Gene Reports 39 (2025) 102174

Contents lists available at ScienceDirect

Gene Reports

journal homepage: www.elsevier.com/locate/genrep

Somatic mutation profiles in non-tobacco smoking and non-alcohol drinking South African female esophageal squamous cell carcinoma patients of African ancestry

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ARTICLE INFO

Edited by Dominic Voon

Keywords:
Esophageal cancer
African ancestry
Whole exome sequencing

ABSTRACT

Background: Esophageal squamous cell carcinoma (ESCC) accounts for 85 % of the global esophageal cancer incidence, with a high disease burden in Eastern and Southern Africa. The etiology of ESCC is complex, with several factors such as tobacco smoking and alcohol consumption contributing to disease risk. Somatic mutations involved in the development of ESCC have been described but are under-studied in Africa. Our study reports the first whole-exome sequencing analysis of ESCC in a South African population who were not exposed to tobacco or alcohol.

Methods: Fifteen female patients of African ancestry with ESCC who had never smoked or consumed alcohol were enrolled. Demographic and epidemiological data were obtained. DNA was extracted from matched blood and tumor tissue and subjected to whole exome sequencing. Bioinformatic analysis was used to identify somatic mutations, somatic copy number variations, and identify mutation signatures.

Results: The genes *TP53*, *MUC2*, *KMT2D*, and *KMT2C* were the most commonly mutated among genes previously reported to be mutated in ESCC tumors from other populations. The most common copy number variations detected were amplifications of chromosome 11q13, containing the *CCND1* gene; 3q26, containing *PIK3CA* and *SOX2*; and 8q24, containing *MYC*. The most common mutation signature detected in the tumors was SBS5, associated with aging.

Conclusions: Despite the lack of two major risk factors for ESCC in our patients, DNA sequencing detected somatic mutation profiles comparable to those found in patients from other populations, suggesting similar molecular pathways. This may stimulate their access to new therapeutic approaches developed in high-income countries.

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https://doi.org/10.1016/j.genrep.2025.102174
Received 19 November 2024; Received in revised form 8 February 2025; Accepted 16 February 2025
Available online 20 February 2025

1. Introduction

Esophageal squamous cell carcinoma (ESCC) is common in Eastern and Southern Africa, where tobacco and alcohol are known risk factors (Morgan et al., 2022). The molecular etiology of ESCC is heterogeneous, with multiple somatic mutations implicated (Murphy et al., 2017).

While multiple factors have been implicated in the development of ESCC, tobacco and alcohol are considered the strongest environmental risk factors, and the two factors combined have been shown to have a synergistic effect (Businello et al., 2020). Despite this, a large number of female patients from Africa, diagnosed with ESCC, have no history of consumption of either (Ferndale et al., 2020). This lack of exposure to these well-known carcinogens has been observed in other high-incidence areas (Tran et al., 2005). The reasons for this observation are unknown, but genomic factors may play a role.

We investigated the somatic mutation profiles of a subgroup of female patients with ESCC with no exposure to tobacco and alcohol.

2. Methods

Female patients with no history of tobacco use or alcohol consumption, with a histologically diagnosed ESCC, presenting to Grey's Hospital, Pietermaritzburg, South Africa, were enrolled in this study. None of the patients in the cohort had any co-morbidities or family history of esophageal cancer. Data collection and bio-sample processing are described in Ferndale et al. (Ferndale et al., 2022). Detailed methodology is available in the Supplementary Methods.

In brief, whole-exome sequencing was performed on DNA libraries prepared from tumor and germline DNA using the Agilent SureSelect Human All Exon V6 kit (Agilent, USA). The libraries were quantified by Qubit (Thermofisher, USA) and sized by Bioanalyzer (Agilent, USA). Quantified libraries were pooled and sequenced on the Illumina NovaSeq (Illumina, USA) with the PR150 strategy, with a target sequencing depth of 100× for tumor DNA and 50× for germline DNA.

