Received: 21 January 2021 | Revised: 28 May 2021 | Accepted: 22 June 2021

DOI: 10.1002/mgg3.1744

Molecular Genetics & Genomic Medicine Open Access WILEY

ORIGINAL ARTICLE

STAT3 polymorphisms in North Africa and its implication in breast cancer

Wafa Ziadi[1] | **Sami Boussetta**[1] | **Sarra Elkamel**[1] | **Andrew J. Pakstis**[2] | **Kenneth K. Kidd**[2] | **Imen Medimegh**[1] | **Amel Ben Ammar Elgaaied**[1] | **Lotfi Cherni**[1,3]

[1]Laboratory of Genetics, Immunology and Human Pathology, Faculty of Science of Tunis, University of Tunis El Manar, Tunis, Tunisia

[2]Department of Genetics, Yale University School of Medicine, New Haven, Connecticut, USA

[3]High Institute of Biotechnology, University of Monastir, Monastir, Tunisia

Correspondence
Wafa Ziadi, Laboratory of Genetics, Immunology and Human Pathology, Faculty of Sciences of Tunis, University of Tunis El Manar, 2092 Tunis, Tunisia.
Email: wefetou@gmail.com

Funding information
This work was partially supported by the Tunisian Ministry of Higher Education and Scientific Research as well as by the University of Tunis El Manar. Data generation at Yale was supported by grants from the U.S. National Institute of Justice.

Abstract

Background: Only a few studies have investigated the association of single nucleotide polymorphisms in *STAT3* gene with the susceptibility to cancer and response to chemotherapy. Our aim was to determine the allele frequencies of rs3869550, rs957971, and rs7211777 at the *STAT3* gene in North African populations and compare them to 1000 genomes populations, and to investigate their relation with cancer.

Methods: The targeted SNPs have been analyzed in six Tunisian populations and a sample of Libyans using TaqMan® Assay. The results were compared to 1000 Genomes Project population samples. Targeting of the regions encompassing the three SNPs by micro-ARN was assessed using miR databases.

Results: The analysis of the 3 SNPs showed that North African populations were close to South Asians. As expected, African populations presented a significant frequency of the ancestral CCG haplotype in contrast to other populations where the fully derived TGA haplotype was more frequent. The presence and diversity of rare haplotypes at *STAT3* in North African populations could have been generated by recombination between the two major haplotypes. A screening of the micro-RNA databases showed that the *STAT3* region with the mutated allele of rs7211777 (G>A) could be targeted by miR hsa-miR-3606-5p, which also targets genes involved in breast cancer.

KEYWORDS
breast cancer, miR-3606-5p, North Africa, rs7211777, *STAT3*

1 | INTRODUCTION

Signal transducer and activator of transcription protein 3 (*STAT3*) (OMIM accession number: *102582) is encoded by one of seven STAT family genes located in chromosomal band 17q21.2 and extends over 75kb (Aggarwal et al., 2009). Its expression is induced by cytokines, hormones, and growth factors. *STAT3* protein is activated by phosphorylation of its tyrosine and serine residues via signaling from upstream regulators (Klemm et al., 1998). *STAT3* is reported to regulate the expression of many genes such as Bcl-xL, cyclin D1, c-myc, VEGF, IL-10, IL-2, subsequently leading

Amel Ben Ammar Elgaaied and Lotfi Cherni have equal contribution.

to cellular proliferation and slowing-down of apoptosis (Herrmann et al., 2010; Rane & Reddy, 2000). Moreover, experimental models revealed important immune functions for *STAT3*, including innate and adaptative immunity (Hillmer et al., 2016).

In addition to its physiological role, studies have revealed the role of *STAT3* in diseases. Indeed, some mutations in the *STAT3* gene are associated with human immune disorders (Gutiérrez et al., 2018; Milner et al., 2015; Velayos et al., 2017). *STAT3* is also implicated in tumorigenesis by enhancing tumor growth, survival, invasion, immune suppression, and angiogenesis on the one hand and, decreasing tumor cell apoptosis on the other hand. Moreover, the Jak-STAT3 signaling pathway has been shown to have central roles in obesity (Priceman et al., 2013) and/or metabolism and in inflammation-mediated cancer, including cancer stem cells (CSCs) (Schroeder et al., 2014) and pre-metastatic niche formation (Deng et al., 2012).

In fact, many proteins whose expression is driven by overexpression of unphosphorylated *STAT3* have been implicated in many cancers (Yang et al., 2005). Activated *STAT3* has been implicated in multiple human cancers including lung (Du et al., 2012), gastric (Wu et al., 2012), ovarian, breast (Hsieh et al., 2005) (Sansone et al., 2007), colon (Calon et al., 2012) (Liang et al., 2013), prostate (Kroon et al., 2013), hepatocellular carcinoma (Hatziapostolou et al., 2011), and lymphoma (Liu et al., 2012).

A fundamental role for *STAT3* in the normal development of the mammary gland and the pathogenesis of human breast cancer (BC) has been established (Clevenger, 2004; Watson, 2001). Several studies have demonstrated increased levels of *STAT3* in primary mammary tumors. Immunohistochemical approaches in humans have found increased levels of nuclear-localized *STAT3* in malignant BCs when compared with normal tissues (Watson, 2001). A recent study has identified that *STAT3* expression was found to have a significantly higher correlation with luminal breast cancer (Eroglu et al., 2020). Hence, according to these data and because of its implication in cancer development and progression, Signal transducer and activator of transcription protein 3 (*STAT3*), has been recognized as a type of oncogene (Bromberg et al., 1999).

Despite the identification of around fifteen single nucleotide polymorphisms (SNPs) in the *STAT3* gene, only a few studies have investigated the association of SNPs in this gene with the susceptibility to cancer. For example, Vaclavicek et al. (2007) reported that the STAT5B rs6503691 and the *STAT3* rs7211777 polymorphisms were associated with an increased risk for breast cancer in German patients with familial breast cancer (Vaclavicek et al., 2007). Wang et al. (2011) detected an association between *STAT3* polymorphism rs4769793 and cervical cancer. Indeed, women with a G allele appeared to have a higher risk for cervical cancer. Further, the G allele was associated with poor tumor differentiation and positive parametrial invasion (Wang et al., 2011). Moreover, Jiang et al. (2011) have shown that a haplotype in the *STAT3* gene may have a protective role in the development of non-small cell lung cancer (NSCLC) (Jiang et al., 2011). Hence, Zhao et al. (2015) proposed that *STAT3* polymorphisms might be a candidate pharmacogenomic factor to assess susceptibility and prognosis of cancer (Zhao et al., 2015). Moreover, it has been shown that rs957971 polymorphism in *STAT3* gene may predict an unfavorable response to first-line platinum-based therapy for women with advanced serous epithelial ovarian cancer in an American population sample of European ancestry (Permuth-Wey et al., 2016).

In the present study, we characterized the genetic variation of *STAT3* gene in Tunisian and Libyan populations. The association status of *STAT3* polymorphism and cancer in Tunisian populations is still unknown. Only two case/control studies on *STAT3* polymorphism have been conducted in these populations. The first on rs744166, in Pemphigus patients, with no association was carried out (Ben Jmaa et al., 2018), and the second on rs1053023 and rs1053004 in relation to the Idiopathic Recurrent Miscarriage (IRM) showing that *STAT3* rs1053023 was positively associated with IRM in Tunisian women (Messoudi et al., 2013).

