Computational analysis of high-risk SNPs in human CHK2 gene responsible for hereditary breast cancer: A functional and structural impact

Nutan V. Badgujar, Bhoomi V. Tarapara, Franky D. Shah

Stem Cell Biology Lab, Department of Cancer Biology, The Gujarat Cancer & Research Institute, Ahmedabad, Gujarat, India

*franky.shah@gcriindia.org, frankyjha@yahoo.com

Abstract

Nowadays CHK2 mutation is studied frequently in hereditary breast and ovarian cancer patients in addition to BRCA1/BRCA2. CHK2 is a tumor suppressor gene that encodes a serine/threonine kinase, also involved in pathways such as DNA repair, cell cycle regulation and apoptosis in response to DNA damage. CHK2 is a well-studied moderate penetrance gene that correlates with third high risk susceptibility gene with an increased risk for breast cancer. Hence before planning large population study, it is better to scrutinize putative functional SNPs of CHK2 using different computational tools. In this study, we have used various computational approaches to identify nsSNPs which are deleterious to the structure and/or function of CHK2 protein that might be causing this disease. Computational analysis was performed by different in silico tools including SIFT, Align GVGD, SNAP-2, PROVEAN, Poly-Phen-2, PANTHER, PhD-SNP, MUpro, iPTREE-STAB, Consurf, InterPro, NCBI Conserved Domain Search tool, ModPred, SPARKS-X, RAMPAGE, Verify-3D, FT Site, COACH and PyMol. Out of 78 nsSNP of human CHK2 gene, seven nsSNPs were predicted functionally most significant SNPs. Among these seven nsSNP, p.Arg160Gly, p.Gly210Arg and p.Ser415Phe are highly conserved residues with conservation score of 9 and three nsSNP were predicted to be involved in post translational modification. The p.Arg160Gly and p.Gly210Arg may interfere in phosphopeptide binding site on FHA conserved domain. The p.Ser415Phe may interfere in formation of activation loop of protein-kinase domain and might interfere in interactions of CHK2 with ligand. The study concludes that mutation of serine to phenylalanine at position 415 is a major mutation in native CHK2 protein which might contribute to its malfunction, ultimately causing disease. This is the first comprehensive study, where CHK2 gene variants are analyzed using in silico tools hence it will be of great help while considering large scale studies and also in developing precision medicines related to these polymorphisms in the era of personalized medicine.
Introduction

Of all cancers, one of the main cause of cancer related deaths is breast cancer among women worldwide, with 5% to 10% of cases being due to hereditary risk [1]. The CHK2 gene is moderately penetrance gene most extensively studied as possible third high risk susceptibility gene in hereditary breast and ovarian cancer. CHK2 gene is the human homolog of Rad53 (Saccharomyces cerevisiae) and Cds1 (Schizosaccharomyces pombe). Human CHK2 gene is a tumor suppressor gene, located on long arm of chromosome 22 at q12.1 and encoded by CHK2 serine/threonine kinase. It consists of three major domains. 1) N-terminal has SQ/TQ cluster domain that serves as a site for phosphorylation in response to DNA damage, 2) forkhead-associated protein interaction domain (FHA) which is essential for activation in response to DNA damage and is rapidly phosphorylated in response to replication blocks and DNA damage. In FHA domain residues 112–175 are involved in dimerization of CHK2 molecules in phosphorylation manner, for full activation of CHK2 by trans-autophosphorylation of the activation loop. The major function of FHA domain is to regulate the kinase activities in CHK2 by interacting with other proteins thus mediates protein-protein interactions [2, 3] and 3) C-terminal which has serine/threonine kinase activity [4]. CHK2 is activated by the kinases ATM and ATR in response to DNA double-strand breaks or replicative stress [5]. These proteins catalyze the phosphorylation of threonine 68 of CHK2 causing its transient dimerization via the FHA domain leading to CHK2 trans-autophosphorylation and its full activation. In response to DNA damage, CHK2 gene is involved in different pathways such as cell cycle regulation, DNA repair and apoptosis. CHK2 phosphorylates downstream cell cycle regulators such as p53, Cdc25, and BRCA1 to activate checkpoint repair or recovery responses, as well as concurrently delay entry into mitosis [6, 7]. Deviation from its normal physiological function is likely to contribute to disease pathogenesis. In particular, the missense variants of CHK2 p.Ile157Thr, p.Asp252Gly, c.1100delC, p.His371Tyr, p.Glu161del, p.Arg117Gly, p.Ser428Phe, c.591delA, p.Thr476Met and p.Asp438Tyr were significantly associated with germ-line variants in hereditary breast and ovarian cancer [8–11]. Finnish population (1.4%) and Polish population (0.2%) confer a relative risk for developing breast tumors of about 2 for women and 10 for men if c.1100delC mutation is present [12, 13]. Variant p.Ile157Thr, present in 5.3% of the Finnish population and in 4.8% of the Polish population, confers a relative risk of breast cancer of 1.5 [14, 15]. A recent analysis by the Breast Cancer Association Consortium (BCAC) estimated a relative risk of 2.26 for p.Thr367MetfsTer15 (rs555607708) [16]. Limited data is available for whole CHK2 gene for hereditary breast and ovarian cancer. Further, impact of missense variants on protein function is not known fully, although substitutions in the FHA domain and the kinase domain have been shown to abolish activity [17–19].

Single nucleotide polymorphism is a common genetic variant in human and about 93% SNPs are present in human genes [20]. SNPs can be present in coding, noncoding or intergenic regions [21, 22]. Both non-coding and intergenic SNPs may have slight impact, but non-synonymous coding SNPs (nsSNPs) have more impact on protein [20]. Identification of the impact of variants on structure, stability and function of the protein is an important task as not all reported polymorphisms are deleterious [23]. Therefore there is a need to understand the deleterious impact of nsSNPs on protein structure and function using different recent molecular biology techniques. Till now large numbers of SNPs are reported in NCBI data, to screen these nsSNPs for their impact on biological function through experimental work is very tedious and costly. However, utilization of computational methods could be an efficient alternative for the same.

Nowadays, different computational tools have been extensively used for predicting deleterious nsSNP and their role in protein function, stability and structure maintenance. Taking all
these in consideration, the present study is aimed to determine various deleterious nsSNPs of human CHK2 gene using SIFT, Align GVGD, SNAP-2, PROVEAN, PolyPhen-2, PANTHER, PhD-SNP, I-Mutant, iPTREE-STAB, Mupro etc. Conservation of amino acid residues was predicted using ConSurf. ModPred was used to identify post-translational modification site present in protein. The 3D structure of the CHK2 protein was generated using SPARK-X and refined using ModRefiner. The quality of model was checked using RAMPAGE and Varify3D. The ligand binding sites were predicted using FTsite and COACH. The visualization of 3-D structure and labelling of native as well mutant amino acid was done using Pymol and Swiss PDB viewer.

Materials and methods

SNP dataset

The data related to human CHK2 gene was retrieved from following databases: Uniport database (https://www.uniprot.org) (UniprotKB ID 096017), the NCBI database SNP (rsIDs) and FASTA nucleotide sequence (NC_000022.11) and amino acid sequence (NP_001005735) sequence from (https://www.ncbi.nlm.nih.gov) for further computational analysis [24, 25].

Prediction of functional consequence of non-synonymous SNPs

The functional consequences of the nsSNP of human CHK2 gene were analysed using different computational tools.

**SIFT.** SIFT (Sorting intolerant from tolerant) predicts whether an amino acid substitution affects protein function based on sequence homology and the physical properties of amino acids. SIFT can be applied to naturally occurring nonsynonymous polymorphisms and laboratory-induced missense variants. SIFT (http://siftdna.org/www/SIFT_dbSNP.html) determines if an amino acid substitution is deleterious to protein function [26]. A SIFT score predicts whether an amino acid substitution affects protein function. The SIFT score ranges from 0.0 (deleterious) to 1.0 (tolerated). The input query for SIFT algorithm is rsIds of SNPs from dbSNP.

