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Unveiling the Genomic Landscape: Architectural Insights into Triple-Negative Breast Cancer in Moroccan Patients Through Whole-Exome Sequencing

Meryem Fakhkhari[a], Sihame Lkhoyaali[b], Amina Gihbid[c,d], Maroua Boujemaa[e], Ikram Salih[a], Ahmed Rebai[f], Yosr Hamdi[e,g], Meriem Khyatti[d], Fouzia Radouani[h], Hassan Errihani[b], Khalid Sadki*[a]

[a]*Research Laboratory in Oral Biology and Biotechnology, Faculty of Dental Medicine, Mohammed V University in Rabat, Morocco*

[b]*Department of Medical Oncology, National Institute of Oncology, Rabat, Morocco*

[c]*Laboratory of Pathophysiology, Molecular Genetics and Biotechnology, Faculty of Sciences Ain Chock, Hassan II University, Casablanca, Morocco*

[d]*Laboratory of Viral Oncology, Institut Pasteur du Maroc, Casablanca, Morocco*

[e]*Laboratory of Biomedical Genomics and Oncogenetics, Institut Pasteur de Tunis, University of Tunis El Manar, Tunis, Tunisia*

[f]*Centre of Biotechnology of Sfax, University of Sfax, Sfax, Tunisia*

[g]*Laboratory of Human and Experimental Pathology, Institut Pasteur de Tunis, Tunis, Tunisia*

[h]*Research Department, Institut Pasteur du Maroc, Casablanca, Morocco*

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ABSTRACT

Background: Triple-negative breast cancer (TNBC) is a highly aggressive subtype of breast cancer characterized by the absence of hormone receptors and *HER2* expression, resulting in limited treatment options and poorer prognoses. This study investigates the genetic landscape of TNBC in Moroccan patients through high-throughput whole-exome sequencing (WES) to identify genetic alterations that could enhance diagnostic accuracy and inform targeted therapies.

Case Presentation: This study included 10 unrelated Moroccan female patients diagnosed with TNBC, recruited from the National Institute of Oncology in Rabat, Morocco. Clinical data, including tumor location, Scarff-Bloom-Richardson (SBR) grade, histological type, lymph node involvement, and Ki-67 index, were collected. Tumor grades varied from SBR grade II to IV, with some cases demonstrating metastasis to distant organs. The Ki-67 index ranged from 10% to 80%, indicating a range of tumor proliferative activity across the cohort.

Conclusion: This study provides insights into the genetic landscape of TNBC in Moroccan patients. It highlights novel genetic variants in *CTBP2*, *IGSF3*, *ZNF334*, *TPRG1*, and *NMNAT1*, suggesting their potential as biomarkers and therapeutic targets for TNBC. These findings emphasize the importance of investigating genetic alterations in underrepresented populations, which could help refine treatment strategies and predict treatment responses.

***Address for correspondence:**
Khalid Sadki,
Research Laboratory in Oral Biology and Biotechnology, Faculty of Dental Medicine, Mohammed V University in Rabat, Morocco
Email: ksadki1@yahoo.fr

INTRODUCTION

Breast cancer is the most prevalent malignancy worldwide and continues to be the primary cause of cancer-associated deaths in women. Recent statistics from *GLOBOCAN 2022* indicate that breast cancer is

the fourth deadliest cancer type overall, preceded only by lung, colorectal, and liver cancers. With approximately 2.3 million new diagnoses and over 666 000 fatalities reported in 2022, the disease represents a major global health challenge (Figure 1).[1-3]

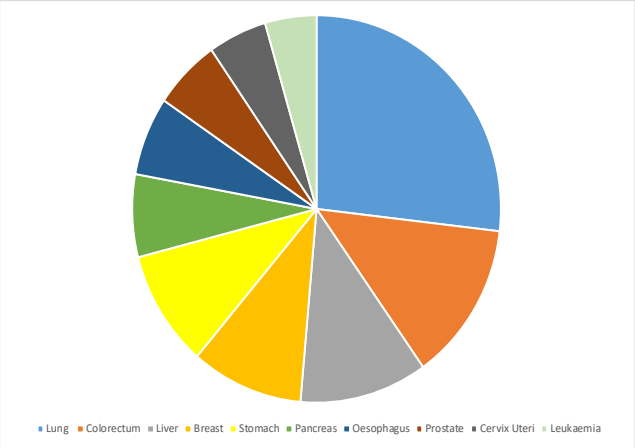

Figure 1. Global Estimate of Cancer-Related Deaths in Both Sexes in 2022[3]

