MOLECULAR EPIDEMIOLOGY OF GASTRIC AND ESOPHAGEAL CANCER SURVIVAL

by

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ABSTRACT

Introduction

Gastric and esophageal cancers are among the deadliest forms of cancer. Studies of human cancer susceptibility examine factors associated with the incidence of disease. Studies of human cancer prognosis and prediction examine factors associated with disease outcomes. This dissertation is about molecular and other factors that affect survival of gastric and esophageal cancer patients.

Methods

Population-based registry data linked with patient outcome data was used to describe the epidemiology of gastric and esophageal cancers in BC; to compare survival of cancer patients in BC, and Ardabil, Iran and to describe differences in survival of BC patients of different ethnicity. The ethnicity of patients was determined based on lists of names corresponding to each ethnic group. A prospective cohort study was conducted to examine the effect of genetic polymorphisms in *TIMP (1-4)* and *MMP (2, 7 and 9)* genes. Results

Analysis of cancer registry data points to several factors associated with gastric and esophageal cancer survival. Patients with gastric cardia experience worse survival compared to other gastric cancers. Ethnicity of gastric and esophageal cancer patients is associated with their survival. Gastric and esophageal cancer patients diagnosed in British Columbia have better survival compared to those daignosed in Adabil, Iran. Genetic polymorphisms are also associated with survival. My prospective study identified 4 genetic polymorphisms at *TIMP-*

3 associated with survival of esophageal adenocarcinoma and gastroesophageal junction (GEJ).

Conclusion

Besides established prognostic indicators, other factors affect survival of gastric and esophageal cancers. Differences in survival by ethnicity support the importance of ethnicity as a prognostic factor. Survival differences between BC and Ardabil are likely due to disease characteristics and patient factors, in addition to differences in healthcare systems. *TIMP3* genetic polymorphisms are promising prognostic factors for adenocarcinoma of esophagus and GEJ. Modeling prognosis based on host factors, including ethnicity and genetic polymorphisms, is an emerging field of translational cancer research. More research is needed to fully explore the functional effects of *TIMP3* polymorphisms, and to identify both genetic and lifestyle factors underlying the effect ethnicity on survival.

PREFACE

CHAPTER 2

A version of this chapter has been submitted for publication and is under review.

Bashash M, Shah A, Hislop G, Le N, Bajdik C, Brooks-Wilson A. Review: Genetic variants associated with esophageal and gastric cancer survival (Submitted June 19, 2010)

Author contribution: MB designed and conducted the study, and wrote 100% of this manuscript. AS assisted in the intepretation and reviewed the manuscript. GH assisted in the intepretation and reviewed the manuscript. NL assisted in the intepretation and reviewed the manuscript. CB and ABW supervised all aspects of the study, contributed to interpretation of the findings, and reviewed and edited the manuscript.

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Author contribution: MB designed the study, collected the BC data, performed the analysis and wrote 100% of the manuscript. AS supervised clinical aspects of the study and reviewed the manuscript. GH supervised epidemiological aspects of the study and reviewed the manuscript. NL supervised statistical aspects of study and reviewed the manuscript. CB and ABW supervised all aspects of study, contributed to interpretation of the findings, and reviewed and edited the manuscript. PY and RM supervised the Iranian investigators and corresponding portions of the study.

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CHAPTER 6

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Author contribution: MB designed the study, performed patient recruitment, performed data collection, designed the genetic analysis, performed the statistical analysis and wrote 100% of the manuscript. AS supervised clinical aspects of the study and reviewed the manuscript. GH supervised epidemiological aspects of the study and reviewed the manuscript. NL supervised statistical aspects of study and reviewed the manuscript. CB and ABW supervised all aspects of the study, contributed to interpretation of the findings, and reviewed and edited the manuscript. All sample preparation, DNA extraction and genotyping preparation was performed in A Brooks-Wilson's lab. Genotyping was sponsored by ABW.

Ethics approval: This study was approved by the UBC/BCCA Ethics Board (certificate number H07-2807)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

3' UTR	Three prime untranslated region
5FU	Fluorouracil
ADAM	Adamalysins
ADAMTS	Adamalysin-thrombospondin
AJCC	American Joint Committee on Cancer
Arg	Arginine
ASR	Age-standardized incidence rate
BC	British Columbia
BCCA	British Columbia Cancer Agency
BCCR	British Columbia Cancer Registry
BRCA	Breast cancer (gene)
CEU	Utah residents with Northern and Western European ancestry
cGy	centi-Gray
CI	Confidence interval
del	Deletion polymorphism
DNA	Deoxyribonucleic acid
EAPC	Estimated annual percent change
ECM	Extracellular matrix
ECRG	Esophageal cancer-related gene
EGFR	Epidermal growth factor receptor
ERCC1	Excision repair cross-complementing
FA	Folinic acid

GEJ	Gastroesophageal junction
GERD	Gastro-esophageal reflux disease
GI	Gastrointestinal
GSH	Glutathione
GST	Glutathione-S-transferases
GWAS	Genome-wide association studies
HapMap	Human haplotype map
HR	Hazard ratio
HWE	Hardy-Weinberg equilibrium
ICDO	International Classification of Diseases for Oncology
IL	Interleukin
Ile	Isoleucine
ins	Insertion polymorphism
MDR	Multidrug resistance
MMP	Matrix Metalloproteinases
MSP	British Columbia Medical Services Plan
MTHFR	5, 10-methylenetetrahydrofolate reductase
mTOR	Mammalian target of rapamycin
NS	Non-significant
OGG1	8-oxoguanine glycosylase
Pgp	P-glycoprotein
PI3K	Phosphoinositide 3-kinases
Pro	Proline

PTEN	Phosphatase and tensin homolog
SCC	Squamous cell carcinoma
SD	Standard deviation
SMPBC	Screening Mammography Program of British Columbia
SNP	Single nucleotide polymorphisms
STR	Short tandem repeat (microsatellite) polymorphism
TIMP	Tissue inhibitors of metalloproteinases
TNF	Tumour necrosis factor
TYMS	Thymidylate synthase
Val	Valine
VEGFR	Vascular endothelial growth factor receptor
WHO	World Health Organization
WHOSIS	WHO Statistical Information System
XP	Xeroderma pigmentosum
XRCC	X-ray repair cross complementing group

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DEDICATION

To Maryam, Deniz and Doreen!

In the loving memory of my mom Khadijeh, my dad Samad Bashash, My grandmother Robabeh and my sister in law Adeleh!

CHAPTER 1: Introduction

1.1 Background

It is estimated that in the year 2010 more than 4,210 cases of gastric and esophageal cancers will be diagnosed in Canada and more than 3,220 will die of this disease [1]. In spite of being infrequent in Canada, these cancers have significant contribution to cancer mortality. After lung, prostate and colorectal, this combination of cancer is the 4th cause of cancer death among men in Canada. The morbidity of these cancers makes them extremely important to general health and of interest to researchers. Increased knowledge about early detection, better treatments and influences on survival are essential. This dissertation concentrates on factors that might influence survival of these cancers.

Gastric cancer

Overall, gastric cancer incidence and mortality have fallen dramatically over the past 70 years[2]. Despite its recent decline, gastric cancer is the fourth most commonly-diagnosed cancer and the second leading cause of cancer-related death worldwide [2]. In 2000, about 880,000 people globally were diagnosed with gastric cancer and approximately 650,000 died of the disease [3]. Several different types of cancer can occur in the stomach. Adenocarcinoma, which starts in the glandular cells, is the most common histological type, accounting for 90-95% of all gastric malignancies. It can spread to nearby lymph nodes and other areas of the body, such as the liver, pancreas, colon, lung, and ovaries [4]. The two main sites of gastric adenocarcinoma are cardia (proximal) and non-cardia (lower). Despite a decline in lower gastric cancers, proximal tumours have been increasing in incidence since the 1970s, especially among males in the Western countries [5]. Gastric cardia tumours now account for nearly half of all stomach cancers among men in the US and UK [6]. Risk factors for gastric cancers include: Helicobacter pylori infection[7];

heredity, genetic and individual immunological factors; diet and lifestyle; tobacco smoking; obesity; ionizing radiation, pernicious anemia, blood type A, prior gastric surgery for benign conditions, and Epstein-Barr virus [3]. Gastric cancer is also classified based on the Lauren classification[8] into two major types: (1) intestinal and (2) diffuse gastric cancer (DGC). It has been shown that germline mutations in the E-cadherin (CDH1) gene are the cause of hereditary diffuse gastric cancer (HDGC). It is estimated that about 40% of HDGC families may harbour the CDH1 mutation [9]. The mutation's penetrance is reported to be about 70% [10 11].

Esophageal cancer

80% of the worldwide esophageal cancers occur in developing countries with high incidence areas including Asia, south-eastern Africa, eastern South America and some areas of Western Europe. It includes, most commonly, squamous cell carcinoma (SCC) in the upper and mid esophagus and adenocarcinoma in the lower esophagus. The incidence of SCC of the esophagus is decreasing in western countries, while that of gastroesophageal adenocarcinoma is increasing [2,12]. What is disturbing (but also fascinating) about esophageal cancer, is the remarkable variation in its geographic incidence in different parts of the world. While the incidence of this disease in North America is about 5 to 10/100,000, the corresponding figure in some areas of Iran is more than 100/100,000. It is often lethal, most commonly presenting in an advanced stage as a swallowing disorder (dysphagia) in elderly patients [13]. Risk factors for SCC include: a chronic interaction between tobacco smoking or chewing and alcohol intake together with low intake of fresh fruit and vegetables, resulting in vitamin and mineral deficiencies; repeated intake of high-temperature drinks such as tea in China, Calvados in France, or maté in Brazil; chronic exposure to tobacco, opium pipe residue, betel quid; contaminants such as mycotoxins, nitrosamines in foods and drinks; rare conditions, such as tylosis, a genetic change leading to thickening of the skin (hyperkeratosis) of the palms and soles; chronic esophageal stasis, e.g. achalasia, peptic

strictures; Plummer–Vinson syndrome; and previous or concomitant head or neck squamous cell cancer [2,12]. Until recently, most epidemiological and biological studies of esophageal cancer have focused on SCC. Prior to 1980, approximately 90% of cases of esophageal cancers were of squamous cell histology. During the past two decades, however, there has been a dramatic increase in the incidence of adenocarcinoma of both the esophagus and gastroesophageal junction (GEJ) in North America and Western Europe [14]. In many western countries, adenocarcinoma is now more common than SCC [15]. The first report of an esophageal adenocarcinoma is credited to White in 1898 [16]. A review of the literature in 1900 revealed only six cases, and at the time most physicians believed that these cancers represented extension of gastric tumours into the esophagus [16]. Once a rare tumour, adenocarcinoma of the esophagus currently has higher incidence than SCC in America. The rate of increase of adenocarcinoma of the esophagus is outpacing the next closest cancer, melanoma, by nearly three times [17]. The current average yearly rise in incidence in the United States exceeds 20% and, among white men, the incidence has increased >800% since the mid-1970s[18]. Risk factors for adenocarcinoma include: gastroesophageal reflux, Barrett's esophagus, asthma medications, LES (lower esophageal sphincter)-relaxing medications, removal of the gallbladder (cholecystectomy), obesity and cigarette smoking [16].

The anatomy of the esophagus is shown in Figure 1.1 [19]. The organ bridges three anatomic regions: the neck, thorax, and abdomen. The esophagus extends from the cricopharyngeus muscle to the gastroesophageal junction (GEJ). The cervical esophagus is defined as the ~3 cm portion between the cricopharyngeus and the thoracic inlet. The remainder of the esophagus is commonly divided into thirds. This includes the upper third (upper thoracic ~ 6 cm) extending from the thoracic inlet to the carina, the middle third (mid thoracic ~ 8 cm) extending from the

carina to the inferior pulmonary veins, and the lower esophagus (lower thoracic/abdominal ~ 8 cm) traversing the remaining distance into the abdomen to the gastroesophageal junction. Adenocarcinoma predominates in the lower esophagus[19,20]. The cardia is the portion of the stomach surrounding the cardioesophageal junction, or cardiac orifice (the opening of the esophagus into the stomach).

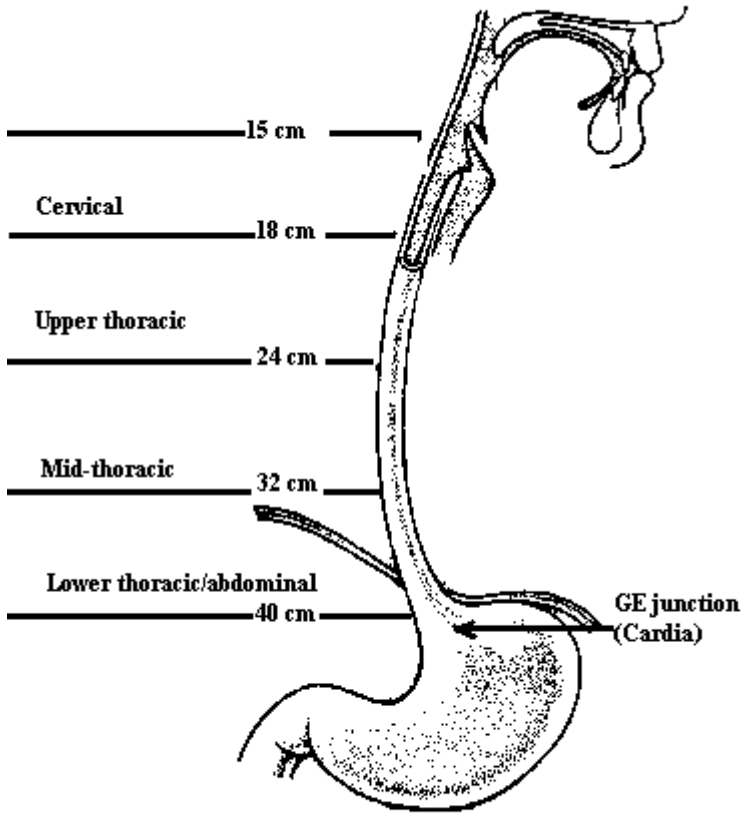

Figure 1.1 Anatomy of the esophagus with landmarks and recorded distance from the incisors used to divide the esophagus into topographic compartments.

1.2 Prognostic factors

A prognostic factor is used to help define patients with high and low risks of death. Knowledge of prognostic factors helps us understand the natural history of cancer [21]. Prognostic factors (predictors) could be classified into three broad groups: i) tumour-related prognostic factors, ii) host-related prognostic factors, and iii) environment-related prognostic factors [21-23].

Tumour-related prognostic factors

Tumour biology includes a large and ever growing field of research that includes molecular and biological prognostic factors. These factors predict the natural history of the disease as well as the likelihood of response to treatment [22]. Molecular cancer research has generated information concerning the progression of adenocarcinoma of the lower esophagus and GEJ [24]. Molecular prognostic factors are still not included in current clinical prognostic models, such as the TNM classification. The main reason for this is that molecular biological research is rapidly evolving and an astonishing number of biomarkers have been described, but sufficiently large studies regarding the prognostic value of each specific gene or protein are still lacking [24].

The histological grade of a tumour refers to an evaluation of whether a tumour is malignant and how aggressive it is likely to be. There can be variation in grade within the same tumour, and the highest (ie, worst) grade is usually recorded for prognostic purposes[20]. According to grade classification: grade 1 tumours are well differentiated with similar characteristics as the original tissue, grade 2 are moderately well differentiated, grade 3 are poorly differentiated, and grade 4 are undifferentiated tumours, which cannot be recognized as having any characteristics of their tissue of origin.

Tumour pathology is crucial to the determination of prognosis in cancer. The Lauren classification is an independent prognostic factor in adenocarcinoma of the esophagus and GEJ; some studies show worse survival of diffuse-type [24].

Stage is a fundamental prognostic factor related to the anatomical extent of disease [21]. Current staging systems (American Joint Committee on Cancer [AJCC] and Union Internationale Contre le Cancer [UICC]), for gastric esophagus and adenocarcinoma of the esophagus and GEJ are TNM based [25,26]. (T), or increasing depth of tumour invasion is associated with lymphatic involvement and is a known important independent prognostic parameter [20]. Pathologic lymph node (pN) staging was evaluated according to the number and site of nodal metastasis. The number-based classification is based on criteria provided by the UICC and AJCC rules [25,26]. Stage groupings are strong independent prognostic parameters, with a higher stage implying more advanced disease [25,26]. Recently, adenocarcinoma of the lower esophagus and GEJ are staged following the same rules as esophageal cancer [25,26]. Before that, the UICC suggests classifying adenocarcinoma of the GEJ as esophageal carcinoma if more than 50% of the tumour mass involves the esophagus and as gastric carcinoma if more than 50% involves the stomach [27,28].This obviously caused problem for classification of GEJ tumours because of the borderline location. GEJ tumours were staged as esophageal cancer by some authors and as gastric cancer by others [20].

For gastric cancer AJCC definedprimary tumour (T), regional lymph node (N) and distant metastasis (M) status is shown in table 1.1 [25,26]. TNM staging group of gastric cancer based on AJCC guidelines is shown in table 1.2 [25,26]. For Esophageal cancer AJCC categorizes primary tumour (T), regional lymph node (N) and distant metastasis(M) status as described in table 1.3 [25,26]. AJCC recommends separate staging systems for squamous cell carcinoma and

adenocarcinoma. In addition to the TNM classifications, for squamous cell carcinoma, the classification is subdivided based on the location of the original tumour as well as the grading of tumour. TNM staging of esophageal squamous cell carcinoma is shown in table 1.4[25,26]. For adenocarcinoma, AJCC uses the T, N, and M classifications, as well as the tumour grade. Adenocarcinomas of the GEJ are staged following the same rules as esophageal adenocarcinoma. TNM staging of esophageal and GEJ adenocarcinoma is shown in table 1.5:

In BC, the general treatment for esophageal cancer is surgery in stage I. 50% of esophageal cancer patient with stage II/III disease receive radiation (4500 cGy in 25 fractions) and chemotherapy (5-fluorouracil [5FU] + cisplatin) followed by surgery, followed by chemotherapy (5FU + cisplatin). The other 50% of patients first undergo surgery followed by radiation (4500 cGy in 25 fractions) and chemotherapy (5FU + cisplatin). 75% of Stage IV esophageal cancer patients recieve chemotherapy (5FU + cisplatin), the other 25% receives only symptomatic care. Generally, treatment for gastric cancer is surgery in stage I, II, III; chemotherapy (5FU or 5FU+cisplatin) in 65% of stage IV cases; and symptomatic care only in the other 35% of stage IV.

Host-related prognostic factors

Patient (or host-related) prognostic factors include inherent and demographic characteristics such as age, gender, and ethnicity. Other factors, such as performance status, comorbidity, and immune status, are also important. All of the host-related factors are also significantly impacted by constitutional genetic polymorphisms. Even the complex process of metastasis might be significantly impacted by host genetics [29]. Ethnicity is a possible prognosis factor for cancer in upper GI other than GEJ [30].

Environment-related prognostic factors

Environment-related prognostic factors are those that are external to the patient, such as choice of treatment, quality of treatment, access to care, health-care policy, and access to drugs or technology that may impact outcome [22]. Treatment is likely the greatest determinant of cancer patients' survival. These factors lend themselves to immediate modification in the interest of improved outcome.

1.3 Research objectives

Our **primary objective** was to assess genetic polymorphism of TIMPs (1,2,3,4) and their prognostic effects in esophagus and GEJ adenocarcinomas in newly-diagnosed patients with esophageal and GEJ adenocarcinomas in BC.

Our **secondary objectives** were to assess the prognostic effects of i) genetic polymorphisms in MMPs 2, 7, and 9; and ii) the environmental and healthcare variables, and iii) ethnicity.

1.4 The thesis sections

Chapter 2 reviews genetic studies of gastric and esophageal cancer prognosis and prediction.

Chapter 3 describes the incidence and survival for gastric and esophageal cancer in the population of BC, Canada between 1990 and 1999.

Chapter 4 compares one-year survival of gastric and esophageal cancers between the populations of British Columbia (BC), Canada and Ardabil, Iran.

Chapter 5 compares survival of gastric and esophageal cancer patients among Chinese, South Asian and Iranian and other ethnic groups in BC.

Chapter 6 assesses genetic polymorphism of TIMPs and MMPs and their prognostic effects for esophagus and GEJ adenocarcinomas.

Table 1.1 Stage category definitions for gastric cancer [25,26].

PRIMARY TUMOR (T)	**TX**	Primary tumor cannot be assessed
	T0	No evidence of primary tumor
	Tis	Carcinoma in situ: intraepithelial tumor without invasion of the lamina propria
	T1	Tumor invades lamina propria, muscularis mucosae, or submucosa
	T1a	Tumor invades lamina propria or muscularis mucosae
	T1b	Tumor invades submucosa
	T2	Tumor invades muscularis propria
	T3	Tumor penetrates subserosal connective tissue without invasion of visceral peritoneum or adjacent structures*,**,***
	T4	Tumor invades serosa (visceral peritoneum) or adjacent structures**,***
	T4a	Tumor invades serosa (visceral peritoneum)
	T4b	Tumor invades adjacent structures
		*A tumor may penetrate the muscularis propria with extension into the gastrocolic or gastrohepatic ligaments, or into the greater or lesser omentum, without perforation of the visceral peritoneum covering these structures. In this case, the tumor is classified T3. If there is perforation of the visceral peritoneum covering the gastric ligaments or the omentum, the tumor should be classified T4. **The adjacent structures of the stomach include the spleen, transverse colon, liver, diaphragm, pancreas, abdominal wall, adrenal gland, kidney, small intestine, and retroperitoneum. ***Intramural extension to the duodenum or esophagus is classified by the depth of the greatest invasion in any of these sites, including the stomach.
REGIONAL LYMPH NODES (N)	**NX**	Regional lymph node(s) cannot be assessed
	N0	No regional lymph node metastasis *
	N1	Metastasis in 1 to 2 regional lymph nodes
	N2	Metastasis in 3 to 6 regional lymph nodes
	N3	Metastasis in 7 or more regional lymph nodes
		* A designation of pN0 should be used if all examined lymph nodes are negative, regardless of the total number removed and examined.
DISTANT METASTASIS (M)	**M0**	No distant metastasis
	M1	Distant metastasis

Table 1.2 Staging group of gastric cancer [25,26].

Group	T	N	M
0	Tis	N0	M0
IA	T1	N0	M0
IB	T2	N0	M0
	T1	N1	M0
IIA	T3	N0	M0
	T2	N1	M0
	T1	N2	M0
IIB	T4a	N0	M0
	T3	N1	M0
	T2	N2	M0
	T1	N3	M0
IIIA	T4a	N1	M0
	T3	N2	M0
	T2	N3	M0
IIIB	T4b	N0	M0
	T4b	N1	M0
	T4a	N2	M0
	T3	N3	M0
IIIC	T4b	N2	M0
	T4b	N3	M0
	T4a	N3	M0
IV	Any T	Any N	M1

Table 1.3 Stage category definitions for esophageal cancer[25,26].

PRIMARY TUMOR (T)	**TX**	Primary tumour cannot be assessed
	T0	No evidence of primary tumour
	Tis	High-grade dysplasia *
	T1	Tumour invades lamina propria, muscularis mucosae, or submucosa
	T1a	Tumour invades lamina propria or muscularis mucosae
	T1b	Tumour invades submucosa
	T2	Tumour invades muscularis propria
	T3	Tumour invades adventitia
	T4	Tumour invades adjacent structures
	T4a	Resectable tumour invading pleura, pericardium, or diaphragm
	T4b	Unresectable tumour invading
		*High-grade dysplasia includes all non-invasive neoplastic epithelium that was formerly called carcinoma in situ, a diagnosis that is no longer used for columnar mucosae anywhere in the gastrointestinal tract.
REGIONAL LYMPH NODES (N)	**NX**	Regional lymph nodes cannot be assessed
	N0	No regional lymph node metastasis
	N1	Regional lymph node metastases involving 1 to 2 nodes
	N2	Regional lymph node metastases involving 3 to 6 nodes
	N3	Regional lymph node metastases involving 7 or more nodes
DISTANT METASTASIS (M)	**M0**	No distant metastasis
	M1	Distant metastasis

Table 1.4 TNM staging of esophageal squamous cell carcinoma[25,26].

GROUP	T	N	M	Grade	Tumour Location
0	Tis	N0	M0	1	Any
IA	T1	N0	M0	1, X	Any
IB	T1	N0	M0	2-3	Any
	T2-3	N0	M0	1, X	Lower, X
IIA	T2-3	N0	M0	1, X	Upper, middle
	T2-3	N0	M0	2-3	Lower, X
IIB	T2-3	N0	M0	2-3	Upper, middle
	T1-2	N1	M0	Any	Any
IIIA	T1-2	N2	M0	Any	Any
	T3	N1	M0	Any	Any
	T4a	N0	M0	Any	Any
IIIB	T3	N2	M0	Any	Any
IIIC	T4a	N1-2	M0	Any	Any
	T4b	Any	M0	Any	Any
	Any	N3	M0	Any	Any
IV	Any	Any	M1	Any	Any

Table 1.5 TNM staging of esophageal and GEJ adenocarcinoma[25,26].

GROUP	T	N	M	Grade
0	Tis	N0	M0	1, X
IA	T1	N0	M0	1-2, X
IB	T1	N0	M0	3
	T2	N0	M0	1-2, X
IIA	T2	N0	M0	3
IIB	T3	N0	M0	Any
	T1-2	N1	M0	Any
IIIA	T1-2	N2	M0	Any
	T3	N1	M0	Any
	T4a	N0	M0	Any
IIIB	T3	N2	M0	Any
IIIC	T4a	N1-2	M0	Any
	T4b	Any	M0	Any
	Any	N3	M0	Any
IV	Any	Any	M1	Any

CHAPTER 2: Review: Genetic variants associated with esophageal and gastric cancer survival[1]

2.1 Introduction

Esophageal and gastric cancers are among the most aggressive and deadliest malignancies in the world. Esophageal cancer is the eighth most common cancer worldwide, and the sixth most common cause of death from cancer [2]. Gastric cancer is the fourth most common cancer worldwide, and the second most common cause of death from cancer [2]. During 2009, it is estimated that 16,470 cases of esophageal cancer and 21,130 cases of gastric cancer were diagnosed in the US, and more than 25,000 people died because of these cancers [31].

