Recent Advances in Gastroenterology

Edited by **Chris Probert**

Contents

Chapter 1

Genetic epidemiology of oesophageal cancer

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INTRODUCTION

The majority of oesophageal cancers are carcinomas–either squamous cell (SCC) or adenocarcinoma (AC). Rarer types include small cell cancer and nonepithelial tumours such as sarcomas (e.g. leiomyosarcoma and rhabdomyosarcoma), malignant melanoma and lymphoma. Oesophageal cancer is the 10th commonest cancer in the world overall, but it is the 5th commonest cancer in less developed countries, most of these being SCCs. It has the distinction of having the widest variation in incidence worldwide of any cancer, there being a 15-fold geographical variation in men and a 20-fold variation in women. The highest rates are found in China, Iran and South Africa and lowest rates in central Africa and central America. In these high-risk countries, the majority of oesophageal cancer is due to SCC, but in low-risk countries, particularly certain Western countries, the incidence of AC is rising rapidly and it is the most rapidly increasing of all cancers.

Worldwide, 482,000 people are diagnosed with oesophageal cancer and 407,000 people die from it each year [1]. In the United States, the comparable figures are an estimated 17,990 cases and 15,210 deaths in 2013 [2], whilst in the European Union, for the year 2010, the estimated number of cases was 43,700 and 27,700 deaths. Within Europe, the United Kingdom has the highest rate of oesophageal cancer, with approximately twice the average European age-adjusted rates.

RISK FACTORS (TABLE 1.1)

Age and gender

Both SCC and AC are associated with increasing age, and are diseases of middle to late life. SCC has roughly an equal gender incidence, particularly in areas of high incidence; however, in areas of low incidence, men predominate just as with AC.

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Table 1.1 Risk factors for oesophageal cancer

	Oesophageal cancer	
Factor	**Squamous cancer**	**Adenocarcinoma**
Age	> 50	> 50
Gender	M = F	M > F
Race	A-C > Cauc > Hisp	Caucasian
Socioeconomic (lower) status	✓	?
Smoking	✓	✓
Alcohol	✓	x
Increased BMI		✓
Dietary—Reduced fruit and vegetables	✓	?
Deficiency of Se, Zn and folate	✓	?
Poor oral hygiene	✓	?
Drinking maté	✓	x
Drinking hot liquids	✓	x
Pre-existing diseases		✓
Hiatus hernia/GOR	x	✓
Barrett's oesophagus	x	✓
Atrophic gastritis	✓	x
Achalasia	✓	x
Caustic injury	✓	x
Thoracic radiation	✓	x
Coeliac disease	✓	x
Previous head and neck cancer	✓	x
HPV infection	✓	x
Previous cholecystectomy	x	✓
H. pylori absence	x	✓
Heredity—Tylosis	✓	x
Alcohol metabolism	✓	x
Medications—Lower LOSP	✓	✓
NSAIDs/aspirin reduced risk	✓	✓
Bisphosphonates	✓	x

A-C, Afro-Caribbean; GOR, gastro-oesophageal reflux—increased frequency and duration; Hisp, Hispanic; NSAIDs, nonsteroidal anti-inflammatory drugs.

Smoking and alcohol

Both tobacco and alcohol have been identified as major risk factors for oesophageal SCC and are synergistic. Smoking cigarettes increases the risk three- to sevenfold [2], whilst alcohol confers a three- to fivefold risk. Individuals who smoke and drink have a ninefold increased risk. Cigar and pipe smokers have a risk similar to cigarette smokers [2].

Smoking is less strongly associated with oesophageal AC. Population-based case–control and cohort studies have shown a less than twofold increase, but several of these studies have shown a dose-related effect, increasing the suspicion of a relationship between the two [3]. Alcohol is not a risk factor for oesophageal AC.

Race

In the United States, age-adjusted incidence of oesophageal cancer is almost twice as high in African American races than in Caucasians and exceeds that for Hispanics, Asian Americans and Native Americans; however, AC is mainly a disease of Caucasian males [4].

Socioeconomic status

SCC was found to be associated with lower socioeconomic status in one study.

Dietary factors

Carcinogens

N-Nitroso compounds

N-Nitroso compounds are known carcinogens, thought to act by causing the development of alkyl adducts in DNA. In animals, they have been implicated in the development of several cancers, including in the nasal cavity, oesophagus and stomach [5], although direct evidence for human cancers is lacking. Furthermore, 45–75% of exposure comes from endogenous synthesis of ingested nitrites or nitrates and the remainder from tobacco smoking, occupational exposure and food sources such as vegetables. Pickled vegetables and other foodstuffs may be a source of nitroso compounds, particularly in endemic areas such as China, either directly or secondary to contamination by toxin-producing fungi.

