Journal of Advanced Research 24 (2020) 149–157

Contents lists available at ScienceDirect

Journal of Advanced Research

journal homepage: www.elsevier.com/locate/jare

Targeted next generation sequencing identifies somatic mutations in a cohort of Egyptian breast cancer patients

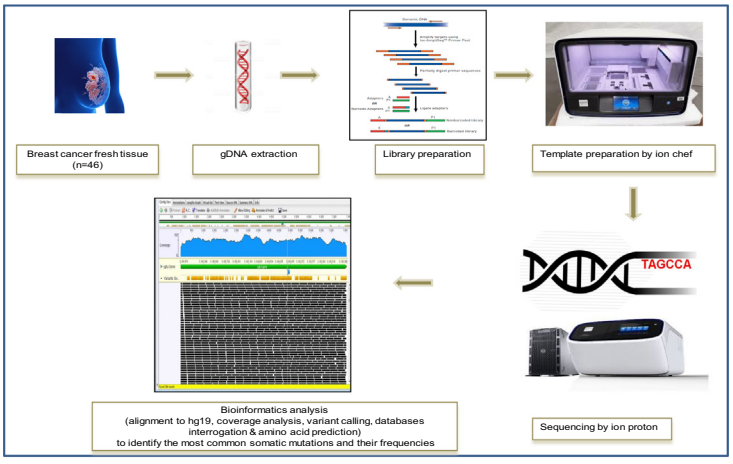
Auhood Nassar [a], Mohamed Abouelhoda [b], Osman Mansour [c], Samah A. Loutfy [a], Mohamed M. Hafez [a], M. Gomaa [d], Abeer Bahnassy [e], Amira Salah El-Din Youssef [a], Mai M. Lotfy [a], Hoda Ismail [e], Ola S. Ahmed [a], Amany Abd-Elhameed Abou-Bakr [e], Abdel-Rahman N. Zekri [a,*]

[a] Cancer Biology Department, National Cancer Institute, Cairo University, Cairo, Egypt
[b] Faculty of Engineering, Cairo University, Cairo, Egypt
[c] Medical Oncology Department, National Cancer Institute, Cairo University, Cairo, Egypt
[d] Radiology Department, National Cancer Institute, Cairo University, Cairo, Egypt
[e] Pathology Department, National Cancer Institute, Cairo University, Cairo, Egypt

HIGHLIGHTS

- Identifying somatic mutations associated with Egyptian breast cancer tumors.
- Identifying breast cancer mutation driver genes in the studied Egyptian patients.
- Identify genetic mutations in BC tumors help developing personalized treatment protocols or combination therapies.
- Identifying novel variants that may be associated with Egyptian breast cancer patients.
- Help in customization of Egyptian related breast cancer panels as a routine work

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 19 December 2019
Revised 17 February 2020
Accepted 1 April 2020
Available online 3 April 2020

Keywords:
Breast cancer
Somatic mutations
Target sequencing
Ion torrent sequencing
Next Generation Sequencing

ABSTRACT

Breast cancer (BC) incidence is progressively increasing in Egypt. However, there is insufficient knowledge of the acquired somatic mutations in Egyptian BC patients which limit our understanding of its progression. To the best of our knowledge, this is the first Egyptian cohort to sequence a multiple-gene panel of cancer related genes on BC patients. Four hundred and nine cancer related genes were sequenced in 46 fresh breast tumors of Egyptian BC patients to identify somatic mutations and their frequencies.*TP53* and *PIK3CA* were the most top two frequently mutated genes. We detected 15 different somatic mutations in *TP53* and 8 different ones in *PIK3CA*, each in 27 samples (58.7%). According to Clinvar database; we found 19 pathogenic somatic mutations: 7 in *Tp53*, 5 in *PIK3CA*, and single variants of *VHL*, *STK11*, *AKT1*, *KRAS*, *IDH2*, *PTEN* and *ERBB2*. We also identified 5 variants with uncertain significance (4 in *TP53* and 1 in *CEBPA*) and 4 variants with conflicting interpretations of pathogenicity (2 in *TP53* and 1 in each of *APC* and *JAK3*). Moreover, one drug response variant (p.P72R) in *TP53* was detected in 8 samples.

Peer review under responsibility of Cairo University.

* Corresponding author at: Molecular Virology and Immunology Unit, Cancer Biology Department, National Cancer Institute, Cairo University, Kasr Al-Aini st., Fom El-Khaleeg, Cairo 11976, Egypt.

E-mail addresses: auhood.nassar@nci.cu.edu.eg (A. Nassar), osman.mansour@nci.cu.edu.eg (O. Mansour), amira.salah@nci.cu.edu.eg (A.S. El-Din Youssef), abdelrhman.zekri@nci.cu.edu.eg (A.-R.N. Zekri).

https://doi.org/10.1016/j.jare.2020.04.001
2090-1232/

Furthermore, four novel variants were identified in *JAK2*, *MTOR*, *KIT* and *EPHB*. Further analysis, by Ingenuity Variant Analysis software (IVA), showed that PI3K/AKT signaling is altered in greater than 50% of Egyptian BC patients which implicates PI3K/AKT signaling as a therapeutic target. In this cohort, we shed the light on the most frequently detected somatic mutations and the most altered pathway in Egyptian BC patients.

Introduction

Breast Cancer (BC) is the second most common lethal malignancy in women and the leading cause of cancer-related death in women worldwide [1]. It has been reported that over half (52%) of new BC cases and 62% of deaths occur in economically developing countries [2]. In Egypt, BC is the most common cancer among females accounting for 37.7% of about 12,000–13,000 new cases per year. These estimates have been confirmed in many regional Egyptian cancer registries [3,4].

Recently, the Next Generation Sequencing (NGS) technology has provided a fast and cost effective means to characterize the mutations in the individual patient genome, which shed light on the mutated cancer genes involved in cancer progression [5]. NGS has been used to study the mutation pattern in BC patients from different ethnic groups and at different disease stages. In many studies, whole genome and whole exome sequencing have been used to study BC mutations on large number of patients [6,7].

Further focused analysis using targeted sequencing was later conducted in many studies: Pereira et al. studied the somatic mutation profile conducted on 2433 patients using a custom gene panel of 173 genes and identified 40 mutation-driver genes [8]. Meric-Bernstam et al. used panel of 182 genes and determined the spectrum of genomic alterations in primary and metastatic BC [9]. Also, Wiesman et al. used a custom gene pane of 229 genes and identified the key somatic mutations previously reported in triple negative BC [10]. Moreover, Smith et al. could detect clinically actionable mutations in BC solid tumors using the MammaSeq [11]. On the other hand, Liu et al. and Bai et al. used Ion Torrent Ampliseq Cancer Panel to identify genetic mutations in BC tumors to help developing personalized treatment protocols or combination therapies [12,13]. The previous studies covered mostly European and North American populations. Little is done to study the somatic profile for other ethnic groups; we could only locate the work on the Chinese [14,15], Mexico and Vietnam [16] populations. To great extent, these studies were successful in understanding the molecular basis of the disease. Therefore, it was necessary to conduct such targeted sequencing studies on the Egyptian population to answer an important question: how the Egyptian patients are different in terms of mutations and affected genes from other populations? Answering this question helps understanding the Egyptian BC profiling which will help in the future evaluating drug efficacy and treatment protocols for that population. So, we developed this cohort study to explore the landscape of somatic mutations in Egyptian BC patients. We used the Ion Torrent sequencing technology (Ion AmpliSeq Comprehensive Cancer Panel) to sequence 409 tumor suppressor genes and oncogenes from 46 BC tumors of various subtypes.