In this paper, we focused on two single nucleotide polymorphisms rs7211777 (g.42382057G>A) and rs3869550 (g.42340869T>C), chosen for their association with cancer in German and Chinese populations (Jiang et al., 2011; Vaclavicek et al., 2007). In addition to these two SNPs, we also investigated rs957971 (g.42367907C>G), located in the *STAT3* gene between these two SNPs; rs957971 has been associated with the response to chemotherapy (Permuth-Wey et al., 2016). Results will be discussed according to haplotypic diversity in this gene among North African populations.

2 | MATERIALS AND METHODS

2.1 | Ethical compliance

This work is approved by Ethics Committee for Research in Life Sciences and Health of the ISBM (CER-SVS/ISBM).

2.2 | DNA samples and *STAT3* SNP typing

A total of 349 North African individuals were collected including 279 Tunisians from 6 populations well distributed throughout Tunisia: Kesra (n = 42) to the north, Sousse (n = 46), Mahdia (n = 45) and Kairouan (n = 40) to the center, Smar (n=62) to the south, and a population of Kerkennah island (n = 44), in addition to 70 Libyans (Figure 1). All

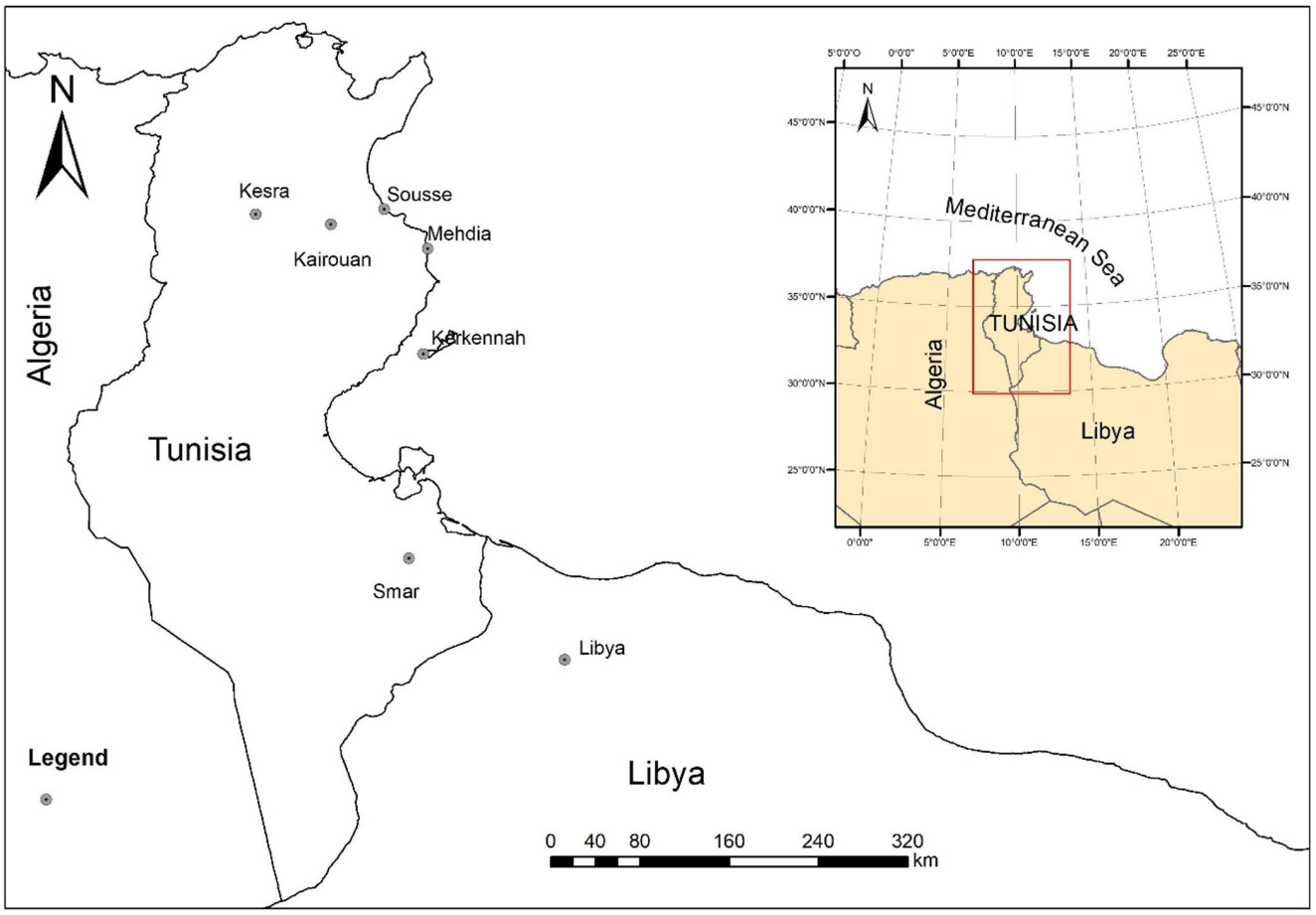
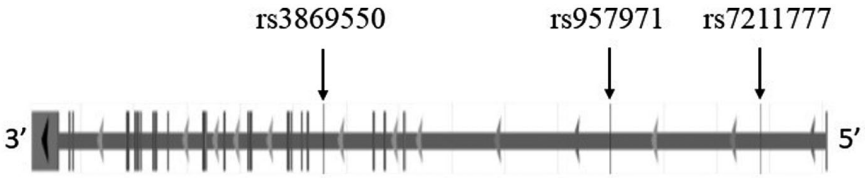


FIGURE 1 Localization of the seven North African populations analyzed in this study

FIGURE 2 SNPs (rs7211777, rs957971, rs3869550) positions on *STAT3*



	SNP	Chromosome	Symbol	GRCh38.7	Intron
3	rs7211777	17	STAT3	42 382 057	intron 1
2	rs957971	17	STAT3	42 367 907	intron 1
1	rs3869550	17	STAT3	42 340 869	intron 4

individuals sampled were unrelated and healthy persons and all individuals gave informed consent for the study of DNA sequence variants.

Total human genomic DNA was isolated from peripheral blood samples collected into EDTA tubes using the phenol-chloroform method.

The 3 SNPs (rs3869550, rs957971, rs7211777) of the *STAT3* gene have been typed in 3 µl reactions using TaqMan® Assay-on-demand following the manufacturer's protocol. Assays were obtained from Applied Biosystems, Thermo Fisher AB TaqMan Catalog Numbers C___7530575_10 C___1952199_10 C___1952182_10, respectively. 384-well plates were read on an AB7900 thermocycler using SDS software. The SNP frequency results are in Appendix Table A; see also the ALFRED database (Cherni et al., 2016; Rajeevan et al., 2012) at https://alfred.med.yale.edu.

The Reference sequence gene of STAT3 is: RefSeqGene (LRG_112) on chromosome 17 (Accession NG_007370, Region: 5001..80171, Version NG_007370.1).

