



# Whole Exome Sequencing Reveals a Combination of Rare High and Low Penetrance Variants that Correlate with Familial Breast Cancer Relative Risk

Ben Rekaya Mariem<sup>1\*</sup>, Hamdi Yosr<sup>1,2</sup>, El Benna Houda<sup>1,3</sup>, Mejri Nessrine<sup>1,3</sup>, Jaidane Olfa<sup>4</sup>, Ayari Jihene<sup>5</sup>, Ben Nasr Sonia<sup>1,5</sup>, Dallali Hamza<sup>1</sup>, Messaoud Olfa<sup>1</sup>, Meddeb Rym<sup>6</sup>, Mighri Najah<sup>1</sup>, Boujemaa Maroua<sup>1</sup>, Boubaker Mohamed Samir<sup>1,2</sup>, Haddaoui Abderazek<sup>5</sup>, Mrad Ridha<sup>6</sup>, Boussem Hamouda<sup>1,3</sup>, Abdelhak Sonia<sup>1</sup>, Labidi Soumaya<sup>1</sup>

<sup>1</sup>Laboratory of Biomedical Genomics and Oncogenetics, Tunis, Tunisia; <sup>2</sup>Laboratory of Human and Experimental Pathology, Tunis, Tunisia; <sup>3</sup>Abderrahmane Mami Hospital, Ariana, Tunisia; <sup>4</sup>Institute Salah Azaiez of Oncology, Tunis, Tunisia; <sup>5</sup>Military Hospital of Instruction of Tunis, Tunis, Tunisia; <sup>6</sup>Charles Nicolle Hospital, Tunis, Tunisia

## ABSTRACT

**Objective:** We aimed to investigate and identify simultaneously all rare pathogenic and common variants in unrelated Breast Cancer (BC) cases.

**Methods:** All frequent mutations in BRCA genes previously identified in Tunisia have been excluded by Sanger sequencing in 42 women affected with high family risk having at least 3 cancer affected related individuals. Two unrelated cases having two different family histories have been selected for whole exome sequencing. Selected high risk variants were confirmed and segregation analysis was performed.

**Results:** We identified a pathogenic frame-shift loss of function variant in BRCA2 p.Val1283Lysfs in three cases and a pathogenic rare variant in OGG1, p.Arg46Gln that co-segregates with a rare non sense variant in BRCA2, p.K3326X, in two breast cancer affected cases. These variants have never been described in Tunisia or North Africa.

**Conclusion:** Family history and the young age at onset for patient F1.1 correlate with the presence of a high penetrant variant (p.Val1283Lysfs) in BRCA2 gene. However, the late age at onset and the less severe phenotype for patient F2.2 are the consequence of the presence of a low penetrant variant Lys3326X in BRCA2 that co-segregate with a pathogenic variant p.Arg46Gln in OGG1 gene only in BC cases.

**Keywords:** OGG1; BRCA2; Polygenic inheritance; Rare variants ; BER

## INTRODUCTION

In Tunisia, Breast Cancer (BC) is a public health problem with at least 2300 new cases per year. Many studies suggest that it is more aggressive than in Western countries, with notably large proportions of young patients [1]. Both non-genetic and genetic factors are involved in the etiology of BC. Genetic counseling should incorporate family history profiles, the gene involved and the mutation location [2]. A measure of the familial clustering is the Familial Relative Risk (FRR). International guidelines for BRCA testing use (i) breast or ovarian family, (history, (ii) young age at diagnosis  $\leq 36$  years and (iii) triple-negative breast cancer as the most risk factors). Many studies suggest that the commonly used guidelines for testing were insufficient to detect

all mutation carriers in the BC cohorts [3,4]. Indeed, a higher rate of both BRCA1 and BRCA2 mutations has been observed in affected patients from North Africa without or unknown family history (8.0% in North Africa versus 1.1% in France for BRCA1 mutations,  $P=0.02$ ; 7.2% in North Africa vs. 1.1% in France for BRCA2 mutations:  $P<0.05$ ) [5]. Genetic variants associated with breast cancer risk can be classified as high-penetrant mutations that are rare in the population but associated with a high risk; moderate penetrant variants associated with a moderate risk; and low-penetrant polymorphisms which are common and associated with a small risk [6].

Common variants in genes involved in DNA repair pathways especially Base Excision Repair (BER) have a synergistic functional

**Correspondence to:** Dr. Ben Rekaya Mariem, Laboratory of Biomedical Genomics and Oncogenetics, Tunis, Tunisia, Tel: 0021697885235; E-mail: rekayamariem@gmail.com

**Received:** 01-Mar-2022, Manuscript No. JDMGP-22-14666; **Editor assigned:** 04-Mar-2022, PreQC No. JDMGP-22-14666 (PQ); **Reviewed:** 18-Mar-2022, QC No. JDMGP-22-14666; **Revised:** 25-Mar-2022, Manuscript No. JDMGP-22-14666 (R); **Published:** 04-Apr-2022, DOI: 10.4172/2153-0602.22.13.248.

**Citation:** Mariem BR, Yosr H, Houda EB, Nessrine M, Olfa J, Jihene A, et al. (2021) Whole Exome Sequencing Reveals a Combination of Rare High and Low Penetrance Variants that Correlate with Familial Breast Cancer Relative Risk. J Data Mining Genomics Proteomics. 13:248.

**Copyright:** © 2022 Mariem BR, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

effect increasing cancer risk susceptibility in BRCA2 mutation carriers [7,8]. Among 144 SNPs analyzed in a two stage study involving 23,463 carriers from the CIMBA consortium, eleven SNPs showed evidence of association with breast and/or ovarian cancer at  $p < 0.05$  in the combined analysis. Four of the five genes for which strong evidence of association was observed in DNA glycosylases, OGG1, TDG and NEIL2. Most of these SNPs are common non-coding, present in regulatory regions. Unfortunately, we have no idea on the role of rare coding modifiers variants located in these genes.

In another side, a large proportion of all breast cancers arises in a genetically susceptible minority of women that are not carriers of BRCA1 or BRCA2. Susceptibility to breast cancer is likely to be the result of at risk alleles in many different genes. Disease susceptibility in non-carriers of BRCA1/2 mutations is explainable, in a polygenic model, by large numbers of susceptibility polymorphisms that are multiplicatively acting on risk [9-11]. These SNP associations are specific to some ethnicity or estrogen receptor [12]. A largest BC study has identified a total of 172 risk-associated SNPs that account for an estimated  $\sim 18\%$  of FRR [13-15]. A portion of missing heritability in familial BC is likely represented by rare functional coding variants in genes not currently present on Genome Wide Association Study (GWAS) available panels [16]. Whole Exome Sequencing (WES) technology has been demonstrated to be efficient in the discovery of novel BC predisposition genes such as those that encode proteins involved in the DNA damage response or DNA repair and to identify novel genetic modifiers of risk for early-onset BC predisposition in carriers of high-risk mutations [17,18].