Prognostic factors estimate the risk of various outcomes based on clinical and non-clinical characteristics [32], but do not consider treatment. In contrast, predictive markers consider which patients may benefit from a specific treatment [33] and classify populations into groups for which different treatment options can be recommended. The majority of prognostic factors in cancer are directly related to the tumour [22,33], including tumour stage, tumour size and, in some cases, other markers related to the presence of a tumour. Molecular research has generated an immense amount of information about the progression of cancers, including gastric and esophageal cancers [24], but molecular markers are not yet routinely included in prognostic models. Other variables affecting the outcome include host-related and environment-related factors [34]. Environment-related factors **a**re external to the patient, such as choice of treatment, quality of treatment, access to care, health-care policy and access to drugs or technology [22]. Host-related

[1] A version of this chapter has been submitted for publication and is under review.
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prognostic factors include inherent and demographic characteristics such as age, gender and ethnicity. Other factors such as performance status, co-morbidity and immune status are also important [22]. Many host factors are inherited and likely to be, at least in part, due to genetic variants [35]. Some of these factors directly affect tumour cell growth and may modulate the host immune response to allow tumour growth [36]. For example, genes influence the risk of progression and/or metastasis from cancer (permissive metastasis genotypes), and similar cancers in different patients may exhibit different tendencies to metastasize [29].

Genetic studies of human cancer susceptibility determine alleles associated with the incidence of disease. Genetic studies of human cancer prognosis try to determine alleles associated with the outcomes of disease. This article reviews genetic studies of esophageal and gastric cancer prognosis.

2.2 Material and methods

Using PubMed, MEDLINE has been searched for the keywords “gastric cancer”, “esophageal cancer”, “survival”, “outcome”, “polymorphism”, “SNP” and “genetic”. Searches were limited to English language articles on human studies published between 1957 and 2009. Abstracts of articles were reviewed and those that were only related to disease incidence excluded. Studies of mutations in tumour DNA were also excluded, as were studies that did not consider genetic variants in any way. Genetic polymorphisms were divided into two classes: single nucleotide polymorphisms (SNPs) and structural variants [35]. The dbSNP database was used to standardize variation in genetic names [37].

2.3 Results

The literature search identified 36 articles that examined 129 polymorphisms in 63 genes for their effect on esophageal and gastric cancer survival. These included 20 articles on gastric cancer, 14 on esophageal cancer and two regarding the gastroesophageal junction. Of 14 articles about esophageal cancer, 5 specifically studied adenocarcinoma [38,39] and 2 studied squamous cell carcinoma [40] [41]. A summary of the esophageal cancer studies is given in Table 2.1; a summary of the gastric cancer studies is given in Table 2.2. Note that one study considered esophageal and gastric cancer patients combined [42]and is included in both Tables 2.1 and 2. The gastric cancer articles included studies of between 44 [43] and 503 [44] cases. For esophageal cancer, the corresponding numbers of cases were 39 [38] and 371 [45]. Blood was the source of DNA in 10 esophageal cancer studies [40,42,45-52] and 14 gastric cancer studies [43,44,48,53-63]; the other studies used normal tissue from tumour samples (blocks, slides or fresh tissue). Most of the articles reported on North American studies [38-40,42,45,46,49,64-68] although 6 articles were about German populations [41,47,51,63,69,70] and 5 were about Chinese populations [43,55-57,61]. All of the samples were collected as part of routine clinical procedures and some patients already had received adjuvant and neoadjuvant treatment [43,48,51,55-57,63,70].

Results from these studies indicate that 6 polymorphisms in 5 genes were consistently associated with esophageal cancer prognosis, 7 polymorphisms in 7 genes showed an association in some but not all studies, and 55 polymorphisms in 41 genes did not show any effects. For gastric cancer, 9 polymorphisms in 8 genes showed a prognostic effect, 10 polymorphisms in 7 genes showed discordant results and 39 polymorphisms in 21 genes did not influence gastric cancer patients' survival. Finally, 8 polymorphisms in 8 genes had prognostic effects on both gastric and

esophageal tumours (Figure 2.1). This includes 3 genetic polymorphisms in 3 genes for esophageal and stomach cancer combined.

These polymorphisms can be categorised according to the genetic pathways in which they are involved.

Cytokines

Cytokines are small intercellular signalling peptides that act within the autocrine, paracrine or endocrine system to promote growth, differentiation and activation of cells. The anti-inflammatory activity and immunosuppressive activity of cytokines depends on the microenvironment [71]. Cytokines that are predominantly produced by monocytes include tumour necrosis factor (TNF) and interleukin (IL) molecules IL1, IL6, IL8, IL12, IL15, IL18 and IL23 [71]. Genetic polymorphisms of cytokines have been shown to be important in cancer. In a review of 161 meta-analyses and pooled analyses of candidate SNPs in 99 genes, nearly one-third (98/344) of variants were significantly associated with various cancers, including 6 cytokine gene variants [72]. Among patients with advanced cancer, increased expression of pro-inflammatory cytokines also are associated with anorexia and cachexia, pain, toxicity and resistance to treatment [72]. Tumour necrosis factor-α (TNFα) is a highly multifunctional cytokine involved in immune and inflammatory responses and affecting angiogenesis and tumour growth [73]. Deans *et. al.* conducted a study of 203 gastroesophageal (i.e., gastric, esophageal or gastroesophageal junction) cancer patients showing that interaction of three genotypes (*IL6* −174 G/C, *IL10* -1082A/G and *TNFα* -308G/A) resulted in a cumulative reduction in survival [42]. In a small study of 44 gastric and gastroesophageal junction cancer patients, Jatoi *et al.* examined *IL1β* and found a survival benefit for patients with +3954C/T or +3954T/T genotypes (hazard ratio (HR) 0.3; p=0.04) compared to patients with the C/C genotype [66]. In a study of 123 patients

with relapsed or metastatic gastric cancer, Graziano *et al*. reported that short tandem repeat (microsatellite) polymorphism (STR) of *IL1RN, IL1β* -511C/T and *IL1β* -31T/C polymorphisms influence prognosis [74]. In an earlier study, Shimura *et al.* examined an NcoI restriction fragment length polymorphism of the tumour necrosis factor-β (*TNFβ*) gene among gastric cancer patients and reported better 3-year survival among patients homozygous for the 10.5 kb fragment (87.1% 3-year survival) compared to other alleles (5.5 kb homozygote, 52.5% 3-year survival; heterozygote, 79.1% 3-year survival) [75]. There was no prognostic significance of *IL1β* -31T/C, *IL1β* -511C/T or *IL1RN* STR [66], *IL1β* -511C/T, *LTα* +252 [42], *IL8* -251, *IL1β* -511, *IL1RN* or *TNFα* -857 [62] on gastric and esophageal cancer patients.

Matrix Metalloproteinases (MMPs) and inhibitors

MMPs contribute to multiple steps of tumour progression including invasion, promotion, angiogenesis, and the establishment and growth of metastatic lesions in distant organ sites [76]. It is recognized that MMPs can be synthesized by tumour cells, but are frequently produced by surrounding stromal cells including fibroblasts and infiltrating inflammatory cells [76]. Finally, MMPs solubilize cell surface and matrix-bound factors that can influence cellular properties such as growth, death, migration and metastasis [76]. An important mechanism for the regulation of MMP activity is binding to a family of homologous proteins referred to as the tissue inhibitors of metalloproteinases (TIMPs). These are natural inhibitors of MMP proteolytic activity *in vitro* as well as *in vivo* [77]. Tang *et al*. conducted a study of 74 patients in China showing an association between the *MMP9* Gln279Arg-Pro574Arg haplotype and 1 year postoperative mortality of gastric cancer patients [52]. Kubben *et al.* showed association of *MMP7* -181A/G and *TIMP2* 303C/T with poor outcome in patients from the Netherlands [78]. In contrast, a recent study on 313 esophageal cancer patients in the U.S did not find any association between polymorphisms of *MMP1, MMP3* and *MMP12* and outcome [45]. There were no significant associations between

MMP2 -1306C/T, *MMP7* -153C/T, *MMP8* -799C/T, *MMP8* +17C/G, *MMP9* -1562C/T, *TIMP1* 372C/T and *TIMP2* -418G/C [78], *MMP9* R279Q [52] polymorphisms and survival of gastric cancer patients.

Xenobiotic metabolism

Glutathione-S-transferases (GSTs) are a family of Phase II detoxification enzymes that catalyse the conjugation of glutathione (GSH) to a wide variety of xenobiotics [79]. Glutathiones play a role in detoxifying, and consequently protecting cells from alkylating agents and products of reactive oxidation. GSTP1 is known to detoxify platinum compounds including oxaliplatin [80] and cisplatin [79]. Polymorphisms of these genes are well studied in the literature, however, only a few studies have found significant results for GST gene variants and survival [57 69 51 59]. Huang *et al*. conducted a study of 102 gastric cancer patients who were treated with oxaliplatin-based adjuvant chemotherapy, and showed an association between *GSTP1* Ile105 Ile genoytpe and survival [57]. Goekkurt *et al*., in a study of 52 gastric cancer patients who received at least one complete cycle of 5FU/cisplatin/FA as first-line chemotherapy, showed that patients possessing the *GSTP1* Val105 Val genotype demonstrated a significantly superior median survival time of 15.0 months (95% CI 7.8-22.0) compared to 6.0 months (95% CI 5.1-7.0) in patients with at least one GSTP1 105 Ile allele [69]. In a study of 110 patients with locally advanced gastric carcinomas who received preoperative chemotherapy, Ott *et al*. examined the effect of GST polymorphism on outcome and found improved survival for patients with the *GSTM1*- (non- conjugators) genotype compared to patients with the *GSTM1* null [51]. Finally, Lee *et al*. conducted a study of 233 patients with esophageal cancer and showed that patients carrying *GSTP1* 105 Val variants had poorer survival [59]. In these and other studies there were no statistically significant associations between survival and *GSTP1*/rs1138272, *GSTP1*/rs1695, *GSTP1* Ile105Val, *GSTP1* Ala114Val, *GSTT1* +/- or *GSTM1* +/- polymorphisms [51 57 68 61 69 70 59 57].

A polymorphism of the thymidylate synthase (TYMS) gene was one of the most studied variants for prognostic and predictive effect on gastric and esophageal cancer. TYMS is the main target of 5FU [80], a major chemotherapeutic agent for gastric and esophageal cancers. One of the most highly studied polymorphisms is a tandem repeat, of which TSER*2 and TSER*3 are the most common alleles [80]. Huang *et al*. conducted a study of 116 patients with gastric cancer treated with 5FU-based adjuvant chemotherapy and reported significantly better survival among patients with *TYMS* 3′UTR (1494del6) ins6/ins6 compared with patients with the del6/del6 or heterozygous genotypes [57]. Keam *et al*. conducted a study of 73 patients with metastatic or relapsed gastric adenocarcinoma who were enrolled in a prospective phase II clinical trial. The study reported that the del6/del6 variant in *TYMS* 3'UTR was significantly associated with prolonged overall survival [48]. In the same and other studies, the *TYMS* variants rs2790, rs699517 [70 68 57 39], *TYMS* tandem repeat polymorphisms, rs45445694 [41 81 57 39] and TYMS 3'UTR del6/del6 [67 69 57 39] did not show significant prognostic effects.

Multidrug resistance (MDR) describes the phenomenon of simultaneous resistance to unrelated drugs, possibly because of reduced drug accumulation involving the P-glycoprotein (Pgp; mdr1 gene) [82]. Wu *et al*. studied four *MDR* variants (3435C/T, Ala892Ser, Ala892Ser, 3425C/T) of the *MDR1* gene and reported a significant association of the *MDR1* 3435C/T variant allele with reduced recurrence risk among esophageal cancer patients receiving platinum-based drugs [68].

5, 10-methylenetetrahydrofolate reductase (MTHFR) is a key enzyme for intracellular folate homeostasis and metabolism. Because activity of 5FU is dependent on a competitive interaction with folate metabolism, the *MTHFR* polymorphism has been suggested to have an effect on 5FU-based chemotherapies [57]. Wu *et al*. examined the effect of polymorphism of this gene among esophageal cancer patients and concluded that *MTHFR* 677C/T (Ala222Val, rs1801131)

and 1298A/C (rs1801131) SNPs influence disease recurrence and survival; and patients with variant alleles at both loci had a significantly reduced recurrence risk and better survival [68]. Huang *et al*. also reported that *MTHFR* 677CC genotype was associated with shorter overall survival among gastric cancer patients [57].

Cell cycle

Alteration of the p53 gene is found in about half of human cancers, and most other cancers deactivate the p53 pathway by increasing its inhibitors, reducing its activators or inactivating its downstream targets [83]. p53 is best characterized as a transcription factor that binds to specific DNA sequences and transactivates a number of genes with a variety of functions including cell cycle arrest, apoptosis and metabolism [83]. As a result, this gene might be prognostic for gastric and esophageal cancer patients. Huang *et al*. conducted studies on 110 gastric cancer patients treated with 5FU-based adjuvant chemotherapy [43] and 102 with oxaliplatin-based adjuvant chemotherapy [57], showing a roughly two-fold prognostic decrease for patients carrying *p53* codon Pro72Pro. The same result has been shown by Cescon *et al.* in a recent study of 371 esophageal carcinoma patients [45]. Other studies failed to show significant prognostic effects of *p53* +62 A/G(rs1625895), *p53* Pro72Arg [68] or other *p53* variants (rs1788332, rs1042522, rs1625895 or rs1801173/rs2273953) [53] among gastric and esophageal cancer patients.

The p53 binding protein homolog 2 (MDM2) is a key element in the activation of p53. In two relatively large studies, Ohmiya *et al.* and Cescon *et al.* studied the prognostic effect of *MDM2*. In one, analysis of 410 gastric cancer patients showed that *MDM2* 309G/G is an independent marker of poor overall survival in advanced carcinomas [62]. In the other study, of 371 patients with esophageal carcinoma, *MDM2* 309T/G was associated with markedly reduced survival in squamous cell carcinoma [45].

The cyclin D1 proto-oncogene is a powerful control element that regulates the mitotic cell cycle, and excessive cyclin D1 expression and/or activity is common in human cancers. Polymorphism of *cyclin D1* is also suggested to influence cancer risk and outcome in general [84]. In a study of 124 esophageal cancer patients, Izzo *et al.* observed polymorphism of *cyclin D1* 870G/A was statistically associated with overall survival [65]. However, in a smaller study of 69 esophageal squamous carcinomas patients, no significant association of this polymorphism was observed [40].

The esophageal cancer-related gene 2 (ECRG2) has been shown to be related to cell proliferation and induction of apoptosis in esophageal cancer cells [85]. Short tandem repeat polymorphism of *ECRG2* have been studied in 86 patients with primary esophageal cancer, showing that the *ECRG2* TCA3/TCA3 genotype was the factor most strongly associated with negative outcome [85].

DNA repair genes

Cancer cells are often defective in a DNA repair pathways such as mismatch repair, base excision repair, nucleotide excision repair, homologous recombination, nonhomologous end-joining and trans-lesion synthesis [86]. Polymorphisms in *OGG1, XRCC1, ERCC1, XPC, XPD, XPF, BRCA2* and *XRCC3* have been shown to affect other cancer risks outcomes [87]. Wu *et al.* conducted a study of genetic polymorphisms on clinical outcomes in 210 esophageal cancer patients and reported that variant alleles of *XRCC1* Gln399Arg were significantly associated with poor survival [68]. In another study of 62 gastric cancer patients, Liu *et al*. determined that patients with the *XRCC1* Gln399Arg genotype demonstrated a significant worse survival [61]. Other studies of *ERCC1*/rs3212986, *ERCC1*/rs11615, *MGMT*/rs12917, *XRCC1*/rs25487, *XRCC1*/rs1799782, *XRCC3*/rs861539 [70], *ERCC1* 3'UTR, *ERCC6* Met1097Val, *ERCC6* Arg1230Pro, *APEX1* Asp148Glu, *XRCC1* Gln399Arg [68], *ERCC1* Asn118Asn, *ERCC1* 8092C/A [48], *ERCC1* 118C/T,

[55,57], *ERCC1* C118C/T *ERCC2* Gln751Lys [69] polymorphisms did not find significant associations with survival.

Signalling pathways and growth factor genes

Signalling through the PI3K/PTEN/AKT/mTOR pathway is responsible for balancing cell survival and apoptosis [64]. The signal is initiated by growth factors and hormones that bind receptor tyrosine kinases such as epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR) [64]. Hildebrandt *et al*. studied polymorphisms of the PI3K/PTEN/AKT/mTOR pathway in 210 esophageal cancer cases who received chemotherapy. They described an association of mTOR pathway polymorphisms *FRAP1*/rs11121704 and *FRAP1*/rs2295080 on the overall study group and *AKT1*/rs1130214 and *AKT2*/rs892119 on patients who received taxane [64]. Jain *et al*. conducted a study of 69 patients and reported that *EGF* 61A/G genotype independently influenced survival in squamous cell esophageal cancer [40]. In a recent study Bradbury *et al*. reported the variant allele of *VEGF* 936C/T was associated with improved overall survival compared with the wild type allele among esophageal cancer patients [88]. Kim *et al*. examined 503 gastric cancer patients for polymorphism of *VEGF* and reported that the +936C/T variant TT allele was associated with worse overall survival compared to the C/C allele [89]. Other studies did not show any association between *AKT1*/rs4375597 [70], *AKT1*/rs3803304, *AKT1*/rs2498804, *AKT1*/rs2494738, *AKT2*/rs8100018, *PIK3CA*/rs7651265, *PIK3CA*/rs7640662, *PIK3CA*/rs7621329, *PIK3CA*/rs6443624 [64], *VEGF* -460T/C, *VEGF* 405G/C [88] polymorphisms and survival.

2.4 Discussion

Gastric and esophageal cancer are among the most deadly of all gastrointestinal malignancies worldwide, with 5-year mortality rates exceeding 80% [90]. This review examined 36 articles in

which prognostic effects of 62 genes were studied and among them, 31 polymorphisms affected gastric and esophageal cancer survival. In general, the literature review identified some recurring problems with published studies. First, most studies concentrated on a group of gastric and esophageal cancer patients that was not population-based. Second, most studies described patients who received a specific treatment and, as a result, reported prognostic effects might be predictive. Third, the small number of cases in most studies limits statistical power. Fourth, most studies did not discriminate between cardia and lower gastric cancers, and some studies did not discriminate between squamous cell carcinoma and adenocarcinoma of the esophagus. Two large studies considered all gastric, esophageal and gastroesophageal junction cancer cases as a single group. Finally, several different kinds of biological samples were used in these studies and therefore comparisons between the studies might not be optimal.

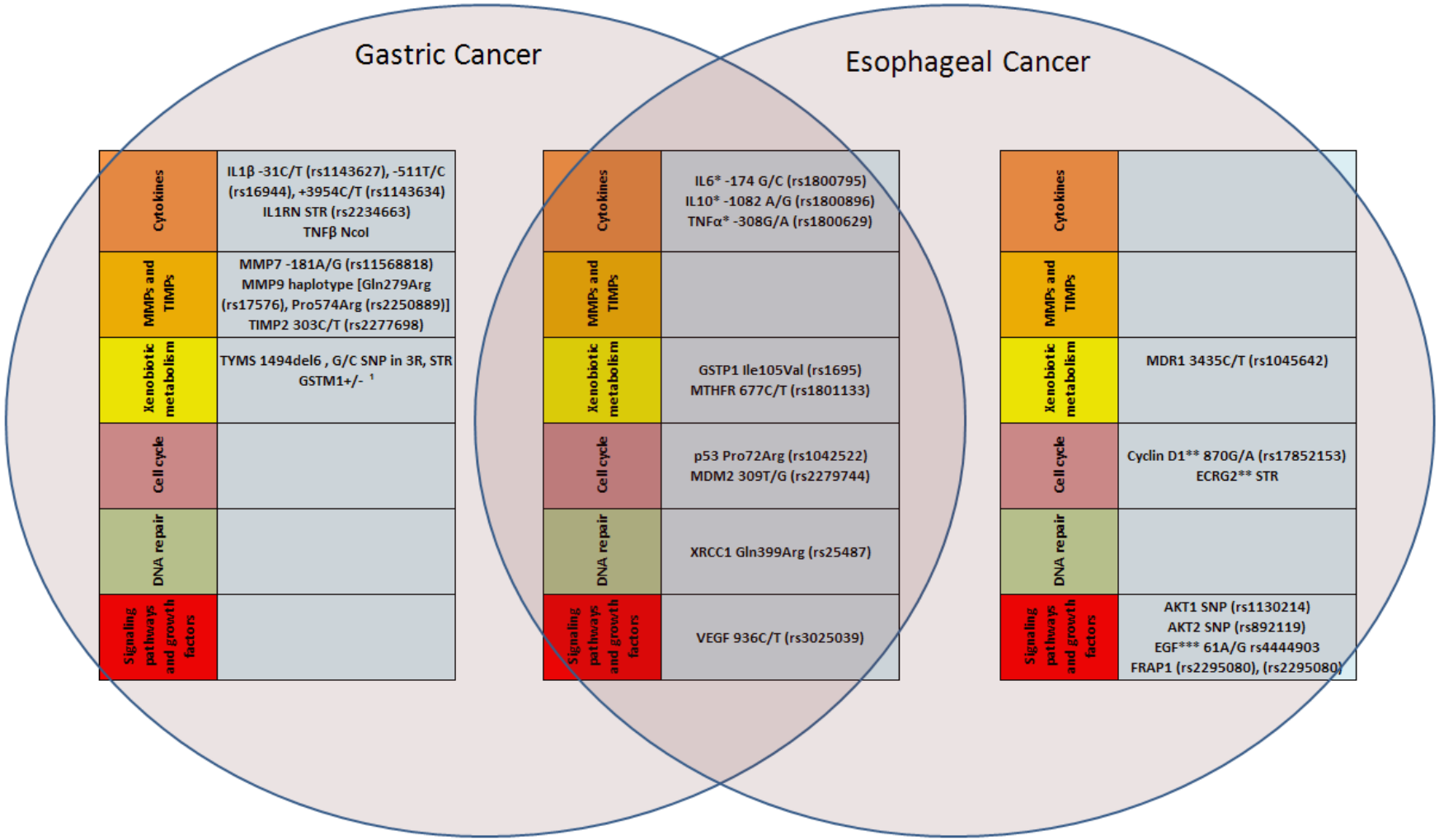
Generally, most of the papers summarized here examined only one or a very small number of polymorphisms in each gene. This candidate polymorphism strategy fails to examine the bulk of the genetic variation. Newer studies examine genes more comprehensively using haplotype tags based on HapMap data and provide more meaningful results. For single SNP studies, a negative result can mean that the SNP examined is not involved in disease or outcome, but the gene could still have an effect. No genome-wide association studies (GWAS) of survival in gastric and esophageal cancer have been reported.

2.5 Conclusion

This review shows that genetic polymorphisms in cell cycle (*Cyclin D1, ECRG2, MDM2* and *p53*), DNA repair (*XRCC1*), signalling and growth factors (*AKT1, AKT2, EGF, FRAP1*, and *VEGF*) and xenobiotic metabolism (*MDR1, GSTP1*, and *MTHFR*) are associated with esophageal cancer survival. Similarly, genetic polymorphisms in cell cycle (*p53, MDM2*),

cytokines (*IL1β, IL1RN,* and *TNFβ*), DNA repair (*XRCC1*), matrix metalloproteinases and their inhibitors (*MMP7, MMP9* and *TIMP2*), signalling and growth factor genes (VEGF) and xenobiotic metabolism *(TYMS, GSTM1, GSTP1* and *MTHFR*) had prognostic value for gastric cancer. Genetic polymorphisms in the cytokines *IL10, IL1β, IL6* and *TNFα* were significant in gastric and esophageal cancer patients combined (Figure 2.1). Our results indicate that polymorphisms in genes associated with cell cycle, xenobiotic metabolism, DNA repair and signalling and growth factors have prognostic significance for both gastric and esophageal cancer. Understanding the mechanism of each polymorphism, and pathway-based analyses, might help identify markers for gastric and esophageal cancer survival.

There is an increasing interest in the effect of host genetic polymorphisms on the survival of cancer patients. Conventional techniques generally do not adequately predict the heterogeneity of patient outcomes. In many cancers, tumour markers have been used as a factor for survival models and guiding treatment decisions. However in gastric and esophageal cancer, adequate tumour samples for these assays may not be easily available. Modeling prognosis based on host factors including genetic polymorphisms is an emerging field of translational research. Compared to tumour, constitutional genetic material is relatively easy to obtain, and can be assessed before treatment is started. To definitively evaluate prognostic biomarkers, however, a large sample is required. A consortium of research groups with a large numbers of samples would allow the optimal detection of predictive and prognostic effects.



[1]conjugators vs non- conjugators,*gastric and esophageal cancer combined, ** adenocarcinoma only, *** squamous cell carcinoma only

Figure 2.1: Genetic polymorphism associated with esophageal and gastric cancer survival

Table 2.1: Studies of prognostic significance for genetic polymorphisms in esophageal cancer patients.