TABLE 1 Allelic frequencies in North African populations at 3 SNPs (rs3869550, rs957971, rs7211777) of the STAT3 gene (2n= number of analyzed chromosomes, *ancestral reference allele)

SNP alleles	Kairouan	Kerkennah	Kesra	Mahdia	Smar	Sousse	Libya
rs3869550	2n = 80	2n = 86	2n = 78	2n = 86	2n = 116	2n = 86	2n = 118
C*	**47 (0.59)**	**47 (0.55)**	**22 (0.29)**	**45 (0.52)**	**54 (0.47)**	**40 (0.47)**	**58 (0.49)**
T	**33 (0.41)**	**39 (0.45)**	**56 (0.71)**	**41 (0.48)**	**62 (0.53)**	**46 (0.53)**	**60 (0.51)**
rs957971	2n = 76	2n = 84	2n = 84	2n = 90	2n = 122	2n = 92	2n = 134
C*	**44 (0.58)**	**44 (0.52)**	**29 (0.35)**	**46 (0.51)**	**59 (0.48)**	**43 (0.47)**	**66 (0.49)**
G	**32 (0.42)**	**40 (0.48)**	**55 (0.65)**	**44 (0.49)**	**63 (0.52)**	**49 (0.53)**	**68 (0.51)**
rs7211777	2n = 70	2n = 88	2n = 84	2n = 90	2n = 124	2n = 92	2n = 140
G*	**39 (0.56)**	**49 (0.56)**	**29 (0.35)**	**45 (0.5)**	**57 (0.46)**	**45 (0.49)**	**73 (0.52)**
A	**31 (0.44)**	**39 (0.44)**	**55 (0.65)**	**45 (0.5)**	**67 (0.54)**	**47 (0.51)**	**67 (0.48)**

TABLE 2 Linkage disequilibrium between pairs of the studied SNPs (rs3869550, rs957971, rs7211777) of the *STAT3* gene in North African, African, and European populations

Population	L1	L2	D′	R^2	Distance basepairs
Kairouan	rs3869550	rs957971	1.0	1.0	27,038
	rs3869550	rs7211777	1.0	1.0	41,188
	rs957971	rs7211777	1.0	1.0	14,150
Kerkennah	rs3869550	rs957971	1.0	1.0	27,038
	rs3869550	rs7211777	0.892	0.757	41,188
	rs957971	rs7211777	0.944	0.77	14,150
Kesra	rs3869550	rs957971	0.935	0.875	27,038
	rs3869550	rs7211777	0.935	0.875	41,188
	rs957971	rs7211777	0.947	0.897	14,150
Mahdia	rs3869550	rs957971	1.0	0.907	27,038
	rs3869550	rs7211777	1.0	0.864	41,188
	rs957971	rs7211777	1.0	0.957	14,150
Smar	rs3869550	rs957971	0.963	0.896	27,038
	rs3869550	rs7211777	0.964	0.897	41,188
	rs957971	rs7211777	1.0	0.936	14,150
Sousse	rs3869550	rs957971	1.0	0.952	27,038
	rs3869550	rs7211777	0.951	0.905	41,188
	rs957971	rs7211777	1.0	0.915	14,150
Libya	rs3869550	rs957971	1.0	0.932	27,038
	rs3869550	rs7211777	0.931	0.866	41,188
	rs957971	rs7211777	1.0	0.942	14,150
Africa	rs3869550	rs957971	1.0	0.976	27,038
	rs3869550	rs7211777	0.993	0.868	41,188
	rs957971	rs7211777	1.0	0.859	14,150
Europe	rs3869550	rs957971	1.0	0.996	27,038
	rs3869550	rs7211777	1.0	0.996	41,188
	rs957971	rs7211777	1.0	1.0	14,150

2.3 | Statistical analysis

The analysis of allelic and genotypic frequencies was performed using Plink 1.09 software (Purcell et al., 2007) http://pngu.mgh.harvard.edu/purcell/plink/, and the calculation of haplotypes has been done with the HAPLO program (Hawley & Kidd, 1995) based on the EM algorithm (Dempster et al., 1977). The determination of linkage disequilibrium (LD)

between the studied SNPs was performed with Haploview Software for all the North African populations.

For comparative analysis, we included data from 59 populations from the Kidd Lab (Brissenden et al., 2015; Cherni et al., 2016) and the 26 worldwide populations from The 1000 Genomes Project (1KG) (Consortium, 2015). The haplotypic data of the three SNPs were downloaded from LD link website of the 1000 Genome Project (Machiela & Chanock, 2015). Data obtained from the 7 North African populations analyzed in this study were merged with data from the 1KG project subset (Table S1: Population list file, supplementary material). Principal Component Analysis (PCA) was performed with PAST software (Hammer et al., 2001).

2.4 | miRNA targeting *STAT3* mRNA

On the *STAT3* gene, the three studied SNPs are intronic; rs7211777 and rs957971 are in intron 1 and rs3869550 is in intron 4 (Figure 2). Both normal genomic sequences and those carrying the derived *STAT3* allele were investigated. MiRs targeting the areas with the studied SNPs were identified using the online database miRBase. The investigated sequences cover 50 nucleotides on each side of the studied SNP. This was done by transporting a 100 bp sequence containing the ancestral and derived genotype of the SNP studied in the search application of the miRBase site: http://www.mirbase.org/index.shtml.

Expression information on the identified miRNA and its relationship with breast cancer was obtained using the online databases ONCOMIR and TCGA.BRCA.sampleMap/miRNA HiSeq gene database.

3 | RESULTS

3.1 | Allelic and genotypic frequencies

The analysis of allelic and genotypic frequencies for each of the three SNPs showed no significant deviation ($p < .01$) from Hardy-Weinberg equilibrium in Tunisian and Libyan populations (Appendix Table A). Allelic frequencies do vary across the North African populations. The allelic frequency of ancestral alleles at SNPs rs3869550, rs95797, and rs7211777 were higher in Kairouan, Kerkennah, and Mahdia populations than the alternate alleles. The opposite situation was observed in Kesra, Smar, Sousse, and Libyan populations (Table 1). Globally, average values in the North African populations were close to those of South Asian populations. For all three SNPs the Kesra show the strongest frequency differences with each of the other North African population samples.

Allele frequencies among the three SNPs studied tend to be strongly correlated across populations around the world (Table S2: Supplementary Data).

3.2 | Linkage disequilibrium

We compared the linkage disequilibrium (LD) structure of *STAT3* SNP (rs3869550, rs957971, rs7211777) among the studied populations.

Linkage disequilibrium among the three SNPs of the *STAT3* gene in North African populations was also compared to European populations and African populations in Table 2 which illustrates r^2 and D′ values for each pair of SNPs in these populations. Taken together, results revealed a strong linkage disequilibrium among the 3 SNPs. The lower value of D′ between rs3869550 and rs957971 was observed in the Kesra population which is the only population considered as Berber among the studied populations. We also noticed that the population of Mahdia displayed a total LD similar to European populations while the remaining North African populations studied presented similar LD to African populations.

Linkage disequilibrium is very strong among the 3 SNPs considered pairwise. In Table 2, the r^2 measurements are in the range of 0.85 to 1.00. The high degree of similarity of the SNP frequencies can be seen visually in supplementary Figure (SF1) or by inspecting the SNP frequencies in supplementary Table S2. Overall, the LD is very high and the deviations from complete (D′ = 1.0) LD is attributable to rare haplotypes (Figure 3).

