

Esophageal cancer genetics in South Africa

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Abstract: Esophageal cancer (EC) is an extremely aggressive cancer with one of the highest mortality rates. The cancer is generally only diagnosed at the later stages and has a poor 5-year survival rate due to the limited treatment options. China and South Africa are two countries with a very high prevalence rate of EC. EC rates in South Africa have been on the increase, and esophageal squamous cell carcinoma is the predominant subtype and a primary cause of cancer-related deaths in the black and male mixed ancestry populations in South Africa. The incidence of EC is highest in the Eastern Cape Province, especially in the rural areas such as the Transkei, where the consumption of foods contaminated with *Fusarium verticillioides* is thought to play a major contributing role to the incidence of EC. China is responsible for almost half of all new cases of EC globally. In China, the prevalence of EC varies greatly. However, the two main areas of high prevalence are the southern Taihang Mountain area (Linxian, Henan Province) and the north Jiangsu area. In both countries, environmental toxins play a major role in increasing the chance that an individual will develop EC. These associative factors include tobacco use, alcohol consumption, nutritional deficiencies and exposure to environmental toxins. However, genetic polymorphisms also play a role in predisposing individuals to EC. These include single-nucleotide polymorphisms that can be found in both protein-coding genes and in non-coding sequences such as miRNAs. The aim of this review is to summarize the contribution of genetic polymorphisms to EC in South Africa and to compare and contrast this to the genetic polymorphisms observed in EC in the most comprehensively studied population group, the Chinese.

Keywords: esophageal squamous cell carcinoma, ESCC, adenocarcinoma, alcohol, smoking, diet, South Africa, China

Introduction

Esophageal cancer (EC) is a malignant tumor in the epithelial cells padding the esophagus.¹ Worldwide, EC is the third most frequently diagnosed malignancy of the upper digestive tract. EC is responsible for >400,000 deaths each year, making it the seventh leading cause of cancer-related deaths.^{2–4} The regions with the highest number of cases and most deaths are the south and the east of Africa, central Asia, Turkey, Iran, Kazakhstan and China (Figure 1).⁵ The disease has a poor prognosis with a 5-year survival rate of <10%.⁶ The two most important histological forms of EC are etiologically and pathologically unrelated. These are esophageal squamous cell carcinoma (ESCC), which predominates in non-white populations, and adenocarcinoma, which is more common in white population groups.^{4,7,8} EC occurrence and type are strongly

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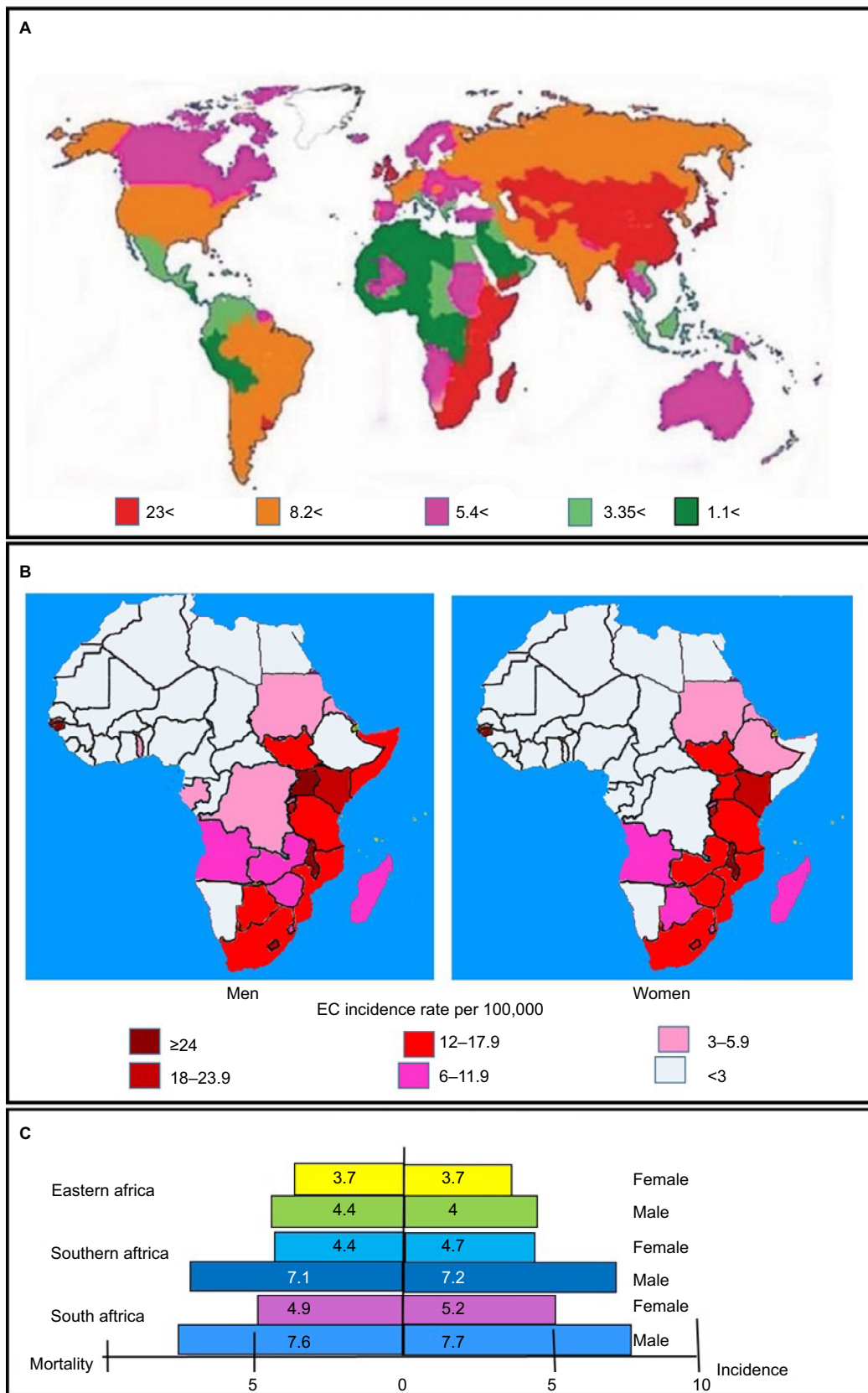


Figure 1 Incidence and mortality rates of EC.

Notes: (A) Worldwide in both sexes and (B) in men and women in Africa. The highest incidence rate is in South Africa and East Africa, with the highest incidence in both sexes being found in Kenya, Uganda and Malawi. A high incidence rate of EC is found in both sexes in Guinea Bissau. (C) The incidence and mortality rates per 100,000 in South Africa and the Eastern Africa and Southern Africa regions is much higher in males than in females.⁴

Abbreviation: EC, esophageal cancer.

influenced by the geographic area and ethnicity.^{4,7,8} Most incidences of esophageal malignancy are ESCC, which makes up >95% of all cases.⁹ However, the number of adenocarcinoma cases is on the rise, with the proportion of adenocarcinomas increasing from 3.5% in 1985 to 17.0% in the year 2000. This is especially true in western countries, where it now accounts for 30%–50% of all diagnosed ECs.^{10–12} One of the main environmental factors that has been investigated as an associative factor contributing to the development of ESSC is nutrition. Nutritional factors that may play a role in the development of ESSC include a diet low in dietary vitamins and minerals, the consumption of hot beverages and pickled food and exposure to foods contaminated with or containing nitrosamines.^{13,14} However, while these environmental risk factors may be factors that strongly contribute to the development of ESCC, they alone may require a genetic component which would predispose an individual to ESCC.^{4,15}