Gene	Polymorphism[1]	SNP ID	Risk allele	Effect[2]	Study population[3]	Reference
VEGF	-460T/C	rs833061			361 esophageal carcinoma patients (293 adenocarcinoma, 56 SCC, 12 poorly differentiated/other) enrolled during 1999-2004, Boston, USA	Bradbury et al., 2009[88]
VEGF	405G/C	rs2010963				
VEGF	936C/T	rs3025039		OS HR=0.70 (95% CI: 0.49-0.99)		
p53	Pro72Arg	rs1042522	Pro/Pro	OS HR=2.05 (95% Cl: 1.30-3.24)	371 esophageal carcinoma patients (300 adenocarcinoma, 63 SCC, 8 poorly differentiated/other) enrolled during 1999-2004, Boston, USA	Cescon et al., 2009[45]
MDM2	309T/G	rs2279744	GG	OS HR=7.9 (95% Cl: 2.4-26.0) in SCC		
AKT1	SNP	rs4375597			52 patients (21 adenocarcinoma, 31 SCC) with locally advanced resectable esophageal cancer (cT2–4, Nx, M0) from a prospective neoadjuvant trial, Cologne, Germany	Warnecke-Eberz et al., 2009[70]
C-ERBB-2	Ile655Val	rs1801200				
ERCC1	8092C/A	rs3212986				
ERCC1	Asn118Asn	rs11615	CC	response to neoadjuvant therapy		
FGFR4	Gly388Arg	rs351855				
GSTP1	Ala114Val	rs1138272				
GSTP1	Ile105Val	rs1695				
MDR1	3435C/T	rs1045642				
MGMT	16286C/T	rs12917				
MTHFR	Glu429Ala	rs1801131				
TERT	SNP	rs6882077				
TYMS	227A/G	rs2790				
TYMS	157C/T	rs699517				
XRCC1	Gln399Arg	rs25487				
XRCC1	Arg194Trp	rs1799782	AA	response to neoadjuvant therapy		
XRCC3	18067C/T	rs861539				
MMP1	1G/2G	rs1799750			313 esophageal adenocarcinoma cases, Boston, MA	Bradbury et al., 2009[46]
MMP3	6A/5A	rs3025058				
MMP12	–82A/G	rs2276109				
MMP12	1082A/G	rs652438				
AKT1	SNP	rs3803304			174 patients with resectable adenocarcinoma and 36 squamous cell carcinoma patients, Houston, TX	Hildebrandt et al., 2009[64]
AKT1	SNP	rs2498804				
AKT1	SNP	rs2494738				
AKT1	SNP	rs1130214	TT	OS HR=8.92 (95% CI: 1.56-51.17) in taxane-treated patients		
AKT2	SNP	rs892119	AG+GG	OS HR=3.5 (95%		

Gene	Polymorphism[1]	SNP ID	Risk allele	Effect[2]	Study population[3]	Reference
				CI: 1.43-8.78) in taxane-treated patients		
AKT2	SNP	rs8100018				
FRAP1	SNP	rs11121704	TT	OS HR=3.53 (95% CI: 1.48-8.39)		
FRAP1	SNP	rs2295080	TT	OS HR=4.19 (95% CI: 1.83-9.61)		
PIK3CA	SNP	rs7651265				
PIK3CA	SNP	rs7640662				
PIK3CA	SNP	rs7621329				
PIK3CA	SNP	rs6443624				
Cyclin D1	870G/A	rs17852153			39 esophageal adenocarcinoma cases, Pittsburgh, PA	Gupta et al., 2008[38]
EGF	61A/G	rs4444903			312 esophageal adenocarcinoma cases, Boston, MA	Lanuti et al., 2008[49]
TYMS	STR	rs34743033			82 esophageal adenocarcinoma patients who underwent esophagectomy, Los Angles, CA	Kuramochi et al., 2008[39]
BCL2	Ala43Thr	rs1800477			69 esophageal squamous carcinoma patients, Lucknow, India	Jain et al., 2007[40]
FAS	-670A/G	rs1800682				
Cyclin D1	870G/A	rs17852153				
EGF	61A/G	rs4444903	61GG	OS HR=31.1 (95% CI: 4.1-224.3)		
EGFR	Arg497Lys	rs11543848				
ECRG2	STR		TCA3/TCA3	OS RR=2.56 (95% CI: 1.53–4.29)	86 patients (48 SCC, 38 adenocarcinoma), Hamburg-Germany	Kaifi et al., 2007[85]
Cyclin D1	870G/A	rs17852153	870 AA	OS HR=3.48 (95% CI: 1.94-6.23)	124 esophageal adenocarcinoma patients, Houston, TX	Izzo et al., 2007[65]
MTHFR	677C/T	rs1801133			68 patients with locally advanced esophageal (SCC) cancer, Essen, Germany	Sarbia et al., 2006[41]
MTR	Asp919Gly	rs1805087				
TYMS	STR	rs34743033				
GSTP1	Ile105Val	rs1695	Val/Val, lle/Val	OS HR=1.36 (95% CI: 1.01-1.84)	233 patients with esophageal cancer (200 SCC, 20 adenocarcinoma, 10 other), Taipei, Taiwan	Lee et al., 2005[59]
GSTT1	GSTT1+/-					
GSTM1	GSTM1+/-					
MTHFR	Glu429Ala	rs1801131	Glu429Ala	OS HR=0.56 (95% CI: 0.35-0.89)	174 adenocarcinoma and 39 SCC esophageal cancer patients, Houston, TX	Wu et al., 2006[68]
MTHFR	1298A/C	rs1801133				
MTR	Asp919Gly	rs1805087				
TYMS	157C/T	rs699517				
TYMS	227A/G	rs2790				
MDR1	3435C/T	rs1045642	3435CT	OS HR=1.92 (95% CI: 0.23-0.85)		
MDR1	Ala892Ser	rs2032582				
GSTP1	Ile105Val	rs1695				

Gene	Polymorphism[1]	SNP ID	Risk allele	Effect[2]	Study population[3]	Reference
GSTP1	Ala114Val	rs1138272				
MPO	-764T/C	rs2243828				
P53	62A/G	rs1625895				
P53	Pro72Arg	rs1042522				
FAS	-670A/G	rs7089946				
FasL	-844C/T	rs763110				
NQO1	Pro187Ser	rs1800566				
XPA	23A/G	rs1800975				
XPC	Lys939Gln	rs2228001				
XPD (ERCC2)	Lys751Gln	rs13181				
XPG (ERCC5)	Asp1104His	rs17655				
ERCC1	8092C/A	rs3212986				
ERCC6	Met1097Val	rs2228526				
ERCC6	Arg1230Pro	rs4253211				
CCNH	Val270Ala	rs2230641				
RAD23B	Ala249Val	rs1805329				
hOGG1	Ser326Cys	rs1052133				
APEX1	Asp148Glu	rs1130409				
ADPRT	Val762Ala	rs1136410				
XRCC1	Gln399Arg	rs25487	Gln399Arg	OS HR=1.92 (95% CI: 1.00-3.72)		
IL1β	−511T/C	rs16944				
IL6	−174 G/C	rs1800795	CC compared with GG or GC	median survival 256 vs. 431 days	91 esophageal, 37 gastroesophageal junction and 75 gastric cancer patients, UK	Deans et al., 2007[42]
IL10	-1082A/G	rs1800896	GG compared with AA/AG	median survival 310 vs. 389 days		
TNFα	-308G/A	rs1800629	AA	OS HR=2.5 (95% CI: 1.3–4.9)		
LTa	252A/G	rs909253				

[1] SNP is an unspecified Single Nucleotide Polymorphism; STR is short tandem repeat (microsatellite) polymorphism; +/- is conjugators vs. non-conjugators
[2] HR is hazard ratio; CI is confidence interval; RR is relative risk; OR is odds ratio; OS is overall survival; PFS is progression free survival
[3] SCC is squamous cell carcinoma

Table 2.2: Studies of prognostic significance of genetic polymorphisms gastric cancer patients.

Gene	Polymorphism[1]	SNP ID	Risk allele	Effect[2]	Study population[2]	Reference
MMP9	Gln279Arg	rs17576	Haplotype double homozygotes	OR=6.5 (95% CI: 1.18-35.7) for 1 year survival	74 patients with gastric carcinoma, Fuzhou, China	Tang et al., 2008[52]
MMP9	Pro574Arg	rs2250889				
GSTT1	GSTT1+/-				110 patients with locally advanced gastric carcinomas who received preoperative chemotherapy, Heidelberg, Germany	Ott et al., 2008[51]
GSTM1	GSTM1+/-		GSTM1+	RR=0.32 (95% CI: 0.12–0.86) for completely resected patients		
GSTP1	Ile105Val	rs1695				
IL6	-634C/G	rs1800796			194 stages II and III gastric adenocarcinoma patients, Taichung, Taiwan	Liao et al., 2008[50]
IL6	−174G/C	rs1800795				
TYMS	STR	rs34743033			73 advanced gastric cancer patients administered a modified FOLFOX-6 regimen Seoul, South Korea	Keam et al., 2008[48]
TYMS	1494del6	rs34489327	6-bp deletion in 3'UTR	OS HR=0.55 (95% CI: 0.29–1.07)		
GSTP1	Ala114Val	rs1138272				
ERCC1	Asn118Asn	rs11615				
ERCC1	8092C/A	rs3212986				
XPD (ERCC2)	Arg156Arg	rs238406				
XPD (ERCC2)	Asp312Asn	rs1799793				
XPD (ERCC2)	Lys751Gln	rs13181				
XRCC	Gln399Arg	rs25487	Pro/Pro	OS HR=2.58 (95% CI: 1.05–6.33)		
p53	Arg72Pro	rs1042522			110 gastric cancer who were treated with 5FU-based adjuvant chemotherapy, Jiangsu, China	Huang et al., 2008[43]
TYMS	STR	rs34743033			116 patients with gastric cancer who were treated with 5FU-based adjuvant chemotherapy, Jiangsu, China	Huang et al., 2009[56]
TYMS	1494del6		ins6/ins6 vs. del6/del6 and ins6/del6	OS HR=2.44 (95% CI: 1.04–5.72)		

Gene	Polymorphism[1]	SNP ID	Risk allele	Effect[2]	Study population[2]	Reference
MTHFR	C677C/T	rs1801133	TT and CT vs. CC	OS HR=1.68 (95% CI: 0.99–2.86)		
p53	Pro72Arg	rs1042522	Pro/Pro	RFS HR=2.48 (95% CI: 1.44–4.27) OS HR=2.0 (95% CI: 1.11–3.64)	102 patients with gastric cancer treated with oxaliplatin-based adjuvant chemotherapy, Jiangsu, China	Huang et al., 2009[57]
ERCC1	Asn118Asn	rs11615				
GSTP1	Ile105Val	rs1695	Ile/Ile	RFS HR=2.00 (95% CI: 1.15–3.48) OS HR=2.13 (95% CI: 1.14–4.00)		
GSTM1	GSTM1+/-					
XRCC1	Gln399Arg	rs25487	Gln/Gln and Arg/Gln	OS HR=2.13 (95% CI: 1.14–4.00)		
p53	SNP	rs1788332			115 gastric cancer patients who underwent curative gastrectomy, Rome, Italy	De Feo et al., 2009[53]
p53	Pro72Arg	rs1042522				
p53	62A/G	rs1625895				
p73	14C/T	rs1801173				
p73	-81C/T	rs2273953				
RANTES	-403G/A	rs 2107538			177 gastric cancer patients, Taiwan	Liou et al., 2008[60]
RANTES	-28G/C	rs2280788				
CCR2	Val64Ile	rs1799864				
ERCC1	Asn118Asn	rs11615			82 patients with gastric cancer treated with oxaliplatin-based adjuvant chemotherapy, Jiangsu, China	Huang et al., 2008[55]
IL1β	−31C/T	rs1143627			44 gastric and gastroesophageal junction adenocarcinoma patients collected by North Central Cancer Treatment Group (NCCTG), Mayo clinic, Rochester, MN	Jatoi et al., 2007[66]
IL1β	−511T/C	rs16944				
IL1β	+3954C/T	rs1143634	CT and TT compared with CC	OS HR=0.3; P=0.04		
IL1RN	STR	rs2234663				

Gene	Polymorphism[1]	SNP ID	Risk allele	Effect[2]	Study population[2]	Reference
XPD (ERCC2)	Lys751Gln	rs13181			62 gastric adenocarcinoma patients, Nanjing, China	Liu et al., 2007[61]
GSTP1	Ile105Val	rs1695				
XRCC1	Gln399Arg	rs25487	G allele	median survival 337 days vs. 370 days (p=0.03)		
VEGF	VEGF -460T/C	rs833061			503 gastric cancer patients who underwent surgical gastrectomy, Daegu, Korea	Kim et al., 2007[89]
VEGF	–116G/A					
VEGF	VEGF 405G/C	rs2010963				
VEGF	936C/T	rs3025039	TT	OS HR=3.23 (95% CI: 1.13–9.25)		
NAT2	Arg64Gln	rs1801279			100 gastric cancer patients, Oman	Al-Moundhri et al., 2007[44]
NAT2	282C/T	rs1041983				
NAT2	Ile114Thr	rs1801280				
NAT2	481C/T	rs1799929				
NAT2	Arg197Gln	rs1799930				
NAT2	Arg268Lys	rs1208				
NAT2	Gly286Glu	rs1799931				
MTHFR	SNP	rs1801133			135 gastric cancer patients that received preoperative chemotherapy, Munich, Germany	Ott et al., 2006[63]
TYMS	STR	rs34743033	3rpt/3rpt	OS HR=4.57 (95% CI: 1.88-11.14)		
MDM2	MDM2 T309G	rs2279744	GG	OS HR=3.16 (95% CI: 1.22-8.20) in stage IB-IV disease	410 gastric cancer patients, Nagoya, Japan	Ohmiya et al., 2006[62]
IL8	IL8 −251A/T	rs4073				
IL1β	−511T/C	rs16944				
IL1RN	STR	rs2234663				
TNFα	SNP	rs1799724				
TYMS	1494del6	rs34489327			146 Caucasian patients with adenocarcinoma of the esophagus, Houston, TX	Liao et al., 2006[67]

Gene	Polymorphism[1]	SNP ID	Risk allele	Effect[2]	Study population[2]	Reference
MMP2	-1306C/T	rs243865			79 patients underwent resection for primary gastric adenocarcinoma Leiden, The Netherlands	Kubben et al., 2006[58]
MMP7	-181A/G	rs11568818	AA vs. AG/GG	OS HR=1.72 (95% CI: 0.97–3.06)		
MMP7	-153C/T	rs12184413				
MMP8	-799C/T	rs11225395				
MMP8	+17C/G					
MMP9	-1562C/T	rs3918242				
TIMP1	372C/T	rs4898				
TIMP2	303C/T	rs2277698	CC vs. CT/TT	OS HR=3.22 (95% CI: 1.57–6.62)		
TIMP2	-418G/C	rs2277698				
GSTP1	Ile105Val	rs1695	Val/Val	median survival 15.0 months (95% CI: 7.8-22.0) vs. 6.0 months (95% CI: 5.1-7.0)	52 Caucasian patients with advanced gastric cancer, Hamburg Eppendorf, Germany	Goekkurt et al., 2006[69]
GSTT1	GSTT1+/-					
TYMS	STR	rs34743033				
TYMS	1494del6	rs34489327				
MTHFR	677C/T	rs1801133				
ERCC1	Asn118Asn	rs11615				
XPD (ERCC2)	Lys751Gln	rs13181				
IL1β	−511T/C	rs16944	wild-type genotypes and IL1RN 2R allele vs. IL1β -511T/IL1β -31C carriers with wild-type IL1RN	OS HR=3.01 (95% CI: 2.15-4.23)	123 patients with relapsed or metastatic gastric cancer, Italy	Graziano et al., 2005[54]
IL1β	−31C/T	rs1143627		PFS HR=3.33 (95% CI: 2.35-4.46)		
IL1RN	STR	rs2234663				

Gene	Polymorphism[1]	SNP ID	Risk allele	Effect[2]	Study population[2]	Reference
TNFβ	NcoI		10.5-kb homozygote vs. 5.5 kb homozygote and heterozygote	OS	143 patients with gastric cancer, Japan	Shimura et al., 1995[75]
IL1β	−511T/C	rs16944			91esophageal, 37 gastroesophageal junction and 75 gastric cancer patients, UK	Deans et al., 2007[42]
IL6	−174 G/C	rs1800795	CC compared with GG or GC	median survival 256 vs. 431 days		
IL10	-1082A/G	rs1800896	GG compared with AA/AG	median survival 310 vs. 389 days		
TNFα	-308G/A	rs1800629	AA	OS HR=2.5 (95% CI: 1.3–4.9)		
LTa	252A/G	rs909253				

[1] SNP is an unspecified Single Nucleotide Polymorphism; STR is short tandem repeat (microsatellite) polymorphism; +/- is conjugators vs. non-conjugators

[2] HR is hazard ratio; CI is confidence interval; RR is relative risk; OR is odds ratio; OS is overall survival; PFS is progression free survival; SCC is squamous cell carcinoma

CHAPTER 3: Incidence and survival of gastric and esophageal cancer diagnosed in British Columbia, 1990 to 1999 [2]

3.1 Introduction

Changing patterns of esophageal and gastric cancer incidence over recent decades have made this subject of increasing interest in cancer epidemiology. Geographic and temporal trends in incidence have been reported to vary according to both tumour morphology and organ subsite [91-93].

Esophageal cancer incidence shows striking variation in different parts of the world . About 80% of esophageal cancers occur in developing countries. High incidence areas include parts of Asia, south-eastern Africa, eastern South America and Western Europe [91,92,94]. Esophageal cancer incidence in North America is about 5 to 10 per 100,000, whereas it is more than 100 per 100,000 in areas of the Iranian east Caspian littoral [13]. Prior to 1980, approximately 90% of all cases of esophageal cancers were squamous cell carcinoma (SCC). Over the past two decades, however, the incidence of esophageal SCC has decreased and that of adenocarcinoma increased [5,95]. In many western countries, adenocarcinoma is now more common than SCC [15]. Esophageal cancer is one of the deadliest types of cancers and the sixth leading cause of death from cancer worldwide [96].

Gastric cancer incidence and mortality have fallen dramatically over the past 70 years [2,93]. Nonetheless, gastric cancer remains the fourth most commonly diagnosed cancer, and the second most common cause of cancer-related death worldwide [2,93]. Adenocarcinoma is the most common histological type of gastric cancer, accounting for 90-95% of all gastric

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malignancies [4,93,94]. Despite a decline in lower gastric cancers, proximal tumour incidence has been increasing since the 1970s, especially among men in Western countries [5]. Tumours of the gastric cardia now account for nearly half of all gastric cancers among men in the US and UK [6].Gastric cancer is a disease of poor prognosis and high mortality. In general, countries with higher incidence rates of gastric cancer have better survival rates than countries with lower incidence [3].

In this paper, incidence and survival of gastric and esophageal cancer in the population of BC, Canada between 1990 and 1999 have been described.

3.2 Methods

Data

Cancer incidence data for invasive primary esophageal and gastric cancers were obtained from the BC Cancer Registry (BCCR) for the period 1990-1999. The topography and histology of cases were coded according to the International Classification of Diseases for Oncology, Second Edition [97]. The topography of esophageal cancers was grouped into four anatomic subsites: esophagus upper third (C15.0-C15.3), esophagus middle third (C15.4), esophagus lower third (C15.5), overlapping lesion and esophagus unknown (C15.8 and C15.9). The topography of gastric cancer was grouped into three subsites: proximal (cardia) in the gastroesophageal junction or upper third of the stomach (C16.0-C16.1), lower stomach or lower two-thirds of the stomach (C16.2-C16.7), and unknown or unspecified/overlapping regions (C16.8-C16.9). Histological categories for esophageal and gastric cancers were squamous cell carcinoma (8050-8082), adenocarcinoma (8140-8573) and others (mainly 8000- 8020) (16). Diffuse gastric tumours were defined by histology codes 8142, 8145 and 8490 [98]. Five years of follow-up information was available for each patient. The stage of

diagnosis was defined according to American Joint Committee on Cancer (AJCC) TNM classification [99].

Statistical analysis

Annual age-adjusted incidence rates were computed by anatomic subsite, histological type and gender. All rates were standardized to the 1996 Canadian population. The Estimated Annual Percent Change (EAPC) was used to measure trends or the change in rates over time by fitting a regression line to the natural logarithm of the rates using calendar year as an independent variable [100]. Overall survival was calculated as the time between cancer diagnosis and death. Cases diagnosed at the time of a patient's death were excluded because they were probably they registered through autopsy or death certificate only. Survival curves were calculated using the Kaplan-Meier method and log-rank statistics were used to compare survival differences between groups. p-values less than 0.05 were considered statistically significant.

3.3 Results

Incidence

Between 1990 and 1999, 1741 cases of esophageal cancer and 3431 cases of gastric cancer were diagnosed in BC. Figure 3.1 shows the frequency of esophageal and gastric cancers according to the age of diagnosis and gender. The average age of diagnosis was 69.0 years (standard deviation (SD) 11.5) for esophageal cancer and 69.5 years (SD 13.2) for gastric cancer. In both esophageal and gastric cancers, men were more commonly affected with 71.0% and 64.5% of diagnoses, respectively. Staging information for 60% (1132) of esophageal and 20% (696) of gastric cancer patients was available. For esophageal cancer, 10% of patients had stage I, 56% had stage II, 11% had stage III and 23% had stage IV

disease. For gastric cancer, 14% of patients had stage I, 16% had stage II, 23% had Stage III (18% III a, 5% IIIb) and 47% had stage IV disease.

Table 3.1 shows the incidence rates for gastric and esophageal cancers by topography, histology and gender. For esophageal cancer, 55% were diagnosed in the lower third, 22% in the middle third, and 10.5% in upper third. Incidence of men's cancers in the lower esophagus increased during the study period (EAPC=4.6 with average incidence 3.7/100,000). 57% of esophageal cancers were SCC, 43% were adenocarcinoma, and 12% were other histological types. There was a substantial increase in incidence of esophageal adenocarcinoma over the study period among men (EAPC=9.0 with average incidence 3.3/100,000). There was almost no increase in esophageal adenocarcinoma incidence among women (EAPC=0.2 with average incidence 0.2/100,000). For gastric cancer, 29% of cases were diagnosed in the proximal third, 33% in the lower two-thirds, and 38% had uspecified topology. There was an increase in incidence of proximal gastric cancers over time for both men (EAPC=3.8 with average incidence 4.4/100,000) and women (EAPC=9.2 with average incidence 1.0/100,000). 84% of gastric cancer patients were diagnosed with adenocarcinoma and 16% had other histological types. Among gastric adenocarcinoma, 20% were the diffuse type (including signet ring cell carcinoma). The rates of diffuse gastric cancer in both men (EAPC=7.5 with average incidence 1.6/100,000) and women (EAPC=8.1 with average incidence 1.3/100,000) increased substantially from 1990 to 1999.

Survival

Figure 3.2 shows overall 5-year survival curves for esophageal and gastric cancer. The patients had overall 5-year survival rates of 8.8% for esophageal cancer and 16.2% for gastric cancer, and gastric cancer survival was significantly better than that for esophageal cancer. Gender did not have any significant effect on gastric cancer survival, but women had

significantly better survival than men for esophageal cancer (Figure 3.3). There was no significant difference in survival for cancers affecting the lower, middle and upper third of the esophagus, however, patients with cancer of the upper third had a slightly better survival when compare with patients having cancer in the lower third (Figure 3.4A). For gastric cancer, lower tumours had a significantly better survival than proximal tumours ($p<0.001$) (Figure 3.4B) however proximal gastric (cardia) cancers had significantly better survival compared to adenocarcinoma tumours in the lower third of the esophagus ($p<0.001$). There were no significant differences by tumour histology for esophageal cancer survival.

3.4 Discussion

Over the last decades the epidemiology of upper GI cancers has changed. There are some similarities and interesting differences between changing pattern of cancers in the gastroesophageal junction area. The most notable similarity was the incidence of proximal gastric cancer and esophageal adenocarcinoma. Major differences in survival and incidence of these cancers were seen between men and women. Increased incidence of esophageal adenocarcinoma is only substantial in men, but women had a greater incidence than males for proximal gastric cancer. This trend is different than in other parts of Canada [101].

The incidence of esophageal cancer in men showed an increase over the study period. The pattern of histological changes for esophageal cancer in BC is compatible with patterns in other western countries. The incidence of esophageal adenocarcinoma in men is rising in most countries, although this trend is highly varied among ethnicities [102,103]. Our study did not analyze ethnicity because the BC registry does not collect this information. The highest incidence rates for white men in the year 2000 were found in Great Britain (5.0- 8.7 cases per 100,000 population) and Australia (4.8 cases per 100,000 population) followed by The

Netherlands (4.4 cases per 100,000 population), the US (3.7 cases per 100,000 population), and Denmark (2.8 cases per 100,000 population) (21). Regional differences between esophageal adenocarcinoma incidence rates have been reported in the US (22). BC has shown a decrease in incidence of esophageal SCC, however this decrease was not significant and different than the trend in Ontario, Canada [101].

Gastric cancer incidence has decreased over the past several decades worldwide [94]. In 1900, gastric cancer was the leading cause of death in United States [104]. The incidence of lower gastric in BC have been almost steady over time, however the incidence of proximal (cardia) cancer has increased. The increasing incidence rate for proximal gastric in men follows an increasing trend elsewhere in Ontario [101]. Like adenocarcinoma of the esophagus, the incidence of gastric cardia cancer has increased significantly since the 1970s [16]. In the US, this increasing trend has stabilized since the late 1980s [105]. Our study indicates the incidence and increase in incidence of proximal gastric is higher in BC than in the US and Ontario. Unlike other major tumours of the upper GI tract, incidence of diffuse gastric cancer was not influenced by gender in BC. The increase of diffuse gastric cancer in the US increased from 0.3 cases per 100,000 persons in 1973 to 1.8 cases per 100,000 persons in 2000 [98].

The 5-year overall survival for esophageal cancer in BC (9%) is very poor. In our study, women had better esophageal cancer survival compared with men. This result agrees with a report from Europe [106]. It should be reminded that rates in this paper should be compared with caution to those in other reports because of the possible effects of standardizing with different populations. Topology in esophageal cancer patients did not significantly influence survival. However, cancers in the upper esophagus have a small survival benefit compared with cancer in the lower esophagus, possibly because they are detected or present earlier.

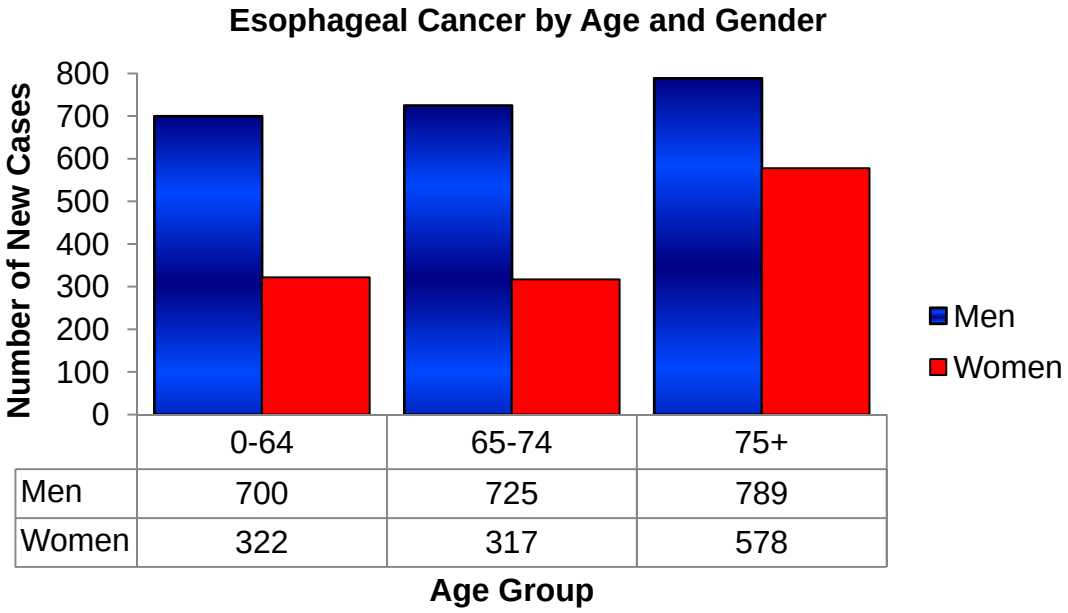
According to our data, there was no significant difference in survival between SCC and adenocarcinoma of esophagus. These results are in contrast with studies in UK (1987-2000) and Germany (1982-2000) that have shown adenocarcinoma has a more favorable prognosis than SCC [107,108] in esophageal cancer patients. In general, gastric cancer 5-year survival (16%) is poor but it was significantly better than that for esophageal cancer. This result shows a slightly better survival comparing with a previous report of gastric cancer patients treated in BC between 1978 to 1997 [30]. In our study, a significant difference in 5-year survival was observed between lower and proximal cancers of the stomach with a worse outcome for patients with cancer of cardia. This agrees with other studies, which have shown the same prognosis pattern for cardia cancers compared with other gastric cancers [109,110].