In this study, we report and discuss the role of rare pathogenic mutations in BC candidate genes and known risk common variants for predisposition to BC.

## MATERIALS AND METHODS

### Clinical manifestations and family history

This study was conducted according to the principles of the declaration of Helsinki and has obtained the ethics approval from the institutional review board of Pasteur Institute of Tunisia Registration number 2017/16/E/hospital a-m/V1. Forty-two BC unrelated affected patients were recruited from the Oncology Department of XXX or from the surgical oncology department of XXXX, or the Oncology XXXX of Tunis. Clinical and epidemiological characteristics of the five reported BC affected members (Tables 1 and 2). The mean age at onset of BC in F1 family is 42.6 years however it is 66 years for the F2 family. For the F2 family, there are 4 other cancer diseases in family members (2 colorectal cancers, 1 cervical cancer, and 1 testicular cancer and the father died by Larynx cancer at age of 49) (Figure 1).

### Exome sequencing

WES was performed for patients F1.1 and F2.2. Exome was captured from genomic DNA using Agilent Sure Select Protocol Version 1.2 (Agilent Technologies; Santa Clara, CA, USA) and then sequenced on an Illumina HiSeq 2000 sequencer. We used BWA to align sequence reads to the hg19 reference genome and GATK to call SNVs and indels. Control quality showed that 88% of targeted bases were covered at  $>20\times$ .

### Exome sequencing data analysis

The results were analyzed using the VarAft software version 1.6,

(<http://varaft.eu/index.php>). For analysis, dominant models of inheritance have been selected. Given the number of variants identified in WES, and in order to prioritize them, variants were filtered according to several stringent criteria. Indeed, we kept only rare functional variants (missense, nonsense, splice site variants, and indels) that were heterozygous in the index cases and we discarded variants with a Minor Allele Frequency (MAF)  $\geq 1\%$  according to 4 databases (1000 Genomes, Exome Variant Server, Exome Aggregation Consortium (ExAC) and a local database encompassing 48 exomes of Tunisian individuals with no personal nor familial BC history). Mutation Taster, PolyPhen, SIFT were used to predict the functional impact and pathogenicity of the missense variants. We selected all rare coding variants (frequency  $< 0.01$ ) described at least once as pathogenic in ClinVar. Candidate genes are matched with BC disorders according to the VarElect prioritization tool (<http://varelect.genecards.org>) [19]. We also extracted common at risk variants reported to contribute in increasing BC risk (Tables S1-S4).

### Variant validations and co-segregation

Two exons with a bad coverage (i.e. Exon 5 in BRCA2 and Exon 13 in BRCA1) as well as 3 exons contain potential at risk variants, 11 and 27 in BRCA2 and exon 1 in OGG1 gene, were amplified and Sanger sequenced.

## RESULTS

### Exome analysis strategies

**Screening for rare and common variants in BRCA genes:** All heterozygous and homozygous variants in BRCA genes, which have been identified in the two cases, are listed in Table 3. The structure of the genetic profile between the two patients showed significant difference in terms of frequency and function (Figure 2).

The patient F1.1 has 6 variants in BRCA1 and 6 variants in BRCA2. Among them, two interesting variants: the frame-shift deletion c.3847\_3848delGT (p.Val1283Lysfs) (BIC: 4075delGT), classified as rare pathogenic variant and the frequent variant rs799905 predicted putative functional according to regulomdb software (Score=2b).

However the patient F2.2 has 16 variants in BRCA1 and 11 variants in BRCA2. None of them is known, as a pathogenic variant. However, in silico analysis showed the presence of 4 frequent regulatory functional variants in BRCA1 gene (rs16940, rs3092994, rs1060915, rs3765640) that are responsible for cis regulation expression according to Encode data and Regulome DB software (Score=1F). In addition, she has the rare non-sense coding variant p.Lys3326Ter that results in a 92 amino acid truncation of BRCA2 protein. This rare variant and other 3 frequent non synonymous variants (rs16942, rs1799966, rs144848) are classified as low penetrant BC variants and could together generate a Polygenic Risk Score (PRS) [20] (Table S2).

### Screening for rare and pathogen variants out BRCA genes

The patient F1.1, has four rare missense variants at heterozygous state (APC:p.I1307K; SPTA1:p.D791E; GCH1:p.K224R; FREM1:p.R498Q) classified as conflicting interpretations of pathogenicity and located in different genes having variable Varelect Score ranging from 670,03 to 14,31 (Table 2).

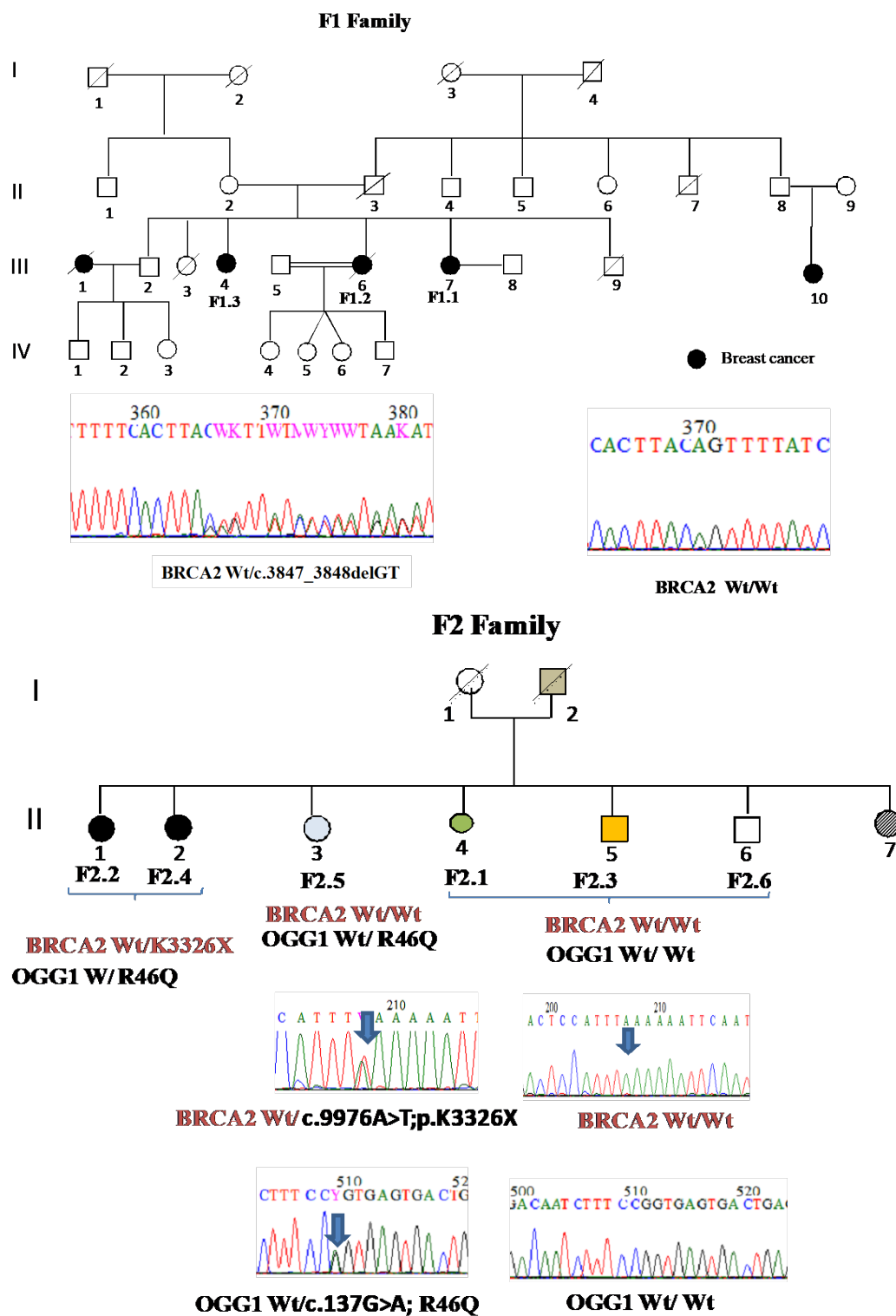
Table 1: Epidemiological and clinical features of breast cancer affected family members.