Patients and methods

Ethics statement

All human subject protocols and procedures were approved by the Institutional Review Board (IRB number: IRB00004025) of National Cancer Institute (NCI), Cairo University, Egypt which conducted the study in accordance with ICH-GCP guidelines (approval number: MD2010014038.3). A written informed consent was obtained from each patient during the enrollment into this study.

Patient samples

Tissue samples used in this cohort were recruited from the Egyptian National Cancer Institute from October 2016 to March 2018. Forty-six fresh tissue samples from Egyptian BC female patients were collected at surgery. Included patients were naïve to treatment and those receiving neoadjuvant chemotherapy were excluded. Patients were classified according to age, histological type, histological grade, hormone receptor status (estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (Her2)) and molecular subtype (Luminal A, Luminal B, Her2- over-expressing and triple negative). All the clinicopathological features of the studied patients were collected from the clinical records.

DNA preparation

Twenty-five mg of fresh tissues were collected from 46 BC female patients. DNA was isolated using QIAamp® DNA Mini Kit (Qiagen, Germany: Cat. No. 51304) following manufacturer's instructions. For each sample, the isolated DNA was quantified using Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific) and Qubit 4 Fluorometer (Thermo Fisher Scientific). Moreover, DNA was checked by electrophoresis using 2% Ethidium-Bromide-stained agarose gel and visualized under UV *trans*-illuminator to confirm its integrity.

Ion AmpliSeq™ DNA library preparation, template preparation and sequencing

Ion AmpliSeq™ DNA Library was constructed using the Ion AmpliSeq™ Library Kit 2.0 (Cat. No. 4480441) which is designed for preparation of amplicon libraries using Ion AmpliSeq Comprehensive Cancer panels (Ion AmpliSeq CCP, Life Technologies, Cat. no. 4477685). For this, four amplicon pools per sample covering the 409 genes were quantified by qPCR with the Ion Library Quantitation Kit (Life Technologies, Cat. no. 4468802). The concentration and size of the library were determined by Agilent 2100 BioAnalyzer and DNA High-Sensitivity Lab Chip (Agilent Technologies). The quality of the libraries was assessed by QIAxcel advanced (QIAGEN). Then, the quantified libraries were preceded to template preparation on the ion chef using the Ion PI Hi-Q Chef Kit (Life Technologies, Cat. No. A27198) and loaded into an Ion PI Chip (Life Technologies, Cat. No. A26770) to be sequenced on the Ion proton using the Ion Proton Sequencing 200 Kit v2 (Life Technologies, Cat. No. 4485149).

Variant calling and variant classification

The bioinformatics analysis pipeline started with checking the QC step where the reads of each NGS run were examined and low quality parts were trimmed out. We then ran the alignment of the reads to the human reference genome (version hg19). For that step, we used the Torrent Suite as recommended by the man-

ufacturer. A run was accepted only if the total number of aligned reads covered at least 95% of the target regions with an average depth of coverage of 668X. After alignment, we ran the TSVC module of the Torrent Suite to call the variants, using the options for calling somatic variants with hotspots. The qualified variants were annotated using an in-house developed system composed of different databases: we used the ANNOVAR [17] package to annotate the variants with all available public population information. For cancer Few variants databases, we used the Catalogue of Somatic Mutations in Cancer COSMIC [18] and CIViC [19]. The possible impact of amino acid changes was assessed with PolyPhen-2 [20], Sift [21], and CADD [22] prediction tools to understand their potential role in carcinogenesis. The identified variants were classified as benign or pathogenic according to Clin Var database [23]. For identifying somatic mutations and filtering out germline variants in the absence of normal tissues, we used the in silico methods of [24,25] using our database sets. The filtration algorithm has the following steps: the variants with accepted quality, depth of coverage, and existence in our hotspot regions are retained for further analysis. Variants that exist in COSMIC database or those that exist in population databases (including our in-house one) with minimum allele frequency (MAF) less than 1% were retained. Variants that do not exist in cancer CiViC or COSMIC were filtered out. Finally, the remaining variants were inspected manually on IGV to revise their alignments and neighboring sequences.

Results

In this cohort study, we sequenced 46 BC samples from Egyptian patients ranging from 29 to 73 years of age. Patients classification was based on their age, histological type, histological grade, receptor status (ER, PR, and Her-2 Neu), and molecular classification as shown in Table 1. In this study, we used Ion AmpliSeq™ Comprehensive Cancer Panel which was designed to target 409 tumor suppressor genes and oncogenes across multiple gene families to identify somatic mutations among Egyptian BC patients and their frequencies.

Our analysis showed that there were 44 out of 46 patients had somatic mutations. Initial filtering yielded 79 variants. By looking up these variants in the most recent version of the COSMIC database (version 90), we found that 28 of them have been reclassified as SNP. Therefore, they were excluded from further discussion. This reclassification was due to their frequencies in the ExAC and GnomAD databases. From the remaining 51 variants, there were 10 benign ones according to Clinvar database with frequency higher than 1% in ExAC and GnomAD databases except for one variant (p.E168D) in *MET* gene. The final remaining set of somatic variants includes 38 variants; out of them there were 4 novel variants, as they did not show up in any of the public databases. Three of these novel variants (p.F151V, p.H263Q & p.T600I) were predicted to be damaging by CADD-phred and PolyPhen2 prediction tools.

We detected different somatic mutations (Substitution – missense, Frame shift deletion, Substitution – coding silent, Substitution – nonsense, In Frame shift insertion, and Substitution-intronic). Summary of types and numbers of the detected somatic mutations is shown in Fig. 1a. Fifty one somatic mutations were detected in 22 genes, out of them there were: 19 pathogenic or likely pathogenic variants, 10 benign or likely benign variants, 5 variants of uncertain significance, 4 variants with conflicting interpretation of pathogenicity, 8 variants not reported in Clinvar database, 4 novel variants, an 1 drug response variant as shown in Fig. 1b. The distribution of somatic mutations in the studied BC patients was shown in the Oncoplot (Fig. 2**).** We also analyzed variants with Ingenuity Variant Analysis software (IVA; QIAGEN) for further variant annotation and interpretation. IVA showed that PI3K/AKT signaling was up- regulated in 54% of our patients as shown in Fig. 3a and 3b.

Table 1
Clinical features of the studied 46 Egyptian patients.