**Align GVGD.** Align GVGD is a web based program available at http://agvgd.hci.utah.edu/. It combines the biophysical characteristics of amino acids, protein multiple sequence alignments to predict whether the missense substitution is deleterious or not [27]. The input query is FASTA sequence of protein and amino acid substitution.

**SNAP2.** SNAP2 (Screening of non-acceptable Polymorphism 2) predicts the functional consequences of amino acid variation based on neutral network classification method [28]. It is a web based tool available at https://www.rostlab.org/services/SNAP/ in which the input query is a protein sequence of CHK2 in FASTA format.

**PROVEAN.** PROVEAN (Protein variation effect analyzer) predicts whether single nucleotide variant affects protein function through alignment based score [29]. It is an online software available at http://provean.jcvi.org/index.php produced by J Craig Venture Institute. Based on this, if the score is below threshold value of 2.5, variant is predicted deleterious whereas the variant is neutral if the score is above 2.5. The input query is the FASTA sequence of protein CHK2 and amino acid variants.

**PolyPhen-2.** PolyPhen-2 (Polymorphism Phenotyping V2) predicts the impact of amino acid substitution on protein structure and function by using straight forward physical and comparative consideration [30]. It is a web based tool available online at http://genetics.bwh.harvard.edu/pph2/. It calculates the PSIC (Position-Specific independent score). If score is >0.85, then variant is probably damaging and score is >0.15 possibly damaging and rest are
considered as benign. The input query for PolyPhen-2 is FASTA sequence of protein CHK2 and amino acid variants.

**PANTHER.** PANTHER cSNP (Protein analysis through evolutionary relationship-coding SNP) predicts functional consequences of variants on the protein. It is an online tool available at [http://pantherdb.org/tools/csnpscoreForm.jsp](http://pantherdb.org/tools/csnpscoreForm.jsp). It compares the sequence of protein with a family of evolutionarily related protein. Longer the preservation time, higher the functional impact of amino acid variant. It calculates the subPSEC (Substitution Position Specific evolutionary conservation) score on the basis of alignment of evolutionary related proteins [31]. The input query is plain protein sequence, amino acid variants and human organism.

**PhD-SNP.** PhD-SNP (Predictor of human deleterious single nucleotide polymorphism) server is a Support Vector Machine (SVM) based method to discriminate between neutral and disease-related single point protein variants [32]. It is an online tool available at [http://snps.biofold.org/phd-snp/phd-snp.html](http://snps.biofold.org/phd-snp/phd-snp.html). Results were obtained through evolutionary information and using hybrid predictive model. The input query is plain protein sequence, position of SNP along with new residue.

**MUpro.** MUpro is a set of machine learning programs which predicts the protein stability changes for single nucleotide variation in amino acid sequence [33]. It is a web based server available at [http://mupro.proteomics.ics.uci.edu/](http://mupro.proteomics.ics.uci.edu/). Prediction of result based on both value and sign of energy change using SVM and sequence information only. The input query for this is also a plain sequence of protein followed by original and substituted amino acid.

**iPTREE-STAB.** iPTREE-STAB is a web based server available at [http://203.64.84.190:8080/iPTREEr/iptree.html](http://203.64.84.190:8080/iPTREEr/iptree.html) which is based on decision tree. It predicts the impact of single amino acid change on protein stability [34]. The input query is original amino acid as well as mutated amino acid residue followed by three flanking residues from both sides of the mutated residue.

**Phylogenetic conservation**

Consurf is a computational tool available at [http://consurf.tau.ac.il](http://consurf.tau.ac.il) which calculates the evolutionary conservation of amino acid position through phylogenic relations between homologous sequences [35]. Consurf calculates conservation score from 0 to 9 which is classified into variable, average and highly conserved. The input query for consurf is FASTA sequence of protein CHK2.

**Prediction of post translational modification sites**

The ModPred server is available at [http://www.modpred.org](http://www.modpred.org) which is used to predict post translational modification sites within CHK2 protein sequence. ModPred is a sequence-based predictor of potential post-translational modification (PTM) sites in proteins. It consists of 34 ensembles of logistic regression models, trained separately on a combined set of 126,036 non-redundant experimentally verified sites for 23 different modifications, obtained from public databases and an ad-hoc literature search [36].

**ExAC browser beta**

ExAC browser is freely available at [http://exac.broadinstitute.org](http://exac.broadinstitute.org). The minor allele frequency (MAF) was retrieved from ExAC Browser Beta for the nsSNPs of human CHK2 gene. The Exome Aggregation Consortium (ExAC) is a coalition of investigators seeking to aggregate and harmonize exome sequencing data from a variety of large-scale sequencing projects and to make summary data available for the wider scientific community. The ExAC browser provides gene and transcriptcentric displays of variation, a critical view for clinical applications.
Additionally, it provides a variant display, which includes population frequency and functional annotation data as well as short read support for the called variant. ExAC has already been used extensively by clinical laboratories worldwide [37]. The input query is name of human CHK2 gene.

**Prediction of nsSNPs position in different protein domains**
NCBI Conserved Domain Search tool (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi and InterPro (https://www.ebi.ac.uk/interpro/) were used to locate the position of SNPs in different domains of CHK2 protein structure [38, 39]. Input query for InterPro is a plain sequence of CHK2 and for NCBI Conserved Domain Search tool the input query is FASTA amino acid sequence of protein CHK2.

**Protein 3D modelling and structural analysis**
The 3D structure of full length CHK2 protein is not available in protein data bank. The 3D structure of protein CHK2 was generated using SPARKS-X fold recognition server (http://sparks-lab.org/yueyang/server/SPARKS-X) [40]. The input query for SPARKS-X server is FASTA amino acid sequence of protein CHK2. The degree of similarity of templates used by SPARKS-X server for 3D model prediction was checked by BLASTp. The 3D structure predicted by SPARKS-X server was further refined using Modrefiner (https://zhanglab.ccmb.med.umich.edu/ModRefiner) [41]. The quality of refined model was checked using Varify3D (http://services.mbi.ucla.edu/Verify3D) and RAMPAGE (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) [42]. Input query for Varify3D and RAMPAGE analysis is refined structure predicted using SPARKS-X.

**Ligand binding site prediction**
The ligand binding sites within CHK2 protein were predicted using FT site server (http://ftsite.bu.edu/) and COACH server (https://zhanglab.ccmb.med.umich.edu/COACH/). FT site is freely available online tool which predicts ligand binding sites of CHK2 protein. FT site accurately identifies binding sites in over 94% of apoproteins, including structure based prediction of protein, the explanation of functional relationships among proteins, protein engineering and drug designing [43]. COACH is a meta-server based approach used for protein-ligand binding site prediction. Using two comparative methods, TM-SITE and S-SITE COACH predicts complementary ligand binding sites [44]. The input query for COACH is refined structure generated by modrefiner. PyMol and Swiss PDB viewer were used to visualize 3D structure of protein.

**Results**

**SNP database**
The CHK2 gene investigated in the present study was retrieved from dbSNP database (dbSNP-NCBI: https://www.ncbi.nlm.nih.gov/snp/?term=chek2). It contained a total of 13929 SNPs out of which 753 are missense (nsSNP), 105 are frame shift, 642 in 5’UTR, 55 in 3’ UTR, 50 nonsense, 13062 intronic, 50 stop gained, 19 in 3’ splice site, 24 in 5’ splice site and 266 in coding synonymous SNPs (Fig 1). Only nsSNP of CHK2 were selected for this investigation.