Raw unmapped reads that passed quality checks were mapped to GRCh38 using Burrow-Wheeler Aligner (BWA-MEM) (v0.7.17) (Li and Durbin, 2009) SAMtools (v1.8) was used for sorting the BAM output files and Picard was used to mark duplicate reads (Danecek et al., 2021). GATK germline short variant discovery workflow (v4.0) was used for germline variant discovery. MuTect (v1.1.4) was used for somatic SNV discovery and Strelka (v2.9.4) was used for somatic Indel dis. Tumor mutation burdens (TMB) were compared against 33 datasets from The Cancer Genome Atlas (TCGA) covery (Sougnez et al., 2013; Kim et al., 2018). Control-FREEC (v11.4) was used for somatic CNV discovery (Boeva et al., 2012). SNVs and Indels with a minimum sequencing depth of 10× or more were retained for further analysis. Mutant variant allele frequencies (MAF) were calculated as the number of mutant-allele reads divided by the total number of reads for each variant. Variants with an MAF of 10 % or more were retained for analysis.

ANNOVAR (2015Dec14) was used for variant annotation (Wang et al., 2010). Mutation annotations were visualized using Maftools (v2.14.0) (Li and Durbin, 2009) using the Maftools package (Mayakonda et al., 2018). Somatic mutations were defined as protein-truncating SNVs and/or Indel where MAF $\geq$ 0.1, not present in germline DNA. A two-sided Wilcoxon rank-sum test was used to evaluate whether the tumor mutation burdens of our ESCC tumors differed from the TCGA-esophageal cancer dataset (TCGA-ESCA). Mutation signature profile for each sample were fitted into known Single-Base Substitution (SBS96), Doublet-Base Substitution (DINUC), and Small Insertion and Deletion (ID) Signatures, as defined by the COSMIC Mutational Signatures classification (https://cancer.sanger.ac.uk/signatures/). The best fitting mutation signatures were assigned per sample using SigProfiler-Assignment (v0.1.4) (Islam et al., 2022). The study was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (UKZN), Durban, South Africa (Certificate number BF270/15), and the Human Research Ethics Committee (Medical) at the University of the Witwatersrand (Certificate number M170871).

3. Results

Fifteen patients with a median age of 63 (IQR: 60–73) were enrolled. Demographic and clinical data are shown in Supplemental Table 1.

The average total effective yield was 11,599.57 Mb (SD. 3008.29) and 7221.84 Mb (SD. 714.54 Mb) per sample for tumor and blood DNA whole exome sequencing (WES), respectively. The most common type of somatic mutations identified were single nucleotide missense mutations, with C>T changes being the predominant type. The median number of mutations observed per sample was 101 (IQR: 62–280) (Supplemental Fig. 1).

The most commonly mutated genes were *SPATA31D1* in 60 % of patients and *TP53* in 53 % of patients (Fig. 1). *TP53*, *MUC2*, *KMT2D*, and *KMT2C* were the most commonly mutated genes among those previously described in ESCC (Supplemental Fig. 2 & Supplemental Table 2). Five of the patients had mutations in one or more members of the *FAT* gene family. Two patients' tumors did not carry somatic mutations in the top 22 genes, as described in the remaining 13 tumors.

Regions of the genome known to be amplified or deleted frequently in ESCC from other populations were assessed and are presented in Supplemental Fig. 3. Eleven of the 15 tumors (73 %) contained CNVs in one or more of these regions.

The most common SBS96 signature was SBS5, of unknown etiology. SBS5 was assigned to 14/15 tumors as the top SBS96 signature with cosine similarity ranging from 0.82 to 0.97 (Fig. 2). One tumor was assigned the SBS40b signature (cosine similarity: 0.93) as its top SBS96 signature. DBS signatures were assigned to 12/15 tumors with DBS17 present in 7 of 12. However, most DBS had low cosine similarities and high L2 errors. ID signatures were assigned to all 15 tumors, but similarly to DBS signatures, large variations in cosine similarities were observed. (Fig. 2).

4. Discussion

To our knowledge, this is the first report of an exome-wide study of the genes mutated in ESCC from a female, non-smoking, and non-drinking South African population. Previous studies investigated candidate genes and copy number changes (Brown et al., 2020; Moodley et al., 2007; Gamieldien et al., 1998).

Although the TMB in our study was lower than the esophageal cancer data in The Cancer Genome Atlas, the TCGA data included both squamous cell carcinoma and adenocarcinoma. The wide variability in TMB in our cohort has been found in other studies (Erkizan et al., 2021; Liu et al., 2016) and may be typical for ESCC.