Family code	Affected cases code	Age at Diagnosis (years)	births number	Breast feeding duration (months)	Menarche age (years)	Menopausal Age (years)	Hormonal contraception (duration)	Histological subtype	SBR grade	Tumor size (mm)	TNM Classification	Hormone receptors status (HR)	HER2 status	Ki67 index value	Treatment	Other disorders
F2	F1.1	46	1	>12	ND	ND	NO	Invasive Ductal carcinoma / Comedocarcinoma	I	ND	T2,N1,M0	RE+RP-	Negative	25%	Mastectomy, Chemotherapy, Hormonal therapy	Infertility
	F1.2	50	4	>12	11	50	3 years	Atypical ductal carcinoma with in situ component	III	30	T1,N0,M0	RH+	Negative	ND	Mastectomy Chemotherapy Radiation therapy Hormonal therapy	ND
F2	F1.3	31	0 (not married)	No	12	No	No	Multifocal Infiltrating ductal carcinoma of the left breast	I	24,10,20	T2N0M0	RH+,	Negative	15%	Mastectomy Chemotherapy Radiation therapy Hormonal therapy	ND
	F2.2	62	3	>12 months	13	52	No	Invasive Ductal carcinoma	III	6	11N-	ER+ (100%)/ PR+(20%)	Negative	5%	-Mastectomy -3FEC,3Taxotel (6 cycles for 4 months) - aromatase inhibitors HT (5 years)	Diabetes type 2
F2	F2.4	70	3	≤2 months	10	42	No	Invasive Ductal carcinoma	III	10	9N-	ER+/PR+	Negative	5%	Mastectomy -3FEC,3Taxotel (6 cycles for 4 months) - aromatase inhibitors HT (5 years)	Biliary lithiasis, thyroid adenoma, osteoporosis, osteoarthritis, hypertension, cholesterol

Table 2: List of all rare and pathogenic variants found in F1.1 and F2.2

Chr	Ref	Gene Descriptio	AAChange.refgene	esp500 siv2_all	1000g 2015 aug_all	avsnp144	SIFT_pred	Polyphen2_HVAR_pred	MutationTaster_pred	cosmic70	CLINSIG	CLNDBN	ExAC_ALL	ExAC_AFR	Varelect Score
chr13	TG/-	BRCA2, DNA Repair Associated	NM_000059.3: exon11: c.3847_3848del: p.T1282fs	0.0004	NA	rs746229647/rs80359405	NA	NA	NA	NA	Pathogenic	Breast-ovarian_cancer,_familial_predisposing_syndrome	0.0001	0	920,64
chr5	T/A	APC, WNT Signaling Pathway Regulator	APC: NM_001127510: exon17: c.3920T>A: p.I1307K	0.0011	NA	rs1801155	T	B	D	ID=COSM26697; OCCURENCE=1 (large_intestine)	Conflicting interpretations of pathogenicity	Adenomatous_polyposis_coli,_susceptibility_to Breast_cancer,_susceptibility_to	0.0017	0	670,03
F1.1 chr1	G/T	Spectrin Alpha, Erythrocytic 1	SPTA1: NM_003126: exon17: c.2373C>A: p.D791E	0.0155	0.0133786	rs7418956	T	P	A	NA	Conflicting interpretations of pathogenicity	Elliptocytosis_2	0.0043	0.05	33,86
chr14	T/C	GTP Cyclohydrolase 1	GCHI: NM_001024024: exon6: c.671A>G: p.K224R	0.0002	0.000399361	rs41298442	T	B	A	NA	Conflicting interpretations of pathogenicity	Dystonia,_dopa-responsive,_with_or_without_hyperphenylalaninemia,_autosomal_recessive	0.0004	0	23,94
chr9	C/T	FRAS1 Related Extracellular Matrix 1	FREMI: NM_144966: exon10: c.1493G>A: p.R498Q	0.0007	0.000599042	rs184394424	D	D	D	NA	Conflicting interpretations of pathogenicity	Trigonocephaly_2 not_provided Peters_anomaly Rieger_anomaly	0.0003	0.0019	14,31
chr3	G/A	8-Oxoguanine DNA Glycosylase	OGG1: NM_016829: exon1: c.137G>A: p.R46Q	0.0022	0.000599042	rs104893751	D	D	D	NA	Pathogenic	Clear_cell_carcinoma_of_kidney	0.0021	0.0004	236,97
F1.2 chr17	G/A	Glucagon Receptor	GCCR: NM_000160: exon3: c.118G>A: p.G40S	0.0081	0.00419329	rs1801483	T	B	A	NA	Pathogenic	Diabetes_mellitus_type_2	0.0084	0.0012	22,16
chr21	-/C	Formimi doyltransferase Cyclodeaminase	FTCD: NM_206965: exon9: c.990dupG: p.P331fs	0.0034	NA	rs35208133/rs398124234	NA	NA	NA	NA	Conflicting interpretations of pathogenicity	Glutamate-formiminotransferase_deficiency not_provided	0.0024	0.0036	12,80

Variant in red are confirmed by Sanger sequencing



**Figure 1:** Pedigrees of the F1 and F2 families and DNA-sequence electropherograms for unaffected (wt) and affected family members. The frame shift deletion BRCA2:c.3847\_3848delTG is present at heterozygote state only in affected BC cases of F1. The BRCA2:c.9976A>T and the OGG1: c.137G>A are present together only in the BC affected cases of F2 family.

