Thesis for doctoral degree (Ph.D.) 2007

Risk Indicators for Esophageal Cancer

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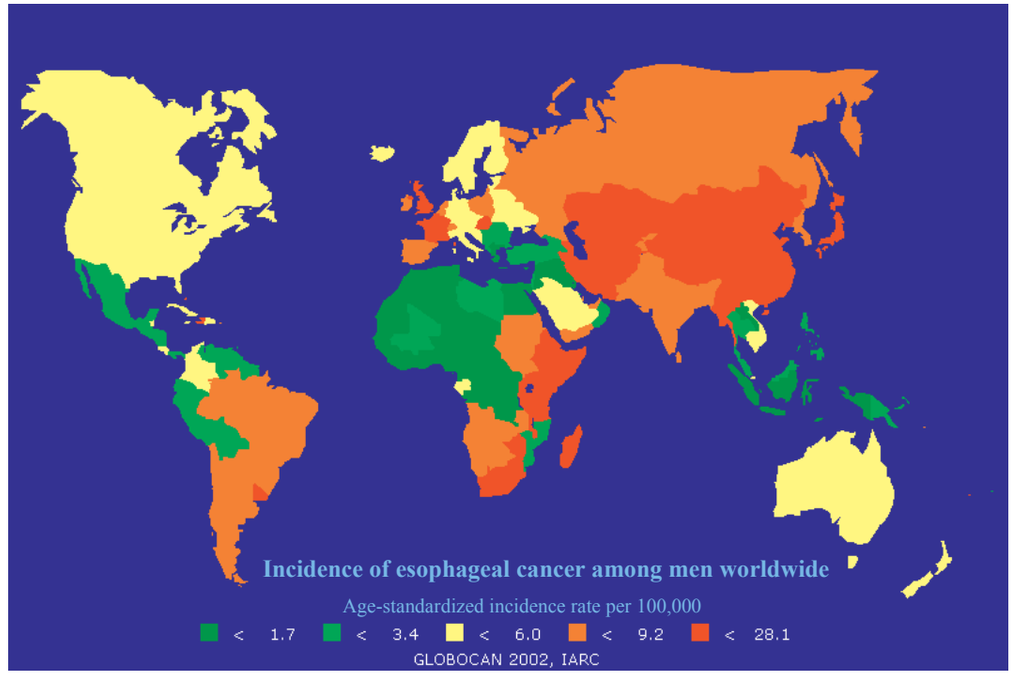

Kazem Zendehdel

Karolinska Institutet

From the Department of Medical Epidemiology and Biostatistics,
Karolinska Institutet, Stockholm, Sweden

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بسم الله الرّحمن الرّحيم

In the name of God, the beneficent, the merciful

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Summary

Because of little progress in the prognosis and survival of esophageal cancer patients, the early diagnosis and prevention have been prioritized. Alcohol consumption and tobacco smoking are the main risk factors of squamous cell carcinoma, and high body mass index and gastroesophageal reflux are strongly linked to adenocarcinoma of the esophagus. However underlying mechanisms for the observed associations between these risk factors and esophageal cancer are not fully understood. This thesis was aimed to shed further light on the etiology of this cancer through a series of epidemiological studies.

An inverse relation between *H. pylori* infection and the risk of esophageal adenocarcinoma, and a positive link with the risk of squamous cell carcinoma is suggested. We identified retrospective cohorts of patients hospitalized for gastric and duodenal ulcers – both strongly linked to *H. pylori* infection – between 1965 and 2003 through the Swedish Inpatient Register. We found a 70% excess risk of esophageal adenocarcinoma in duodenal ulcer patients (SIR=1.7 95% CI 1.1-2.5) compared to the general Swedish population. This finding was plausible because duodenal ulcer is associated with hyperacidity and gastroesophageal reflux, a strong risk factor for esophageal adenocarcinoma. However, it was not consistent with the reported inverse relationship between *H. pylori* and adenocarcinoma of the esophagus. On the other hand, gastric ulcer patients exhibited 80% higher risk of squamous cell carcinoma (SIR=1.8 95% CI 1.4-2.3), supporting the postulated hypothesis in which bacterial overgrowth in an atrophic stomach may lead to the generation of N-nitroso compounds, a suspected risk factor for esophageal squamous cell carcinoma.

In a large cohort study among achalasia patients, we found a strong association between achalasia and risk of esophageal cancer (SIR=10.5 95% CI 7.0-15.9). The excess risk was evident for both adenocarcinoma and squamous cell carcinoma, particularly among men. We also found that the risk of esophageal cancer was high among both operated and unoperated achalasia patients. However, there was some indication that the risk of squamous cell carcinoma may decrease among patients undergoing esophagogastric myotomy. This study showed that achalasia surgery does not increase the risk of esophageal adenocarcinoma.

Scandinavian moist snuff (snus) is increasing in Sweden. There are strong forces from tobacco lobbies to encourage snus use as a safer alternative to smoking and to lift the ban put on snus use in most Euopean countries. Using information from 336,381 male Swedish construction workers, we studied the associations between snus use and tobacco smoking and the risk of esophageal cancer. In an analysis among smokers, we found no convincing evidence to support that additional snus use among smokers may decrease the risk of esophageal adenocarcinoma and squamous cell carcinoma compared to those who were only smoking. Moreover, the risk of esophageal squamous cell carcinoma was 3.5-fold higher among never-smoking snus users compared to never-users of any tobacco (95% CI 1.6-7.6). The latter analysis was restricted to never smokers to discard the confounding by smoking appropriately. We therefore concluded that snus cannot be considered an entirely safe alternative to smoking and should not be marketed as a means for harm reduction until strong evidence is able to refute its carcinogenicity. Strong associations between tobacco smoking and the risks of esophageal adenocarcinoma (RR=2.3 95%CI 1.4-3.7) and squamous cell carcinoma (RR=5.2, 95% CI 3.1-8.6) were also noted.

Finally, in a population based case-control study we studied the association between polymorphisms of some tobacco-metabolizing genes (*GSTP, GSTT1* and *GSTM1*) and the risk of esophageal cancer. Although there were no associations between these polymorphisms and the risk of adenocarcinoma, the variant *GSTP1* Val^{105} was associated with an increased risk of squamous cell carcinoma (OR=1.7, 95% CI 1.0-2.9). The association tended to be stronger among smokers and homozygotes with the variant allele. Together with the combined literature, we concluded that carriage of the variant *GSTP1* Val^{105} allele may be associated with the risk of both histological types of esophageal cancer among Caucasian populations.

خلاصه فارسی

بالاترین میزان بروز سرطان مری در دنیا در منطقه "کمربند سرطان مری" قرار دارد که از گنبدکاووس در شمال شرقی ایران شروع شده و تا شمال چین امتداد دارد. نوع سلول سنگفرشی (squamous cell carcinoma) عمده ترین نوع سرطان مری در این مناطق می‌باشد. با افزایش نوع دیگر سرطان مری از نوع سلول غددی (adenocarcinoma) در کشورهای غربی اپیدمی جدید دیگری در مورد سرطان مری در سالهای اخیر مطرح شده است. در بعضی از کشورها بروز نوع سلول غددی حتی از نوع سلول سنگفرشی نیز بیشتر شده است. علیرغم پیشرفتهای بسیار زیاد در تشخیص و درمان سرطان مری، متاسفانه بهبود رضایت بخشی در طول عمر و پیش آگهی سرطان مری ایجاد نشده است. لذا محققین برآنند تا از طریق مطالعات اپیدمیولوژیک و تعیین عوامل خطرزا و در نتیجه گروههای پرخطر، اقدام به غربالگری و پیش گیری این سرطان نمایند. در حالیکه مصرف سیگار و الکل عمده ترین عوامل اصلی خطرزا برای نوع سلول سنگفرشی می‌باشند، چاقی و بازگشت محتویات معده به مری (ریفلاکس) مهمترین علل شناخته شده برای نوع سلول غددی سرطان مری هستند. با این حال مکانیسم سرطانزایی این عوامل به خوبی معلوم نیست. در این پایان نامه با انجام یک سری مطالعات اپیدمیولوژیک علل سرطان مری مورد بررسی قرار گرفته است.

زخم‌های معده و دوازدهه (اثنی‌عشر) ارتباط بسیار زیادی با عفونت میکروبی *هلیکوباکترپیلوری* دارند که براساس مطالعات انجام شده این عفونت میکروبی خطر بروز نوع سلول غددی سرطان مری را کاهش ولی خطر نوع سلول سنگفرشی سرطان مری را افزایش می‌دهد. در مطالعه اول، پیگیری ۶۱,۵۴۸ بیمار بستری شده به علت زخم دوازدهه و ۸۱,۳۷۹ نفر کوهورت بیماران بستری شده به علت زخم معده نشان داد که خطر بروز نوع سلول غددی سرطان مری در زخم دوازدهه ۷۰٪ افزایش می‌یابد، در حالیکه ارتباطی بین زخم معده و این سرطان مشاهده نشد. با توجه به افزایش ترشح اسید معده در زخم دوازدهه، احتمال ایجاد ریفلاکس دراین بیماران بسیار بالاست. و از آنجا که ریفلاکس جزو عوامل اصلی خطرزا برای نوع غددی سرطان مری است، این یافته از نظر علمی قابل توجیه می‌باشد. از طرف بروز سرطان مری نوع سلول سنگفرشی در بیماران مبتلا به زخم معده ۸۰٪ بیشتر از افراد معمول جامعه بود. این یافته نیز فرضیه ای را تقویت می‌کند که بر اساس آن کاهش ترشح اسید معده در بیماران زخم معده ای محیط مناسبی را برای رشد انواع میکروبها فراهم می‌کند که احتمالاً منجر به تولید مواد سرطانزایی (ترکیبات نیتروزآمین) می‌شوند که بروز نوع سلول سنگفرشی سرطان مری را افزایش می‌دهد.

در مطالعه دوم، پیگیری ۲,۸۹۶ بیمار مبتلا به بیماری آشالازی که طی سالهای ۱۹۶۵ الی ۲۰۰۴ میلادی در بیمارستانهای سوئد بستری شده بودند نشان داد که خطر بروز سرطان مری در این بیماران بیش از ده برابر افراد عادی می‌باشد. جراحی (میوتومی تحتانی) که به عنوان موثر ترین روش درمانی برای این ارائه می‌شود ممکن است باعث ریفلاکس شده و خطر سرطان مری نوع غددی را افزایش دهد. با مطالعه تاثیر عمل جراحی روی خطرسرطان مری، مشخص شد که جراحی نه تنها خطر سرطان مری را در بیماران آشالازی افزایش نمی‌دهد، بلکه به علت رفع فشار از اسفنکتر ممکن است در دراز مدت خطر سرطان مری نوع سلول سنگفرشی را نیز کاهش دهد.

مصرف نوعی از تنباکو که ''اسنوس'' یا ''اسناف'' نام دارد و بصورت مستقیم در زیر لب قرار می‌گیرد در کشور سوئد در حال افزایش می‌باشد. همچنین کمپانی های تنباکو در تلاشند تا اسنوس را به عنوان روش کم خطرتر از سیگار تبلیغ نمایند و با تلاش فراوان در اتحادیه اروپا سعی دارند که ممنوعیت مصرف آن را در کشورهای اروپایی حذف نمایند که در این صورت بی‌تردید در آینده مصرف آن در سایر کشورها نیز فراگیر خواهد شد. این در حالیست که هنوز مطالعات کافی در مورد خطرات آن انجام نشده است. در مطالعه سوم این پایان نامه، با استفاده از اطلاعات ۳۳۶,۳۸۱ نفر کوهورت کارگران ساختمانی در سوئد، مشخص شد که خطر سرطان نوع سلول سنگفرشی در کسانیکه فقط اسنوس مصرف می‌کردند سه و نیم برابر کسانی بود که سیگار و یا هیچ نوع تنباکوی دیگری مصرف نمی‌کردند. بنابراین نتیجه این مطالعه نشان داد که نمی‌توان اسنوس را کاملاً بی خطر تلقی نموده و به عنوان یک ماده کم خطر و یا بی خطر به مردم تجویز نمود. از دیگر یافته های این مطالعه این بود که مصرف سیگار خطر نوع سلول سنگفرشی سرطان مری را به میزان بیش از پنج برابر و نوع غددی را بیش از دو برابر افزایش می‌دهد.

در مطالعه آخر، ارتباط سرطان مری با پلی‌مورفیسم ژنهای مربوط به متابولیسم تنباکو و سیگار شامل *GSTP1*، *GSTT1* و*GSTM1* بررسی گردید. بر اساس این بررسی پلی‌مورفیسم ژن *GSTP1* با نوع سلول سنگفرشی سرطان مری ارتباط داشت. البته این ارتباط در میان مصرف کنندگان سیگار بیشتر بود. با اینکه در این مطالعه خطر بروز ابتلا به نوع سلول غددی با این ژنها ارتباطی نداشت، بررسی تمامی مطالعات انجام شده تا کنون به روش متا آنالیز نشان داد که احتمالا پلی‌مورفیسم ژن *GSTP1* با هر دو نوع سرطان مری در نژاد اروپایی ارتباط دارد.

List of Publications

I. **Bahmanyar S., Zendehdel K., Nyrén O., Ye W.** Risk of esophageal cancer by histology among patients hospitalized for gastroduodenal ulcers. ***Gut 2007; 56: 464-8.***

II. **Zendehdel K., Nyrén O., Edberg A., Ye W.** Risk of esophageal adenocarcinoma in achalasia patients, a retrospective cohort study in Sweden.
***Am J Gastroenterol 2007; in press*.**

III. **Zendehdel K., Nyrén O., Luo J., Dickman P. , Boffetta P., Englund A., Ye W.** Risk of gastroesophageal cancer among smokers and users of Scandinavian moist snuff (snus).
Submitted.