3.3 | Analysis of haplotype frequencies

The three *STAT3* SNPs can occur in eight possible combinations. Direct gene counting evidence supports the occurrence of all eight haplotypes among the populations sampled from around the world. As shown in Figure 3 and Table S3 (Supplementary Data), there are, in the 92 populations (>5600 individuals) studied, two haplotype alleles CCG, the ancestral allele, and TGA, the fully derived allele, that occur at very common frequencies worldwide. The other six haplotypes usually occur at low to rare frequencies (<5%) but in some populations, they do occur at moderately common frequencies (5%–21%). The ancestral allele occurs at the highest frequencies (often at 80%–90%) in sub-Saharan Africa and in some populations in the Pacific region. The fully derived allele is found at very common frequencies (>90%) in Native American populations. In other world regions (North Africa, Europe, and Asia) the TGA and CCG haplotypes both occur at very common frequencies with TGA usually being the more frequent allele.

In Figure 4, a Network summarizes this relationship between the haplotypes constructed by these three SNPs of the STAT3 gene.

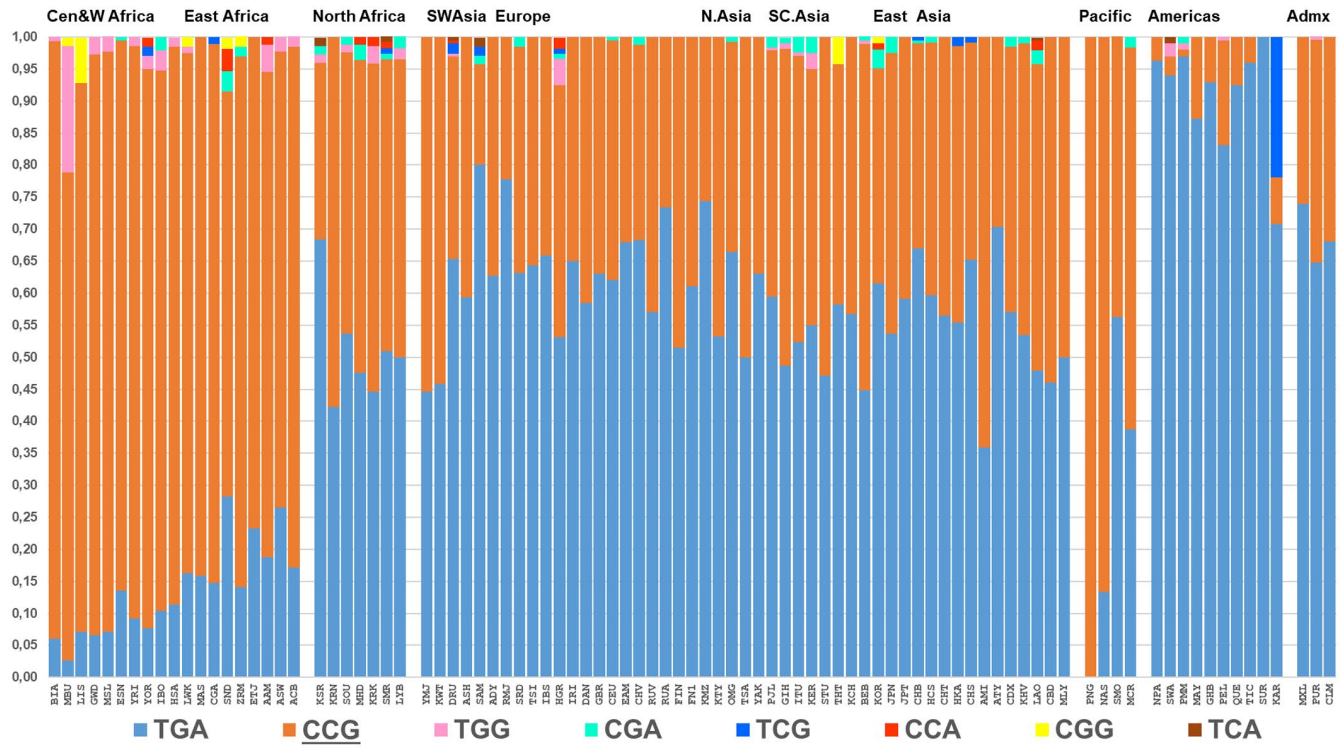


FIGURE 3 STAT3 *haplotype frequency estimates based on 3 SNPs in 92 populations*

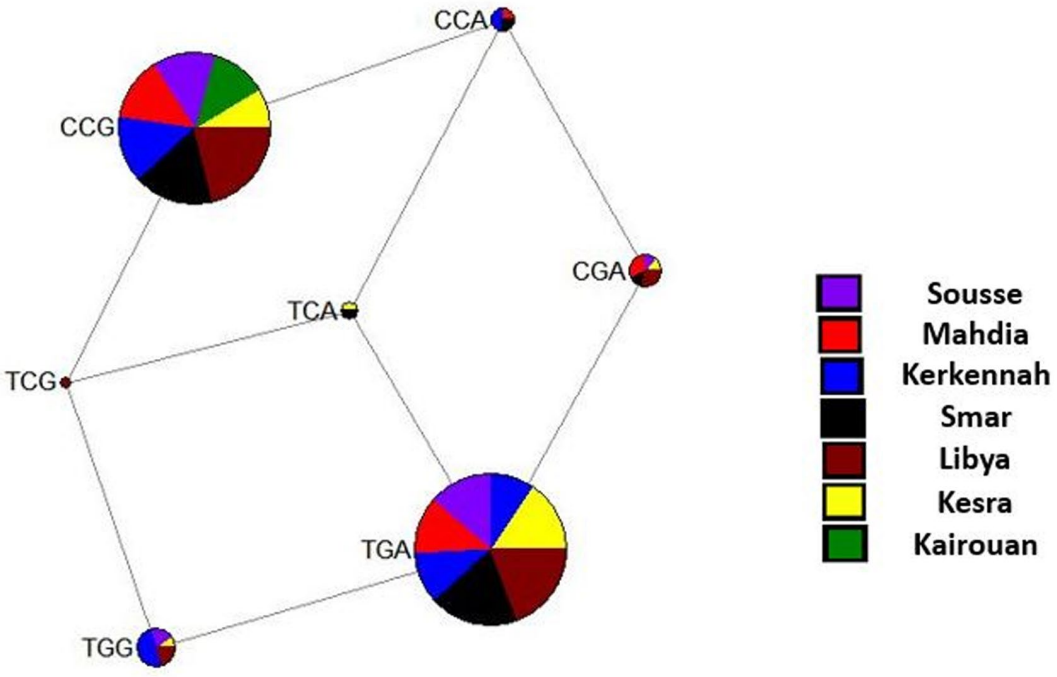


FIGURE 4 Network of the relationship between the haplotypes constructed by the three SNPs (rs7211777, rs957971, rs3869550) of the *STAT3* gene

3.4 | miRNA targeting *STAT3* mRNA

Considering the functional aspects of the studied SNPs that are located at the intronic level, we hypothesized that sites containing these SNPs could be the target of microRNAs with a possible effect on the splicing or stability of *STAT3* mRNA. So, we sifted the databases for possible miRs that could target these regions at the level of human *STAT3* gene. No target miR was identified for rs3869550 and rs957971, but the derived allele of rs7211777 was targeted by a miR centric

hsa-miR-3606-5p when G is replaced by A with a score of 65 which can be significant (Figure 5).

4 | DISCUSSION

STATs are ligand-induced transcriptional factors that are activated in response to a wide range of cytokines, growth factors, and hormones. *STAT3* is constitutively activated in various cancers including breast cancer. The association of *STAT3* polymorphism and cancer in the Tunisian population is still unknown.