Cancer of the esophagus was an uncommon disease in South African during the 1920s and 1930s.¹⁶ This was followed by a rapid rise in the number of diagnosed cases of the disease.^{17–19} EC has become the third most commonly diagnosed cancer in Black South Africa. Age occurrence values are 22.3 per 100,000 in black males and 11.7 per 100,000 in black females, respectively.²⁰ It is also the fourth highest

cause of death in males of the colored or mixed ancestry group.²¹ EC rates increase noticeably in South Africa between the average age of 60 and 70 years, along with cigarette fuming and extreme alcohol use (Table 1).²² The Transkei region is considered as the center of the malady in South Africa,²³ with a standard incidence rate of 46.7/100,000 for males and 19.2/100,000 for females.²⁴ The increase in EC rates in South Africa over the last decades can largely be attributed to changes in environmental factors and exposure to carcinogens.²³ Two of the most important factors are increased alcohol intake (Table 1)¹⁶ and increased tobacco use (Table 1).²⁵ However, other weak carcinogens also play a role, such as the exposure to environmental smoke²⁶ mainly in the form of cooking fires, the use of wild herbs such as *Solanum nigrum*, human papillomavirus (HPV) infection²³ and the consumption of traditional beer brewed with maize that may contain fungal mycotoxins or nitrosamines.¹⁶ Another important factor contributing to the increase in EC rates is the adoption of a western diet. This includes increased consumption of fats and animal protein as well as a decrease in the vitamin content of traditional beer that is now brewed almost exclusively with maize.¹⁶

Another region of the world with a high incidence rate of EC is China. Over half of all new cases of EC are diagnosed

Table 1 Alcohol consumption and tobacco use in China and South Africa

	Males			Females		
	Number of new cases	Crude incidence/100,000	ASIRW	Number of new cases	Crude	ASIRW
China ¹⁸⁴	13,161	26.46	18.03	5,763	10.85	5.81
South Africa ¹⁸⁵	848	3.2	4.7	650	2.35	2.67
China						
Alcohol per capita (15+) consumption (in liters of pure alcohol)²						
	Average 2003–2005		Average 2008–2010	Males	Females	
	4.9		6.7	10.9	2.2	
Prevalence of smoking any tobacco product among persons aged ≥15 years¹⁸⁶						
	Male		Female			Total 2015
	2000	2015	2000	2015		
	55.8	47.6	3.5	1.8		25.3
South Africa						
Alcohol per capita (15+) consumption (in liters of pure alcohol)²						
	Average 2003–2005		Average 2008–2010	Males	Females	
	10.1		11.0	11.4	4.2	
Prevalence of smoking any tobacco product among persons aged ≥15 years¹⁸⁶						
	Male		Female			Total 2015
	2000	2015	2000	2015		
	35.3	31.4	12.5	6.5		18.4

Notes: The amount of pure alcohol consumed per capita and the prevalence of tobacco use in China and South Africa are presented in the above table. For alcohol consumption, data are shown for the periods 2003–2005 and 2008–2010 and for males and females in 2010. The trend observed in both countries is an increase in alcohol consumption over time.¹⁸⁷ In terms of tobacco use, data are given as percentages of the population that uses tobacco.

Abbreviations: ASIRW, age-standardized incidence rate by world standard population.

in China. In South Africa, EC is the eighth most common cancer in men and the eleventh in women, while in China, it is the fourth most frequently diagnosed cancer.⁴ Like South Africa, the areas in China with the highest incidence rates are the rural areas.²⁷ Areas such as Henan, Hebei and Shanxi in Central North China have the highest incidence rates in the world (over 100 per 100,000).^{28,29} Additionally, the provinces of Sichuan, Anhui, Jiangsu, Hubei, Fujian, Guangdong and Xinjiang have age-adjusted rates >30 per 100,000 (Figure 2).³⁰ Overall, EC is the fourth leading cause of cancer-related deaths in China.⁴ However, in Xi'a city in Shaanxi Province, it is the leading cause of cancer-associated mortality with a mortality rate of 24 per 100,000.³¹ Once again, environmental factors similar to those suspected of playing a role in South African cases of EC are also thought to be important contributing factors in China. These include nutritional factors such as the consumption of fatty meat, salted and pickled vegetables and moldy food, as well as nutritional deficiencies. Lifestyle factors include passive smoking, esophageal lesions, infection with *Helicobacter pylori*, low socioeconomic status and poor oral hygiene. However, a family history of cancer is also an important risk factor associated with an increased risk of ESCC,³² and this points to a genetic component.

Despite the known dominant role played by environmental factors in increasing the rates of EC, the occurrence

of high rates of EC in members of the same blood family observed in both China and South Africa suggests that genetics may also play an important role.²⁶

Environmental risk factors for EC

Environmental risk factors for EC are complex and have a complicated relationship with genetic and etiological elements concerning the development of ESCC and adenocarcinoma.³³ The influence of these different factors on the risk of developing adenocarcinoma and ESCC is given in Table 2. The geographic variation in the occurrence of EC is likely caused by genetic polymorphism and unstable environmental factors.⁶ Worldwide, the primary risk factors for ESCC are alcohol use and tobacco smoking. Other factors rely on the region of the world. These include hot brews in Iran and South America, and smokeless tobacco and alimentary deficits in China, Central Asia and South Africa.³⁴ Other risk factors include the extensive utilization of wood and charcoal for cooking and heating,^{35,36} along with nutritional factors that are related to the growth of the EC.⁶

Analyses carried out within the areas of the Transkei region and KwaZulu-Natal Province have demonstrated that people at highest risk are commonly poorer, have inadequate diets and consume some class of alcohol and tobacco.^{31,32,37} Furthermore, bacteria from the family Helicobacteraceae were discovered in roughly 50% of South African patients

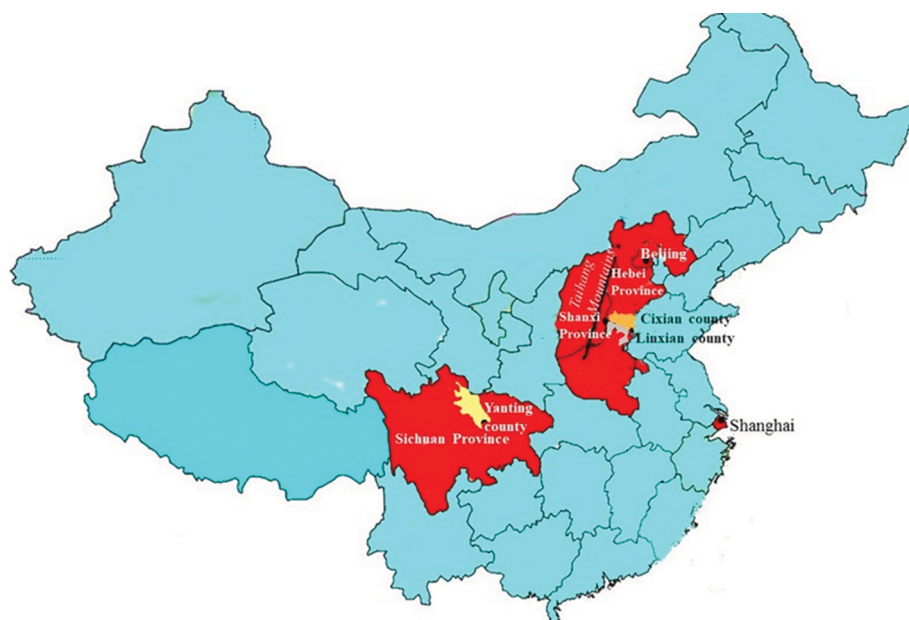


Figure 2 Geographic locations of the four high-risk areas in China.

