Whole exome sequencing reveals a combination of rare high and low penetrance variants that correlates with familial breast cancer relative risk

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Research

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Abstract

Background: Genetic risk factors of breast cancer are very heterogeneous and complex. They vary according to the familial relative risk, the age of cancer diagnosis of the index case and the age of the affected relatives.

Objectives: We aimed to investigate and identify simultaneously all rare pathogenic and common variants in unrelated BC cases with different relative risk ratios for breast cancer and evaluate the contribution of these variants in genetic susceptibility to breast cancer.

Patients and 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 (in terms of different numbers of affected first-degree relatives and young age onset) have been selected for whole exome sequencing. The first family is composed of three sisters F1.1, F1.2 and F1.3 affected at 46, 50, and 32 years old, respectively. The second has only two breast cancer cases, F2.2 and F2.4, affected at late age 61 and 70 years old, respectively, in addition to other 5 members affected by different kinds of cancer. Selected high risk variants were confirmed and segregation analysis was performed using Sanger sequencing.

Results and discussion: For F1.1 case, we identified a pathogenic frame-shift loss of function variant in *BRCA2* p.Val1283Lysfs. For F2.2 we identified a pathogenic rare variant in *OGG1*, p.Arg46Gln that co-segregates with a rare non sense variant in *BRCA2* p.K3326X, only in the breast cancer affected cases. Moreover, F2.2 patient has 9 other common low penetrant variants in different loci known to represent independently minor, but cumulatively significant, increased risk for breast cancer.

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

Background

Cancer is one of the leading causes of death world-wide. There were 6.7 million new cancer cases and 3.5 million deaths among females worldwide in 2012. Among them, 56% of cases and 64% of deaths were in under-developed countries. These numbers are expected to increase to 9.9 million cases and 5.5 million deaths among females annually by 2030 as a result of the lifestyle changes and aging of the populations. [1].

Breast cancer (BC) is the most frequently diagnosed cancer worldwide especially in economically developed countries. In Tunisia, BC is a major 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 [2].

Both non-genetic and genetic factors are involved in the etiology of breast cancer. About 15% of cases exhibit a family history of the disease which represents the strongest risk factor for mutation carriers. Its risk varies according to the mutation location and the genes involved. Therefore, genetic counseling should incorporate both family history profiles and mutation location [3]. A measure of this familial clustering is the familial relative risk (FRR), defined as the ratio of the risk of disease for a relative of an affected individual to that for the general population. Current International guidelines for BRCA testing use (i) breast or ovarian family, (history, (ii) young age at diagnosis ≤ 36 years and (iii) triple-negative breast cancer as the most risk factors. However, the commonly used guidelines for testing were insufficient to detect all mutation carriers in the BC cohorts [4]. Indeed, a higher rate of both *BRCA1* and *BRCA2* mutations has been observed in affected patients from North Africa without 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].

The genetic variants associated with breast cancer risk can be classified as high-penetrant mutations that are rare in the population but associated with a very high risk (relative risk of carriers versus non carriers of 5 to >20); moderate penetrant variants associated with a moderate risk (5>relative risk >1.5) ; and low-penetrant polymorphisms which are common and associated with a small risk (relative risk <1.5) [6].

At the age of 80, cumulative cancer risk for *BRCA1* and *BRCA2* mutation carriers ranges from 69% to 72% for breast cancer development, and from 17% to 44% for ovarian cancer [3]. This variability is explained by other genetic modifiers and/or environmental factors [7]. Studies of the genetic variants influencing the risk of breast cancer in *BRCA1/2* mutation carriers have been conducted first by the Consortium of Investigators of Modifiers of *BRCA1* and *BRCA2* (CIMBA) and then by the Collaborative Oncological Gene–environment Study (COGS). More than one hundred SNPs have been so far identified that are associated with the risk of developing breast or ovarian cancer for *BRCA1* or *BRCA2* carriers [8-13]. More recent studies have shown that common variants in genes involved in DNA repair pathways especially Base Excision Repair (BER) have a synergistic functional effect increasing cancer risk susceptibility in *BRCA2* mutation carriers [14, 15]. 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 were DNA glycosylases especially, *OGG1*, *TDG* and *NEIL2*. Most of these SNPs are common and 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. Previous studies suggested that 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 [16-18]. It is estimated that 28% of familial breast cancer risk is explained by common breast cancer susceptibility loci. In some cases, SNP associations may be specific to some ethnicity or estrogen receptor [13]. The most recent and largest breast cancer Genome Wide Association study (GWAS) using the Illumina OncoArray BeadChip has identified a total of 172 risk-associated SNPs that account for an estimated ~18% of familial relative risk [19-21].