The importance of this transcription factor and its involvement in various biological processes and different types of pathologies justifies its attention from the point of view of its activity, which is often a function of genetic polymorphism. *STAT3* SNP data in human populations show frequency differences that need to be clarified especially for North African populations on which little data is available.

Our results on the genetic diversity of *STAT3*, considering 3 SNPs associated with cancer in populations, show the distinctiveness of Sub-Saharan Africans and Native American populations from each other and from populations in other world regions. Although there is very strong linkage disequilibrium present among these *STAT3* SNPs in the many populations examined, the allele and haplotype frequency levels do vary around the world. Two of the eight possible haplotypes (the ancestral CCG and the fully derived TGA) are observed to occur at predominant frequencies in the 92 populations studied. This could have arisen by random genetic drift or it could be related to positive selection but studies differentiating among these possibilities have not yet been carried out. However, to the extent that beneficial or harmful genetic variants for the development of various types of cancers or for the response to therapeutic interventions exist in linkage disequilibrium with these observed haplotypes, it is clear from the genetic variation demonstrated in this report that we should expect that population differences will be observed.

Our results on the genetic diversity of *STAT3* showed a high level of diversity in North African populations with seven haplotypes observed. In the PCA plot, North African populations resemble South Asians populations and occupy an intermediate position between Sub-Saharan African and the rest of worldwide populations, with a particular behavior of Berber population from Kesra which was close to Europeans, and the island of Kerkennah population which was isolated (Supplementary Figure SF2). This feature seems to be related to the presence of rare *STAT3* haplotypes such CCA and TCA which were specific to North African populations. Two rare haplotypes CGG and TGG could have been generated from the ancestral haplotype CCG by point mutation or recombination, then the four others (CGA, TCG, CCA, TCA) could be mostly obtained by recombination between the two major haplotypes (CCG and TGA), excepted for TCA haplotype that could have been generated by recombination between the two rare haplotypes (Figure 6). One has to ask for the cause of such haplotype diversity in North-African populations. Since the two major haplotypes are present in all human populations, heterozygous genotypes CCG/TGA could lead to the possibility of recombination.

Moreover, the presence of recombinant specific haplotypes seems to be characteristic of the North African populations studied. This has been reported for other genes, such as BRCA1, in Tunisian breast cancer patients that displayed several distinct SNP haplotypes, corresponding to different evolution forms, which were less numerous than haplotypes observed in US patients (Troudi et al., 2007). In fact, the American melting pot is recent compared to the very ancient admixture that occurred in North Africa and shown by genetic analysis of actual populations (Ennafaa et al., 2011; Frigi et al., 2010) and also genome analysis of Neolithic fossils (Fregel et al., 2018). Indeed, according to these studies, four components of diverse origins (Sub-Saharan, European, Middle Eastern, and North-African) have been found to be present in North African genomes since at least the Neolithic period. This high level of admixture from prehistoric times should have given possibilities for recombination between distinct haplotypes, leading to new combinations. These conditions may not have been met in other ancient human communities, whose low numbers would have generated a tendency to consanguinity and homozygosity. Considering the pleiotropic role of *STAT3* as a transcription factor particularly involved in inflammation and immune response on one hand and the impact of the studied SNPs at the functional level, we can argue that positive selection should have played a role out of Africa, when human migrants settled in a new infectious environment toward which their immune system

FIGURE 5 The mutated sequence at rs7211777 targeted by mir-3606-5p

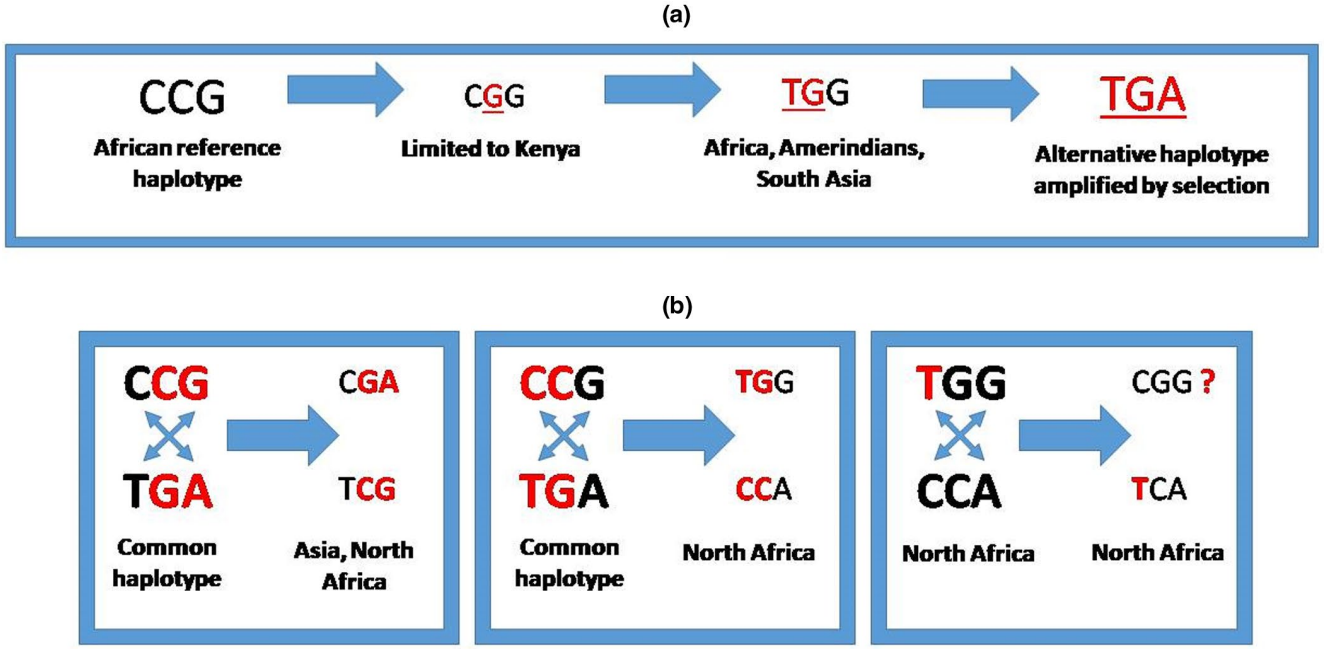


FIGURE 6 (a) Haplotype generated by point mutation, (b) Haplotypes generated by combination

TABLE 3 Targeted genes by miR-3606-5p in breast cancer (BRCA type) according to ONCOMIR

Gene	Gene Description	Correlation	Correlation P-value	Correlation FDR	miRDB Score
MKL2	MKL/myocardin-like 2	−0.0824	1.65e-02	9.46e-01	53
RMND1	required for meiotic nuclear division 1 homolog (S. cerevisiae)	−0.0822	1.69e-02	9.46e-01	83
NFAT5	nuclear factor of activated T-cells 5, tonicity-responsive	−0.0763	2.65e-02	9.46e-01	73
MEF2D	Myocyte Enhancer factor 2D	−0.0729	3.41e-02	9.46e-01	90
EDNRB	Endothelin Receptor type B	−0.0720	3.64e-02	9.46e-01	58
MED12L	Mediator complex subunit 12-like	−0.0697	4.28e-02	9.46e-01	60