Alarmingly, 2020 data revealed a disproportionate burden of breast cancer in low-resource settings, with developing regions accounting for over 50% of global cases and nearly 70% of mortality. This disparity highlights critical healthcare inequities in cancer management worldwide.[2] In North Africa, the incidence of breast cancer has been rising, with rates increasing from 24.3 to 43.6 cases per 100 000 individuals between 2000 and 2015.[4] In Morocco, according to the Casablanca Cancer Registry (2013–2017), breast cancer accounted for 22.5% of all cancer cases across both sexes. Among women specifically, breast cancer represented 38.1% of all cancer diagnoses. The age-adjusted breast cancer incidence among women reaches 45.6 cases annually per 100 000 women.[5]

According to the Global Cancer Observatory (https://gco.iarc.fr/today/en/dataviz/bars?types=0&mode=cancer&group_populations=1&sort_by=value0&key=crude_rate&populations=504&sexes=0&age_end=14), the crude incidence rate of breast cancer in Morocco for individuals aged 0–74 years across both sexes was 65.4 per 100 000 in 2022.

Furthermore, Africa exhibits a higher incidence of hormone receptor-negative and triple-negative breast cancers (TNBC)[6], with North Africa showing particularly high rates of these aggressive forms of breast cancer.[7,8] This trend highlights the urgent need for comprehensive research on TNBC, which is particularly deadly. It accounts for approximately 10-17% of all breast cancer cases worldwide, with a higher prevalence in North Africa. In Morocco, TNBC accounts for around 16.5% to 17.5% of breast cancer cases.[9,10]

The aggressive nature of TNBC poses significant challenges in terms of prognosis and treatment, primarily due to the absence of estrogen receptors, progesterone receptors, and *HER2* amplification, which limits treatment options and results in higher rates of recurrence and metastasis. In addition, TNBC tends to affect younger women and is associated with poorer survival outcomes, making it imperative to focus on targeted research in this area.[11-13]

The genomic landscape of TNBC is characterized by unique genetic alterations, which may serve as potential biomarkers and therapeutic targets. In this context, several relevant genes and variants associated with TNBC have been identified.[14,15] Currently, data regarding these associations in Moroccan and North African populations remain sparse.[10,16,17] This gap in knowledge emphasizes the importance of conducting studies that specifically address the genetic characteristics of TNBC within these populations.

The focus on whole-exome sequencing (WES) specifically for TNBC, as opposed to other forms of breast cancer, is paramount to unraveling its unique genetic underpinnings and finding more effective and tailored therapeutic strategies.[18]

Given this context, the primary objective of this case series is to investigate the genetic variations associated with TNBC in a Moroccan cohort through WES. By elucidating the genetic signatures of TNBC, this research aims to advance the understanding of this aggressive breast cancer subtype and contribute to the development of more effective, tailored therapeutic strategies for diverse populations.

CASE PRESENTATION

This study included 10 unrelated female patients diagnosed with TNBC, all recruited from the National Institute of Oncology in Rabat, Morocco, between June 2022 and June 2023. Informed consent was obtained from all participants, and the study protocol was approved by the Ethics Committee for Biomedical Research at the Faculty of Medicine and Pharmacy of Rabat (Approval No. N° C53/20). The clinical characteristics of the patients, including tumor location, Scarff-Bloom-Richardson (SBR) grade, lymph node involvement that tested for metastatic cancer cells, histological type, and Ki-67 index, were collected from their medical records. All patients had a confirmed diagnosis of TNBC, characterized by negative hormone receptor (estrogen and progesterone) and HER2 status, determined through immunohistochemistry. Detailed demographic, epidemiological, and clinical features

are provided in Table 1, which is intended as a descriptive summary.

Table 1. Distribution of Epidemiological and Clinicopathological Features of the Recruited Moroccan Triple-Negative Breast Cancer Patients

Characteristics	Cases (N=10)
Mean age	50.5
Consanguinity of parents	
Yes	0%
No	100%
Menopausal status	
Premenopausal	30%
Premenopausal	10%
Postmenopausal	60%
Family history of cancer	
Yes	50%
No	50%
Tumor location	
Right breast	50%
Left breast	50%
SBR grade	
Grade II	50%
Grade III	40%
Grade IV	10%
Lymph node involvement	
No lymph node invasion	70%
Lymph node invasion	30%
Histological type	
Invasive carcinoma of no special type	80%
Invasive secretory breast carcinoma	10%
Invasive florid metaplastic carcinoma	10 %
Ki-67 index	
Not reported	30%
Ki-67 index <30%	10%
Ki-67 index 30–60 %	40%
Ki-67 index 75–80 %	20%

SBR, Scarff-Bloom-Richardson (tumor grading system)

Case 1: A 63-year-old postmenopausal female with a family history of cancer presented with a right breast nodule, initially classified as cT2N0M0. Histopathological examination revealed an invasive carcinoma of no special type (NST), classified as Scarff-Bloom-Richardson (SBR) grade III, with no lymph node involvement. Hormone receptor status (estrogen receptor [ER]: 0%, progesterone receptor [PR]: 0%) and HER2 were negative (Score 0, HercepTest).