**Note:** ( ● )Breast cancer; ( ■ ) Testicular cancer; ( ● ) Cervical cancer; ( ■ ) Larynx cancer; ( ● ) Colorectal cancer; ( ○ ) High Blood Pressure

**Table 3:** Variants in BRCA1 and BRCA2 genes in F1.1 and F2.2 patients and their functional annotations.

Gene	avsnp144	Ref	Alt	Genotype	Func	ExonicFunc	AA Change	1000 genomes	ExAC	esp 6500	Mutation Taster	Polyphen2	SIFT	CLINSIG	cosmic70	Regulome DB Score	Regulome DB data	
BRCA1	rs799917	G	A	het	exonic	Misense	NM_007300: exon10:c.2612C>T;p.P87IIL	0.54393	0.4100	0.4932	P	B	T	Benign Likely benign Uncertain significance	ID-COSM148278; OCCURENCE=1 (large_intestine),1 (stomach)	4		
	rs36808376	AAAT	-	het	intronic	NA	NA	0.000798722	NA	NA	NA	NA	NA	NA	NA	6		
	rs799916	T	G	het	intronic	NA	NA	0.502396	NA	NA	NA	NA	NA	Benign	NA	6		
	rs368252296/rs80308573	A	-	het	intronic	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6		
	rs799905	G	C	het	intronic	NA	NA	0.545128	0.4805	NA	NA	NA	NA	Benign	NA	2b	TF binding + any motif + DNase Footprint + DNase peak	
	rs80358329	AGA	-	het	exonic	nonframeshift deletion	NM_007300: exon10:c.1846_1848del;p.616_616del	0.00119808	0.0003	0.0016	NA	NA	NA	NA	other Benign Likely benign	NA	No Data	
	rs543304	T	C	het	exonic	synSNV	NM_000059: exon11:c.3807T>C	0.168131	0.1898	0.1911	NA	NA	NA	NA	Benign	NA	6	
	rs169547	T	C	het	exonic	Misense	BRC2: NM_000059: exon14:c.7397T>C;p.V2466A	0.975839	0.9937	0.9777	P	.	T	Benign Uncertain significance	NA	No Data		
	rs11571744	C	T	het	intronic	NA	NA	0.0161741	0.0044	0.0152	NA	NA	NA	NA	Benign	NA	6	
	BRCA2	rs206076	G	C	het	exonic	synSNV	NM_000059: exon11:c.6513G>C;p.V217IV	0.973642	0.9930	0.9755	NA	NA	NA	Benign Likely benign Uncertain significance	ID-COSM4147690; OCCURENCE=1 (thyroid)	6	
rs80359405/rs746229647		TG	-	het	exonic	frameshift deletion	BRC2: NM_000059: exon11:c.3846_3847del;p.T1282fs	NA	0.0001	0.0004	NA	NA	NA	Pathogenic	NA	No Data		
rs206075		A	G	het	exonic	synSNV	NM_000059: exon11:c.4563A>G;p.L152IIL	0.974042	0.9931	0.9758	NA	NA	NA	Benign, Likely benign	NA	No Data		
rs273902772		A	-	het	intronic	NA	NA	0.334864	NA	0.2784	NA	NA	NA	Benign	NA	No Data		
rs3765640		A	G	hom	intronic	NA	NA	0.353634	NA	NA	NA	NA	NA	Benign	NA	1f	cQTL + TF binding / DNase peak	

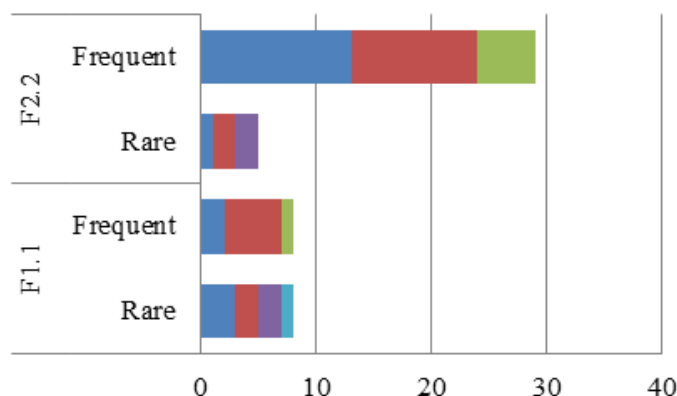




rs543304	T	C	het	exonic	synSNV	NM_000059; exon11: c.380T>C; p.V1269V	0.168131	0.1898	0.1911	NA	NA	NA	Benign	NA	6
BRCA2:															
rs206076	G	C	hom	exonic	synSNV	NM_000059; exon11: c.651G>C; p.V2171V	0.973642	0.9930	0.9755	NA	NA	NA	Benign   Likely benign   Uncertain significance	ID=COSM4147690; OCCURENCE=1 (thyroid)	6
BRCA2:															
rs169547	T	C	hom	exonic	Misense	NM_000059; exon14: c.7397T>C; p.V2466A	0.975839	0.9937	0.9777	P	.	T	Benign   Uncertain significance	NA	No Data
BRCA2:															
rs11571744	C	T	het	intronic	NA	NA	0.0161741	0.0044	0.0152	NA	NA	NA	Benign	NA	6
BRCA2:															
rs11571833a	A	T	het	exonic	stopgain	NM_000059; exon27: c.9976A>T; p.K3326X	0.00439297	0.0070	0.0065	A	.	.	Benign	NA	6
BRCA2:															
rs206073	G	A	hom	intronic	NA	NA	0.974042	NA	NA	NA	NA	NA	Benign	NA	5
rs144549870	TATCT	-	het	intronic	NA	NA	0.0157748	NA	NA	NA	NA	NA	Benign	NA	No Data
BRCA2:															
rs144848a	A	C	het	exonic	Misense	NM_000059; exon10: c.1114A>C; p.N372H	0.249401	0.2779	0.2332	P	.	T	Benign   Uncertain significance	ID=COSM3753646; OCCURENCE=1 (thyroid),1 (large_intestine)	5
BRCA2:															
rs206075	A	G	hom	exonic	synSNV	NM_000059; exon11: c.4563A>G; p.L1521L	0.974042	0.9931	0.9758	NA	NA	NA	Benign   Likely benign	NA	No Data
BRCA2:															
rs11571818	T	C	het	intronic	NA	NA	0.00439297	0.0076	0.0059	NA	NA	NA	Benign   Other	NA	6
rs206080	T	C	hom	intronic	NA	NA	0.974042	NA	NA	NA	NA	NA	Benign	NA	No Data



## Variants in BRCA1 and BRCA2 genes



**Figure 2:** Genetic profile of BRCA genes in F1.1 and F2.2 patients: The total number of variant in BRCA genes is higher in F2.2 case than in F1.1 case. F2.2 has more frequent and less rare variants than F1.1 case.

**Note:** ( ■ ) Intronic; ( ■ ) Exonic; ( ■ ) Functional Regulatory; ( ■ ) Exonic functional; ( ■ ) Pathogene

The APC gene has the highest Varelect score (670,03). The APC: p.I1307K, is known as a functional variant converting the DNA sequence to a homo-polymer region (A8) that is genetically unstable and prone to somatic mutation [21]. It could predict the prevalence of breast, lung, urologic, pancreatic, and skin cancers and it has been associated with an increased risk of colorectal cancer among Ashkenazi Jewish, Croatian, and Egyptian patients [22-26].