Polycyclic aromatic hydrocarbons

These compounds are contained in a drink called *maté* which is made by making infusions of the herb *Ilex paraguayensis* with hot water–a practice common in certain parts of South America, which also has high rates of oesophageal cancer [5]. It is not certain whether it is the polycyclic aromatic hydrocarbons (PAH) alone or the fact that the maté is often drunk very hot (see below) which is the important factor.

Nutritional deficiencies

Fresh fruit and vegetables

Diets low in fresh fruit and vegetables have been postulated to be conducive to the development of oesophageal cancer, most of the evidence coming from case–control studies. Conversely, diets high in fresh fruit and vegetables have been shown to lower the risk of oesophageal cancer although the vast majority of the evidence has been in relation to SCC [6]. What evidence there is does not support a reduced risk of AC with diets high in fruit and vegetables.

Vitamins and minerals

Low levels of selenium, zinc and folic acid have been associated with increased risk of SCC. Diets high in folic acid have been shown to reduce the risk of oesophageal cancer.

Poor oral hygiene

Initial evidence supporting a link between oesophageal cancer and poor oral hygiene appear to be confirmed by later studies. However, most of the studies did not take account of other, potentially confounding, factors.

Repetitive thermal injury

Repetitive thermal injury to the oesophageal mucosa from the ingestion of hot food and drinks has been considered a cause of oesophageal cancer. More than half of 59 case–control and cohort studies have shown a significantly increased risk with ingestion of hot liquids although the methodology has been criticised and, in the majority of studies, oesophageal SCC and AC were not differentiated.

Occupation

Traditionally, oesophageal SCC was said to be increased in certain occupations related to alcohol production, such as draymen and publicans, although the evidence for this was contaminated by confounding factors which were not adjusted for in the analysis. Occupations characterised by exposure to asbestos, silica and other substances such as sulphuric acid have been associated with an increased risk of this cancer [6].

Oesophageal disease

Achalasia

Case–control studies in patients with achalasia have indicated that oesophageal SCC occurs in 3–7% of this population, compared with 2% in the general population [7].

Gastro-oesophageal reflux

Symptomatic gastro-oesophageal reflux is one of the strongest risk factors for oesophageal AC. The risk is increased depending on the length of the history, the frequency of symptoms and presence of nocturnal reflux [8].

Barrett's oesophagus

Barrett's oesophagus (BO) is a condition in which the normal squamous mucosa is replaced with columnar epithelium in the distal oesophagus as a result of chronic gastro-oesophageal reflux and is strongly associated with the development of oesophageal AC. The prevalence of BO is 1.6% in the general population and 10–15% in patients with reflux who have undergone upper gastrointestinal endoscopy. The risk of AC in patients with Barrett's oesophagus is 0.5% per year. Barrett's oesophagus is most commonly diagnosed in middle to late life, with the average age at diagnosis being 55. More men are diagnosed than women and it is most common in Caucasian populations, less common in black and Hispanic populations and least common in Asian populations. Barrett's oesophagus is associated with smoking and obesity. The risk of AC in BO is approximately 1% [9].

Obesity

The mechanism whereby obesity increases the risk of oesophageal AC is probably indirect, through the promotion of gastro-oesophageal reflux as a result of increased intra-

abdominal pressure and the subsequent development of BO. A meta-analysis showed an increased odds ratio (OR) of 2.78 (1.85–4.16) for AC in patients with a body mass index greater than 30 [10,11].

Coeliac disease

Coeliac disease is associated with an increased risk for a number of malignancies including oesophageal SCC for which the risk is increased eightfold [12].

Previous aerodigestive cancers

Approximately 2% of patients who have had a SCC of the upper aerodigestive tract will go on to develop a metachronous SCC of the oesophagus [13].

Caustic injury

This is due to the ingestion of strong alkalis such as sodium or potassium hydroxide contained in drain-cleaning fluid and other household cleaning products. Most cases occur in children under the age 5 when they are accidental and the rest in adults as a result of psychotic or suicidal behaviour. It results in scarring and stricture formation in the oesophagus. SCC develops after approximately 40 years [14].

Thoracic radiation

Radiotherapy to the mediastinum for the treatment of a number of cancers including breast cancer, lung cancer and lymphoma increases the risk of both types of oesophageal cancer [15].