The strength of this study was the availability of population-based data with details of histology and pathology. Furthermore, the Gastrointestinal Tumour Group at the BC Cancer Agency (BCCA) provides province-wide treatment guidelines in BC so that most patients receive similar treatment. This is important because treatment is one of the greatest determinants of cancer survival. Over the study period, the general treatment for esophageal cancer was surgery in stage I cancer; preoperative radiation (4500 cGy in 25 fractions and chemotherapy (5-fluorouracil [5FU] and cisplatin) followed by surgery and further chemotherapy (5FU+cisplatin) in 50% of stage II and III cancers, or surgery followed by radiation (4500 cGy in 25 fractions) and chemotherapy (5FU+cisplatin) for the other 50%; and chemotherapy (5FU+cisplatin) in 75% of stage IV with the remainder receiving symptomatic care only. General treatment for resectable gastric cancer (Stages I, II or III) was surgery; chemotherapy chemotherapy with 5FU or 5FU plus cisplatin was prescribed for about 65% Stage IV pateints, while the rest had symptomatic care only. A weakness of this

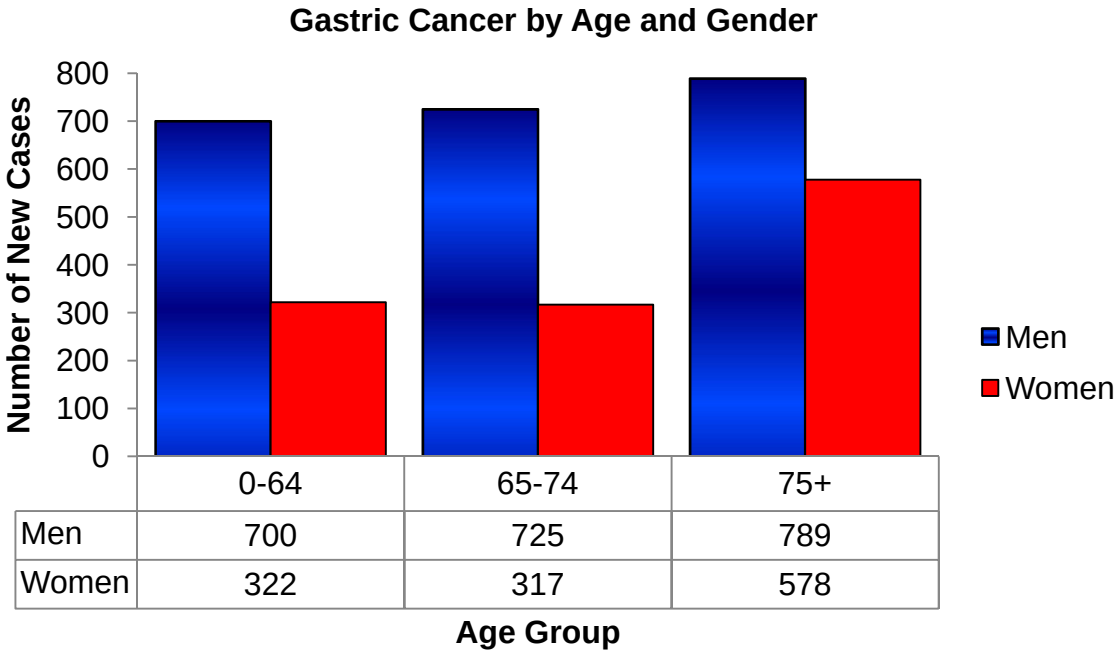
study is the number of cases with unspecified histology and pathology, and the lack of ethnicity information.

Although gastric and esophageal cancers are relatively infrequent in Canada, their epidemiology is changing. The trends are most evident when tumours are classified by histology and anatomic location. While incidence of squamous cell carcinoma of the esophagus and adenocarcinoma of the lower stomach appears to be stable or decreasing, the incidence of esophageal adenocarcinoma and gastric cardia cancer are increasing.There are three possible explanations for this trend: (i) increased exposure to one or more risk factors, (ii) misclassification and over-diagnoses, and (iii) immigration and the changing population of BC. Risk factors for gastric cancer include Helicobacter pylori infection [7], heredity, genetic and immunological variables [4], diet and lifestyle [111], tobacco smoking [112], obesity [113], ionizing radiation [114] and exposure to the Epstein-Barr virus [115]. Risk factors for esophageal cancer include gastroesophageal reflux [116], Barrett's esophagus [117], asthma medication use, LES (lower esophageal sphincter-relaxing) medication use [118], cholecystectomy [119], obesity [113], and cigarette smoking [16]. It is unlikely that misclassification or over-diagnosis is responsible for the temporal changes because all of the cancers were invasive, and different survival patterns between cardia and esophageal adenocarcinoma indicate that these are separate diseases. Finally, the number of immigrants living in BC in the 1996 Census was 903,190 [120]. This is a 25% increase since 1991 [120]. Among these, immigrant Chinese was the largest ethnic group, representing 14% of the BC population. Esophageal SCC and lower gastric cancers are more common in China, but there is no indication of an increase in the incidence rate of cardia or lower esophagus adenocarcinoma in China.

Tumours in the upper gastro-intestinal (GI) tract are heterogeneous but share some epidemiological features. Gastric and esophageal cancers arise in advanced age, both predominantly occur in men, they are silent until advanced stages and therefore they have very poor survival. Gastric cardia cancers share epidemiologic features with adenocarcinoma of the lower esophagus and gastroesophageal junction. Both gastric and esophageal cancer patients have poor survival. Factors associated with the poor survival rates are the absence of symptoms in early cancer, lack of effective screening tools, and lack of effective treatment options. Examining these cancers together might elucidate new etiologic and prognostic factors.



3.1 A



3.1 B

Figure 3.1: New diagnoses of esophageal (A) and gastric (B) cancer in BC during 1990-1999 by age and gender

Table 3.1: Age standardized incidence rate per 100,000 (ASR) and estimated annual percentage change (EAPC), with 95% confidence interval (95% CI), for esophageal and gastric cancer by topology and histology, BC 1990-1999 .

Gender	Men		Women	
Cancer	ASR (95% CI)	EAPC (95% CI)	ASR (95% CI)	EAPC (95% CI)
ESOPHAGEAL TOPOLOGY				
Upper Third	0.6 (0.5,0.7)	-1.2 (-8.8,7.1)	0.4 (0.3,0.4)	6 (-1.5,14.1)
Middle Third	1.3 (1.1,1.5)	0.4 (-5,6.1)	0.5 (0.5,0.6)	-1.9 (-6.1,2.5)
Lower Third	3.7 (3.3,4.1)	4.6 (1.1,8.3)	0.7 (0.7,0.8)	0.1 (-4.6,5.1)
GASTRIC TOPOLOGY				
Cardia	4.4 (4,4.8)	3.8 (0.3,7.4)	1 (0.8,1.2)	9.2 (2.6,16.2)
Lower	3.8 (3.6,4)	0.4 (-1.9,2.7)	2.2 (2,2.4)	-1.8 (-6,2)
ESOPHAGEAL HISTOLOGY				
SCC	2.9 (2.6,3.2)	-2.9 (-6.6,1)	1.7 (1.6,1.8)	0.4 (-2.8,3.7)
AC	3.3 (2.7,3.9)	9.5 (5.1,14.2)	0.2 (0.2,0.3)	6.7 (-4.9,19.7)
GASTRIC HISTOLOGY				
AC Diffuse	1.6 (1.3,1.9)	7.5 (1,14.4)	1.3 (1.1,1.5)	8.1 (2.5,14)
AC (other)	9.1 (8.6,9.5)	-0.3 (-2.3,1.8)	2.9 (2.6,3.2)	-2.7 (-6,1)

SCC (Squamous Cell Carcinoma), AC (Adenocarcinoma)

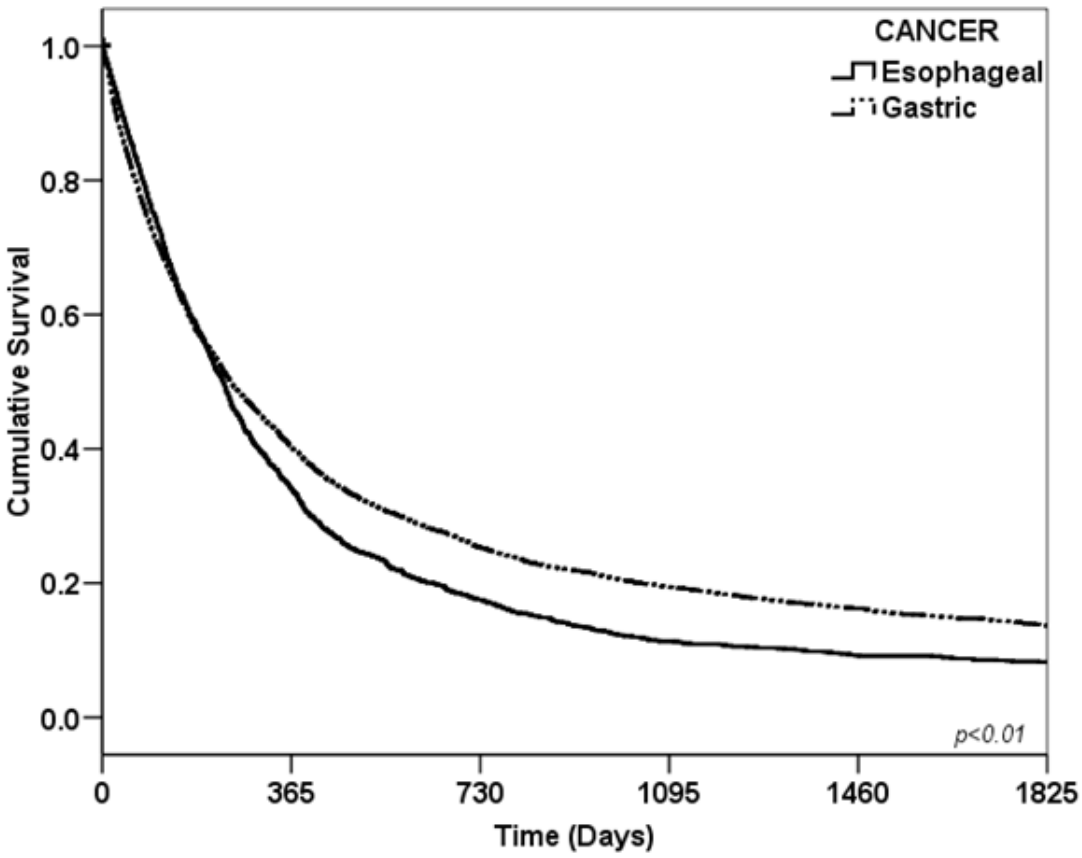
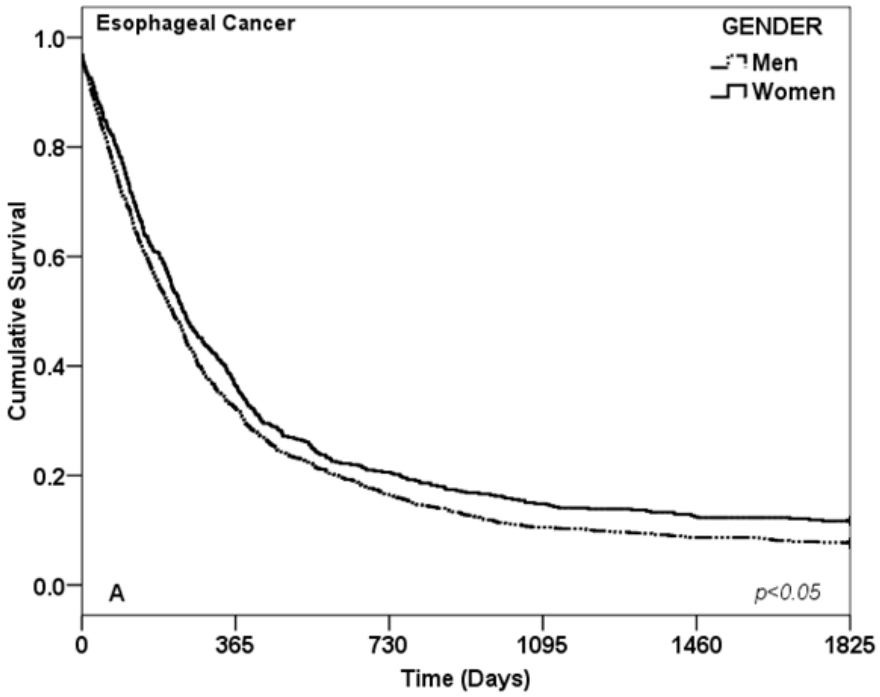


Figure 3.2: Five-year survival of esophageal and gastric cancer in BC.



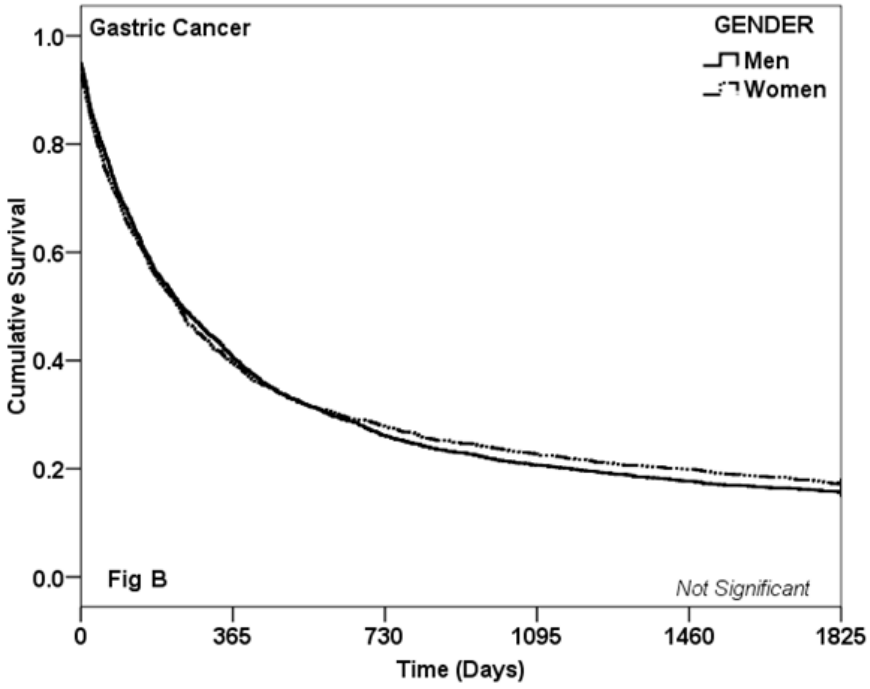


Figure 3.3: Five-year survival by gender for (A) esophageal and (B) gastric cancer in BC.

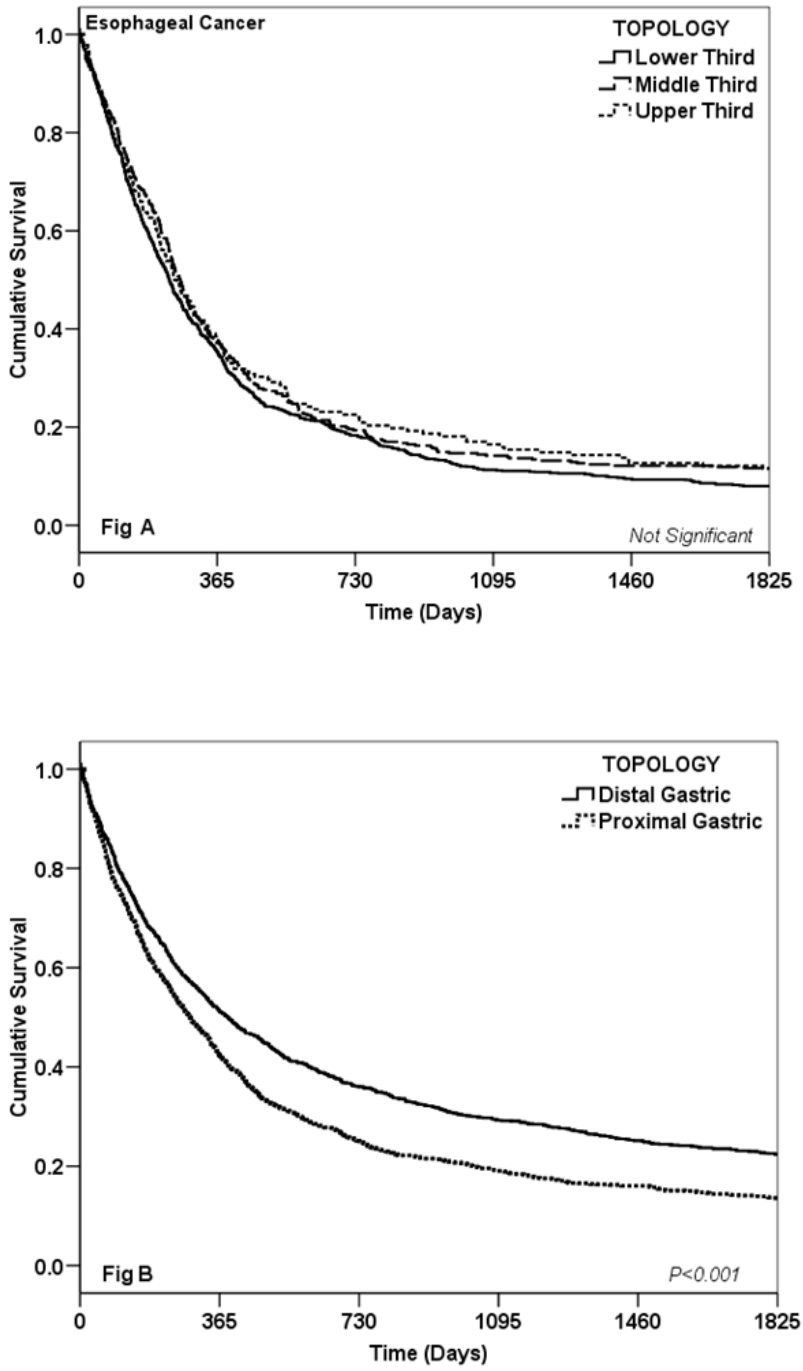


Figure 3.4 Five-year survival by tumour location for (A) esophageal and (B) gastric cancer in BC.

CHAPTER 4: Comparison of two diverse populations, British Columbia, Canada and Ardabil, Iran, indicates several variables associated with gastric and esophageal cancer survival [3]

4.1 Introduction

Geographic variation and temporal trends in the epidemiology of esophageal and gastric cancers vary according to tumour morphology and organ subsite [121]. Both diseases are among the deadliest forms of cancer. Gastric cancer incidence and mortality have fallen dramatically over the past 70 years in western countries, but it is the fourth most commonly diagnosed cancer and the second most common cause of cancer related death worldwide [2]. The majority of esophageal carcinoma patients in the world die within a year of diagnosis and only 8-20% are alive after 5 years [122]. Gastric and esophageal cancers are relatively infrequent in Canada, but common in Iran [123].

This study compares one-year survival of gastric and esophageal cancers between the populations of British Columbia (BC), Canada and Ardabil, Iran. BC and Ardabil have been choosen because both areas have high-quality population-based cancer registries. The BC Cancer Registry has been in existence since 1969 (www.bccancer.bc.ca/HPI/CancerStatistics accessed November 4, 2009) and the Ardabil Cancer Registry is the first such registry in the Islamic Republic of Iran [124]. This study does not compare survival rates for different types of esophageal or gastric cancer within Ardabil or within BC.

[3] A version of this chapter is accepted for publication:
Bashash M, Yavari P, Hislop G, Shah A, Sadjadi A, Babaei M, Le N, Brooks-Wilson A, Malekzadeh R, Bajdik C. Journal of Gastrointestinal Cancer (In press)

4.2 Methods

Data for invasive primary esophageal and gastric cancer patients diagnosed in 2004 were obtained from the cancer registries for BC and Ardabil. For the BC registry, completeness of case ascertainment was 86.8% and completeness of other information was 99.8%[125]. For the Ardabil registry, overall completeness was 89% based on reports from pathology centers, identity information, demographic information and percentage of coded cancer cases [126]. Dates in the Ardabil registry were converted to equivalent values in the western calendar. For both registries, the topography and histology of cases were coded according to the International Classification of Diseases for Oncology, Third Edition (ICD-O) [127]. Similar methods were used for the collection and classification of breast cancer data from BC and Ardabil data in an earlier report [128]. Esophageal cancers were grouped into four anatomic subsites: upper third (ICD-O codes C15.0-C15.3), middle third (C15.4), lower third and overlapping lesions (C15.5), and unknown (C15.8 and C15.9). Gastric cancers were grouped into three anatomic subsites: proximal third (i.e., cardia) in the gastroesophageal junction or upper third of the stomach (C16.0 and C16.1), lower stomach or lower two thirds of the stomach (C16.2–C16.7), and unknown or unspecified/overlapping lesion (C16.8 and C16.9). Histological categories for esophageal cancers were squamous cell carcinoma (ICD-O codes 8050-8082), adenocarcinoma (8140-8573) and others (mainly 8000-8020). Gastric cancers were categorized as diffuse or intestinal according to the Lauren classification system [128]. Diffuse gastric tumours are defined by ICD-O histology codes 8142, 8145 and 8490 [8]; other gastric tumours are defined as intestinal.

In BC, the vital status and date of death for cancer patients is routinely collected from government statistics. At least one year of follow-up information was available for each patient in BC. In Ardabil, information on a patient's survival and date of death was obtained by

interviewing cases or their families. Interviews were conducted by members of the Ardabil Cancer Registry whenever possible. The death registry in Ardabil was used to confirm this information and obtain data for cases that could not be interviewed. Based on this approach, 83.3% of Ardabil patients had complete one-year follow-up information.

Survival time was defined as the time between cancer diagnosis and death. The relative survival rate [129] was calculated for various subgroups of each population using WHO Statistical Information System (WHOSIS) life-tables for each country [130]. Chi-square and Fisher's exact tests were used to compare differences in one-year survival proportions between BC and Ardabil. T-tests, chi-square tests and Fisher's exact test were used to compare patient characteristics and tumour factors between the populations. p-values less than 0.05 were considered statistically significant. Other p-values were denoted non-significant (NS).

4.3 Results

In 2004, 357 and 261 cases of gastric cancer were diagnosed in BC and Ardabil, respectively. Characteristics of the cases are summarized in Table 4.1. The mean age of patients was 69.1 years in BC and 66.1 years in Ardabil ($p<0.01$). Women comprised about one third of gastric cancer patients in both BC and Ardabil (NS). Approximately 34.5% of gastric cancer cases (49% of cases with known topography) in BC and 41.8% (60% of cases with known topography) in Ardabil were diagnosed with proximal disease ($p<0.05$). Adenocarcinoma was the predominant histological type of gastric tumour, accounting for 87.4% and 79.7% of cases in BC and Ardabil, respectively ($p<0.01$). About 16.0% of gastric tumours in BC and 30.3% in Ardabil were the diffuse type ($p<0.05$).

In 2004, 232 and 124 cases of esophageal cancer were diagnosed in BC and Ardabil, respectively. Characteristics of cases are summarized in Table 4.2. The mean age of cases was

69.7 years in BC and 63.3 years in Ardabil ($p<0.01$). Women accounted for about one third of cases in BC and half of cases in Ardabil ($p<0.01$). Most tumours in BC cases were located in the lower third of the esophagus, while the lower and middle thirds of the esophagus had nearly equal incidence in Ardabil ($p<0.01$). Adenocarcinoma was the leading type of tumour in BC cases (50% of all cases) while only 10% of cases in Ardabil had this histology type ($p<0.01$).

Figures 4.1 and 4.2 show the overall one-year age-standardized relative survival of gastric and esophageal cancers in BC and Ardabil. Details of the survival rates for gastric and esophageal cancer in BC and Ardabil are shown in Tables 4.3 and 4.4. Overall and separately for each gender, age group, tumour location and histology, there was greater one-year survival of gastric cancer patients in BC compared to Ardabil. Patients under age 65, patients with tumours in the middle or upper third of the esophagus, and patients with squamous cell carcinoma had significantly better esophageal cancer survival in BC than in Ardabil. In BC, there were significant 1-year survival differences among age and tumour location groups. In Ardabil there were no significant differences in one-year survival within groups. However, in esophageal cancer patients one-year survival difference have seen in age and tumour location groups among Ardabil patients , but not in BC patients.

4.4 Discussion

Based on available registry and follow-up information, this study compares one-year survival of gastric and esophageal cancer in two populations. Results indicate major differences and some interesting similarities between the populations. In general, overall one-year relative survival was better in BC than Ardabil. There were significant differences between the populations in gastric cancer survival according to patient gender, age, tumour location and tumour histology. For esophageal cancer; patients under age 65, patients with tumours in the middle or upper third of

esophagus, and patients with squamous cell carcinoma had significantly better survival in BC than in Ardabil. Survival differences between the populations might be based on other tumour-related factors, other patient characteristics, cancer control measures and treatment factors.

Stage at diagnosis is likely the main tumour-related factor affecting a patient's prognosis, and stage at diagnosis determines the course of a patient's treatment[22]. Unfortunately, stage could not be included in our analysis because both cancer registries provided only limited information about it. Cell histology is another tumour-related factor that might affect patient survival [108]. In this study, the Lauren classification (based on tumour histology) did not have prognostic significance for one-year survival of gastric cancer patients in either population. Several clinical studies report better survival for adenocarcinoma of esophagus [107,108,131] but it has not been observed at this study. In both BC and Ardabil, tumour histology did not have a substantial influence on the survival of esophageal cancer patients. In Ardabil, tumours in middle third of the esophagus were associated with worse survival than tumours located in lower third. Low number of cases with tumour at the upper third of esophagus in Ardabil makes it impossible to make any statistical conclusion. This result is consistent with a recent report from Turkey[132]. However, results from BC and other North American studies [121,122] indicate that survival of patients with cancers in the upper, middle and lower third of the esophagus are similar.