For the variant in the SPTA1 gene (Score=33,86), it is associated with the elliptocytosis disease which is a heterogeneous Red Blood Cell (RBC) membrane disorder. The gene encodes for an actin crosslinking and molecular scaffold protein. According to the My Genome Cancer database, somatic missense mutations in this gene are observed in cancers such as esophageal, genital tract, and endometrial. This mutation has been previously found in a Tunisian family and recently in two other asymptomatic patients but having ektacytometry profile consistent with mild hereditary elliptocytosis; this phenotype called "Jendouba spectrine phenotype" [27,28].

For the two variants, GCH1:p.K224R and FREM1:p.R498Q, none relevant data have been described in relation with cancer. The first gene is associated with the autosomal recessive Dystonia dopa responsive with or without hyperphenylalaninemia disease and is associated with an increased risk for Parkinson's disease [29]. The second gene is associated with the autosomal dominant Trionocephaly\_2 phenotype with nonsyndromic metopic craniosynostosis.

The patient F2.2 have two rare pathogenic variants in OGG1:p.R46Q and in GCGR:p.G40S and one variant in FTCD:p.P331fs classified as conflicting interpretation of pathogenicity. The OGG1 gene has the highest Varelect score matching with BC disorders (236,97).

The second variant GCGR: p.G40S in the Glucagon Receptor, has been associated with type 2 diabetes in various white Europeans and with hypertension in both European whites and Australians [30-32]. Experimental evidence showed that hyper glucagonemia

in type 2 diabetes promotes colon cancer progression via GCGR-mediated regulation of AMPK and MAPK pathways [33]. It is known that women with diabetes mellitus are at higher risk of BC-specific and all-cause mortality after initial BC diagnosis [34].

For the third variant, FTCD:p.P331fs, it is associated with the mild phenotype of the Glutamate form aminotransferase deficiency, an autosomal recessive disorder and the second most common inborn error of folate metabolism. There is a conflicting epidemiological evidence on the role of folate in BC risk. A recent metanalysis review has shown that BC does not appear to be associated with folate intake, and this did not vary by menopausal status or hormonal receptor status [35].

**Screening for common at risk variants out BRCA genes:** Investigation of common at risk variants could contribute to estimate and refine each individual risk and help to identify the highest risk patient. So, among a published list of 182 risk associated SNPs that have displayed genome-wide significant associations with BC, we have extracted those present in each of our patients [12,13].

For the patient F1.1, we found only one SNP rs11374964, however for F2.2, we found 6 SNPs (rs2992756, rs4971059, rs4245739, rs6964587, rs11374964, rs2236007) (Table S3). These six SNPs added to the four variants that are present in BRCA genes could contribute together to increase the individual risk for developing BC.

### Sanger confirmation and validation

The BRCA2 c.3847\_3848delGT frame-shift mutation was confirmed by Sanger sequencing and co-segregation analysis was performed in the two other affected sisters.

We confirmed that the second variant in BRCA2 p.Lys3326Ter, was present in the index case and also in her BC affected sister (F2.4) but absent in the healthy sister (F2.3) and healthy brother (F2.6) and also absent in the affected colorectal cancer sister (F2.1) and in the affected testicular cancer brother (F2.5). Thus, confirming that this variant segregate only with the BC phenotype.

These two BRCA2 variants are also absent in other 40 BC affected Tunisian patients suggesting that are rare or are may not be founder mutations in Tunisia.

For the OGG1 variant, co-segregation analysis was performed. It was found at a heterozygous state in the two BC affected patients (F2-2 and F2-4) and in one clinically healthy sister F2-3 and absent in the 3 remaining family members. F2-3 has 68 years old, she reached menopause at the age of 48 years old. Co-segregation of the two variants in BRCA2 and OGG1 was observed only in the two BC affected cases, suggesting an additive risk.

## DISCUSSION

The identification of mutations responsible for BC through clinical genetic testing enables patients to benefit from early screening and prevention strategies, some of which provide generally survival benefit. Using WES allowed the identification of all coding rare and common variants that could be linked to BC predisposition.

According to the international guideline for BRCA testing, female members in F1 family should undertake BRCA test because of the positive family history and the young age at onset for the third sister F1.3 (31 years old) (Table 1). Results showed a highly penetrant variant BRCA2:c.3847\_3848delGT. This mutation described in BC males and associated with the young form of prostate cancer and colorectal cancer among Finns [36]. It is found in 5% of BRCA2 positive in Danish families and is one of the four founder mutations in BRCA2 in Norway but it has never been described among Arab or African populations. It is also present among Japanese patients and other Asian populations but it is rare elsewhere [37-39]. The index case has, in addition, the variant, APC:Ile1307Lys, known as a risk factor for breast, lung, urologic, pancreatic, and skin cancers and has been associated with an increased risk of colorectal cancer among Ashkenazi Jewish, Croatian, and Egyptian patients [22-26]. This protein can modulate the BER pathway through an interaction with the DNA polymerase  $\beta$  (Pol- $\beta$ ) and the flap endonuclease 1 (Fen-1) and consequently might play an important role in carcinogenesis and chemotherapy by determining whether cells with DNA damage survive or undergo apoptosis [40-43]. Furthermore, we have identified 3 other conflicting pathogen variants in three different genes, SPTA1, GCH1, FREM1, with unknown role in the tumorigenesis process neither a relationship with the BC disorders.

For the F2.2 patient and her affected sister, they have a late onset age ranging from 62 to 70 years with non-aggressive tumor according to histopathology test. They responded well to treatment without signs of recidivism. We found a rare pathogen variant, OGG1:R46Q, in the two affected sisters and also in the clinically healthy sister who is currently 68 years old. This variant has been described as a risk allele for the Human clear cell carcinoma of kidney that impairs the enzymatic activity of the OGG1 DNA glycosylase observed in a patient with a familial form of small intestinal neuroendocrine tumors and also in a putative clinically healthy carrier member [44,45]. In addition, significant associations between other OGG1 germ line variants and BC risk have been shown by meta-analysis and experimental data. For some missense variants in OGG1, the risk increases by 14-fold and reach 18-fold in BC patients compared with controls [46]. Also, some common regulatory variants in OGG1 and other DNA glycosidase are classified as potential cancer risk modifiers for BRCA mutations carriers because they exert a synergetic effect with BRCA mutations on DNA damage and telomere shortening [7,47].

Also, we have identified a rare non sense variant in BRCA2:p.K3326X, combined with 4 common regulatory functional variants in BRCA1 gene (rs16940, rs3092994, rs1060915, rs3765640) predicted to have a functional role for cis-regulation expression and also 9 other common at risk SNPs: rs144848, rs1799966, rs1042522, , rs2992756, rs4971059, rs4245739, rs6964587, rs11374964, rs2236007 respectively in genes BRCA2, BRCA1, TP53, and loci 1p36.13, 1q22, 1q32.1, 7q21.2, 11q22.3, 14q13.3, reported to represent independently minor, but cumulatively significant, increased risk for BC [12,14,15,20] (Table S3).

