Cancer Genetics 235–236 (2019) 13–17

Cancer Genetics

CASE REPORT

A novel CHEK2 variant identified by next generation sequencing in an Indian family with hereditary breast cancer syndrome ☆

Pratibha Bhai*, Renu Saxena, Samarth Kulshrestha, Ishwar Chander Verma
Institute of Medical Genetics and Genomics, Sir Ganga Ram Hospital, Rajinder Nagar, New Delhi 110060, India

Abstract

Genetic variations in *CHEK2* (checkpoint kinase 2) gene have been associated with hereditary predisposition to various cancers including breast and ovarian cancer. *CHEK2* tumor suppressor gene encodes for a checkpoint kinase that responds to breaks in DNA, regulates DNA repair and cellular proliferation. We report a *BRCA* negative family with multiple affected women having breast cancer, with a novel, missense, likely pathogenic variant in the *CHEK2* gene (c.1376T>G; p.Ile459Ser) that segregated with subjects with breast cancer. This case provides insight into the role of the *CHEK2* gene in causing breast cancer susceptibility in families and supports the use of multigene panel testing in cases with hereditary predisposition to breast cancer.

Keywords Breast cancer, CHEK2, Next generation sequencing.

Introduction

Approximately 5–10% of all breast and ovarian cancers are hereditary. Classically, deleterious variants in *BRCA1* and *BRCA2* genes have been associated with hereditary breast and ovarian cancer (HBOC) syndrome, and account for 20–50% of cases [1]. Next-generation sequencing (NGS) technologies have helped in understanding the role of other moderate penetrance breast cancer susceptibility genes in non BRCA1/2 families. One of these genes is *CHEK2* (checkpoint kinase 2), implicated in various types of cancers including breast cancer. *CHEK2* gene codes for a serine/threonine kinase involved in DNA repair and cell cycle regulation in response to DNA damage [2]. Several variants are reported in the *CHEK2* gene conferring risk to breast and other types of cancers including prostate, colon and thyroid cancer [3–4]. The contribution of *CHEK2* variants to breast cancer varies in different countries [3–8]. The most widely studied variant is c.1100delC. None of the patients in Rwanda had a variant in the *CHEK2* gene [7]. Currently little is known about the variants in the *CHEK2* gene and associated cancer risks among Asian Indian women [9,10]. We report here, the identification of a novel missense variation in the *CHEK2* gene in a non BRCA1/2 family with HBOC, using NGS based multi-gene panel testing.

Presentation of family

The index patient was a 68-year-old female (Fig. 1(A); II.5) of Asian Indian origin. She was diagnosed with breast ductal carcinoma in situ at 58 years of age. She underwent surgery and currently is doing well. One of the proband's sisters (72 years) was diagnosed with infiltrating adenocarcinoma of the breast at the age of 60 years (II.3). She was operated followed by chemotherapy and now doing well. The other sister (72 years old; II.3) and two brothers (II.2, age: 74 years and II.7, age; 66 years) are healthy and alive. One of the brother's (II.2) daugh-

☆ Novel Insight brought forward by this case report. This case has identified a novel variant in the CHEK2 gene in an Asian Indian family with hereditary breast cancer. Next-generation sequencing identified three novel variants in three different breast cancer predisposition genes. We performed segregation studies of the three identified variants with several members of the family and performed in silico analysis to identify the family-specific variants associated with increased risk. The novel variant identified have contributed to knowledge of the molecular basis of this disorder.

Received November 11, 2018; received in revised form April 1, 2019; accepted May 29, 2019

* Corresponding author.
E-mail addresses: pratibha.bhai@lhsc.on.ca, samarth.kulshrestha@sgrh.com

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https://doi.org/10.1016/j.cancergen.2019.05.003