Characteristics			Number
Age (Years)	Mean: 54.75 Median: 55 Range: 29–77	<40 y 40–50 y 50–60 y ≥60 y	5 8 18 15
HR status		ER+ ER- PR+ PR-	33 13 27 19
Grade		I II III	3 33 10
Histological type		Invasive duct carcinoma Invasive tubular carcinoma Invasive micropapillary carcinoma Invasive tubulolobular carcinoma Invasive duct & Invasive lobular carcinoma (mixed)	41 1 1 2 1
Molecular classification		Luminal A Luminal B Her2- enriched Basal like (triple negative)	25 9 8 4

Tumor protein TP53 (*TP53*), phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*), proto-oncogene c-Kit (*KIT*), Phosphatase and Tensin Homolog (*PTEN*), proto-oncogene MET (*MET*), and Kirsten Rat Sarcoma (*KRAS*) were the most common mutated genes (≥2 variants per gene). *TP53* was the most mutated one (15 different variants), followed by *PIK3CA* (8 different variants), *KIT* (3 variants), *PTEN* (2 variants), *KRAS* (2 variants), *TSC1* (2 variants) and *MET* (2 variants). The detailed list of the affected genes, incurred somatic mutations, mutation type, frequency, zygosity and other data are listed in Table 2.

Fifteen different somatic mutations were detected in TP53 gene; all, except only one, were within known hotspot regions and most of them were classified as pathogenic. Notably, one *TP53* drug response variant (p.P72R) was detected in 8 samples.

Eight different somatic mutations were detected in *PIK3CA* gene. p.H1047R, p.E545K, p.E542K, p.E80K, and p.Q546R were found at known hotspot regions and classified as pathogenic. p. H1047R is the most frequently detected pathogenic somatic mutation in this study. Another *PIK3CA* variant (p.I391M) was detected in 7 samples and one more *PIK3CA* substitution coding silent variant (p.T1025T) was detected in 4 samples.

Three different somatic mutations were detected in *KIT* gene; p. L862L, p.M541L, p.K546K in 9, 6, and 5 samples respectively. On the other hand, two different *PTEN* somatic mutations were detected; one variant (p.E288fs) in 6 samples as homozygous mutation and in one sample as heterozygous mutation and the other variant (p.R130X) in one sample. While *KRAS* gene had one substitution intronic splicing variant in 6 samples and 1 sample harbored one missense variant (p.G12V). Interestingly, we detected 2 frame shift deletions (p.F148fs and p.G279fs) in *VHL* and *STK11* genes in 5 and 4 samples, respectively. These two variants were recorded as pathogenic in NCBI Clin Var database. In addition, 3 frame shift deletions were detected; 2 (p.F608Lfs*21 and p.L203Cfs*7) in *TSC1* gene and 1 (p.F298Lfs*65) in *TSC2* gene. Moreover, one substitution-intronic variant in platelet derived growth factor receptor alpha (PDGFRA) was detected in 6 samples and this variant is pathogenic according to FATHMM score.

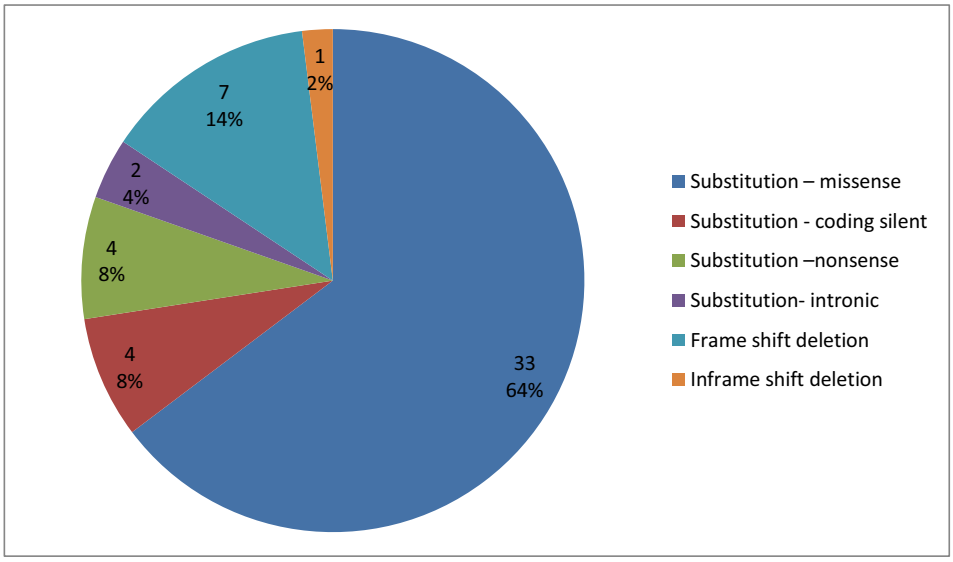


Fig. 1a. Summary of types and numbers of the detected somatic mutations.

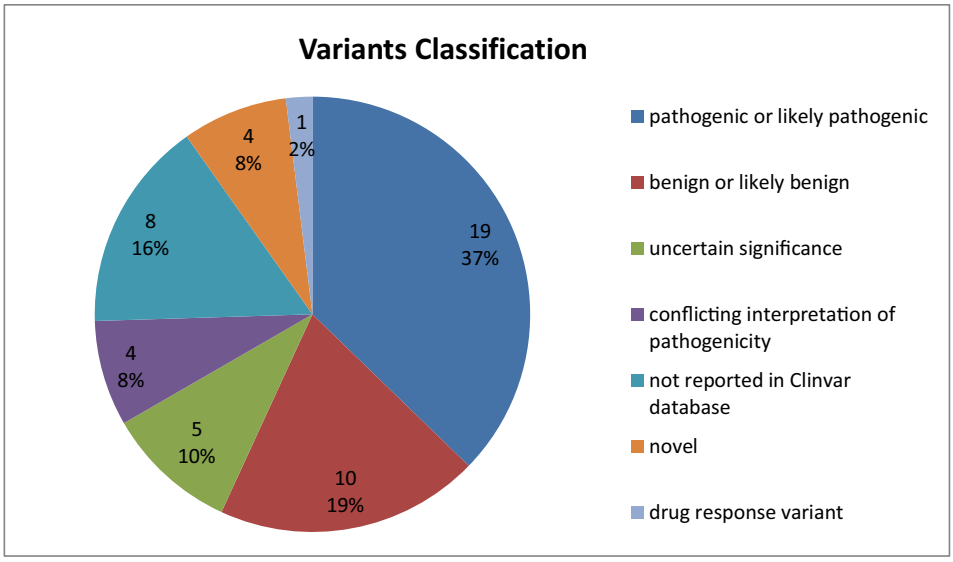


Fig. 1b. Classification of the identified variants.

Discussion

The identification of somatic mutations analyzed by Next- Generation Sequencing is increasingly used in clinical research as it allows deepening the knowledge of cancer progression. In this work we used Ion AmpliSeq Comprehensive caner panel to target most frequently cited and mutated cancer related genes and to report the frequency of the detected somatic mutations among 46 Egyptian BC patients. To the best of our knowledge, this is the first Egyptian cohort to sequence a multiple-gene panel of cancer related genes on BC patients.

In this cohort, we detected somatic mutations in genes previously reported to be frequently mutated in BC: *TP53*, *PIK3CA*, *PTEN*, *AKT1*, and *MAP2K4*. In addition, other somatic mutations were detected in genes recently reported to be mutated in BC; *KRAS*, *PDGFRA, VHL*, *STK11*, *APC*, *MET*, *JAK3*, *SMARCB1*, *ERBB2*, and *IDH2* [26]. Matching with several previous studies, *TP53* and *PIK3CA* were the most top two frequently mutated genes in BC proving their importance in carcinogenic process [14]. On the contrary, we detected variants at a relatively high frequency in *STK11*, *KRAS*, and *ERBB2* genes, which were previously reported to occur infrequently in BC [7,27].

