Received: 12 November 2021 | Revised: 4 October 2022 | Accepted: 7 October 2022

DOI: 10.1002/mgg3.2074

Molecular Genetics & Genomic Medicine Open Access WILEY

ORIGINAL ARTICLE

Gene polymorphisms of *cyclin-dependent kinase inhibitor* and *matrix metalloproteinase-9* in Sudanese patients with esophageal squamous cell carcinoma

Majdolin Mohammed Eltayeb[1] | Mohamed M. Ali[1] | Saeed M. Omar[2] | Nouh Saad Mohamed[3] | Ishag Adam[4] | Hamdan Z. Hamdan[5,6]

[1]Faculty of Medicine, Bahri University, Khartoum, Sudan

[2]Faculty of Medicine, Gadarif University, Gadarif, Sudan

[3]Molecular Biology Unit, Sirius Training and Research Centre, Khartoum, Sudan

[4]Department of Obstetrics and Gynecology, Unaizah College of Medicine, Qassim University, Unaizah, Kingdom of Saudi Arabia

[5]Department of Basic Medical Sciences, Unaizah College of Medicine, Qassim University, Unaizah, Kingdom of Saudi Arabia

[6]Department of Biochemistry and Molecular Biology, Faculty of Medicine, Al-Neelain University, Khartoum, Sudan

Correspondence
Majdolin Mohammed Eltayeb, Department of Biochemistry, Faculty of Medicine, Bahri University, Khartoum, Sudan.
Email: majdo.m.eltayeb@gmail.com

Abstract

Background: The polymorphisms of the *cyclin-dependent kinase inhibitor* (*CDKN1A*) gene and *matrix metalloproteinase-9* (*MMP9*) gene may increase one's susceptibility to malignancies. In this study, the association of the single nucleotide polymorphisms (SNPs) *CDKN1A* rs1059234 c.70C>T at the 3′ untranslated region and *MMP9* rs17576 (c.836A>G, p.Gln279Arg) with esophageal squamous cell carcinoma (ESCC) in Sudanese individuals were investigated.

Materials and Methods: A case-control study involving age- and gender-matched groups were conducted in a cancer center in eastern Sudan (Gadarif) between April and October 2020. The case group consisted of ESCC patients, whereas the control group comprised healthy subjects. Polymerase chain reaction-restriction fragment length polymorphism was performed for the genotyping of the *CDKN1A* rs1059234 and *MMP9* rs17576 SNPs. The genotyping results were confirmed by Sanger sequencing.

Results: The genotype distributions for *CDKN1A* rs1059234 and *MMP9* rs17576 were in agreement with the Hardy–Weinberg equilibrium. The variant allele T in *CDKN1* rs1059234 c.70C>T was significantly more prevalent in the ESCC patients than in the healthy controls [51.3% vs. 19.2%; OR = 4.4; 95% CI (2.6–7.4); $p < 0.001$]. Moreover, in *CDKN1A* rs1059234, the genotype TC + TT [76.9% vs. 38.4%; OR = 5.3; 95% CI (2.6–10.7); $p < 0.001$] was more frequent in the cases than in the controls, and it was significantly associated with ESCC risk. In *MMP9* rs17576, the variant allele G was also significantly prevalent in the cases relative to the controls, and it was significantly associated with increased ESCC risk in the cases compared with the controls [27.5% vs. 1.9%; OR = 19.4; 95%CI (5.8–64.1); $p < 0.001$]. Both genotypes containing the allele G (AG + GG) were the most common genotypes in the cases [48.7% vs. 3.8%; OR = 23.7; 95%CI (6.8–81.7); $p < 0.001$], and they significantly increased the risk of ESCC.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
© 2022 The Authors. *Molecular Genetics & Genomic Medicine* published by Wiley Periodicals LLC.

Conclusion: A significant increase in ESCC risk is associated with the SNPs *CDKN1A* rs1059234 and *MMP9* rs17576.