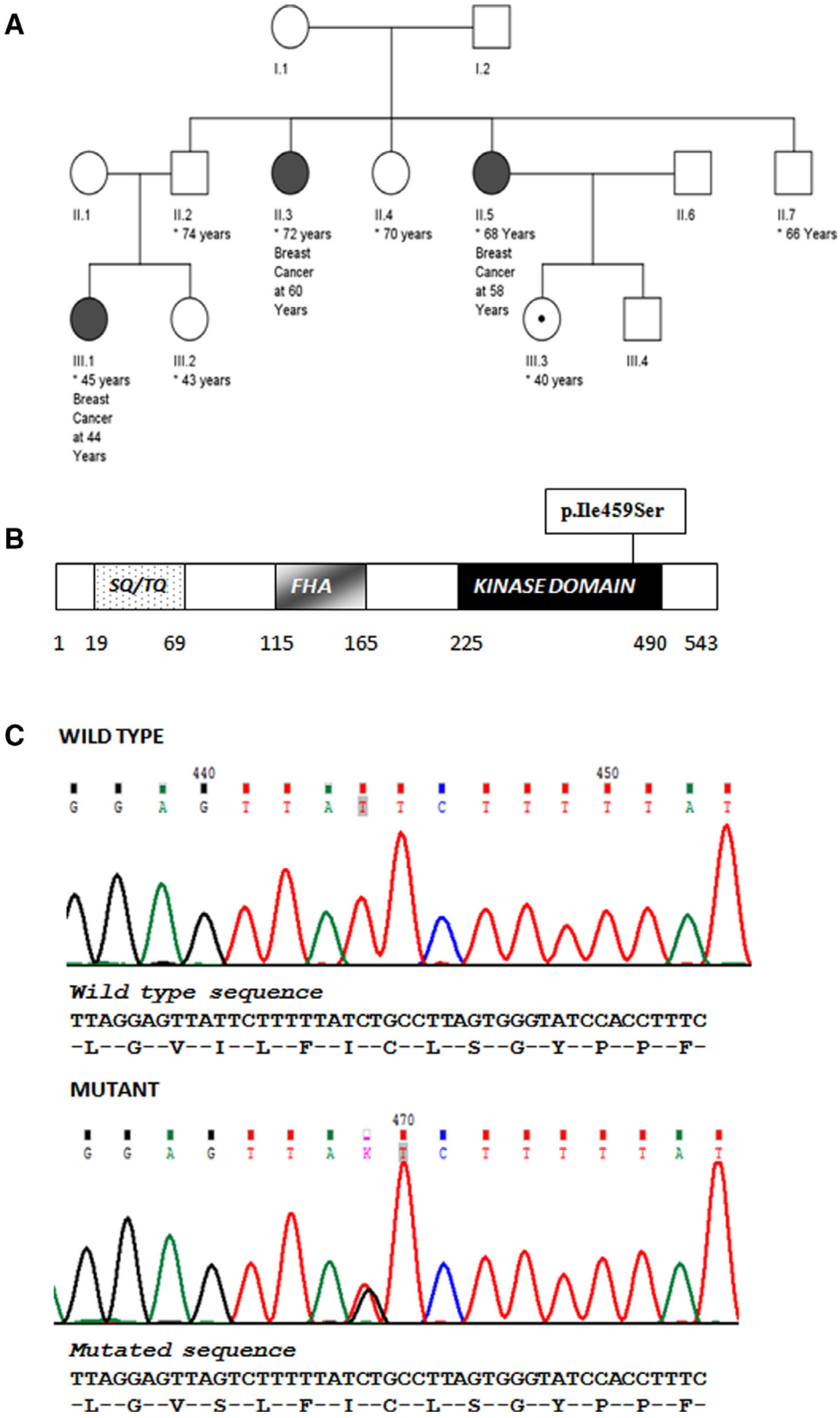

Fig. 1 Novel CHEK2 variant (Ile459Ser) identified in the family.
(A): Pedigree chart of the family.
(B): Domain organisation of CHEK2 protein.constitutes of three distinct functional domains: the SQ/TQ-rich domain (residues 19–69), the forkhead-associated domain (residues 115–165), and the Serine/threonine kinase domain (residues 225–490).
(C): Chromatogram showing wild type and mutant sequences.

ter (III.1) has been recently diagnosed with infiltrating ductal carcinoma breast at 44 years of age. She underwent surgery followed by chemotherapy and radiotherapy. Based on the observation of cases of breast cancer in the family, HBOC was diagnosed. The proband was advised to undergo targeted multigene panel testing for known breast cancer susceptibility genes *ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, MRE11A, MUTYH, NBN, PALB2, PTEN, RAD50, RAD51C, RAD54L, STK11, TP53, MLH1, MSH2, MSH6, EPCAM, PPM1D*. Genomic DNA extracted from blood was used to perform targeted gene sequencing for Hereditary cancer gene panel using a custom capture kit and sequenced on the Illumina sequencing platform in a paired-end mode. Sequencing of protein coding regions were performed to a mean depth of 80–100X. Reads were obtained in FASTQ format and were aligned against human reference genome (GRCh37/hg19). Standard bioinformatics tools were used to align and detect variants in the NGS dataset. We applied Burrows-Wheeler Aligner (BWA v0.7.5a-r405) to align the short length paired-end data. Further, reads with mapping quality (MAPQ) greater than 30 were retained and were processed for downstream analysis. PICARD toolkit was used to remove PCR duplicates and Genome Analysis Tool Kit (GATK) best practice guidelines were followed to detect high quality DNA variants. Identified variants were further annotated using Annovar . Common variants were filtered out based on minor allele frequency (MAF >0.01) in 1000 G phase 3 and Exome aggregation Consortium (ExAC) database. Missense variants were further screened for their pathogenicity using available In-silico prediction analysis tools. Three heterozygous variants of unknown significance (VUS) were identified in three different moderate or low penetrance breast cancer predisposition genes (CHEK2:c.1376T>G, RAD54L:c.1250C>T, FANCM:c.4931G>A) (Table 1). Sanger sequencing was performed to confirm the presence of variants in *CHEK2, FANCM* and *RAD54L* genes in the proband. Additional family members were then screened for the three validated variants. Based on these results variants (*FANCM* and *RAD54L*) were excluded as being causative, as they did not segregate with thecancer phenotype. The *CHEK2* variant (c.1376T>G; p.Ile459Ser) segregated completely with disease status of the three affected and 2 unaffected individuals (Fig. 1 and Table 2).