TP53 is a key transcription factor that participates in repair of DNA damage, cell cycle check point control, and apoptosis induction. *TP53* is mutated in BC in around 30–35% of cases and losing its normal functions causes tumorigenesis. In this cohort, 15 different *TP53* somatic mutations were present in 58.7% (27 out of 46) of patients and they were all (except one) within known hotspot regions. Of interest, two polymorphic variants of *TP53* gene were detected; the most frequent one was TP53 p.P72R (COSM250061) in 8 patients (17.4%) followed by TP53 p.P72A (COSM3738520) in 4 patients (8.7%). There are contradictory results about if *TP53* codon 72 polymorphism is associated with BC risk or not. In meta-analysis by Zhang et al., it was reported that TP53 P72R contributes to BC susceptibility [28]. On the contrary, Ma et al. found that TP53 P72R showed no significant association with BC risk [29]. This null significant association was verified again in updated meta-analysis by Cheng et al. [30]. Therefore, an additional large

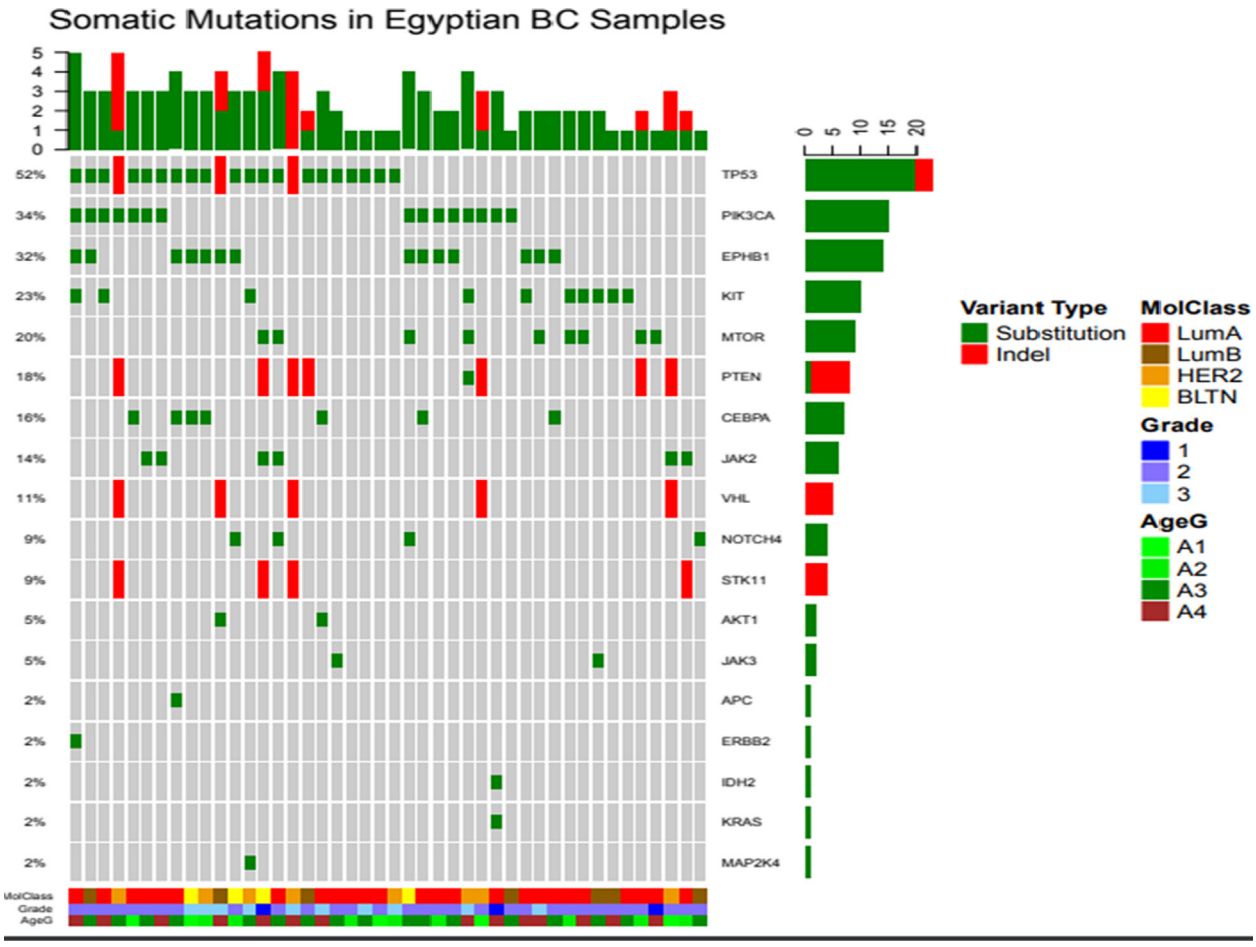


Fig. 2. Oncoplot showing the distribution of somatic mutations in the studied breast cancer patients. The Oncoplot provided an overview of somatic mutations in particular genes (rows) affecting individual samples (columns). According to the logic of oncoplot, if a sample has more variants, it is counted once, and not with the total frequencies. The plot shows total positive 44 samples. The substitution mutations were shown in green, indels were shown in red.

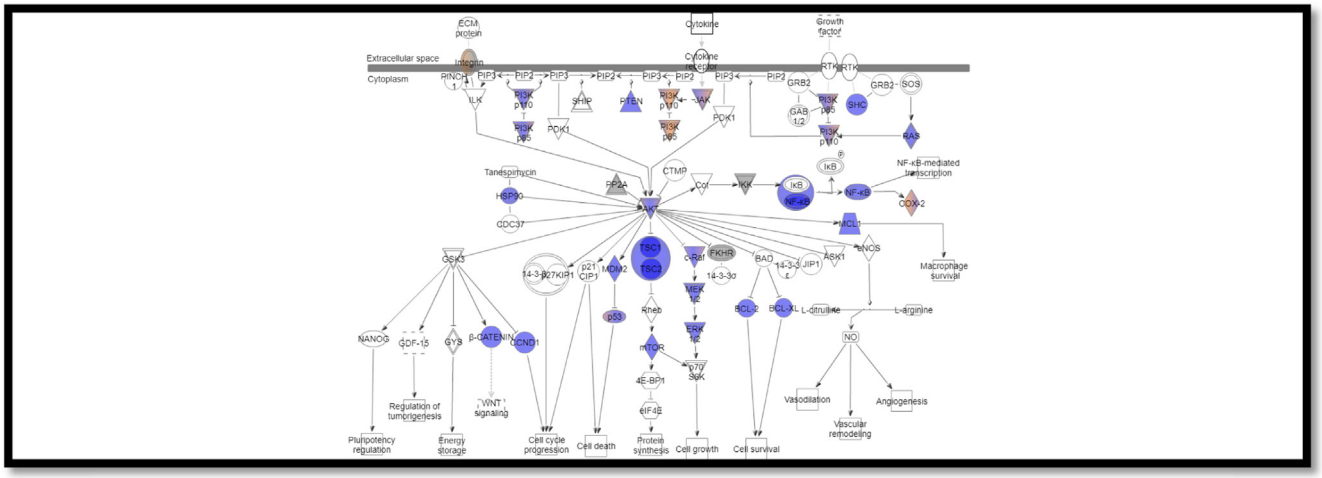


Fig. 3. a. PI3K/AKT signaling pathway identified using ingenuity variant analysis (IVA). Blue represents loss of function, orange represents gain of function, and grey inferred normal.

study is required to validate this association in our Egyptian BC patients.