Case 2: A 41-year-old premenopausal female, with no family history of cancer, was evaluated for a right breast nodule, initially classified as cT1N0M0. Biopsy confirmed invasive NST, classified as SBR grade II, with no lymph node involvement. Hormone receptor status (ER: 0%, PR: 0%) and HER2 were negative (Score 0, HercepTest).

Case 3: A 49-year-old premenopausal female with a family history of breast cancer, with 2 of her sisters having been diagnosed with the disease (1 of whom had passed away), presented with a left breast nodule, initially classified as cT3N0M0. Histopathology confirmed NST, classified as SBR grade II, with no lymph node involvement. The patient's hormone receptor status (ER: 0%, PR: 0%) and HER2 were negative (Score 0, HercepTest).

Case 4: A 68-year-old postmenopausal female with no family history of cancer was evaluated for a left breast nodule, initially classified as cT2N0M0. Biopsy confirmed the diagnosis of invasive secretory breast carcinoma, categorized as SBR grade II, with no lymph node involvement. Hormone receptor status (ER: 0%, PR: 0%) and HER2 (Score 0, HercepTest Negative) were negative. The Ki-67 index was recorded at 10%.

Case 5: A 52-year-old postmenopausal female with a family history of cancer was referred for a right breast nodule evaluation, initially classified as cT2N1M0. Biopsy confirmed invasive florid-appearing metaplastic breast carcinoma, classified as SBR grade III, with no lymph node involvement. Hormone receptor status (ER: 0%, PR: 0%) and HER2 were negative (Score 0, HercepTest). The Ki-67 index was notably high at 75%.

Case 6: A 30-year-old premenopausal female with a family history of cancer presented with a left breast nodule, initially classified as cT1N0M0. Biopsy confirmed the diagnosis of NST, categorized as SBR grade II, with no lymph node involvement. Hormone receptor status (ER: 0%, PR: 0%) and HER2 were negative (Score 0, HercepTest). The Ki-67 index was recorded at 30%, indicating moderate tumor proliferation.

Case 7: A 40-year-old premenopausal female with no family history of cancer presented with a left breast nodule, initially classified as cT3N0M0. Histopathology confirmed NST, classified as SBR grade III, with no lymph node involvement. Hormone receptor status (ER: 0%, PR: 0%) and HER2 were negative (Score 0, HercepTest). The Ki-67 index was notably high at 80%, indicating an aggressive proliferative rate.

Case 8: A 49-year-old postmenopausal female was referred for a right breast nodule evaluation, initially classified as cT2N0M0. She had no family history of cancer. Biopsy confirmed NST, categorized as SBR grade IV, with lymph node involvement and metastatic spread to the brain and bones. Hormone receptor status (ER: 0%, PR: 0%) and HER2 were negative (Score 0, HercepTest). The Ki-67 index was high at 60%.

Case 9: A 52-year-old postmenopausal female was referred for evaluation of a right breast nodule, initially classified as cT2N0M0. She reported a family history of cancer, with a maternal aunt having passed away from breast cancer. Biopsy confirmed

the diagnosis of NST, classified as SBR grade II, with lymph node involvement. Hormone receptor status (ER: 0%, PR: 0%) and HER2 were negative (Score 0, HercepTest). The Ki-67 index was recorded at 40%, indicating moderate tumor proliferation.

Case 10: A 60-year-old postmenopausal female with no family history of cancer was referred for a left breast nodule, initially classified as cT2N0M0. Histopathology examination confirmed NST, categorized as SBR grade III, with lymph node involvement and metastatic spread to the brain. Hormone receptor status (ER: 0%, PR: 0%) and HER2 (Score 1+, HercepTest) were negative. The Ki-67 index was recorded at 44%, indicating moderate tumor proliferation.

Genetic analysis

Genomic DNA was extracted from the blood of each patient using the Invitrogen PureLink Genomic DNA Mini Kit (K182001), following the manufacturer's protocol. DNA quality and quantity were assessed using a Thermo Scientific NanoDrop 2000/2000c Spectrophotometer. WES was performed using the Twist Human Core Exome kit, and libraries were sequenced on an Illumina NovaSeq 6000 platform. The raw sequencing data were aligned to the human genome reference assembly GRCh37/hg19 using Burrows-Wheeler Aligner (BWA-mem version 0.7.17-cegat) (Li and Durbin 2009).A comprehensive analysis was conducted on the cohort of 10 patients to identify common variants (Table 2). Variants were filtered based on a minor allele frequency (MAF) below 1% across public databases (1000G, ExAC, gnomAD, and ClinVar), with a focus on protein-altering variants predicted to be deleterious or damaging, by bioinformatics tools such as SIFT, PolyPhen2, FATHMM, MutationTaster, MutationAssessor, CADD, PROVEAN, MetaSVM, and MetaLR.