TABLE 4 Linkage disequilibrium between pairs of the SNPs (rs3869550, rs957971, rs7211777, rs3736164, rs4796793) of the *STAT3* gene worldwide populations

All populations					
Rs number	**rs3869550**	**rs957971**	**rs7211777**	**rs3736164**	**rs4796793**
D′					
rs3869550	1.0	0.999	0.983	1.0	0.996
rs957971	0.999	1.0	1.0	1.0	0.996
rs7211777	0.983	1.0	1.0	1.0	0.996
rs3736164	1.0	1.0	1.0	1.0	0.996
rs4796793	0.996	0.996	0.996	0.996	1.0
R^2					
rs3869550	1.0	0.98	0.96	0.623	0.557
rs957971	0.98	1.0	0.976	0.635	0.567
rs7211777	0.96	0.976	1.0	0.619	0.553
rs3736164	0.623	0.635	0.619	1.0	0.894
rs4796793	0.557	0.567	0.553	0.894	1.0

had to adapt. One has to ask how the location of these SNPs in *STAT3* introns might impact the function of the protein. Analysis performed using micro-RNA databases allowed to assess the possibility of targeting these SNPs regions by specific miR. Our results showed the *STAT3* region with the derived allele of rs7211777 (G>A) was targeted by miR hsa-miR-3606-5p.

The previous study of *STAT3* polymorphism rs3869550 of Jiang et al. conducted on (NSCLC) non-small cell lung cancer showed that the *STAT3* protective haplotype G**G**CGGC contains the ancestral allele (G) instead of the derived allele A (Jiang et al., 2011). Analysis of haplotypes deduced by Vaclavicek et al (Vaclavicek et al., 2007) that the rare haplotype C**A**GCC which contained the derived allele from each SNP (STAT3 rs721177 and STAT5B rs6503691), was associated with an increased risk of Breast Cancer (OR = 5.83, 95% CI 1.51–26.28, $p = .002$).

Interestingly, several genes are targeted by miR-3606-5p in breast cancer according to ONCOMIR (Table 3). Expression of hsa-miR-3606-5p has been quantified in normal and breast cancer tissues (TCGA.BRCA.sampleMap/miRNA HiSeq gene database). According to these results, miR-3606-5p might explain the association of rs7211777 at *STAT3* gene with Breast cancer. This hypothesis should be confirmed particularly in North African populations where the risk allele (A) is associated with different STAT3 haplotypes and also by assessment of *STAT3* mRNA expression and mir-3606-5p in normal and pathological conditions along with *STAT3* SNP genotype.

Moreover, miR-3606-5p is not the only one that regulates *STAT3*, it is also regulated by other miRs. For example, Mir-520 blocks the progression of EMT by targeting *STAT3*, in addition, mir-544 inhibits Bcl6 and *STAT3* in parallel to decrease cell growth in TNBC (Wang et al., 2017; Zhu et al., 2016).

Moreover, rs7211777, as located in intron 1, is close to exon 1 and the promoter region. Indeed, the investigation about LD with two other common SNPs in the *STAT3* promoter region (rs 3736164, rs4796793) revealed a strong linkage disequilibrium among the 3 SNPs in worldwide populations (LD Supplementary Data). Table 4 illustrates r^2 and D′ values for each pair of SNPs. Hence this strong LD does not exclude that another mechanism could be associated with the regulation of *STAT3* haplotypes expression at the transcriptional level due to functional variants affecting the promoter region of the gene. Indeed, the rs4796793 SNP was previously shown to be associated with cervical cancer, women with a G allele at rs4769793 being submitted to a higher risk for cervical cancer (K. Wang et al., 2011).

In conclusion, previous research has shown that polymorphisms at the STAT3 gene appear to have functional effects on the development and pathological course of various cancers. Assessment of such effects should be investigated in North African populations, considering the presence of specific recombinant *STAT3* haplotypes.

ACKNOWLEDGMENTS

Special thanks go to the thousands of individuals around the world who volunteered to give blood and saliva samples to make this study possible and all the authors who contributed to this study.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Wafa Ziadi https://orcid.org/0000-0002-1174-9469
Sarra Elkamel https://orcid.org/0000-0002-1110-5225

REFERENCES

Aggarwal, B. B., Kunnumakkara, A. B., Harikumar, K. B., Gupta, S. R., Tharakan, S. T., Koca, C., Dey, S., & Sung, B. (2009). Signal transducer and activator of transcription-3, inflammation, and cancer: how intimate is the relationship? *Annals of the New York Academy of Sciences*, *1171*, 59–76. https://doi.org/10.1111/j.1749-6632.2009.04911.x

Ben Jmaa, M., Abida, O., Fakhfakh, R., Bahloul, E., Sellami, K. H., Gaddour, L., Elloumi, N., Ben Ayed, M., Masmoudi, A., Dhouib, M., Abdelmoula, M., Mahfoudh, N., Makni, H., Turki, H., & Masmoudi, H. (2018). Involvement of the IL23/Th17 pathway in the pathogenesis of Tunisian pemphigus foliaceus. *Mediators of Inflammation*, *2018*, 1–12. https://doi.org/10.1155/2018/8206983

Brissenden, J. E., Kidd, J. R., Evsanaa, B., Togtokh, A. J., Pakstis, A. J., Friedlaender, F., Kidd, K. K., & Roscoe, J. M. (2015). Mongolians in the genetic landscape of Central Asia: Exploring the genetic relations among Mongolians and other world populations. *Human Biology*, *87*(2), 73–91. https://doi.org/10.13110/humanbiology.87.2.0005

Bromberg, J. F., Wrzeszczynska, M. H., Devgan, G., Zhao, Y., Pestell, R. G., Albanese, C., & Darnell, J. E. Jr. (1999). Stat3 as an oncogene. *Cell*, *98*(3), 295–303. https://doi.org/10.1016/s0092-8674(00)81959-5

Calon, A., Espinet, E., Palomo-Ponce, S., Tauriello, D. V. F., Iglesias, M., Céspedes, M. V., Sevillano, M., Nadal, C., Jung, P., Zhang, X.-F., Byrom, D., Riera, A., Rossell, D., Mangues, R., Massagué, J., Sancho, E., & Batlle, E. (2012). Dependency of colorectal cancer on a TGF-β-driven program in stromal cells for metastasis initiation. *Cancer Cell*, *22*(5), 571–584. https://doi.org/10.1016/j.ccr.2012.08.013

Cherni, L., Pakstis, A. J., Boussetta, S., Elkamel, S., Frigi, S., Khodjet-El-Khil, H., Barton, A., Haigh, E., Speed, W. C., Ben Ammar Elgaaied, A., Kidd, J. R., & Kidd, K. K. (2016). Genetic variation in Tunisia in the context of human diversity worldwide. *American Journal of Physical Anthropology*, *161*(1), 62–71. https://doi.org/10.1002/ajpa.23008

Clevenger, C. V. (2004). Roles and regulation of stat family transcription factors in human breast cancer. *American Journal of Pathology*, *165*(5), 1449–1460. https://doi.org/10.1016/s0002-9440(10)63403-7

Consortium, G. P. (2015). A global reference for human genetic variation. *Nature*, *526*(7571), 68–74.

Dempster, A. P., Laird, N. M., & Rubin, D. B. (1977). Maximum likelihood from incomplete data via the EM algorithm. *Journal of the Royal Statistical Society: Series B (Methodological)*, *39*(1), 1–22.