Plummer—Vinson/Patterson—Kelly syndrome

Patients with this condition, which comprises a triad of features, namely dysphagia, iron deficiency anaemia and oesophageal webs, have a greater risk of SCC [16] although some cases of this syndrome may be due to undiscovered coeliac disease.

Helicobacter pylori infection and atrophic gastritis

A meta-analysis of 19 studies suggested an inverse relationship between the presence of CagA-positive *Helicobacter pylori* (*H. pylori*) and the risk of AC [17]. It has been suggested that the decline of *H. pylori* infection may be partly responsible for the increase in AC over the past decades.

Previous cholecystectomy

A population-based, cohort study of cholecystectomised patients in Sweden found an increased risk of AC (standardized incidence ratio, 1.3; 95% CI, 1.0–1.8) but not of SCC (SIR, 0.9; 95% CI, 0.7–1.1) [18]. The rationale behind the study was that duodenogastric reflux of bile occurred after cholecystectomy and gastro-oesophageal reflux of bile is associated with Barrett's oesophagus.

Human papilloma virus

Human papilloma virus (HPV) has been implicated in the development of both benign and malignant squamous cell tumours of the oesophagus; 21.3% of squamous cell papillomas and 22.9% of SCCs of the oesophagus were positive for HPV infection [19].

Medications

Medicines that reduce the tone of the lower oesophageal sphincter

A number of medications may increase the risk of AC by causing relaxation of the lower oesophageal sphincter, thereby promoting gastro-oesophageal reflux. These include β-adrenergic agonists (bronchodilators), calcium channel blockers, nitrates, phosphodiesterase inhibitors (aminophylline), anticholinergics, γ-aminobutyric acid agonists (baclofen), benzodiazepines and hormone replacement therapy (HRT). The available evidence has been conflicting.

Bisphosphonates

Early studies on the possible link between bisphosphonate use and oesophageal cancer showed conflicting results. In a series of population-based case–control studies in two large primary care databases, exposure to bisphosphonates was not associated with increased risk [20], although a limitation of this study was that no attempt was made to differentiate the two main types of oesophageal cancer.

Medications that may protect against oesophageal cancer

Nonsteroidal anti-inflammatory drugs and aspirin

There is epidemiological evidence to indicate that these medications may reduce the risk of oesophageal cancer, particularly AC, by approximately 40%, although one study found that using the selective COX inhibitor, celecoxib, did not appear to have any effect [21]. The mechanism of this action is thought to be by the inhibition of the enzyme cyclooxygenase.

Proton pump inhibitors and H2 receptor antagonists

Unfortunately, studies looking at the effects of these medications in oesophageal cancer have not produced any consistent results. Theoretically, they could work by reducing the acid content of the gastric refluxate, the stimulant to mucosal change in Barrett's oesophagus. Alternatively, by reducing gastric acid, they may encourage gastric colonisation by bacteria that are capable of producing gastric carcinogens such as *N*-nitrosamines.

Hormone replacement therapy

It has been speculated that HRT may be implicated in a number of cancers including cancers of the oropharynx, stomach and colon. A meta-analysis of three case–control studies from Europe provided evidence for a beneficial effect of HRT in oesophageal cancer. This result is all the more surprising considering the fact that HRT has been shown to increase the risk of reflux (*vide supra*).

Heredity

Molecular studies

Oesophageal SCC, like most solid tumours, has been shown to harbour many somatic mutations, which may be driver or bystander events. Early whole genome searches for causative genes used the technologies of the time, namely, restriction fragment-length polymorphisms (RFLP) and loss of heterozygosity (LOH) or allelic imbalance (AI) of microsatellite alleles, to identify commonly deleted or duplicated regions. However, by today's standards, these studies used widely spaced markers and fairly modest tumour

numbers. The most commonly identified regions by these methods were 3p (35–41%), 5q (36–53%), 9p (36–65%), 9q (31–60%), 13q (43–53%), 17p (55–62%), 17q (33–71%), 18q (38–46%) and 21q (65%). Studies investigating the progressive accumulation of genetic defects, ranging from low-grade to high-grade dysplasias and carcinoma, demonstrated early loss of genetic regions at 3p21, 9p22 and 17p13 in dysplastic lesions that mirrored similar evidence from head and neck cancers [22,23] and suggests some commonality in the mutational pathway of these squamous cell aerodigestive tract cancers.