Stringent filtering revealed four common variants in the *IGSF3* gene across all patients: a synonymous single nucleotide variant (SNV), p.S579S, and a nonsynonymous SNV, p.I619T, both with no previous clinical associations, and 2 stopgained variants, p.W575X, classified as pathogenic and linked to familial congenital nasolacrimal duct obstruction, and p.Q212X, a variant with no previous clinical associations. Both mutations result in truncated proteins, which may potentially disrupt the normal function of *IGSF3*.

Table 2. Study Workflow for Sample Preparation, Sequencing, Analysis, and Variant Validation

Step	Description
Samples	DNA was extracted from the blood of 10 unrelated Moroccan TNBC patients.
Sequencing	Libraries were prepared and sequenced on the Illumina NovaSeq 6000 platform using an S4 flow cell.
Mapping	Reads aligned to the human genome reference assembly GRCh37/hg19.
Variant annotation	Variants were annotated using various databases, including Ensembl v109, RefSeq Curated (20230628), CCDS r24, MANE Select 1.0, dbSNP156, GnomAD 2.1.1 (exonic), GnomAD 3.1.2 (genomic), Gencode 43, and HGNC (20230628).
Variant Filtration	Variants filtered based on the following criteria: Minor Allele Frequency < 0.01 in various databases, classified as Pathogenic or VUS or those with unavailable clinical significance but predicted to be functionally "Deleterious" or "Damaging" or Probably Damaging by bioinformatic tool.
Variant Review	Manual Review of raw sequencing (BAM) files conducted using IGV software (Version 2.5.3).

Additionally, a common nonsynonymous variant was identified in all patients in the *CTBP2* gene, located in exon 4. This mutation, p.D112A, substitutes aspartic acid with alanine, a hydrophobic amino acid, and has not been previously reported in the literature. These common variants in *IGSF3* and *CTBP2* suggest potential roles in breast cancer pathogenesis and warrant further investigation as genetic candidates in TNBC.

Further analysis revealed 4 additional variants in the *NMNAT1*, *TPRG1*, *LRRK2*, and *ZNF334* genes, which were classified as pathogenic or of uncertain significance (VUS) in all databases. However, these variants were not common in all patients and were only identified in Cases 8 and 10, as summarized in Figure 2.

DISCUSSION

The average age of TNBC patients in this study was 50.5 years, which is consistent with earlier studies in Morocco. Aznag *et al.* [19] reported an average age of around 48 years, while Khalis *et al.* [20] found the highest incidence rates in Moroccan women aged 45 to 59 years. This alignment not only reinforces the demographic trends observed in our cohort but also underscores the significant burden of TNBC among middle-aged women in Morocco. Such findings point to the importance of early screening and targeted interventions in this age group.

Table 3. Relevant Variants Identified in Triple-Negative Breast Cancer Patients

Patient No.	Gene	Chr	Description	Variant	rsID	Genotype	Variant type	Clinical significance
8 and 10	*NMNAT1*	1	Nicotinamide nucleotide adenylyltransferase 1	NM_001297778:exon2:c.G37A:p.A13T	rs138613460	Het	Nonsynonymous SNV	Pathogenic (Ensembl, ClinVar, UniProt)
All patients	*IGSF3*	1	Immunoglobulin superfamily member 3	NM_001007237:exon7:c.G1737A:p.S579S	rs552602059	Het	Synonymous SNV	NA
				NM_001007237:exon7:c.T1856C:p.I619T	rs75067537	Het	Nonsynonymous SNV	NA
				NM_001007237:exon7:c.G1724A:p.W575X	rs61730489	Het	Stopgain	Pathogenic (Ensembl, ClinVar)
				NM_001542:exon3:c.C634T:p.Q212X	rs139013364	Het	Stopgain	NA
8 and 10	*TPRG1*	3	Tumor protein p63 regulated 1	NM_198485:exon2:c.G191A:p.R64Q	rs143701531	Het	Nonsynonymous SNV	VUS (Ensembl)
All patients	*CTBP2*	10	C-terminal binding protein 2	NM_001321013:exon4:c.A335C:p.D112A	rs796433756	Het	Nonsynonymous SNV	NA
8 and 10	*LRRK2*	12	Leucine-rich repeat kinase 2	NM_198578:exon41:c.G6055A:p.G2019S	rs34637584	Het	Nonsynonymous SNV	Pathogenic (Ensembl, ClinVar, UniProt)
8 and 10	*ZNF334*	20	Zinc finger protein 334	NM_018102:exon5:c.A1016G:p.H339R	rs201084413	Het	Nonsynonymous SNV	VUS (Ensembl)

Chr, chromosome; Het, heterozygous; NA, not available; rsID, reference single-nucleotide polymorphism cluster ID; SNV, single-nucleotide variant; VUS, variant of uncertain significance.