Deng, J., Liu, Y., Lee, H., Herrmann, A., Zhang, W., Zhang, C., Shen, S., Priceman, S. J., Kujawski, M., Pal, S. K., Raubitschek, A., Hoon, D. S. B., Forman, S., Figlin, R. A., Liu, J., Jove, R., & Yu, H. (2012). S1PR1-STAT3 signaling is crucial for myeloid cell colonization at future metastatic sites. *Cancer Cell*, *21*(5), 642–654. https://doi.org/10.1016/j.ccr.2012.03.039

Du, L., Subauste, M. C., DeSevo, C., Zhao, Z., Baker, M., Borkowski, R., Schageman, J. J., Greer, R., Yang, C.-R., Suraokar, M., Wistuba, I. I., Gazdar, A. F., Minna, J. D., & Pertsemlidis, A. (2012). miR-337-3p and its targets STAT3 and RAP1A modulate taxane sensitivity in non-small cell lung cancers. *PLoS One*, *7*(6), e39167. https://doi.org/10.1371/journal.pone.0039167

Ennafaa, H., Fregel, R., Khodjet-el-khil, H., González, A. M., Mahmoudi, H. A. E., Cabrera, V. M., Larruga, J. M., & Benammar-Elgaaïed, A. (2011). Mitochondrial DNA and Y-chromosome microstructure in Tunisia. *Journal of Human Genetics*, *56*(10), 734–741. https://doi.org/10.1038/jhg.2011.92

Eroglu, M., Kokenek-Unal, T. D., Akin-Bali, D. F., & Kirimlioglu, S. H. (2020). STAT3 expression is correlated with pathological stage in luminal subtypes of breast carcinoma. *Bratislavske Lekarske Listy*, *121*(1), 51–61. https://doi.org/10.4149/bll_2020_008

Fregel, R., Méndez, F. L., Bokbot, Y., Martín-Socas, D., Camalich-Massieu, M. D., Santana, J., Morales, J., Ávila-Arcos, M. C., Underhill, P. A., Shapiro, B., Wojcik, G., Rasmussen, M., Soares, A. E. R., Kapp, J., Sockell, A., Rodríguez-Santos, F. J., Mikdad, A., Trujillo-Mederos, A., & Bustamante, C. D. (2018). Ancient genomes from North Africa evidence prehistoric migrations to the Maghreb from both the Levant and Europe. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(26), 6774–6779. https://doi.org/10.1073/pnas.1800851115

Frigi, S., Cherni, L., Fadhlaoui-Zid, K., & Benammar-Elgaaied, A. (2010). Ancient local evolution of African mtDNA haplogroups in Tunisian Berber populations. *Human Biology*, *82*(4), 367–384. https://doi.org/10.3378/027.082.0402

Gutiérrez, M., Scaglia, P., Keselman, A., Martucci, L., Karabatas, L., Domené, S., Martin, A., Pennisi, P., Blanco, M., Sanguineti, N., & Bezrodnik, L. (2018). Partial growth hormone insensitivity and dysregulatory immune disease associated with de novo germline activating STAT3 mutations. *Molecular and Cellular Endocrinology*, *473*, 166–177.

Hammer, Ø., Harper, D., & Ryan, P. (2001). PAST-palaeontological statistics, ver. 1.89. *Palaeontologia Electronica*, *4*(1), 1–9.

Hatziapostolou, M., Polytarchou, C., Aggelidou, E., Drakaki, A., Poultsides, G. A., Jaeger, S. A., Ogata, H., Karin, M., Struhl, K., Hadzopoulou-Cladaras, M., & Iliopoulos, D. (2011). An HNF4α-miRNA inflammatory feedback circuit regulates hepatocellular oncogenesis. *Cell*, *147*(6), 1233–1247. https://doi.org/10.1016/j.cell.2011.10.043

Hawley, M. E., & Kidd, K. K. (1995). HAPLO: a program using the EM algorithm to estimate the frequencies of multi-site haplotypes. *Journal of Heredity*, *86*(5), 409–411. https://doi.org/10.1093/oxfordjournals.jhered.a111613

Herrmann, A., Kortylewski, M., Kujawski, M., Zhang, C., Reckamp, K., Armstrong, B., Wang, L., Kowolik, C., Deng, J., Figlin, R., & Yu, H. (2010). Targeting Stat3 in the myeloid compartment drastically improves the in vivo antitumor functions of adoptively transferred T cells. *Cancer Research*, *70*(19), 7455–7464. https://doi.org/10.1158/0008-5472.can-10-0736

Hillmer, E. J., Zhang, H., Li, H. S., & Watowich, S. S. (2016). STAT3 signaling in immunity. *Cytokine & Growth Factor Reviews*, *31*, 1–15. https://doi.org/10.1016/j.cytogfr.2016.05.001

Hsieh, F. C., Cheng, G., & Lin, J. (2005). Evaluation of potential Stat3-regulated genes in human breast cancer. *Biochemical and Biophysical Research Communications*, *335*(2), 292–299. https://doi.org/10.1016/j.bbrc.2005.07.075

Jiang, B., Zhu, Z. Z., Liu, F., Yang, L. J., Zhang, W. Y., Yuan, H. H., Wang, J. G., Hu, X. H., & Huang, G. (2011). STAT3 gene polymorphisms and susceptibility to non-small cell lung cancer. *Genetics and Molecular Research*, *10*(3), 1856–1865. https://doi.org/10.4238/vol10-3gmr1071

Klemm, J. D., Schreiber, S. L., & Crabtree, G. R. (1998). Dimerization as a regulatory mechanism in signal transduction. *Annual Review of Immunology*, *16*, 569–592. https://doi.org/10.1146/annurev.immunol.16.1.569

Kroon, P., Berry, P. A., Stower, M. J., Rodrigues, G., Mann, V. M., Simms, M., Bhasin, D., Chettiar, S., Li, C., Li, P.-K., Maitland, N. J., & Collins, A. T. (2013). JAK-STAT blockade inhibits tumor initiation and clonogenic recovery of prostate cancer stem-like cells. *Cancer Research*, *73*(16), 5288–5298. https://doi.org/10.1158/0008-5472.can-13-0874

Liang, J., Nagahashi, M., Kim, E. Y., Harikumar, K. B., Yamada, A., Huang, W. C., Hait, N. C., Allegood, J. C., Price, M. M., Avni, D., & Takabe, K. (2013). Sphingosine-1-phosphate links persistent STAT3 activation, chronic intestinal inflammation, and development of colitis-associated cancer. *Cancer Cell*, *23*(1), 107–120. https://doi.org/10.1016/j.ccr.2012.11.013

Liu, Y., Deng, J., Wang, L., Lee, H., Armstrong, B., Scuto, A., Kowolik, C., Weiss, L. M., Forman, S., & Yu, H. (2012). S1PR1 is an effective target to block STAT3 signaling in activated B cell-like diffuse large B-cell lymphoma. *Blood*, *120*(7), 1458–1465. https://doi.org/10.1182/blood-2011-12-399030

Machiela, M. J., & Chanock, S. J. (2015). LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics*, *31*(21), 3555–3557. https://doi.org/10.1093/bioinformatics/btv402

Messoudi, S., Al-Sulaiti, M. A., Al-Busaidi, A. S., Dendana, M., Nsiri, B., Almawi, W. Y., & Mahjoub, T. (2013). Contribution of JAK2 and STAT3 variants to the genetic susceptibility of recurrent miscarriage among Bahraini and Tunisian Arabs. *Molecular Biology Reports*, *40*(1), 585–589. https://doi.org/10.1007/s11033-012-2096-8