The most common type of somatic, C>T substitution, in our study, is consistent with results from other studies on ESCC genomics (Erkizan et al., 2021). It is related to spontaneous cytosine deamination, a known mutagenic process in ESCC (Barnes and Lindahl, 2004; Song et al., 2014). The significance of the high mutation rate in the *SPATA31D1* gene is unclear since this gene is only expressed in testes tissue (Cancer Genome Atlas Research Network T, 2017).

The frequency of *TP53* mutations in our study (53 %) was low compared to many other studies of ESCC, although it was also relatively low in other studies from Africa (Brown et al., 2020). The mutations like *KMT2D*, *KMT2C*, and the *NOTCH* and *FAT* gene families are consistent with other studies (Liu et al., 2016; Gao et al., 2014; Van Loon et al., 2018).

Our finding that amplification of chromosome 11q13.3, which contains the *CCND1* gene, was the most frequent CNV in our study is consistent with previous studies on South African ESCC patients (Brown

et al., 2020). *CCND1* gene is a key regulator of cell cycle progression through its interaction with CDK4/6 (Montalto and Amicis, 2020). *CCND1* amplification is associated with increased proliferation and has been implicated in resistance to endocrine therapy in hormone receptor-positive breast cancer (Montalto and Amicis, 2020). Given the clinical relevance, CDK4/6 inhibitors (e.g., palbociclib, ribociclib, abemaciclib) have been developed to target this pathway, demonstrating significant efficacy in advanced breast cancer (Braal et al., 2021). Moreover, since 11q13 amplifications frequently co-occur with *PIK3CA* mutations, combination therapies with PI3K inhibitors (e.g., alpelisib) may provide an enhanced therapeutic benefit (Chang et al., 2021). Future studies should explore the predictive value of *CCND1* amplification for response to CDK4/6 inhibitors and the potential for rational combination strategies to overcome resistance (Ying et al., 2012) Several tumors in our study had copy number losses at chromosomes 3p and 9p, which is consistent with findings in the Malawian study (Liu et al., 2016). The overlap of mutated genes and CNVs between our patients and other populations suggests that progression from dysplasia to carcinoma may involve similar molecular pathways.

The SBS5 signature, which was the most common mutational signature in our patients, is commonly associated with ESCC in a global, multicentre study of mutational signatures in ESCC (Moody et al., 2021). The etiology of SBS5 is unknown but it is a shared signature between patients from the two regions where ESCC is most common, the Asian esophageal cancer belt and the African esophageal cancer corridor (Liu et al., 2016; Schaafsma et al., 2015). In a global study of 552 ESCC cases, SBS5 was among the top six COSMIC reference signatures that were responsible for >80 % of the average ESCC mutation burden. SBS5 and SBS40, both thought to be age-related were present in 76 % of the cases and on average accounted for 38 % of the mutation burden (Moody et al., 2021).

The presence of the SBS92 signature, associated with tobacco smoking, suggests that despite our cohort not smoking tobacco or its related products, they may have been exposed to tobacco smoke. A possible connection between mutation signatures and exposure to cooking fires has not yet been established, but it is worthy of further investigation.

The main limitations of this study were sample size and lack of data on other risk factors for ESCC, particularly exposure to indoor wood fire smoke. These limitations make it difficult to draw firm conclusions and apply the data to other populations with ESCC. A follow-up study, with a larger sample size and data on additional risk factors for ESCC is currently underway.

5. Conclusions

Although our patients were not exposed to some of the established risk factors for ESCC, there appears to be significant overlap in the molecular pathways involved in our patients and other populations. Thus, if high-income countries develop targeted therapies for some of these shared molecular pathways, it is possible that local patients with known genomic profiles could be enrolled in clinical trials of such treatments. This would go some way to redress the degree to which populations of African ancestry are underserved regarding access to new therapeutic advances in cancer.

Abbreviations

ESCC	Esophageal squamous cell carcinoma
DNA	deoxyribonucleic acid
CNV	copy number variant
TMB	Tumor mutation burden
TCGA	The Cancer Genome Atlas
SBS	Single base substitution
ID	insertion deletion
DINUC	Dinucleotide