IV. **Zendehdel K., Bahmanyar S., McCarthy S., Nyrén O., Andersson B., Ye W.** Genetic polymorphisms of glutathione S-transferase genes, *GSTP1*, *GSTM1*, and *GSTT1* and risk of esophageal and gastric cardia cancers.
Submitted.

Contents

List of abbreviations

EAC	Esophageal Adenocarcinoma
ASR	Age Standardized Incidence Rate (per 100,000 World Population)
BMI	Body Mass Index [weight (kg) divided by height (m^2)]
CI	Confidence Interval
ESCC	Esophageal Squamous Cell Carcinoma
GST	Glutathione S-Transferase
H. pylori	*Helicobacter pylori*
ICD	International Classification of Disease
IR	Incidence Rate
LES	Lower Esophageal Sphincter
NRN	National Registration Number
OR	Odds Ratio
PAR	Population Attributable Risk Percent
RR	Relative Risk
SECC	Swedish Esophageal and Cardia Cancer Study
SIR	Standardized Incidence Ratio

1 Introduction

Esophageal cancer is the 8th most common malignancy and 6th most common cause of cancer death worldwide. It is one of the most deadly cancers with overall 5-year survival less than 16% in US and 10% in Europe. An infamous “esophageal cancer belt” stretching from the northeastern part of Iran to northern China has presented extremely high incidence of esophageal cancer, mainly squamous cell carcinoma (ESCC) type. South America, southern and eastern Africa are other high risk areas for esophageal cancer.

While extensive research has been carried out to understand the etiology of this lethal cancer and explain such surprising geographical differences, a new enigma in epidemiology of esophageal cancer has emerged, as the incidence of another histological type, esophageal adenocarcinoma (EAC), has increased in Western countries since the 1970s. A cancer with previously low incidence has increased during the last three decades and now surpasses ESCC incidence in some Western populations.

In spite of considerable advances in the diagnostic methods and treatment of esophageal cancer, little improvement has been achieved in the prognosis and survival of these patients. Moreover, because of the wide geographical differences and substantial changes in the incidence of esophageal cancer over time, it has been suggested that the environmental risk factors play a major role in the etiology of esophageal cancer. Therefore, epidemiological studies prioritize identifying the risk factors and high risk groups, as necessary steps to accomplish effective screening and prevention programs. While alcohol and smoking are the main risk factors for squamous cell carcinoma, high body mass index and gastroesophageal reflux is strongly linked to adenocarcinoma of the esophagus. However, underlying biological mechanisms are not fully explained. This thesis was aimed to study the risk indicators for esophageal cancer in order to shed further light on etiology of this deadly disease.

2 Background

2.1 Descriptive Epidemiology

Esophageal cancer is the 8th most common malignancy, and 6th most common cause of cancer death worldwide (1). It is a rapidly fatal disease in the great majority of cases, so that the mortality and incidence rates are comparable. A wide geographic variation in the risk of esophageal cancer exhibits 20-fold higher age-standardized incidence rate (ASR) in southern Africa and China compared to the low risk southern Europe (Figure 1). There is an infamous "Asian esophageal cancer belt" which stretches to the east from the northeastern part of Iran to Henan province in north-central China passing through Turkmenistan, Uzbekistan and Kazakhstan. The highest incidence rates in the world are reported from the Gonbad region in northeastern Iran (206 and 262 per 100,000 person-years in men and women, respectively), Cixian (209 and 120 per 100,000 person-years in men and women, respectively) and Linxian (138 and 99 per 100,000 person-years in men and women, respectively) counties in China. Other high incidence areas are found in parts of South America and in Southern and Eastern Africa. There are striking local variations even within these geographical regions. A considerable variation exists also in Europe; it varies from the ASR of 3.1 and 1.0 per 100,000 person-years in Swedish men and women, respectively, to 22.3 (men) and 1.1 (women) per 100,000 person-years in Calvados, France (2). In spite of numerous investigations, the reasons for such a wide geographic variation are not explained. Migrant studies have shown that the risk decreases when high risk population relocates in low-risk areas, indicating the importance of local environment in the etiology (3).

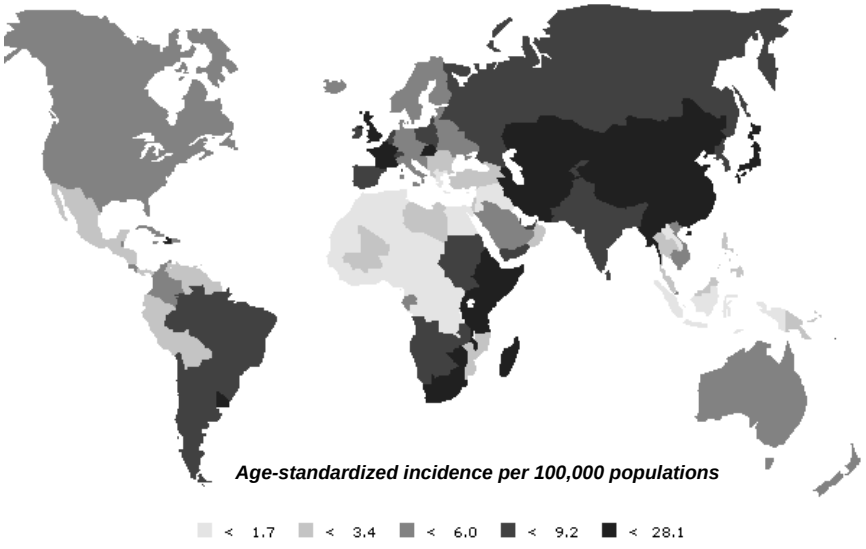

Figure 1. The global burden of esophageal cancer among men. Northern Iran, the Central Asian republic, North-Central China, parts of South America, and Southern and Eastern Africa are the high risk areas (source: Globocan 2002, IARC)

The dominating histological type of esophageal cancer in the high risk regions and many other areas in the world is esophageal squamous cell carcinoma (ESCC), accounting for over 90% of the cases in most populations. Although the incidence of ESCC has been almost stable over time, a new epidemic has emerged recently, as many Western societies reported an increasing trend in the incidence of esophageal adenocarcinoma (EAC). The incidence of EAC has increased rapidly since the 1970s in the United States and several other developed countries, particularly in North America, Europe, Australia and New Zealand (4-9). The percentage of increase per year varied from 2.3% (Sweden) to 8.6% (US whites). However, EAC is still a rare cancer and ASR below 5 per 100,000 was reported in most countries. The highest incidence rates were demonstrated in Scotland (about 10 per 100,000 person-years) and England and Wales (7 per 100,000 person-years) (2). The incidence of EAC has surpassed ESCC and has become the most common esophageal cancer type among US white men (Figure 2) as well as among Australian, Scottish, and Swedish men.

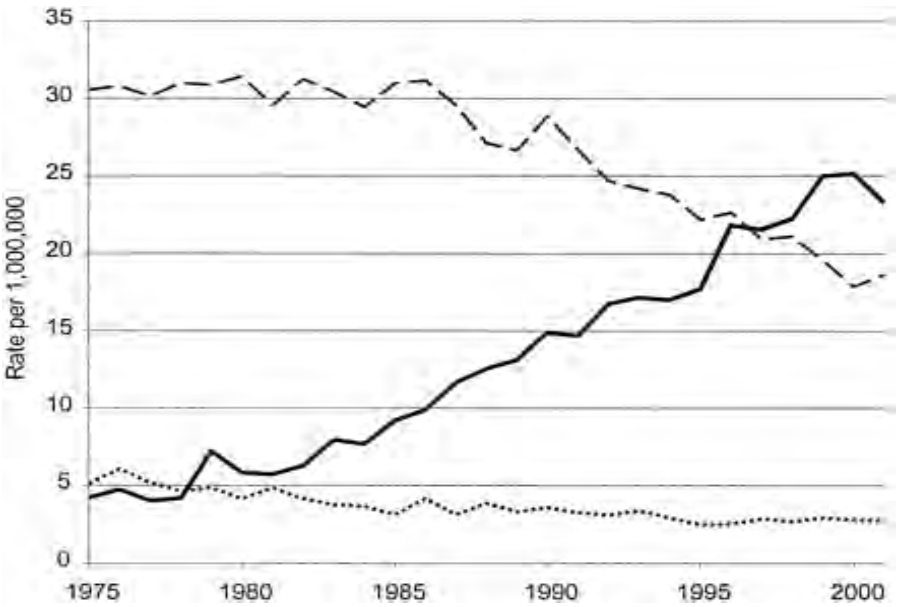

Figure 2. Histology and esophageal cancer incidence (1975–2001). Data from the National Cancer Institute's Surveillance, Epidemiology, and End Results program with age-adjustment using the 2000 U.S. standard population. **Solid black line** = adenocarcinoma; **dashed line** = squamous cell carcinoma; **dotted line** = not otherwise specified. *Reprinted from Pohl H et al., The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence, J Nat Cancer Inst 2005;97(2):142-146, by permission of Oxford University Press.*

2.2 Risk Factors

Esophageal squamous cell carcinoma (ESCC)
Tobacco smoking is strongly and dose-dependently associated with the risk of ESCC. A more thorough summary about the risk of tobacco use is provided in section 2.3.3. Alcohol consumption is another strong risk factor for ESCC with an established dose-risk linear trend. Although alcohol use is an independent risk factor of ESCC, the combined effect of tobacco and alcohol use seems to increase the risk more than multiplicatively in most population. The risk among the heaviest users of both alcohol and tobacco is typically 20- to 50-fold higher than among non-users of both (2). In Western Europe and North America, over 90% of the ESCC risk can be attributed to alcohol and tobacco use (1).

The relation between dietary factors and ESCC have come into focus because smoking, alcohol use and also genetic factors are not strongly linked to esophageal cancer in the Asian high risk area, where ESCC is the dominating histological type and low socioeconomic status, lack of variation in diet, low consumption of fruits and vegetables have been typical attributes. Overall, associations of food groups other than fruits and vegetables with ESCC are inconsistent. Consumption of poly- or monounsaturated fatty acids has been repeatedly found to be a protective factor for ESCC (10). The local excess in the butter consumption in the high incidence northwestern France, has suggested the butter consumption as possible risk factor for esophageal cancer (11), even though, in South Africa, butter and margarine emerged as protective factors (12). A strong association between high intake of pickled vegetables, which contain a high concentration of N-nitroso compounds, and the risk of ESCC is suggested (13). However, several other Chinese studies could not verify this finding. In a systematic review, the author found no convincing evidence on the relationship between the risk of esophageal cancer and exposure to nitrosamine, estimated from external sources (processed meat, beer, pickled and dried vegetables, smoked fish, or meat as well as salted or dried fish or meat) or endogenous nitrosamine formation based on the intake of haem-containing red meat. But the majority of the reviewed studies showed a point estimate that supported nitrosamine hypothesis, particularly for processed meat (14). Cereal, fiber intake, and green tea were inversely associated with risk of ESCC (2).

Infection with human papillomavirus (HPV), especially HPV type 16, has been suggested as a risk factor for ESCC, although published data are inconsistent (2). An association between *Helicobacter pylori (H. pylori)* infection and gastric atrophy with ESCC is suggested (see section 2.3.1). Some medical conditions have been suggested to be in causal pathways for esophageal cancer, e.g. Plummer-Vinson, celiac disease, achalasia, scleroderma, and tylosis (15).

Occupational exposures have attracted the attention of researchers. Individuals working with vulcanization in the rubber or automobile industry were at substantially higher risk of ESCC. Moreover, an excess risk of ESCC was reported among chimney sweeps, mine workers, chemical product workers, medical x-ray workers, as well as among workers in plastic and composites industry, dye production industry, and bookbindery (2). Positive associations with butchers (16) and workers in cement industry (17) were also reported. Specific workplace exposures linked to ESCC include metal dust, asbestos, silica dust, combustion products, organic solvents, and polycyclic aromatic hydrocarbons (PAHs) (2) .

Male sex has been a risk factor in almost all study populations. However, in spite of the male predominance of esophageal cancer, neither EAC nor ESCC was perceived as hormone-dependent cancers. Only two small studies evaluated the possible effect of parity on risk of ESCC, but found no association (18, 19).

Esophageal Adenocarcinoma (EAC)

Due to the rarity of EAC, only a few studies have studied the etiology of this cancer, mostly published from Western populations. Gastroesophageal reflux and high body mass index (BMI) are the main risk factors for EAC. Although reflux and high BMI could be in the same causal pathway, an independent relationship between EAC and high BMI after adjustment for gastroesophageal reflux has been confirmed in numerous studies (2). Concerning the increasing prevalence of high BMI in the Western countries along with the epidemic increase in the incidence of EAC, this BMI-EAC link sounds plausible. Gastroesophageal reflux symptom was found to be the strongest independent risk factor for EAC. Those with the most severe and longstanding symptoms were at more than 40-fold higher risk in comparison with individuals without any reflux symptoms (20). Barrett's esophagus, a columnar cell metaplasia that replaces the normal squamous cell epithelium of the distal esophagus, has been strongly associated with EAC risk. It arises most commonly in the setting of chronic gastroesophageal reflux and because of the strong link to EAC (50- to 100-fold increased risk), Barrett's esophagus is labeled as a precancerous lesion instead of a risk factor (21).