For the variant K3326X, it has previously been found in linkage disequilibrium with the variant rs144848, BRCA2 p.N372H, in 32 patients sharing the same ancestor haplotype [48]. These two variants are present in our patient suggesting that they could be in linkage disequilibrium. A recent meta-analysis study reveals that the rs144848 H allele could be a low-penetrant risk factor enhancing carcinogenesis in BC [49]. The BRCA2:K3326X, which co-segregates in some cases with other deleterious BRCA2 mutations, is described as a low penetrant variant associated with a modestly high risk of BC [48,50]. This variant confers susceptibility to multi-organ cancers: ovarian, breast, larynx and bladder (Table S4) [51-53]. This is consistent with the family history of our patient that reveals that her father died of larynx cancer at 49 years old. Unfortunately, we could not verify if he was carrying this variation. Its functional effects have been reported in different studies. It is classified pathogenic class 5 according to the *in vitro* splicing assays [54]. Indeed, the BRCA2 COOH terminus interacts with Rad51. Hence, homozygous germ-line deletion of BRCA2 exon 27 disrupts homologous recombination-mediated DNA repair and results in hypersensitivity to ionizing radiation and rapid senescence [55,56]. A recent study has shown that the K3326X acts as a trans-eQTL involved in DNA repair pathway. In addition, it exhibits statistically significant association with expression of TRPC6 gene and 4q21 locus [57]. The 4q21 has been recently identified as a novel BC susceptibility locus associated with differential allelic expression [58]. This locus has been identified among the most frequent candidate loci with at high risk haplotype (haplotype frequency >5%) in the general Tunisian [59]. In our study, we support the role of rare functional variant located in OGG1 and APC as BC factor risk. The "rare variant hypothesis" for susceptibility to common diseases propose that a significant proportion of the inherited component might be due to the addition of the effects of a series of low frequency and independently acting variants from a variety of genes, each conferring a moderate but detectable increase in the relative risk [60]. Accumulation of rare genetic variants in DNA repair genes will increase the deleterious effect of low or high penetrant mutations in BRCA genes by their ability to weaken response of the DNA repair system to oxidative damage. Actually, most of them are often referred as unclassified variants with uncertain clinical significance, thus creating a serious challenge to genetic testing. Familial segregation analysis, functional studies and *in vitro* assays could help to better assess analytical and clinical interpretation of these variants [61,62].

Our study suggest that the presence of only one high penetrant variant in BRCA2 gene is correlated to the early age at onset of the BC disease in the F1 family. However, cumulative of many low penetrant variants in BRCA genes combined to rare functional variant in OGG1 gene, are associated to the late age at onset of the BC disease in the F2 family.

Functional rare variations in DNA repair genes and common

variants in BRCA genes should be investigated in future large studies to understand their potential role in Tunisian population.

## CONCLUSION

WES has been successful in identifying rare coding variants involved in BC etiology. There is strong evidence that rare variants in DNA repair gene have an important role in BC genetic background. These variants although individually rare, are collectively frequent, and even though their effect size are greater than those observed for common variants. Most large studies should be considered to confirm the role and the interaction between these rare variants in BC genetic etiology.

## ACKNOWLEDGEMENT

The authors are very grateful to the patients and their families whose participation made this work possible.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

## CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## FUNDING

This work was supported by the Tunisian Ministry of Health (PEC-4-TUN), the Tunisian Ministry of Higher Education and Scientific Research (LR11IPT05 and LR16IPT05) and by the E.C. Grant Agreement No 295097 for FP7 project GM-NCD-Inco. MBR is recipient of a Mobidoc Post-Doc Fellowship under the Programme d'Appui au Système de recherche et d'Innovation (PASRI-Europe Aid). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Written informed consents were obtained from all participants. Ethical approval according to the Declaration of Helsinki Principles was obtained from the biomedical ethics committee of Institut Pasteur de Tunis (2017/16/E/hospital a-m/V1).

## AUTHOR'S CONTRIBUTION

Mariem Ben Rekaya (MBR), Yosr Hamdi (YH), Houda El Benna (HEB), Nessrine Mejri (NM), Olfa Jaidane(OJ), Jihene Ayari(JA), Sonia Ben Nasr (SBN), Hamza Dallali(HD), Olfa Messaoud (OM), Rym Meddeb(RM), Najah Mighri (NMI), Maroua Boujemaa(MB), Abderazek Haddaoui(AH), Ridha Mrad(RMD), Hamouda Boussen(HB), Sonia Abdelhak(SA), Soumaya Labidi (SL).

Study conception and design: MBR, HB, SA and SL; Data acquisition: MBR, YH, HEB, SL, NM, OJ, JA, SBN, NMI, MB; Analysis and interpretation of molecular data: MBR, YH; Analysis and interpretation of the patient clinic-pathological data: OJ, HEB, SL, JA SBN, RM, and NM; Bioinformatic analysis: HD, MBR, YH. Contribution to the interpretation of the results: HB, SA, SL, NJ,

and YH Technical experiment: MBR, OM, YH. Drafting of the full article: MBR; Editing and critical revision of the manuscript: SA, YH, SL, NM, OJ, JA, SBN, RM, RMD, AH, HB, and OM. Submission: MBR All authors read and approved the final manuscript.