**Prediction of functional nsSNPs in CHK2**
The CHK2 single nucleotide variants obtained from dbSNP analysis were subjected to computational analysis through variety of tools. According to SIFT result out of 753 nsSNPs of CHK2
gene total 78 SNP were predicted to be tolerated or deleterious and rest of 675 were not found in SIFT results. From these 78 SNPs, SIFT classified 35 nsSNPs as damaging, 43 as tolerated. To increase the accuracy of computational techniques, all the 78 SNPs predicted in SIFT were further validated by Align GVGD, SNAP2, PROVEAN, PolyPhen2 and PANTHER tools. Align GVGD is a method that combines Grantham Variation (GV) and Grantham Deviation (GD) scores to predicts whether the missense substitution is deleterious or not. In Align GVGD, if GD score is less than C15 then substitution is less likely affected and score is greater than C65 then substitution is most likely affected. Out of 78 nsSNP Align GVGD predicted 43 SNPs as most likely affected and 10 nsSNPs as less likely affected. SNAP2 predicts whether the impact of amino acid variation is neutral or has effect on a query protein function by evaluating mutability landscape of the entire query protein sequence. Out of 78 SNPs subjected to SNAP2 prediction, 41 showed effect on protein function and 37 predicted as neutral SNPs. Among 78 SNPs subjected to PROVEAN analysis, 35 SNPs were predicted as deleterious and 43 SNPs were predicted as neutral. Out of 78 SNPs subjected to PolyPhen2 analysis 41 were predicted probably damaging, 10 predicted possibly damaging, 26 predicted benign and 1 was not predicted by PolyPhen2. For every input variant PolyPhen2 calculates PSIC (Position specific independent score). Out of 78 nsSNPs, 37 SNPs were predicted probably damaging, 17 predicted possibly damaging and rest 24 SNPs predicted probably benign by PANTHER cSNP. The nsSNP predicted as probably damaging by PolyPhen and PANTHER were considered as damaging and used for further analysis.

All the 78 nsSNPs of CHK2 gene were further analyzed for correlation with disease after functional impact through PhD-SNP. PhD-SNP is a SVM based classifier which predicts the result through evolutionary information and hybrid predictive method with the accuracy of 78% of human protein [29]. PhD-SNP revealed the most unique results showing only 20 nsSNPs as diseased and rest of 58 SNPs as neutral.

We predicted any stability alteration in the CHK2 protein with the help of MuPro and iPTREE-STAB which predict the result by considering single site variant. MuPro predicted 56
nsSNP which decrease stability of CHK2 protein and rest of 22 SNPs increase stability. iPTREE-STAB result revealed to decrease stability of 74 nsSNP and 4 nsSNP showed increase in protein stability. According to some studies, decreased protein stability causes increase in degradation, misfolding and aggregation of proteins. We shortlisted those nsSNP which are common in all 9 different algorithm tools and predicted as deleterious SNPs. Total 7 SNPs out of 78 SNPs met the criteria and classified them as high risk and selected for further analysis. Result of SIFT, Align GVD, SNAP2, PROVEAN, PANTHER, Ph-D SNP, MuPro and iPTREE-STAB is shown in Table 1.

**Conservation profile of deleterious nsSNP in CHK2**

Evolutionary information is used to predict whether the substitution of amino acid affects the protein functions or not. Consurf web server was used to calculate the conservation score of amino acid residue of CHK2 protein to further analyze possible effect of 7 most deleterious nsSNP predicted through different computational tool. Results were obtained in the form of structural representation of the protein (S1 Fig). Highly conserved residues are predicted as either functional or structural based on their location either on protein surface or inside its core. Results obtained via conSurf represented all residues of CHK2 showing their structural and functional conservation levels. But we focused only on those residues which matched their positions with 7 high risk nsSNPs which we have identified. Taking this into consideration, those nsSNPs which are located at these conserved regions are considered immensely damaging to protein as compared to those at non-conserved sites [45, 46]. According to consurf output, p.Arg160Gly, p.Gly210Arg, p.Ser415Phe are highly conserved residues with conservation score of 9. Four amino acids were predicted average conserved. The result of consurf is shown in Table 2. The summary of deleterious prediction for each SNP is shown in Fig 2.

**Prediction of post translational modification sites**

Post translational modification sites present within human CHK2 protein were predicted using ModPred. Out of 7 most significant nsSNPs, three amino acids p.Arg160Gly, p.Arg223Cys, p.Arg188Trp were predicted to be involved in post translational modification sites including proteolytic cleavage and ADP ribosylation. The results of modpred are shown in Table 2.

**ExAC**

The minor allele frequency (MAF) was retrieved from ExAC Browser Beta (http://exac.broadinstitute.org/gene/ENSG00000183765) for the nsSNPs of human CHK2 gene. The result of minor allele frequency of nsSNPs is shown in Table 2.

**Prediction of nsSNPs position in different protein domains**

According to Interpro and NCBI Conserved Domain Search tool two major domains were predicted in CHK2 protein. One was STKc_Chk2 domain (serine/threonine kinase, cell cycle checkpoint kinase 2) which comprises 256–529 amino acids and another one was FHA domain (Forkhead associated domain) which comprises 156 to 244 amino acids. In CHK2 amino acid sequences 269–411 were predicted catalytic domain of ATP Binding site; 264–471 were predicted catalytic domain of dimer interface; 269–434 amino acid sequences were present in active site; 273–434 amino acid sequences were present in polypeptide substrate binding site. The 22 amino acid residues present in activation loop (Thr, Asp, Phe, Gly, His, Ser at 415, Lys, Ile, Leu, Gly, Glu, Thr, Ser, Leu, Met, Arg, Thr, Leu, Cys, Gly, Thr, Pro, Thr) of
Table 1. Prediction of functional consequences of nsSNP in human CK2.