KEYWORDS

cyclin-dependent kinase inhibitor (*CDKN1A*), esophageal squamous cell carcinoma, *matrix metalloproteinase 9* (*MMP9*), single nucleotide polymorphism, Sudan

1 | INTRODUCTION

Esophageal carcinoma (EC) is the eighth most commonly diagnosed cancer worldwide (Malhotra et al., 2017). According to the National Cancer Registry report, the EC incidence rate is 5.8/100,000, making EC the seventh among the top 10 cancers in Sudan (Saeed et al., 2014). EC has two major histopathological subtypes: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma. A geographical variation has been observed between these major subtypes, with ESCC being predominant in south and east African regions, including Sudan (Malhotra et al., 2017). The development of EC has been associated with many risk factors, such as personal habits (e.g., smoking and alcohol consumption), dietary habits (particularly red meat consumption), and genetic susceptibility (Kamangar et al., 2009; Steccanella et al., 2017).

Several genes' variants such as rs4767364 (NC_000012.12:g.112083644 G>A) in the *aldehyde dehydrogenase-2* gene (*ALDH2*) (Chen et al., 2019), rs1229984 (NP_000659.2:p.His48Arg) in *acetaldehyde dehydrogenase* gene (*ADH1B*) (Abnet et al., 2018) and many others (Simba et al., 2019) were found to be associated with EC among the African population. In the Sudanese population in particular, a single study has reported an association of *P53* gene variants with EC (Eltaher et al., 2019). However, up to date, there is no single genome-wide association study (GWAS), or whole-genome sequencing study conducted among Sudanese or even African patients that investigated the association with EC. Therefore, we followed the candidate gene approach in this study, and the selected genes have a prognostic impact on the patient's outcome (Liu et al., 2015).

Human cell growth is well controlled by the cell cycle. The cell cycle may be promoted by oncogenes and tumor suppressor genes; however, they may also arrest cell growth and instead induce apoptosis (Velez & Howard, 2015). Gene polymorphisms in tumor suppressor genes or downstream signaling proteins are well associated with cancer development (Velez & Howard, 2015).

Cyclin-dependent kinase inhibitor-1 (CDN1A) is a downstream signaling protein that is regulated by the P53 protein (guardian of the genome), a tumor suppressor (Sharp et al., 2014). CDN1A expression is markedly increased in response to DNA damage, resulting in cell cycle arrest at the transition between the G_1 and S phases (El-Deiry et al., 1994). It is encoded by the *CDKN1A* gene (ID: 1026, OMIM: 116899), which is located in chromosome number 6 within the cytogenetic band 6p21.2 (El-Deiry et al., 1993). Apparently, a decrease in CDN1A expression may contribute to uncontrolled cellular growth and carcinogenesis (Li et al., 2005; Roninson, 2002). This phenomenon provides the rationale for the association of increased risk of malignancies with the SNP rs1059234 c.70C>T (NC_000006.12:g.36685820 C>T) (Kaya et al., 2022), which is located at the 3′ untranslated region (3′-UTR). Studies have shown that the variant allele T of rs1059234 generates an unstable mRNA, which is susceptible to rapid hydrolysis, leading to low CDN1A expression levels (Amara et al., 1995; Fan et al., 1996).

Tissue remodeling is an important process that enables malignant cells to invade and metastasize to other tissues (Liu et al., 2015). Zinc-containing matrix metalloproteinases (MMPs) are among the protein families that are actively involved in tissue remodeling. A total of 26 MMPs have been identified, and these are coded by different genes in the human genome (Fields, 2015). The *MMP9* gene (ID: 4318, OMIM:120361) is mapped to chromosome 20 and is located within the cytogenetic band 20q12.2-13.1. It is a large gene containing 13 coding exons and 12 non-coding introns (Wu et al., 2013). *MMP9* encodes the matrix metalloproteinase protein-9 (MMP-9), which is an enzyme that catalyzes the degradation of extracellular matrix structures. A report has indicated that MMP is involved in angiogenesis and cancer metastasis (Wieczorek et al., 2012). Meanwhile, SNPs may substitute amino acids in enzyme sequences, and as a result, enzyme activity may be altered. Among the SNPs sequenced from the *MMP9* gene is rs17576 c.836A>G (NC_000020.11:g.46011586A>G) (Hunt et al., 2018), which is a missense mutation wherein glutamine is substituted with arginine p.Gln279Arg. This substitution increases the MMP-9 activity, resulting in increased levels of active enzymes that induce neovascularization and metastasis (Okada et al., 2017; Yang et al., 2014).

Although genetic susceptibility to EC has been studied before (Chen et al., 2021), there is a scarcity of published data on the association of EC with *CDKN1A* rs1059234

and *MMP9* rs17576 (Taghavi et al., 2010). Therefore, this study aimed to investigate the association of these SNPs with EC.