Notes: The counties Cixian, Shexian and Linxian are located on the borders of the Henan and Hebei provinces. These three counties are located in an area with a radius of 60 km. The Taihang Mountains straddle the border of the Shanxi and Hebei provinces and have some of the highest mortality rates of esophageal cancer in the world. Another high-risk area for esophageal cancer is Yanting in Sichuan Province. Data from Lin et al.³⁷

Table 2 Comparison of the risk factors for adenocarcinoma and ESCC

Factor	Adenocarcinoma	ESCC
Gastroesophageal reflux disease	Approximately 6-fold increased risk, mainly in young adults ¹⁸⁸	50 times greater risk ¹⁸⁸
Obesity	Strong increase in associated risk, linked to abdominal adiposity ¹⁸⁹	Increased BMI commonly associated with a decreased risk of developing ESCC. May be due to reverse causation or other confounding factors ¹⁹⁰
Tobacco smoking ^{115,118,121}	Strong associated risk. Quitting smoking for 10 years or longer reduces the risk greatly ¹⁸⁹	Greatly increased risk. From 3- to 9-fold increase in relative risk ¹⁹⁰
Alcohol	No increase in risk ¹⁸⁹	Consumption of alcohol increases the risk of developing ESCC between 2- and 6-fold ¹⁹⁰
Diet	Increased dietary fat increases the risk of adenocarcinoma. Nutrients such as vitamin C, magnesium, folate, vitamin B6 and iron decrease the risk of adenocarcinoma ¹⁸⁹	Most studies on specific foodstuffs show no relationship with ESCC risk. Fruits and vegetables decrease the risk of developing ESCC. Hot beverages and foods increase the risk of developing ESCC. ¹⁹⁰ Pickled vegetable consumption leads to an increase in the risk of developing ESCC. It is suspected that mycotoxin contamination occurs during the pickling process. ¹⁹⁰ Consumption of foods contaminated with <i>Fusarium</i> mycotoxins may lead to increased risk of ESCC
Exercise	Reduced risk ¹⁹¹	No clear association ¹⁹¹
<i>Helicobacter pylori</i> infection	<i>H. pylori</i> decreases the risk of adenocarcinoma ¹⁹²	No significant association between <i>H. pylori</i> infection and ESCC ¹⁹³
Reproductive, sex-related factors	Menopause, oral contraceptives and hormone therapy all reduce the risk ¹⁹⁴	Hormone therapy and menopause may have protective effects ¹⁹⁰
Medicines	Both NSAIDs and statins decrease the risk of adenocarcinoma ¹⁸⁹	Aspirin has been shown to reduce the risk and mortality of ESCC ¹⁹⁵

Abbreviations: BMI, body mass index; ESCC, esophageal squamous cell carcinoma.

with EC.³⁸ The use of any form of tobacco was found to increase the risk of developing EC by as much as four times in the Eastern Cape Province of South Africa.³⁹ Currently, the prevalence of tobacco consumption among adult South Africans is 17.6%, with males being four times more likely (29.2%) than females (7.3%) to use tobacco products.⁴⁰ Cigarette smoke is composed of over 4,000 chemical compounds, and >60 of these are recognized to cause diseases, including cadmium, nicotine/cotinine and benzo[a]pyrene.⁴¹ In South Africa, the consumption of >53 g of ethanol per day led to a risk of developing EC five times greater in comparison to non-drinkers, while a combination of both alcohol, consumption and smoking led to an 8.5 times greater risk of developing EC. The attributable fractions in high-risk populations in South Africa were 58% for smoking, 48% for alcohol consumption and 64% for both factors combined.³⁹ In comparison, tobacco use in China is much higher in Chinese males than females. Currently, the prevalence of smoking in China is 56.1% among males and 2.2% among females in rural areas. In urban regions, the occurrence was 49.2% among males and 2.6% among females.⁴² The attributable fraction for both tobacco and alcohol consumption was lower

in high-risk Chinese populations compared to their South African counterparts. The attributable fractions of esophageal patients who were smokers were 17.9% in Chinese men and 1.9% in Chinese women,⁴³ and for alcohol consumption they were 15.2% in Chinese men and 1.3% in Chinese women.⁴⁴ This higher tobacco use and alcohol consumption among males in both South Africa and China (Table 1) is most likely the major factor contributing to the higher incidence of EC observed in males.

In the Eastern Cape of South Africa, an increase in the risk of developing EC is associated with a diet low in green leafy vegetables and fruit.⁴⁵ When examining the diet of those in high-risk, remote rural areas of South Africa, it was found that low plasma concentrations of vitamins A, E, B12, folic acid and selenium were also associated with an increased risk of developing EC.⁴⁶ Similarly, reports in Linxian, China showed that general undernourishment, as well as deficiencies in selenium, zinc, folate, riboflavin and vitamins A, C, E and B12 were linked with amplified risk of ESCC.⁴⁷ Another dietary cause of EC in South Africa is the production of carcinogenic nitrosamines resulting from fungal contamination of food. The shift in the diet of the black population of

South Africa from sorghum to maize is one of the factors that have been identified as contributing to the current high levels of EC in this population group. Unlike sorghum, maize is frequently contaminated with fungi such as *Fusarium verticillioides*. These fungi produce toxins such as fumonisins, which reduce nitrates to nitrites and synthesize nitrosamines.⁴⁸ Homemade traditional beer is commonly consumed in these regions of South Africa. This beer was once brewed mainly from sorghum, but is now generally brewed from maize. This traditional beer has been found to contain high amounts of carcinogenic *N*-nitrosamines.⁴⁹ The maize-rich diet also leads to high levels of prostaglandin E2 (PGE2) in gastric fluid. This enzyme activates the Wnt signaling pathway. PGE2 also stimulates proliferation and may predispose esophageal cells exposed to gastric fluid to developing ESCC.⁵⁰ Linoleic acid is used to synthesize prostaglandins, and therefore, high levels of linoleic acid lead to increased PGE2 activity in the stomach.⁵¹ PGE2 represses gastric acid secretion and leads to a reduction in the tone of the muscles that control pyloric and lower esophageal sphincters. This results in chronic hyperchlorhydria duodenogastroesophageal reflux (DGOR).⁵² Chronic alkali DGOR is thought to lead to the denaturation of proteases due to the altered pH. The inhibition of proteases leads to increased growth factor activity as they are no longer broken down by proteases. This leads to an increase in the risk of developing ESCC.⁵³ The cyclooxygenase enzyme (COX-2) is required to convert a linoleic acid derivative into PGE. Like PGE2, the level of COX-2 is elevated in EC.^{36,54-57} It has previously been confirmed that gastric fluid out of specimens from Transkei contained higher levels of PGE2.⁵⁰ In China, analysis was performed on local sources of drinking water and samples and in food samples, in order to determine the level of nitrates and nitrites in these samples. It was determined that these concentrations were abnormally high and, therefore, indicated that high levels of nitrosamine were present.⁵⁸ Another common causative agent is infection with *H. pylori* and HPV. However, case studies in South Africa found no association between EC and an increase in *H. pylori* infection.⁵⁹ However, in Chinese populations, the relationship between *H. pylori* infection and ESCC was significant.⁶⁰ This shows an important difference between environmental factors that may lead to ESCC in Chinese and western populations. In terms of HPV infection and its relationship to EC in South Africa, HPV DNA could only be isolated from 9% of ESCC patients. HPV infection appears to only play a minor role in EC.⁶¹ However, looking for HPV biomarkers (E6, E8 and p16) in ESCC tissue samples from China led to the conclusion that there was no association between HPV infection and ESCC.⁶²

Epidemiologic studies in Chinese populations have studied the link between EC and other factors such as the consumption of warm beverages in the form of green tea. While there seemed to be no relationship between drinking tea and ESCC, either directly or inversely, there was a relationship between drinking hot green tea and the development of ESCC. This implies that consumption of any high-temperature beverage could increase the chances of developing ESCC.⁶³ Another factor that has been studied is gastric atrophy. This refers to dying of gastric cells and their replacement with intestinal tissues. Gastric atrophy is indicated by lower serum ratios of pepsinogen I/pepsinogen II. In Chinese populations, gastric atrophy increases the risk of ESCC as indicated by lower Pepsinogen I to Pepsinogen II ratios being positively associated with ESCC.¹³

These risk factors have the capacity to influence the stability of fragile sites.⁶⁴ Tobacco amplifies the expression of regular sensitive locations;⁶⁵ HPV fuses within fragile site loci.⁶⁶ Alcohol and fumonisin each influence folate levels, which facilitates the expression of fragile sites.^{64,67,68} The addition of fumonisin to cell cultures increases the frequency of chromosomal damage.⁶⁹ The combination of these risk factors leads to the initiation of genetic instability at the early stages of carcinogenesis, with the fragile sites being prime targets.⁶⁴ It would be of great benefit, for diagnostic and prognostic purposes, if the genes within the most common fragile sites could be determined in South African cases of ESCC.⁶⁴