The present study on TNBC in Moroccan patients reveals significant insights into the genetic variants that may influence the pathogenesis of the disease. The application of WES in this study allowed for an in-depth exploration of the genetic underpinnings of TNBC in Moroccan patients. Through a rigorous filtering process, we identified 9 variants across 6 genes: *IGSF3*, *CTBP2*, *LRRK2*, *NMNAT1*, *TPRG1*, and *ZNF334*. These genetic variants are likely involved in the pathogenesis of TNBC, providing valuable insights into the molecular mechanisms driving this aggressive subtype of breast cancer.

This aggressive subtype is characterized by a dysregulation of key signaling pathways, including Wnt and Notch, both of which are implicated in cancer progression. In particular, the Notch signaling pathway plays a central role in regulating tumor growth, metastasis, and chemoresistance in TNBC. [21, 22] Aberrant activation of this pathway is associated with poor prognosis and therapeutic resistance in TNBC. For example, mutations in *NOTCH1* can lead to increased *MYC* expression, contributing to tumor initiation and metastasis. [23, 24] In TNBC, mutations can trigger abnormal activation of the Notch pathway, leading to tumor growth.[21,25] Notch1 alterations in TNBC upregulate target genes like *MYC*, contributing to an oncogenic phenotype.[26-28]

Our findings suggest that the CTBP2-MYC axis may be a critical contributor to TNBC progression, with potential therapeutic implications for targeting this pathway. The *CTBP2* gene, in particular, encodes a transcriptional co-repressor that regulates gene expression. The nonsynonymous p.D112A mutation in *CTBP2* identified in the 10 patients could lead to the disruption of its normal regulatory functions, potentially contributing to tumorigenesis by affecting key signaling pathways like Wnt/β-catenin.[29] The mutation is a key finding because *CTBP2* functions as a transcriptional co-repressor that regulates gene expression. Disruption of its normal activity may lead to tumorigenesis, particularly in breast cancer.[29-31] Additionally, CTBP2 modulates signaling pathways such as Wnt/β-catenin to promote cancer progression.[32] Its inhibition has been shown to reduce cancer cell proliferation by decreasing c-Myc expression, a key oncogenic driver.[29] In TNBC, where Notch1 mutations amplify MYC activity, a CTBP2 mutation could further dysregulate MYC signaling, contributing to cancer progression through Notch1-MYC crosstalk. This suggests that the CTBP2-MYC axis may serve as a promising therapeutic target in TNBC, highlighting the need for further investigation into its role in cancer biology.

Table 3 shows predicted and experimentally validated interactions between these proteins (https://string-db.org/cgi/input?sessionId=bRWgpaQCqNr6&input_page_show_search=on).

The *ZNF334* gene, a member of the zinc-finger protein family, plays a critical role in regulating various biological processes, including differentiation and development.[33] In TNBC, *ZNF334* has been shown to suppress cancer progression by modulating the Wnt/β-catenin pathway, which is often aberrantly activated in many cancers.[34] The aberrant activation of the Wnt/β-catenin signaling pathway is closely linked to the development and progression of various cancers.[35,36] Numerous studies have highlighted its association with tumor proliferation, metastasis, stemness maintenance, and drug resistance in breast cancer.[37,38]

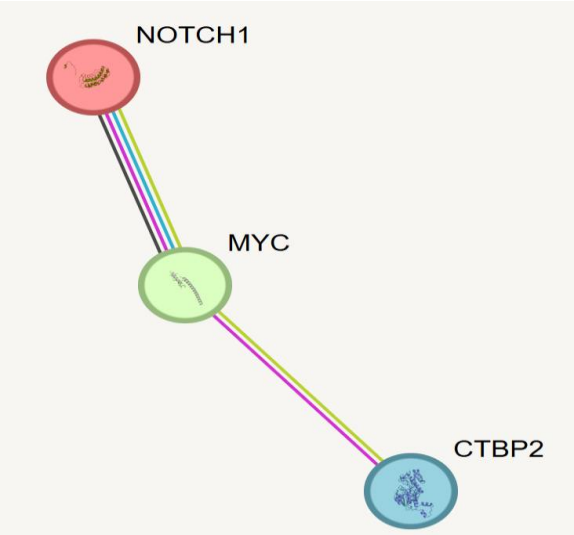

Figure 3. Interactome of *MYC*, *CTBP2*, and *NOTCH1* Generated Using STRING (v12.0)