2 | MATERIALS AND METHODS

2.1 | Study subjects

An age- and gender-matched case-control study involving newly diagnosed and untreated EC patients and healthy controls was conducted between April and October 2020. The patients were histologically confirmed as ESCC cases at a cancer center in Eastern Sudan (Gadarif). The controls were recruited from the same community and were matched for age and gender. The participants were interviewed after they have signed informed consent. Data were collected using a questionnaire, which were filled out by the investigators after they have interviewed the participants. The questionnaire collects the participants' socio-demographic data (age, gender, weight, height, and place of residence), personal habits (smoking history and alcohol consumption), and clinical data (symptoms and tissue diagnosis). Then, peripheral venous blood (5 ml) was collected in EDTA-containing vacutainer tubes; DNA was immediately extracted, and the aliquot was stored at −20°C until genotyping through polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) analysis.

2.2 | DNA extraction

Genomic DNA was extracted from peripheral blood leucocytes by a salting-out procedure. In brief, 5 ml of blood was mixed with a lysis buffer (0.32 M sucrose, 1% TritonX-100, 5 mM $MgCl_2$, 10 mM Tris–HCl, pH 7.5). Leucocytes were spun down and washed with H_2O; the pellet was incubated with proteinase K at 37°C and subsequently salted out at 4°C using a saturated NaCl solution. The precipitated proteins were removed by centrifugation, and the DNA was precipitated with ethanol.

2.3 | SNP selection and genotyping

The genotypes for *CDKN1A* rs1059234 and *MMP9* rs17576 were determined by PCR-RFLP, as described previously (Huang et al., 2004; Wu et al., 2013). The PCR reaction mixtures (25 μl) for the *CDKN1A* (NG_009364.1) and *MMP9* (NG_011468.1) genes consisted of 0.1 μM each of the forward and reverse primers, 100 μM each of the dNTPs, 0.5 U *Taq polymerase* (i-Taq iNtRON Biotechnology, Korea), and 2 μl of the extracted DNA. The PCR conditions for *CDKN1A* rs1059234 were as follows: an initial denaturation step at 95°C for 5 min followed by 35 cycles of denaturation at 96°C for 30 s, annealing at 65°C for 30 s, an extension at 72°C for 30 s, and a final extension at 72°C for 6 min. This reaction yielded an amplicon product of 298 bp. This product was digested by (*Pst1*, Thermo Scientific) after overnight incubation at 37°C. For *MMP9* rs17576, the PCR reactions were as follows: an initial denaturation step at 95°C for 5 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 66°C for 1 min, an extension at 72°C for 1 min, and a final extension at 72°C for 10 min. This reaction yielded a PCR product of 300 bp, which was subjected to digestion by (*MSPI*, Thermo Scientific) after overnight incubation at 37°C. The digestion products for both SNPs are detailed in Table S1.

The digested products were visualized by running them in 2.5% gel electrophoresis and staining them with ethidium bromide.

The genotypes of the two SNPs were sequenced to confirm the PCR-RFLP results by using the Sanger deoxyribonucleic acid sequencing method. Sequencing was provided by the BGI Company in China.

The sample size of 78 was calculated based on a 1:1 case-to-control ratio and based on the predicted difference in the rate of *MMP9* rs17576 polymorphism between the cases and the controls. Based on a previous report on EC in Sudan (Eltaher et al., 2019), we assumed that the investigated SNPs would be present in at least 30% of the EC cases and 10% of the controls, giving this study an 80% power to detect differences at the α-level of 0.05. The sample size was calculated using the OpenEpi calculator (OpenEpi, n.d.)

2.4 | Statistics

The collected data were entered into SPSS (Statistical Package for Social Science) version 22.0 for Windows for data analysis. Continuous data (age) were checked for normality using the Shapiro–Wilk test. The clinical data for the two groups were compared using the Mann–Whitney *U* test (for not normally distributed data) and Pearson's chi-square (χ^2) test for continuous and categorical data. The observed and expected genotype distributions and their agreement with the Hardy–Weinberg equilibrium (HWE) were assessed by using the chi-square (χ^2) goodness-of-fit test. Differences in allele frequencies between the EC patients and the controls were compared using Pearson's chi-square test. The risk associated with a specific genotype was estimated using the Chi-square or

Fisher's exact test and using odds ratios (ORs), and it is expressed with a 95% confidence level. p-values of <0.05 were considered significant.