| SNPs rs ID  | AA Variant | SIFT   | Align GVGD | SNAP2 | PROVEAN | Poly Phen-2 | Panther | Ph-D SNP | MuPro | iPTREE-STA |
|------------|------------|--------|------------|-------|----------|-------------|---------|----------|-------|------------|
| rs17879961 | I200T      | C65    | Effect     | PosD  | PosD     | N-1         | ↓       | -ve      |
| rs17882942 | L555V      | C25    | Neutral    | N     | Ben.     | ProB N-9    | ↓       | -ve      |
| rs17883172 | E544K      | C55    | Neutral    | N     | Ben.     | ProB N-3    | ↓       | -ve      |
| rs17883862 | P85L       | C65    | Effect     | N     | ProD     | PosD N-6    | ↑       | -ve      |
| rs17886163 | I491S      | C65    | Neutral    | N     | Ben.     | PosD N-5    | ↓       | -ve      |
| rs28909980 | D390N      | C15    | Effect     | D     | ProD     | ProD N-0    | ↓       | -ve      |
| rs28909982 | R160G      | C65    | Effect     | D     | ProD     | ProD Di-5   | ↓       | -ve      |
| rs27552322 | G210R      | C65    | Effect     | D     | ProD     | ProD Di-7   | ↓       | -ve      |
| rs27552323 | I203T      | C65    | Effect     | D     | ProD     | ProD Di-2   | ↓       | -ve      |
| rs77130927 | R223C      | C65    | Neutral    | D     | PosD     | PosD Di-10  | ↓       | -ve      |
| rs121908694| S41F       | C65    | Effect     | N     | ProD     | ProD N-6    | ↓       | -ve      |
| rs121908701| R224H      | C25    | Neutral    | N     | Ben.     | ProB Di-2   | ↓       | -ve      |
| rs121908702| E282K      | C55    | Neutral    | N     | ProD     | PosD Di-2   | ↓       | -ve      |
| rs121908703| S399L      | C65    | Neutral    | N     | PosD     | PosD N-2    | ↑       | -ve      |
| rs121908704| T444A      | C55    | Neutral    | N     | Ben.     | ProB N-6    | ↓       | -ve      |
| rs121908705| N489D      | C15    | Neutral    | N     | Ben.     | ProB N-7    | ↓       | -ve      |
| rs121908706| RS17H      | C25    | Effect     | D     | ProD     | ProD Di-7   | ↓       | -ve      |
| rs13783007 | R188W      | C65    | Effect     | D     | ProD     | ProD Di-2   | ↓       | -ve      |
| rs13783008 | A175       | C65    | Neutral    | N     | Ben.     | ProB N-5    | ↓       | -ve      |
| rs13783009 | R223H      | C25    | Neutral    | D     | Ben.     | PosD Di-4   | ↓       | -ve      |
| rs13783010 | R224C      | C65    | Neutral    | N     | Ben.     | ProB Di-4   | ↓       | -ve      |
| rs13783011 | S471F      | C65    | Neutral    | N     | ProD     | PosD Di-1   | ↑       | -ve      |
| rs13804061 | E571K      | C55    | Neutral    | D     | ProD     | PosD N-6    | ↓       | -ve      |
| rs13908861 | V494A      | C55    | Neutral    | N     | Ben.     | ProB Di-0   | ↓       | -ve      |
| rs139366548| Y467H      | C65    | Effect     | D     | ProD     | ProD N-8    | ↓       | -ve      |
| rs141568342| E64K       | C55    | Effect     | N     | Ben.     | ProB N-6    | ↓       | -ve      |
| rs141776984| C286R      | C65    | Effect     | D     | ProD     | ProD Di-1   | ↓       | -ve      |
| rs142243299| V25I       | C25    | Neutral    | N     | Ben.     | ProB N-5    | ↓       | -ve      |
| rs142763740| TS19M      | C55    | Neutral    | D     | ProD     | PosD N-7    | ↓       | -ve      |
| rs14361174 | R361H      | C25    | Neutral    | N     | Ben.     | PosD N-6    | ↓       | -ve      |
| rs143965148| D540N      | C15    | Neutral    | N     | Ben.     | ProB N-8    | ↓       | -ve      |
| rs144850845| G210E      | C65    | Effect     | D     | ProD     | ProD N-1    | ↓       | -ve      |
| rs145324174| C428Y      | C65    | Effect     | D     | ProD     | ProD N-1    | ↓       | -ve      |
| rs146198085| N229H      | C65    | Neutral    | D     | ProD     | ProD Di-8   | ↓       | -ve      |
| rs147877722| S415F      | C65    | Effect     | D     | ProD     | ProD Di-5   | ↓       | -ve      |
| rs148053495| R361C      | C65    | Effect     | D     | ProD     | ProD Di-3   | ↓       | -ve      |
| rs149501505| R566C      | C65    | Effect     | N     | ProD     | ProB N-5    | ↓       | -ve      |
| rs149991239| T59K       | C65    | Effect     | D     | ProD     | ProD N-4    | ↓       | -ve      |
| rs199708878| R3W        | C65    | Effect     | N     | ProD     | ProD N-8    | ↓       | -ve      |
| rs199749372| I264V      | C25    | Neutral    | N     | Ben.     | ProD N-8    | ↓       | -ve      |
| rs199859140| D404H      | C65    | Neutral    | N     | PosD     | ProB Di-7   | ↓       | -ve      |
| rs200050883| D481Y      | C65    | Effect     | D     | ProD     | PosD Di-7   | ↓       | -ve      |
| rs200432447| R562G      | C65    | Effect     | D     | Ben.     | ProD N-2    | ↓       | -ve      |
| rs200451612| I264M      | C0     | Neutral    | N     | PosD     | ProD N-3    | ↓       | -ve      |
| rs200649225| R449H      | C25    | Neutral    | N     | ProD     | PosD N-3    | ↓       | -ve      |
| rs200928781| Y433C      | C65    | Effect     | D     | ProD     | ProD N-6    | ↓       | -ve      |

(Continued)
STKc_Chk2 domain. The 160 to 210 amino acid sequences present in polypeptide binding site on conserved domain of FHA domain (Arg at 160th and Gly at 210th position).

Protein 3D modeling and structural analysis

The 3D structure of full length CHK2 protein was not available in protein data bank. SPARKS-X modeled 3D structure of CHK2 protein by submitting FASTA amino acid sequences, where 10 best full length models were generated using different similar templates. The quality of full length models were predicted based on (>6) Z-score. All the templates were subjected to BLASTp analysis to identify the sequence similarity of the templates with CHK2 protein. The 3D structure generated using 3i6wA as a template was used for further analysis.

Table 1. (Continued)

| SNPs rs ID | AA Variant | SIFT | Align GVGD | SNAP2 | Poly Phen-2 | Panther | Ph-D SNP | MuPro | iPTREE-STA |
|------------|------------|------|------------|-------|-------------|---------|---------|-------|-----------|
| rs201084748 | SSL | T | C65 | Effect | N | Ben | ProB | N-8 | ↓ | -ve |
| rs201206424 | R389C | D | C65 | Effect | D | ProD | ProD | N-7 | ↓ | -ve |
| rs202051128 | I387M | T | C0 | Effect | N | ProD | ProD | N-7 | ↓ | -ve |
| rs202089930 | T426I | D | C65 | Effect | D | ProD | ProD | N-1 | ↓ | -ve |
| rs267606211 | S422F | D | C65 | Effect | D | PosD | ProD | N-2 | ↑ | +ve |
| rs366570187 | R180Q | T | C35 | Neutral | N | Ben | ProB | N-7 | ↓ | -ve |
| rs3696070738 | N448S | T | C45 | Neutral | N | Ben | ProB | N-2 | ↓ | -ve |
| rs369223840 | N229S | T | C45 | Neutral | D | ProD | ProD | N-4 | ↓ | -ve |
| rs369256181 | Q11R | T | C35 | Neutral | N | Ben | ProD | N-7 | ↓ | -ve |
| rs370968992 | F518I | T | C15 | Neutral | N | Ben | ProB | N-7 | ↓ | -ve |
| rs371207635 | H382Y | D | C65 | Effect | D | ProD | ProD | N-6 | ↓ | -ve |
| rs371657037 | S53T | D | C55 | Neutral | N | ProD | ProD | N-5 | ↓ | -ve |
| rs372874441 | D177H | T | C65 | Neutral | N | ProD | ProB | N-8 | ↓ | -ve |
| rs373073383 | A435V | D | C55 | Effect | D | ProD | ProD | N-8 | ↓ | -ve |
| rs373648967 | K162R | T | C25 | Neutral | N | Ben | ProD | N-6 | ↓ | +ve |
| rs373959274 | R564Q | D | C35 | Effect | N | ProD | ProD | N-3 | ↓ | -ve |
| rs374395284 | E364A | D | C65 | Effect | D | ProD | ProD | N-4 | ↓ | -ve |
| rs374660293 | L381H | D | C65 | Effect | D | ProD | ProD | N-4 | ↓ | -ve |
| rs375130261 | M424V | D | C15 | Effect | D | ProD | ProD | N-8 | ↓ | -ve |
| rs17880867 | N489K | T | C65 | Neutral | N | Ben | ProB | N-4 | ↓ | -ve |
| rs17881473 | F490I | D | C15 | Effect | D | Ben | ProD | N-8 | ↓ | -ve |
| rs17882922 | L479M | D | C0 | Neutral | N | ProD | ProD | N-1 | ↓ | -ve |
| rs112032663 | G30D | T | C65 | Effect | N | ProD | ProB | N-3 | ↓ | +ve |
| rs113947614 | I264T | T | C65 | Neutral | N | Ben | ProD | N-4 | ↓ | -ve |
| rs137926355 | R144Q | T | C35 | Neutral | N | - | - | N-8 | ↓ | -ve |
| rs141502354 | I386V | T | C25 | Neutral | N | PosD | PosD | N-6 | ↓ | -ve |
| rs142966756 | R191M | D | C65 | Effect | D | ProD | PosD | N-5 | ↓ | -ve |
| rs150677496 | L173Q | T | C65 | Effect | D | ProD | PosD | Di-5 | ↓ | -ve |
| rs151218932 | C18Y | T | C65 | Effect | N | Ben | ProB | N-4 | ↓ | -ve |
| rs32168051 | P225H | D | C65 | Effect | D | ProD | ProD | Di-5 | ↓ | -ve |
| rs375507194 | Q20H | D | C15 | Neutral | N | ProD | ProB | N-6 | ↓ | -ve |
| rs376736188 | Q27E | T | C25 | Effect | N | PosD | ProB | N-9 | ↓ | +ve |