Interpretation of CHEK2 variant

The identified *CHEK2* missense variant (p.Ile459Ser) identified in this asian indian family is novel and not been previously reported in public database like gnomAD (The Genome Aggregation Database http://gnomad.broadinstitute.org), Human Genome Mutation Database (HGMD; http://www.hgmd.cf.ac.uk) and dbSNP databases (https://www.ncbi.nlm.nih.gov/projects/SNP/). Publically available Somatic cancer mutation databases: Sanger COSMIC, TCGA (The Cancer Genome Atlas) were also checked for this variant but no data is available for the identified variant. The variant was predicted to be damaging by in silico tools like MutationTaster [11] (Score: 1; score closer to 1 indicates Damaging nature) PolyPhen-2 [12] (Score:0.99; probably damaging nature if score is between 0.957 and 1), Mutation Assessor [13], Provean [14] (Score: −5.42; score equal or

Table 1 Variant(s) of uncertain significance (vus) identified in Ngs based multigene panel testing.

Gene (Transcript)	*Variant*	*Amino acid Change*	*Zygosity*	*Depth*	*Allele frequency reported in gnomAD**	*In Silico tools Predicting the variant as Damaging*	*ACMG Classification*
CHEK2 ((ENST00000382580)	c.1376T>G	p.Ile459Ser	Het	150	Not reported	PROVEAN Mutation Taster2 Polyphen2	Uncertain Significance
RAD54L (ENST00000371975)	c.1250C>T	p.Thr417Ile	Het	293	0.00002	PROVEAN Mutation Taster2 Polyphen2	Uncertain Significance
FANCM (ENST00000267430)	c.4931G>A	p.Arg1644Gln	Het	132	0.001	PROVEAN Polyphen2	Uncertain Significance

* The Genome Aggregation Database (gnomAD) http://gnomad.broadinstitute.org.

Table 2 Analysis of CHEK2, FANCM and RAD54L variants among the family members.

Family Member	Age	Diagnosed with breast cancer (age)	CHEK2 (c.1376T>G)	FANCM (c.4931G>A)	RAD54L (c.1250C>T)
Proband	68	Yes (58)	+	+	+
Sister	72	Yes (60)	+	+	–
Niece	45	Yes (44)	+	–	+
Sister	70	Not affected	–	+	+
Niece	43	Not affected	–	–	+
Proband's daughter	40	Not affected	+	+	+

below −2.5 have a "Deleterious" effect) and mutpred2 (http://mutpred2.mutdb.org). MutPred2 score for this variant is 0.882 (The closer the score is to 1, the more likely it is pathogenic or disease-associated (https://www.biorxiv.org/content/early/2017/05/09/134981). MutPred2 report suggests that *CHEK2* protein properties predicted to be affected by this variant is the protein–protein interface, and thus, protein–protein interactions (altered ordered interface with *P* value is 0.00019). This is possibly predicted due to the increased propensity for allosteric interactions in the neighborhood of the variant (at Trp454; *P*-value is 0.009). All the affected members of the family were positive for this variant and this confirmed the association of this variant with susceptibility to breast cancer in this family.

Discussion

Here, we describe a *BRCA* negative Asian Indian family with a history of breast cancer. Three variants of unknown significance, in three different genes, were identified in the index patient (Fig. 1(A); II.5) by next-generation sequencing based targeted multi-gene panel testing (Table 1). Segregation of a novel *CHEK2* gene variant (p.Ile459Ser) correlated with the breast cancer phenotype in the affected family member (Table 2) while the other two variants did not. *CHEK2* is a tumor suppressor gene that encodes for a serine/threonine kinase, involved in DNA repair and cell cycle regulation in response to DNA damage. *CHEK2* is phosphorylated by kinases ATM/ATR, followed by homodimerization enabling it to acquire kinase activity. It regulates cell cycle by arresting the cell cycle in gap 1 phase (G1), in response to DNA damage [2]. In absence of functional *CHEK2* protein, uncontrolled cell division may cause cancer. Several studies have reported the association of *CHEK2* variants with increased predisposition to breast cancer [3–8,15–18].