As focusing on common non coding variants, GWAS studies have limited the capacity to identify interactions between *BRCA1* and *BRCA2* mutations and rare coding modifiers variants [22]. A portion of missing heritability in familial breast cancer is likely represented by rare functional coding variants in genes not currently present on available panels [23]. These rare coding variants could be identified using Whole Exome Sequencing (WES) or Multi Genes High Throughput Sequencing.

Recently, WES technology has been demonstrated to be efficient in the discovery of novel breast cancer predisposition genes such as those that encode proteins involved in the DNA damage response or DNA repair [24] and has helped to determine the frequency of causal germline mutations [23, 25] and to identify novel possible genetic modifiers of risk for early-onset breast cancer predisposition in carriers of high-risk mutations [26].

In the present study, we used WES to explore potential rare pathogenic variants for two cases having different relative risk ratios for breast cancer and evaluate the contribution of these variants in the genetic susceptibility to the disease.

Patients And Methods

1. 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 breast cancer unrelated affected patients were recruited from the Oncology Department of Abderrahman Mami Hospital of Ariana or from the surgical oncology department of Salah Azaiez Institute, or the Oncology department of Military hospital of Tunis.

Clinical and epidemiological characteristics of the available Breast cancer affected members are summarized in Table 1. The first patient, F1.1, diagnosed at the age of 46 years, has two affected sisters (F1.1 and F1.3) diagnosed respectively at the age of 50 and 32 years. The second patient, F2.2, diagnosed at 61 years, has a sister, F2.4, affected by breast cancer at 71 years, another sister, F2.1, affected by colorectal cancer and diagnosed at 66 years, a third sister affected by cervical cancer, one brother ,F2.5, affected by testicular cancer diagnosed at 60 years and the father who died of Larynx cancer at age of 49 in addition to one related individual who died of colorectal cancer.

2. Exome sequencing

Whole-exome sequencing was performed for patients F1.1 and F2.2. Exome was captured from genomic DNA using Agilent SureSelect 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 >20X.

3. Exome sequencing data analysis

The results were analyzed using the VarAft software version 1.6, (http://varaft.eu/index.php). For exome 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) ≥1% according to 4 databases (1000 Genomes Project, Exome Variant Server (EVS), Exome Aggregation Consortium (ExAC) and a local database encompassing 48 exomes of Tunisian individuals with no personal nor familial breast cancer history). We also excluded variants with low sequencing quality. A number of online tools were used to predict the functional impact and pathogenicity of the missense variants such as Mutation Taster, PolyPhen, SIFT. Of the variants that met these criteria, we selected all rare coding variants (frequency <0.01) described at least once as pathogenic in ClinVar and located in a gene that matched with breast cancer disorders according to the VarElect prioritization tool (http://varelect.genecards.org) [27]. We also extracted common at risk variants previously reported to contribute in increasing breast cancer risk.

4. Variant validations and co-segregation

Exons with a bad coverage (i.e. Exon 5 in *BRCA2* and Exon 13 in *BRCA1)* as well as exons 11 and 27 in *BRCA2* and exon1 in *OGG1* gene, which contain potential at risk variants, were amplified and sequenced by Sanger sequencing using the primers listed in (Supplementary Table 1) in order to confirm the variations and to analyze co-segregation for available related affected and healthy individuals.

Results

1. Exome Analysis and interpretation strategies

1.1 Screening for variants in BRCA genes

All heterozygous and homozygous variants in *BRCA1* and *BRCA2* genes, that 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 (Fig1).