Where D: Deleterious; T: Tolerated; GD ≥ C65 = most likely affected; GD ≥ C0 = less likely affected; N: Neutral; D: Deleterious; ProD: Probably damaging; ProB: Probably benign; PosD: Possibly damaging; Ben: Benign; N: Neutral; Di: Disease; ↓: Decrease; ↑: Increase; -ve: negative; +ve: positive

https://doi.org/10.1371/journal.pone.0220711.t001
The 3D structure was further refined by submitting structure in ModRefiner server which showed RMSD value 2.821 and TM-score of 0.9685 to initial model. After that refined structure was further validated using Verify3D and RAMPAGE tools. Ramachandran plot analysis by RAMPAGE for the native protein model showed 541 (92.6%) residues in favoured region, 38 (6.5%) residues in allowed region and 5 (0.9) residues in outlier region. Varify-3D showed 66.21% of the amino acids have scored $\leq 0.2$ in 3D-1D profile. The results of both tools are shown in Table 3.

Ligand binding site prediction

FT site server predicted 3 binding sites present in CHK2 protein. First binding site consisted residues Lys at 292$^{\text{th}}$, Leu at 320$^{\text{th}}$, Ile at 329$^{\text{th}}$, Ile at 342$^{\text{th}}$, Ile at 331$^{\text{th}}$, Leu at 344$^{\text{th}}$, Thr at

![Graphical representation of nonsynonymous SNPs in CHK2 gene and protein](https://doi.org/10.1371/journal.pone.0220711.g002)
410th, Asp at 411th, Phe at 412th, Gly at 413th, His at 414 and Ser at 415th position. Second binding site consisted residues Trp at 93th, Tyr at 199th, Ile at 200th, Ala at 201th, Pro at 225th, Leu at 226th, Asn at 227th and Asp at 246th position. Third binding site constituted Trp at 93th, Asn at 197th, Ser at 198th, Tyr at 199th, Asp at 246th, Thr at 248th and Val at 249th position. Two binding sites are presented using PyMOL in Fig 3. Coach server also predicted Ser at 415th position within ligand binding sites. The detailed results of COACH prediction are shown in Table 4.

**3D structure prediction of mutant and model validation**

The 3D structure of mutant of CHK2 protein was generated by substituting serine with phenylalanine at 415th position in wild type sequence and the sequence was submitted to SPARKS-X server. The 3D structure generated was further refined by submitting structure in ModRefiner server which showed RMSD value 2.310 and TM score of 0.9517. The prediction of TM score suggested the structural deviation of mutant protein as compared to native. After that refined structure was further validated using Verify3D and RAMPAGE. Verify 3D showed 60.75% of the amino acids have scored ≥0.2 in 3D-1D profile. Mutant model is a good quality as having more than 90% region in favoured region. Mutant model showed (93.3%) residues in favoured region, 31 residues (5.3%) residues in allowed region and 8 (1.4%) residues in outlier region.

**Discussion**

The CHK2 gene is a tumor suppressor gene, involved in cell-cycle regulation, in response to DNA damage, DNA repair and apoptosis pathway. Variants of CHK2 have been implicated in various types of cancer including breast cancer [47]. Single nucleotide polymorphism plays an important role in most of the diseases. About more than 4 million unique human single nucleotide polymorphism (SNPs) have been described by dbSNPs and 2% of the reported SNPs associate with monogenic diseases are present in protein coding region and hence predicted that these SNPs can be related to complex inherited disease traits [48]. Testing the functional
consequences of variant by using functional assay can be the best approach but it is quite costly and time consuming too. Hence, for this purpose we have exploited computational approach by using various in silico tools of different algorithms for the analysis of SNVs in \textit{CHK2} gene. To date, 13929 human \textit{CHK2} gene SNPs are reported in NCBI dbSNP (database) which have been located in non-coding, coding and regulatory regions. The coding SNVs cause amino acid variation which further alters the protein function and leads to disease susceptibility. All the nsSNPs may not have major deleterious effect on protein function, some may have neutral effect. Therefore it is necessary to differentiate deleterious SNPs from the neutral SNPs to analyze susceptibility of individual SNPs to diseases, and also to focus on those SNVs which are responsible for structural and functional consequences of \textit{CHK2} protein [49]. However, to

### Table 4. Prediction of ligand binding sites within \textit{CHK2} protein using COACH.

| COACH Result | C-Score | Cluster Size | Name of ligands | Residue number |
|--------------|---------|--------------|-----------------|----------------|
| TM-site      |         |              |                 |                |
|              | 0.58    | 308          | ANP, ADP, ATP   | 267,269,270,271,272,273,274,275,277,279,290,292,329,344,345,346,347,348,350,351,354,394,395,397,410,411 |
|              | 0.29    | 11           | AMP,FMM,76      | 269,277,290,292,316,320,329,342,344,345,346,347,350,357,411,412 |
|              | 0.20    | 30           | III             | 271,272,273,274,351,353,357,392,393,394,415,431,432,434,462,466,467,468,471,472,473,479,480 |
|              | 0.19    | 9            | Mg, ANP, B11    | 292,395,411    |
|              | 0.18    | 5            | AF3, MG, PO4    | 272,273,274,390,392,395,411 |
|              | 0.11    | 9            | III, TAR        | 351,353,354,355,356,357,358,359,392,393,394,411,414,427,428,429,430,431,432,434,438,439,440,441,462,466,467,468,469,470,471,476,477,479,480 |
|              | 0.13    | 18           | PDY, IMD, AGX   | 353,354,355,356,357,358,359,392,393,394,433,462,466,467,468,469,470,471,476,477,479,480,482,488,489,491,492,493,494 |
|              | 0.11    | 9            | III, TAR        | 351,353,354,355,356,357,360,390,392,393,394,411,414,427,428,429,430,431,432,434,438,439,440,441,462,466,467,468,469,470,471,476,477,479,480,482,488,489,491,492,493,494 |