## REFERENCES

1. Chouchane L, Boussen, H, Sastry KS. Breast cancer in Arab populations: molecular characteristics and disease management implications. *Lancet Oncol.* 2013;14(10):e417-424.
2. Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips KA, Mooij TM, Roos-Blom MJ, et al. Risks of breast, ovarian, and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. *JAMA.* 2017;317(23):2402-2416.
3. Grindedal EM, Heramb C, Karsrud I, Ariansen SL, Maehle L, Undlien DE, et al. Current guidelines for BRCA testing of breast cancer patients are insufficient to detect all mutation carriers. *BMC Cancer.* 2017;17(1):438.
4. McCarthy AM. Persistent underutilization of BRCA1/2 testing suggest the need for new approaches to genetic testing delivery. *J Natl Cancer Inst.* 2019;111(8):751-753.
5. Corsini C, Henouda S, Nejima DB, Bertet H, Toledano A, Boussen H, et al. Early onset breast cancer: differences in risk factors, tumor phenotype, and genotype between North African and South European women. *Breast Cancer Res Treat.* 2017;166(2):631-639.
6. Mavaddat N, Antoniou AC, Easton DF, Garcia-Closas M. Genetic susceptibility to breast cancer. *Mol Oncol.* 2010;4(3):174-191.
7. Benitez-Buelga C, Vaclava T, Ferreira S, Urioste M, Inglada-Perez L, Soberon N, et al. Molecular insights into the OGG1 gene, a cancer risk modifier in BRCA1 and BRCA2 mutations carriers. *Oncotarget.* 2016;7(18):25815-25825.
8. Osorio A, Milne RL, Kuchenbaecker K, Vaclava T, Pita G, Alonso R, et al. DNA glycosylases involved in base excision repair may be associated with cancer risk in BRCA1 and BRCA2 mutation carriers. *PLoS Genet.* 2014;10(4):e1004256.
9. Antoniou AC, Easton DF. Polygenic inheritance of breast cancer: Implications for design of association studies. *Genet Epidemiol.* 2003;25(3):190-202.
10. Mavaddat N, Pharoah PD, Michailidou K, Tyrer J, Brook MN, Bolla MK, et al. Prediction of breast cancer risk based on profiling with common genetic variants. *J Natl Cancer Inst.* 2015;107(5).
11. Pharoah PD, Antoniou A, Bobrow M, Zimmern RL, Easton DF, Ponder BA. Polygenic susceptibility to breast cancer and implications for prevention. *Nat Genet.* 2002;31(1):33-36.
12. Maxwell KN, Nathanson, KL. Common breast cancer risk variants in the post-COGS era: a comprehensive review. *Breast Cancer Res.* 2013;15(6):212.
13. Lilyquist J, Ruddy KJ, Vachon CM, Couch FJ. Common Genetic Variation and Breast Cancer Risk - Past, present, and future. *Cancer Epidemiol Biomarkers Prev.* 2018;27(4):380-394.
14. Michailidou K, Lindstrom S, Dennis J, Beesley J, Hui S, Kar S, et al. Association analysis identifies 65 new breast cancer risk loci. *Nature.* 2017;551(7678): 92-94.
15. Milne RL, Kuchenbaecker KB, Michailidou K, Beesley J, Kar S, Lindstrom S, et al. Identification of ten variants associated with risk of estrogen-receptor-negative breast cancer. *Nat Genet.* 2017;49(12):1767-1778.



16. Stafford JL, Dyson G, Levin NK, Chaudhry S, Rosati R, Kalpage H, et al. Reanalysis of BRCA1/2 negative high risk ovarian cancer patients reveals novel germline risk loci and insights into missing heritability. *PLoS One*. 2017;12(6):e0178450.
17. Banerjee T, Brosh RM. RECQL: a new breast cancer susceptibility gene. *Cell Cycle*. 2015;14(22): 3540-3543.
18. Nguyen-Dumont T, Teo ZL, Hammet F, Roberge A, Mahmoodi M, Tsimiklis H, et al. Is RNASEL:p.Glu265 a modifier of early-onset breast cancer risk for carriers of high-risk mutations? *BMC Cancer*. 2018;18(1):165.
19. Stelzer G, Plaschkes I, Oz-Levi D, Alkelai A, Olender T, Zimmerman S, et al. VarElect: the phenotype-based variation prioritizer of the GeneCards Suite. *BMC Genomics*. 2016;17:444.
20. Johnson N, Fletcher O, Palles C, Rudd, M, Webb E, Sellick G, et al. Counting potentially functional variants in BRCA1, BRCA2 and ATM predicts breast cancer susceptibility. *Hum Mol Genet*. 2007;16(9):1051-1057.
21. Woodage T, King SM, Wacholder S, Hartge P, Struwing JP, McAdams M, et al. The APC11307K allele and cancer risk in a community-based study of Ashkenazi Jews. *Nat Genet*. 1998;20(1):62-65.
22. Leshno A, Shapira S, Liberman E, Kraus S, Srur M, Harlap-Gat A, et al. The APC I1307K allele conveys a significant increased risk for cancer. *Int J Cancer*. 2016;138(6):1361-1367.
23. Redston M, Nathanson KL, Yuan ZQ, Neuhausen SL, Satagopan J, Wong N, et al. The APC11307K allele and breast cancer risk. *Nat Genet*. 1998; 20(1):13-14.
24. Abdel-Malak C, Darwish H, Elsaid A, El-Tarapely F, Elshazli R. Association of APC I1307K and E1317Q polymorphisms with colorectal cancer among Egyptian subjects. *Fam Cancer*. 2016;15(1): 49-56.
25. Kapitanovic S, Cacev T, Radosevic S, Spaventi S, Spaventi R, Pavelic K. APC gene loss of heterozygosity, mutations, E1317Q, and I1307K germ-line variants in sporadic colon cancer in Croatia. *Exp Mol Pathol*. 2004; 77(3):193-200.
26. Liang J, Lin C, Hu F, Wang F, Zhu L, Yao X, et al. APC polymorphisms and the risk of colorectal neoplasia: a HuGE review and meta-analysis. *Am J Epidemiol*. 2013;177(11):1169-1179.
27. Alloisio N, Wilmotte R, Morle L, Baklouti F, Marechal J, Ducluzeau M, et al. Spectrin Jendouba: an alpha II/31 spectrin variant that is associated with elliptocytosis and carries a mutation distant from the dimer self-association site. *Blood*. 1992;80(3):809-815.
28. Niss O, Chonat S, Dagaonkar N, Almansoori MO, Kerr K, Rogers ZR, et al. Genotype-phenotype correlations in hereditary elliptocytosis and hereditary pyropoikilocytosis. *Blood Cells Mol Dis*. 2016;61:4-9.
29. Mencacci NE, Isaias IU, Reich MM, Ganos C, Plagnol V, Polke JM, et al. Parkinson's disease in GTP cyclohydrolase 1 mutation carriers. *Brain*. 2014;137:2480-2492.
30. Hager J, Hansen L, Vaisse C, Vionnet N, Philippi A, Poller W, et al. A missense mutation in the glucagon receptor gene is associated with non-insulin-dependent diabetes mellitus. *Nat Genet*. 1995;9(3):299-304.
31. Brand E, Bankir L, Plouin PF, Soubrier F. Glucagon receptor gene mutation (Gly40Ser) in human essential hypertension: the PEGASE study. *Hypertension*. 1999;34(1):15-17.
32. Strazzullo P, Iacone R, Siani A, Barba G, Russo O, Russo P, et al. Altered renal sodium handling and hypertension in men carrying the glucagon receptor gene (Gly40Ser) variant. *J Mol Med (Berl)*. 20017;9(10):574-580.
33. Yagi T, Kubota E, Koyama H, Tanaka T, Kataoka H, Imaeda K, et al. Glucagon promotes colon cancer cell growth via regulating AMPK and MAPK pathways. *Oncotarget*. 2018;9(12):10650-10664.
34. Zhou Y, Zhang X, Gu C, Xia J. Influence of diabetes mellitus on mortality in breast cancer patients. *ANZ J Surg*. 2015;85(12):972-978.
35. Tio M, Andrici J, Eslick GD. Folate intake and the risk of breast cancer: a systematic review and meta-analysis. *Breast Cancer Res Treat*. 2014;145(2):513-524.
36. Garre P, Martin L, Sanz J, Romero A, Tosar A, Bando I, et al. BRCA2 gene: a candidate for clinical testing in familial colorectal cancer type X. *Clin Genet*. 2015;87(6):582-587.
37. Thomassen M, Hansen TV, Borg A, Lianee HT, Wikman F, Pedersen IS, et al. BRCA1 and BRCA2 mutations in Danish families with hereditary breast and/or ovarian cancer. *Acta Oncol*. 2008; 47(4):772-777.
38. Moller P, Hagen AI, Apold J, Maehle L, Clark N, Fiane B, et al. Genetic epidemiology of BRCA mutations-family history detects less than 50% of the mutation carriers. *Eur J Cancer*. 2007; 43(11):1713-1717.
39. Sugano K, Nakamura S, Ando J, Takayama S, Kamata H, Sekiguchi I, et al. Cross-sectional analysis of germline BRCA1 and BRCA2 mutations in Japanese patients suspected to have hereditary breast/ovarian cancer. *Cancer Sci*. 2008;99(10):1967-1976.
40. Das D, Preet R, Mohapatra P, Satapathy SR, Siddharth S, Tamir T, et al. 5-Fluorouracil mediated anti-cancer activity in colon cancer cells is through the induction of Adenomatous Polyposis Coli: Implication of the long-patch base excision repair pathway. *DNA Repair (Amst)*. 2014;24:15-25.
41. Jaiswal AS, Narayan S. A novel function of Adenomatous Polyposis Coli (APC) in regulating DNA repair. *Cancer Lett*. 2008;271(2):272-280.
42. Narayan S, Jaiswal AS, Law BK, Kamal MA, Sharma AK, Hromas RA. Interaction between APC and Fen1 during breast carcinogenesis. *DNA Repair (Amst)*. 2016;41:54-62.
43. VanKlompbergen MK, Bedalov CO, Soto KF, Prosperi JR. APC selectively mediates response to chemotherapeutic agents in breast cancer. *BMC Cancer*. 2015;15:457.
44. Audebert M, Chevillard S, Levalois C, Gyapay G, Vieillefond A, Kljanienco J, et al. Alterations of the DNA repair gene OGG1 in human clear cell carcinomas of the kidney. *Cancer Res*. 2000; 60(17):4740-4744.
45. Dumanski JP, Rasi C, Bjorklund P, Davies H, Ali AS, Gronberg M, et al. A MUTYH germline mutation is associated with small intestinal neuroendocrine tumors. *Endocr Relat Cancer*. 2017; 24(8):427-443.
46. Ali K, Mahjabeen I, Sabir M, Mehmood H, Kayani MA. OGG1 Mutations and risk of female breast cancer: meta-analysis and experimental data. *Dis Markers*. 2015;690878.
47. Benitez-Buelga C, Baquero JM, Vaclova T, Fernandez V, Martin P, Inglada-Perez L, et al. Genetic variation in the NEIL2 DNA glycosylase gene is associated with oxidative DNA damage in BRCA2 mutation carriers. *Oncotarget*. 2017;8(70):114626-114636.
48. Mazoyer S, Dunning AM, Serova O, Dearden J, Puget N, Healey CS, et al. A polymorphic stop codon in BRCA2. *Nat Genet*. 1996;14(3):253-254.
49. Li Q, Guan R, Qiao Y, Liu C, He N, Zhang X, et al. Association between the BRCA2 rs144848 polymorphism and cancer susceptibility: a meta-analysis. *Oncotarget*. 2017;8(24):39818-39832.
50. Wu K, Hinson SR, Ohashi A, Farrugia D, Wendt P, Tavtigian SV, et al. Functional evaluation and cancer risk assessment of BRCA2 unclassified variants. *Cancer Res*. 2005;65(2):417-426.