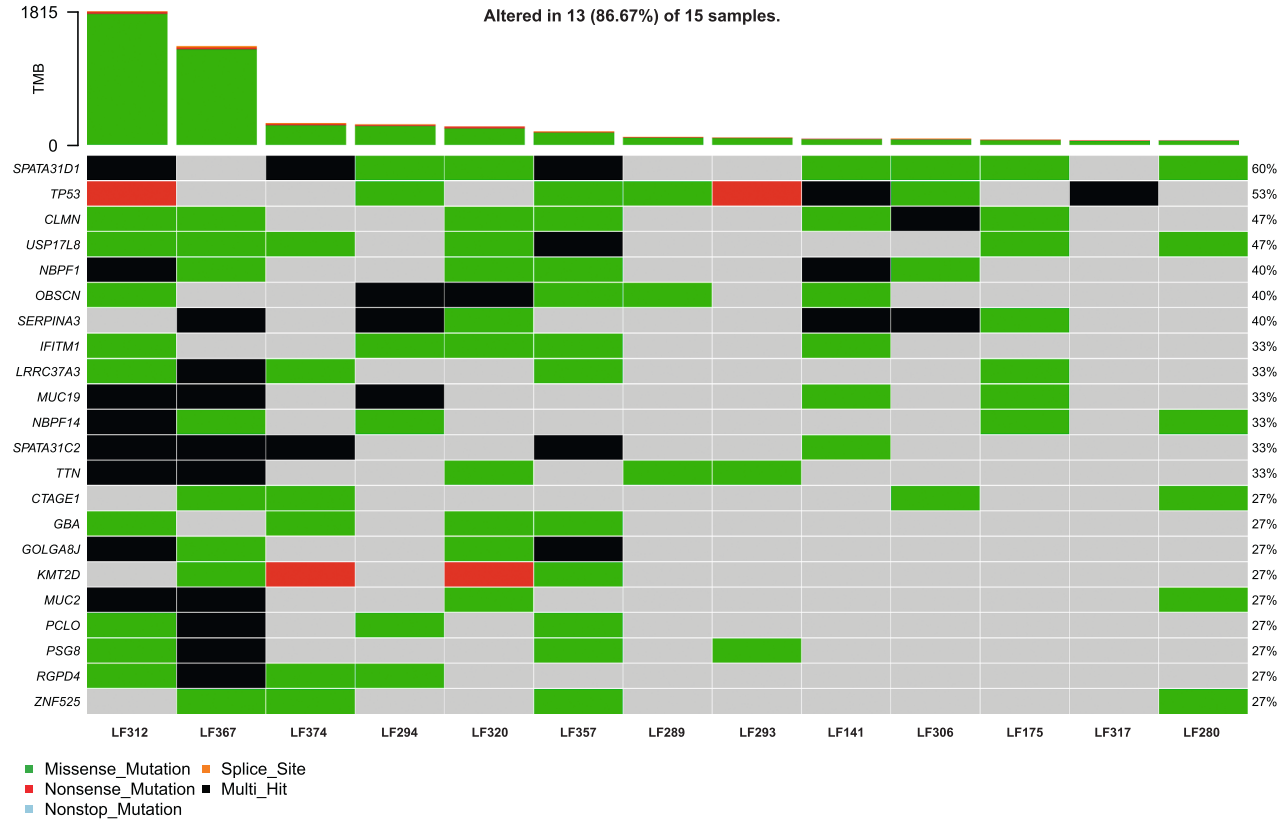

Fig. 1. Oncoplot of the top 22 mutated genes found in 13 out of 15 SA ESCC tumors.