To date, 13929 human \textit{CHK2} gene SNPs are reported in NCBI dbSNP (database) which have been located in non-coding, coding and regulatory regions. The coding SNVs cause amino acid variation which further alters the protein function and leads to disease susceptibility. All the nsSNPs may not have major deleterious effect on protein function, some may have neutral effect. Therefore it is necessary to differentiate deleterious SNPs from the neutral SNPs to analyze susceptibility of individual SNPs to diseases, and also to focus on those SNVs which are responsible for structural and functional consequences of \textit{CHK2} protein [49]. However, to
predict the pathogenic effect of nsSNP using single bioinformatic tool may not be reliable [50]. In present study prediction of CHK2 genetic variants was accomplished by utilizing sequence and structure based bioinformatics tools- SIFT, Align GVGD, SNAP2, PolyPhen 2, PROVEAN, PANTHER, PhD SNP, MuPro and iPPTREE-STAB. According to study of Hicks et al., and Thusberg and Vihinen, to identify most deleterious nsSNPs, SIFT and PolyPhen 2 were reported as best performing tools [51, 52]. To check the stability of protein, MuPro and iPPTREE-STAB were used. Out of 79 nsSNP subjected to functional analysis 7 SNPs (p.Arg160Gly, p.Arg188Trp, p.Ile203Thr, p.Gly210Arg, p.Arg223Cys, p.Pro225His and p.Ser415Phe) were predicted to be most deleterious nsSNP in human CHK2 protein. To the best of our knowledge none of the studies showed the genetic risk of p.Arg160Gly, p.Arg188Try, p.Ile203Thr, p.Gly210Arg, p.Arg223Cys, p.Pro225His and p.Ser415phe with any known disease condition. p.Arg160Gly, p.Arg188Try, p.Ile203Thr, p.Gly210Arg, p.Arg223Cys and p.Pro225His all nsSNP are part of FHA domain which is activated in response to DNA damage. p.Arg160Gly marks the substitution of arginine (basic amino acid) by glycine (non-polar amino acid) and vice-versa in p.Gly210Arg substitution. p.Ile203Thr entails the substitution of isoleucine (non-polar) to threonine (-OH containing amino acids) leading to decrease in stability of protein. In FHA domain, 2 SNPs (p.Arg223Cys and p.Arg188Try) leads to substitution of arginine (basic amino acids) to cysteine (sulphar containing amino acid) and tryptophan (non-polar aromatic amino acid) which decrease protein stability. R160, G210, S415 are highly conserved residues with conservation score of 9. Four amino acids (R160, I203, R223 and P225) were predicted average conserved. p.Arg160Gly, p.Gly210Arg and p.Arg223Cys might interfere in post-translational modification of CHK2 protein as these residues were predicted to be involved in post translational modifications through ModPred. The Arg at 160 and Gly at 210 residue present in polypeptide binding site on conserved site of FHA domain. Ser 415 residue is present in STKc_Chk2 domain. In p.Ser415Phe substitution of serine (-OH containing amino acid) to phenyl alanine (non-polar amino acid). This residue is part of aimer interface, catalytic domain of ATP binding site and active site of STKc_Chk2 domain. Any change in this residue alters the stability of protein which is predicted by Mupro and iPPTREE_STAB. Two amino acids S415 and P225 were predicted to be involved in ligand binding site interactions. These suggest that p.Pro225His and p.Ser415Phe might interfere in ligand binding site interactions. Several studies have investigated the role of CHK2 polymorphism as a genetic determinant for susceptibility to diseases. Several polymorphisms (p.Ile157Val, p.Asp252Gly, c.1100delC, p.Asp438Tyr and p.His371Tyr) have been reported for the CHK2 gene [53, 54]. Pritzlaff and their colleagues assessed multi-gene panel testing using male breast cancer patients and identified pathogenic variants i.e.c.591delA, p.Arg117Gly, p.Thr476Met, p.Ser428Phe, p.Ile517Thr, p.Gln29* and c.1100delC in different population [55]. CHK2 c.1100delC and p.Ile577Thr were most studied in populations all over the world. The inherited variants CHK2 c.1100delC truncates the kinase domain of the CHK2 protein and is responsible for a two fold increase in breast cancer risk in families of northern and northwestern European ancestry [12, 13, 56]. According to Delimitsou and his colleagues study, p.Ile160Arg and p.Ile160Thr variants were characterized as damaging and p.Asp203Gly variant was characterized as benign. All these variants were located within the kinase domain [57]. p.Ile160Arg was characterized as intermediate according to study done by Roeb et al in 2012 [58]. Different CHK2 variants were categorized as damaging according to in silico tools and yeast based assay i.e.p.Trp93Arg, p.Cys108Arg, p.Arg117Gly, p.Arg145Trp, p.Arg148Gly, p.Ile160Arg, p.Ile160Thr, p.Asp162Gly, p.Asn166Ser, p.Gly167Arg, p.Leu183Ser, p.Leu183Phe, p.Leu236Pro, p.Ile251Phe, p.Arg346Cys, p.Arg346His, p.Asp347Ala, p.Asn352Asp, p.Gly370Glu, p.Cys385Arg, p.Thr387Ser, p.Tyr390Ser, p.Ala392Pro, p.Ala392Val, p.Glu394Lys, p.Cys420Thr, p.Tyr424His, p.Arg474Cys and p.His483Arg [57]. Avraham Shaag and his team
discovered two novel amino acid substitutions, p.Ser428Phe in the kinase domain and p.Pro85Leu in the N-terminal region [59]. The individual having CHK2 sequence variants (c.1100delC) may contribute to the Li-Fraumeni syndrome in Dutch families [60]. In Pakistan, two novel mutations p.Gln20X and p.Glu85X at exons 1 and 2 respectively have been identified in breast cancer patients [61]. However the results were contradictory among different studies. By means of in silico, deleterious prediction done in the present study, the p.Gln20His and p.Pro85Leu were not predicted highly deleterious. However, Pro at 85 and Gln at 20th were predicted as conserved residue with conservation score of 7. However none of the study till now available that identify p.Pro225His and p.Ser415Phe nsSNPs as damaging. CHK2 variants found in this study have not been reported earlier so they need to be validated to check its significance. The major limitation of this study is the fact that it is in silico study thus the results cannot be blindly extrapolated to humans without validation by wet lab study. When we predict pathogenicity of CHK2 variants, it is important to conduct functional assay in cell-lines. In addition to this, analysing data from epidemiological and genetic studies as well as segregation analysis would provide more accurate classification.

As multiple CHK2 variants of unknown clinical significance emerge every day when performing genetic testing analyses in patients with cancer, a rapid variant assessment is of great importance. Therefore, the in silico assay used herein provides essential, fast and low-cost evaluation for the largest series of tested CHK2 variants to date, thus providing valuable information that can be ultimately implemented in clinical practice. Thus, the present study indicates that the procedure of computational approach provides an alternative approach to select SNPs targets by considering the role of SNPs on the functional attributes or molecular phenotype of protein. These results may be helpful for further understanding of CHK2 SNPs in disease susceptibility by laboratory experiments.

**Conclusion**

The present study suggests that structure and function of CHK2 can be distributed by various nsSNPs. In native protein of CHK2 gene, out of 79 SNPs, seven major variants found were: p.Arg160Gly, p.Arg188Trp, p.Ile203Thr, p.Gly210Arg, p.Arg223Cys, p.Pro225His and p.Ser415Phe. Among seven most significant SNPs, 3 were highly conserved and 4 SNPs were averaged conserved residues. Among 7 most significant SNPs, 3 were predicted to be involved in post translational modifications. A variant of Serine—Phenyl alanine at position 415 occurs in activation loop of protein-kinase domain of CHK2 protein hence is of particular concern as this is the functional domain of the protein. The one SNP p.Ser415Phe might interfere in interactions of CHK2 with ligand. Therefore, these nsSNPs can be strongly considered as key candidates in causing diseases related to CHK2 malfunction and hence will help in effective drug discovery and developing precision medicines. Wet lab experiments are needed to explore the effects of these polymorphisms on structure and function of protein.

**Supporting information**

S1 Fig. Consurf prediction showing conservation profile of amino acids in CHK2 gene. (PDF)

**Acknowledgments**

We acknowledge Dr Prabhudas S. Patel, Professor & Head of Cancer Biology Department of GCRI for all the support, assistance, facilities and constant encouragements to carry out this work.
Author Contributions

Formal analysis: Bhoomi V. Tarapara.
Investigation: Franky D. Shah.
Writing – original draft: Nutan V. Badgujar.
Writing – review & editing: Nutan V. Badgujar, Franky D. Shah.