The identification of chromosomal areas of genetic alteration in oesophageal SCC led to an explosion of targeted AI studies investigating those regions most commonly altered in an attempt to localise the causative genes, but with limited initial success owing to the infancy of the human genome project. More definitive results were obtained by targeting candidate genes previously implicated in other SCCs, such as *APC* (5q), *p16* (CDKN2A; 9p), *Rb* (13q), *BRCA2* (13q) and *p53* (17p), which have all been demonstrated to be altered in many oesophageal SCCs.

The advent of high-density microarray-based genome-wide scanning methods such as comparative genome hybridisation (CGH) and single-nucleotide polymorphism (SNP) arrays has revolutionised the field of genomics. Several relatively large-scale CGH studies in oesophageal SCC have been undertaken and have confirmed the previous allelic imbalance studies in identifying losses at 3p, 5q, 9p and 18q and gains at 13q [24]. Additional amplifications have also been frequently observed at 3q and 8q. Interestingly, chromosome 17 alterations are rarely observed by CGH, implying that no net loss or gain of genomic material results during AI on this chromosome.

The use of SNP arrays has been much more extensive, given the additional, gene-specific information that can be gleaned from such studies. Indeed, over 5000 cases and more than 7000 controls have been analysed in this way, with validation in a further 30,000 individuals [25,26]. These studies, together with meta-analyses, [27] have identified SNPs having highly significant associations with oesophageal SCC and allowed the identification of candidate genes on 10q23 (*PLCE1*), 20p13 (C20orf54; *SLC52A3*) and loci located at 2q33, 5q11, 6p21, 12q24 and 21q22.

Much of this more recent work has been undertaken in Asian populations, where the incidence of the disease is relatively high; therefore, it is gratifying that there is a degree of crossover in the chromosomal regions identified by both AI (predominantly in Caucasian populations) and CGH or SNP arrays (mainly Asian populations) and in the identification of *PLCE1* and C20orf54 as candidate oesophageal SCC-specific aberrations [28].

Family history

In areas of high incidence, such as Iran and China, familial aggregation of squamous cancer has been recorded [29], but this is not observed in areas of low incidence suggesting that common environmental rather than hereditary risk factors are responsible.

There has been a steady accumulation of evidence pointing to a genetic component in hiatus hernia, reflux oesophagitis, Barrett's oesophagus and oesophageal AC. Familial clustering of cases of hiatus hernia, Barrett's oesophagus and gastro-oesophageal reflux have all been documented [30], with each condition cosegregating in different families. In some families, the familial tendency has been so strong as to suggest autosomal dominant inheritance [31]. Twin studies have confirmed significantly greater concordance rates in monozygotic, compared with dizygotic, twin pairs–implying that genetic factors are playing an important role in these conditions.

Alcohol-metabolising enzymes

Many gastro-oesophageal cancers are strongly associated with environmental and life style risk factors. For oesophageal SCC, alcohol is an important risk factor and, in Asian populations, even light alcohol use appears to be associated with susceptibility [32]. Because exposure to high levels of acetaldehyde, the principal metabolite of alcohol, may be the cause, it is not a surprise that genome-wide SNP array studies identified chromosomes 4q23 and 12q24, where the major alcohol-metabolising enzymes, *ADH* cluster and *ALDH2* genes, respectively, are located.

Several large studies have been carried out investigating genetic susceptibility in Asian and Western populations, including meta-analyses, and have identified increased risk of oesophageal cancer in individuals with polymorphisms in *ADH1B* and *ALDH2* that code for proteins with lower metabolising activity. Thus, the less active G allele of rs1229984 in *ADH1B* is associated with an increased risk of oesophageal SCC, with the OR increasing from a modest effect to an OR of 20 in heavy drinkers, whilst the A allele of rs671 in *ALDH2* shows a protective effect in nondrinkers but is strongly associated with oesophageal SCC in heavy drinkers [33–35].

Similarly, polymorphisms in other detoxifying enzymes, such as *NAD(P)H* dehydrogenase, quinone 1 (*NQO1*), which has been proposed to stabilise p53, have been shown to confer an increased risk in Asian populations but not in Europeans [36].