Alcohol and tobacco use are less strongly associated with the risk of EAC than with the ESCC risk. Published studies suggest a relative risk around 2.0 for tobacco smoking and almost no association with alcohol consumption. The patterns of association between dietary factors and EAC are almost similar to that observed in ESCC; a clear inverse relationship between a high consumption of fruits and vegetables and EAC risk was reported (2). In a US study, consumption of the saturated fat appeared to be risk factor for EAC, which was reported to be a risk factor also for ESCC (13). However, in a US case-control study that included both histological type of esophageal cancer, the intake of dietary fat was a risk factor for EAC, but not for ESCC (22). A positive association between the EAC risk and total meat intake, processed meat, and poultry intake was observed in the EPIC study (23).

A 6-fold or higher incidence of EAC among men compared to women has been repeatedly reported in almost all studied populations (2). Although it has been hypothesized that estrogens may protect against this cancer (24), based on the available data, neither exposure to exogenous estrogens nor reproductive factors are associated with EAC risk (24-27). Breast feeding was associated with a 60% reduction in EAC risk among Scottish women (26). Notwithstanding an enigmatic higher risk of EAC among men compared to women, the prevalence of high BMI and gastroesophageal reflux as well as Barrett's esophagus is almost similar among both genders (28, 29), or even more common among females (30).

2.3 Risk Factors Studied in This Thesis

2.3.1 *Helicobacter pylori* (*H. pylori*) Infection

Robin Warren and Barry Marshall were awarded the Nobel Prize of 2005 because of their discovery of *H. pylori*, an important achievement in Medicine, which revolutionized the treatment of peptic ulcer. *H. pylori*, designated as a class I human carcinogen by the International Agency for Research on Cancer (IARC) (31), has a central role in the etiology of peptic ulcer disease. Ninety percent of patients with duodenal ulcer and 70-90% of those with gastric ulcer harbor *H. pylori* in their stomachs (32). Duodenal ulcer is associated with colonization of *H pylori* and gastritis in the antrum. A higher release of gastrin in these patients leads to an increased acid secretion by stimulating the healthy body mucosa. Gastric ulcer is, on the other hand, linked to infection of acid-secreting body mucosa (pangastritis or a body-predominant gastritis) which develops into gastric atrophy and hypochlorhydria through disappearance of the parietal cells (Figure 3) (33).

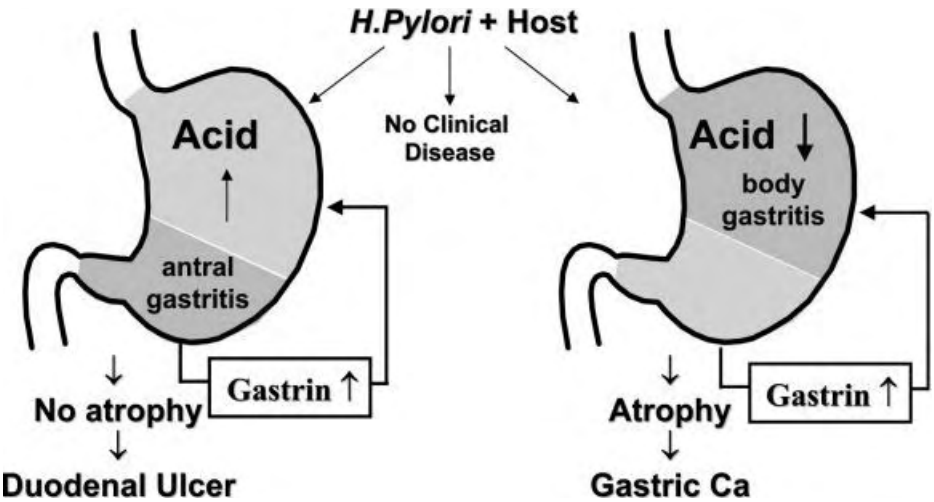

Figure 3. The relationship between the pattern of gastritis induced by *H pylori* infection and subsequent gastroduodenal disease. *Reprinted from Gillen D., McColl KE Gastroduodenal Disease, Helicobacter pylori, and Genetic Polymorphisms. Clin Gastroenterol Hepatol. 2005;3(12):1180-6.by permission of Elsevier*

Several epidemiologic studies have shown a positive association between *H. pylori* infection and risk of stomach cancer (34). Moreover, *H. pylori* infection has also been involved in diseases outside the stomach including EAC and ESCC. *H. pylori* infection could induce atrophic gastritis, leading to bacterial overgrowth which, in turn, may increase intragastric nitrosamine production which is known to be a risk factor for ESCC (35). This hypothesis was supported by a Swedish population based case-control study (36).

Based on an intriguing secular concurrence of the rise in EAC incidence and an obvious decrease in *H. pylori* infection prevalence (and fall in *H. pylori*-related diseases such as duodenal ulcer and gastric ulcer), it has been proposed that these two trends are causally related (37). Moreover, there is strong epidemiologic evidence that *H. pylori* infection is associated with a reduced risk of EAC (36, 38-41). The protective effect of *H. pylori* was hypothesized to be associated with gastric atrophy and hypochlorhydria among these patients leading to a lower acid reflux, the strong risk factor for EAC (42). However, the inverse association between *H. pylori* infection and risk of EAC, was seemingly independent of presence or absence of significant gastric atrophy (36).

2.3.2 Achalasia

Achalasia is characterized by aperistalsis and failure of the lower esophageal sphincter (LES) to relax on swallowing. Histologically, it has long been recognized that the clinical syndrome of achalasia occurs with a loss of ganglion cells in the intermyenteric (Auerbach's) plexus. Onset and progression of symptoms from achalasia are insidious, often dating back many years from the time of presentation. In addition, clinical symptoms are unreliable in the diagnosis, being poorly correlated with severity of disease. Therefore, the diagnosis depends on radiographic, histologic, and manometric findings in combination with clinical signs and symptoms. In these patients, barium swallow demonstrates a smooth tapering stenosis of the distal esophagus (bird's beak narrowing) with a variable degree of proximal dilatation (Figure 4.) Consequently, the esophagus may become dilated and filled with food debris and fluid (43).

The relation between achalasia and esophageal cancer was first reported by Fagge in 1872 (44). An association between achalasia and esophageal cancer has been reported repeatedly since then (43, 45-48). However, most of the evidence comes from case reports and small series with small sample size or short follow-up. The development of esophageal cancer in achalasia patients is probably not a direct consequence of neuronal deterioration, and the longstanding mucosal exposure to noxious substances is a more likely explanation. The food retention, increased bacterial growth, and esophagitis increase sensitivity to the carcinogens in achalasia. Several epithelial abnormalities, e.g. lymphatic esophagitis, diffuse squamous hyperplasia, and high grade dysplasia, have been noted repeatedly in achalasia patients (49). Moreover, evaluation of esophagectomy specimens from patients with end-stage achalasia has demonstrated that squamous hyperplasia, increased numbers of CD3+ cells, and p53 immunoreactivity – some of the molecular steps behind the mutational sequence of normal mucosal to ESCC – are common also among the achalasia patients (50, 51). While the link between achalasia and ESCC seems well established, the basis for risk elevation vis-à-vis Barrett's esophagus and EAC rests only on case reports and small case series (52), and a causal relationship has been doubted (47).

Because the core functional aberration in achalasia is a spasm of LES, gastroesophageal reflux – the main risk factor for Barrett's esophagus and EAC – is expected to be rare. However, heartburn and acid reflux among these patients is fairly common (43, 53, 54) and there is a variation in the pressure of the lower esophageal sphincter among achalasia patients; patients with heartburn symptoms had a lower pressure in comparison to those without the reflux symptoms (54).

Based on the proposed mechanisms of cancer development in a dilated esophagus, many have suggested preventive measure in achalasia patients through early treatment of stasis by pneumatic dilation or surgery (55). Although commonly used procedures like chemical paralysis of LES with botulinum toxin injection and dilation with an inflatable balloon offer good short-term relief, surgical myotomy of the gastroesophageal sphincter is needed for long-term results (47). Whether surgery results in any protection against esophageal cancer remains debatable (43, 47, 48, 52, 56, 57). On the other hand, it has been a matter of discussion whether iatrogenic reflux after the surgery increases the risk of Barrett's esophagus and EAC (52). However, because of the rarity of both achalasia and EAC no epidemiological study has evaluated this question.

The problem in detecting esophageal cancer in patients with achalasia is that the dilated esophagus compensates readily for the partial obstruction by esophageal cancer. Moreover, symptoms of esophageal cancer – dysphagia and weight loss – are associated with the achalasia and these patients are adapted to such symptoms. Therefore, the cancer is diagnosed in a very advanced stage and the prognosis of cancer among achalasia patients is considered poor (58).

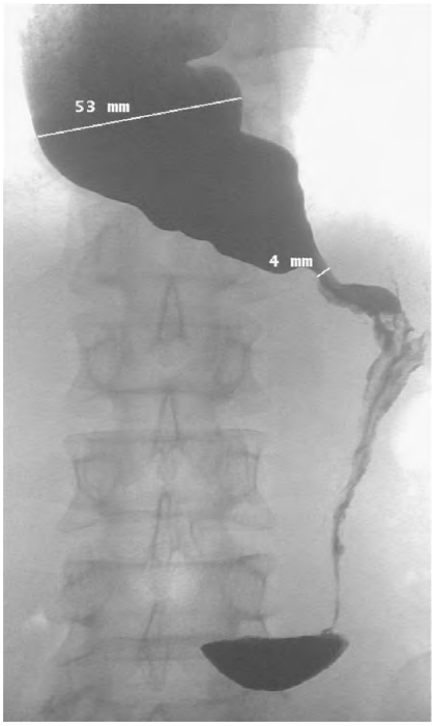

Figure 4. Contrast swallow study , a dilated esophageal body to 53 mm and a tight gastroesophageal junction of 4 mm. These are classic findings in a patient with achalasia. *Reprinted from St Peter SD, Swain JM. Achalasia: a comprehensive review. Surg Laparosc Endosc Percutan Tech 2003;13:227-40. By permission of Elsevier.*

Some institutions that implemented a surveillance endoscopy program – at least for those with a longstanding disease – reported a similar prognosis for achalasia-cancer compared to esophageal cancer without achalasia, however (48). The need for endoscopic surveillance in achalasia patients has been suggested by some researchers. Such a surveillance program was undermined because of cost-effectiveness and safety. There are also conservative proposals on using brush cytology (47), follow-up of selected group with severe and longstanding achalasia (55), or extension of screening intervals into two to three years (45).

2.3.3 Tobacco Smoking and Use of Scandinavian Snuff (Snus)

Tobacco smoking is widely acknowledged as the main known cause of cancer-related death worldwide and estimated to be responsible for approximately 25% of all cancers in men and 4% in women (3). Its relation to cancer of the esophagus (both ESCC and EAC) is well established (59). N-nitrosonorinocotine (NNN), the tobacco specific nitrosamines, is known as the responsible carcinogen for the association between

tobacco smoking and esophageal cancer (60). With a clear trend for dose and duration, tobacco smoking is strongly associated with the risk of ESCC (61). In a US case-control study, the estimated population attributable risk percent (PAR) for smoking vis-à-vis ESCC was 57% (62). In other words, 57% of ESCC cases can be prevented by elimination of tobacco smoking. Combination of tobacco and alcohol was estimated to be responsible for 90% of ESCC in an American case-control study (63). There are consistent indications that different smoking products, e.g. pipe, cigar, hand-rolled and/or high-tar cigarettes, bidi, or black tobacco, which all are perceived as strong and unrefined tobacco, is associated with steeper risk increase than using the commercially available cigarettes made from blond tobacco (63, 64). Moreover, chewing of different tobacco products, e.g. betel quid, pan, and nass, was linked to a higher risk of ESCC (2, 65). However, in the high risk area of China the association between smoking and ESCC seems to be weaker and only up to 30% significant excess risk due to smoking is reported (66) and smoking and alcohol use are not common in the high risk area of northern Iran (67). The positive association between tobacco smoking and EAC appeared to be weaker than ESCC, although a moderate approximately 2-fold excess risk was consistently reported (61, 68-72).

Scandinavian Snuff (Snus)

Snus use was common in the 19th century in Sweden, which is known as the world leader in per capita consumption of moist snuff. After a decline in sales in the 1920s, snus use increased again in the late 1960s. The Swedish Tobacco Company claims that Swedish snuff is a 'less harmful' alternative to cigarettes (73). The safety of snus use is generally referred to its lower risk in comparison to tobacco smoking. Prevalence of cigarette smoking has declined significantly from 36% to 17% among Swedish men – the lowest rate in Europe. This decrease has been linked to snus use and a corresponding small increase in its prevalence from 17% to 19% between 1980 and 2000 (74, 75), dismissing all other efforts made against tobacco use. A similar dramatic decrease in smoking prevalence has been observed without snus use in UK, California and Massachusetts (74). Snus use was also suggested as a reason to give up smoking or a means to prevent young people from the inclination to take up smoking (75-78).

Few prospective studies have examined the health risks associated with snuff use. Specifically, Scandinavian moist snuff, with comparably low levels of tobacco-specific nitrosamines (79), has been brought forward as a particularly safer alternative. Indeed, with a few exceptions (80, 81), studies of snus have found no demonstrable risk (79). Faced with this convincing evidence, the tobacco industry has started marketing smokeless tobacco products as substitutes for smoking with the explicit goal of reducing harm among smokers. While fervent attempts are presently being made to lift the ban put on snus in several European countries, many anti-tobacco activists warn that the existing literature may not be sufficiently strong to refute important carcinogenic risks; particularly in view of a clear conclusion by the International Agency for Research on Cancer that smokeless tobacco (including also Scandinavian snus) is “carcinogenic to humans” (82).