3 | RESULTS

The *CDKN1A* rs1059234 and *MMP9* rs17576 SNPs were successfully genotyped in 78 EC patients and 78 controls. These two groups were well-matched in terms of age and gender. The mean (SD) age was 60.0 (18.0) years for the cases and 58.0 (13.0) years for the controls ($p = 0.687$). The number of individuals with first-degree relatives who have had cancers and with a history of being alcoholic was significantly higher in the patient group than in the control group (Table 1).

The *CDKN1A* rs1059234 polymorphism was significantly associated with EC (OR = 5.3; 95% CI = 2.6–10.7; $p < 0.001$). The proportion of heterozygotes (TC) was significantly higher in the patient group (51.3%) than in the control group (38.5%) ($p < 0.001$; OR = 3.5; 95%CI = 1.7–7.3). The proportion of T allele was also significantly higher in the patient group than in the healthy control group (51.3% vs. 19.2%; OR = 4.4; 95% CI = 2.6–7.4; $p < 0.001$) (Table 2).

Similarly, the *MMP9* rs17576 polymorphism was significantly associated with EC (OR = 23.7; 95% CI = 6.8–81.7; $p < 0.001$). The proportion of heterozygotes (AG) was also significantly higher in the patient group (42.3%) than in the control group (3.8%) ($p < 0.001$; OR = 20.6; 95% CI = 5.95–71.46). Additionally, the proportion of the G allele was significantly higher in the patient group than in the healthy control group (27.5% vs. 1.9%; OR = 19.4; 95% CI = 5.8–64.1; $p < 0.001$) (Table 3).

The distribution of the genotypes and alleles of *MMP9* (rs17576) (goodness of fit $\chi^2 = 1.050$, $df = 1$, $p = 0.305$) and *CDKN1A* (rs1059234) (goodness of fit $\chi^2 = 0.045$, $df = 1$, $p = 0.830$) was consistent with the HWE.

4 | DISCUSSION

To our best knowledge, this study is the first to investigate the association of the gene polymorphisms of *MMP9* and *CDKN1A* with ESCC in the African–Sudanese population. The major finding of this study is that there is a significant association of *CDKN1A c.70C>T* rs1059234 with ESCC development. This finding is contrary to that reported by Taghavi et al., who investigated Iranian ESCC patients (Taghavi et al., 2010). Taghavi and colleagues have found smoking as the major risk factor for ESCC, and it may have a synergistic interaction with *CDKN1A* rs1059234, leading to the development of ESCC. This gene-environment interaction was not investigated in our study. Another factor that may have contributed to this disagreement is the ethnic group variations between the African–Sudanese patients and the Iranian patients, who belong to the Persian ethnic group. The dominant genotype in Iranian patients was homozygous CC (wild genotype), whereas TT (variant genotype) was dominant in our study population. Of note, the exact chromosomal location of the c.70C>T polymorphism is just 20 nucleotides after the stop codon. This is a critical position in the 3′-UTR of the gene containing the variant allele T, as the occurrence of a mutation in this region results in a physiologically unstable mRNA that is prone to rapid degradation; consequently, CDKN1A levels are affected and ultimately

TABLE 1 Clinical characteristics of esophageal cancer (EC) patients and healthy controls from eastern Sudan (2020)

Variables	EC patients ($n = 78$) frequency (%)	Controls ($n = 78$) frequency (%)	p-value
Gender			
Female	40 (51.2)	39 (50.0)	0.872
Male	38 (48.8)	39 (50.0)	
Smoking			
No	68 (87.2)	70 (89.7)	0.803
Yes	10 (12.8)	8 (10.3)	
Alcohol			
No	69 (88.5)	77 (98.7)	0.018*
Yes	9 (11.5)	1 (1.3)	
Family history of cancer			
No	69 (88.5)	78 (100)	0.003
Yes	9 (11.5)	0 (0)	

*p-value <0.05.