Ethnicity has been suggested to be a possible prognosis factor for cancer in upper GI tract [22,30]. BC has an ethnically diverse population. The 2006 census reported that only 52% of people living in BC at that time had a single ethnic origin [133]. In contrast, the population of Ardabil is homogeneous, with 95% being of Azeri ethnic background, which is of Aryan Caucasoid ancestry [124]. Family history also has been shown to be a prognostic factor in gastric and esophageal cancer. Gastric cancer patients with a family history of this tumour have unique

clinicopathologic characteristics [134]. Poor survival among young patients from Ardabil could be explained by the presence of higher proportion of familial cases of the disease. This is consistent with reports from other high incidence areas [135]. Also, the high incidence of diffuse gastric cancer in the ethnically homogeneous Ardabil population is consistent with some cases having inherited mutations in the E-cadherin gene that underlie hereditary diffuse gastric cancer [136].

Treatment is likely to be the greatest external determinant of cancer patients' survival, and province-wide treatment guidelines in BC result in nearly uniform treatment (http://www.bccancer.bc.ca/HPI/CancerManagementGuidelines/Gastrointestinal/default.htm).

Patient characteristics include inherent and demographic characteristics such as age, sex, ethnicity, physical performance status, comorbidity and immune status. These variables are usually unrelated to the presence of tumour but may have a profound impact on treatment choices as well as direct influence on survival. The general treatment for esophageal and respectable gastric cancer was surgery. Some patients also received radiation and chemotherapy depending on the stage of disease [121]. In Ardabil, guidelines do not exist and treatment is not uniform. Based on previous reports, only 28% of patients with gastric and esophageal cancer in Ardabil received curative resectional surgery and about 25% of patients did not receive any treatment [137].

tumourOne of the interesting differences between BC and Ardabil is the survival pattern for proximal and lower gastric cancers. In BC, patients with proximal gastric tumours had poorer survival than patients with lower ones. In Ardabil, there was very little difference between survival for proximal and lower gastric cancers. In BC, only about one-third of tumours occurred in the middle and upper third of the esophagus. In Ardabil, more than half of tumours with known topology were located in this anatomic region. More men than women had gastric and

esophageal cancers in BC; however, the incidence of esophageal cancer in Ardabil seemed independent of gender (i.e., the same in both sexes).

The strength of this study was the availability of population-based data with details of tumour histology and pathology. This study's limitations include the lack of complete staging information, incomplete follow-up data, and the relatively large proportion of esophageal tumours with unspecified histology. Differences in the quality of registry data between two populations could also have influenced survival comparisons. As noted in the Report of National Cancer Registration in Iran [126], there are challenges in interpreting registry information regarding the health care system in Iran. There is vast, uncontrolled population movement in and out of Ardabil, an uncoordinated medical services system, and inconsistent referrals to different centers for diagnosis and treatment [126]. It is also possible that patients with better socioeconomic status are referred to better medical facilities in central cities.

Population-based survival studies cannot assess specific treatments but can quantify the effect of cancer control measures at population level [138]. Neither BC nor Ardabil has a screening program for gastric and esophageal cancers. In BC, these cancers are infrequent and feasibility of screening is questionable. However, Ardabil has the highest rates of gastric and esophageal cancers in the world, and a screening program should be considered.

4.5 Conclusion

Gastric and esophageal cancers are heterogeneous diseases, but they share important features. They remain clinically asymptomatic until late in the disease process with consequent poor prognoses and high mortality rates. This study points to differences in disease characteristics and patient factors, not solely differences in healthcare systems, as being responsible for the survival

difference in these populations. Even so, the outcomes of these cancers are poor for both populations and improvements in diagnosis and management are urgently needed.

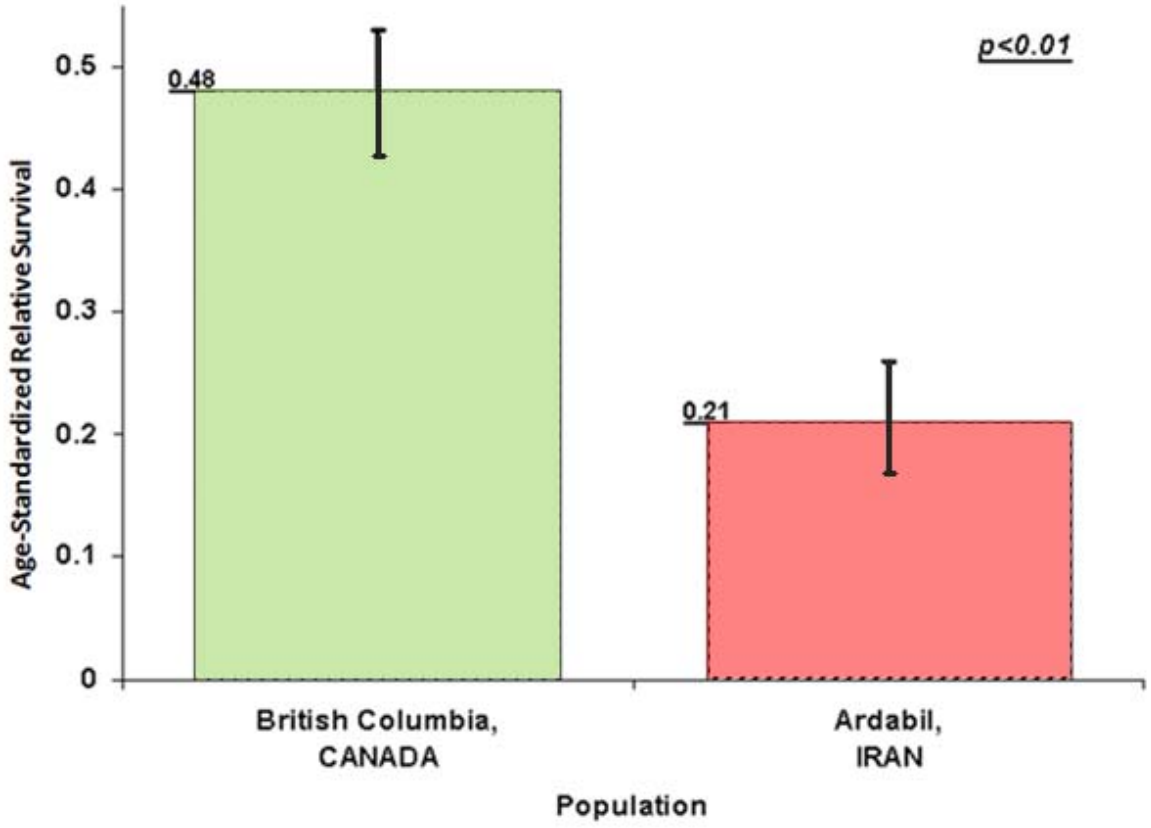


Figure 4.1. Overall one-year age-standardized survival rates for gastric cancer cases in Ardabil (Iran) and British Columbia (Canada). Bars are ± standard error.

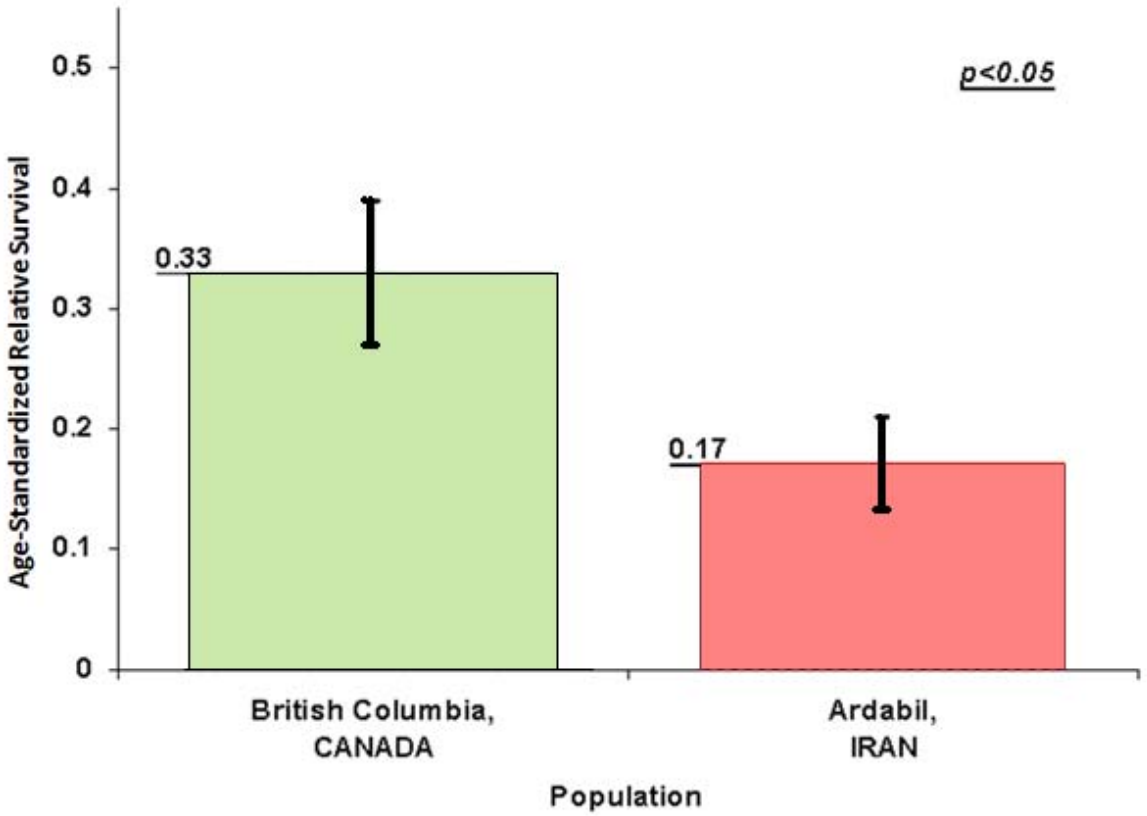


Figure 4.2. Overall one-year age-standardized survival rates for esophageal cancer cases in Ardabil (Iran) and British Columbia (Canada). Bars are ± standard error.

Table 4.1. Gastric cancer patients in BC (Canada) and Ardabil (Iran).

	British Columbia		Ardabil	
Gender				
Women	119	(33.3%)	75	(28.7%)
Men	238	(66.7%)	185	(70.9%)
Unknown			1	(0.4%)
Age Group				
Less than 65	118	(33.1%)	108	(41.4%)
65 or more	239	(66.9%)	153	(58.6%)
Location				
Lower	127	(35.6%)	72	(27.6%)
Proximal	123	(34.5%)	109	(41.8%)
NOS / Overlapping lesion	107	(30.0%)	80	(30.7%)
Lauren Classification*				
Intestinal	262	(84.0%)	145	(69.7%)
Diffuse	50	(16.0%)	63	(30.3%)

NOS = not otherwise specified.
*Adenocarcinomas only

Table 4.2. Esophageal cancer patients in BC (Canada) and Ardabil (Iran).

	British Columbia		Ardabil	
Gender				
Women	64	(27.6%)	61	(49.2%)
Men	168	(72.4%)	62	(50.0%)
Unknown			1	(0.8%)
Age Group				
Less than 65	76	(32.8%)	67	(54.0%)
65 or more	156	(67.2%)	57	(46.0%)
Tumour Location				
Upper third	23	(9.9%)	6	(4.8%)
Middle third	40	(17.2%)	35	(28.2%)
Lower third	112	(48.3%)	38	(30.6%)
NOS / Overlapping lesion	57	(24.6%)	45	(36.3%)
Tumour Histology				
Squamous Cell Carcinoma	88	(37.9%)	89	(71.8%)
Adenocarcinoma	116	(50.0%)	13	(10.5%)
Other	28	(12.1%)	22	(17.7%)

NOS = not otherwise specified.
*Adenocarcinomas only

Table 4.3. One-year relative survival for gastric cancer patients in BC (Canada) and Ardabil (Iran).

		BC	Ardabil	*p*
Gender	Male	0.48 ± 0.074	0.18 ± 0.09	*<0.01*
	Female	0.46 ± 0.09	0.26 ± 0.08	*<0.01*
p		NS	NS	
Age	Less than 65	0.62 ± 0.068	0.22 ± 0.09	*<0.01*
	65 or more	0.41 ± 0.04	0.20 ± 0.05	*<0.01*
p		<0.01	NS	
Tumour Location	Lower	0.61 ± 0.07	0.23 ± 0.06	*<0.01*
	Proximal	0.45 ± 0.08	0.19 ± 0.06	*<0.01*
p		<0.05	NS	
Lauren Classification*	Intestinal	0.48 ± 0.07	0.18 ± 0.04	*<0.01*
	Diffuse	0.50 ± 0.10	0.24 ± 0.04	*<0.05*
p		NS	NS	

*Adenocarcinomas only
Values are age-standardized rates ± standard error
NS=Non-significant.

Table 4.4. One-year relative survival for esophageal cancer patients in BC (Canada) and Ardabil (Iran).

		BC	Ardabil	*p*
Gender	Male	0.32 ± 0.07	0.25 ± 0.1	*NS*
	Female	0.34 ± 0.07	0.18 ± 0.06	*NS*
p		*NS*	*NS*	
Age	Less than 65	0.44 ± 0.11	0.19 ± 0.06	*<0.01*
	65 or more	0.27 ± 0.03	0.26 ± 0.05	*NS*
p		*NS*	*<0.05*	
Tumour Location	Upper third	0.50 ± 0.14	0	*<0.05*
	Middle third	0.42 ± 0.12	0.1 ± 0.05	*<0.05*
	Lower third	0.33 ± 0.10	0.33 ± 0.09	*NS*
p		*NS*	*<0.01*	
Histology	SCC	0.40 ± 0.10	0.20 ± 0.06	*<0.01*
	Adenocarcinoma	0.34 ± 0.08	0.38 ± 0.16	*NS*
p		*NS*	*NS*	

Values are age-standardized rates ± standard error
NS=Non-significant.

CHAPTER 5: The prognostic effect of ethnicity for gastric and esophageal cancer: the population-based experience in British Columbia, Canada [4]

5.1 Introduction

Gastric and esophageal cancers are among the most lethal human malignancies. Worldwide, gastric cancer is the fourth most common cancer, but the second most common cause of death from cancer [2]. Esophageal cancer is the eighth most common cancer, but the sixth most common cause of cancer death [2]. The epidemiology of these cancers is geographically diverse. Incidence rates for gastric cancer vary from 3.4 per 100,000 among women in North America to 26.9 per 100,000 among men in Asia. The 5-year survival is usually about 20% [139]; however, countries with higher incidence rates of gastric cancer generally have better survival rates than countries with lower incidence [3]. Incidence rates for esophageal cancer range from 5-10 per 100,000 in North America to more than 100 per 100,000 in Eastern Iran near the Caspian Sea [13]. These differences between populations reflect different environmental and lifestyle (including healthcare) factors, as well as different genetic profiles of the tumours and the patients.

In order to investigate population characteristics such as ethnicity and to reduce or eliminate environmental confounding, it is preferable to conduct a study in a single geographic area with a heterogenous population rather than to conduct international comparisons [140]. British Columbia (BC), Canada, has a multi-ethnic population. Based on 2006 census data, about one in every four 4,428,400 British Columbians (24.8%) belongs to a visible minority, representing about one million people in the province. Of these, approximately 75% were born outside Canada, and about 60% immigrated to BC from 1991 to 2006 [133]. That indicates about 676,000 immigrants

[4] A version of this chapter has been submitted for publication and is under review.
Bashash M, Shah A, Hislop G, Le N, Brooks-Wilson A, Bajdik C.

and 297,000 non-immigrants in BC belonged to a visible minority group in 2006 [133]. Chinese was the largest group, accounting for 40% of all visible minorities in the province, followed by South Asians (26%) [133]. Iranians represent a relatively small but growing percentage of the BC population (0.5%, or 19,000 people) in 2001 [141], although they originate from a geographic region with the world's highest incidence of gastric and esophageal cancers [124,142]. This study compares survival of gastric and esophageal cancer patients among Chinese, South Asian and Iranian and other ethnic groups in BC.

5.2 Methods

Cancer incidence and survival data for invasive primary esophageal and gastric cancers were obtained from the population-based BC Cancer Registry for all BC patients diagnosed between 1984 and 2006. The topology and histology of cases were coded according to the International Classification of Diseases for Oncology, Third Edition (ICD-O) [127]. The topography for esophageal cancers was then grouped into four categories: esophagus upper third (ICD-O codes C15.0-C15.3), esophagus middle third (ICD-O codes C15.4), esophagus lower third and overlapping lesions (ICD-O codes C15.5), and esophagus unknown (ICD-O codes C15.8 and C15.9). The topography for gastric cancer was grouped into three categories: proximal third (cardia) in the gastroesophageal junction or upper third of the stomach (ICD-O codes C16.0 and C16.1), lower stomach or lower two thirds of the stomach (ICD-O codes C16.2–C16.7), and unknown or unspecified/overlapping lesion (ICD-O codes C16.8 and C16.9). Histological categories for esophageal cancers were squamous cell carcinoma (ICD-O codes 8050-8082), adenocarcinoma (ICD-O codes 8140-8573) and others (mainly ICD-O codes 8000-8020). Histology for gastric cancer was also categorized based on the Lauren classification system as diffuse or intestinal type [8] (diffuse gastric tumours defined by histology codes 8142, 8145 and

8490) [98]. For both esophageal and gastric cancers, nonepithelial tumours (ICD-O codes 8800-9759) were excluded.

Primary treatment was categorized as surgery, chemotherapy and radiotherapy, with only therapeutic (i.e., not diagnostic) surgeries being considered as treatment. Overall survival was the primary study outcome, and was calculated as the time between diagnosis and death. Complete follow-up information was available for all patients to 31 August 2007.

The ethnicity of patients was determined according to their names and categorized as Chinese, South Asian or Iranian. This method for identification of ethnicity was necessary because the BC Cancer Registry does not record ethnicity; the method has been described elsewhere [30,98,141,143-145]. Patients not classified as belonging to any of these three ethnic groups were categorized as "Other." Based on the ethnic distribution of the BC population, more than 80% of "Other" are British and Western Europeans [120]. British and Western Europeans could not be separated as a group because corresponding name lists do not exist.

Univariate comparisons of demographic, tumour and treatment variables between ethnic groups were performed using Chi-square tests. Survival was calculated using the Kaplan-Meier method and log-rank tests were used to compare survival differences among groups. All analyses were performed separately for nonmetastatic (Stage I-III) and metastatic (Stage IV) disease. Cox proportional hazards regression was used to estimate the effect of ethnicity adjusted for patient sex, age (less than 55 years, 55-64 years, 65-74 years and 75+ years), date of diagnosis (1984-1990, 1991-1995, 1996-2000, 2001-2006), tumour histology (intestinal and diffuse for gastric cancer; adenocarcinoma and squamous cell carcinoma for esophageal cancer), tumour location, disease stage and treatment received. The hazard ratio (HR) was calculated for each ethnic group

and is the ratio of the hazard rate in each ethnic group compared to the "Other" group. For each HR, a 95% confidence interval (95%CI) was calculated. p-values less than 0.05 were considered statistically significant.

5.3 Results

Gastric cancer

3136 cases of invasive gastric cancer were diagnosed during the study period. Descriptive information for the cases is shown by ethnicity in Table 5.1. The age and sex distributions were significantly different among the ethnic groups ($p<0.01$). A higher proportion of Chinese and South Asian gastric cancer patients were female as compared to the other ethnic groups. The average age at diagnosis was 61.0 years for Iranians, 62.6 years for Chinese, 61.7 years for South Asians, and 65.4 years for Other ethnicities. There were significant differences among the year of diagnosis by ethnicity ($p<0.01$). Tumour location was significantly different among the ethnic groups ($p<0.01$). Tumours in the proximal 1/3 of the stomach were more common in South Asians and Other ethnicities as compared to Chinese and Iranians. Histology based on the Lauren classification was also significantly different among ethnic groups ($p=0.03$). The diffuse type of gastric cancer was most common among the Chinese compared to the other ethnic groups. The distribution of stage and proportion with metastatic disease was not significantly different among the ethnic groups; however, treatment by surgery and chemotherapy were significantly different among the ethnic groups. The Chinese and Iranian groups received surgery more often than the South Asian or Other ethnicities group ($p<0.01$), and the South Asian and Iranian groups received chemotherapy more often than Chinese or Others ($p<0.01$). Figure 5.1 shows survival curves for gastric cancer patients according to ethnic group. Survival was significantly different between ethnic groups ($p<0.01$). When considered separately by presence

or absence of metastatic disease, significant differences were only found for non-metastatic disease ($p<0.01$), as shown in Figure 5.3. Furthermore, the association between survival and ethnicity was only significant for patients with non-metastatic disease who received therapeutic surgery ($p<0.01$).

In multivariate analyses adjusting for patient factors, disease factors and treatment, there was a significant difference among ethnic groups. Only Chinese had significantly longer survival as compared to the Other ethnicities, as shown in Table 5.2. This survival advantage in Chinese was only seen for non-metastatic disease (HR=0.78, 95% CI=0.64-0.95).

Esophageal cancer

2873 cases of esophageal cancer were diagnosed during the study period. Descriptive characteristics of these patients are presented by ethnicity in Table 5.3. The majority of South Asians were women whereas the majority in the other ethnic groups was men ($p<0.01$). There was no significant difference in age at diagnosis among the ethnic groups, the average age being 73.0 years, 68.0 years, 65.5 years and 68.4 years for Iranians, Chinese, South Asians and Other ethnicities, respectively. There was no significant difference among ethnic groups based on date of diagnosis.

Tumour location was significantly different among ethnic groups ($p<0.01$). More than half of tumours in Iranians and Other ethnicities were located in the lower third of esophagus whereas this location was less common in Chinese and South Asians. Histology was significantly different among the ethnic groups ($p<0.01$), with Chinese and South Asians having higher proportions of squamous cell carcinoma compared to Iranians and Other ethnicities.

There were no significant differences in stage or the proportion with metastatic disease among ethnic groups. Treatment received was not different, except for chemotherapy which had

significant differences among the ethnic groups ($p<0.01$), with the Chinese, Iranian and South Asian patients accessing chemotherapy more often than Other ethnicities. Figure 5.2 shows the survival curves for esophageal cancer patients by ethnic group ($p=0.029$). In multivariate analyses, there was no overall significant difference among ethnic groups, however South Asians showed better survival compared to the Other ethnicity group, as shown in Table 5.4. A significant survival difference only was observed among ethnic groups for patients with non-metastatic disease ($p=0.0498$), as shown in Figure 5.4. Again, South Asians showed better survival compared to the Other ethnicity group (HR=0.74, 95%CI=0.56-0.97) in the multivariate analysis.

5.4 Discussion

An earlier population-based study in BC reported overall five-year survival rates of 8.8% for esophageal cancer and 16.2% for gastric cancer [121]. The current study was conducted to examine the effect of ethnicity on survival. Our results indicate that patient ethnicity is a prognostic factor for both gastric and esophageal cancer; however ethnicity is only an independent prognostic factor for gastric cancer patients.

Prognostic factors can be classified into three broad groups: i) tumour-related, ii) host-related, and iii) environment-related (health care, treatment, lifestyle) factors [22,34]. Among tumour-related prognostic factors, disease stage is the most important [34] and often strongly influences the treatment plan. There were no significant differences in the stage distributions among ethnic groups; however, survival differences among ethnic groups were only significant for non-metastatic (i.e., stage I-III) disease. After adjustment for other factors (such as stage), the prognostic effect of ethnicity was significant only for gastric cancer patients.

Tumour topography is another prognostic factor, and there were significant differences in tumour location among different ethnic groups. It has been shown previously in Western countries that gastric cardia tumours are associated with worse survival as compared to lower gastric tumours [146-148]. In addition, for studies of esophageal cancer, the location of tumours also showed differences in survival. Tumours in the middle 1/3 of esophagus [132,149] showed worse survival for Turkey and Ardabil (Iran), but for BC and the United States where tumours in the lower 1/3 of esophagus are reported to have worse survival [121,122].

Among host-related prognostic factors, ethnic differences were found for sex and age in both gastric and esophageal cancer. Of environment-related factors, treatment is likely the most powerful determinant of survival. There were significant ethnic differences in the proportions of gastric cancer patients who received surgery and chemotherapy. The reason for treatment differences among ethnic groups is not clear in a system where all patients have equal access to cancer care, but the differences might be explained by disease factors, other patient characteristics or patient preferences.

It has been suggested that lower quality care and disparities in treatment are major contributors to differences in survival between minority and non-minority populations [150]. BC residents have access to publicly-funded healthcare, and the BC Cancer Agency (BCCA) has developed province-wide treatment guidelines and protocols [151].

The main strength of this study is the availability of reliable population-based data with details on tumour histology and pathology, treatment, disease stage and survival outcomes. The main weakness is the lack of self-reported ethnicity information, requiring the use of a proxy method (i.e., name lists) to assign ethnicity. The weakness of using name lists as proxy for ethnicity is

greater for women, who may change their surnames after marriage. This method has been developed, however, based on the names of women participating in the Screening Mammography Program of British Columbia (SMPBC) [143]. Further, women account for only 30% of gastric and esophageal cancer cases. The difference between proportion of ethnicities in this study and general population might be due to age distribution differences.

Our study investigated ethnicity as a prognostic factor for gastric and esophageal cancer patients. It has been shown that for gastric cancer, patient ethnicity is significant and Chinese patients experience better survival than other people in BC. It has also been shown that, for esophageal cancer, South Asian patients experience better survival than others in BC. The result for gastric cancer is consistent with several US studies in which all other ethnic groups had better survival compared to the non-Hispanic white population [152], and a Los Angeles study that showed that Asians with gastric adenocarcinoma had superior outcomes compared to other ethnic groups [153]. Our study also confirms the findings of an earlier study in BC that reported better survival outcomes for gastric cancer patients with Asian ethnicity compared to the general population [30]. Our findings are consistent with international population-based cancer survival data that indicate that the 5-year survival for gastric cancer in China is higher than in India [154]. A comparison between registries from Shanghai (China) and Madras (India) shows that the 5-year relative survival for gastric (20% versus 7.5%) and esophageal cancer (9.0% versus 6.9%) is better in Shanghai [155]. These survival rates for both cancers are also higher than those reported in Iran [137].

Ethnicity may represent biological characteristics of patients. Genetic variation may be responsible for differences in tumour-host interactions, such as the micro-architecture of tumours [156] and the complex process of metastasis, both of which are influenced by host genetic polymorphisms [29]. Ethnicity may also determine lifestyle and environmental characteristics

including cultural, socioeconomic, and religious practices. Such differences are expected to be less apparent with increasing generations after immigration. The difference observed in patient survival is not likely to be due to healthcare disparities among minority groups, as all BC residents are covered for healthcare through the BC Medical Services Plan (MSP). Interestingly, survival was found to be better in minority groups compared to the BC general population.