Genetic polymorphisms

The importance of genetic factors in contributing to increased risk of developing ESCC was demonstrated by a study undertaken in the Tenwek Mission Hospital in Western Kenya. Kenya is a very high-risk area for ESCC and is part of the ESCC corridor (Figure 1). In this study, ESCC patients younger than 30 were found to have a high incidence of cancer within their family with 20 out of 60 patients having a family history of EC. All these patients also originated from the same community (Kipsigis).⁷⁰ Many of the genes that have been linked to the development of EC^{71,72} were found to be associated with DNA maintenance and repair, alcohol folate and carcinogen metabolism, cell cycle regulation and apoptosis.⁷³ Nevertheless, just a few of these genes have shown to be associated with disease vulnerability.⁷² Therefore, some additional genetic factors assist in predisposing individuals to EC.⁷¹ The most common genetic polymorphisms are single-nucleotide polymorphisms (SNPs). A large percentage of the genetic variations that occur in the human genome are due to SNPs.⁷⁴

A xenobiotic is any chemical substance that is foreign to an organism in its normal physiological function. Humans are exposed to multiple environmental conditions that may result in the buildup of xenobiotics within the body tissues. Many dietary and environmental xenobiotics would require metabolic activation by the Phase I or Phase II enzymes to exert their carcinogenic effect.⁷⁵ The Phase I enzymes catalyze the conversion of a toxic or insoluble compound into a polar, water-soluble metabolite through oxidation. The resulting metabolites contain functional groups such as OH, NH₂ and SH. These groups are sites for reactions catalyzed by the Phase II enzymes.⁷⁵ The Phase II enzymes give rise to compounds that are more hydrophilic and thus easily excreted in the urine.⁷⁵ Although the Phase I and Phase II enzymes act in the detoxification pathway, they usually generate unstable and more reactive intermediate compounds that, if not quickly removed, can bind covalently to DNA and generate DNA adducts with mutagenic and/or carcinogenic properties.⁷⁶ The presence of xenobiotics and polymorphisms in xenobiotic-metabolizing enzymes (XMEs) can increase the risks of sensitivity to ESCC.⁷⁷ These XMEs include Phase I enzymes such as CYP1A1, CYP1B1, CYP2A6 and CYP2E1 and Phase II enzymes such as GSTM1, GSTT1 and GSTP1 (Table 3).³¹ Variations at 10q23 in PLCE1 were linked with ESCC (Table 3).⁷⁸ Alteration in the riboflavin transporter C20 ORF54 on chromosome 20p13 is a risk factor for ESCC.⁴³ ESCC is related to polymorphisms of the ADH enzymes ALDH2 and ADH1B1. These enzymes are engaged in alcohol metabolism, and with CYP1A1, are involved in xenobiotics' detoxification.⁷⁹ NQO1 is one of the enzymes belonging to the NAD(P)H dehydrogenase (quinone) family that are involved in protecting cells from oxidative damage.⁸⁰ A significant polymorphism dealing with a single C to T replacement at nucleotide 609 of exon 6 in the NQO1 cDNA affects the NQO1 enzyme activity that induces a Pro187Ser amino acid substitution.⁸¹ The NQO1 C609T polymorphism has been related to ECs.^{82–84} NQO1 is involved in cellular antioxidant defense systems by detoxifying quinines.⁸⁵ A meta-analysis showed that the NQO1 C609T polymorphism considerably increases the risk of developing EC.⁸⁵

The role of polymorphisms in alcohol metabolising enzymes: ADH and ALDH in the risk of developing EC

The WHO classifies alcohol as a Group 1 carcinogen. In the body, alcohol is initially metabolized by the ADHs to acetaldehyde. This is then further metabolized to acetic

acid by ALDH2. Mutations and polymorphisms that affect the functioning of these enzymes may affect the ability of the individual to detoxify ethanol, leading to increased exposure of cells to carcinogens such as acetyl aldehyde which is dissolved in the saliva following consumption of alcohol or smoking tobacco (Table 4).⁸⁶ There is a guanine to adenine SNP within ALDH2 at position 1510, which leads to a lysine at codon position 487. This leads to a catalytically inactive subunit, and the allele containing this polymorphism is termed ALDH2*two (wild-type ALDH*1). Individuals homozygous for this mutation (ALDH2 *1/*2) have only 6.25% of normal ALDH2 *1/*one activity.⁸⁷ The ADH 1B *two mutant allele results from an SNP in Exon 3 of *ADH1B*, resulting in a His to Arg substitution at codon 48. This results in a protein with a much higher activity than the wild type encoded by the *ADH1B* *one allele. The homozygous *ADH1B**2/*two polymorphism has a V_{max} 40 times higher than the wild-type *ADH1B**1/*1. This results in greater amounts of alcohol being oxidized by the more active enzyme.⁸⁸ Both these polymorphisms are frequently found in individuals originating from East Asia. There is a strong association between the ALDH2 *2/*two genotype and EC (OR =4.42). The lower activity of *ADH1B* *one allele is also associated with an increase in the risk of developing EC. This occurs in a manner associated with the number of *ADH1B* *one alleles present, with *1/*one homozygous having a greater risk for developing EC than the *1/*two allele. In South Africa, the role played by mutations in the genes coding for these enzymes has not been studied intensively. The ALDH2*two allele is present in black and mixed ancestry South African populations, where it is significantly associated with an increased risk for EC (OR =2.35; Table 4).^{89,90} The *ADH1B* *two allele was also detected in the mixed ancestry population in South Africa. As in other population groups, this allele is associated with a decreased ESCC risk.⁹⁰ Mutations affecting the function of ALDH2 are so prevalent among East Asian population groups that it is common for individuals in these population groups to have a flushed skin or red blotches on their face, neck and shoulders following consumption of alcohol. This is known as the alcohol flush reaction. People displaying this reaction have an increased risk of developing EC. This rash results from excess acetyl aldehyde, with the polymorphism most commonly associated with the flush response being the rs671 allele of *ALDH2*. This mutation in *ALDH2* and the flush reaction occur in 30%–50% of South East Asians, but it is extremely rare in Europeans and sub-Saharan Africans (Tanzania =0.0025%). In South Africa, it is, therefore, of no use as a diagnostic or

Table 3 Genetic polymorphisms affecting the genes related to changing the risk of developing esophageal cancer in South Africa and China