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 rs80359405 and the frequent variant rs799905 predicted putative functional according to regulomdb software (Score=2b).

However for the patient F2.2, she 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 RegulomeDB software (Score=1F). In addition, she has the rare non sense coding variant rs11571833, c.9976A>T; 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 breast cancer variants and could together generate a polygenic risk score (PRS) [28] (Supplementary Table 2).

1.2 Screening for rare and pathogen variants

For the patient F1.1, we found one rare variant classified as pathogenic in ClinVar rs746229647/ rs80359405 in *BRCA2*. This gene has the highest Varelect Score 920,64 matching with the breast cancer disease. In addition, she has four rare missense variants at heterozygous state (rs1801155 APC c.3920T>A; p.I1307K; rs7418956 SPTA1:c.2373C>A, p.D791E; rs41298442 GCH1 c.671A>G p.K224R; rs184394424 FREM1 c.1493G>A, 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).

The variant, c.3846_3847del in *BRCA2*, was previously described as deleterious and associated with the hereditary cancer-predisposing syndrome. It is a frame-shift deletion c.3847_3848delGT (p.*Val1283Lysfs*) (BIC: 4075delGT), described to be causing breast cancer in males among Finns and associated with the young form of prostate cancer and colorectal cancer [29]. This mutation, classified as a breast cancer high penetrant variant, is found in 5% of *BRCA2* positive in Danish families [30] and is one of the four founder mutations in *BRCA2* gene in Norway [31] but it has never been described among African populations.

The *APC* gene has the highest Varelect score (670,03). The APC variant, c.3920T>A, is predicted as damaging by two *in silico* prediction softwares. It is described as a functional variant converting the DNA sequence to a homo-polymer region (A8) that is genetically unstable and prone to somatic mutation [32]. Previous studies have shown that it could predict the prevalence of breast, lung, urologic, pancreatic, and skin cancers [33, 34] and it has been associated with an increased risk of colorectal cancer among Ashkenazi Jewish, Croatian, and Egyptian patients [35-37].

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" [38, 39].

For the two variants, *GCH1* c.671A>G:p.K224R and *FREM1* c.1493G>A; 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 [40]. The second gene is associated with the autosomal dominant Trigonocephaly_2 phenotype with nonsyndromic metopic craniosynostosis.

For patient F2.2, we have identified two rare pathogenic variants in *OGG1* and *GCGR* genes, rs104893751 c.137G>A, p.R46Q and rs1801483 c.118G>A, p.G40S respectively and one variant in *FTCD* gene (rs35208133/rs398124234 c.990dupG, p.P331fs) classified as conflicting interpretation of pathogenicity.

The *OGG1* gene has the highest Vareclect score matching with breast cancer disorders (236,97). The variant R46Q has been previously described as a risk allele for the Human clear_cell_carcinoma_of_kidney that impairs the enzymatic activity of the OGG1 DNA glycosylase [41] and recently observed in an affected member by a familial form of small intestinal neuroendocrine tumors (SI-NETs) and also in a putative clinically healthy carrier member [42].

The second variant c.118G>A; p.G40S is present in the *GCGR* gene that encodes for the Glucagon Receptor. It has been associated with Type 2 diabetes in various white European populations [43] and with hypertension in both European whites and Australians [44, 45]. Interestingly, a recent study has provided experimental evidence that hyperglucagonemia in type 2 diabetes promotes colon cancer progression via GCGR-mediated regulation of AMPK and MAPK pathways [46]. It is known that women with diabetes mellitus are at higher risk of breast cancer-specific and all-cause mortality after initial breast cancer diagnosis [47]. So we hypothesize that this variant could have increased the risk of breast cancer in our case.