None of the previous epidemiological studies on snus and esophageal cancer, including one cohort study (81) and two population based case-control studies (80, 83), have shown significant excess risks. But the point estimates, multivariately adjusted for smoking dose, were above unity in all, ranging between 1.2 (80) and 1.4 (81, 83). In the only study that distinguished between the major histological types of esophageal cancer (83), the point estimate of relative risk for squamous cell carcinoma (1.4) tended to be

higher than that for adenocarcinoma (1.2). In order to evaluate the carcinogenic effect of snus appropriately, analyses need to be done among never smokers, where the residual confounding by smoking is ignorable. But none of the previous studies had sufficient power to analyze relative risk of esophageal cancer specifically in the strata of never-smokers.

2.4 Genetic and Molecular Epidemiology

It is thought that multifactorial etiology and a web of causation is involved including endogenous and exogenous environment, genetic and epigenetic modulators, and also gene-environment interaction. Alteration in three types of genes – oncogenes, tumor-suppressor genes and stability genes – are responsible for tumorigenesis. Fortunately there are multiple safeguards to protect the human cells against potentially lethal effects of cancer gene mutations. Therefore, cancer develops if only several genes are mutated, indicating that mutation contributes to, instead of causing, cancer (84).

Somatic event

Studies of somatic mutations could provide valuable information on the pattern of cancer progression and may lead to identification of critical markers for the early diagnosis and prevention. Cascades of somatic mutations initiate the neoplastic process with mutations in an oncogene or tumor suppressor gene leading to clonal expansion. Subsequent somatic mutations cause further clonal expansion and tumor progression (84). In ESCC, mutations in *P53* gene, deregulation of cell cycle control in G1 by disturbance of the cell cyclin-dependent kinas-Rb pathway and also alteration of oncogenes with ensuing deregulation of signal transduction have been consistently observed, regardless of patient origin and suspected etiologic factors (85). Mutation of the *P53* gene suggested as the early event in ESCC as the alterations in *P53* are frequently observed in esophageal precursor lesions (86).

A stepwise process of metaplasia-dyplasia-carcinoma sequence is suggested for the development of EAC (87). Because of Barrett's esophagus as an intermediate condition, EAC is a nice model to study the cancer progression from normal cell to the intermediate step (Barrett's), and, then, to the cancer. Mutation of P53 plays a major role in EAC carcinogenesis which is observed in Barrett's and with a prevalence of up to 90% in EAC (88). *CDKN2A* and increased cyclin D1 expression are other common aberrations of cell cycle genes observed in EAC and also in Barrett's esophagus (88).

Germline mutation

Germline mutations of cancer genes lead to predisposition of cancer instead of causing cancer *per se*. People with these mutations have a "head start" on the neoplastic process and they carry these component causes of cancer in every one of their cells (84). In the classic approach, the genetic studies would provide promising support if there is evidence on the genetic effect through heritability and familial aggregation of the disease of interest.

Although familial aggregation of ESCC has been demonstrated from different studies in the high-risk regions (89, 90) there is no evidence in favor of heritability of EAC (91). However, a familial aggregation of gastroesophageal reflux disease which is an established risk factor for this cancer was reported (92). A genetic component was also detected for gastroesophageal reflux in a twin study (93). Several candidate genes have been evaluated in association with esophageal cancer, mostly selected based on

biological understanding of the involved molecular mechanisms. The review of the vast literature on associations between different genetic poymorphisms and risk of esophageal cancer is beyond the scope of this summary. Relevant to this piece of work, studies published on associations between *Gluthathione S-transferase* polymorphisms (*GSTT1, GSTM1*, and *GSTP1*) and esophageal cancer are reviewed.

2.4.1 Polymorphisms of *Gluthathione S-Transferase* Genes

Tobacco smoking is an established risk factor for esophageal cancer and nitrosamines are the most likely candidates for ESCC carcinogenesis. N-nitrosonorinocotine (NNN) is the most abundant nitrosamine in tobacco and tobacco smoke, which after being metabolized by phase-I enzymes including P450, acts as an activated carcinogen to form DNA adducts (60). Expression of various kinds of the metabolizing enzymes in the human esophagus has been reported including the phase I activating enzymes (e.g., CYP1A2/1, 2E1, 2B6, 2C11, and 3A4/3) and the phase II detoxifying enzymes (e.g., GSTπ, μ, and α) (94, 95), suggesting a local activation and detoxification of the carcinogens in the esophagus.

Glutathione S-transferase genes – *GSTP1*, *GSTM1*, and *GSTT1* – found to be polymorphic in humans (96). There is a single nucleotide polymorphism (SNP) (313 A→G) in exon 5 of the *GSTP1* gene that leads to substitution of isoleucine (Ile) by valine (Val) at codon 105, altering the conjugating activity of some substrates (97). The Val variant has been linked to increased risks for bladder, testicular, and prostate cancer (98). *GSTM1* and *GSTT1* null genotypes found in, respectively, 42-60% and 13-26% of the Caucasian population worldwide (99), have been implicated as risk factors for several human malignancies, including gastric, colorectal and lung cancer (100-105). However, null or even inverse associations were also reported (103, 104, 106-109). In the following section all the published literature on associations between *GST* polymorphisms and esophageal cancer was reviewed. Due to the rarity of esophageal cancer, particularly EAC, all previous studies suffered from insufficient statistical power and few were truly population based. These findings need to be replicated in larger studies with more careful design (110).

GSTM1

According to 19 published epidemiological investigations, the results concerning the role of the *GSTM1* deletion polymorphism on risk of esophageal cancer are conflicting (Table 2). These studies are mainly from China and Japan but also from India, the Netherlands, France, and Canada (111-117). The odds ratios for esophageal cancer among individuals with the *GSTM1* 0/0 genotype varied between 0.4 and 13.2. The summary estimate among 12 studies included in a meta-analysis was 1.07 (95% CI 0.76-1.51), in which the histological types of esophageal cancer, ESCC and EAC, were combined (105). Moreover, the variation between Asian and Caucasian populations was not taken into consideration.

Table 1. Relative risks of published studies about association of *GSTM1* deletion and risk of esophageal cancer by histology.

Study	Country, year	Number of controls		Number of cases		Odds ratio (95% CI)
		Null	Active	Null	Active	Null vs. Active
Squamous cell carcinoma						
Hori H (118)	Japan, 1997	196	232	41	53	1.1 (0.6-2.1)
Morita S (119)	Japan, 1998	55	77	23	30	1.1 (0.6-2.0)
Yokoyama A (120)	Japan, 2002	321	313	103	131	0.8 (0.6-1.1)*
Nimura Y (121)	China, 1997	74	63	42	47	0.8 (0.4-1.3)*
Lin DX (122)	China, 1998	21	24	20	25	1.0 (0.4-2.3)*
Tan W (123)	China,2000	76	74	46	104	0.4 (0.3-0.7)
Gao CM (124)	China, 2002	133	90	106	35	2.2 (1.4-3.5)
Wang LD (125)	China, 2003	19	19	27	35	0.9 (0.3-2.3)*
Wang AH (126)	China, 2002	44	57	74	53	1.8 (1.0-3.2)
Lu X (113)	China 2005	4	100	36	68	13.2 (4.5-38.9)
Lu X (114)	China 2006	310	344	44	72	0.7 (0.4-1.0)*
Jain M (127)	India 2006	51	86	30	46	1.1 (0.6–2.0)
Van Lieshout EM (128)	Netherlands, 1999	128	119	5	8	0.6 (0.1-2.1)*
Abbas A (129)	France 2004	59	61	27	16	1.8 (0.9-3.8)
Adenocarcinoma						
Jain M. (127)	India 2005	51	86	5	4	2.1 (0.5-8.6)
Abbas A(129)	France 2004	59	61	12	13	1.0 (0.4-2.4)
Casson AG (130)	Canada, 2003	25	20	26	19	1.1 (0.5-2.7)
Casson AG (115)	Canada, 2006	54	41	34	22	0.9 (0.4-1.8)
Van Lieshout EM (128)	Netherlands	128	119	12	9	1.2 (0.5-3.5)*

* Odds ratio was calculated from the genotype frequency provided by authors.

GSTT1

With only one borderline exception (117), 11 studies on the importance of the *GSTT1* 0/0 deletion polymorphism, conducted in China, India, France, and Canada, were consistently null (111, 112, 115-117, 129). The summary relative risk among 6 of these studies included in the meta-analysis was 0.99 (95% CI 0.80-1.22) (105). Interestingly though, the null *GSTT1* genotype was observed significantly less often in 26 French patients with EAC than in 130 control subjects (odds ratio 0.1) (129), but this inverse association was not confirmed among 9 Indian, 21 Dutch, or 101 Canadian patients with this histological type of esophageal cancer (115, 116, 128, 130).

Table 2. Relative risks of published studies about association of *GSTT1* deletion and risk of esophageal cancer by histology.

Study	Country, year	Number of controls		Number of cases		Odds ratio (95% CI)
		Null	Active	Null	Active	Null vs. Active
Squamous cell carcinoma						
Lin DX (122)	China, 1998	23	22	19	26	0.7 (0.3-1.5)
Wang LD (125)	China, 2003	20	18	34	28	1.1 (0.4-2.7)
Gao CM (124)	China, 2002	119	104	74	67	0.9 (0.6-1.4)
Tan W. (123)	China, 2002	59	91	60	90	0.9 (0.7-1.2)
Wang Z (117)	China 2006	-	-	-	-	1.7 (1.0-3.0)
Jain M. (127)	India 2005	37	100	20	56	0.9 (0.5-1.7)
Van Lieshout EM (128)	Netherlands, 1999	49	198	5	8	0.7 (0.1-3.5)*
Abbas A (129)	France 2004	30	85	13	31	1.0 (0.4-2.0)
Adenocarcinoma						
Jain M. (127)	India, 2005	37	100	3	6	1.2 (0.3-5.2)
Abbas A (129)	France, 2004	30	85	1	25	0.1 (0.0-0.6)
Casson AG (130)	Canada, 2003	12	33	8	37	0.6 (0.2-1.6)
Casson AG (115)	Canada, 2006	15	80	14	42	0.9 (0.4-3.2)
Van Lieshout EM (128)	Netherlands, 1999	49	128	4	17	1.0 (0.2-3.5)*

* Odds ratio was calculated from the genotype frequency provided by authors.

GSTP1

The *GSTP1* Ile105Val polymorphism has been evaluated in relation to esophageal cancer risk (mostly ESCC) in 13 studies (111, 112, 115-117, 129, 131), but the results were highly variable with odds ratios ranging between 0.1 and 4.6 among heterozygous or homozygous carriers of the variant Val allele, relative to homozygotes for the wild-type Ile allele. The summary estimate of relative risk in the meta-analysis including 7 of these studies was 1.01 (95% CI 0.6-1.70). However, this analysis combined ESCC and EAC and also pooled the results of studies from Caucasian and Asian populations (105).

The highest odds ratio (4.6; 95% CI 1.5-14.6) was noted for Dutch patients with EAC (128) and the other studies that could evaluate EAC separately showed point estimates between 1.2 and 1.9 (115, 116, 129, 130). These findings are in line with the higher expression of *GSTP1* in the esophagus compared to other *GST* genes. It may also partly explain the highest relative risk observed for association between smoking and esophageal smoking in Western countries but not in the Asian high risk areas.

Table 3. Summary of published studies on association between *GSTP1* polymorphism and esophageal cancer by histology.

Study	Country, year	Number of contrls			Number of cases			Odds ratio (95% Confidence Interval)		
		Ile/Ile	Ile/Val	Val/Val	Ile/Ile	Ile/Val	Val/Val	Heterozygous IL/Val	Homozygous Val/Val	Mutant IL/Val Val/Val
Squamous cell carcinoma										
Morita S. (184)	Japan, 1998	113	48	3	61	5	0	0.2 (0.1-0.5)*	0.0 (0.0-3.6)*	0.1 (0.0-0.5)*
Lin DX (122)	China, 1998	22	11	3	29	12	1	0.8 (0.3-2.5)*	0.3 (0.0-3.5)*	0.7 (0.3-1.8)
Tan W. (123)	China, 2000	91	53	6	93	48	9	0.9 (0.5-1.5)*	2.2 (0.6-10.1)*	1.0 (0.8-1.3)
Wang LD (125)	China, 2003	24	13	1	29	30	3	1.9 (0.8-4.9)*	2.5 (0.2-135.7)*	2.0 (0.8-4.9)*
Cai L (131)	China 2004	143	58	3	265	116	12	0.5 (0.1-1.8)*	0.9 (0.6-1.4)*	0.9 (0.6-1.3)*
Lee JM (185)	Taiwan, 2000	160		94	65		25	NA	NA	0.7 (0.4-1.1) †
Jain M.(127)	India, 2005	72	56	9	46	23	7	0.6 (0.3-1.1)	1.5 (0.5-4.3)	0.7 (0.4-1.1)*
Abbas A (129)	France, 2004	59	56	9	21	21	3	1.0 (0.5-2.3)*	0.2 (0.0-0.6)*	1.0 (0.5-1.9)
Van Lieshout EM (128)	Netherlands, 1999	146	89	12	5	6	2	2.0 (0.6-9.2)*	4.9 (0.4-33.4)*	4.6 (1.5-16.6)*
Adenocarcinoma										
Jain M.(127)	India, 2005	72	56	9	4	4	1	1.2 (0.3-5.1)	1.6 (0.2-15.7)	1.6 (0.2-15.7)*
Abbas A (129)	France, 2004	59	56	9	10	12	3	1.3 (0.5-3.5)*	0.3 (0.1-1.3)*	1.2 (0.5-3.0)
Van Lieshout EM (128)	Netherlands, 1999	146	89	12	5	15	1	2.4 (0.0-24.3)*	4.9 (1.6-17.8)*	4.6 (1.5-16.6)*
Casson AG (130)	Canada, 2003	26	12	7	19	22	4	2.5 (1.0-6.3)	0.8 (0.2-3.1)	1.8 (0.8-4.3)*
Casson AG (115)	Canada, 2006	40	44	11	18	27	11	1.4 (0.6-3.0)	2.2 (0.7-6.8)	1.5 (0.8-3.1)

* Odds ratio was calculated from the genotype frequency provided by the authors.
† If authors used different reference group, ORs and corresponding 95% CIs were reversed to have the Ile/Ile variant as the reference group for all studies.