References
1. Rahner N, Steinke V. Hereditary cancer syndromes. Deutsches Ärzteblatt International. 2008; 105(41):706. https://doi.org/10.3238/arztebl.2008.0706 PMID: 19623293
2. Durocher D, Jackson SP. The FHA domain. FEBS letters. 2002; 513(1):58–66. https://doi.org/10.1016/s0014-5793(01)03294-x PMID: 11911881
3. Oliver AW, Paul A, Boxall KJ, Barrie SE, Aherne GW, Garrett MD, et al. Trans-activation of the DNA-damage signalling protein kinase Chk2 by T-loop exchange. The EMBO journal. 2006; 25(13):3179–90. https://doi.org/10.1038/sj.emboj.7601205 PMID: 16794575
4. Bartek J, Falck J, Lukas J. CHK2 kinase—a busy messenger. Nature reviews Molecular cell biology. 2001; 2(12):877. https://doi.org/10.1038/35103059 PMID: 11733767
5. Matsuoka S, Huang M, Elledge SJ. Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. Science. 1998; 282(5395):1893–7. https://doi.org/10.1126/science.282.5395.1893 PMID: 9836640
6. Hirao A, Kong Y-Y, Matsuoka S, Wakeham A, Ruland J, Yoshida H, et al. DNA damage-induced activation of p53 by the checkpoint kinase Chk2. Science. 2000; 287(5459):1824–7. https://doi.org/10.1126/science.287.5459.1824 PMID: 10710310
7. Falck J, Mailand N, Sylijusänen RG, Bartek J, Lukas J. The ATM–Chk2–Cdc25A checkpoint pathway guards against radioresistant DNA synthesis. Nature. 2001; 410(6830):842. https://doi.org/10.1038/sj.emboj.7601209 PMID: 11298456
8. Toss A, Tomasello C, Razzaboni E, Contu G, Grandi G, Cagnacci A, et al. Hereditary ovarian cancer: not only BRCA 1 and 2 genes. BioMed research international. 2015; 2015.
9. Baloch AH, Daud S, Raheem N, Luqman M, Ahmad A, Rehman A, et al. Missense mutations (p. H371Y, p. D438Y) in gene CHEK2 are associated with breast cancer risk in women of Balochistan origin. Molecular biology reports. 2014; 41(2):1103–7. https://doi.org/10.1007/s11033-013-2956-x PMID: 24390236
10. Baysal BE, DeLoia JA, Willett-Brozick JE, Goodman MT, Brady MF, Modugno F, et al. Analysis of CHEK2 gene for ovarian cancer susceptibility. Gynecologic oncology. 2004; 95(1):62–9. https://doi.org/10.1016/j.ygyno.2004.07.015 PMID: 15385111
11. Hines SL, Mohammad AN, Jackson J, Macklin S, Caulfield TR. Integrative data fusion for comprehensive assessment of a novel CHEK2 variant using combined genomics, imaging, and functional–structural assessments via protein informatics. Molecular omics. 2019.
12. Bell DW, Varley JM, Szydlo TE, Kang DH, Wahrer DC, Shannon KE, et al. Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. Science. 1999; 286(5449):2528–31. https://doi.org/10.1126/science.286.5449.2528 PMID: 10617473
13. Vahteristo P, Bartkova J, Eerola H, Syunjakoski K, Ojala S, Kilpivaara O, et al. A CHEK2 genetic variant contributing to a substantial fraction of familial breast cancer. The American Journal of Human Genetics. 2002; 71(2):432–8. https://doi.org/10.1086/341943 PMID: 12094328
14. Kilpivaara O, Vahteristo P, Falck J, Syunjakoski K, Eerola H, Easton D, et al. CHEK2 variant I157T may be associated with increased breast cancer risk. International journal of cancer. 2004; 111(4):543–7. https://doi.org/10.1002/ijc.20298 PMID: 15239132
15. Staalesen V, Falck J, Geisler S, Bartkova J, Barresen-Dale A-L, Lukas J, et al. Alternative splicing and mutation status of CHEK2 in stage III breast cancer. Oncogene. 2004; 23(52):8535. https://doi.org/10.1038/sj.onc.1207926 PMID: 15361853
16. Schmidt MK, Hogervorst F, Van Hien R, Cornelissen S, Broeks A, Adank MA, et al. Age-and tumor subtype–specific breast cancer risk estimates for CHEK2* 1100delC carriers. Journal of clinical oncology. 2016; 34(23):2750. https://doi.org/10.1200/JCO.2016.66.5844 PMID: 27269948
17. Bell DW, Kim SH, Godwin AK, Schiripo TA, Harris PL, Hasefmat SM, et al. Genetic and functional analysis of CHEK2 (CHK2) variants in multiethnic cohorts. International journal of cancer. 2007; 121(12):2661–7. https://doi.org/10.1002/ijc.23026 PMID: 17721994
18. Schwarz JK, Lovly CM, Pwinnica-Worms H. Regulation of the Chk2 Protein Kinase by Oligomerization-Mediated cis-and trans-Phosphorylation1 1 NIH. Note: HPW is an investigator of the Howard Hughes Medical Institute. CML and JKS are members of the Medical Scientist Training Program at Washington University School of Medicine. Note: JKS and CML contributed equally to this work. Molecular Cancer Research. 2003; 1(8):598–609. PMID: 12805407

19. Sodha N, Mantoni TS, Tavtigian SV, Eeles R, Garrett MD. Rare germ line CHEK2 variants identified in breast cancer families encode proteins that show impaired activation. Cancer research. 2006; 66(18):8966–70. https://doi.org/10.1158/0008-5472.CAN-06-1990 PMID: 16982735

20. Gassoum A, Abdelraheem NE, Elsadig N. Comprehensive Analysis of nsSNPs Associated with Hypertension Using In-Silico Bioinformatics Tools. Open Access Library Journal. 2016; 3(07):1.

21. Nachman MW. Single nucleotide polymorphisms and recombination rate in humans. TRENDS in Genetics. 2001; 17(9):481–5. PMID: 11525814

22. Desai M, Chauhan J. In silico analysis of nsSNPs in human methyl CpG binding protein 2. Meta Gene. 2016; 10:1–7.

23. Desai M, Chauhan J. Computational analysis for the determination of deleterious nsSNPs in human MTHFD1 gene. Computational biology and chemistry. 2017; 70:7–14. https://doi.org/10.1016/j.compbiolchem.2017.07.001 PMID: 28734179

24. Bhagwat M. Searching NCBI's dbSNP database. Current protocols in bioinformatics. 2010; 32(1):1.19. 1–1.

25. Abdelraheem NE, El-Tayeb GM, Osman LO, Abedlrhman SA, Ali AS, Elsadig AH, et al. A comprehensive in silico analysis of the functional and structural impact of non-synonymous single nucleotide polymorphisms in the human KRAS gene. American Journal of Bioinformatics Research. 2016; 6(2):32–55.

26. Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. Nucleic acids research. 2003; 31(13):3812–4. https://doi.org/10.1093/nar/gkg509 PMID: 12844245

27. Tavtigian SV, Deffenbaugh AM, Yin L, Judkins T, Scholl T, Samollow PB, et al. Comprehensive statistical study of 452 BRCA1 missense substitutions with classification of eight recurrent substitutions as neutral. Journal of medical genetics. 2006; 43(4):295–305. https://doi.org/10.1136/jmg.2005.033878 PMID: 16014699

28. Yachdav G, Hecht M, Pasmanik-Chor M, Yeheskel A, Rost B. HeatMapView: interactive display of 2D data in biology. F1000Research. 2014; 3.

29. Choi Y, Sims GE, Murphy S, Miller JR, Chan AP. Predicting the functional effect of amino acid substitutions and indels. PloS one. 2012; 7(10):e46688. https://doi.org/10.1371/journal.pone.0046688 PMID: 23056405

30. Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. Nucleic acids research. 2002; 30(17):3894–900. https://doi.org/10.1093/nar/gkf493 PMID: 12202775

31. Tang H, Thomas PD. PANTHER-PSEP: predicting disease-causing genetic variants using position-specific evolutionary preservation. Bioinformatics. 2016; 32(14):2230–2. https://doi.org/10.1093/bioinformatics/btw222 PMID: 27193693

32. Capriotti E, Calabrese R, Casadio R. Predicting the insurgence of human genetic diseases associated to single point protein mutations with support vector machines and evolutionary information. Bioinformatics. 2006; 22(22):2729–34. https://doi.org/10.1093/bioinformatics/btl423 PMID: 16895930

33. Cheng J, Randall A, Baldi P. Prediction of protein stability changes for single-site mutations using support vector machines. Proteins: Structure, Function, and Bioinformatics. 2006; 62(4):1125–32.