TABLE 2 Genotype and allele frequencies for the *CDKN1A* rs 1059234 gene polymorphism in esophageal cancer patients and healthy controls from eastern Sudan (2020)

Genotypes	Patients (*n* = 78) frequency (%)	Controls (*n* = 78) frequency (%)	OR (95% CI)	*p*-value
CC	18 (23.1)	48 (61.5)	Reference	
TC	40 (51.3)	30 (38.5)	TC vs. CC 3.5 (1.7–7.3)	<0.001***
TT	20 (25.6)	0 (0)	TT vs. CC 53.3 (6.6–426.5)	<0.001***
TC + TT	60 (76.9)	30 (38.4)	TC + TT vs. CC 5.3 (2.6–10.7)	<0.001***
Allele C	76 (48.7)	126 (80.7)	Reference	<0.001***
Allele T	80 (51.3)	30 (19.2)	T vs. C 4.4 (2.6–7.4)	

****p*-value <0.001.

TABLE 3 Genotype and allele frequencies for *MMP9* rs17576 gene polymorphism in esophageal cancer patients and healthy controls from eastern Sudan (2020)

Genotypes	Patients (*n* = 78) frequency (%)	Controls (*n* = 78) frequency (%)	OR (95% CI)	*p*-value
AA	40 (51.2)	75 (96.3)	Reference	
AG	33 (42.3)	3 (3.8)	AG vs. AA 20.6 (5.95–71.46)	<0.001***
GG	5 (6.4)	0 (0)	GG vs. AA 10.54 (1.18–93.5)	<0.001***
AG + GG	38 (48.7)	3 (3.8)	AG + GG vs. AA 23.7 (6.8–81.7)	<0.001***
Allele A	113 (72.4)	153 (98.1)	Reference	<0.001***
Allele G	43 (27.5)	3 (1.9)	G vs. A 19.4 (5.8–64.1)	

****p*-value <0.001.

cells' ability to divide, grow, and undergo apoptosis is reduced (Amara et al., 1995; Fan et al., 1996). Another finding of this study is that the minor allele frequency (MAF) for the variant allele was $T = 0.352$ in our population, and it is associated with ESCC development, as observed in this study. This allele frequency is far from $T = 0.099$ that was reported by Taghavi et al. However, according to the 1000 Genomes Project, the MAF among African populations is generally $T = 0.23$, and that among the Gambian populations is $T = 0.31$, which is considered close to the frequency we reported (Hunt et al., 2018). Such MAF variations are not astonishing, as African populations are well known as having a considerably high intra- and inter-African population diversity (Bereir et al., 2003). Interestingly, the highest MAF reported in the 1000 Genomes Project is $T = 0.50$, which was observed in the Chinese Han population; this ethnic group is found in Southeast Asia, where the highest frequency of ESCC has been reported, followed by the East African region (Malhotra et al., 2017).

In this study, we observed a significant association between the *MMP9* rs17576 p.Gln279Arg and ESCC development. The AG and GG genotypes, which possess the variant allele G, were significantly higher in the cases than in the controls. To date, no published studies have investigated the role of rs17576 p.Gln279Arg in EC. However, many studies have investigated this polymorphism in association with gastric and other types of malignancy (Oliveira et al., 2020; Tang et al., 2008). Our finding is consistent with that of Tang and colleagues, who have found a significant association between rs17576 and gastric cancer (Tang et al., 2008). It is worth mentioning that this missense mutation leads to the replacement of uncharged glutamine with a positively charged arginine. Structurally, arginine is a huge amino acid compared with glutamine. Moreover, this substitution occurs at position 279 in the MMP-9 protein sequence; this position is functionally important as it is located within the catalytic domain of the enzyme MMP-9, and it bears the enzyme active site for its substrate (Zhang et al., 1999). Biophysically, the variant residue 279R forms strong salt bridges with the 280D residue through its side chains. The formation of such a bridge will change the 3D conformation of the enzyme and hence the activity of the substrate-binding cleft. These changes do not occur in the wild-type 279Q residue (Tang et al., 2008). Perhaps, this evidence may provide the rationale for the observed overactivity of MMP-9 in patients suffering from different types of cancer.

The frequency of the variant allele G, which is also the minor allele, was significantly higher in the cases than in the controls ($G = 0.275$ vs. $G = 0.019$). However, the overall MAF in this study is $G = 0.147$, which is lower than that reported for the African population ($G = 0.34$) in the

1000 Genomes Project, and it is nearly half that reported in the Gambian population (G = 0.27). This variation is expected, as the African population is considerably diverse, and the Sudanese population is not an exception (Bereir et al., 2003).