For the third variant, c.990dupG: p.P331fs on *FTCD* gene. Mutations in *FTCD* represent the molecular basis for the mild phenotype of the Glutamate form iminotransferase 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 breast cancer risk. A recent metanalysis review has shown that breast cancer does not appear to be associated with folate intake, and this did not vary by menopausal status or hormonal receptor status. In addition, folate blood levels also do not appear to be associated with breast cancer risk [48]

1.3 Screening for Common at risk Variants

Investigation of common at risk variants could contribute to estimate and refine each individual risk and help to identify the highest risk patient. For this, among a published list of 182 risk associated SNPs that have displayed genome-wide significant associations with breast cancer [13, 21], we have extracted those present in each of our patients.

For the patient F1.1, we found only one SNP rs11374964, however for the patient F2.2, we found 6 SNPs (rs2992756, rs4971059, rs4245739, rs6964587, rs11374964, rs2236007) (Supplementary Table 3). These six SNPs added to the four variants that are present in *BRCA* genes could contribute together to increase the individual risk for developing breast cancer.

2. 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 (F1.2 and F1.3). However, It was absent in 40 available affected with high family risk women having at least 3 affected related individuals.

We also confirmed that the second variant in *BRCA2* gene, c.9976A>T; 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 brother (F2.6) and also in the affected colorectal cancer sister (F2.1) and the affected testicular cancer brother (F2.5). Thus, confirming that this variant segregate only with the breast cancer phenotype.

For the *OGG1* variant, it was confirmed by Sanger sequencing and 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 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. Cosegregation 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 breast cancer through clinical genetic testing enables patients to benefit from early screening and prevention strategies, some of which provide generally survival benefit. Using next generation sequencing allowed the identification of all rare and common variants that could be linked to breast cancer 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 of WES showed a highly deleterious variant c.3847_3848delGT in *BRCA2* gene. This mutation has been described as founder and frequent in the Danish population [49]. It is also present among Japanese patients and other Asian populations but it is rare elsewhere [50]. The index case has, in addition, a variant, Ile1307Lys in *APC* gene, previously described as a risk factor for breast, lung, urologic, pancreatic, and skin cancers [33, 34] and has been associated with an increased risk of colorectal cancer among Ashkenazi Jewish, Croatian, and Egyptian patients [35-37]. This gene encodes a tumor suppressor protein that acts as an antagonist of the Wnt signaling pathway that regulates crucial aspects of cell fate determination, cell migration, cell polarity, neural patterning and organogenesis during embryonic development. This protein can modulate the BER pathway through an interaction with the DNA polymerase β (Pol-β) 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 [51, 52]. In breast tumors, the transcriptional silencing of the *APC* gene by promoter hypermethylation has been detected in up to 70% of inflammatory human breast tumors [53] and 7% of metaplastic breast carcinomas [54]. In addition, screening of *APC* and *Fen1* polymorphic and/or mutational variations could provide an important tool in the assessment of individual DNA repair capability and the risk for breast cancer development [51]. It is also suggested that APC selectively mediates response of chemotherapeutic agents in breast cancer. Since cisplatin and doxorubicin cause oxidative DNA damage, the chemotherapeutic resistance of the MMTV-PyMT/ApcMin+ mammary tumors could also be due to increased BER because mutant APC have a decreased DNA damage activity which enhances cell survival [55, 56]. 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 breast cancer 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, R46Q *OGG1,* in the two affected sisters and also in the clinically healthy sister who is currently 73 years. This variant has been previously described as a risk allele for the Human clear_cell_carcinoma_of_kidney that impairs the enzymatic activity of the OGG1 DNA glycosylase [41]. R46Q *OGG1* has been recently observed in a patient with a familial form of SI-NETs and also in a putative clinically healthy carrier member [42].

In addition, significant associations between other *OGG1* germline variants and breast cancer risk have been shown by meta-analysis and experimental data. For some missense variants in *OGG1*, the risk increases by 14-fold ($p < 0.01$) and reach 18-fold ($p < 0.004$) in breast cancer patients compared with controls [57]. Also, some common regulatory variants in *OGG1* and other DNA glycosidase like NEIL2 are classified as potential cancer risk modifiers for *BRCA1* and *BRCA2* mutations carriers because they exert a synergetic effect with BRCA mutations on DNA damage and telomere shortening [15, 58].

For this patient, we have identified a rare non sense low penetrant variant in *BRCA2* gene, 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 breast cancer [13, 19-21, 28].