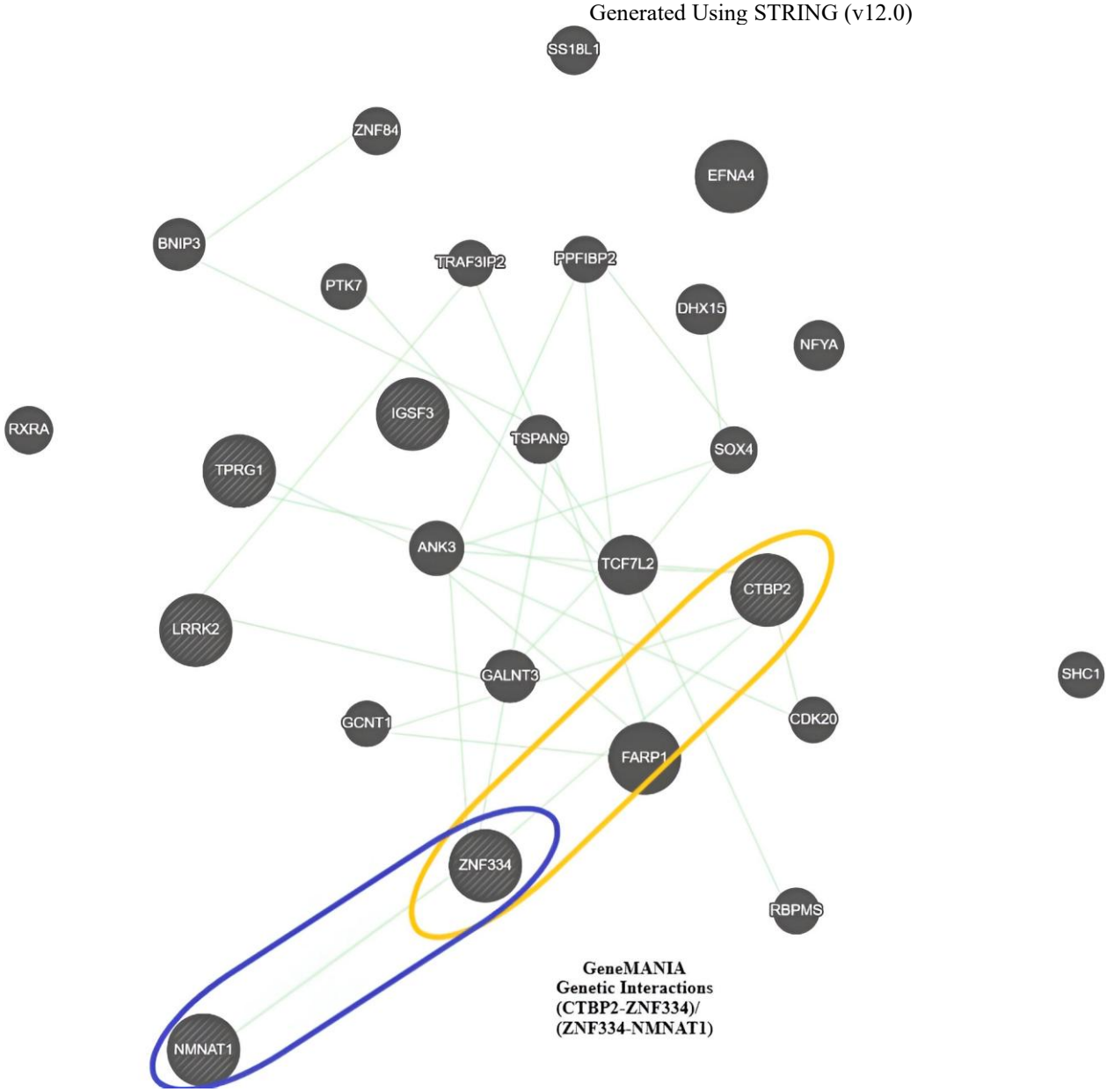

Figure 4. Molecular Interactions and Pathways Associated with Each Gene, for GeneMANIA (https://genemania.org/search/homo-sapiens/CTBP2/IGSF3/ZNF334/NMNAT1

The involvement of the Wnt/β-catenin pathway in TNBC is particularly notable, as its activation leads to the expression of specific target genes, supporting the role of *ZNF334* in modulating this pathway.[39]

The nonsynonymous variant identified in Exon 5 leads to a histidine-to-arginine substitution at amino acid position 339. This alteration may disrupt *ZNF334*'s suppressive function, potentially impacting the Wnt/β-catenin pathway.

Given that ZNF334 is involved in regulating cancer progression and metastasis through the Wnt/β-catenin pathway, the presence of this variant in Cases 8 and 10, both with brain metastasis, raises the possibility that the disrupted function of *ZNF334* could contribute to enhanced metastatic potential. It is possible that the alteration in *ZNF334* function may allow for unchecked activation of the Wnt/β-catenin pathway, leading to increased tumor invasiveness and metastatic spread, particularly to distant organs like the brain.