To the best of our knowledge, this is the first African study to investigate the association of rs17576 and rs1059234 with the development of EC. The variant alleles in the investigated SNPs were found to be significantly associated with ESCC. Our current findings are valuable to academics, clinical oncologists, and molecular oncologists. Our results are in addition to the current African literature on EC. Moreover, our results might help in the screening and stratification of cases with a poor-prognosis and poor clinical outcome. Furthermore, it consolidates the current understanding of the association of the investigated SNPs with EC, and this could be lay-out a base for a targeted molecular drug. However, for a better understanding of our results, some limitations should be mentioned. First, this study employed the case-control study design, so causality could not be determined. Second, we did not assess the expression level of *CDKN1A* or *MMP9* or their enzymatic activity, which would affirm the functional impact of the investigated SNPs. Therefore, further studies employing cohort design that evaluate the expression level and enzymatic activity for *CDKN1A* and *MMP9* are warranted.

AUTHOR CONTRIBUTIONS

Majdolin Mohammed Eltayeb, Mohamed M. Ali, and Nouh Saad Mohamed carried out the study and participated in the statistical analysis and procedures. Majdolin Mohammed Eltayeb and Nouh Saad Mohamed carried out the practical part of the study. Hamdan Z. Hamdan and Ishag Adam coordinated and participated in the design of the study, statistical analysis, and drafting of the manuscript. All the authors read and approved the final version.

ACKNOWLEDGMENT

The authors thank all the study participants.

CONFLICT OF INTEREST

All authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The dataset generated and/or analyzed during the current study are available upon reasonable request from the corresponding author.

ETHICS STATEMENT

This study received ethical clearance from the Ethics Review Board, Faculty of Medicine, Bahri University, Sudan. All participants signed informed consent prior to enrolment.

ORCID

Hamdan Z. Hamdan https://orcid.org/0000-0001-9269-8239

REFERENCES

Abnet, C. C., Arnold, M., & Wei, W. Q. (2018). Epidemiology of esophageal squamous cell carcinoma. *Gastroenterology*, *154*, 360–373. https://doi.org/10.1053/J.GASTRO.2017.08.023

Amara, F., Chen, F., & Wright, J. (1995). Defining a novel cis element in the 3′-untranslated region of mammalian ribonucleotide reductase component R2 mRNA: Role in transforming growth factor-beta 1 induced mRNA stabilization. *Nucleic Acids Research*, *23*, 1461–1467. https://doi.org/10.1093/NAR/23.9.1461

Bereir, R., Mohamed, H., Seielstad, M., el Hassani, A. M., Khalil, E. A., Peacock, C. S., Blackwell, J. M., & Ibrahim, M. E. (2003). Allele frequency and genotype distribution of polymorphisms within disease-related genes is influenced by ethnic population sub-structuring in Sudan. *Genetica*, *119*, 57–63. https://doi.org/10.1023/A:1024486716497

Chen, H., Xu, X., Hua, C., Zhang, H., Jian, J., Ge, T., Xie, J., & Yu, Z. (2021). Polymorphisms of matrix metalloproteinases affect the susceptibility of esophageal cancer: Evidence from 20412 subjects, systematic review and updated meta-analysis. *Medicine (Baltimore)*, *100*, e27229. https://doi.org/10.1097/MD.0000000000027229

Chen, W. C., Bye, H., Matejcic, M., Amar, A., Govender, D., Khew, Y. W., Beynon, V., Kerr, R., Singh, E., Prescott, N. J., Lewis, C. M., Babb de Villiers, C., Parker, M. I., & Mathew, C. G. (2019). Association of genetic variants in CHEK2 with oesophageal squamous cell carcinoma in the south African black population. *Carcinogenesis*, *40*, 513–520. https://doi.org/10.1093/CARCIN/BGZ026

El-Deiry, W. S., Harper, J. W., O'Connor, P. M., Velculescu, V. E., Canman, C. E., Jackman, J., Pietenpol, J. A., Burrell, M., Hill, D. E., & Wang, Y. (1994). WAF1/CIP1 is induced in p53-mediated G1 arrest and apoptosis. *Cancer Research*, *54*, 1169–1174.