For the variant K3326X, it has been previously found in linkage disequilibrium with the variant rs144848, *BRCA2* p.N372H, in 32 patients sharing the same ancestor haplotype [59]. These two variants are present in our patient suggesting that they could be in linkage disequilibrium. A recent metanalysis study reveals that the rs144848 H allele could be a low-penetrant risk factor enhancing carcinogenesis in breast cancer [60]. The 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 breast cancer [59, 61]. This variant confers susceptibility to multi-organ cancers: ovarian, breast, larynx and bladder (Supplementary Table 4) [62-64]. 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. Functional effects of the rs11571833 have been reported in different studies, e.g. it is classified pathogenic class 5 according to the i*n vitro* splicing assays [65]. 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. [66, 67]. 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 [68]. The 4q21 has been recently identified as a novel breast cancer susceptibility locus associated with differential allelic expression [69]. This locus has been identified among the most frequent candidate loci with at high risk haplotype (haplotype frequency > 5%), through a genome wide haplotype study in the general Tunisian population [70]. Little is known about the involvement of common variants and their association with breast cancer risk in Tunisia.

The "rare variant hypothesis" for susceptibility to common diseases postulates 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 [71]. Accumulation of rare genetic variants in DNA repair genes probably increases the deleterious effect of mutations in the high penetrant *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 [72, 73].

Our results join those of previous studies and support the suggestion of screening variants in DNA repair genes for *BRCA2* carriers of low or high penetrant variations. This investigation allows the assessment of the individual DNA repair capacity and help to refine the breast cancer risk. At risk individuals who are carriers of functional variants in DNA repair genes might lower their breast cancer risk by reducing exposure to environmental carcinogens [51].

Combination of rare pathogen variants in DNA repair genes with high or low penetrant variants in *BRCA* genes among Tunisian breast cancer cases might explain the relatively young onset age and the aggressive tumor types observed in some cases described in local epidemiological reports. Our previous studies have shown a relatively high rate of consanguinity [74, 75] that increases the frequency of monogenic diseases such autosomal recessive DNA repair disorders predisposing to cancer and raise the prevalence of healthy carriers [76, 77] ; (Ben Rekaya unpublished data).

In summary, using WES and segregation analysis, we have identified a low penetrant variant, K3326X in *BRCA2* gene, that co-segregates with a rare pathogenic variant, R46Q in *OGG1* gene, only in breast cancer affected cases. The OGG1 variation is a candidate risk factor predisposing to the disease. In addition, other rare variants such as the variant in *GCGR* gene should be investigated in future studies to understand their potential role. It is also recommended that the ten risk common variants found in *BRCA* genes and in others candidate loci be investigated through a large association study to better understand their (synergic) implication in the disease. For the F1 family, all affected members have benefited from a specific oncogenetic counseling. Further investigation should be considered to calculate its frequency in North African population.

Conclusion

WES has been successful in identifying rare coding variants involved in breast cancer etiology. There is a strong evidence that rare variants in DNA repair gene have an important role in breast cancer 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 breast cancer genetic etiology.

List Of Abbreviations

Base Excision Repair BER

BC Breast cancer

Consortium of Investigators of Modifiers of *BRCA1* and *BRCA2* CIMBA

Collaborative Oncological Gene–environment Study COGS.

DNA polymerase β Pol-β

Exome Aggregation Consortium ExAC

Exome Variant Server EVS

Flap Endonuclease 1 Fen-1

Familial Relative Risk FRR

Genome Wide Association study GWAS

Red Blood Cell RBC

Small Intestinal Neuroendocrine Tumors SI-NETs

Whole Exome Sequencing WES

Declarations

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).

Competing interests: The authors declare that they have no competing interests.

Authors' contributions

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Study conception and design: MBR, HB and SA; Data acquisition: MBR, YH, SL, NM, OJ and JA, SBN; Analysis and interpretation of molecular data: MBR, YH; Analysis and interpreted the patient clinic-pathological data: OJ, 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. Redaction of the full article: MBR; Involvement in the drafting of the manuscript: SA, YH, SL, NM, OJ, JA, SBN, RM. Critical revision of the article: RMD, AH, HB, and OM. Submission procedure: MBR All authors read and approved the final manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its additional files.