While direct studies on *ZNF334* variants in breast cancer are limited, its role as a transcriptional regulator implies potential effects on gene expression linked to cancer progression. The genetic interaction between *ZNF334* and *CTBP2*, highlighted by GeneMANIA (Figure 4), suggests a regulatory network influencing *MYC* activity. As *MYC* drives cell proliferation and survival, mutations in *ZNF334* or *CTBP2* may enhance tumor progression and metastasis, pointing to these interactions as potential therapeutic targets in TNBC.

The *LRRK2* gene, located on chromosome 12, encodes a large multi-domain protein involved in cellular signaling and neuronal functions. Mutations in *LRRK2*, particularly the G2019S variant, are primarily linked to Parkinson's disease (PD), where they enhance kinase activity, contributing to neurodegeneration.[40,41] Notably, G2019S is the most common PD-associated variant, with age-related penetrance increasing from 17% at age 50 to 85% by age 70.[42] However, penetrance is influenced by factors such as ethnicity, sex, and polygenic risk scores, which modulate the likelihood of PD onset. For instance, carriers with a high PD polygenic risk score have up to a 27-fold increased risk compared to non-carriers with a low score.[43-45]

In our cohort of TNBC patients, Cases 8 and 10 carried the G2019S mutation without exhibiting PD symptoms, consistent with incomplete penetrance. Interestingly, both cases had metastatic spread to the brain, a feature that raises intriguing questions about the role of *LRRK2* in cancer metastasis. Although G2019S-related PD typically manifests later in life or with atypical features, such as a lack of prodromal symptoms[45], the presence of brain metastases could suggest that the G2019S mutation may influence tumor progression or metastatic potential in addition to its neurodegenerative effects. This may reflect the dual role of *LRRK2* in both neurodegeneration and oncogenesis, with its mutation potentially promoting tumor growth and facilitating metastasis.

Beyond neurodegeneration, recent studies have implicated *LRRK2* mutations in cancer, including hormone-related cancers such as breast cancer.[40] The G2019S variant has been shown to promote tumor growth by increasing cell proliferation, inflammation, and immune response alterations, as demonstrated in colitis-associated cancer models.[40] Consistently, targeting *LRRK2* with specific inhibitors like LRRK2-IN-1 has been found to reduce cancer cell viability in breast cancer cell lines by downregulating key signaling proteins such as STAT3 and AKT, which are critical for cancer progression.[46] Our findings suggest a potential association between the *LRRK2* G2019S mutation and TNBC in individuals without PD, raising questions about the dual role of *LRRK2* in neurodegeneration and oncogenesis. This mutation may act through distinct pathways, influencing tumorigenesis independently of its neurodegenerative effects. Larger studies, particularly within the Moroccan population, are needed to confirm these associations and explore whether G2019S could serve as a biomarker for cancer risk. Such research may provide insights into *LRRK2*'s dual functionality and inform the development of targeted therapies for TNBC and other cancers.

The *NMNAT1* gene, which encodes an enzyme involved in nicotinamide adenine dinucleotide (NAD+) biosynthesis, plays a crucial role in cellular energy metabolism, redox balance, and stress responses.[47,48] The p.A13T variant in *NMNAT1*, identified in Cases 8 and 10, raises questions about its potential involvement in tumorigenesis. While this variant has been previously linked to Leber congenital amaurosis type 9 (LCA9), an early-onset autosomal recessive retinal degeneration disorder, it has not been directly associated with cancer.[49,50] The heterozygous state of this variant in TNBC patients raises the question of whether *NMNAT1* dysfunction could indirectly contribute to tumorigenesis. NAD+ metabolism is crucial for cellular survival, particularly in stress conditions such as those in tumors, where disruptions in NAD+ biosynthesis have been implicated in cancer cell proliferation and resistance to apoptosis.[51,52] Given *NMNAT1*'s involvement in pathways like tryptophan catabolism and nicotinamide metabolism, its dysregulation could influence the tumor microenvironment by altering

energy homeostasis, immune responses, or metabolic adaptation in cancer cells.[53,54] Additionally, interactions between *NMNAT1* and *ZNF334* (Figure 4) suggest broader roles for *NMNAT1* beyond retinal health. These interactions may point to potential mechanisms linking *NMNAT1* mutations to cancer susceptibility, especially in conditions involving viral etiology or metabolic stress. Although *NMNAT1* has not been widely reported in cancer, its central role in metabolic pathways relevant to cell survival suggests it may influence tumor progression. Further research, including functional studies, is warranted to explore whether *NMNAT1* variants act as modifiers or interact with other genetic alterations in cancer. The identification of this variant in TNBC patients underscores the need for larger cohort studies to clarify its role in oncogenesis and its potential as a therapeutic target.