On the other hand, it was reported that *TP53* polymorphism may influence individual responsiveness to cancer chemotherapy via modulation of *TP73*-dependent apoptosis [31]. The ability of mutant *TP53* to bind to *TP73*, the *TP53*-family member, and inactivate it is influenced by *TP53* codon 72 [32]. Xu et al., indicated that BC patients with the Pro/Pro genotype were less sensitive to a

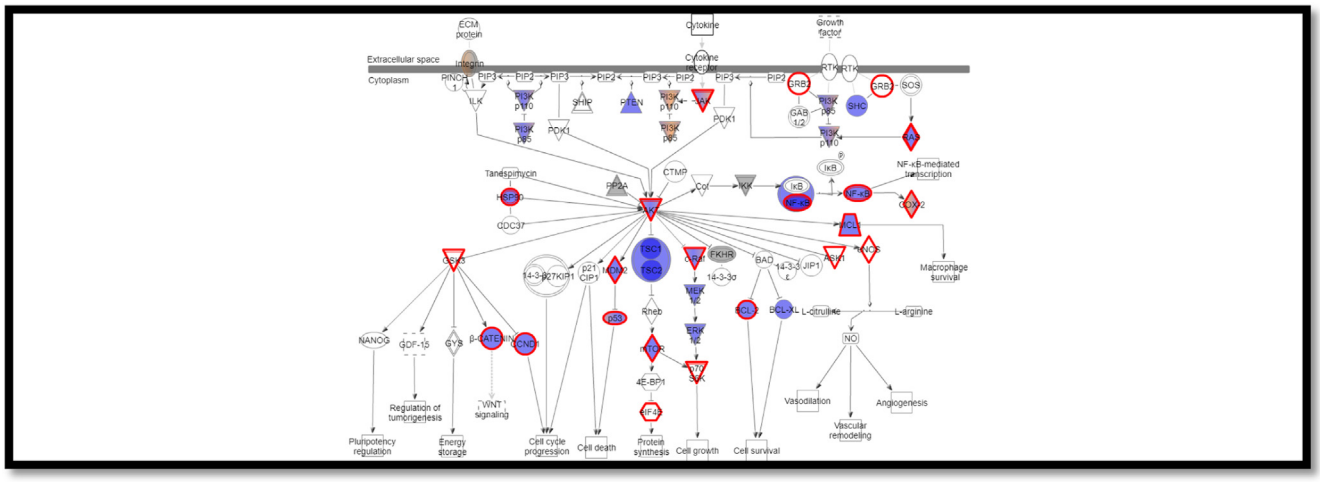


Fig. 3b. PI3K/AKT signaling pathway identified using ingenuity variant analysis (IVA). Blue represents loss of function, orange represents gain of function, grey inferred normal, and entities outlined in red are potential drug targets.

neoadjuvant chemotherapy regimen that included 5-fluorouracil, cyclophosphamide, and the anthracycline-based neoadjuvant chemotherapy than those with the Arg/Arg or Arg/Pro genotypes [33]. Similar results were reported in head and neck carcinoma [sullivan 2004]. It was also reported that Arg/Arg genotype induces apoptosis more effectively than Pro/Pro genotype, which may be due to enhanced mitochondrial localization of *TP53* protein in cells with the Arg/Arg genotype [34]. Furthermore, it was reported that mutant *TP53* with the R72 variant was significantly associated with poor prognosis in women with BC [35]. Thus, it is suggested that *TP53* codon 72 might be a strong predictive marker for chemotherapy response in BC patients.

Genetic alteration causes abnormalities in PI3K/AKT/mTOR pathway and results in deregulated kinase activity and malignant transformation. Thus, target therapies are being actively investigated to inhibit this pathway including; PI3K inhibitors such as pictilisib, alpelisib, copanlisib, and taselisib; AKT inhibitors such as ipatasertib; and mTOR inhibitors such as everolimus [36]. *PIK3CA* gene is an important component of the phosphoinositide-3 kinase (PI3K) pathway which is frequently altered in human cancers. Deregulation of the PI3K pathway, through the acquired somatic mutations, contributes to tumors development and progression. In The Cancer Genome Atlas (TCGA) and COSMIC databases, it was reported that *PIK3CA* gene is mutated in ~36% of BC [37]. In patients with *PIK3CA* mutations, recent study showed promising results in progression-free survival when using buparlisib, PI3K inhibitor; in combination with endocrine therapy [38]. Many other studies revealed that patients with *PIK3CA* mutations might benefit from PI3K-selective inhibitor treatment [39,40]. On the other hand, *AKT* gene is the PI3K effector and AKT signalling leads to increased cellular growth and survival. A somatic mutation (E17K) in *AKT*1 gene was discovered in 8% of BC [41]. At least one component of PI3K/AKT/mTOR pathway is altered in more than 50% of ER/PR positive tumors, and these alterations cause tumor growth and develop resistance to antihormone therapies. Thus, hormone receptor positive tumors will benefit from using PI3K/AKT inhibitors in combination with endocrine therapy [42]. We detected eight different *PIK3CA* mutations in 27 patients (58.7%). H1047R, E545K, E542K, Q546R, p.E80K hotspots accounted for 55.6% of all *PIK3CA* mutations in our cohort. We also detected *PIK3CA* I391M polymorphism; which is far from the binding site but can affect the protein function and change its dynamic action. It was suggested by Ahmadi et al. that this polymorphism may be involved in BC invasion [43]. This variant was present in 7 patients (15.2%) and may be used as marker for BC tumorigenesis. Another remaining *PIK3CA* (p.T1025T) polymorphism in our cohort is thought to be Arab specific variant. This SNP rs17849079 (p.T1025T) was reported at high prevalence among Arab BC patients and suggested to be used as a molecular marker for early diagnosis in this population [44]. So, further studies are needed to validate the use of such structural variants as SNP marker for BC early detection and invasion. Moreover, we found one hotspot mutation in *AKT1* gene (exon3:c.49G > A: p.E17K) in two patients of luminal A and Triple negative subtypes. It was reported that BC patients with AKT p.E17K mutation are sensitive to AKT inhibitors [45]. Thus Egyptian BC patients carrying this mutation may represent good candidates for AKT inhibitors treatment. In this study and according to IVA, we identified many mutated genes that commonly up-regulate PI3K/AKT signaling pathway and promote carcinogenesis. Thus, we propose that Egyptian BC patients might benefit from PI3K/AKT inhibitors in combination with endocrine therapy.