Consent for publication

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Tables

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

Family code	Affected cases code	Age at Diagnosis (years)	births number	Breastfeeding duration (months)	Menarche age (years)	Menopausal Age (years)	Hormonal contraception(duration)	Histological subtype	SBR grade	Tumor size (mm)	TNM Classification	Hor rece statu
F1	F1.1	46	1	>12	ND	ND	NO	Invasive Ductal carcinoma / Comedocarcinoma	I	ND	T2,N1,M0	RE
	F1.2	50	4	>12	11	50	3 years	Atypical ductal carcinoma with in situ component	III	30	T1,N0,M0	R
	F1.3	31	0 (not married)	No	12	No	No	Multifocal Infiltrating ductal carcinoma of the left breast	I	24,10,20	T2N0M0	RI
F2	F2.2	62	3	>12 months	13	52	No	Invasive Ductal carcinoma	III	6	11N-	E (100% (2
	F2.4	70	3	<2 months	10	42	No	Invasive Ductal carcinoma	III	10	9N-	ER+

SBR: Scarff-Bloom et Richardson, TNM: Tumor, Node, metastases classification, HER2: Humain epidermal growth factor receptor 2 ER : Estrogen Receptor ; PR : Progesterone Receptor ; HT : Hormonal Therapy ; ND: Not determined

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

	Chr	Ref	Gene Description	AAChange.refgene	esp6500siv2_all	1000g2015aug_all	avsnp144	SIFT
1	chr13	TG/-	BRCA2, DNA Repair Associated	BRCA2: NM_000059.3:exon11:c.3847_3848del:p.T1282fs	0.0004	NA	rs746229647/ rs80359405	NA
	chr5	T/A	APC, WNT Signaling Pathway Regulator	APC:NM_001127510:exon17:c.3920T>A:p.I1307K	0.0011	NA	rs1801155	T
	chr1	G/T	Spectrin Alpha, Erythrocytic 1	SPTA1:NM_003126:exon17:c.2373C>A:p.D791E	0.0155	0.0133786	rs7418956	T
	chr14	T/C	GTP Cyclohydrolase 1	GCH1:NM_001024024:exon6:c.671A>G:p.K224R	0.0002	0.000399361	rs41298442	T
	chr9	C/T	FRAS1 Related Extracellular Matrix 1	FREM1:NM_144966:exon10:c.1493G>A:p.R498Q	0.0007	0.000599042	rs184394424	D
2	chr3	G/A	8-Oxoguanine DNA Glycosylase	OGG1:NM_016829:exon1:c.137G>A:p.R46Q	0.0022	0.000599042	rs104893751	D
	chr17	G/A	Glucagon Receptor	GCGR:NM_000160:exon3:c.118G>A:p.G40S	0.0081	0.00419329	rs1801483	T
	chr21	-/C	Formimidoyltransferase Cyclodeaminase	FTCD:NM_206965:exon9:c.990dupG:p.P331fs	0.0034	NA	rs35208133/ rs398124234	NA