Country and polymorphism	OR	Environmental factors	Postulated mechanisms/effect
<i>PLCE1</i> is involved in the regulation of cell growth, differentiation, apoptosis and angiogenesis			
China			
rs10882379 ¹⁹⁶ GG => GA GG => AA GG => A/AA	At 95% CI 0.68 0.69 0.75	No association with smoking and alcohol use	SNP is in the promoter region/decreased ESCC risk
rs829232 ¹⁹⁶ GG => GA GG => AA GG => GA/AA	At 95% CI 1.32 1.88 1.42	No association with smoking and alcohol use.	SNP is in the promoter/increased ESCC risk
rs2274223 at 10q23 ⁴³ AA => AG AA => GG AA => AG/GG	At 95% CI 1.27 1.47 1.30	No association with environmental factors	SNP in exon 26 of <i>PLCE1</i> gene is associated with increased risk of ESCC. AG and GG genotypes lead to lower <i>PLCE1</i> mRNA and protein levels
rs11187870 ¹⁹⁷ GG => CG GG => CC GG => CG/CC	At 95% CI 1.1 1.54 1.21	No association with environmental factors	SNP is in the 3'UTR. Associated with increased risk of ESCC
South Africa			
Arg548Leu (rs17417407) ¹⁹⁸	0.75 at 95% CI	No evidence for an association with smoking or alcohol	Associated with a reduced risk of ESCC in the black population
NAT1 and NAT2 are the main Phase II xenobiotic metabolizing enzymes involved in either detoxification or activation of carcinogenic arylamines			
China			
NAT2 rs1565684 ¹⁹⁹ T > C SNP CC = TC/CC	At 95% CI 1.14 1.77	Alcohol consumption had no effect on ESCC risk	Genotype was associated with an increased risk for ESCC
South Africa			
NAT2 341 CC (rs1801280) ²⁰⁰ Mixed ancestry black	At 95% CI 0.31 0.55	In the black population group, smokers with this mutation had a higher risk of ESCC	Mutation resulted in decreased acetylation activity and reduced the risk for EC in mixed ancestry group
NAT1 and NAT2 slow/intermediate acetylation phenotype ²⁰⁰	0.44 at 95% CI		NAT2 slow/intermediate acetylation/reduced risk of ESCC in mixed ancestry group
CASP8 is an initiator of CASP3, a key regulator of apoptosis, and is important in cancer development and progression			
China			
rs1035142 G>T ²⁰¹ Heterozygote Homozygote	At 95% CI 1.55 2.34		Associated with an increased risk of ESCC by negatively affecting the cells' ability to undergo apoptosis
South Africa			
(-652 6Ndel:302His) ⁹⁰	2.37 at 95% CI	Associated with ESCC in current smokers, but not in former smokers	Haplotype associated with ESCC in mixed ancestry population
Asp302His (rs1045485) ⁹⁰	1.42 at 95% CI		Suggestive association with increased ESCC risk in mixed ancestry population
p53 can induce cell cycle arrest for DNA repair and/or apoptosis in response to cellular stress such as DNA damage or hypoxia			
China			
Arg72Pro (rs1042522) ^{6,9,97,202} Pro/Arg vs Pro/Pro Pro vs Arg Pro/Pro vs Arg/Arg + Arg/Pro Pro/Pro vs Arg/Arg Pro/Pro vs Arg/Pro	At 95% CI 1.01 1.83 1.17 1.32 1.35	No significant association with tobacco use	Increased risk of EC in Han Province. Associated with Burkitt's lymphoma
rs2909430 ²⁰³	1.94 at 95% CI		Commonly observed in ESCC patients in high-risk area of China
rs78378222 A => C ²⁰³	3.22 at 95% CI		

(Continued)

Table 3 (Continued)

South Africa			
Polymorphisms in exon 4 ²⁰⁴ C => A, codon 34 G => C, codon 36 G => C, codon 72	At 95% CI 1.03 1.86 0.8	Reactive mutagenic compounds form DNA adducts, which can cause nucleotide changes. TP53 plays an important role responding to DNA damage. Loss of function mutations will increase the threat posed by carcinogens	Strongly associated with EC
Small deletions, insertions and point mutations resulting in frame shifts or amino acid changes in exons 5–8 ²⁰⁴			Commonly observed in ESCC patients
16 bp duplication in intron 3 resulting in loss of heterozygosity ²⁰⁴	14 at 95% CI		Commonly observed in ESCC patients

Abbreviations: EC, esophageal cancer; ESCC, esophageal squamous cell carcinoma; rs, reference SNP cluster ID; SNP, single-nucleotide polymorphism.

Table 4 Genetic polymorphisms in alcohol metabolizing enzymes associated with EC in South Africa and China

Country and polymorphism	OR	Environmental factors	Postulated mechanisms/effect
ALDH2 is responsible for the conversion of acetaldehyde to nontoxic acetate			
China			
rs671 at 12q24 ^{205,206} GG => AA GG => GA	At 95% CI 0.67 0.85	No evidence for an association with smoking or alcohol	Mutation increases the risk of ESCC in Kazak and Han populations and leads to a poor ESCC prognosis in Kazak population. The variant A allele of rs671 has a reduced ability to metabolize acetaldehyde
ALDH2*2 E487K ^{87,88} AA => GG	At 95% CI 0.26	There was a difference in tobacco and alcohol use between patients and controls	ALDH2*2 allele results in a glutamate to lysine substitution, leading to an increased risk for EC
ALDH2 (*1/*2) ²⁰⁷ 1510G > A487	At 95% CI 2.89	Inactive ALDH2 with moderate to heavy drinking led to a 8.58-fold higher ESCC risk	Genotype frequencies of the inactive enzyme (GA heterozygote) are higher in male patients with ESCC
South Africa			
ALDH2*2 results in an allele E487K results in change of glutamate to lysine ⁸⁹	2.35 at 95% CI	Associated with increased sensitivity to alcohol consumption	The low activity of ALDH2*2 allele is significantly associated with increased risk for EC among black subjects
ALDH2 (rs886205) ⁹⁰ +82 G > A	At 95% CI 0.70	Nominal evidence of association with ESCC in current smokers	Significantly associated with a reduced risk of ESCC in the mixed ancestry population
ADH catalyzes the reversible oxidation of alcohols to corresponding aldehydes or ketones			
China			
ADH1B (*1/*1) (rs1229984) *2 mutant allele results from an SNP in Exon 3 of ADH1B, resulting in histidine to arginine change at codon 48 ²⁰⁷ (Arg) at codon 48 8 ⁷	3.67 at 95% CI 1.67 at 95% CI	Moderate-to-heavy drinkers with the less-active ADH1B *1/*1 genotype had significantly increased risk of cancer There was a significant risk for ESCC attached to alcohol consumption	The polymorphism influences the enzyme activity. The less-active enzyme is associated with an increased ESCC risk in males Increases the risk of EC
ADH1C rs1789924 ²⁰⁵	1.20 at 95% CI	Significantly associated with smoking	The variant T allele significantly increases ESCC risk. Rs1789924 is located at 5' near the gene ADH1C, and is predicted to affect the binding of transcription factor
South Africa			
ADH3*2/*2 ⁸⁹	2.19 at 95% CI		Is significantly associated with increased risk for EC in black patients
ADH1B Arg48His (rs1229984) ⁹⁰	0.52 at 95% CI	Associated with ESCC in current smokers	Is associated with decreased ESCC risk in a mixed ancestry population

Abbreviations: EC, esophageal cancer; ESCC, esophageal squamous cell carcinoma; rs, reference SNP cluster ID; SNP, single-nucleotide polymorphism.

predictive tool in assessing the susceptibility of an individual to alcohol-related risks of developing ESCC.⁹¹

The role of genetic polymorphisms in the androgen receptor (AR) gene in the risk of developing EC

The AR is a nuclear receptor that translocates to the nucleus and acts as a transcription factor once it has bound its ligand. This ligand is generally any of the androgenic hormones. As a transcription factor, it controls pathways associated with cellular proliferation and differentiation.⁹² The receptor is expressed in multiple cancer tissues and cell lines. These include neoplastic colon tissues,⁹³ breast tumors,⁹⁴ hepatocellular cancer⁹⁵ and ESCC.⁹⁶ AR is a part of the steroid receptor family and the AR gene is situated at q11-12 on the X chromosome.⁹⁷ The AR gene is 75,000–90,000 nucleotides in length, consisting of eight exons.⁹⁸ The various protein isoforms range in size from 910 to 919 amino acids and consist of four domains. The first is an NH₂-terminal transactivation domain. A central C4 zinc finger DNA-binding domain and a nuclear-localizing short hinge region follow this. Finally, the C terminal consists of a steroid hormone ligand-binding domain.⁹⁹ The gene for the AR is highly polymorphic in human populations due to the fact that the first exon contains two polymorphic poly amino acid tracts, a glutamine (CAG) 7–31 repeat^{96,100,101} and a glycine (GGC)^{102–105} 8–20 repeat^{106,107} ~1.1 kb apart. Examination of data concerning the (GGC) n allele indicates a genetic sensitivity element for EC in black males. A short (GGC) n allele was involved in the disease in this population, with a GGC shorter than 16 being associated with the disease with an OR of 2.7, 95% CI, and a GGC shorter than 14 being associated with EC with an OR of 3.3, 95% CI.¹⁰⁸ Among the Chinese population in Beijing, shorter CAG repeats were observed in male EC patients, implicating this polymorphism in predisposing individuals to a greater risk of developing EC.¹⁰⁹

The role of genetic polymorphisms in glutathione S-transferase (GST) genes in increased EC risk

The GSTs are Phase I enzymes that introduce reactive or polar groups onto a compound as the initial step to detoxify or remove a reactive or dangerous compound. GSTs accomplish this by catalyzing the conjugation of glutathione (GSH) to electrophilic compounds.¹¹⁰ These compounds include

carcinogens that may lead to the development of cancers such as ESCC.