Gastric and esophageal cancers are deadly diseases that are often diagnosed at a stage when the treatment options are limited and less effective. It have been shown that for gastric and esophageal cancers, there are significant differences in survival among ethnic groups. Ethnicity may represent underlying genetic factors. Such factors could influence host-tumour interactions by altering tumour etiology and therefore its chance of spreading. Alternatively, genetic factors may determine response to treatments. Finally, ethnicity may represent non-genetic factors that affect survival. Differences in survival by ethnicity support the importance of ethnicity as a prognostic factor, and may provide clues for the future identification of genetic or lifestyle factors that underlie these observations.

Table 5.1. Descriptive characteristics for gastric cancer by ethnicity

		Iranian	Chinese	South Asian	Other	p
Sex (N=3136)	Male	15 (78.9%)	168 (62.2%)	57 (58.8%)	1974 (71.8%)	$4.4x10^{-4}$
	Female	4 (21.1%)	102 (37.8%)	40 (41.2%)	776 (28.2%)	
Age in years (N=3136)	Less than 55	7 (36.8%)	86 (31.9%)	26 (26.8%)	515 (18.7%)	$8.0x10^{-6}$
	55-64	3 (15.8%)	49 (18.1%)	20 (20.6%)	652 (23.7%)	
	65-74	7 (36.8%)	65 (24.1%)	35 (36.1%)	884 (32.1%)	
	75 and More	2 (10.5%)	70 (25.9%)	16 (16.5%)	699 (25.4%)	
Years of Diagnosis (N=3136)	1984-1990	0 (0.0%)	32 (11.9%)	11 (11.3%)	643 (23.4%)	$3.3x10^{-5}$
	1991-1995	7 (36.8%)	54 (20.0%)	16 (16.5%)	481 (17.5%)	
	1996-2000	4 (21.1%)	63 (23.3%)	27 (27.8%)	626 (22.8%)	
	2001-2006	8 (42.1%)	121 (44.8%)	43 (44.3%)	1000 (36.4%)	
Tumour Histology - Lauren classification (N=3136)	Intestinal	14 (73.7%)	205 (75.9%)	74 (76.3%)	2188 (79.6%)	0.032
	Diffuse	3 (15.8%)	55 (20.4%)	13 (13.4%)	382 (13.9%)	
	Other	2 (10.5%)	10 (3.7%)	10 (10.3%)	180 (6.5%)	
Tumour Location (N=3136)	Proximal 1/3	6 (31.6%)	52 (19.3%)	47 (48.5%)	1302 (47.3%)	1.93×10^{-22}
	Lower 2/3	10 (52.6%)	171 (63.3%)	28 (28.9%)	894 (32.5%)	
	NES/NOS*	3 (15.8%)	47 (17.4%)	22 (22.7%)	554 (20.1%)	
Tumour Stage (N=2567)	I	1 (5.6%)	14 (6.1%)	3 (3.7%)	108 (4.8%)	0.85
	II	6 (33.3%)	65 (28.5%)	29 (35.8%)	702 (31.3%)	
	III	6 (33.3%)	96 (42.1%)	29 (35.8%)	829 (37.0%)	
	IV	5 (27.8%)	53 (23.2%)	20 (24.7%)	601 (26.8%)	
Surgery (N=3080)	Yes	14 (73.7%)	178 (66.7%)	56 (57.7%)	1502 (55.7%)	0.0027
	No	5 (26.3%)	89 (33.3%)	41 (42.3%)	1195 (44.3%)	
Chemotherapy (N=3065)	Yes	10 (52.6%)	116 (43.6%)	44 (45.4%)	906 (33.8%)	$5.3x\ 10^{-4}$
	No	9 (47.4%)	150 (56.4%)	53 (54.6%)	1777 (66.2%)	
Radiotherapy (N=3058)	Yes	6 (31.6%)	99 (37.1%)	43 (44.3%)	1203 (45.0%)	0.061
	No	13 (68.4%)	168 (62.9%)	54 (55.7%)	1472 (55.0%)	

* NES not elsewhere specified; NOS not otherwise specified.

Table 5.2. Hazard ratio (HR) and 95% confidence interval (CI) from Cox proportional hazards regression analysis for overall survival of gastric cancer patients adjusted for patient sex, patient age, year of diagnosis, tumour histology (Lauren), tumour location, tumour stage and treatment.

Ethnicity	**N**	**HR**	**95% CI**		**p**
Iranian	16	0.64	0.34	1.18	*P=0.006*
Chinese	214	0.76	0.65	0.90	
South Asian	72	0.88	0.68	1.14	
Other	2038	Reference			

Table 5.3. Descriptive characteristics for esophageal cancer by ethnicity

		Iranian	Chinese	South Asian	Other	P
Sex (N=2873)	Male	10 (71.4%)	94 (74.6%)	57 (47.9%)	1821 (69.7%)	5.0×10^{-6}
	Female	4 (28.6%)	32 (25.4%)	62 (52.1%)	793 (30.3%)	
Age in years (N=2873)	Less than 55	0 (0.0%)	14 (11.1%)	21 (17.6%)	314 (12.0%)	0.12
	55-64	1 (7.1%)	35 (27.8%)	32 (26.9%)	610 (23.3%)	
	65-74	9 (64.3%)	41 (32.5%)	35 (29.4%)	858 (32.8%)	
	75 and More	4 (28.6%)	36 (28.6%)	31 (26.1%)	832 (31.8%)	
Years of Diagnosis (N=2873)	1984-1990	3 (21.4%)	16 (12.7%)	22 (18.5%)	486 (18.6%)	0.164
	1991-1995	1 (7.1%)	26 (20.6%)	15 (12.6%)	580 (22.2%)	
	1996-2000	3 (21.4%)	38 (30.2%)	33 (27.7%)	637 (24.4%)	
	2001-2006	7 (50%)	46 (36.5%)	49 (41.2%)	911 (34.9%)	
Tumour Histology (N=2874)	SCC *	5 (35.7%)	103 (81.7%)	81 (68.1%)	1389 (53.1%)	1.53×10^{-11}
	AC **	7 (50.0%)	19 (15.1%)	27 (22.7%)	1101 (42.1%)	
	Other	2 (14.3%)	4 (3.2%)	11 (9.2%)	124 (4.8%)	
Tumour Location (N=2874)	Upper 1/3	2 (14.3%)	23 (18.3%)	17 (14.3%)	314 (12.0%)	7.35×10^{-4}
	Middle 1/3	1 (7.1%)	45 (35.7%)	34 (28.6%)	605 (23.1%)	
	Lower 1/3	9 (64.3%)	40 (31.7%)	51 (42.9%)	1383 (52.9%)	
	NES/NOS***	2 (14.3%)	18 (14.3%)	17 (14.3%)	312 (12.0%)	
Tumour Staqe (N=2594)	I	1 (8.3%)	12 (10.3%)	8 (7.6%)	212 (9.0%)	0.84
	II	6 (50.0%)	66 (56.9%)	56 (53.3%)	1363 (57.8%)	
	III	3 (25.0%)	27 (23.3%)	26 (24.8%)	459 (19.4%)	
	IV	2 (16.7%)	11 (9.5%)	15 (14.3%)	326 (13.8%)	
Surgery (N=2830)	Yes	2 (15.4%)	24 (19.2%)	35 (29.9%)	630 (24.5%)	0.23
	No	11 (84.6%)	101 (80.8%)	82 (70.1%)	1944 (75.5%)	
Chemotherapy (N=2820)	Yes	0 (0.0%)	39 (31.2%)	25 (21.6%)	526 (20.5%)	*0.0084*
	No	13 (100.0%)	86 (68.8%)	91 (78.4%)	2039 (79.5%)	
Radiotherapy (N=2853)	Yes	13 (100.0%)	112 (89.6%)	111 (93.3%)	2240 (86.3%)	0.052
	No	0 (0.0%)	13 (10.4%)	8 (6.7%)	355 (13.7%)	

*SCC squamous cell carcinoma
**AC adenocarcinoma
*** NES not elsewhere specified; NOS not otherwise specified

Table 5.4. Hazard ratio (HR) and 95% confidence interval (CI) from Cox proportional hazards regression analysis for overall survival of esophageal cancer patients adjusted for patient sex, patient age, year of diagnosis, tumour histology, tumour location, tumour stage and treatment.

Ethnicity	**N**	**HR**	**95 % CI**		**P**
Iranian	10	1.13	0.61	2.12	0.14
Chinese	95	0.90	0.72	1.13	
South Asian	81	0.80	0.59	0.98	
Other	1947	Reference			

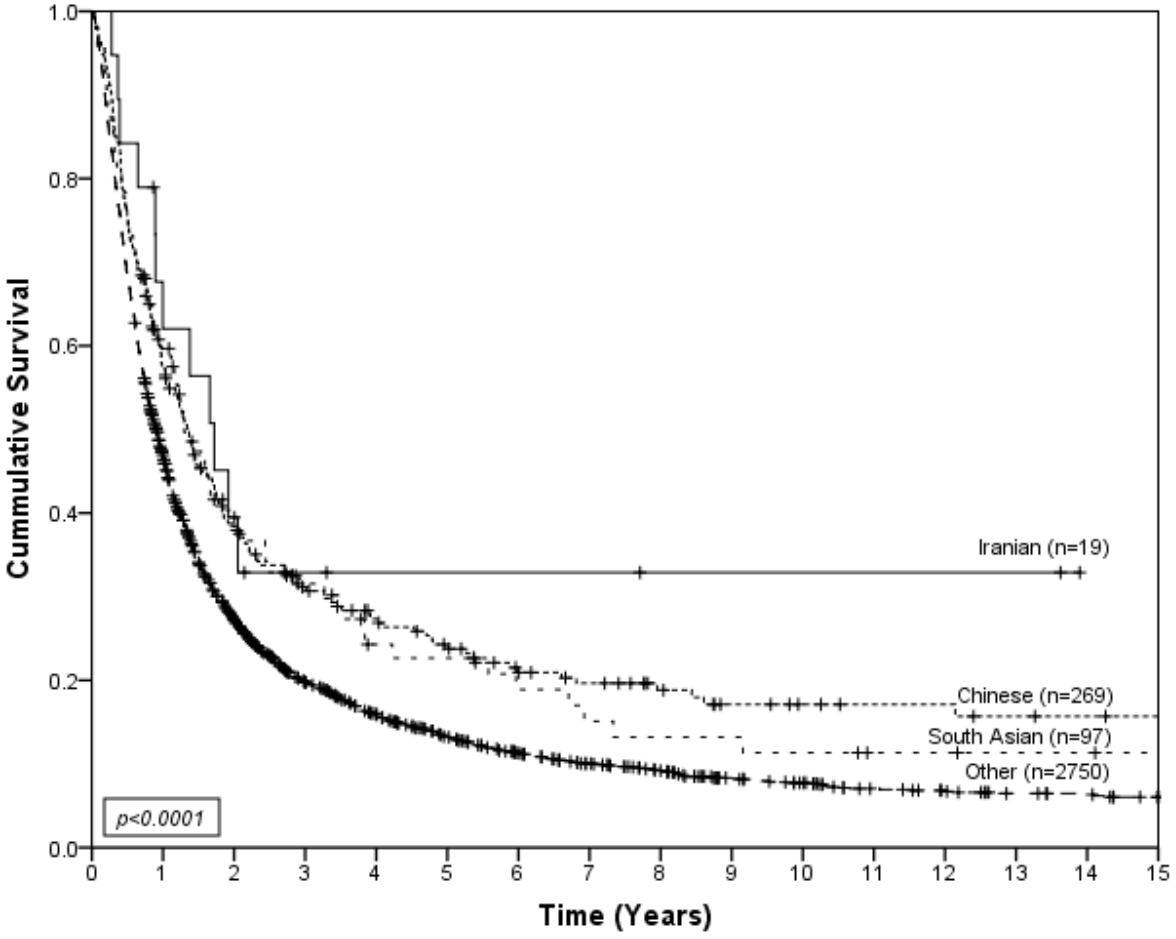


Figure 5.1. Survival of gastric cancer patients by ethnic group

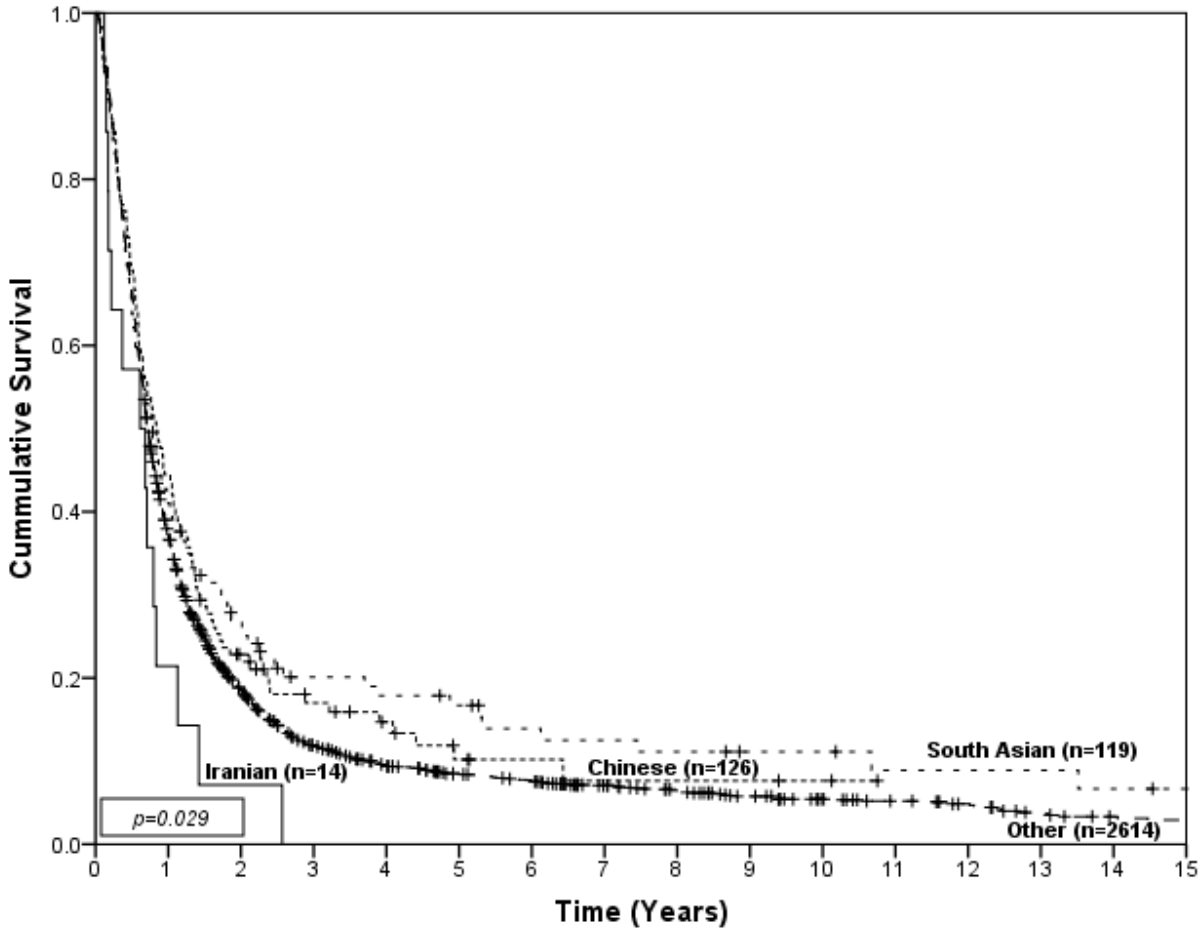


Figure 5.2. Survival of esophageal cancer patients by ethnic group.

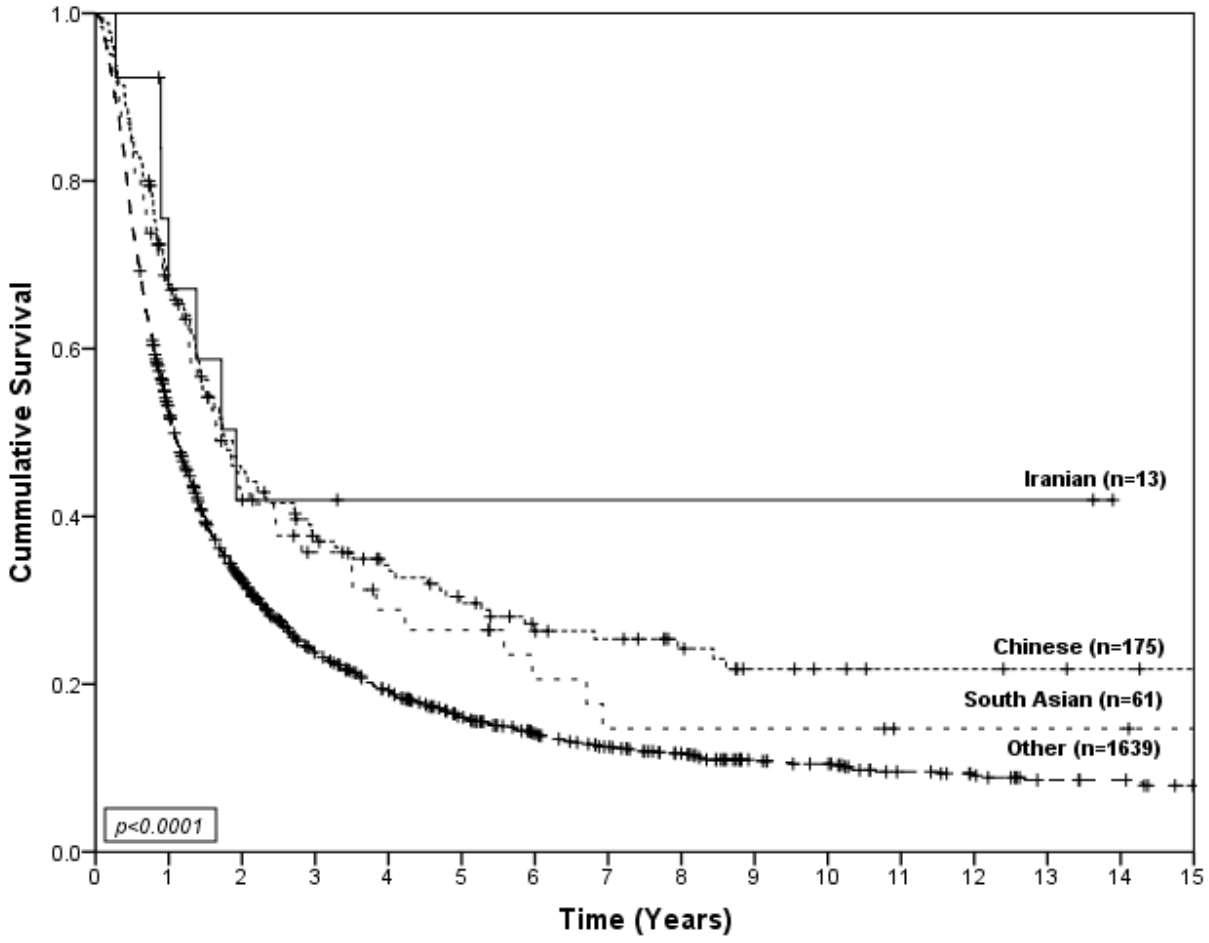


Figure 5.3 . Survival of gastric cancer patients by ethnic group for non-metastatic disease.

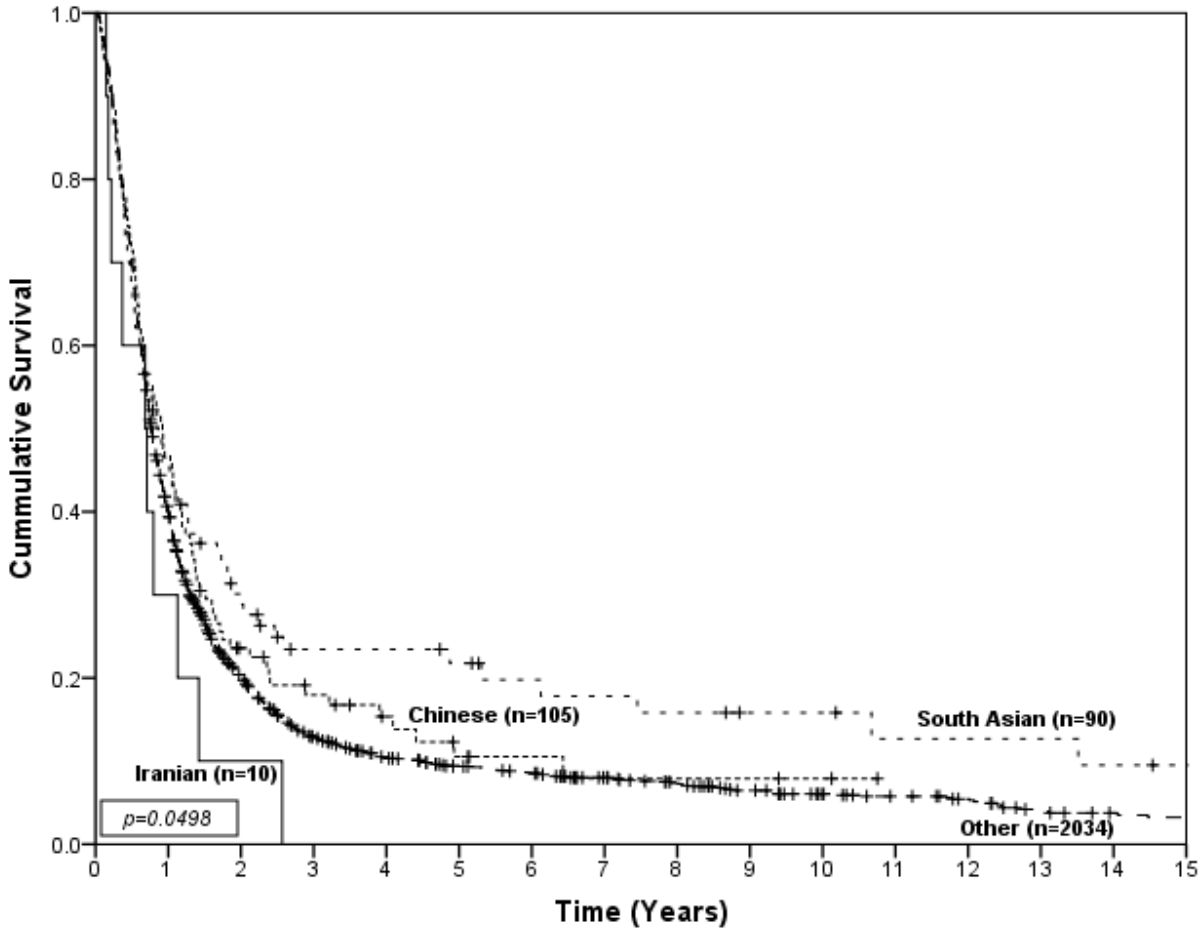


Figure 5.4. Survival of esophageal cancer patients by ethnic group for non-metastatic disease.

CHAPTER 6: Genetic polymorphism at *TIMP-3* predicts survival for patients with adenocarcinoma of the esophagus and gastroesophageal junction [5]

6.1 Introduction

During the past two decades, there has been a dramatic increase in the incidence of adenocarcinoma of both the esophagus and gastroesophageal junction (GEJ) in North America and Western Europe [5,18,96,121]. Because of this increase and also generally poor survival, lower esophageal and GEJ adenocarcinomas are clinically important cancers [18,121]. Similarities and shared prognostic factors suggest these cancers can be considered a single neoplastic entity in many contexts [5].

Adenocarcinomas of the lower esophagus and GEJ originate from the approximately 10 cm segment around the GEJ, including the lower 5 cm of the esophagus and proximal 5 cm of the stomach [25]. Lesions with their centre between 1 and 5 cm above the GEJ are classified as type I or esophagus tumours; lesions between 1 cm above or 2 cm below GEJ are type II or cardia tumours; and lesions between 2 and 5 cm below GEJ are type III or subcardia tumours [157,158] . In spite of this subclassification, the American Joint Committee on Cancer (AJCC) Cancer Staging Manual uses "single-stage grouping" across the entire lower esophagus and GEJ area [25].

Cancer occurs more frequently at the junction of two different tissue microarchitectures, and anatomic sites that have this characteristic are highly influenced by microarchitecture disruption[156]. Loss of structure is a prerequisite and defining characteristic of most cancers and precancerous lesions [159]. The extracellular matrix (ECM) influences tissue and organ architecture, as well as the growth of neoplastic cells [160]. Malignant cells acquire the ability to remodel the ECM and modulate the expression of ECM receptors [161]. The balance between

[5] A version of this chapter will be submitted for publication.
Bashash M, Shah A, Hislop G, Le N, Brooks-Wilson A, Bajdik C.

activated matrix metalloproteinase (MMP) and tissue inhibitors of metalloproteinase (TIMP) controls ECM remodelling [162]. MMPs also contribute to the invasion, promotion, angiogenesis, and the establishment and growth of metastatic lesions in distant organ sites [76]. MMPs can be synthesized by tumour cells, but are frequently produced by surrounding stroma, including fibroblasts and infiltrating inflammatory cells [76]. Finally, MMPs solubilize cell surface and matrix-bound factors that can influence cellular properties, such as growth, death, and migration and metastasis [76]. It is possible that a tissue microenvironment giving rise to malignancy also could allow a malignant cell to metastasize. Many phenotypes, including cancer, are significantly affected by constitutional genetic polymorphisms, and the complex process of metastatic dissemination might be significantly affected by host genotypes [29]. As a result, assessment of genetic polymorphisms in the TIMP gene family has been chosen to evaluate prognosis in esophagus and GEJ adenocarcinoma patients.

This study used a prospective cohort of patients with adenocarcinoma of the esophagus and GEJ in British Columbia (BC) who were admitted to the BC Cancer Agency. Our objective was to assess genetic polymorphisms of TIMP genes for association with prognosis for adenocarcinomas of the esophagus and GEJ. This study was reviewed and approved by the joint University of British Columbia / British Columbia Cancer Agency Research Ethics Board. All subjects provided written informed consent.