The *IGSF3* gene, involved in cell adhesion and immune responses, has been implicated in various cancers, including hepatocellular carcinoma (HCC).[55,56] Increased expression of *IGSF3* is associated with poor prognosis and tumor progression, promoting cell migration, invasion, and growth through the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway. In HCC, silencing *IGSF3* reduces these processes.[56] Additionally, in vivo studies in mouse melanoma models further show that *IGSF3* enhances metastasis by promoting cancer cell adhesion to the vascular endothelium, aiding in lung colonization.[55] While its role in TNBC remains unexplored, these findings suggest *IGSF3* may contribute to cancer progression through similar mechanisms.

In our TNBC cohort, we identified four *IGSF3* variants: p.W575X, p.S579S, p.I619T, and p.Q212X. The p.W575X and p.Q212X truncations likely impair tumor-suppressive functions and enhance NF-κB-driven tumor growth and metastasis. While the synonymous p.S579S variant may affect mRNA stability or splicing, the p.I619T missense variant could disrupt critical protein interactions. The presence of these variants in all TNBC patients suggests a cumulative effect on tumor progression, with the pathogenic p.W575X mutation playing a key role. These findings underscore *IGSF3*'s potential as a TNBC biomarker and therapeutic target, particularly through NF-κB signaling. Further research is needed to elucidate its role in TNBC pathogenesis and treatment, especially in this aggressive subtype with limited targeted therapy options.

The *TPRG1* gene, located near TP63 on chromosome 3q28, is recognized as a tumor suppressor involved in cellular inflammatory responses. Dysregulation of *TPRG1* is associated with early tumor recurrence.[57] While the precise molecular role of *TPRG1* is still under investigation, recent studies on its antisense RNA, TPRG1-AS1, suggest important contributions to cancer progression. For example, TPRG1-AS1 has been shown to promote apoptosis in liver cancer by regulating RBM24 through a competing endogenous RNA (ceRNA) mechanism.[58] In this case series, a nonsynonymous variant (p.R64Q) was identified. This mutation could potentially disrupt *TPRG1*'s function or regulation, and potentially disrupt similar pathways or interacting factors within breast cancer cells. Although direct links between *TPRG1* and TNBC are limited, the presence of the p.R64Q variant suggests that *TPRG1* may influence tumor progression, possibly through mechanisms similar to those of TPRG1-AS1 in other cancers. Large-scale studies could further explore whether mutations in *TPRG1* affect its interaction with noncoding RNAs or tumor suppressive networks, as seen with *TPRG1*-AS1. In fact, despite its tumor-suppressive functions, the complexity of *TPRG1* interactions in cancer suggests that its role may vary across different cancer types. This indicates a need for further research to fully understand its implications in oncogenesis.

Among the TNBC patients in our cohort, variants in *NMNAT1*, *TPRG1*, *ZNF334*, and *LRRK2* were identified exclusively in Cases 8 and 10, who had the highest tumor grades (III and IV) and brain metastasis. The presence of these variants in these cases suggests a potential link to tumor aggressiveness and metastasis. However, it is important to note that the common variants in *CTBP2* and *IGSF3* were identified in all patients, regardless of tumor grade, highlighting their potential role in the overall pathogenesis of TNBC. Although direct evidence is limited, these findings highlight the need for functional studies to clarify their impact on disease pathogenesis and progression.

CONCLUSION

WES has significantly advanced our understanding of the genetic landscape associated with aggressive breast cancer subtypes, uncovering novel genetic factors that may influence cancer etiology across diverse populations. This study identified previously-unreported genetic variations in *CTBP2*, *IGSF3*, *ZNF334*, *TPRG1*, and *NMNAT1*, suggesting their potential roles as biomarkers and therapeutic targets. These findings emphasize the necessity of investigating genetic variations in underrepresented populations to refine breast cancer treatment strategies. Moreover, understanding these genetic alterations may help predict treatment responses, particularly to chemotherapeutic agents commonly used in TNBC, such as platinum-based

compounds or poly (ADP-ribose) polymerase (PARP) inhibitors, which are more effective in tumors with specific genetic vulnerabilities. Furthermore, the involvement of these genetic alterations in key oncogenic pathways suggests potential therapeutic implications, including the use of Wnt-targeted regimens or demethylation therapies, which may enhance treatment efficacy for TNBC patients. Future research should focus on validating these findings in larger cohorts to confirm their clinical relevance and support the development of personalized therapeutic approaches.

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CONFLICTS OF INTEREST

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

ETHICAL CONSIDERATIONS

This study adhered to the principles of the Declaration of Helsinki and was approved by the Ethical Committee for Biomedical Research at the Faculty of Medicine and Pharmacy of Rabat (12/23/21 /N° C53/20). Written informed consent for participation, including permission for data publication, was obtained from the patients at the time of recruitment.

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DATA AVAILABILITY

Data are available from the corresponding author upon request.

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