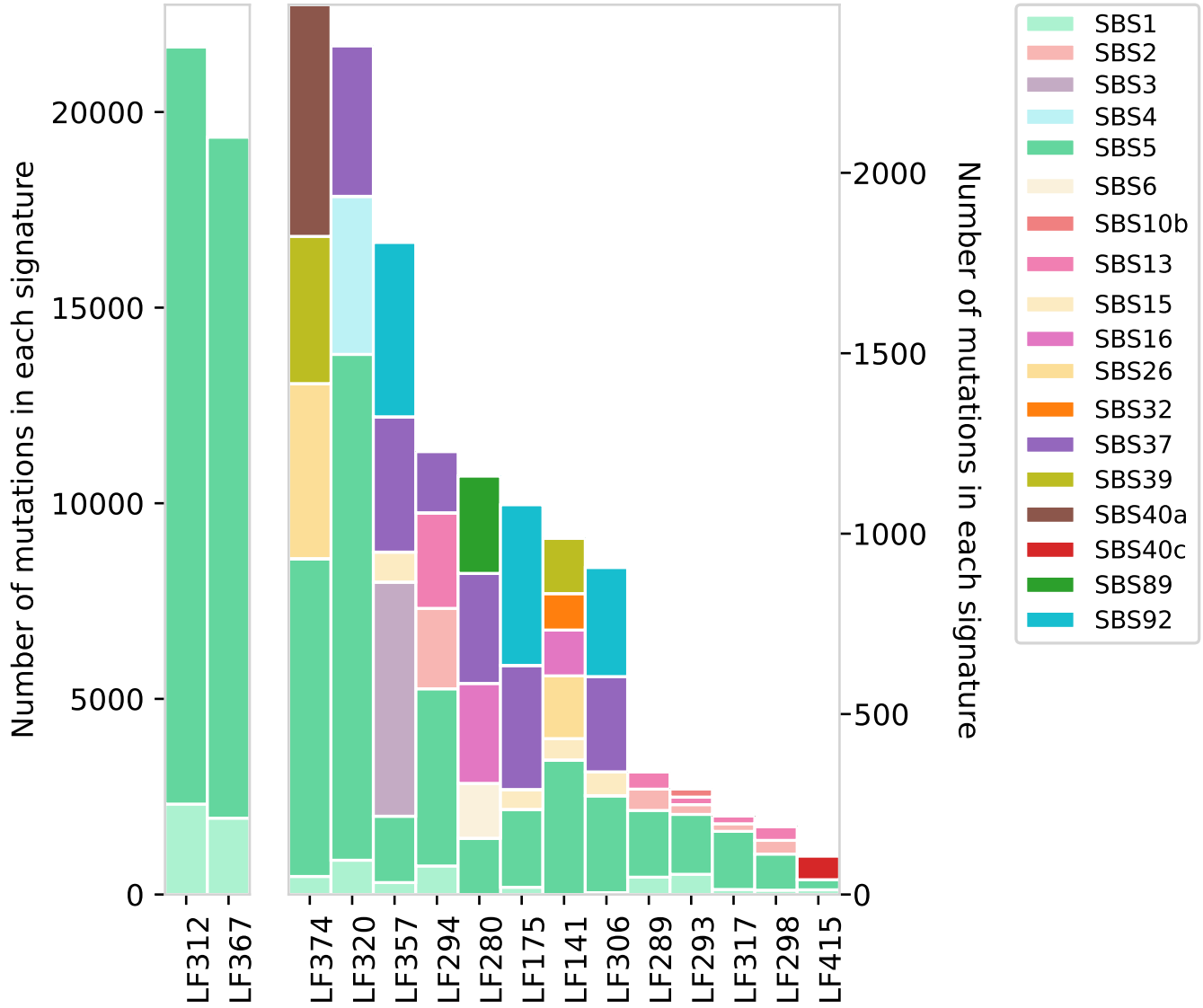

Fig. 2. Single base substitution (SBS) 96 mutation signatures found in the 15 SA ESCC tumors. SBS mutation signatures fitted using COSMIC version 3.4 exome reference signatures, with the artificial signature subgroup removed.

IQR	inter-quartile range
SD	standard deviation
WES	Whole exome sequencing

Supplementary data to this article can be found online at https://doi.org/10.1016/j.genrep.2025.102174.

CRediT authorship contribution statement

Lucien Ferndale: Writing – original draft, Methodology, Funding acquisition, Conceptualization. **Wenlong Carl Chen:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Phelelani Thokozani Mpangase:** Methodology. **Jean-Tristan Brandenburg:** Methodology. **Lamantha Nerija Ngundu:** Methodology. **Mishalan Moodley:** Methodology. **Reubina Wadee:** Methodology. **Colleen A. Wright:** Methodology. **M. Iqbal Parker:** Writing – review & editing, Funding acquisition. **Pascale Willem:** Writing – review & editing, Methodology. **Colleen Aldous:** Writing – review & editing, Methodology. **Christopher G. Mathew:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Data curation, Conceptualization.

Ethics statement

All patients in this study provided written informed consent. Ethical approval was obtained from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (UKZN), Durban, South Africa, and the Human Research Ethics Committee (Medical) at the University of the Witwatersrand, Johannesburg, South Africa.

Funding information

This work was supported by grant #046 (M.I.P., C.G.M., M.M., P.W.) from the South African Medical Research Council (SAMRC) received from the South African National Department of Health, the Medical Research Council (United Kingdom) with funds from the UK Government's Newton Fund and GSK; Self-initiated research grant from the SAMRC (L.F.); National Research Foundation (SA) Thuthuka grant (W.C.C.); National Research Foundation student bursary (LNN).