Variant in red are confirmed by Sanger sequencing

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	AAChange	1000genomes	ExA
F1.1	BRCA1	rs799917	G	A	het	exonic	Misence	NM_007300:exon10:c.2612C>T:p.P871L	0.54393	0.41
		rs368080376	AAAT	-	het	intronic	NA	NA	0.000798722	NA
		rs799916	T	G	het	intronic	NA	NA	0.502396	NA
		rs368252296/rs80308573	A	-	het	intronic	NA	NA	NA	NA
		rs799905	**G**	**C**	het	**intronic**	**NA**	**NA**	**0.545128**	**0.48**
		rs80358329	AGA	-	het	exonic	nonframeshift deletion	NM_007300:exon10:c.1846_1848del:p.616_616del	0.00119808	0.00
	BRCA2	rs543304	T	C	het	exonic	synSNV	BRCA2:NM_000059:exon11:c.3807T>C:p.V1269V	0.168131	0.18
		rs169547	T	C	het	exonic	Misence	BRCA2:NM_000059:exon14:c.7397T>C:p.V2466A	0.975839	0.99
		rs11571744	C	T	het	intronic	NA	NA	0.0161741	0.00
		rs206076	G	C	het	exonic	synSNV	BRCA2:NM_000059:exon11:c.6513G>C:p.V2171V	0.973642	0.99
		rs80359405/rs746229647	**TG**	**-**	**het**	**exonic**	**frameshift deletion**	**BRCA2:NM_000059:exon11:c.3846_3847del:p.T1282fs**	**NA**	**0.00**
		rs206075	A	G	het	exonic	synSNV	BRCA2:NM_000059:exon11:c.4563A>G:p.L1521L	0.974042	0.99
F2.2	BRCA1	rs273902772	A	-	het	intronic	NA	NA	0.334864	NA
		rs3765640	**A**	**G**	**hom**	**intronic**	**NA**	**NA**	**0.353634**	**NA**
		rs8176233	T	C	het	intronic	NA	NA	0.354633	NA
		rs799917	G	A	het	exonic	Misence	NM_007300:exon10:c.2612C>T:p.P871L	0.54393	0.41
		rs1060915	**A**	**G**	**het**	**exonic**	synSNV	**NM_007300:exon12:c.4308T>C:p.S1436S**	**0.336262**	**0.34**
		rs16941	T	C	het	exonic	Misence	NM_007300:exon10:c.3113A>G:p.E1038G	0.335663	0.34
		rs799905	**G**	**C**	**het**	**intronic**	**NA**	**NA**	**0.545128**	**0.48**
		rs1799949	**G**	**A**	**het**	**exonic**	synSNV	**NM_007300:exon10:c.2082C>T:p.S694S**	**0.336462**	**0.34**
		rs1799965	G	A	het	exonic;splicing	synSNV	NM_007300:exon8:c.591C>T:p.C197C	0.000399361	0.00
		rs273900734/rs8176212	G	C	het	intronic	NA	NA	0.353435	NA
		rs8176234	T	C	het	intronic	NA	NA	0.354633	NA
		rs16942 [a]	T	C	het	exonic	Misence	NM_007300:exon10:c.3548A>G:p.K1183R	0.352636	0.34
		rs3092994	C	T	het	intronic	NA	NA	0.342452	NA
		rs1799966[a]	T	C	het	exonic	Misence	NM_007300:exon16:c.4900A>G:p.S1634G	0.355831	0.34
		rs80308573/rs368252296	A	-	het	intronic	NA	NA	NA	NA
		rs8176235	C	T	het	intronic	NA	NA	0.306909	NA
		rs16940[a]	**A**	**G**	**het**	**exonic**	synSNV	**NM_007300:exon10:c.2311T>C:p.L771L**	**0.335264**	**0.34**
	BRCA2	rs543304	T	C	het	exonic	synSNV	BRCA2:NM_000059:exon11:c.3807T>C:p.V1269V	0.168131	0.18
		rs206076	G	C	hom	exonic	synSNV	BRCA2:NM_000059:exon11:c.6513G>C:p.V2171V	0.973642	0.99
		rs169547	T	C	hom	exonic	Misence	BRCA2:NM_000059:exon14:c.7397T>C:p.V2466A	0.975839	0.99
		rs11571744	C	T	het	intronic	NA	NA	0.0161741	0.00
		rs11571833[a]	A	T	het	exonic	stopgain	BRCA2:NM_000059:exon27:c.9976A>T:p.K3326X	0.00439297	0.00
		rs206073	G	A	hom	intronic	NA	NA	0.974042	NA
		rs144549870	TATCT	-	het	intronic	NA	NA	0.0157748	NA
		rs144848[a]	A	C	het	exonic	Misence	BRCA2:NM_000059:exon10:c.1114A>C:p.N372H	0.249401	0.27
		rs206075	A	G	hom	exonic	synSNV	BRCA2:NM_000059:exon11:c.4563A>G:p.L1521L	0.974042	0.99
		rs11571818	T	C	het	intronic	NA	NA	0.00439297	0.00
		rs206080	T	C	hom	intronic	NA	NA	0.974042	NA