The most commonly studied *CHEK2* gene variant is c.1100delC in association with an increased risk of developing breast cancer. Its prevalence among the population of different countries is variable [3–5,7,9,10,16]. Other commonly studied variants include Ile157Thr, Arg117Gly, Ile160Met, Gly167Arg/Ala, IVS2+1G >A, del5395, Gln20Ter, Glu85Ter His371Tyr and Asp438Tyr [3,8,17]. About 75 different variants in *CHEK2* gene have been reported in HGMD (Human Genome Mutation Database) public database (http://www.hgmd.cf.ac.uk) and more than 50% (42 out of 75 variants) are associated with breast cancer. More than 60% (50/75) variants reported in the HGMD database in the *CHEK2* gene are missense. We identified a missense variant in the kinase domain region in this family (Fig. 1(B)). Although the impact of missense variants on *CHEK2* protein function is not extensively studied but substitutions in the FHA domain and the kinase domain have been shown to affect protein function [17,18].

CHEK2 is a multi-organ cancer predisposition gene, and increases risks for other cancers like prostate, colon, thyroid and ovarian, in addition to breast cancer [8]. In our family, only breast cancer was observed. *CHEK2* variants have been associated with moderate breast cancer risk which depends on the specific mutation. Carriers of the *CHEK2* variants are reported to have a cumulative breast cancer risk of 28–37% [15,16].

The contribution of the *CHEK2* gene towards genetic susceptibility to breast cancer in Asian Indian patients has been documented in a few studies. Common *CHEK2* variant, c.1100delC was studied in 22 and 91 patients by Rajkumar et al. and Soumitra et al., respectively, and all were negative for this variant [9,10]. In three recent studies, *CHEK2* gene was studied by Next generations sequencing based multigene panel testing [19–21]. In a recent study, Rajkumar et al. studied 91 patients and no disease-causing CHEK2 variant was identified [19]. Mannan et al. studied 141 HBOC patients and found one patient positive for a deleterious *CHEK2* variant (p.Cys284Ter) [20]. In a recent study by Singh et al. over 1000 Asian Indian patients with an indication of breast and/or ovarian cancer, were studied by NGS based multigene panel. Only 4 patients were positive for pathogenic variants and 6 were positive for variants of unknown significance in the *CHEK2* gene [21].

Counseling and management of *CHEK2* positive families is not straightforward because the risk of cancer is evaluated not on the mutation status alone but also on the history of cancer occurrence in the family. It has been observed that risk of breast cancer for *CHEK2* variant carriers, with a positive family history of breast cancer is greater than that for a carrier of the same variant who has no family history of breast cancer [22]. The National Comprehensive Cancer Network Guideline recommends annual mammogram beginning at 40 years of age, with consideration of annual breast MRI and risk-reducing mastectomy (based on family history) for *CHEK2* mutation carriers (www.nccn.org). In the present case, proband's daughter (III.3) is positive for the likely pathogenic CHEK2 variant but has not yet developed breast cancer owing to her younger age (40 years) and incomplete penetrance of CHEK2 gene variants [8]. Considering the history of breast cancer in the family we advised her to follow regular breast cancer screening as per NCCN guidelines.

Conclusion

Use of next-generation sequencing based multi-gene panel testing in families with a hereditary predisposition to cancer has increased in the last few years. This has resulted in many challenges in the clinic like the interpretation of novel variants and risk management guidelines in subjects with variants in genes other than BRCA. This case discusses that moderate penetrance genes like *CHEK2* make a significant contribution to breast cancer susceptibility, and their identification could help in the clinical management of families with a history of breast cancer. Although it has been suggested that risk assessment in *CHEK2* positive families must incorporate variation status and family history of cancer but lifetime risks can be determined by performing larger studies with adequate follow up to define appropriate guidelines for management.

Conflict of interest

All the authors declare that there is no conflict of interest with regards to the preparation and submission of the manuscript.

Compliance with ethical statement

No ethical clearance was required in the study as the molecular tests were carried out as per standard of care, for diagnostic purpose after obtaining informed consent to carry out genetic studies and share the variant data information.

Funding

There was no source of funding for the study.

Informed consent

Informed consents were obtained from all families for the genetic tests.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2019.05.003.

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