3 Aims of this thesis

The overall aim of this thesis was to examine risk of esophageal squamous cell carcinoma and adenocarcinoma in relation to medical conditions (achalasia and gastroduodenal ulcers) and tobacco related factors. It was specifically aimed:

I. To determine risk of ESCC and EAC in relation to duodenal and gastric ulcers, both *H. pylori*-related diseases but diverse in the pattern of gastric acid secretion.

II. To elucidate the risk of ESCC and EAC among achalasia patients and also to examine whether releasing the pressure from the lower esophageal sphincter by esophagogastric myotomy modifies the risk of esophageal cancer among these patients.

III. To examine the associations of tobacco smoking and use of Scandinavian moist snuff (snus) and the risk of ESCC and EAC.

IV. To evaluate the associations of polymorphisms of tobacco-metabolizing genes (*GSTT1, GSTM1, GSTP1*) with the risk of esophageal cancer by histology.

4 Subjects and Methods

4.1 Subjects

The long tradition of collecting information on health and social conditions of the population provides an excellent base for monitoring diseases and social problems in Sweden. Statistics Sweden keeps the population registers and The Centre for Epidemiology (EPC) in the Swedish National Board of Health and Welfare is responsible for collection and maintenance of epidemiological registers up to date and with high quality.

We used data from the Swedish Inpatient Register to establish cohorts of patients hospitalized for peptic ulcer disease and achalasia (studies I and II). A large cohort of construction workers was used in study III. The national registration numbers (NRNs), unique personal identifiers assigned to all residents in Sweden, enabled us to perform unambiguous record linkages of the defined cohorts with Total Population, Cancer, Migration, and Causes of Death Registers to follow the cohort members in studies I, II, and III. Finally, material from the Swedish Esophageal and Cardia Cancer (SECC) study was used in study IV. After a brief description of the individual data sources, study specific methods will be discussed.

4.1.1 Swedish Hospital Discharge (Inpatient) Register (Study I and II)

The Swedish Inpatient Register was established by The National Board of Health and Welfare in Sweden in 1965 to document individual hospital discharges. The coverage of the Swedish Inpatient Register was 60% of the Swedish population in 1969, 85% in 1983 and 100% since 1987 and onwards. Each patient record contains:

a. The patient's unique national registration number (NRN)
b. The date of hospital admission and discharge
c. One primary discharge diagnosis and up to seven differential diagnoses coded according to the International Classification of Diseases (ICD7, ICD8, ICD9, and ICD10)
d. Surgical procedures coded according to the Swedish Classification of Operations and Major Procedures through 1996 and the Nordic medico – statistical committee (NOMESCO) classification of surgical procedures thereafter.

Because almost no private institutional care has been available in Sweden, and people are required to seek medical care at a hospital in their county of residence, studies based on this register can be considered as population based. Using the International Classification of Diseases (ICD) codes from the Inpatient Register, we identified cohorts of patients hospitalized for duodenal ulcer (ICD-7: 541, ICD-8 and ICD-9: 532, and ICD-10: K26), gastric ulcer (ICD-7: 540, ICD-8 and ICD-9: 531, and ICD-10: K25), both restricted to un-operated patients, as well as the cohort of patients hospitalized for achalasia (ICD7: 530,01, ICD8: 509,01 ICD9: 530A and ICD10: K22.0) between 1965 and 2003.

4.1.2 Swedish Construction Workers Cohort (Study III)

The construction industry's Organization for Working Environment, Safety and Health, "Bygghälsan", offered outpatient medical services to all blue- and white-collar workers in the Swedish building industry between 1969 and 1993. The organization was a joint venture launched by the relevant trade union and the Swedish Construction Employer's Association. The basic units were stationary or mobile clinics, typically staffed by a few nurses and a physician. The main activity was preventive health check-ups to all construction workers, through regular personal invitation (every second year during the first years, every third year thereafter) as well as through visits to or advertisements at virtually all major building sites. Beginning with visits in 1971, data from these check-ups were compiled in a computerized central registry.

During 1971-75 each cohort member filled out a 200-item questionnaire which included detailed questions about smoking and snus use. During the visits, answers were double-checked by attending staff. After a pause during 1976 through 1977, the collection of smoking and snus information was resumed in 1978 but on a new form filled out directly by the staff. The data quality has been reviewed previously and was deemed to be satisfactory. Briefly, data on height and smoking were examined. For individuals with more than one measurement, the difference between highest and lowest values of height – which is not usually subject to changes – was 1 cm. Moreover, only 2.6% inconsistencies were found on smoking data, e.g. people who indicated that they were current or former smokers in the first visit but asserted that they had never smoked in the second questionnaire (132). Because 95% of the participants were men and repeat visits were variable in number and timing among the cohort members, to a large extent driven by self selection, only the data from the first registered visit among men was used in this study.

4.1.3 Follow-up (Study I-III)

The national registration numbers (NRNs), unique personal identifiers assigned to all residents in Sweden, permitted follow-up through linkages to nationwide and essentially complete registers of Cancer, Causes of Death, as well as to registers of the Total Population and Migration. These registers enabled complete follow-up of the cohort members from entry into the cohort until the date of emigration, death, or cancer diagnosis, whichever occurred first (studies I-III). If a NRN could not be found in any of the latter three registers it was deemed to be erroneous and the record was excluded.

The Swedish Cancer Register
The Swedish Cancer Register was founded in 1958 and is reported to be more than 98% complete (133). There are six regional registries associated with the oncological centers in each medical region of Sweden where the registration, coding and the major check-up are performed. The regionalization implies a close contact between the registry and the reporting physician, which in turn simplifies the task of correcting and checking the material. Malignant neoplasms have been coded according to the 7th revision of International Classification of Diseases (ICD7) since the beginning (1958) and onward. In this thesis, the Swedish Cancer Register was used to identify the patients who developed esophageal cancer during the follow-up. The ICD7 code 150

(esophageal cancer) was further broken down into EAC (code 096) and ESCC (code 146) using WHO/HS/CANC/24.1 histology codes (134).

4.1.4 Swedish Esophageal and Cardia Cancer Study (SECC) (Study IV)

To study associations of *GST* polymorphisms and risk of esophageal cancer, we used DNA samples of cancer patients and population based controls recruited in the SECC study. Study design and characteristics of the participants in the SECC study have been described elsewhere (20, 135). In brief, the study base was the entire Swedish population below 80 years old, born and still living in Sweden during a 3-year period (December 1, 1994 through December 1, 1997). All newly diagnosed native Swedish patients with EAC or cardia cancer and half of the ESCC cases (those born on even dates) were recruited. Cancer patients were identified and accrued via a comprehensive organization for rapid case ascertainment with contact persons at all 195 departments in the entire country. Controls were cancer-free native Swedes who were selected randomly from the study base, frequency matched on age (in 10-year categories) and sex distribution among the EAC cases. Information about demographic characteristics and several risk factors including smoking habits were collected from both cases and controls by means of face-to-face interviews conducted by professional interviewers.

4.2 Statistical Analyses

In survival analysis (time-to-event analysis) the time from exposure of interest until the outcome is compared between the exposed and unexposed group. Follow-up of the cohort members until their exit from the cohort (censoring) is very important concept in the cohort analysis. Censoring may occur due to several reasons including loss to follow-up because of emigration, death before the outcome of interest, etc. Because of the national registration number (NRN), unambiguous linkage across the Cancer, Death and Migration Registers ensures a complete follow-up of the cohorts in Sweden.

In cancer epidemiology, cancer may occur among patients who have suffered from another cancer in past, but have survived because of an appropriate treatment. These patients usually receive various treatments including surgery, chemotherapy and radiotherapy. Such patients will very likely change their lifestyle. Therefore, the second cancer may arise from a different background of risk factors. A self-screening may occur because these patients, who had experienced the cancer symptoms in the past, are more likely to seek the health care system for any suspicious symptoms. Therefore, first primary cancer cases were studied and the follow-up was ended upon the development of any cancer diagnosis during the study period. Similarly, all individuals with a history of any cancer diagnosis prior to the entry into the cohort were excluded.

4.2.1 Study I and II: Standardized Incidence Rate (SIR)

Standardized incidence ratio (SIR) is the simplest statistics when the data represents incidence in a single study group. It is a common method in occupational and environmental epidemiology where the excess morbidity and mortality is measured in a single workplace. In such studies, the observed number of outcomes is compared with the expected number derived from a large reference population, e.g. vital statistics of the state or country. This method has been described in details in

standard epidemiologic textbooks (136). In Sweden, cohorts of patients with different diseases can be identified from the Swedish Inpatient Register. These cohorts usually represent groups of people highly exposed to specific exposures among whom study of cancer risk (or other outcomes) provides important information on disease etiology. Moreover, the unique NRNs allows for linkage of the defined cohorts into the Cancer, Causes of Death, and Migration register for a complete follow-up and estimation of the cancer risk. Therefore, it is possible to estimate SIRs and compare the cancer incidence in the defined cohorts and compare it with the corresponding incidence rates in the Swedish general population.

The SIR method was used to estimate the risk of esophageal cancer among cohorts of patients hospitalized for peptic ulcer (study I) and achalasia (study II). Because of a long follow-up time, three time scales were considered in these studies, e.g. attained age, calendar period, and time since entry into the cohort. In order to consider the changes in the time scales, they were then divided into shorter time-bands and the incidence rates were estimated for each specific time-band separately. Figure 5, known as lexis diagram, illustrate schematic contribution of a few patients from our achalasia cohort on different time-bands (137). We used a SAS macro to estimate the person-years according to the method recommended by Clayton D. and Macaluso M. (138-140). In order to estimate SIRs, the observed age, sex and calendar specific incidence rates were compared with corresponding rates (expected rates) derived form the Swedish general population as the reference rates. In order to calculate the incidence rates both in the study cohorts and the reference population, the second primary cancers and cancers found incidentally, first at autopsy, were excluded. Confidence intervals (CIs) of SIRs were calculated assuming that the observed number of events followed a Poisson distribution (141). We further stratified the cancer risks based on the time since entry into the cohort (index hospitalization for peptic ulcer or achalasia). In the achalasia cohort (study I), additional stratification split the cohort to operated and unoperated patients. Operated patients contributed person-years until the day of operation and, subsequently, were switched to the latter sub-cohort. Noteworthy, the direct comparison between SIRs is not fully meaningful as they are differently standardized. A Poisson or Cox regression (the time-dependent modelling, if relevant) can compare the risks between the two or more strata.

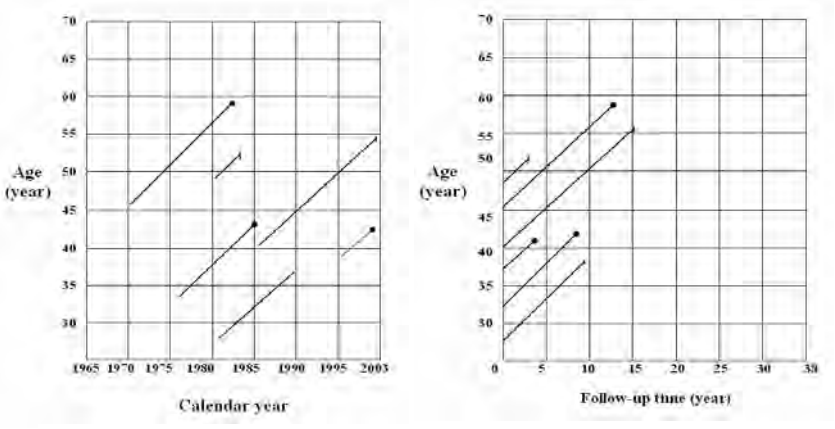

Figure 5. Lexis diagram for a few patients from the achalasia cohort; the left diagram shows the person-years contribute by age and calendar period and the right diagram illustrates the same individuals by age and time since entry into the cohort.

4.2.2 Study III: Cox Proportional Hazards Model

Among different statistical methods, the Cox proportional hazards model is the most commonly applied model in medical time-to-event studies (142). The hazards function, $\lambda(t)$, is another name for the incidence rate which is more commonly used in epidemiology. It is the instantaneous event rate at time t, conditional on survival up to time t. The hazard function could be decreasing, increasing, or be constant with exponential distribution. However, Cox proportional hazards model does not make any assumption about the shape of underlying hazards, but it assumes that the hazards for patient subgroups are proportional over follow-up time. As it is a strong assumption, its appropriateness should always be evaluated. There are different methods to assess the proportionality assumption like 1) plotting the log cumulative hazards functions over time and checking for parallelism (the crude presentation), 2) plotting Schoenfeld's residuals against time to identify pattern, or 3) including time-by-covariate interaction in the model and testing statistical significance.