Two pathogenic frame shift deletion variants in *VHL* and *STK11* were detected in 5 and 4 samples, respectively. Other two frame shift deletion variants in *TP53* and *PTEN* were identified. Interestingly, 2 patients were found to concomitantly harbor these four frame shift variants. This combination of mutation may contribute the BC development in those patients.

Loss of *PTEN* function, on basis of somatic mutations, mostly affects tumor development across tissues. In the nucleus, *PTEN* promotes chromosome stability and DNA repair. Consequently, loss of *PTEN* function increases genomic instability [46]. Also, improper *PTEN* function leads to uncontrolled activation of its downstream signals. One frame shift deletion and one stop gain variants in *PTEN* gene were identified. A deletion in codon 288, exon 8 of *PTEN*, resulting in a frame shift mutation (p.E288fs) was detected in 7 samples of Luminal A subtype. The stop gain variant (p.R130X) was detected in one sample which was stage I. A combination mutation in *PIK3CA* (p.H1047R) and *PTEN* (p.R130X) was also identified.

In addition, we identified rare hotspot point mutation in KRAS (exon2:c.35G > T: p.G12V) that have been previously reported in ductal carcinomas [47]. In our sample set, this mutation was found in one case co-occurred with another PIK3CA point mutation (p.T1025T) in luminal B (Her2+) subtype. This might explain the contribution of this co-occurrence in BC susceptibility as a driver mutation in tumor development. Furthermore, we identified an important pathogenic *ERBB2* variant (p.V777L). In a study by Cocco

Table 2
Somatic mutations detected in 46 Egyptian BC patients:

Gene	Function	AA mutation	CDS mutation	Mutation type	Samples with mutation	Zygosity	CADD Phred prediction	Hot spot	Clinvar
KIT	Exonic; splicing	p.L862L	c.2586G > C	Substitution – coding silent	10	Het.	–	–	Benign
	Exonic; splicing	p.M541L	c.1621A > C	Substitution – Missense	6	Het.	–	4-55593464	Benign
	Exonic; splicing	p.K546K	c.1638A > G	Substitution – coding silent	5	Het.	–	4-55593481	Benign
TSC1	Exonic	p.F608Lfs*21	c.1824del	Frame shift deletion	12	Hom.	D	–	Not reported
	Exonic	p.L203Cfs*7	c.608del	Frame shift deletion	2	Hom.	D	–	Not reported
TSC2	Exonic	p.F298Lfs*65	c.894del	Frame shift deletion	3	Hom.	D	–	Not reported
CEBPA	Exonic	p.A176V	c.527C > T	Substitution – Missense	7	Het.	D	–	Uncertain significance
KRAS	Splicing	–	c.575-9G > A	Substitution- Intronic	6	Het.	–	–	Benign
	Exonic	p.G12V	c.35G > T	Substitution – Missense	1	Het.	D	12-25398284	Pathogenic
PDGFRA	Exonic	–	c.2472C > T	Substitution – coding silent	6	Het.	–	4-55152040	Benign
PTEN	Exonic	p.E288fs	c.863delA	Frame shift deletion	6	Hom.	–	10-89720712	Not reported
					1	Het.	–		
	Exonic	p.R130X	c.388C > T	Substitution – Nonense (stopgain)	1	Het.	D	10-89692904	Pathogenic
VHL	Exonic	p.F148fs	c.440delT	Frame shift deletion	5	Hom.	–	3-10188297	Pathogenic
NOTCH4	Exonic	p.K117Q	c. 349A > C	Substitution – Missense	4	Hom.	–	–	Not reported
STK11	Exonic	p.G279fs	c.837delC	Frame shift deletion	4	Het.	–	19-1221314	Pathogenic
P53	Exonic	p.R175H	c.524G>A	Substitution – Missense	1	Het.	D	17-7578406	Pathogenic
		p.R282W	c.844C>T	Substitution – Missense	1	Het.	D	17-7577094	Pathogenic
		p.C176W	c.528C>G	Substitution – Missense	1	Het.	D	17-7578402	Uncertain significance
		p.Y234C	c.701A>G	Substitution – Missense	1	Het.	D	17-7577580	Pathogenic
		p.R280G	c.838A>G	Substitution – Missense	1	Het.	D	17-7577100	conflicting interpretations of pathogenicity
		P.G245D	c.734G>A	Substitution – Missense	1	Het.	D	17-7577547	Pathogenic
		p.P72A	c.214C>G	Substitution – Missense	4	Het.	–	17-7579473	Uncertain significance
		p.P72R	c.215C>G	Substitution – Missense	8	Hom.	–	17-7579472	Drug response
		p.C135fs	c.403delT	Frame shift deletion	2	Hom.	–	17-7578527	Uncertain significance
					1	Het.			
		p.A276P	c.826G>C	Substitution – Missense	1	Het.	D	17-7577112	conflicting interpretations of pathogenicity
	Exonic; splicing	p.Y220C	c.659A>G	Substitution – Missense	1	Het.	D	17-7578190	Pathogenic
		p.Q331*	c.991C>T	Substitution – Nonense (stopgain)	1	Het.	D	–	Not reported
		p.K132R	c.395A>G	Substitution – Missense	1	Het.	D	17-7578535	Uncertain significance
		p.R306*	c.916C>T	Substitution –Nonense (stopgain)	1	Het.	D	17-7577022	Pathogenic
	Splicing	–	c.376-1G>A	Substitution- Intronic	1	Het.	D	17-7578555	Pathogenic
PIK3CA	Exonic	p.H1047R	c.3140A>G	Substitution – Missense	10	Het.	D	3-178952085	Pathogenic
		p.I391M	c.1173A>G	Substitution – Missense	7	Het.	–	–	Benign
		p.T1025T	c.3075C>T	Substitution – coding silent	4	Het.	–	3-178952020	Benign
		p.E542K	c.1624G>A	Substitution – Missense	1	Hom.	D	3-178936082	Pathogenic
With		p.E80K	c.238G>A	Substitution – Missense	1	Het.	D	3-178916851	Not reported
		p.Q546R	c.1637A>G	Substitution – Missense	1	Het.	D	3-178936095	Pathogenic
		p.E545K	c.1633G>A	Substitution – Missense	2	Het.	D	3-178936091	Pathogenic
		p.E365K	c.1093G>A	Substitution – Missense	1	Het.	D	–	Pathogenic

(*continued on next page*)