Declaration of competing interest

The authors declare that they have no potential conflict of interest to disclose.

Acknowledgements

The authors thank Dr. M Govender and Dr. V Nair for their assistance in collecting endoscopic tissue samples. The authors thank Mrs. Fazana Dessai for the germline DNA isolation and the Sydney Brenner Institute for Molecular Bioscience for sample storage.

Data availability

The African ESCC WES data used in this study are available to interested researchers through the European Genome-Phenome Archive (EGA: pending), subject to controlled access review by the Data and Biospecimen Access Committee of the University of the Witwatersrand. Analysis scripts and mutation signature matrices are available at https://github.com/phelelani/nf-cancerwes/tree/main/bin.

References

Barnes, D.E., Lindahl, T., 2004. Repair and genetic consequences of endogenous DNA base damage in mammalian cells. Annu. Rev. Genet. 38, 445–476. https://doi.org/10.1146/annurev.genet.38.072902.092448.

Boeva, V., Popova, T., Bleakley, K., et al., 2012. Control-FREEC: a tool for assessing copy number and allelic content using next-generation sequencing data. Bioinformatics 28 (3), 423–425. https://doi.org/10.1093/bioinformatics/btr670.

Braal, C.L., Jongbloed, E.M., Wilting, S.M., Mathijssen, R.H.J., Koolen, S.L.W., Jager, A., 2021. Inhibiting CDK4/6 in breast cancer with Palbociclib, Ribociclib, and Abemaciclib: similarities and differences. Drugs 81 (3), 317–331. https://doi.org/10.1007/s40265-020-01461-2.

Brown, J., Stepien, A.J., Willem, P., 2020. Landscape of copy number aberrations in esophageal squamous cell carcinoma from a high endemic region of South Africa. BMC Cancer 20 (1), 1–10. https://doi.org/10.1186/s12885-020-06788-3.

Businello, G., Parente, P., Mastracci, L., et al., 2020. The pathologic and molecular landscape of esophageal squamous cell carcinogenesis. Cancers (Basel) 12 (8), 1–19. https://doi.org/10.3390/cancers12082160.

Cancer Genome Atlas Research Network T, 2017. Integrated genomic characterization of oesophageal carcinoma molecular separation of ESCC and EAC. Nat Publ Gr. 541 (7636), 169–175. https://doi.org/10.1038/nature20805.Integrated.

Chang, D.Y., Ma, W.L., Lu, Y.S., 2021. Role of alpelisib in the treatment of pik3ca-mutated breast cancer: patient selection and clinical perspectives. Ther. Clin. Risk Manag. 17, 193–207. https://doi.org/10.2147/TCRM.S251668.

Danecek, P., Bonfield, J.K., Liddle, J., et al., 2021. Twelve years of SAMtools and BCFtools. Gigascience 10 (2), 1–4. https://doi.org/10.1093/gigascience/giab008.

Erkizan, H.V., Sukhadia, S., Natarajan, T.G., et al., 2021. Exome sequencing identifies novel somatic variants in African American esophageal squamous cell carcinoma. Sci. Rep. 11 (1). https://doi.org/10.1038/s41598-021-94064-0.

Ferndale, L., Aldous, C., Hift, R., Thomson, S., 2020. Gender differences in oesophageal squamous cell carcinoma in a South African tertiary hospital. Int. J. Environ. Res. Public Health 17 (19), 1–9. https://doi.org/10.3390/ijerph17197086.

Ferndale, L., Moodley, M., Chen, W.C., et al., 2022. Processing and analysis of tissue samples from esophageal cancer patients in an African setting. Biopreserv Biobank. 20 (2), 185–194. https://doi.org/10.1089/bio.2021.0030.

Gamieldien, W., Victor, T.C., Mugwanya, D., et al., 1998. p53 and p16/CDKN2 gene mutations in esophageal tumors from a high-incidence area in South Africa. Int. J. Cancer 78 (5), 544–549. https://doi.org/10.1002/(SICI)1097-0215(19981123)78:5<544::AID-IJC3>3.0.CO;2-T.

Gao, Y.B., Chen, Z.L., Li, J.G., et al., 2014. Genetic landscape of esophageal squamous cell carcinoma. Nat. Genet. 46 (10), 1097–1102. https://doi.org/10.1038/ng.3076.