The risk of developing ESCC in South African and Chinese populations is a result of genetic polymorphisms in an individual's GST-coding genes and their exposure to environmental factors such as tobacco smoke and alcohol consumption (Table 5).⁷¹ There are four major subfamilies of human GST genes, which are divided into the categories GST A, M, T and P.¹¹¹ Deletion polymorphisms have been observed in two of these families, GSTM1 and GSTT1.¹¹² Polymorphisms of GSTM1, GSTT1 and GSTP1 have been demonstrated to be linked to susceptibility to various forms of cancer. This is especially true concerning cancer triggered by exposure to carcinogens such as cigarette smoke,¹¹³ and they also play a role in the resistance to chemotherapy treatment¹¹⁴ and in disease outcomes.¹¹⁵

GSTP1 is expressed in hepatic tissues, lungs and the esophagus and at very low levels in the liver.¹¹¹ Moreover, it has been shown to be overexpressed in several malignant tissues compared to normal tissues. Polymorphisms of GSTP1 take part in EC within a pathway of malfunctions in the p53 malignant tumor suppressor gene, which is prevalent in ESCC.¹¹⁶ The human GSTM1 gene is composed of seven exons, is 5.9 kb in length and is found on chromosome 1p13.¹¹⁷ The human GSTT2 gene, located on chromosome 22q11.2, is made up of five exons spanning 3.8 kb in length.¹¹⁸

miRNA polymorphisms in EC development

Altered miRNA transcription changes the expression of oncogenes and tumor suppressor genes. These genes affect processes such as proliferation, apoptosis, as well as the motility and invasiveness of cancer cells. This has been found to apply to ESCC as well.^{119–121} Different studies have identified different numbers of miRNA whose transcription levels change in ESCC. Ogawa et al reported that 22 miRNAs were upregulated in ESCC tissue, while 4 miRNAs were downregulated.¹²² Yao et al found 27 downregulated and 16 upregulated miRNAs in ESCC tissue, while Ren et al detected 51 upregulated and 17 downregulated miRNAs.¹²³ Next-generation sequencing has identified 78 diversely expressed miRNAs in ESCC.¹²⁴ Silencing of cancer genes mainly occurs through DNA methylation. DNA methylation can, therefore, affect a range of cellular processes. These include the regulation of the cell cycle checkpoint, apoptosis, signal transduction, cell adhesion and angiogenesis. Hence, DNA methylation has a role in the deregulation of miRNAs in

Table 5 GST polymorphisms involved in esophageal cancer

Gene/mutation	OR	Interaction with environmental factors
GSTP1		
rs1695 ²⁰⁸		
rs1138272 ²⁰⁸		
South Africa		
<i>GSTP1</i> *D (rs1138272) Ala113Val (341C4T) in exon 6 ^{209,210}	At 95% CI 4.98	Increased risk in both black Xhosa and mixed ancestry populations in South Africa
341 C/T	10.9	
341 T/T		
313G divergence ^{211,212}	3.60	Decreased activity of the <i>GSTP1</i> variant within the tobacco smokers, alcohol users or those utilizing wood or charcoal for cooking and heating has a higher risk of developing ESCC
GSTM1		
South Africa		
<i>GSTM1</i> null genotype (<i>GSTM1</i> *0) 15 kb omission ^{209,213}	1.71 at 95% CI	The homozygous <i>GSTM1</i> *0 genotype is correlated with a decreased risk for ESCC among mixed ancestry people, whereas no expression of <i>GSTM1</i> is associated with an increased risk of ESCC occurrence in the Black Xhosa population
China		
<i>GSTM1</i> null genotype (<i>GSTM1</i> *0) 15 kb omission ²¹¹	2.17 at 95% CI	<i>GSTM1</i> null genotype is associated with an increased risk for ESCC in Jiangsu Province
GSTT2 gene		
South Africa		
<i>GSTT2B</i> null genotype (<i>GSTT2B</i> *0) 37 kb omission ^{210,214}	0.71 At 95% CI	<i>GSTT2B</i> deletion allele is observed at frequencies of 37% and 50% in black Xhosa and mixed ancestry individuals, respectively. The <i>GSTT2B</i> null genotype does not alter the risk for ESCC in the black Xhosa population, but it is linked with a significantly reduced risk in the mixed ancestry group

Note: The table lists the polymorphisms affecting the three GST genes *GSTP1*, *GSTM1* and *GSTT2* in South Africa and China and relates how these polymorphisms affect the risk for esophageal cancer.

Abbreviations: ESCC, esophageal squamous cell carcinoma; rs, reference SNP cluster ID; SNP, single-nucleotide polymorphism.

cancer, and miR-145, miR-30a-3p, miR133a and miR-133b are the possible tumor promoters.¹²⁵

In South Africa, the polymorphisms in miRNA genes have been connected to many cancer types along with ESCC.^{126,127} In black South Africans, a polymorphism in miRNA 3184, known as rs6505162, results in associated risks for ESCC. The location of the rs6505162 SNP allows it to influence two different miRNAs and a single protein coding gene. These include miR-423, miR-3184 and NSRP1. The two miRNAs, miR-423 and miR-3184, are oppositely oriented and overlap at rs6505162. The SNP is situated downstream of miR-423 and upstream of miR-3184.¹²⁸ Therefore, it is more likely that rs6505162 influences the transcription of miR-3184 rather than that of miR-423.¹²⁹ The increased risk of ESCC related to this SNP is also linked to environment. It was found that the rs6505162 polymorphism was related to increased cancer risk in black African patients who lived in an environment where they were subjected to high levels of smoke inhalation.¹²⁸ The risk of developing ESCC was greater in those individuals who possessed the SNP and used solid fuels for cooking than in those individuals with the SNP and who used electricity or

gas for cooking. Due to population heterogeneity, this relationship does not seem to be present in the mixed ancestry group.¹²⁸ Two other SNPs previously identified as playing a role in EC, rs213210 (miR219-1) and rs7372209 (miR26a-1), were found to interact in both the black and mixed ancestry populations to reduce the risk of cancer development. Individuals with the genotype AArs213210-CTrs7372209 had a reduced risk of developing ESCC.¹²⁸

Polymorphisms in miRNA genes related to ESCC risk have also been identified in Chinese populations.¹³⁰ The SNP rs2910164 C > G in miR-146a increases the risk of individuals developing ESCC within the Han Chinese population, and the increased risk posed by the rs2910164 GG genotype was more notable in cigarette smokers.¹³¹ The rs11614913 TC polymorphism in miR-196a2 is predicted to reduce ESCC risk among females who have never smoked or consumed alcohol. However, in males or those who smoke and drink, the miR-196a2 rs11614913 TC, CC or TC/CC genotype may play a role in increasing the risk of developing ESCC.¹³² Similarly, individuals who did not smoke or drink alcohol and possessed the hsa-miR-34b/c rs4938723 CC genotype had a

reduced threat of ESCC.^{130,133} The risk of developing ESCC is increased by the SNPs rs6505162 C.A in Hsa-miR-423¹³³ and rs531564 CG SNP in pri-miR-124-1.^{130,134}

The role of polymorphisms in the DNA mismatch repair (MMR) genes and tobacco smoking in increasing EC risk