JAK3	Exonic	p.V722I	c.2164G>A	Substitution – Missense	2	Het.	D	19-17945696	conflicting interpretations of pathogenicity
APC	Exonic	p.G2502S	c.7504G>A	Substitution – Missense	2	Het.	D	–	Benign
		p.E1317Q	c.3949G>C	Substitution – Missense	1	Het.	–	5-112175240	Conflicting interpretations of pathogenicity
MET	Exonic	p.N375S	c.1124A>G	Substitution – Missense	1	Het.	–	7-116340262	Benign
		p.E168D	c.504G>T	Substitution – Missense	1	Het.	–	7-116339642	Benign
Gene	Function	AA mutation	CDS mutation	Mutation type	Samples with mutation	Zygosity	CADD Phred prediction	Hot spot	Clinvar
AKT1	Exonic	p.E17K	c.49G>A	Substitution – Missense	2	Het.	D	14-105246551	Pathogenic
MAP2K4	Exonic ; splicing	p.Q80*	c.238C>T	Substitution – Nonense (stopgain)	1	Het.	D	–	Not reported
IDH2	Exonic	p.R140Q	c.419G>A	Substitution – Missense	1	Het.	D	15-90631934	Pathogenic
ERBB2	Exonic	p.V777L	c.2329G>T	Substitution – Missense	1	Het.	D	17-37881000	Pathogenic
EPHB1	Exonic	p.F151V	c.451T>G	Substitution – Missense	14	Het.	D	–	Novel
KIT	Exonic	p.H263Q	c.789T>G	Substitution – Missense	10	Het.	D	–	Novel
MTOR	Exonic; splicing	p.T600I	c.1799C>T	Substitution – Missense	9	Het.	D	–	Novel
JAK2	Exonic	–	c.2041_2042delinsTA	inframeshift substitution	6	Het.	–	–	Novel

et al, it was proposed that Neratinib is effective in breast tumors bearing both amplification and mutation of ERBB2 [48].

In conclusion NGS is a very useful tool to evaluate the mutational status of oncogenes and tumor suppressor genes to help identify the mutation drivers of BC [49]. In this cohort we shed the light on the most frequently detected somatic mutations and most altered pathways in Egyptian BC patients.

Recommendation

We recommend following up the patients until diagnosis of recurrence or metastasis and following up their response to treatment to give more focus on the association between survival data and the identified somatic mutations which may have important clinical implications for personalized medicine, target therapy, therapeutic guidance, and monitoring of recurrence or metastasis. Moreover, we recommend sequencing the most frequently detected genes from this preliminary study to confirm our findings on large number of BC patients. In addition, giving more focus on triple negative BC patients as it is the most aggressive and has a poor prognosis.

Author contributions

Abdel-Rahman N Zekri designed the study. **M. Gomaa, Osman Mansour, Amany Abd-Elhameed Abou-Bakr and Samah A. Loutfy** recruited patients and collected clinical data. **Auhood Nassar, Mai M. Lotfy** and **Amira Salah El-Din Youssef** performed the library preparation and NGS workflow. **Ola s. ahmed**, helped in NGS. **Mohamed Abouelhoda and Auhood Nassar** performed the bioinformatic analysis. **Auhood Nassar** drafted the manuscript. **Mohamed M. Hafez**, **Hoda Ismail**, and **Abeer Bahnassy** revised the manuscript. All authors read and approved the final version.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

References

[1] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136(5):E359–86.

[2] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87–108.

[3] The National Cancer Registry Program of Egypt (NCRPE). Reports and Statistics: Aswan, Damietta & El-Minia; 2013 http://www.cancerregistry.gov.eg/reports.aspx [accessed June 2013].

[4] The Gharbiah Population-based Cancer Registry (GPCR). Cancer in Egypt, Gharbiah; 2007 http://www.emro.who.int/ncd/pdf/cancer_registry_Egypt.pdf [accessed June 2013].

[5] Nik-Zainal S, Davies H, Staaf J, Ramakrishna M, Glodzik D, Zou X, et al. Landscape of somatic mutations in 560 BC whole genome sequences. Nature 2016;534(7605):47–54.

[6] Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature 2012;490(7418):61–70. doi: https://doi.org/10.1038/nature11412.

[7] Stephens PJ, Tarpey PS, Davies H, Van Loo P, Greenman C, Wedge DC, et al. The landscape of cancer genes and mutational processes in breast cancer. Nature 2012;486(7403):400–4. doi: https://doi.org/10.1038/nature11017.

[8] Pereira B, Chin SF, Rueda OM, Vollan H-KM, Provenzano E, Bardwell HA, et al. The somatic mutation profiles of 2,433 breast cancers refines their genomic and transcriptomic landscapes. Nat Commun 2016;7:11479. doi: https://doi.org/10.1038/ncomms11479.

[9] Meric-Bernstam F, Frampton GM, Ferrer-Lozano J, Yelensky R, Pérez-Fidalgo JA, Wang Y, et al. Concordance of genomic alterations between primary and recurrent breast cancer. Mol Cancer Ther 2014;13(5):1382–9. doi: https://doi.org/10.1158/1535-7163.MCT-13-0482.

[10] Weisman PS, Ng CK, Brogi E, Eisenberg RE, Won HH, Piscuoglio S, et al. Genetic alterations of triple negative breast cancer by targeted next-generation sequencing and correlation with tumor morphology. Mod Pathol 2016;29(5):476–88. doi: https://doi.org/10.1038/modpathol.2016.39.

[11] Smith NG, Gyanchandani R, Shah OS, Gurda GT, Lucas PC, Hartmaier RJ, et al. Targeted mutation detection in breast cancer using MammaSeq™. Breast Cancer Res 2019;21(1):22. doi: https://doi.org/10.1186/s13058-019-1102-7.

[12] Bai X, Zhang E, Ye H, Nandakumar V, Wang Z, et al. PIK3CA and TP53 gene mutations in human breast cancer tumors frequently detected by ion torrent DNA sequencing. PLoS ONE 2014;9(6):e99306.

[13] Liu S, Wang H, Zhang L, Tang C, Jones L, Ye H, et al. Rapid detection of genetic mutations in individual breast cancer patients by next-generation DNA sequencing. Hum Genom 2015;9(1):2. doi: https://doi.org/10.1186/s40246-015-0024-4.

[14] Li G, Guo X, Chen M, Tang L, Jiang H, Day JX, et al. Prevalence and spectrumof AKT1, PIK3CA, PTEN and TP53 somatic mutations in Chinese breast cancer patients. PLoS ONE 2018;13(9):e0203495. doi: https://doi.org/10.1371/journal. pone.0203495.

[15] Zhang Y, Cai Q, Shu X-O, Gao Y-T, Li C, Zheng W, et al. Whole-exome sequencing identifies novel somatic mutations in Chinese breast cancer patients. J Mol Genet Med: Int J Biomed Res 2015;9(4). doi: https://doi.org/10.4172/1747-0862.1000183.

[16] Banerji S, Cibul Skis K, Rangel-Escareno C, Brown KK, Carter SL, Frederick AM, et al. Sequence analysis of mutations and translocations across breast cancer subtypes. Nature 2012;486(7403):405–9. doi: https://doi.org/10.1038/nature11154.

[17] Yang H, Wang K. Genomic variant annotation and prioritization with ANNOVAR and wANNOVAR. Nat Protoc 2015;10:1556±1566. doi: https://doi.org/10.1038/nprot.2015.105. PMID: 26379229.

[18] Tate JG, Bamford S, Jubb HC, Sondka Z, et al. COSMIC: the catalogue of somatic mutations in cancer. Nucleic Acids Res 2019;47(D1):D941–7.

[19] Griffith M, Spies NC, Krysiak K, McMichael JF, Coffman AC, Danos AM, et al. CIViC is a community knowledgebase for expert crowdsourcing the clinical interpretation of variants in cancer. Nat Genet 2017;49(2):170–4. doi: https://doi.org/10.1038/ng.3774.