In s*tudy III*, Cox proportional hazards regression models estimated relative risks (RRs) and corresponding 95% confidence intervals (CIs) using attained age (in years) as the time scale. All models were further adjusted for body mass index (BMI) at entry, categorized into quartiles. In this study, risk of stomach cancer was also studied. Evaluation of the proportional hazards assumption with graphs of scaled Schoenfeld residuals revealed that the assumption did not hold for the association of snus use with non-cardia stomach cancer. Moreover, test of time-by-covariate interaction showed a significant interaction specifically for non-cardia stomach cancer. Although due to small power the interaction terms were not statistically significant for ESCC, EAC, and cardia cancer, there was similar pattern for the snus-related risk in relation to all the studied cancers. The cancer risks among snus users were diverging after the age of about 70 and the risks were higher than that among non-snus users after age 70 (Figure 6). Therefore, because of the effect modification by age, age-specific relative risks were estimated when studying the snus effect.

To study the effects of snus use, we first compared the cancer risks among all snus users to non-snus users, with adjustments only for attained age and BMI. Although pre-existing smoking dose could have been linked to the inclination to take up snus use, the analyses that were unadjusted for smoking were thought to accommodate the assumed dose-limiting effect of adding snus use to the smoking habit. Hence, the estimates were interpreted as the net effect of the combined habit. To disentangle the independent effect of snus use, the models were additionally adjusted for smoking. These unadjusted and adjusted analyses were then repeated in the substratum of ever-smokers at time of entry. This was because it was assumed that any positive net effect of snus use would be particularly evident among smokers. In addition to attained age and BMI, adjustment was done also for smoking status, dose and type of smoking tobacco. In order to control more efficiently for smoking and appropriately evaluate the carcinogenic effect of the snus use, we estimated the relative risks among never smoking snus users in comparison to never users of any tobacco and adjusted only for attained age and BMI. Due to the apparent time-by-covariate interaction, age-specific relative risks were estimated in all the stratified analyses of the snus effect.

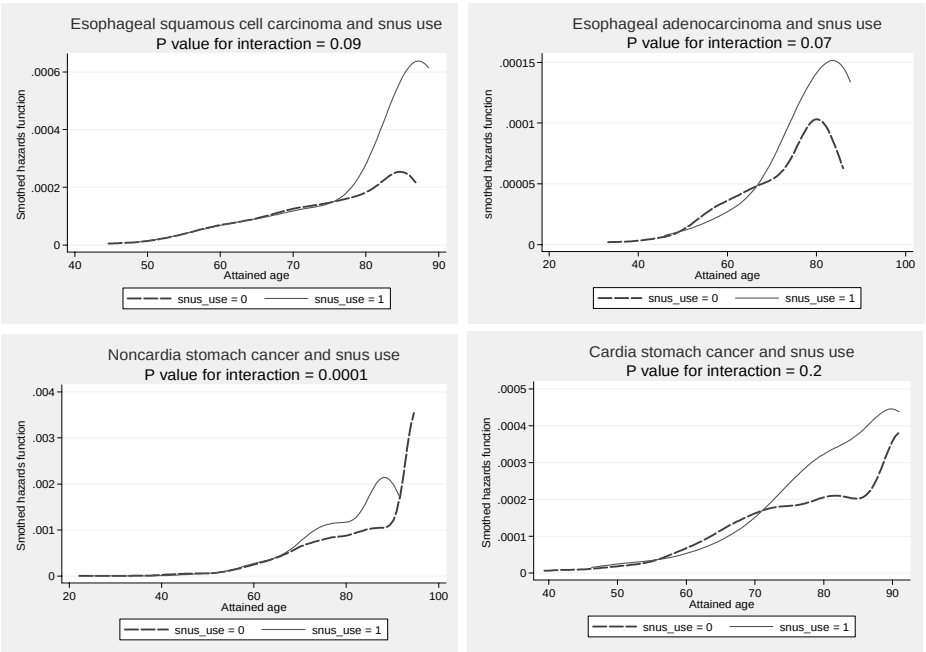

Figure 6. Hazards graphs for esophageal and stomach cancer among snus users **(solid lines)** and non-snus users **(dashed line)**. All the cohort members were included in these analyses. P value for interaction with age is provided.

4.2.3 Study IV: Genetic Association Study

GSTP1 was genotyped with Pyrosequencing, while a multiplex PCR method was used to genotype *GSTM1* and *GSTT1*. The detailed genotyping methods are described in the corresponding paper enclosed in the thesis (study IV). Allele and genotype frequencies for *GSTP1* were determined and deviation from Hardy-Weinberg equilibrium among controls was tested, using the Chi-square method with one degree of freedom. Unconditional logistic regression estimated odds ratios with 95% confidence intervals (CIs). Established risk factors of esophageal cancer were considered in the analyses including smoking status (never, former or current smokers two years before interview), intake of fruits or vegetables (in three categories), alcohol consumption (total amount of all types of alcoholic beverages in four categories), BMI (in quartiles), reflux (occurring at least once per week), and socioeconomic status (reflected by number of years of formal education categorized into three levels). Chi-square test showed no associations between *GST* polymorphisms and these risk factors. Moreover, adjustment in the regression models did not change the gene-cancer odds ratios materially. Therefore, only matching variables – sex and age (in 10-year age bands) – were included in the final models. Due to insufficient power, the interaction between *GST* polymorphisms and smoking status (ever- or never-smoking) was not statistically significant. However, due to the biological understanding of the effect of GST enzymes on tobacco-specific carcinogens, we performed additional analyses restricted to smokers only.

4.3 Validation and Sensitivity Analyses

In observational studies we usually try to control for the random error and confounding. However, controllable confounding and random error are sometime only a fraction of the total error, and rarely an important source of uncertainty. Potential bias due to unmeasured confounding, misclassification and selection bias needs to be discussed thoroughly in all studies. Most biases can be fully analyzed if additional "validation" data are available, but such data are usually absent or very limited. Most investigators try to address them qualitatively in the discussion of their papers. Nearly all epidemiologic studies suffer from some degree of measurement error which is known as misclassification. The impact of even modest amounts of error can be profound, although it is rarely quantified. There are simple and also sophisticated methods to estimate the degree of bias because of this misclassification (143). Quantitative assessments can provide valuable insight into the importance of various sources of error. They provide a more realistic picture of the uncertainty of the study results. In study II, a validation study was performed to ensure the validity of achalasia diagnosis and in study III, a sensitivity analysis evaluated the impact of potential misclassification of smoking status on the observed association between snus use and gastroesophageal cancers.

Study II: Validation study

As achalasia served as exposure in this study, an exposure misclassification could produce a biased estimate if the misclassification was related to the outcome. From clinical practice, there are other achalasia-like diseases that could be erroneously diagnosed as achalasia. For instance, patients with gastroesophageal reflux may suffer from post reflux-stricture in the gastroesophageal sphincter and present with achalasia-like symptoms. A great extent of the misclassification of such patients with achalasia in this study could potentially explain the excess risk observed for EAC. This was probably one of the most important challenges in this study. As the records of all 2,896 achalasia patients registered from 1965 through 2003 were used, validation of the achalasia diagnosis through records review of every single case was not feasible. Therefore, a validation study was designed to evaluate the extent of such a bias in a random sample of patients.

Staff at the National Board of Health and Welfare (that keeps the register) randomly selected 3 achalasia patients from each department of general surgery and otorhinolaryngology at all county and regional referral hospitals in Sweden. According to the register, these departments managed the overwhelming majority of the recorded achalasia patients. In order to facilitate the field work, the sample was restricted to patients seen in the past 10 years. A questionnaire was sent out to the chairman or the specialist in charge of these patients at the respective units. We asked about the general policies, if any, in relation to investigations and treatment of achalasia at their unit, e.g., diagnostic and therapeutic practices, criteria for diagnosis, when hospitalization was considered, and the percentage of achalasia patients who were managed exclusively as outpatients. Moreover, these local experts were also asked to review the case records of the selected patients in order to answer questions about the time sequence of onset of symptoms — first diagnosis — treatments given, diagnostic tests used, and results of

Table 4. Diagnostic and treatment information for achalasia patients in the validation study (n=83)

	Frequency
Achalasia diagnosis unambiguously correct	67 (100%)
Diagnostic approach	
Single procedure	13 (19%)
Combination of two or more procedures	54 (81%)
M, BS and E[†]	24 (36%)
BS and E	15 (23%)
M and E	10 (15%)
M and BS	5 (7%)
Excellent and long-lasting response to treatment [‡]	
Myotomy	18/27 (67%)
Forceful dilation	22/53 (41.5%)

***** Since information about diagnostic approach was not meaningful in patients who were incorrectly recorded as achalasia patients (n=16, 19%), they were not included in the analyses.
[†] M: Manometry, BS: Barrium swallow, E: Endoscopy
[‡] Based only on patients who received such treatment and for whom an assessment was given. There were 16 (30%) patients who were treated with both forceful dilation and myotomy. Information for 3 (7.5%) patients was missing.

dilatations and esophagomyotomies. The confidence in the diagnosis was evaluated, from unambiguously correct to doubtlessly incorrect. The responses from hospitals were sent to the National Board of Health and Welfare, where the responses were registered, de-identified and forwarded to the investigators. Based on the records of 83 patients received from the contacting units, 16 (19%) patients were incorrectly classified among whom 4 (5%) patients had a post-reflux stricture and 1 (1%) had status post-surgery for obesity, while the other had diagnoses that were unrelated to esophageal cancer risk, like e.g., cricopharyngeal achalasia. This validation study reassured us that the strong association observed between achalasia and esophageal cancer is unlikely to be importantly biased conspicuously by misclassifcation. Potential misclassification due to diseases that are not related to esophageal cancer would only lead to an underestimation of the risk.

Study III: Sensitivity Analysis

In this study, since exposure information was collected at entry into the cohort, there is a possibility that non-smoking snus users were more inclined to take up smoking in the follow-up period, compared to never-users of any tobacco,. Because majority of workers were visited only once (40%), the cross-sectional data across successive repeat visits was more likely to be sensitive to selection bias. Bearing such a concern in mind, the main analyses were confined to the information collected only at the entry into the cohort. However, we used such a data from the repeat visits among workers who were reported never smokers at entry into the cohort and who had at least two visits to evaluate potential confounding by smoking. This analysis revealed that differential misclassification of smoking status is indeed a valid concern as 6.7% of never-users of any tobacco and 13.2% of never-smoking snus users were found to be smokers in their subsequent visits. In a sensitivity analysis, these proportions were extrapolated to the entire subcohorts of never-users of any tobacco and never-smoking snus users and assumed that workers with a positive smoking record at any point in time during the follow-up were, in fact, smokers. Using the magnitude of smoking-disease association obtained from this data, the observed estimate were corrected as proposed by Schneeweiss (144). Taking the suspected misclassification into account, after the correction, the relative risk for ESCC among never-smoking snus users decreased from

3.5 to 2.88 and, correspondingly, relative risks for non-cardia stomach cancer decreased from 1.4 to 1.37. It was also estimated that at least 60% of the snus users would have to be smokers to cancel the observed association between ESCC and snus use, provided that no smoking misclassification exists among never users of any tobacco. Not even 100% smoking prevalence among snus users would fully explain the observed association between exclusive snus use and non-cardia stomach cancer.

5 Results

5.1 Study I

Risk of esophageal cancer among patients hospitalized for gastroduodenal ulcers

In total 61,546 duodenal ulcer patients and 81,379 patients with gastric ulcer contributed 524,960 and 576,458 person-years after excluding the first year of follow-up. Corresponding average duration of follow-up was 9.1 and 7.2 years, respectively. The reasons for hospitalization (bleeding, perforation, or other) in duodenal ulcer and gastric ulcer patients were fairly similar, although gastric ulcer patients were older (on average 66.7 years) than duodenal ulcer cohort (on average 62.1 years) at the index hospitalization. Due to the selection bias, the risk of ESCC was high in the first year of follow-up in both duodenal ulcer (SIR= 2.9, 95% CI 1.4-5.1) and gastric ulcer (SIR= 3.5, 95% CI 2.1-5.5) cohorts. The risk of EAC was also high in the first year of follow-up among both duodenal ulcer (SIR= 6.8, 95% CI 3.3-12.5) and gastric ulcer (SIR= 7.8, 95% CI 4.4-12.8) patients.

Squamous cell carcinoma

After excluding the first year of follow-up, we found a borderline significant 30% excess risk of ESCC among duodenal ulcer patients in comparison with the general Swedish population (SIR=1.3, 95% CI 1.0-1.8) (Table 5). We also observed a significant 80% increase in the relative risk of ESCC (SIR=1.8, 95% CI 1.4-2.3) in gastric ulcer patients. Since *H. pylori* eradication, prescribed since the 1990s, could modify the cancer risk, analyses were further stratified by the calendar period of follow-up (before versus after 1995). However, we found no differences in the cancer risks in these two periods.

Adenocarcinoma

Among duodenal ulcer patients, 27 EAC cases rendered a 70% excess risk after the first year of follow-up (SIR=1.7; 95% CI 1.1-2.5) (Table 5). The relative risk tended to be higher among patients with ulcer complications (SIR=2.6; 95% CI 1.5-4.3) compared to those with uncomplicated disease. We found no excess risk for EAC among gastric ulcer patients.

Table 5. Standardized incidence ratios (SIRs) and their 95% confidence intervals (CIs) for ESCC and EAC among non-operated peptic ulcer patients in Sweden (1965-2003) after excluding the first year of follow-up.