Islam, S.M.A., Díaz-Gay, M., Wu, Y., et al., 2022. Uncovering novel mutational signatures by de novo extraction with SigProfilerExtractor. Cell Genomics 2 (11). https://doi.org/10.1016/j.xgen.2022.100179.

Kim, S., Scheffler, K., Halpern, A.L., et al., 2018. Strelka2: fast and accurate calling of germline and somatic variants. Nat. Methods 15 (8), 591–594. https://doi.org/10.1038/s41592-018-0051-x.

Li, H., Durbin, R., 2009. Fast and accurate short read alignment with burrows-wheeler transform. Bioinformatics 25 (14), 1754–1760. https://doi.org/10.1093/bioinformatics/btp324.

Liu, W., Snell, J.M., Jeck, W.R., et al., 2016. Subtyping sub-Saharan esophageal squamous cell carcinoma by comprehensive molecular analysis. JCI Insight 1 (16), 1–11. https://doi.org/10.1172/jci.insight.88755.

Mayakonda, A., Lin, D.C., Assenov, Y., Plass, C., Koeffler, H.P., 2018. Maftools: efficient and comprehensive analysis of somatic variants in cancer. Genome Res. 28 (11), 1747–1756. https://doi.org/10.1101/gr.239244.118.

Montalto, F.I., Amicis, F. De, 2020. Cycle control, adhesion and invasion in tumor. Cells 9, 1–15.

Moodley, R., Reddi, A., Chetty, R., Naidoo, R., 2007. Abnormalities of chromosome 17 in oesophageal cancer. J. Clin. Pathol. 60 (9), 990–994. https://doi.org/10.1136/jcp.2006.042416.

Moody, S., Senkin, S., Islam, S., Wang, J., Nasrollahzadeh, D., Penha, R., Fitzgerald, S., 2021. Mutational signatures in esophageal squamous cell carcinoma from eight countries with varying incidence. Nat. Genet. 53 (11), 1553–1563. https://doi.org/10.1038/s41588-021-00928-6.

Morgan, E., Soerjomataram, I., Rumgay, H., et al., 2022. The global landscape of esophageal squamous cell carcinoma and esophageal adenocarcinoma incidence and mortality in 2020 and projections to 2040: new estimates from GLOBOCAN 2020. Gastroenterology 163 (3), 649–658.e2. https://doi.org/10.1053/j.gastro.2022.05.054.

Murphy, G., McCormack, V., Abedi-Ardekani, B., et al., 2017. International cancer seminars: a focus on esophageal squamous cell carcinoma. Ann. Oncol. 28 (9), 2086–2093. https://doi.org/10.1093/annonc/mdx279.

Schaafsma, T., Wakefield, J., Hanisch, R., et al., 2015. Africa's oesophageal cancer corridor: geographic variations in incidence correlate with certain micronutrient deficiencies. PloS One 10 (10), 1–13. https://doi.org/10.1371/journal.pone.0140107.

Song, Y., Li, L., Ou, Y., et al., 2014. Identification of genomic alterations in oesophageal squamous cell cancer. Nature 508 (7498), 91–95. https://doi.org/10.1038/nature13176.

Sougnez, C., Gabriel, S., Meyerson, M., Lander, E.S., 2013. MuTect. Nat. Biotechnol. 31 (3), 213–219. https://doi.org/10.1038/nbt.2514.Sensitive.

Tran, G.D., Di, Sun X., Abnet, C.C., et al., 2005. Prospective study of risk factors for esophageal and gastric cancers in the Linxian General Population Trial cohort in China. Int. J. Cancer 113 (3), 456–463. https://doi.org/10.1002/ijc.20616.

Van Loon, K., Mwachiro, M.M., Abnet, C.C., et al., 2018. The African esophageal cancer consortium: a call to action. J Glob Oncol. 2018 (4), 1–9. https://doi.org/10.1200/JGO.17.00163.

Wang, K., Li, M., Hakonarson, H., 2010. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 38 (16), 1–7. https://doi.org/10.1093/nar/gkq603.

Ying, J., Shan, L., Li, J., et al., 2012. Genome-wide screening for genetic alterations in esophageal cancer by aCGH identifies 11q13 amplification oncogenes associated with nodal metastasis. PloS One 7 (6). https://doi.org/10.1371/journal.pone.0039797.