34. Huang L-T, Gromiha MM, Ho S-Y. iPTREE-STAB: interpretable decision tree based method for predicting protein stability changes upon mutations. Bioinformatics. 2007; 23(10):1292–3. https://doi.org/10.1093/bioinformatics/btm100 PMID: 17379687

35. Ashkenazy H, Erez E, Martz E, Pupko T, Ben-Tal N. ConSurf 2010: calculating evolutionary conservation in sequence and structure of proteins and nucleic acids. Nucleic acids research. 2010; 38(suppl_2): W529–W33.

36. Pejaver V, Hsu WL, Xin F, Dunker AK, Uversky VN, Radijovac P. The structural and functional signatures of proteins that undergo multiple events of post-translational modification. Protein Science. 2014; 23(8):1077–93. https://doi.org/10.1002/pro.2494 PMID: 24888500

37. Karczewski KJ, Weisburd B, Thomas B, Solomonson M, Ruderfer DM, Kavanagh D, et al. The ExAC browser: displaying reference data information from over 60,000 exomes. Nucleic acids research. 2016; 45(D1):D840–D5. https://doi.org/10.1093/nar/gkw971 PMID: 27896911

38. Hunter S, Apweiler R, Attwood TK, Bairoch A, Bateman A, Binns D, et al. InterPro: the integrative protein signature database. Nucleic acids research. 2008; 37(suppl_1):D211–D5.

39. Marchler-Bauer A, Derbyshire MK, Gonzales NR, Lu S, Chitsaz F, Geer LY, et al. CDD: NCBI's conserved domain database. Nucleic acids research. 2014; 43(D1):D222–D6.
40. Yang Y, Faraggi E, Zhao H, Zhou Y. Improving protein fold recognition and template-based modeling by employing probabilistic-based matching between predicted one-dimensional structural properties of query and corresponding native properties of templates. Bioinformatics. 2011; 27(15):2076–82. https://doi.org/10.1093/bioinformatics/btr350 PMID: 2166270

41. Xu D, Zhang Y. Improving the physical realism and structural accuracy of protein models by a two-step atomic-level energy minimization. Biophysical journal. 2011; 101(10):2525–34. https://doi.org/10.1016/j.bpj.2011.09.024 PMID: 21998752

42. Lovell SC, Davis IW, Arendall WB III, De Bakker PI, Word JM, Prisant MG, et al. Structure validation by Co geometry: φ, ψ and Cβ deviation. Proteins: Structure, Function, and Bioinformatics. 2003; 50(3):437–50.

43. Ngan C-H, Hall DR, Zerbe B, Grove LE, Kozakov D, Vajda S. FTSite: high accuracy detection of ligand binding sites on unbound protein structures. Bioinformatics. 2011; 28(2):286–7. https://doi.org/10.1093/bioinformatics/btr651 PMID: 22113084

44. Yang J, Roy A, Zhang Y. Protein–ligand binding site recognition using complementary binding-specific substructure comparison and sequence profile alignment. Bioinformatics. 2013; 29(20):2588–95. https://doi.org/10.1093/bioinformatics/btt447 PMID: 23975762

45. Doniger SW, Kim HS, Swain D, Corcuera D, Williams M, Yang S-P, et al. A catalog of neutral and deleterious polymorphism in yeast. PLoS genetics. 2008; 4(8):e1000183. https://doi.org/10.1371/journal.pgen.1000183 PMID: 18769710

46. Miller MP, Kumar S. Understanding human disease mutations through the use of interspecific genetic variation. Human molecular genetics. 2001; 10(21):2319–28. https://doi.org/10.1093/hmg/10.21.2319 PMID: 11689479

47. Nevanlinna H, Bartek J. The CHEK2 gene and inherited breast cancer susceptibility. Oncogene. 2006; 25(43):5912. https://doi.org/10.1038/sj.onc.1209777 PMID: 16998506

48. Desai M, Chauhan J. Computational analysis for the determination of deleterious nsSNPs in human MTHFR gene. Computational biology and chemistry. 2018; 74:20–30. https://doi.org/10.1016/j.compbiochem.2018.02.022 PMID: 29524840

49. AlaaNazi M, Abduljaleel Z, Khan W, Warsy AS, Elroob M, Khan Z, et al. In silico analysis of single nucleotide polymorphism (SNPs) in human β-globin gene. PloS one. 2011; 6(10):e25876. https://doi.org/10.1371/journal.pone.0025876 PMID: 22028795

50. Abdelraheem NE, Osman MM, Elgemaabi OM, Alla AAF, Ismail MM, Osman SA, et al. Computational Analysis of Deleterious Single Nucleotide Polymorphisms (SNPs) in Human MutS Homolog6 (MSH6) Gene. American Journal of Bioinformatics Research. 2016; 6(2):56–97.

51. Thusberg J, Olatubosun A, Vihinen M. Performance of mutation pathogenicity prediction methods on missense variants. Human mutation. 2011; 32(4):358–68. https://doi.org/10.1002/humu.21445 PMID: 21412949

52. Hicks S, Wheeler DA, Plon SE, Kimmel M. Prediction of missense mutation functionality depends on both the algorithm and sequence alignment employed. Human mutation. 2011; 32(6):661–8. https://doi.org/10.1002/humu.21490 PMID: 21480434

53. Jalilvand M, Oloomi M, Najafipour R, Alizadeh SA, Saki N, Rad FS, et al. An association study between CHEK2 gene mutations and susceptibility to breast cancer. Comparative clinical pathology. 2017; 26(4):837–45. https://doi.org/10.1007/s00580-017-2455-x PMID: 28680382

54. Apostolou P, Papasotiriou I. Current perspectives on CHEK2 mutations in breast cancer. Breast Cancer: Targets and Therapy. 2017; 9:331.

55. Pritzlaff M, Summerour P, McFarland R, Li S, Reineke P, Dolinsky JS, et al. Male breast cancer in a multi-gene panel testing cohort: insights and unexpected results. Breast cancer research and treatment. 2017; 161(3):575–86. https://doi.org/10.1007/s10549-016-4085-4 PMID: 28008555

56. Meijers-Heijboer H, Wasielewski M, Wagner A, Hollesteke A, Elstrodt F, van den Bos R, et al. The CHEK2 1100delC mutation identifies families with a hereditary breast and colorectal cancer phenotype. The American Journal of Human Genetics. 2003; 72(5):1308–14. https://doi.org/10.1086/375121 PMID: 12690581

57. Delimitsou A, Fostira F, Kalfakakou D, Apostolou P, Konstantopoulou I, Kroupis C, et al. Functional characterization of CHEK2 variants in a Saccharomyces cerevisiae system. Human mutation. 2017.

58. Roeb W, Higgins J, King M-C. Response to DNA damage of CHEK2 missense mutations in familial breast cancer. Human molecular genetics. 2012; 21(12):2738–44. https://doi.org/10.1093/hmg/dd101 PMID: 22419737

59. Shaag A, Levy-Lahad E, Mandell JB, Offit K, Nafa K, King M-C, et al. Functional and genomic approaches reveal an ancient CHEK2 allele associated with breast cancer in the Ashkenazi Jewish
population. Human Molecular Genetics. 2005; 14(4):555–63. https://doi.org/10.1093/hmg/ddi052 PMID: 15649950

60. Ruijs MW, Broeks A, Menko FH, Ausems MG, Wagner A, Oldenburg R, et al. The contribution of CHEK2 to the TP53-negative Li-Fraumeni phenotype. Hereditary cancer in clinical practice. 2009; 7(1):4. https://doi.org/10.1186/1897-4287-7-4 PMID: 19338683

61. Baloch AH, Khosa AN, Bangulzai N, Shuja J, Naseeb HK, Jan M, et al. Novel Nonsense Variants c. 58C>T (p. Q20X) and c. 256G>T (p. E85X) in the CHEK2 Gene Identified identified in Breast Cancer Patients from Balochistan. Asian Pacific Journal of Cancer Prevention. 2016; 17(3):1089–92. https://doi.org/10.7314/apjcp.2016.17.3.1089 PMID: 27039729