51. Delahaye-Sourdeix M, Anantharaman D, Timofeeva MN, Gaborieau V, Chabrier A, Vallee MP, et al. A rare truncating BRCA2 variant and genetic susceptibility to upper aerodigestive tract cancer. *J Natl Cancer Inst.* 2015;107(5).
52. Ge Y, Wang Y, Shao W, Jin J, Du M, Ma G, et al. Rare variants in BRCA2 and CHEK2 are associated with the risk of urinary tract cancers. *Sci Rep.* 2016;6:33542.
53. Meeks HD, Song H, Michailidou K, Bolla MK, Dennis J, Wang Q, et al. BRCA2 Polymorphic Stop Codon K3326X and the Risk of Breast, Prostate, and Ovarian Cancers. *J Natl Cancer Inst.* 2016;108(2).
54. De Garibay GR, Acedo A, Garcia-Casado Z, Gutierrez-Enriquez S, Tosar A, Romero A, et al. Capillary electrophoresis analysis of conventional splicing assays: IARC analytical and clinical classification of 31 BRCA2 genetic variants. *Hum Mutat.* 2014;35(1):53-57.
55. Atanassov BS, Barrett JC, Davis BJ. Homozygous germ line mutation in exon 27 of murine Brca2 disrupts the Fancd2-Brca2 pathway in the homologous recombination-mediated DNA interstrand cross-links' repair but does not affect meiosis. *Genes Chromosomes Cancer.* 2005;44(4):429-437.
56. McAllister KA, Bennett LM, Houle CD, Ward T, Malphurs J, Collins NK, et al. Cancer susceptibility of mice with a homozygous deletion in the COOH-terminal domain of the Brca2 gene. *Cancer Res.* 2002;62(4):990-994.
57. Quiroz-Zarate A, Harshfield BJ, Hu R, Knoblauch N, Beck AH, Hankinson S, et al. Expression Quantitative Trait loci (QTL) in tumor adjacent normal breast tissue and breast tumor tissue. *PLoS One.* 2017;12(2):e0170181.
58. Hamdi Y, Soucy P, Adoue V, Michailidou K, Canisius S, Lemacon A, et al. Association of breast cancer risk with genetic variants showing differential allelic expression: Identification of a novel breast cancer susceptibility locus at 4q21. *Oncotarget.* 2016;7(49):80140-80163.
59. Hamdi Y, Ben Rekaya M, Jingxuan S, Nagara M, Messaoud O, Benammar Elgaied A, et al. A genome wide SNP genotyping study in the Tunisian population: specific reporting on a subset of common breast cancer risk loci. *BMC Cancer.* 2018;18(1):1295.
60. Bodmer W, Tomlinson I. Rare genetic variants and the risk of cancer. *Curr Opin Genet Dev.* 2010;20(3):262-267.
61. Garrett LT, Hickman N, Jacobson A, Bennett RL, Amendola LM, Rosenthal EA, et al. Family studies for classification of variants of uncertain classification: current laboratory clinical practice and a new web-based educational tool. *J Genet Couns.* 2016;25(6):1146-1156.
62. Moghadasi S, Meeks HD, Vreeswijk MP, Janssen LA, Borg A, Ehrencrona H, et al. The BRCA1 c. 5096G>A p.Arg1699Gln (R1699Q) intermediate risk variant: breast and ovarian cancer risk estimation and recommendations for clinical management from the ENIGMA consortium. *J Med Genet.* 2018;55(1):15-20.