6.2 Methods

Eligible patients were patients between ages 20 and 90 years who were diagnosed with primary invasive adenocarcinoma of esophagus and GEJ between January 1, 2008 and April 30 2009, admitted to the BCCA, and able to provide written informed consent. Patient recruitment started January 1, 2008 and finished July 31 2009, this includes a two-month recruitment follow-up

period. Eligible patients were identified using the electronic appointment list of new gastrointestinal (GI) tumour group patients. Pathology reports of all gastric and esophageal cancer patients were reviewed to identify eligible patients for the study. The anatomic sites esophagus and cardia were defined as International Classification of Diseases for Oncology (ICDO-3) site codes C150-C160, and adenocarcinoma was defined as ICDO-3 histology codes 8140/3-8573/3. Eligibility of identified patients and capability to participate was assessed by a BCCA GI Tumour Group oncologist. Eligible patients were invited to participate in the study at their next BCCA visit. The BCCA operates clinics in five major centres across the province of British Columbia. A patient's participation required their written consent to provide blood or saliva for genetic analysis, and access to their medical chart for demographic and clinical information.

Germline DNA was extracted from blood or saliva (saliva was collected using Oragene® DNA sample collection kit). To minimize the potential for population stratification bias, only patients who identified themselves as white Canadian, British, and Western European were included for genotyping. TagSNPs [163] representing genetic variation in each gene were chosen using Haploview Ver. 4.1[164] on HapMap [165] (phase 3 release 2) western European ancestry (CEU) data. TagSNPs with minimum minor allele frequency of 0.1 were chosen within ≤500 kb of each gene using an r^2 threshhold of 0.9. We also included SNPs reported to be associated with cancer in the literature, and non-synonymous coding SNPs in these genes. The minor allele frequencies of these SNPs were obtained using HapMart (BioMart ver 7. using HapMap release 27) on CEU population data. The list of SNPs genotyped is shown in Table 6.1.

In total, 63 SNPs were genotyped using two Sequenom multiplex iPLEX Gold assays [166] on a single 96-well plate of 90 subject DNA samples. 88 out of 90 samples (98%) were genotyped

successfully, with an average call rate of 96%. For quality control of genotyping, clustering of observed genotypes was reviewed by transferring intensity data to MassArray Typer software (version 7.0.2.5). 15 SNPs with less than 95% call rate were excluded from analysis . Concordance between duplicate samples was 100%. All of the X chromosome markers (rs6609533, rs4898) were consistent with the sex of patients. After all quality control restrictions, 48 SNPs were used in the final analysis.

Patient characteristics and clinical information were obtained from BCCA medical charts and preadmission questionnaires. Patient age was categorized into two groups (<70 and 70+) and BMI was categorized into three groups (normal: 18.5 to <25, overweight: 25 to <30, and obese: 30+). Tumour location was categorized as esophagus (i.e., Siewert I) or gastric cardia (i.e., Siewert II/III). Disease stage was defined according to recent AJCC Guidelines [25] for lower esophagus and GEJ cancers and categorized as metastatic or non-metastatic cancer. Primary treatment was categorized as surgery, chemotherapy and radiotherapy, with only therapeutic (i.e., not diagnostic) surgeries considered as treatment. Overall survival was the primary study outcome, and calculated as the time between diagnosis and death. April 30, 2010 was the end of follow-up and all patients had at least 1 year follow-up information on this date (median follow-up = 16.7 months).

Univariate comparisons of demographic, tumour and treatment variables between living and deceased patient groups were performed using Chi-square tests. Survival estimates were calculated using the Kaplan-Meier method; log-rank tests were used to compare survival differences. Haplotype analysis was performed using HAPSTAT software[167]. SNPs were each fit using additive, dominant, recessive and codominant models. Cox proportional hazards regression was used to estimate the effect of SNPs adjusted for patient age, tumour location,

disease stage and treatment received. For each hazard ratio (HR), a 95% confidence interval (95%CI) was calculated. P-values less than 0.05 were considered statistically significant. The false discovery rate (FDR) method [168] was applied to address multiple comparisons, and FDR values of <0.2 were considered as the least likely to be due to a false positive finding. Given 85 cases and assuming 80% power, 0.05 type I error probability, a range of MAF values was used to calculate the minimum detectable hazard ratios (Figure 6.2).

6.3 Results

During the study period 202 esophageal and gastroesophageal junction (GEJ) adenocarcinomas patients diagnosed from Jan 1 2008 to 30 April 2009 were assessed for eligibility. Fifty-five of these 202 patients were excluded: 15 (24%) patients who were deceased at the time of assessment, 18 (33%) patients who were deceased at time of contact and 22 (40%) patients who were unable to consent because of poor health. The total number of eligible cases for the study was 147 patients. Of these, 4 (2.7%) could not be contacted, 31 (21%) did not reply to after repeated study invitations, and 8 (5.4%) refused to participate. Biological samples appropriate for DNA extraction were received for 93 cases. Genotyping results were available for 88 (98%) of the sample; 85 cases satisfied quality control restrictions and thus were available for analysis.

Men accounted for 91% of the sample, and the median age of diagnosis was 63 years. With regard to BMI, 27% of cases were normal, 43% were overweight and 30% were obese. About half of the patients (48%) had a tumour in the esophagus (Siewert I) and the others had a tumour in the GEJ or cardia. The majority of patients received chemotherapy (65%) and radiation (65%) as their primary treatment, and 45% underwent surgery before the time of recruitment. Stage could be assessed for 94% of the patients: 59% had local/regional disease and 41% had

metastatic disease. The stages of non-metastatic cases were: 6.4% IA, 4.3% IIA, 34.0% IIB, 42.6% IIIA, 6.4% IIIB and 6.4% IIIC.

Table 6.1 lists the SNPs genotyped. Estimates from a Cox model of survival associated with TIMP genetic variations are shown in table 6.2. Variation in *MMP2* markers (rs243842 and rs243847) showed significant association with patient survival in the codominant model. Other models and other MMP gene SNPs were not significant.

Among TIMP genes, significant association of variations in *TIMP3* with the survival of adenocarcinoma of the esophagus and GEJ cancer patients was observed. The SNPs and the linkage disequilibrium (LD) structure plot for *TIMP3* is shown in Figure 6.2. Of 14 SNPs tested in *TIMP3*, there were significant associations for rs130274, rs1962223, rs5754312, rs715572 in all (i.e., codominant, additive, recessive, or dominant) adjusted and unadjusted models. These four SNPs also passed the multiple testing comparison criterion (i.e., FDR < 0.2). Table 6.3 shows the survival model for *TIMP3* variations after adjusting for age, tumour location, disease stage and treatment. A genetic polymorphism in the promoter region of *TIMP3* (rs1962223) was associated with about a 3-fold increase in the HR for patients who carried the CG genotype. This association was observed after adjustment for patient age, tumour location, disease stage and treatment received. rs1962223 and rs130274 were in moderate LD (r^2=0.56). rs130274 showed a more than 3-fold increased HR for the dominant and codominant models and 2-fold increased HR for the additive model. rs715572 was associated with about a 3-fold increased HR for the dominant and codominant models and a 2-fold reduction in the HR for the additive model; rs5754312 was associated with a 4-fold reduction in the HR in the recessive and codominant model, and a 2-fold reduction in the HR for the additive model. In part because of the low number or lack of homozygotes for the minor allele at rs130274, rs715572 and rs1962223, a

dominant model best describes the association of these SNPs with the survival of cancer patients. For rs5754312, a recessive model best describes the association. In haplotype analysis, a block including rs5754312, rs715572 and showed significant association with the patients' survival (p=0.002). Figure 6.3 shows the survival of patients by SNPs and haplotype. There were also associations for rs137485 and rs9619311 with the HR in the dominant and additive models respectively.

6.4 Conclusion

Adenocarcinoma of the esophagus and GEJ is one of the most aggressive forms of human malignancies. In spite of improvements in the management of this disease, the 5-year overall survival after treatment hardly exceeds 25% [20]. Patients with adenocarcinoma of the GEJ and gastric cardia (Siewert II and III) have distinctively worse survival than patients with lower gastric and lower esophageal adenocarcinoma [169]. TNM cancer staging systems predict survival on the basis of the anatomic extent of the tumour. Other factors, however, including host factors, should be considered for determining and predicting the outcome of these patients. We demonstrated that polymorphisms at *TIMP3* are prognostic factors for this cancer.

The TIMP gene products are important in human cancers. The family of homologous proteins corresponding to the tissue inhibitors of metalloproteinases are expressed by a variety of cell types and present in most tissues and body fluids. TIMP proteins are natural inhibitors of proteolysis activity MMP, and adamalysins (ADAMs and ADAMTS) proteins[170]. Numerous reports suggest that TIMP proteins act at various steps of tumour progression including invasion, metastasis, growth and angiogenesis [77]. The protein product of *TIMP3* is a 24-kDa protein that, unlike other TIMP protein family members, binds to the ECM. The protein corresponding to *TIMP3* acts as a suppressor in some cancers by affecting tumour growth, angiogenesis, invasion

and the development of metastases [76,171,172]. TIMP3 also been reported to induce apoptosis of cancer cells [173].

The study has several strengths. A prospective design allows study of current treatments for esophagus and GEJ adenocarcinomas. It also permits the collection of biological samples, allows the verification of clinical and patient information, and greatly simplifies ethical issues regarding patient participation and use of personal information. This study includes patients from the entire province of British Columbia, and is not restricted to a single hospital or clinic. Additionally, our provence-wide approach is not biased by treatment disparity because all BC residents are covered for healthcare through the BC Medical Services Plan (MSP). Moreover, the GI Tumour Group at the BC Cancer Agency (BCCA) provides care for all patients in the province, and devises province-wide treatment guidelines and protocols. Our use of a candidate gene design addresses genetic pathways of known biological relevance, and is based on a prior hypothesis for each gene. This approach simplifies interpretation of findings based on the biological plausibility of each gene and minimizes loss of study power due to correction for multiple tests.

A limitation of the study is that patients with esophagus and GEJ adenocarcinomas have poor survival and are often diagnosed with significant comorbidities or poor physical performance. Because of this limitation, our results do not apply to patients with very short survival (i.e. less than 2 months) or additional substantial health problems. Compared to other cancers, adenocarcinoma of the esophagus and GEJ is a rare disease and a consortium of research groups would be required to obtain enough samples to detect lower predictive and prognostic effect size (i.e., smaller HRs).

This study shows that one polymorphism in the promoter region and three intronic tagSNPs of *TIMP3* predict survival of patients with adenocarcinoma of the esophagus and GEJ.. This

conclusion is consistent with other studies that showed associations of the methylation status of *TIMP3* with cancer outcomes [174-178]. Some reports suggest that reduced expression of the TIMP3 protein has a dual role in esophageal adenocarcinoma. There seems to be both an early role in the development of tumours via Barrett's esophagus, and a later role in the invasive and metastatic phases, the latter leading to worse patient survival [178,179]. It has been also shown that aberrant hypermethylation of the human *TIMP3* is directly responsible for the transcriptional inactivation of its expression in gastric cancer cell lines. This supports the recent extensive accumulation of evidence about *TIMP3* and tumour progression/invasion [180]. SNPs in *TIMP3* have been associated with breast cancer prognosis [181,182]. To our knowledge, this study is the first report of the association of genetic polymorphisms of *TIMP3* with survival of gastric or esophageal cancers.

Adenocarcinoma of the esophagus and GEJ are deadly diseases that are often diagnosed at a stage when the treatment options are limited and have limited effectiveness. Our results do not establish an exact mechanism by which *TIMP3* affects survival. Other reports suggest that *TIMP3* is involved in variety of steps affecting cancer progression, including the induction of apoptosis[183] and anti-angiogenesis[184] The latter might involve directly binding to VEGF receptor 2 or inhibiting ADAM-17 activity [185]. Regardless of the mechanisms, factors that affect regulation of *TIMP3* expression, including promoter methylation or genetic variation, could be a promising prognostic factor or therapeutic target for this cancer. Modeling prognosis based on host factors including genetic polymorphisms is an emerging field of translational research. Compared to tumour, constitutional genetic material is relatively easy to obtain, and can be assessed before treatment is started.

Table 6.1. SNPs in TIMP and MMP genes used for survival analyses.

Gene Name	Reference ID	Chromosome	Genomic position	Alleles	Location in Gene	HapMap MAF	HWE p*	GENOTYPE %*	MAF*	Alleles*
MMP2	rs11541998	16	54094264	C/G	SYNONYMOUS CODING	0.102	1	100	0.1	C/G
MMP2	rs11639960	16	54090771	A/G	INTRONIC	0.35	0.905	98.8	0.286	T/C
MMP2	rs17301608	16	54076111	C/T	INTRONIC	0.385	0.563	98.8	0.304	C/T
MMP2	rs1992116	16	54085392	G/A	INTRONIC	0.469	0.437	100	0.347	C/T
MMP2	rs243842	16	54084923	T/C	INTRONIC	0.36	0.324	100	0.418	T/C
MMP2	rs243847	16	54081499	T/C	INTRONIC	0.398	0.397	100	0.441	A/G
MMP2	rs243865	16	54069307	C/T	UPSTREAM	0.243	1	100	0.2	C/T
MMP2	rs7201	16	54097115	A/C	3PRIME UTR	0.451	0.945	97.6	0.355	T/G
MMP7	rs10502001	11	101903803	C/T	NON SYNONYMOUS CODING	0.205	0.074	100	0.253	G/A
MMP7	rs11225308	11	101904688	T/G	INTRONIC	0.21	0.056	98.8	0.25	A/C
MMP7	rs12184413	11	101894798	C/T	DOWNSTREAM	0.102	1	100	0.118	G/A
MMP7	rs12285347	11	101901817	T/C	INTRONIC	0.467	0.162	100	0.453	A/G
MMP7	rs1996352	11	101901457	C/T	INTRONIC	0.23	1	100	0.206	T/C
MMP7	rs495041	11	101895398	C/T	DOWNSTREAM	0.128	1	100	0.165	G/A
MMP7	rs880197	11	101910881	A/T	UPSTREAM	0.235	1	100	0.206	A/T
MMP9	rs17576	20	44073632	A/G	NON SYNONYMOUS CODING	0.363	0.153	100	0.353	A/G
MMP9	rs3918261	20	44076999	A/G	INTRONIC	0.164	1	100	0.165	T/C
TIMP1	rs4898	X	47329929	T/C	SYNONYMOUS CODING	0.485	0	100	0.494	C/T
TIMP1	rs6609533	X	47330230	A/G	INTRONIC	0.485	0	100	0.494	C/T
TIMP2	rs12452379	17	74427053	C/A	INTRONIC	0.496	1	100	0.429	G/T
TIMP2	rs12600817	17	74413060	G/A	INTRONIC	0.487	1	100	0.476	A/G
TIMP2	rs2277700	17	74378306	C/T	INTRONIC	0.254	1	100	0.188	T/C
TIMP2	rs2377004	17	74382054	C/T	INTRONIC	0.382	0.65	100	0.335	T/C
TIMP2	rs2889529	17	74409070	A/G	INTRONIC	0.429	0.749	100	0.459	A/G
TIMP2	rs4789932	17	74435870	G/A	UPSTREAM	0.442	0.447	98.8	0.393	C/T

Gene Name	Reference ID	Chromosome	Genomic position	Alleles	Location in Gene	HapMap MAF	HWE p*	GENOTYPE %*	MAF*	Alleles*
TIMP2	rs4789936	17	74409569	C/T	INTRONIC	0.496	1	100	0.471	A/G
TIMP2	rs6416835	17	74420830	A/G	INTRONIC	0.433	1	100	0.394	C/T
TIMP2	rs7211674	17	74410660	C/A	INTRONIC	0.438	0.779	100	0.447	A/C
TIMP2	rs7212662	17	74429726	T/G	INTRONIC	0.456	0.704	100	0.494	A/C
TIMP2	rs8064344	17	74388569	C/T	INTRONIC	0.25	1	100	0.188	T/C
TIMP2	rs8068674	17	74419040	C/T	INTRONIC	0.425	0.779	100	0.447	C/T
TIMP3	rs130274	22	31534334	C/T	INTRONIC	0.23	0.974	100	0.259	C/T
TIMP3	rs135029	22	31570290	A/G	INTRONIC	0.314	1	98.8	0.31	C/T
TIMP3	rs137485	22	31584283	T/A	INTRONIC	0.279	0.905	98.8	0.286	A/T
TIMP3	rs137487	22	31589104	A/G	DOWNSTREAM	0.473	0.907	100	0.459	G/A
TIMP3	rs137489	22	31592935	T/C	DOWNSTREAM	0.235	0.855	100	0.241	A/G
TIMP3	rs1427378	22	31582041	A/G	INTRONIC	0.254	1	100	0.276	A/G
TIMP3	rs1962223	22	31523905	G/C	UPSTREAM	0.183	0.139	100	0.165	C/G
TIMP3	rs2040435	22	31593431	C/T	DOWNSTREAM	0.288	1	100	0.165	C/A
TIMP3	rs242072	22	31565517	C/T	INTRONIC	0.478	0.712	100	0.482	T/C
TIMP3	rs242077	22	31559685	T/C	INTRONIC	0.392	0.984	98.8	0.417	C/T
TIMP3	rs5754312	22	31574421	A/T	INTRONIC	0.473	0.948	100	0.482	A/T
TIMP3	rs715572	22	31564931	G/A	INTRONIC	0.221	0.657	100	0.188	C/T
TIMP3	rs738992	22	31540005	C/T	INTRONIC	0.5	0.162	100	0.471	C/T
TIMP3	rs9606994	22	31523050	G/A	UPSTREAM	0.483	0.642	100	0.429	G/A
TIMP3	rs9619311	22	31526693	T/C	UPSTREAM	0.292	0.235	100	0.312	A/G
TIMP4	rs308952	3	12129428	A/G	INTRONIC	0.107	1	97.6	0.133	G/A
TIMP4	rs3755724	3	12175906	C/T	REGULATORY REGION	0.35	0.898	100	0.341	G/A

*Observed at study cohort

Table 6.2. Hazard ratios (HR) and 95% confidence intervals (CI) estimates for the association between TIMP and MMP gene variations and survival. (Unadjusted)

Gene	SNP ID	Alleles	NO (Freq)	Codominant model HR(95%CI)	P	Additive model HR(95%CI)	Dominant Model HR(95%CI)	Recessive model HR(95%CI)
MMP2	rs11541998	CC	69(81.2%)	REF	0.709	0.726(0.316,1.667)	0.747(0.315,1.773)	0.049(0,78936.855)
		CG	15(17.6%)	3183.68(0.00, E)				
		GG	1(1.2%)	2502.46(0.00, E)				
	rs11639960	TT	42(50.0%)	REF	0.412	0.709(0.426,1.179)	0.69(0.374,1.275)	0.518(0.125,2.147)
		TC	36(42.9%)	0.74(0.39, 1.39)				
		CC	6(7.1%)	0.45(0.11, 1.92)				
	rs17301608	CC	39(46.4%)	REF	0.823	0.867(0.525,1.43)	0.884(0.485,1.609)	0.667(0.161,2.768)
		TC	39(46.4%)	0.92(0.50, 1.70)				
		TT	6(7.1%)	0.64(0.15, 2.74)				
	rs1992116	CC	34(40.0%)	REF	0.825	0.862(0.537,1.384)	0.835(0.455,1.533)	0.827(0.295,2.318)
		TC	43(50.6%)	0.85(0.45, 1.60)				
		TT	8(9.4%)	0.76(0.26, 2.24)				
	rs243842	CC	26(30.6%)	REF	0.032	0.995(0.608,1.628)	0.649(0.349,1.208)	1.992(0.953,4.162)
		TC	47(55.3%)	0.52(0.26, 1.02)				
		TT	12(14.1%)	1.33(0.59, 3.02)				
	rs243847	AA	24(28.2%)	REF	0.043	0.988(0.61,1.599)	0.638(0.34,1.197)	1.811(0.891,3.681)
		AG	47(55.3%)	0.51(0.26, 1.01)				
		GG	14(16.5%)	1.18(0.53, 2.64)				
	rs243865	CC	54(63.5%)	REF	0.814	0.841(0.463,1.526)	0.811(0.421,1.56)	0.992(0.135,7.301)
		TC	28(32.9%)	0.80(0.41, 1.57)				
		TT	3(3.5%)	0.92(0.12, 6.86)				
	rs7201	AA	35(42.2%)	REF	0.793	0.857(0.546,1.345)	0.819(0.445,1.509)	0.815(0.32,2.077)
		AC	37(44.6%)	0.84(0.44, 1.61)				
		CC	11(13.3%)	0.75(0.28, 2.01)				

Gene	SNP ID	Alleles	NO (Freq)	Codominant model HR(95%CI)	P	Additive model HR(95%CI)	Dominant Model HR(95%CI)	Recessive model HR(95%CI)
MMP7	rs10502001	GG	51(60.0%)	REF	0.988	0.971(0.618,1.526)	0.975(0.524,1.814)	0.921(0.329,2.583)
		AG	25(29.4%)	0.99(0.50, 1.97)				
		AA	9(10.6%)	0.92(0.32, 2.64)				
	rs11225308	CC	51(60.7%)	REF	0.834	1.041(0.672,1.613)	1.133(0.613,2.093)	0.888(0.317,2.488)
		AC	24(28.6%)	1.21(0.62, 2.36)				
		AA	9(10.7%)	0.94(0.33, 2.71)				
	rs12184413	GG	66(77.6%)	REF	0.021	0.779(0.365,1.666)	0.669(0.298,1.505)	8.823(1.118,69.648)
		AG	18(21.2%)	0.58(0.25, 1.38)				
		AA	1(1.2%)	8.01(1.01, 63.45)				
	rs12285347	AA	29(34.1%)	REF	0.218	0.868(0.586,1.285)	1.083(0.56,2.093)	0.567(0.262,1.227)
		AG	35(41.2%)	1.37(0.69, 2.74)				
		GG	21(24.7%)	0.68(0.28, 1.66)				
	rs1996352	TT	53(62.4%)	REF	0.841	0.893(0.519,1.537)	0.926(0.497,1.724)	0.559(0.076,4.095)
		TC	29(34.1%)	0.97(0.51, 1.83)				
		CC	3(3.5%)	0.55(0.07, 4.10)				
	rs495041	GG	59(69.4%)	REF	0.756	0.932(0.502,1.731)	0.879(0.451,1.714)	1.774(0.24,13.099)
		AG	24(28.2%)	0.84(0.42, 1.68)				
		AA	2(2.4%)	1.69(0.23, 12.58)				
	rs880197	AA	53(62.4%)	REF	0.841	0.893(0.519,1.537)	0.926(0.497,1.724)	0.559(0.076,4.095)
		TA	29(34.1%)	0.97(0.51, 1.83)				
		TT	3(3.5%)	0.55(0.07, 4.10)				
MMP9	rs17576	AA	32(37.6%)	REF	0.84	1.145(0.705,1.859)	1.211(0.639,2.294)	1.098(0.391,3.081)
		AG	46(54.1%)	1.21(0.63, 2.33)				
		GG	7(8.2%)	1.23(0.41, 3.76)				
	rs2274755	CC	59(69.4%)	REF	0.183	0.543(0.279,1.058)	0.556(0.273,1.13)	0.047(0,82.314)
		AC	24(28.2%)	0.61(0.30, 1.25)				
		AA	2(2.4%)	0.00(0.00, E)				

Gene	SNP ID	Alleles	NO (Freq)	Codominant model HR(95%CI)	P	Additive model HR(95%CI)	Dominant Model HR(95%CI)	Recessive model HR(95%CI)
MMP9	rs3918261	TT	59(69.4%)	REF	0.183	0.543(0.279,1.058)	0.556(0.273,1.13)	0.047(0,82.314)
		TC	24(28.2%)	0.61(0.30, 1.25)				
		CC	2(2.4%)	0.00(0.00, E)				
TIMP1	rs4898	CC	40(47.1%)	REF	0.983	1.025(0.752,1.397)	1.055(0.579,1.922)	1.04(0.571,1.894)
		TC	6(7.1%)	1.09(0.32, 3.66)				
		TT	39(45.9%)	1.05(0.57, 1.95)				
	rs6609533	CC	40(47.1%)	REF	0.983	1.025(0.752,1.397)	1.055(0.579,1.922)	1.04(0.571,1.894)
		TC	6(7.1%)	1.09(0.32, 3.66)				
		TT	39(45.9%)	1.05(0.57, 1.95)				
TIMP2	rs12452379	GG	28(32.9%)	REF	0.603	0.927(0.606,1.416)	1.143(0.508,2.57)	0.765(0.392,1.492)
		TG	41(48.2%)	1.39(0.70, 2.79)				
		TT	16(18.8%)	1.08(0.42, 2.74)				
	rs12600817	AA	23(27.1%)	REF	0.962	0.955(0.623,1.464)	0.912(0.476,1.75)	0.978(0.468,2.043)
		AG	43(50.6%)	0.91(0.45, 1.82)				
		GG	19(22.4%)	0.92(0.39, 2.16)				
	rs2277700	TT	56(65.9%)	REF	0.243	1.231(0.7,2.167)	1.115(0.595,2.091)	3.224(0.761,13.657)
		TC	26(30.6%)	1.02(0.53, 1.96)				
		CC	3(3.5%)	3.24(0.75, 13.94)				
	rs2377004	TT	36(42.4%)	REF	0.663	1.249(0.771,2.025)	1.285(0.692,2.388)	1.38(0.489,3.891)
		TC	41(48.2%)	1.25(0.66, 2.36)				
		CC	8(9.4%)	1.56(0.52, 4.72)				
	rs2889529	AA	26(30.6%)	REF	0.733	1.154(0.762,1.748)	1.316(0.663,2.612)	1.114(0.548,2.266)
		AG	40(47.1%)	1.31(0.64, 2.71)				
		GG	19(22.4%)	1.32(0.56, 3.12)				
	rs4789932	CC	33(39.3%)	REF	0.614	0.806(0.52,1.25)	0.746(0.404,1.377)	0.764(0.322,1.815)
		TC	36(42.9%)	0.78(0.40, 1.50)				
		TT	15(17.9%)	0.67(0.27, 1.69)				