	Squamous cell carcinoma				Adenocarcinoma			
	Duodenal ulcer		Gastric ulcer		Duodenal ulcer		Gastric ulcer	
	No. Cases	SIR (95% CI)	No. Cases	SIR (95% CI)	No. Cases	SIR (95% CI)	No. Cases	SIR (95% CI)
Overall	44	1.3 (0.1-1.8)	70	1.8 (1.4-2.3)	27	1.7 (1.1-2.5)	18	1.1 (0.6-1.7)
Follow-up time since entry								
2-10 years	33	1.6 (1.1-2.2)	48	1.8 (1.3-2.4)	17	1.9 (1.1-3.1)	12	1.1 (0.6-2.0)
11+ years	11	0.9 (0.5-1.6)	22	1.9 (1.2-2.9)	10	1.5 (0.7-2.8)	6	1.0 (0.4-2.2)
P for trend		0.01		0.9		0.2		0.9
Calendar year of follow-up								
1965-1994	25	1.2 (0.8-1.8)	38	1.6 (1.1-2.2)	13	2.3 (1.2-4.0)	5	0.8 (0.3-1.9)
1995-2003	19	1.5 (0.9-2.3)	32	2.2 (1.5-3.1)	14	1.4 (0.8-2.3)	13	1.3 (0.7-2.1)
Complication*								
Yes	30	1.4 (1.0-2.0)	39	1.7 (1.2-2.3)	12	1.2 (0.6-2.1)	11	1.1 (0.6-2.0)
No	14	1.2 (0.6-2.0)	31	2.1 (1.4-2.9)	15	2.6 (1.5-4.3)	7	1.1 (0.4-2.2)

* Including bleeding and perforation.

5.2 Study II

Risk of esophageal adenocarcinoma in achalasia patients

A total of 2,896 patients were accrued in the study cohort. Men constituted 54.3% of the achalasia cohort and the mean age at entry was 54.4 and 59.6 years among men and women, respectively. Average length of follow-up was 9.9 years, and 25,766 person-years at risk were accrued after excluding the first year of follow-up. Esophagogastric myotomy was performed in 688 patients who were younger (mean age 42.3), but had a longer follow-up time (14.9 years on average) compared to the main cohort.

Because of the selection bias (or reversed causation), we found an increased risk of all cancer types (SIR=3.9, 95% CI 3.1-4.8) in the first year of follow-up, driven from a considerable excess risk in gastroesophageal cancer. The highest SIR appeared to be for cardia cancer (SIR=171.8, 95% CI 104.9-265.4) followed by that for ESCC (SIR=49.2, 95% CI 19.8-101.4) and EAC (SIR= 79.7, 95% CI 21.7-204.0).

After excluding the first year of follow-up, we observed 238 cancers (any site) during year 2-38, corresponding to a SIR close to unity (SIR=1.1, 95% CI 0.9-1.2) (Table 6). We found more than 10-fold increased risk of esophageal cancer in achalasia patients (SIR = 10.5, 95% CI 7.0-15.9). The excess risks were equally evident for both EAC (SIR=10.4, 95% CI 3.8-22.6) and ESCC (SIR=11.0, 95% CI 6.0-18.4). Although women contributed 46% of the observed person-time in our achalasia cohort, only 9% of (2 of 22) esophageal cancers occurred in women, both EACs.

Table 6. Standardized incidence ratios (SIRs) and 95% confidence intervals (CIs) for esophageal cancer (ESCC and EAC) in achalasia after excluding the first year of follow-up , stratified by sex and follow-up duration (Sweden, 1965-2003)

	No. of cases	SIR (95% CI)
All achalasia patients		
All cancers	238	1.1 (0.9-1.2)
All esophageal cancers	22	10.5 (7.0-15.9)
2-9 yrs of follow-up	12	9.2 (4.8-16.1)
10-38 yrs of follow-up	10	12.7 (6.1-23.3)
Men	20	13.1 (8.1-20.4)
Women	2	3.5 (0.4-12.6)
Squamous cell carcinoma	14	11.0 (6.0-18.4)
2-9 yrs of follow-up	9	11.1 (5.1-21.1)
10-38 yrs of follow-up	5	10.8 (3.5-25.1)
Men	14	16.1 (8.8-26.9)
Women	0	-
Adenocarcinoma	6	10.4 (3.8-22.6)
2-9 yrs of follow-up	2	6.1 (0.7-21.8)
10-38 yrs of follow-up	4	16.3 (4.4-41.8)
Men	4	8.4 (2.3-21.6)
Women	2	19.8 (2.4-71.6)

Analyses stratified by surgical myotomy revealed excess risks of esophageal cancer among both unoperated (SIR=9.1, 95% CI 5.1-15.0) and operated (SIR=16.0, 95% CI 6.4-33.1) achalasia patients (Table 7). Although excess risk among unoperated patients was fairly stable before and after 10 years of follow-up, SIR among operated patients decreased from 20.4 in the first 10 years of follow-up to 12.5 afterwards (95% CI 2.6-16.5). However these differences were not statistically significant. Only 1 of 7 esophageal cancers observed among operated patients was EAC (SIR=8.0, 95% CI 0.2-44.4).

Table 7. Standardized incidence ratios (SIRs) and 95% confidence intervals (CIs) for esophageal cancer (ESCC and EAC) and myotomy in achalasia patients, after excluding the first year of follow-up.

Gastroesophageal myotomy	No. of cases	SIR (95% CI)
Achalasia without esophagomyotomy*	15	9.1 (5.1-15.0)
Follow-up year 2-9	9	8.0 (3.7-15.2)
Follow-up year 10+	6	11.6 (4.2-25.2)
Achalasia with esophagomyotomy*	7	16.0 (6.4-33.1)
Follow-up year 2-9	4	20.4 (5.6-52.2)
Follow-up year 10+	3	12.5 (2.6-36.5)

After excluding the first year of follow-up, and assuming yearly endoscopic examinations, we estimated that 658 (95% CI 447-1,063) endoscopies would be required to detect one esophageal cancer among men. Corresponding estimate for women was 5,975 (95% CI 2,145-49,792).

5.3 Study III

Risk of gastroesophageal cancer among smokers and snus users

The cohort of 336,381 construction workers was followed for up to 33.5 years (mean 22.2) corresponding to 7,475,628 person-years under observation. The mean age at entry was 34.7 years. The prevalence of smoking (current or former users) was 58% at time of entry. Overall, 28% of workers reported being snus users at the entry into the cohort but it was higher among young workers. We observed 130 cases of EAC and 236 cases of ESCC in this cohort. Age-standardized incidence rates stratified by tobacco habits are presented in Table 8.

Squamous cell carcinoma and tobacco smoking

We observed a 5.2-fold excess risk of ESCC (95% CI 3.1-8.6) among ever-smokers relative to never-users of tobacco (Table 9). The dose-response trend was statistically significant (P=0.001). All types of smoking products were strongly related to ESCC risk. The risk among previous smokers was similar to that among never-users of tobacco, even when the analysis was limited to those who quitted smoking less than 5 years before entry into the cohort.

Table 8. Age-standardized incidence rates of esophageal cancer by histology for different tobacco habits.

	Squamous cell carcinoma		Adenocarcinoma	
Tobacco habits	No.	Incidence rate*	No.	Incidence rate*
Never-users of any tobacco *	16	0.8	20	1.0
Ever-smokers †	170	4.4	83	2.2
All snus users	50	3.2	27	1.7
Smoking snus users	40	3.5	26	2.2
Never-smoking snus users	10	2.6	1	0.2

*Incidence rate per 100,000 person years, standardized to the age distribution of person-years experienced by all workers using 5-year age categories. † Snus users were excluded when analyzing the smoking strata.

Table 9. Relative risks (RR) for ESCC and EAC among male Swedish construction workers who were ever-smokers and never-users of snus, relative to never-users of any tobacco.

	Adenocarcinoma		Squamous cell carcinoma	
Tobacco habits	No.	RR (95% CI)	No.	RR (95% CI)
Never-users of any tobacco	20	Reference	16	Reference
Ever-smokers	83	2.2 (1.4-3.7)	170	5.2 (3.1-8.6)
Current smokers	68	2.9 (1.8-4.8)	161	7.6 (4.5-12.7)
Previous smokers	15	1.2 (0.6-2.4)	9	0.9 (0.4-2.0)
Smoking products*				
Cigarette only	52	2.6 (1.5-4.3)	77	4.5 (2.6-7.8)
Pipe only	8	1.1 (0.5-2.4)	62	8.3 (4.8-14.5)
Cigar only	1	1.2 (0.2-9.3)	4	5.8 (1.9-17.4)

*All smokers (both current and former smokers) were used when analyzing relative risks for different smoking products. All RRs were adjusted for attained age and body mass index

Adenocarcinoma and tobacco smoking

The risk of EAC among ever-smokers was 2.2-fold higher than among never users of any tobacco users (95% CI 1.4-3.7) (Table 9). While previous smokers had no increased risk of EAC overall, those who quitted less than five years before entry showed a relative excess close to that observed for current smokers (RR =2.1, 95% CI 0.9-4.9).

Squamous cell carcinoma and snus use

Models based on the entire cohort gave no indication of any overall increased or decreased risk for ESCC among snus users (Table 10). A restriction to smokers yielded a non-significant tendency towards decreased risk among snus users, relative to non-users, but only before the age of 70 years. This risk reduction was attenuated after adjustments for smoking variables. We observed a significant 3.5-fold excess risk (95% CI 1.6-7.6) among isolated snus users relative to never-users of any tobacco.

Adenocarcinoma and snus use

In a model that included the entire cohort, we found no increased risk of EAC among snus users (Table 10). The risk before the age of 70 years tended to be slightly below that among non-users and slightly above the risk among those who were older. In a model restricted to ever-smokers and unadjusted for smoking variables, the relative risk among snus users overall was 1.0 but it was 0.6 (95% CI 0.3-1.1) among workers who had not yet attained age 70 and the relative risk was 2.3 (95% CI 1.1-4.6) above this age. However, among never-smokers the relative risk based on nly one exposed case, tended to be markedly lower than in the reference group, but due to lack of power the estimate was unstable (RR=0.2, 95% CI 0.0-1.9).

Table 10. Association of snus use with esophageal cancer by histology among male Swedish construction workers 1971 to 1993, followed through 2004

	Squamous cell carcinoma		Adenocarcinoma	
Tobacco habit	No.	Relative risk (95% CI)	No.	Relative risk (95% CI)
Among the entire Cohort				
Non-snus user	186	Reference	103	Reference
Snus user, adjusted only for BMI and attained age	50	1.1 (0.8-1.5)	27	1.0 (0.6-1.5)
< 70 years old	28	0.9 (0.6-1.4)	14	0.7 (0.4-1.2)
≥ 70 years old	22	1.4 (0.8-2.2)	13	1.6 (0.8-3.0)
Snus user, additionally adjusted for smoking intensity	50	1.0 (0.8-1.4)	27	1.0 (0.6-1.5)
< 70 years old	28	0.9 (0.6-1.3)	14	0.7 (0.4-1.3)
≥ 70 years old	22	1.4 (0.8-2.2)	13	1.7 (0.9-3.3)
Among ever-smokers				
Non-snus user	170	Reference	83	Reference
Snus user, adjusted only for BMI and attained age	40	0.8 (0.6-1.2)	26	1.0 (0.6-1.5)
< 70 years old	23	0.7 (0.4-1.1)	13	0.6 (0.3-1.1)
≥ 70 years old	17	1.1 (0.6-1.9)	13	2.3 (1.1-4.6)
Snus user, additionally adjusted for smoking variables	40	1.2 (0.8-1.7)	26	1.3 (0.8-2.0)
< 70 years old	23	1.0 (0.6-1.6)	13	0.8 (0.4-1.5)
≥ 70 years old	17	1.6 (0.9-2.8)	13	2.9 (1.4-6.0)
Among never-smokers †				
Never-users of any tobacco	16	Reference	20	Reference
Users of snus only	10	3.5 (1.6-7.6)	1	0.2 (0.0-1.9)
< 70 years old	5	3.7 (1.2-11.4)	1	0.6 (0.1-5.0)
≥ 70 years old	5	3.1 (1.0-9.4)	0	-

† Relative risks were adjusted only for attained age and body mass index

5.4 Study IV

Association of polymorphisms of *GST genes* and risk of esophageal cancer

In the SECC study 88%, and 73%, and 84% of all eligible cases of EAC, ESCC, and cardia cancer were interviewed, respectively. Among 1128 randomly selected control subjects, 820 (73%) were also interviewed. Not all participants donated blood or agreed to participate in genetic studies. We analyzed 96 EAC, 79 ESCC, and 126 cardia cancer patients, as well as 471 controls in this study. Among controls, the frequency of the *GSTP1* variant allele was 31 percent and the genotype distribution was in Hardy-Weinberg equilibrium (p=0.2). *GSTT1* and *GSTM1* null genotypes were observed among 16% and 49% of controls. As esophageal cancer was the focus of this thesis, results on cardia cancer are not presented here (see enclosed paper for the result of cardia cancer).

Squamous cell carcinoma

We observed a 70% excess risk of ESCC (OR=1.7, 95% CI 1.0-2.8) among carriers of the *GSTP1* variant Val105 allele (both homozygote and heterozygote) (Table 11). Further stratification showed that the excess risk was mainly driven by individuals who were homozygotes for the variant allele (OR= 2.4. 95% CI 1.0-5.0). A restriction to ever-smokers unveiled a stronger significant association between *GSTP1* variant genotype and ESCC, while there was no association among non-smokers. The interaction did not attain statistical significance, though. Finally, the prevalence of the *GSTT1* null genotype tended to be lower among patients with ESCC (OR= 0.5, 95% CI 0.2-1.2) than among controls, but the inverse associations were not statistically significant.