Environmental toxins are able to cause cancer due to their ability to damage DNA. An important safeguard against this is the DNA MMR enzyme system. The MMR system restores mistakes within DNA. These mistakes take place during normal DNA metabolism; however, they are also related to cancer caused by exposure to environmental carcinogens.⁷³ Studies suggest that the MMR gene polymorphisms control the etiology of EC. These genes are connected to the processes of DNA preservation and repair, alcohol, folate and carcinogen metabolism, cell cycle control and apoptosis.⁷³ Furthermore, MMR genes and their polymorphisms account

for the increased risk in the development of lung or head and neck cancer, including EC.^{36,135–138} A list of some of the polymorphisms in MMR genes that may contribute to the risk of EC is given in Table 6. The DNA MMR process is made up of MLH1, MSH2, MSH3, MSH6 and PMS2 proteins.¹³⁹ During DNA synthesis, the MMR corrects the microsatellite instabilities (MSIs) in order to sustain genomic integrity.¹⁴⁰ MSI is characterized by increased mutations in microsatellites that arise due to germline MMR gene mutations causing Lynch syndrome. MSI can also be caused through the epigenetic inactivation of the MLH1 gene and the CpG island methylator phenotype as a consequence of MLH1 hypermethylation.¹⁴¹ This may eventually lead to carcinogenesis due to replication errors that remain uncorrected in essential cancer regulating genes.¹⁴²

Studies searching for extensive microsatellite modification in EC have discovered low-level MSI in 16%–67% of adenocarcinomas, while 2%–60% of squamous cell carcinoma tumors

Table 6 Genetic polymorphisms in DNA mismatch repair genes that influence the risk of developing esophageal carcinoma in South Africa and China

Mutation	OR	Effect on ESCC risk
South Africa		
G/G vs A/A or A/G genotype polymorphism in <i>MSH3</i> rs26279 ⁷³	2.71 at 95% CI	Allied with increased ESCC susceptibility in mixed ancestry South Africans
G-allele in <i>PMS1</i> rs5742938 polymorphism (GG vs AA or AG) ⁷³	1.73 at 95% CI	Allied with increased ESCC susceptibility in mixed ancestry South Africans
A-allele in <i>MLH3</i> rs28756991 polymorphism (AA or GA vs GG) Arg797His ⁷³	2.07 at 95% CI	Allied with increased ESCC susceptibility in mixed ancestry South Africans. Compromises the structure and function of MLH3 protein. The ternary complex formed between heterodimers MSH2–MSH3 and MLH1–PMS1 is a critical incident in ESCC development
China		
DNA mismatch repair gene <i>MSH2</i> (<i>MSH2c.2063G</i>) ¹⁵³	3.36 at 95% CI	Polymorphisms increase the risk for EC
DNA mismatch repair gene <i>MLH1</i> (<i>MLH1/VS14-19G</i>) ¹⁵³	1.70 at 95% CI	Polymorphisms increase the risk for EC
DNA lesion repair enzyme <i>MGMT</i> gene <i>MGMT</i> Rs11016879 ^{152,153} AA vs GG AG vs GG Rs12771882 AA vs GG Rs7075748 (CT vs CC) Rs11016878 AG vs AA Rs7069143 CT vs CC Rs7071825 GG vs AA	At 95% CI 3.11 3.93 2.44 1.95 0.39 0.48 0.49	Some polymorphisms in the gene lead to increased risk of developing ESCC, while others have a protective function decreasing the risk for ESCC
<i>MGMT</i> ¹⁵³ A allele Rs10734088 C allele Rs475111	At 95% CI 1.97 2.18	Significantly increase the risk for developing ESCC
<i>MGMT</i> A allele Rs2053139 ¹⁵³	0.51 at 95% CI	Associated with a protective effect on ESCC progression
<i>MGMT</i> C allele Rs7068306 ^{152,153}	2.20 at 95% CI	Increased risk of ESCC metastasis
<i>WRN</i> gene ²¹⁵ (<i>WRN</i> c.4330TC)	2.21 at 95% CI	Significantly increased risk for developing ESCC

Note: The table describes genetic polymorphisms in DNA repair pathway genes that are found in South African and Chinese populations.

Abbreviations: ESCC, esophageal squamous cell carcinoma; rs, Reference SNP cluster ID; SNP, single-nucleotide polymorphism.

were MSI-L positive. These high-incidence populations possessed the maximum MSI abundances, signifying that MMR might be included in the pathogenesis of the esophagus.^{143,144} Genetic conversions in microsatellite regions, a property of a faulty DNA MMR procedure, have been found in ECs.⁷³

In black South Africans, no association between MMR polymorphisms and cancer risk was observed at the SNP level. It was suggested that different linkage disequilibrium patterns are the reason for the lack of significant associations in these individuals.⁷³ On the other hand, three regular polymorphisms were allied with ESCC in mixed ancestry South Africans.⁷³ Epithelial cells of the esophagus are regularly exposed to DNA destructive compounds and should have the capacity to fix the DNA damage provoked by numerous carcinogens in food, tobacco and solid fuel smoke.¹³² A polymorphism within the DNA repair associated gene *MSH3* (Table 6) may interact with cigarette in some cases of carcinogenesis. A model was developed to explain the role played by the interaction between the MSH3 protein and tobacco smoke in EC,¹⁴⁵ as MSH3 is implicated in playing a significant role in DNA double-strand break (DSB) repair.^{146–148} Cigarette smoke has been shown to damage DNA by causing DSBs.¹⁴⁹ Therefore, any mutation or polymorphism decreasing MSH3 activity may increase the risk of developing EC.

Promoter CpG island methylation appears to be a frequent event in ESCC carcinogenesis, with methylated sequences of hMLH1, hMSH2 and MGMT being identified in a large proportion of Chinese ESCC patients.¹⁵⁰ Promoter methylation in the ML1 promoter of male Han Chinese ESCC patients is associated with a poor prognosis.¹⁵¹

Genetic polymorphisms of CYP genes increase the risk of developing EC

CYPs play a considerable role in detoxifying chemicals that the esophagus is exposed to.^{152,153} The CYP enzymes are expressed in the esophageal mucosa, suggesting that this tissue can detoxify DNA-binding chemical carcinogens.^{154–156} CYP2E1 is an 11.4 kb gene containing nine exons positioned on chromosome 10q26.345.¹⁵⁷ The CYP2E1**six* heterozygous genotype is linked to ESCC in South Africa¹⁵⁸ and the *c1/c1* genotype is linked to ESCC in China (Table 7). CYP3A is mostly regulated by genetic polymorphisms in the CYP3A4 and CYP3A5 genes, which exist together in a 231 kb area on chromosome 7q21.1.¹⁵⁹ CYP3A5 is an important enzyme in the esophagus and metabolizes carcinogenic compounds,¹⁵² playing a role in both metabolic catalysis of xenobiotics to produce reactive intermediates

found in the pathogenesis of EC and in the detoxification of carcinogens that the esophagus is exposed to. Functional polymorphisms in the CYP3A5 gene are mainly represented by CYP3A5**three* and CYP3A5**six* alleles.¹⁶⁰ CYP3A5**six* appears to be African specific.¹⁶¹ In South African populations, the CYP3A5**three* allele was found to be present in 14%–16% of the black subjects and 48%–59% of the mixed ancestry population (Table 7).¹⁶²

As mentioned previously, South African maize is contaminated with high levels of aflatoxins and fumonisin.¹⁶³ Fumonisin stimulates xenobiotic metabolizing enzymes along with the CYPs.¹⁶⁴ CYP3A5 could be slightly decreased. CYP3A enzymes metabolize several steroids such as progesterone, estradiol, testosterone and corticosterone.¹⁶⁰ Similar associations between environmental factors and the CYP3A5**three* allele have been found in a high-risk Chinese population from Shanxi Province.¹⁵⁸

Genetic polymorphisms in genes encoding sex hormone metabolizing enzymes increase the risk for EC

Males are two to four times more likely to develop EC than females. Also, while men are more commonly exposed to risk factors, one study seemed to indicate that these factors had an even greater harmful effect on females than they did in males (Table 8).¹⁶⁵ Evidence from epidemiological and experimental studies suggests that sex hormones may play a significant role in the development of ESCC.¹⁵ Since hormone therapy is able to lower the risk of ESCC,^{78,166,167} it has been suggested that low estrogen plays a role in increased risk of ESCC.¹⁶⁸ One line of evidence that suggests this is the increased levels of estradiol, which is an estrogen receptor agonist, in patients with ESCC, further suggesting that low levels of estrogen are associated with an increased risk of ESCC.¹⁶⁸ This implies that mutations in genes that play a role in metabolizing sex hormone may be important in the etiology of ESCC. Genetic variation in at least six of these genes that play a role in the metabolism of sex hormones have been associated with an increase in the risk of developing EC. These include cytochrome P450s (CYP1B1, CYP3A7, CYP3A5 and CYP11A1), SULT2B1 and SHBG. These genes regulate sex hormone activity by catalyzing their breakdown or regulating their bioavailability.¹⁵