Gene	SNP ID	Alleles	NO (Freq)	Codominant model HR(95%CI)	P	Additive model HR(95%CI)	Dominant Model HR(95%CI)	Recessive model HR(95%CI)
TIMP2	rs4789936	AA	24(28.2%)	REF	0.872	0.924(0.604,1.415)	0.847(0.447,1.604)	0.978(0.468,2.043)
		AG	42(49.4%)	0.83(0.42, 1.65)				
		GG	19(22.4%)	0.88(0.38, 2.03)				
	rs6416835	CC	31(36.5%)	REF	0.542	0.993(0.648,1.521)	1.178(0.628,2.206)	0.702(0.276,1.785)
		TC	41(48.2%)	1.30(0.68, 2.50)				
		TT	13(15.3%)	0.82(0.30, 2.25)				
	rs7211674	AA	27(31.8%)	REF	0.344	1.345(0.883,2.047)	1.664(0.82,3.379)	1.329(0.652,2.706)
		AC	40(47.1%)	1.61(0.77, 3.38)				
		CC	18(21.2%)	1.81(0.75, 4.35)				
	rs7212662	AA	23(27.1%)	REF	0.349	1.055(0.704,1.579)	1.455(0.698,3.035)	0.81(0.399,1.647)
		AC	40(47.1%)	1.65(0.77, 3.55)				
		CC	22(25.9%)	1.13(0.46, 2.79)				
	rs8064344	TT	56(65.9%)	REF	0.243	1.231(0.7,2.167)	1.115(0.595,2.091)	3.224(0.761,13.657)
		TC	26(30.6%)	1.02(0.53, 1.96)				
		CC	3(3.5%)	3.24(0.75, 13.94)				
	rs8068674	CC	27(31.8%)	REF	0.882	1.092(0.711,1.678)	1.062(0.544,2.072)	1.199(0.589,2.44)
		TC	40(47.1%)	1.01(0.49, 2.05)				
		TT	18(21.2%)	1.20(0.52, 2.79)				
TIMP3	rs130274	CC	46(54.1%)	REF	*0.025*	1.961(1.191,3.228)	2.113(1.149,3.884)	2.632(0.785,8.82)
		TC	34(40.0%)	2.00(1.07, 3.75)				
		TT	5(5.9%)	3.68(1.04, 12.97)				
	rs135029	CC	40(47.6%)	REF	0.335	1.34(0.846,2.12)	1.291(0.694,2.402)	1.856(0.779,4.424)
		TC	36(42.9%)	1.16(0.60, 2.24)				
		TT	8(9.5%)	2.00(0.79, 5.08)				
	rs137485	AA	42(50.0%)	REF	0.423	1.329(0.817,2.161)	1.297(0.705,2.384)	1.851(0.657,5.218)
		AT	36(42.9%)	1.206(0.638, 2.281)				
		TT	6(7.1%)	2.026(0.685, 5.996)				

Gene	SNP ID	Alleles	NO (Freq)	Codominant model HR(95%CI)	P	Additive model HR(95%CI)	Dominant Model HR(95%CI)	Recessive model HR(95%CI)
TIMP3	rs137487	GG	24(28.2%)	REF	0.127	1.406(0.912,2.167)	2.247(0.999,5.053)	1.151(0.55,2.412)
		AG	44(51.8%)	2.088(0.775, 5.623)				
		AA	17(20.0%)	2.306(1.003, 5.300)				
	rs137489	AA	48(56.5%)	REF	0.152	0.839(0.503,1.398)	1.022(0.553,1.887)	0.044(0,8.164)
		AG	33(38.8%)	1.25(0.676, 2.32)				
		GG	4(4.7%)	0(0.00,)				
	rs1427378	AA	44(51.8%)		0.258	0.735(0.45,1.2)	0.818(0.449,1.492)	0.22(0.03,1.603)
		AG	35(41.2%)	0.22(0.03, 1.60)				
		GG	6(7.1%)	0.96(0.52, 1.76)				
	rs1962223	CC	57(67.1%)	REF	*0.012*	2.155(1.17,3.967)	2.155(1.17,3.967)	NA
		CG	28(32.9%)	2.16(1.17, 3.97)				
		GG	0(0%)					
	rs242072	TT	24(28.2%)	REF	0.869	0.898(0.598,1.35)	0.886(0.461,1.7)	0.837(0.412,1.7)
		TC	40(47.1%)	0.93(0.46, 1.88)				
		CC	21(24.7%)	0.80(0.35, 1.83)				
	rs242077	CC	29(34.5%)	REF	0.833	1.127(0.749,1.696)	1.212(0.64,2.296)	1.135(0.543,2.37)
		TC	40(47.6%)	1.19(0.60, 2.37)				
		TT	15(17.9%)	1.25(0.54, 2.90)				
	rs5754312	AA	22(25.9%)	REF	*0.036*	0.633(0.41,0.979)	0.773(0.395,1.514)	0.281(0.1,0.787)
		TA	44(51.8%)	1.03(0.52, 2.05)				
		TT	19(22.4%)	0.29(0.09, 0.89)				
	rs715572	CC	57(67.1%)	REF	*0.01*	1.709(1.092,2.674)	2.367(1.296,4.322)	1.095(0.264,4.537)
		TC	24(28.2%)	2.53(1.36, 4.71)				
		TT	4(4.7%)	1.49(0.35, 6.35)				
	rs738992	CC	20(23.5%)	REF	0.913	1.106(0.696,1.757)	1.134(0.543,2.369)	1.136(0.545,2.372)
		TC	50(58.8%)	1.11(0.52, 2.37)				
		TT	15(17.6%)	1.22(0.48, 3.09)				

Gene	SNP ID	Alleles	NO (Freq)	Codominant model HR(95%CI)	P	Additive model HR(95%CI)	Dominant Model HR(95%CI)	Recessive model HR(95%CI)
TIMP3	rs9606994	GG	26(30.6%)	REF	0.305	1.037(0.672,1.6)	1.414(0.696,2.873)	0.692(0.291,1.644)
		AG	45(52.9%)	1.59(0.77, 3.28)				
		AA	14(16.5%)	0.95(0.34, 2.62)				
	rs9619311	AA	43(50.6%)	REF	0.082	0.586(0.362,0.949)	0.578(0.313,1.066)	0.272(0.066,1.125)
		AG	31(36.5%)	0.71(0.38, 1.34)				
		GG	11(12.9%)	0.23(0.06, 0.99)				
TIMP4	rs308952	GG	62(74.7%)	REF	0.65	1.149(0.614,2.15)	1.219(0.631,2.356)	0.049(0,89202.191)
		AG	20(24.1%)	1.26(0.65, 2.44)				
		AA	1(1.2%)	0.00(0.00,E.)				
	rs3755724	GG	36(42.4%)	REF	0.383	1.077(0.672,1.727)	0.911(0.498,1.666)	1.705(0.717,4.054)
		AG	40(47.1%)	0.80(0.42, 1.53)				
		AA	9(10.6%)	1.52(0.61, 3.83)				

Table 6.3.. Hazard ratio (HR) and 95% confidence interval (CI) estimated for the association between *TIMP3* gene variations and survival of the study cohort adjusted for patient age, tumour location, disease stage and treatments.

SNP ID	Alleles	No. (Freq)	Codominant model HR(95%CI)p	Additive *model HR(95%CI)	*p*	Dominant model HR(95%CI)	*p*	Recessive model HR(95%CI)	*P*
rs130274	CC	42	REF	1.918(1.157, 3.179)	0.012	3.039(1.455, 6.348)	0.003	1.363(0.39, 4.766)	0.628
	TC	33	3.273(1.503, 7.126)						
	TT	5	3.68(1.04, 12.97)						
rs135029	CC	39	REF	1.464(0.883, 2.427)	0.14	1.359(0.706, 2.615)	0.359	2.555(0.932, 7.001)	0.068
	TC	33	1.163(0.578, 2.34)						
	TT	7	2.724(0.949, 7.82)						
rs137485	AA	41	REF	1.381(0.826, 2.309)	0.218	1.305(0.68, 2.505)	0.423	2.367(0.779, 7.187)	0.129
	AT	32	1.142(0.57, 2.29)						
	TT	6	2.478 (0.794, 7.738)						
rs137487	GG	22	REF	1.633(0.956, 2.79)	0.072	2.689(0.99, 7.303)	0.052	1.368(0.613, 3.055)	0.444
	AG	41	3.09(0.941, 10.145)						
	AA	17	2.608(0.949, 7.167)						
rs137489	AA	46	REF	0.875(0.485, 1.58)	0.658	1.022(0.517, 2.019)	0.951	0(0, .)	0.976
	AG	30	1.195(0.60, 2.38)						
	GG	4	0(0.00,)						
rs1427378	AA	42	REF	0.612(0.332, 1.131)	0.117	0.673(0.333, 1.364)	0.272	0.229(0.028, 1.867)	0.169
	AG	33	0.724(0.361, 1.45)						
	GG	5	0.179(0.0, 1.565)						
rs1962223	CC	53	REF	2.722(1.373, 5.398)	0.004	2.722(1.373, 5.398)	0.004	NA	NA
	CG	27	2.72(1.373, 5.4)						
	GG	0	-						
rs242072	TT	23	REF	0.977(0.615, 1.552)	0.922	1.032(0.48, 2.216)	0.936	0.903(0.404, 2.018)	0.804
	TC	38	0.948(0.37, 2.435)						
	CC	19	1.087(0.473, 2.5)						

SNP ID	Alleles	No. (Freq)	Codominant model HR(95%CI)p	Additive *model HR(95%CI)	*p*	Dominant model HR(95%CI)	*p*	Recessive model HR(95%CI)	*P*
rs242077	CC	28	REF	1.408(0.889, 2.228)	0.144	1.737(0.851, 3.546)	0.13	1.417(0.597, 3.366)	0.43
	TC	38	1.687(0.789, 3.608)						
	TT	13	1.874(0.708,4.963)						
rs5754312	AA	22	REF	0.577(0.349, 0.954)	0.032	0.677(0.332, 1.378)	0.282	0.251(0.078, 0.812)	0.021
	TA	40	0838(0.412, 1.706)						
	TT	18	0.222(0.062, 0.790)						
rs715572	CC	53	REF	2.238(1.328, 3.77)	0.002	2.79(1.455, 5.349)	0.002	2.086(0.477, 9.118)	0.329
	TC	23	2.752(1.415, 5.351)						
	TT	4	3.25(0.714, 14.785)						
rs738992	CC	18	REF	1.013(0.593, 1.728)	0.963	1.316(0.552, 3.136)	0.535	0.777(0.326, 1.853)	0.569
	TC	48	1.387(0.579, 3.322)						
	TT	14	1.016(0.326, 3.172)						
rs9606994	GG	24	REF	0.969(0.607, 1.547)	0.896	1.395(0.64, 3.042)	0.403	0.591(0.237, 1.471)	0.258
	AG	44	0.832(0.283, 2.452)						
	AA	12	1.666(0.744, 3.731)						
rs9619311	AA	41	REF	0.561(0.323, 0.977)	0.041	0.51(0.255, 1.019)	0.057	0.342(0.076, 1.536)	0.161
	AG	29	0.586(0.280, 1.223)						
	GG	10	0.290(0.064, 1.312)						

* The effect of the minor allele on the risk of disease is changed by a factor equal to the number of copies

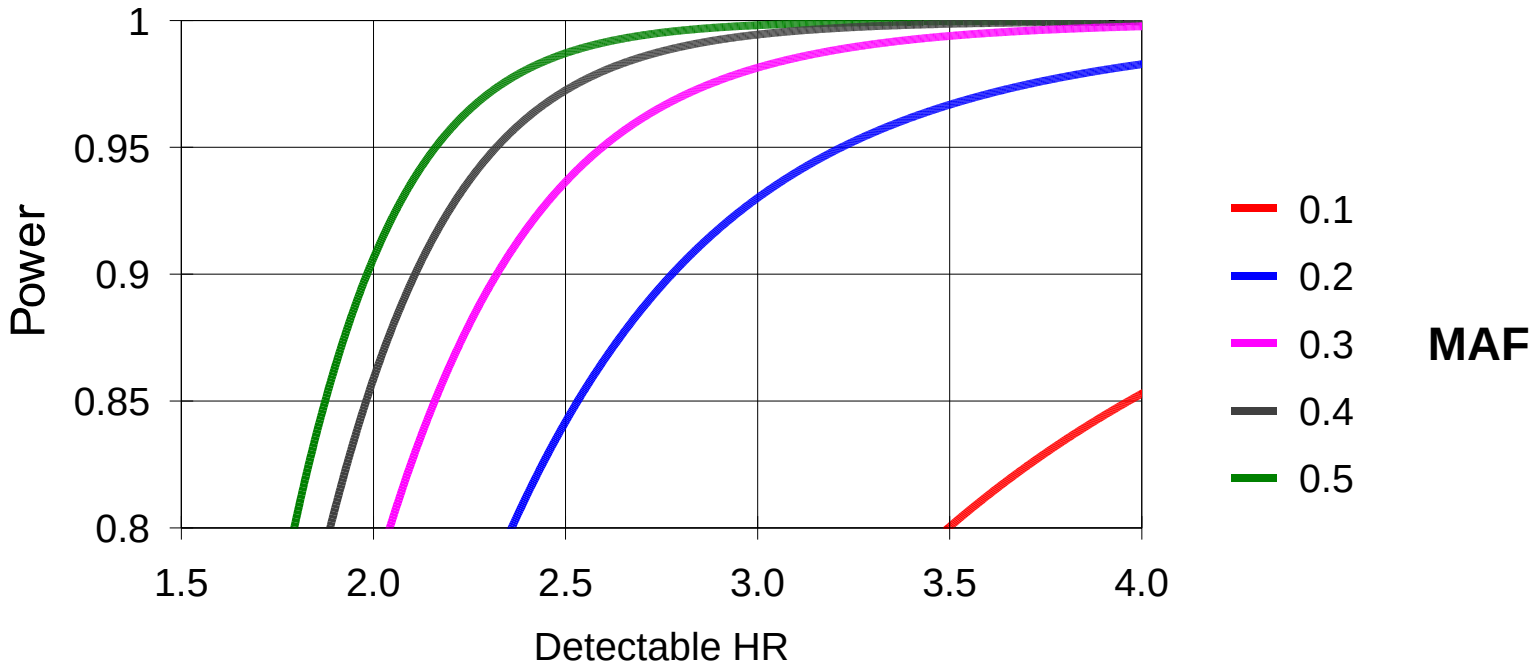


Figure 6.1 Minimum detectable Hazard Ratio by different MAF. Calculation is based on 85 patients, 80% power, Type I error probability =0.05.

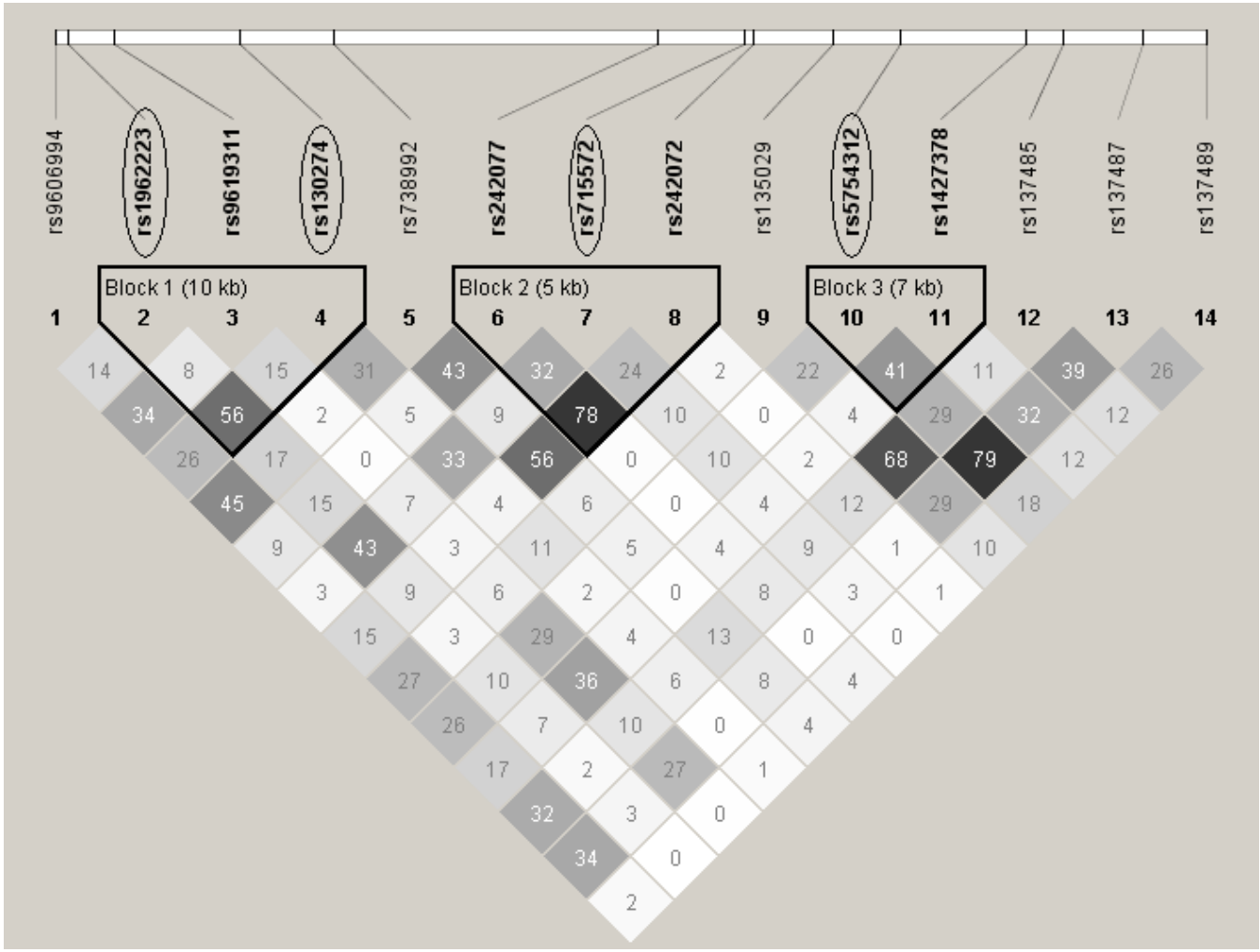


Figure 6.2. Linkage disequilibrium (LD) plot for *TIMP3*. Numbers in the plot are r^2 values; boxes with dark color illustrate high LD; lighter shading represent weak LD. Circled SNPs show significant association with survival in the study cohort.

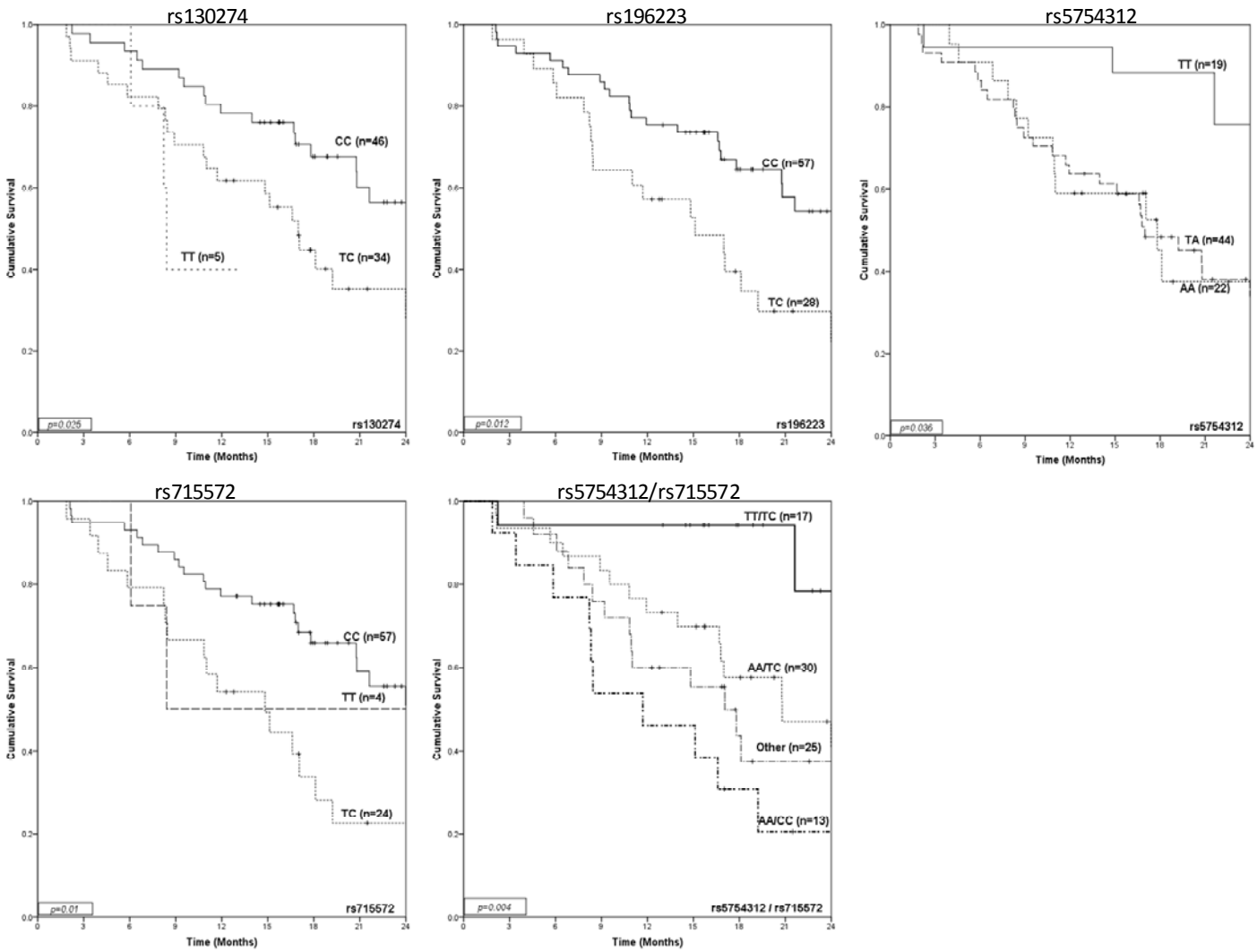


Figure 6.3. Survival of study cohort by *TIMP3* variations, rs130274, rs1962223, rs5754312, rs715572 and rs5754312/rs715572 haplotype

CHAPTER 7: Conclusions

Studies of human cancer susceptibility examine factors associated with the incidence of disease. Studies of human cancer prognosis and prediction examine factors associated with the outcomes of disease. This dissertation is about molecular and related factors that affect gastric and esophageal cancer patients' prognosis (i.e., survival).

Chapter 2 indicated that polymorphisms in genes associated with cell cycle, xenobiotic metabolism, DNA repair and signalling and growth factors have prognostic significance for both gastric and esophageal cancer. Understanding mechanisms of each polymorphism and pathway-based analyses might help identify markers for gastric and esophageal cancer patient survival. There is an increasing interest in the effect of host genetic polymorphisms on the survival of cancer patients. Generally, conventional techniques do not adequately predict the heterogeneity of patient outcomes. In many cancers, tumour markers have been used as a factor for survival models and guiding treatment decisions. However in gastric and esophageal cancer, adequate tumour samples for these assays may not be easily available. Modeling prognosis based on host factors, including genetic polymorphisms, is an emerging field of translational research.

Based on population-based registry data (chapter 3), it is concluded that gastric and esophageal cancers are relatively infrequent in Canada, although their epidemiology is changing. The trends are most evident when tumours are classified by histology and anatomic location. While incidence of squamous cell carcinoma of the esophagus and adenocarcinoma of the lower stomach appears to be stable or decreasing, the incidence of esophageal adenocarcinoma and gastric cardia cancer are increasing. Both gastric and esophageal cancer patients have poor survival.

In an international comparison of gastric and esophageal cancer (chapter 4), differences in survival between patients in BC, Canada and Ardabil, Iran have been found. Based on this study, it is concluded that disease characteristics and patient factors, not solely differences in healthcare systems, are likely responsible for the survival difference in these populations. Even so, the outcomes of these cancers are poor for both populations and improvements in diagnosis and management are urgently needed.

It has shown that for gastric and esophageal cancers, there are significant differences in survival among ethnic groups in BC (chapter 5). Ethnicity may represent underlying genetic factors. Such factors could influence host-tumour interactions by altering the tumour's etiology and therefore its chance of spreading. Alternatively, genetic factors may determine response to treatments. Finally, ethnicity may represent non-genetic factors that affect survival. Differences in survival by ethnicity support the importance of ethnicity as a prognostic factor, and may provide clues for the future identification of genetic or lifestyle factors that underlie these observations.

Based on a prospective cohort study, it demonstrated that genetic polymorphism in the promoter region and three intronic TagSNPs of *TIMP3* predict survival of patients with adenocarcinoma of esophagus and GEJ. Regardless of the mechanisms, factors that affect regulation of *TIMP3* expression, including promoter methylation or genetic variation, could be a promising prognostic factor or therapeutic target for this cancer. Modeling prognosis based on host factors including genetic polymorphisms is an emerging field of translational research. Compared to tumour, the constitutional genetic material is easy to obtain, and can be assessed before treatment is started.

7.1 Opportunities and challenges

In BC, population-based registry data with outcome information are available and make possible studies about prognostic factors at the population level. Additionally, all BC residents are covered for healthcare through the BC Medical Services Plan (MSP) and the BC Cancer Agency (BCCA) provides care for all cancer patients in the province (using province-wide treatment guidelines and protocols). The biggest challenge with BC registry data was the lack of ethnicity information. This required us to use a proxy method (i.e., name lists) to assign ethnicity. With the collaboration of Iranian scientists, access to population-based registry data for Ardabil, Iran was made posible. Ardabil has the highest incidence of gastric and esophageal cancer in the world. There are some meaningful challenges in registry information and health care in Ardabil; including vast immigration, an uncoordinated system of medical services and patients' repeated referral to different centers for diagnosis and treatment. Additionally, lack of complete information did not allow us to adjust our results for staging or follow-up, giving only a very limited view of prognosis.

The biggest challenge in our prospective study was the lack of operational funding thereby causing difficulties in recruitment and genotyping. Despite this, the support of GI tumour group physician, made it possible to recruit patients and collect biological samples with minimal cost. On the other hand, improvements in technology made it possible to genotype our samples at a substantially lower cost.

7.2 Future directions

The dissertation leaves some unanswered questions that would benefit from subsequent research. First, there is a need for prospective studies with longer recruitment period. The prospective GE study successfully set up a patient recruitment system. Continuation of patient ascertainment

would produce a valuable cohort of patient to allow detection of more prognostic markers. In addition, genetic results from this dissertation should be validated in other, national and international, gastric and esophageal patient cohorts. It would be useful to study of TIMP3 expression in tumour to understand the correlation between *TIMP3* polymorphism and gene expression (using a tissue microarray (TMA)) on survival of GEJ adenocarcinoma. One potential study would be the comparison of *TIMP3* polymorphism and expression between BC and Iran. Currently, Iranian collaborators are collecting DNA and tumour specimens from cardia and esophageal cancers. Comparing specimens from the BC and Iranian populations could produce valuable information about these cancers. Finally, there is a need to study factors underlying the effect of ethnicity on survival of gastric and esophageal cancer. This requires studies of both genetic and environment, including quantitative studies on lifestyle and behavior to address the complex concept of ethnicity.

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