Hereditary diseases with oesophageal cancer

Clarke–Howel–Evans–McConnell syndrome (OMIM 1485000)

Tylosis (hyperkeratosis palmaris et plantaris) is a focal, nonepidermolytic form of keratoderma. It is inherited as an autosomal dominant condition. Two types of tylosis are recognised. In type B, the hyperkeratosis appears in the first year of life but, in type A, it does not appear until about puberty. Type B is not associated with malignancy, but type A is associated with SCC of the oesophagus that usually manifests in middle to later life. Type A is also associated with other features such as oral leucokeratosis and follicular hyperkeratosis. The gene for type A tylosis has been localised to chromosome 17q25 and has been identified as *RHBDF2* [37]. This 30.5-kb gene encodes a protein of 856 amino acids that is a member of a class of proteins known as rhomboids. Rhomboid proteins are intramembrane serine proteases that cleave substrates in or near the transmembrane domains. The protein product of *RHBDF2* is classified as an inactive or irhomboid due to the fact that it lacks the typical proteolytic site made up of a serine, a histidine and an aspartic acid residue. Altered expression and localisation of this protein have subsequently also been shown in sporadically occurring oesophageal SCC, thus validating the AI data at 17q and implicating the gene product, iRhom2, in many of these cancers (Risk, unpublished data, 2013). Further, the iRhom2 protein has been shown to control processing of the major 'sheddase,' ADAM17, which controls the membrane shedding of many epidermal growth factor receptor ligands, inflammatory cytokines and adhesion molecules [38]. Indeed, in cell lines derived from tylotic skin, increased ADAM17 processing is detected together with increased production of ADAM17 substrates in response to inflammatory stimuli, whilst increased EGF signalling and cell migration are also observed (Kelsell, personal communication, 2014). Five families have been documented and, in four of these families, a mutation has been found in the *RHBBF2* gene. The risk of developing oesophageal SCC in the Liverpool family has been calculated to be 95% at the age of 65 [39].

Dyskeratosis congenital (OMIM 305000)
This condition is characterised by cutaneous pigmentation, premature greying, nail dystrophy, leucoplakia, thrombocytopenia, anaemia, testicular atrophy and a predisposition to cancer, including oesophageal cancer.

Fanconi's anaemia (OMIM 227650)
Fanconi's anaemia is a rare, mainly autosomal recessive disorder, resulting in failure of DNA repair. It, therefore, leads to the development of haematological (acute myeloid leukaemia) and nonhaematological (head and neck, oesophageal, gastrointestinal, vulvar and anal) malignancies.

Recessive dystrophic epidermolysis bullosa (OMIM 226600)
This is one of a number of inherited disorders resulting in the formation of skin blisters on minimal trauma. Patients have an increased of cancer of the skin and oesophagus.

SUMMARY

The demography of oesopahgeal cancer has been changing over the past few decades. Not only has adenocarcinoma overtaken squamous cancer as the most frequent oesophageal cancer in Western countries, but there has been an improvement in the 5-year survival rate as a result of the earlier recognition and treatment of less advanced lesions. Several predisposing environmental risk factors have been identified, the removal of which should potentially result in further reductions in incidence and mortality, although at present this remains to be seen.

A significant compilation of genetic information now exists for oesophageal SCC, but the challenge of realizing this information in clinical practice remains. The identification and implementation of genetic biomarkers for this disease remains problematic. Perhaps the genetic analysis of this disease should be focussed towards the identification of novel druggable targets to increase treatment options which can then be offered to targeted patient populations of the basis of the genetic analysis of the tumour.

Key points for clinical practice

- Oesophageal cancer is not particularly common, except in certain well-known areas of the world, but it has a poor prognosis, with the 5-year survival being less than 20%.
- Oesophageal SCC has the widest geographical variation in the incidence of any cancer.
- Oesophageal AC is the fastest growing of all cancers, but the cause for this rise is not apparent at present.
- Epidemiological studies indicate that environmental factors play a large part in the pathogenesis of oesophageal cancer. and they may, therefore, be amenable to manipulation in an attempt to reduce the incidence of this condition.
- State-of-the-art investigational technologies have revealed an increasing number of molecular changes in the metamorphosis from benign to malignant epithelium.
- Rare, inherited disorders, which include oesophageal cancer. in the clinical spectrum, are throwing light on the understanding of the sporadic forms of the disease.
- Lessons learnt from oesophageal squamous cancer may be applied to other aerodigestive cancers and vice versa.

- An understanding of the molecular events involved in the conversion of oesophageal squamous to columnar epithelium and their interplay with environmental factors may suggest ways of preventing this development.
- The poor prognosis of oesophageal cancer. is partly due to early, particularly lymphoid, spread. Identification of the earliest molecular events in the development of both SCC and AC may lead to an ability to diagnose these cancers at the earliest stages before any spread has occurred, thereby improving the mortality rate.
- Molecular studies may also help to identify individuals who are especially prone to oesophageal cancer. and who may benefit from surveillance or even chemoprevention.

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