Earlier reports showed that ESCC cells grown in vitro express both estrogen and ARs and the addition of these hormones to the growth media promoted the growth of ESCC cells in vitro.^{96,169}

Table 7 Genetic polymorphisms in CYP/CYP450 genes relating to ESCC in South Africa and China

Country and polymorphism	OR	Interaction with the environment	Effect on ESCC risk
CYP2E1			
1053C > T (rs2031920) ²¹⁶ 1293G > C (rs3813867) ²¹⁶	5.04		Changes in the 5' UTR of the gene changes the transcription of the gene
China			
(*c1/*c1) homozygote (*c1/*c2) heterozygote (*c2/*c2) mutated homozygote ^{207,217}	2.89	In moderate-to-heavy drinkers, the CYP2E1 (*c1/*c1) genotype significantly increased the risk of cancer	The c1/c1 genotype correlated with susceptibility to EC. Differences in CYP gene expression may contribute to individual susceptibility to procarcinogens
South Africa			
CYP2E1 (*c1/*c1) ¹⁵⁸			Genotype frequencies were higher among patients with ESCC
CYP3A			
China			
CYP3A5*3A A6986G, C31611T ¹⁶⁰		Found in 65%–73% of Chinese individuals	Splicing defect preventing expression
CYP3A5*3C A6986G ¹⁶⁰		Found in 70% of Chinese individuals	Splicing defect preventing expression
CYP3A5*4 A14665G Q200R ¹⁶⁰		Found in 0.9% of Chinese individuals	
CYP3A5*5 T12952C ¹⁶⁰		Found in 0.9% of Chinese individuals	Splicing defect leading to alternatively spliced mRNA
South Africa			
CYP3A5 *1/*1	1.00	Black subjects are more likely to convert environmental toxins they are exposed to into more reactive intermediates that may play a role in the initiation of EC	A potentially high CYP3A5 activity may increase EC risk. CYP3A5 mutant alleles are associated with reduced risk for EC ^{158,160,162,164}
CYP3A5 *1/*3	1.97		
CYP3A5 *1/*6	1.37		
CYP3A5 *1/*7	4.67		
CYP3A5 *3/*3	0.84		
CYP3A5 *3/*6	1.17		
CYP3A5 *3/*7	0.90		
CYP3A5 *6/*6	0.81		
CYP3A5 *6/*7	2.92		
CYP3A homozygous (GG) heterozygotes (GA) ¹⁵⁸			Declined ESCC risk in homozygous Increased risk of ESCC in heterozygous
CYP1A1			
CYP1A1 MspI ¹⁸ C vs T CC + CT vs TT	1.25 1.35		Polymorphism increases the risk of ESCC
CYP1A1 exon 7 Val/Val ³¹	3.35	CYP1A1 Val/Val genotype had synergic interactions with tobacco and alcohol consumption	Individuals carrying CYP1A1 Val/Val genotype compared to those with CYP1A1 Ile/Ile genotype had an increased risk for EC

Abbreviations: EC, esophageal cancer; ESCC, esophageal squamous cell carcinoma; rs, reference SNP cluster ID; SNP, single-nucleotide polymorphism.

Table 8 The extent to which different risk factors influence the odds of developing ESCC in Swedish males and females¹⁶⁵

Risk factor	OR at 95% CI	
	Male	Female
Obesity	5.4	10.3
Smoking	2.8	5.3
Reflux	3.4	4.6

Abbreviation: ESCC, esophageal squamous cell carcinoma.

Estrogen is metabolized into 4-hydroxy estrogen by the enzyme CYP/CYP450 1B1 (CYP1B1). This enzyme has a high catalytic activity for this reaction. The resulting

4-hydroxy estrogen is able to induce DNA damage.¹⁷⁰ Mutations in *Cyp1B1* that result in a GG substitution rather than the GA in normal copies decrease the efficacy of the enzyme. South African individuals homozygous for the mutant allele (GG), and therefore possessing a less-efficient CYP1B1, had a lower risk of developing ESCC. These heterozygous individuals (GA) had an increased risk of developing ESCC.¹⁵⁸

SULT2B1 catalyzes the sulfate conjugation of many hormones, and is therefore required for the formation of these hormones. Two SNPs, SULT2B1a and SULT2B1b, in this gene are connected with the risk of ESCC in the Chinese population.¹⁶⁰

The role of dietary selenium in contributing to EC

Selenium is an essential factor in several metabolic activities. This is due to the use of selenium by selenoproteins, proteins containing a selenocysteine amino acid residue. These proteins function as enzymes in many vital processes. These include protecting the cell membranes from lipid peroxidation, defending cells from oxidative damage and regulation of the immune system.¹⁷¹ Selenium has anticarcinogenic and chemoprotective effects, and selenium-containing proteins perform a significant function in the metabolism and detoxification of polycyclic aromatic hydrocarbons.¹⁷² Selenium also protects the DNA from being damaged by oxygen free radicals by scavenging these oxygen molecules. Selenium also promotes the removal of damaged cells by inducing apoptosis. If they are not removed, these damaged cells could potentially develop into cancerous cells.¹⁷³

Selenium compounds stimulate apoptotic death of tumor cells,¹⁷⁴ and selenium regulates p53 in the role it plays in DNA repair or apoptosis.¹⁷⁵ Primarily, selenium is found as selenomethionine. Selenomethionine stimulates the repair of DNA damage through the p53 pathway. An essential component of this signaling pathway is the GSTP1 protein. GSTP1 activates p53 through a redox mechanism.¹⁷⁶ The function and activity of a mutant GSTP1 protein that has an Ile-to-Val substitution (rs947894) are decreased. This mutant protein is the result of a polymorphism in the GSTP1 gene at codon 105.¹⁷⁷ Mutations in the p53 gene are prevalent in all cancers including EC.^{178,179} A single base modification from arginine (CGC) or proline (CCC) is initiated on codon 72 (rs1042522).¹⁸⁰ This polymorphism is connected to tumorigenesis in a variety of cancers. It is also a risk factor for HPV-associated cervical neoplasia and ESCC.^{181,182} The GSTP1 and p53 gene polymorphisms modify selenium–ESCC relation.¹⁸³ Studies showed that p53 Pro/Pro was related to ESCC risk as compared with p53 Arg/Arg homozygotes.¹⁸³ Individuals with both the GSTP1 Ile/Ile and p53 Pro/Pro genotypes have an increased risk of ESCC.¹⁸³ Therefore, dietary selenium intake can alter an individual's risk of developing EC. This risk can then be further increased if the polymorphisms in the GSTP1 and the p53 genes are also present.¹⁸³

Conclusion

This review highlights the contribution that genetic polymorphisms make to the incidence of EC in a South African population, how these genetic polymorphisms are found in other population groups, especially Chinese populations, and the extent to which they contribute to the incidence of

EC in these population groups. The characterization of these genetic polymorphisms may allow us to identify the molecules that can serve as lead drug targets as well as the new diagnostic and prognostic markers. These polymorphisms can best be studied through the use of large cohort studies that take into account the role played by environmental and lifestyle factors that may contribute to or protect an individual from EC. These studies would not only enable us to identify new genetic polymorphisms that are involved in EC, but also allow us to more accurately establish the role played by environmental hazards, such as drinking alcohol, smoking tobacco and consumption of *Fusarium*-contaminated maize. However, the future challenges include combining the results of these multiple studies in such a way so as to obtain a set of genetic signatures that can be used as population-specific prognostic or diagnostic markers, or even as targets for the development of new drugs. Furthermore, simultaneous analysis of multiple polymorphic genes would allow us to obtain a complete picture of their contribution in the development of EC.

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