El-Deiry, W. S., Tokino, T., Velculescu, V., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., & Vogelstein, B. (1993). WAF1, a potential mediator of p53 tumor suppression. *Cell*, *75*, 817–825. https://doi.org/10.1016/0092-8674(93)90500-P

Eltaher, S. M., Idris, A. B., Mahmoud, A. H., Yousif, M. Y. M., Mohamed, N. S., Abdel Hamid, M. M., Elsiddig, K. E., Hassan, M. A., & Yousif, G. M. (2019). Genetic analysis of TP53 gene mutations in exon 4 and exon 8 among esophageal cancer patients in Sudan. *bioRxiv*, 572214. https://doi.org/10.1101/572214

Fan, H., Villegas, C., Huang, A., & Wright, J. A. (1996). Suppression of malignancy by the 3′ untranslated regions of ribonucleotide reductase R1 and R2 messenger RNAs. *Cancer Research*, *56*, 4366–4369.

Fields, G. B. (2015). New strategies for targeting matrix metalloproteinases. *Matrix Biology*, *44–46*, 239–246. https://doi.org/10.1016/J.MATBIO.2015.01.002

Huang, S.-P., Wu, W.-J., Chang, W.-S. W., Wu, M. T., Chen, Y. Y., Chen, Y. J., Yu, C. C., Wu, T. T., Lee, Y. H., Huang, J. K., & Huang, C. H. (2004). p53 codon 72 and p21 codon 31 polymorphisms in prostate cancer. *Cancer Epidemiology, Biomarkers & Prevention*, *13*, 2217–2224.

Hunt, S., McLaren, W., Gil, L., Thormann, A., Schuilenburg, H., Sheppard, D., Parton, A., Armean, I. M., Trevanion, S. J., Flicek, P., & Cunningham, F. (2018). Ensembl variation resources. *Database: The Journal of Biological Databases and Curation*, *2018*, bay119. https://doi.org/10.1093/DATABASE/BAY119

Kamangar, F., Chow, W.-H., Abnet, C. C., & Sanford, M. D. (2009). Environmental causes of esophageal cancer. *Gastroenterology Clinics of North America*, *38*, 27–57. https://doi.org/10.1016/J.GTC.2009.01.004

Kaya, Z., Karan, B. M., & Almalı, N. (2022). CDKN1A (p21 gene) polymorphisms correlates with age in esophageal cancer. *Molecular Biology Reports*, *49*, 249–258. https://doi.org/10.1007/S11033-021-06865-1

Li, G., Liu, Z., Sturgis, E. M., Shi, Q., Chamberlain, R. M., Spitz, M. R., & Wei, Q. (2005). Genetic polymorphisms of p21 are associated with risk of squamous cell carcinoma of the head and neck. *Carcinogenesis*, *26*, 1596–1602. https://doi.org/10.1093/CARCIN/BGI105

Liu, Y., Liu, H., Luo, X., Deng, J., Pan, Y., & Liang, H. (2015). Overexpression of SMYD3 and matrix metalloproteinase-9 are associated with poor prognosis of patients with gastric cancer. *Tumor Biology*, *366*(36), 4377–4386. https://doi.org/10.1007/S13277-015-3077-Z

Malhotra, G. K., Yanala, U., Ravipati, A., Follet, M., Vijayakumar, M., & Are, C. (2017). Global trends in esophageal cancer. *Journal of Surgical Oncology*, *115*, 564–579. https://doi.org/10.1002/JSO.24592

Okada, R., Naito, M., Hattori, Y., Seiki, T., Wakai, K., Nanri, H., Watanabe, M., Suzuki, S., Kairupan, T. S., Takashima, N., Mikami, H., Ohnaka, K., Watanabe, Y., Katsuura-Kamano, S., Kubo, M., Hamajima, N., Tanaka, H., & Japan Multi-institutional Collaborative Cohort Study Group. (2017). Matrix metalloproteinase 9 gene polymorphisms are associated with a multiple family history of gastric cancer. *Gastric Cancer*, *20*, 246–253. https://doi.org/10.1007/S10120-016-0608-2

Oliveira, V., Chagas, D., Amorim, J., Pereira, R. D., Nogueira, T. A., Borges, V. M., Campos-Verde, L. M., Martins, L. M., Rodrigues, G. P., Nery Júnior, E. D., & Sampaio, F. A. (2020). Association between matrix metalloproteinase-9 gene polymorphism and breast cancer in Brazilian women. *Clinics (São Paulo, Brazil)*, *75*, 1–5. https://doi.org/10.6061/CLINICS/2020/E1762