Milner, J. D., Vogel, T. P., Forbes, L., Ma, C. A., Stray-Pedersen, A., Niemela, J. E., Lyons, J. J., Engelhardt, K. R., Zhang, Y. U., Topcagic, N., Roberson, E. D. O., Matthews, H., Verbsky, J. W., Dasu, T., Vargas-Hernandez, A., Varghese, N., McClain, K. L., Karam, L. B., Nahmod, K., … Cooper, M. A. (2015). Early-onset lymphoproliferation and autoimmunity caused by germline STAT3 gain-of-function mutations. *Blood*, *125*(4), 591–599. https://doi.org/10.1182/blood-2014-09-602763

Permuth-Wey, J., Fulp, W. J., Reid, B. M., Chen, Z., Georgeades, C., Cheng, J. Q., Magliocco, A., Chen, D. T., & Lancaster, J. M. (2016). STAT3 polymorphisms may predict an unfavorable response to first-line platinum-based therapy for women with advanced serous epithelial ovarian cancer. *International Journal of Cancer*, *138*(3), 612–619. https://doi.org/10.1002/ijc.29799

Priceman, S. J., Kujawski, M., Shen, S., Cherryholmes, G. A., Lee, H., Zhang, C., Kruper, L., Mortimer, J., Jove, R., Riggs, A. D., & Yu, H. (2013). Regulation of adipose tissue T cell subsets by Stat3 is crucial for diet-induced obesity and insulin resistance. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(32), 13079–13084. https://doi.org/10.1073/pnas.1311557110

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., Maller, J., Sklar, P., De Bakker, P. I., Daly, M. J., & Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*, *81*(3), 559–575. https://doi.org/10.1086/519795

Rajeevan, H., Soundararajan, U., Kidd, J. R., Pakstis, A. J., & Kidd, K. K. (2012). ALFRED: an allele frequency resource for research and teaching. *Nucleic Acids Research*, *40*(Database issue), D1010–1015. https://doi.org/10.1093/nar/gkr924

Rane, S. G., & Reddy, E. P. (2000). Janus kinases: Components of multiple signaling pathways. *Oncogene*, *19*(49), 5662–5679. https://doi.org/10.1038/sj.onc.1203925

Sansone, P., Storci, G., Tavolari, S., Guarnieri, T., Giovannini, C., Taffurelli, M., Ceccarelli, C., Santini, D., Paterini, P., Marcu, K. B., & Chieco, P. (2007). IL-6 triggers malignant features in mammospheres from human ductal breast carcinoma and normal mammary gland. *J Clin Invest*, *117*(12), 3988–4002. https://doi.org/10.1172/jci32533

Schroeder, A., Herrmann, A., Cherryholmes, G., Kowolik, C., Buettner, R., Pal, S., Yu, H., Müller-Newen, G., & Jove, R. (2014). Loss of androgen receptor expression promotes a stem-like cell phenotype in prostate cancer through STAT3 signaling. *Cancer Research*, *74*(4), 1227–1237. https://doi.org/10.1158/0008-5472.can-13-0594

Troudi, W., Uhrhammer, N., Sibille, C., Dahan, C., Mahfoudh, W., Bouchlaka Souissi, C., Jalabert, T., Chouchane, L., Bignon, Y. J., Ben Ayed, F., & Ben Ammar Elgaaied, A. (2007). Contribution of the BRCA1 and BRCA2 mutations to breast cancer in Tunisia. *Journal of Human Genetics*, *52*(11), 915–920. https://doi.org/10.1007/s10038-007-0195-5

Vaclavicek, A., Bermejo, J. L., Schmutzler, R. K., Sutter, C., Wappenschmidt, B., Meindl, A., Kiechle, M., Arnold, N., Weber, B. H. F., Niederacher, D., Burwinkel, B., Bartram, C. R., Hemminki, K., & Försti, A. (2007). Polymorphisms in the Janus kinase 2 (JAK)/signal transducer and activator of transcription (STAT) genes: putative association of the STAT gene region with familial breast cancer. *Endocrine-Related Cancer*, *14*(2), 267–277. https://doi.org/10.1677/erc-06-0077

Velayos, T., Martínez, R., Alonso, M., Garcia-Etxebarria, K., Aguayo, A., Camarero, C., Urrutia, I., de LaPiscina, I. M., Barrio, R., Santin, I., & Castaño, L. (2017). An activating mutation in STAT3 results in neonatal diabetes through reduced insulin synthesis. *Diabetes*, *66*(4), 1022–1029.

Wang, K., Zhou, B., Zhang, J., Xin, Y., Lai, T., Wang, Y., Hou, Q., Song, Y., Chen, Y., Quan, Y. I., Xi, M., & Zhang, L. (2011). Association of signal transducer and activator of transcription 3 gene polymorphisms with cervical cancer in Chinese women. *DNA and Cell Biology*, *30*(11), 931–936. https://doi.org/10.1089/dna.2010.1179

Wang, N., Wei, L., Huang, Y., Wu, Y., Su, M., Pang, X., Ji, F., Zhong, C., Chen, T., & Li, B. (2017). miR520c blocks EMT progression of human breast cancer cells by repressing STAT3. *Oncology Reports*, *37*(3), 1537–1544. https://doi.org/10.3892/or.2017.5393

Watson, C. J. (2001). Stat transcription factors in mammary gland development and tumorigenesis. *Journal of Mammary Gland Biology and Neoplasia*, *6*(1), 115–127. https://doi.org/10.1023/a:1009524817155

Wu, H., Huang, M., Cao, P., Wang, T., Shu, Y., & Liu, P. (2012). MiR-135a targets JAK2 and inhibits gastric cancer cell proliferation. *Cancer Biology & Therapy*, *13*(5), 281–288. https://doi.org/10.4161/cbt.18943

Yang, J., Chatterjee-Kishore, M., Staugaitis, S. M., Nguyen, H., Schlessinger, K., Levy, D. E., & Stark, G. R. (2005). Novel roles of unphosphorylated STAT3 in oncogenesis and transcriptional regulation. *Cancer Research*, *65*(3), 939–947.

Zhao, H., Wang, Z., Wu, H., Xiao, Q., Yao, W., Wang, E., Liu, Y., & Wei, M. (2015). STAT3 genetic variant, alone and in combination with STAT5b polymorphism, contributes to breast cancer risk and clinical outcomes. *Medical Oncology*, *32*(1), 375. https://doi.org/10.1007/s12032-014-0375-z

Zhu, Z., Wang, S., Zhu, J., Yang, Q., Dong, H., & Huang, J. (2016). MicroRNA-544 down-regulates both Bcl6 and Stat3 to inhibit tumor growth of human triple negative breast cancer. *Biological Chemistry*, *397*(10), 1087–1095. https://doi.org/10.1515/hsz-2016-0104

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Ziadi, W., Boussetta, S., Elkamel, S., Pakstis, A. J., Kidd, K. K., Medimegh, I., Ben Ammar Elgaaied, A., & Cherni, L. (2021). *STAT3* polymorphisms in North Africa and its implication in breast cancer. *Molecular Genetics & Genomic Medicine*, 9, e1744. https://doi.org/10.1002/mgg3.1744