Variant in red are confirmed by Sanger sequencing. [a]Variants previously reported to represent independently minor, but cumulatively significant, increased risk for breast cancer. Variants highlighted in bold present functional effect according to RegulomeDB data

Figures

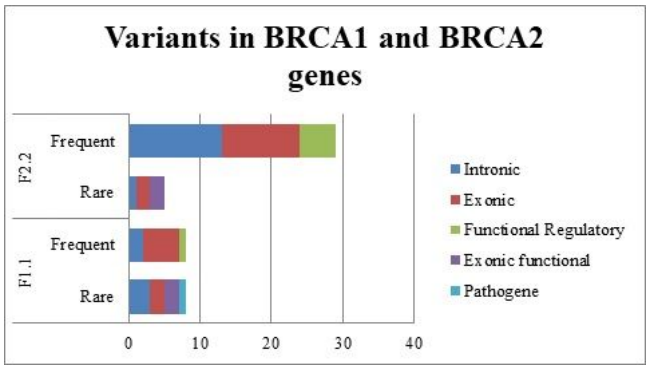

Figure 1

The structure of genetic profile of BRCA1 and BRCA2 genes in F1.1 and F2.2 patients

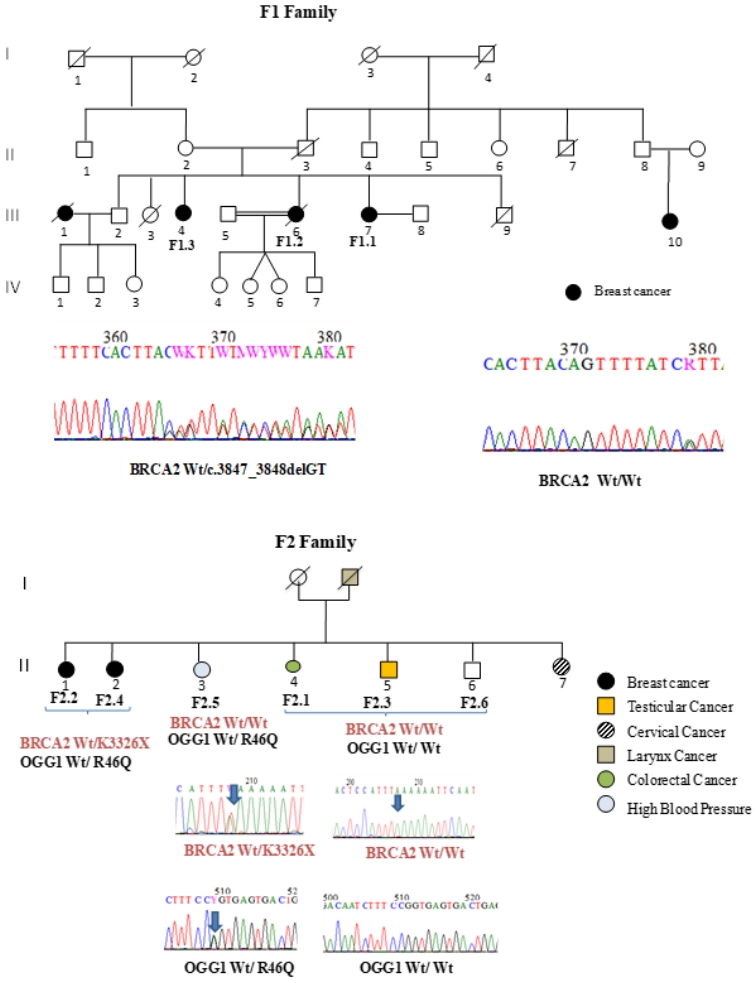

Figure 2

Pedigrees of the F1 and F2 families and DNA-sequence electropherograms for unaffected (wt) and affected family members.

Supplementary Files

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- supplementarytables1231.pdf