[20] Landrum MJ, Lee JM, Benson M, Brown GR, Chao C, Chitipiralla S, et al. ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Res 2018. PubMed PMID: 29165669.

[21] Vaser R, Adusumalli S, Leng SN, Sikic M, Ng PC. SIFT missense predictions for genomes. Nat Protocols 2016;11:1–9.

[22] Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. Nucleic Acids Res 2018. doi: https://doi.org/10.1093/nar/gky1016.

[23] Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nat Methods 2010;7:248–9.

[24] Hiltemann S, Jenster G, Trapman J, van der Spek P, Stubbs A. Discriminating somatic and germline mutations in tumor DNA samples without matching normals. Genome Res 2015;25(9):1382–90. doi: https://doi.org/10.1101/gr.183053.114.

[25] Sukhai MA, Misyura M, Thomas M, Garg S, Zhang T, Stickle N, et al. Somatic tumor variant filtration strategies to optimize tumor-only molecular profiling using targeted next-generation sequencing panels. J Mol Diagn 2019;21(2):261–73. doi: https://doi.org/10.1016/j.jmoldx.2018.09.008.

[26] Ye Q, Qi F, Bian L, Zhang SH, Wang T, Jiang ZF. Circulating-free DNA mutation associated with response of targeted therapy in human epidermal growth factor Receptor 2-positive metastatic breast cancer. Chin Med J 2017;130:522–9.

[27] Loi S, Michiels S, Lambrechts D, Fumagalli D, et al. Somatic mutation profiling and associations with prognosis and trastuzumab benefit in early breast cancer. J Natl Cancer Inst 2013;105:960–7.

[28] Zhang Z, Wang M, Wu D, Tong N, Tian Y. P53 codon 72 polymorphism contributes to breast cancer risk: a meta-analysis based on 39 case–control studies. Breast Cancer Res Treat 2010;120. 509–517 12.

[29] Ma Y, Yang J, Liu Z, Zhang P, Yang Z, Wang Y, et al. No significant association between the TP53 codon 72 polymorphism and breast cancer risk: a meta-analysis of 21 studies involving 24,063 subjects. Breast Cancer Res Treat 2011;125:201–5.

[30] Cheng H, Biao Ma B, Jiang R, Wang W, Guo H, et al. Individual and combined effects of MDM2 SNP309 and TP53 Arg72Pro on breast cancer risk: an updated meta-analysis. Mol Biol Rep 2012;39:9265–74.

[31] Bergamaschi D, Gasco M, Hiller L, Sullivan A, Syed N, Trigiante G. p53 polymorphism influences response in cancer chemotherapy via modulation of p73-dependent apoptosis. Cancer Cell 2003;3(4):387–402.

[32] Marin MC, Jost CA, Brooks LA, Irwin MS, O'Nions J, Tidy JA, et al. A common polymorphism acts as an intragenic modifier of mutant p53 behaviour. Nat Genet 2000;25(1):47–54.

[33] Xu Y, Yao L, Ouyang T, Li J, Wang T, Fan Z, et al. p53 Codon 72 polymorphism predicts the pathologic response to neoadjuvant chemotherapy in patients with breast cancer. Clin Cancer Res 2005;11(20):7328–33.

[34] Dumont P, Leu JI, Della Pietra 3rd AC, George DL, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat Genet 2003;33(3):357–65.

[35] Basu S, Gnanapradeepan K, Barnoud T, Kung CP, Tavecchio M, Scott J, et al. Mutant p53 controls tumor metabolism and metastasis by regulating PGC-1α. Genes Dev 2018;32(3–4):230–43. doi: https://doi.org/10.1101/gad.309062.117.

[36] Araki K, Miyoshi Y. Mechanism of resistance to endocrine therapy in breast cancer: the important role of PI3K/Akt/mTOR in estrogen receptor-positive, HER2-negative breast cancer. Breast Cancer 2018;25(4):392–401.

[37] Chen L, Yang L, Yao L, Kuang XY, Zuo WJ, Li S, et al. Characterization of PIK3CA and PIK3R1 somatic mutations in Chinese breast cancer patients. Nat Commun 2018;9(1):1357. doi: https://doi.org/10.1038/s41467-018-03867-9. PMID: 29636477; PMCID: PMC5893593.

[38] Baselga J, Im SA, Iwata H, Cortes J, De Laurentiis M, Jiang Z, et al. Buparlisib plus fulvestrant versus placebo plus fulvestrant in postmenopausal, hormone receptor-positive, HER2-negative, advanced breast cancer (BELLE-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2017;18:904–16.

[39] Kodahl AR, Ehmsen S, Pallisgaard N, Jylling AMB, Jensen JD, Laenkholm AV, et al. Correlation between circulating cell-free PIK3CA tumor DNA levels and treatment response in patients with PIK3CA-mutated metastatic breast cancer. Mol Oncol 2018;12(6):925–35.

[40] Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K, et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell 2007;12(4):395–402.

[41] Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer 2009;9:550–62. doi: https://doi.org/10.1038/nrc2664.

[42] Polo ML, Riggio M, May M, et al. Activation of PI3K/Akt/mTOR signaling in the tumor stroma drives endocrine therapy-dependent breast tumor regression. Oncotarget 2015;6(26):22081–97.

[43] Ahmadi S, Ramezani S, Ghafouri H, Hosseini SM, Najafi A, et al. Association between the PIK3CA Ile391Met polymorphism and the risk of breast cancer in an Iranian population. J Appl Biotechnol Rep 2018;5(1):8–12.

[44] Karakas B, Colak D, Kaya N, Ghebeh H, Al-Qasem A, Hendrayani F, et al. Prevalence of PIK3CA mutations and the SNPrs17849079 in Arab breast cancer patients. Cancer Biol Ther 2013;14(10):888–96.

[45] Hyman DM, Smyth LM, Donoghue MTA, Westin SN, Bedard PL, Dean EJ, et al. AKT inhibition in solid tumors with AKT1 mutations. J Clin Oncol: Off J Am Soc Clin Oncol 2017;35(20):2251–9.

[46] Dillon LM, Miller TW. Therapeutic targeting of cancers with loss of PTEN function. Curr Drug Targets 2014;15(1):65–79.

[47] Myers MB, Banda M, McKim KL, Wang Y, et al. Breast cancer heterogeneity examined by high-sensitivity quantification of PIK3CA, KRAS, HRAS, and BRAF mutations in normal breast and ductal carcinomas. Neoplasia 2016;18:253–63.

[48] Cocco E, Javier Carmona F, Razavi P, Won HH, Cai Y, Rossi V, et al. Neratinib is effective in breast tumors bearing both amplification and mutation of ERBB2 (HER2). Sci Signal 2018;11(551):eaat9773. doi: https://doi.org/10.1126/scisignal.aat9773.

[49] Uscanga-Perales GI, Santuario-Facio SK, Sanchez-Dominguez CN, Cardona-Huerta S, Muñoz-Maldonado GE, Ruiz-Flores P, et al. Genetic alterations of triple negative breast cancer (TNBC) in women from Northeastern Mexico. Oncol Lett 2019;17(3):3581–8. doi: https://doi.org/10.3892/ol.2019.9984.