Pakistani women get the breast cancer at the earlier age as compared to that of western women [14–20].

The genetic variant c.1111C>T (p.H371Y) in exon 10 was found in an affected patient of Quetta region (Table 2). The p.H371Y amino acid change is within the activation loop of the CHEK2 kinase domain (Fig. 3), which is essential for activation of CHEK2 in response to DNA damage [21]. Bioinformatic analysis suggested that the p.H371Y is likely to alter CHEK2 function as p.H371Y is within the activation loop of the kinase domain of the

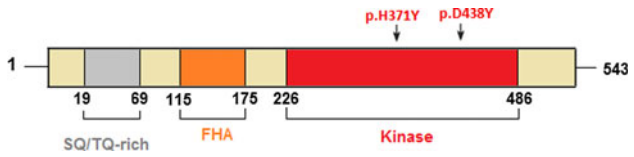

Fig. 3 Showing three domains of CHEK2 protein: N-terminal SQ/TQ cluster domain (*gray*) ranging from amino acids 19–69, fork head associated (FHA) domain (*orange*) ranging amino acid 115–175 and serine/threonine kinase domain (*red*) ranging from amino acid 226–486. Both genetic variants (p.H371Y, p.D438Y) were found in serine/threonine kinase domain in exon 10 and exon 11 of CHEK2 gene. (Color figure online)

CHEK2 protein. These results suggested that CHEK2 p.H371Y associated with tumorigenesis may be through CHEK2 haploinsufficiency, which supports the notion that missense mutation with partial loss of function, may contribute to tumorigenesis via haploinsufficiency [22]. The p.H371Y mutation is therefore highly interesting, and it may alter the CHEK2 kinase activity. In another study, functional analysis suggested that p.H371Y is likely to be pathogenic and the authors suggested that familial breast cancer cases that carried p.H371Y mutation had a nearly six fold of relative risk to development of breast cancer.

The CHEK2 missense mutation p.D438Y (Table 2) was present in catalytic domain of the protein (Fig. 3). Recent studies have been performed to test the functional characteristics and to test protein stability and a partial loss of function is suggested by in vitro analysis of the D438Y mutation.

CHEK2 gene has been identified as a breast cancer susceptibility gene [6, 23]. Mutations in CHEK2 gene may increase the risk of breast cancer to a woman up to 2–5 folds but is still a matter of debate [6, 23, 24]. We sequenced CHEK2 gene along with its intron/exon boundaries and observed 2 different variants in breast cancer cases with two missense mutations which were not observed in a normal individual. The variants c.1111C>T (p.H371Y) and c.1312G>T (p.D438Y) both missense mutations were identified in this study (Fig. 1). Liu et al. [9] also found c.1111C>T in their study and suggested that the p.H371Y was a pathogenic mutation which confer moderate risk of breast cancer in Chinese women. Whereas in European women the 1100delC mutation has been recognized which increase the breast cancer risk in European women up to two fold [6, 8, 25–28]. The c.1312G>T (p.D438Y) identified in a patient with a family history of breast cancer in the present study had also been found in a breast cancer patient with a family history of breast cancer and leukaemia and this alteration had been reported as a germ line mutation in a hereditary prostate cancer patient [29]. To our knowledge, this is first mutation scanning study of gene CHEK2 from Balochistan population.

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