OpenEpi Menu. (n.d.). http://www.openepi.com/Menu/OE_Menu.htm

Roninson, I. B. (2002). Oncogenic functions of tumour suppressor p21(Waf1/Cip1/Sdi1): Association with cell senescence and tumour-promoting activities of stromal fibroblasts. *Cancer Letters*, *179*, 1–14. https://doi.org/10.1016/S0304-3835(01)00847-3

Saeed, I. E., Weng, H.-Y., Mohamed, K. H., & Mohammed, S. I. (2014). Cancer incidence in Khartoum, Sudan: First results from the cancer registry, 2009–2010. *Cancer Medicine*, *3*, 1075–1084. https://doi.org/10.1002/CAM4.254

Sharp, A. N., Heazell, A. E. P., Baczyk, D., Dunk, C. E., Lacey, H. A., Jones, C. J. P., Perkins, J. E., Kingdom, J. C. P., Baker, P. N., & Crocker, I. P. (2014). Preeclampsia is associated with alterations in the p53-pathway in villous trophoblast. *PLoS ONE*, *9*, e87621. https://doi.org/10.1371/JOURNAL.PONE.0087621

Simba, H., Kuivaniemi, H., Lutje, V., Tromp, G., & Sewram, V. (2019). Systematic review of genetic factors in the etiology of esophageal squamous cell carcinoma in african populations. *Frontiers in Genetics*, *10*, 642. https://doi.org/10.3389/FGENE.2019.00642/FULL

Steccanella, F., Costanzo, A., & Petrelli, F. (2017). Editorial on role of p53 in esophageal cancer from a meta-analysis of 16 studies by fisher *et al*. *Journal of Thoracic Disease*, *9*, 1450–1452. https://doi.org/10.21037/JTD.2017.05.72

Taghavi, N., Biramijamal, F., Abbaszadegan, M. R., Khademi, H., Sotoudeh, M., & Khoshbakht, S. (2010). P21(waf1/cip1) gene polymorphisms and possible interaction with cigarette smoking in esophageal squamous cell carcinoma in northeastern Iran: A preliminary study. *Archives of Iranian Medicine*, *13*, 235–242.

Tang, Y., Zhu, J., Chen, L., Chen, L., Zhang, S., & Lin, J. (2008). Associations of matrix metalloproteinase-9 protein polymorphisms with lymph node metastasis but not invasion of gastric cancer. *Clinical Cancer Research*, *14*, 2870–2877. https://doi.org/10.1158/1078-0432.CCR-07-4042

Velez, A. M. A., & Howard, M. S. (2015). Tumor-suppressor genes, cell cycle regulatory checkpoints, and the skin. *North American Journal of Medical Sciences*, *7*, 176–188. https://doi.org/10.4103/1947-2714.157476

Wieczorek, E., Reszka, E., Gromadzinska, J., & Wasowicz, W. (2012). Genetic polymorphism of matrix metalloproteinases in breast cancer. *Neoplasma*, *59*, 237–247. https://doi.org/10.4149/NEO_2012_031

Wu, H., Bai, X., Chen, D., Cao, H. Y., & Qin, L. (2013). Association of genetic polymorphisms in matrix metalloproteinase-9 and coronary artery disease in the Chinese Han population: A case-control study. *Genetic Testing and Molecular Biomarkers*, *17*, 707–712. https://doi.org/10.1089/GTMB.2013.0109

Yang, B., Tang, F., Zhang, B., Zhao, Y., Feng, J., & Rao, Z. (2014). Matrix metalloproteinase-9 overexpression is closely related to poor prognosis in patients with colon cancer. *World Journal of Surgical Oncology*, *12*, 24. https://doi.org/10.1186/1477-7819-12-24

Zhang, B., Henney, A., Eriksson, P., Hamsten, A., Watkins, H., & Ye, S. (1999). Genetic variation at the matrix metalloproteinase-9 locus on chromosome 20q12.2-13.1. *Human Genetics*, *105*, 418–423. https://doi.org/10.1007/S004390051124

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Eltayeb, M. M., Ali, M. M., Omar, S. M., Mohamed, N. S., Adam, I., & Hamdan, H. Z. (2022). Gene polymorphisms of *cyclin-dependent kinase inhibitor* and *matrix metalloproteinase-9* in Sudanese patients with esophageal squamous cell carcinoma. *Molecular Genetics & Genomic Medicine*, *10*, e2074. https://doi.org/10.1002/mgg3.2074