Adenocarcinoma

There were no associations between EAC risk and the studied *GST* polymorphisms (Table 11)

Table 11. Association of polymorphisms of *glutathione S-transferase* genes with esophageal cancer, the Swedish Esophageal and Cardia Cancer Study (SECC)

		Squamous cell carcinoma		Adenocarcinoma	
Genotypes	**No. controls**	**No cases**	**OR (95% CI)**	**No. cases**	**OR (95% CI)**
All Participants					
GSTP1 wild type (Ile/Ile)	208	26	Reference	44	Reference
GSTP1 variant (Ile/Val, Val/Val)	245	52	1.7 (1.0-2.9)	50	1.0 (0.6-1.5)
Heterozygote (Ile/Val)	207	42	1.6 (0.9-2.8)	42	1.0 (0.6-1.6)
Homozygote (Val/Val)	38	10	2.4 (1.0-5.0)	8	1.0 (0.4-2.3)
GSTM1 active	230	35	Reference	52	Reference
GSTM1 null	239	42	1.3 (0.8-2.1)	43	0.9 (0.5-1.3)
*GSTT1*active	394	70	Reference	80	Reference
G*STT1* null	76	7	0.5 (0.2-1.2)	15	1.0 (0.5-1.8)
Only Smokers					
GSTP1 wild type (Ile/Ile)	126	21	Reference	31	Reference
GSTP1 variant (Ile/Val, Val/Val)	135	47	2.1 (1.2-3.9)	34	1.1 (0.6-1.8)
GSTM1 active	127	30	Reference	35	Reference
GSTM1 null	143	38	1.1 (0.6-1.9)	30	0.7 (0.4-1.3)
GSTT1 active	221	62	Reference	56	Reference
GSTT1 null	49	6	0.4 (0.2-1.0)	9	0.8 (0.4-1.7)

6 Methodological Considerations

6.1 Study Design

Cohort Studies

A cohort study is the most straightforward type of epidemiological study design, which is also termed "follow-up study" or "incidence study". In the classical cohort study, incidence of the disease of interest among the exposed group (or several cohorts based on the exposure level) is compared with the incidence in the non-exposed group or reference cohort. Several cohort studies have been designed for specific purposes to collect various information around the main research questions, e.g., "The Nurses' Health Study", "British Doctor Cohort". However, sophisticated organization and substantial financial resources for a prospective cohort has always been the main concern among researchers. Another popular cohort approach is the retrospective cohort study in which the information had already been collected, usually for administrative or health policy purposes, long before the study commences.

The long tradition of collecting information on health and social conditions of the population in Sweden has provided excellent opportunities to use such data for monitoring different diseases and social problems. For instance, the Swedish Hospital Discharge (Inpatient) Register collects clinical and diagnostic information for every patient hospitalized in Sweden (see section 4.1.1 for details). This data has been extensively used to identify cohorts of patients hospitalized for different diseases to study, for instance, cancer risk among a highly exposed group. These diseases serve as an intermediate step between the exposure and cancer. The national registration numbers (NRNs), unique personal identifiers assigned to all residents in Sweden, enables the complete follow-up of the cohort members through unambiguous record linkages with essentially complete nationwide registers, e.g. cancer, causes of death, and emigration registers (see section 5.1).

In the study of peptic ulcer (study I), we recruited a group of patients highly exposed to *Helicobacter pylori*. Using the discharge diagnosis codes we could also distinguish duodenal ulcer from gastric ulcer that are different with regards to location of the ulcer as well as their consequence on stomach function and acid secretion, hypothesized to be important for esophageal cancer risk. The association of esophageal cancer and history of peptic ulcer could be studied in a case-control study in which *H. pylori* infection could be measured directly among the cases and controls. However, interviewees could not address location of their ulcer through an interview, indicating the importance of using the register-based data in this study. This cohort accrued peptic ulcer patients since 1965, when prevalence of *H. pylori* infection as well as peptic ulcer and its complication was high. Since the effective treatment regimens are currently used to eradicate the *H. Pylori* infection and the prevalence of this infection has decreased significantly in Sweden, it would be difficult, thus, to evaluate our hypothesis in a study at the time being.

One of the reasons to use a cohort study approach is when the exposure of interest is rare. In the achalasia cohort, using the data from entire Sweden for a period of about 40 years, there were no more than 2,896 eligible achalasia patients. Table 12 shows the prevalence of achalasia in Sweden since 1980. Although it has been almost doubled

Table 12. Prevalence of achalasia in Sweden since 1980.

Calendar year	No. of achalasia patients	Population size in Sweden	Prevalence in 100,000 person-years
1980	654	8,317,937	7.9
1990	1122	8,590,630	13.1
2000	1559	8,882,792	17.6
2004	1609	9,011,392	17.9

between 1980 and 2000, the absolute number is extremely low. A very low prevalence of achalasia (exposure) and the extremely low incidence of esophageal cancer (approximately 3 per 100,000 in Sweden) indicate that studying the cancer risk in achalasia is quite challenging with other epidemiological approaches.

In study III, detailed information about tobacco was collected in the large cohort of construction workers enabled us to study the cancer risk among snus users with proper control for smoking. Although the association of snus use has been evaluated in case-control studies previously, the estimates were usually derived from multivariate modeling with adjustments for smoking. However, behavioral interaction between snus use and smoking makes the adjustment by smoking difficult and such analyses usually endured the problem of residual confounding. In order to measure independent effects of snus, comparison of non-smoking snus users with the never users of any tobacco would be the best solution. However, such data are sparse. Using the data from the construction workers cohort allowed us to study the independent carcinogenic effect of snus use properly.

Genetic association case-control studies

Both cohort and case-control designs can be used for genetic association studies. Presence or absence of a particular genetic polymorphism is perceived as exposure and the association with the outcome of interest is analyzed with the standard methods. However, collecting biological material from a large number of individuals and also difficulties in the follow-up of cohorts has shifted the attention to the case-control studies. Due to small prevalence of genetic polymorphisms among the general populations, genetic association studies usually demand a large study size. Moreover, in case-control studies, selecting population based controls representing the study base is the most important challenge in most study settings. Hospital-based controls may suffer from diseases or co-morbid conditions related to the studied gene and lead to biased results. Another important element of a good association study is a large study size. Because the chance of finding common genes with large effects is quite low, studies must be powered to detect variants that are common but have low relative risk, or are rare but constitute a higher relative risk. Table 13 presents different study sizes estimated based on various exposure prevalence and effect sizes. Studies involving at least 5000 cases are now being discussed as an essential element of biomedical research. Such research will involve huge national and international investment and incur important opportunity costs (110). In the presence of a strong gene-environment interaction, restriction of study to participants who are exposed to the environmental factor might unveil an association which could not be otherwise detected.

Table 13. Approximate sample sizes necessary to detect significant association (power=90%, two-sided α=0·001) by effect size and allele frequency for predisposing allele.

	Frequency of susceptibility allele in controls					
Allelic odds ratio	**1%**	**5%**	**10%**	**20%**	**30%**	**40%**
1·1	221 927	46 434	24 626	13 987	10 759	9505
1·2	58 177	12 217	6509	3730	2896	2581
1·3	27 055	5702	3051	1763	1380	1240
1·5	10 604	2249	1213	712	566	516
2·0	3193	687	377	229	188	177
4·0	598	134	78	52	46	47

Calculations assume multiplicative effect on disease risk (ie, homozygous susceptibility genotype has penetrance that exceeds that of heterozygote by factor γ, the genotype relative risk, and that of wild-type homozygote by γ^2). Under such model, each allele has independent effects on disease risk, and allelic odds ratio is also equal to γ. Sample sizes presented are total number of cases needed in a case-control study where controls are present in equal numbers. These sample size derivations assume best-case scenario in which susceptibility variant itself (or a perfect proxy) has been typed. *Reprinted from Hattersley, AT., McCarthy,MI.: What makes a good genetic association study? The Lancet 2005; 366 (9493):1315-23. By permission from Elsevier.*

6.2 Precision and Validity

The overall objective of epidemiological studies is to provide an accurate estimate with regards to the main hypothesis. In other words, epidemiologists strive for estimating the value of the parameter that is object of measurement with little error. Source of errors in estimation may be classified as either *random* or *systematic*. The principles of study design emerge to reduce these errors. Random errors in epidemiological studies correspond to the precision problem and systematic errors could arise through different sources, particularly selection bias, confounding and exposure/disease misclassification.

6.2.1 Precision

Precision in measurement and estimation corresponds to the reduction of random error. Increasing study size and improving efficiency are the main elements to reduce the random error (145). Due to the rarity of esophageal cancer, particularly EAC, all studies face the precision problem, especially in stratified analyses. In our register-based studies (studies I and II), especially the achalasia cohort, the small number of cases observed during the follow-up lead to wide confidence intervals and imprecise estimates. The imprecision was further marked in the stratified analyses. However, in spite of the small power, the strong effect of achalasia on esophageal cancer risk led to convincing results. Occurrence of esophageal cancer among women was extremely uncommon so the risk estimates for women were quite unstable.

In the study of snus effects (study III), the estimates among the cohort of never-smoking snus users were quite imprecise. Although the cohort size (40,932 never-smoking snus users compared to 101,959 never-users of any tobacco) and follow-up time was reasonably long in this study, the cohort was not old enough to provide sufficient power in order to study the risk of EAC. Perhaps conducting a similar study in a decade – when the cohort is older – would provide more robust estimates.

In our association study (study IV), in spite of a reasonably high allelic frequency for *GST* genes, our case-control study – the largest study that evaluated association of these polymorphisms and EAC risk to date – was still underpowered to estimate a

mild to moderate effect and especially to measure the gene-environment interaction. Further studies with larger sample sizes are needed. Because of the rarity of EAC in the world not any single research group could afford to collect a reasonably sized study to examine the genetic influence on EAC risk. Combining the data from different countries would probably be the ultimate solution.

6.2.2 Selection Bias

In the retrospective cohorts of peptic ulcer and achalasia patients (studies I and II), we used the records from the Swedish Inpatients Register; a selection bias may occur if undiagnosed cancer patients enter into the cohort before the appearance of cancer symptoms. In fact exposure or disease under study (achalasia) could be secondary to esophageal cancer. Such a cancer would be uncovered soon after the entry and lead to a biased estimate. In fact, analyses of the cancer risk in the first year of follow-up confirmed that such a presumption was a valid concern and the close surveillance of these patients because of their achalasia-like symptoms lead to an overdiagnosis of esophageal and stomach cancer in the first year after entry into the cohort (Table 9). As a standard practice, we have excluded the first year of follow-up to mitigate the influence of such a bias.

Although the SECC study (study IV) was a population based case-control study with a relatively high response rate, selection bias due to non-participation might have distorted our findings. The relatively low proportion of interviewed cases and controls who donated blood may further be a concern. A link between *GST* polymorphisms and poorer prognosis for esophageal cancer was also suggested (146, 147). Given such an association, under-recruitment of the advanced and most aggressive cancers might underestimate the prevalence of the susceptible allele among the cancer patients. However, because of a fairly rapid case ascertainment and similar distribution of demographic and exposure variables obtained at interviews of participants and non-participants, selection bias should not influence our findings notably.

6.2.3 Confounding

Dealing with confounders is an important step for the causal inference in epidemiological studies. Based on classic definition, a confounder is an extraneous variable with three necessary characteristics. A confounding factor:

1. Must be an independent risk factor for the disease.
2. Must be associated with the exposure under study in the source population
3. Cannot be an intermediate step in the causal path between the exposure and disease

The third condition has important implication in the analysis of epidemiological studies. Although statistical adjustment for an intermediate factor is not appropriate, one may consider such an approach when interested in the direct effect of the exposure on the outcome.

One of the important limitations of register-based studies is lack of information on potential confounders. However, the potential impact of confounding factors depends on their prevalence among the study population and also on the strength of their association with the outcome. Although there are quantitative methods to evaluate the possible role of confounders on the observed association (144, 148), it is, sometimes, possible to approach them qualitatively.

In study I, smoking, which is positively linked to both duodenal ulcer and EAC, could attenuate an *H. pylori*-driven inverse association between these two diseases. However, the strength of the associations of smoking with both duodenal ulcer and EAC is comparably moderate and is unlikely to have shifted a 70-80% protection (RR=0.2 to 0.3), as judged from the direct studies on *H. pylori* seroprevalence and EAC risk (36, 38, 40, 41), to a 70% increased risk found in this cohort (RR=1.7). Estimation of SIR for lung cancer (SIR=1.6) revealed that smoking was moderately linked to duodenal ulcer in this population. Ciclooxygenase inhibitors such as aspirin or some other NSAIDs are tentatively associated with both peptic ulcer and EAC (149, 150) and could also be confounders. However, since these drugs seem to protect against EAC, such confounding would tend to strengthen a true inverse association, not to cancel it.

In the gastric ulcer cohort, the combination of confounding by smoking and attenuation due to "misclassification" of the *H. pylori* status in the exposed cohort (approximately 80% infected) and the comparison population (with a mean age of 66.7 years at entry probably close to 70% were infected) could conceivably have wiped out a substantial underlying inverse association between *H. pylori* and EAC. Therefore, the absence of an association should not be over-interpreted. A caveat must be highlighted regarding the moderately positive association between gastric ulcer and ESCC, which could potentially be explained by confounding by smoking, a strong risk factor for ESCC. The differential associations for gastric ulcer and duodenal ulcer, however, somewhat disagree with such confounding as the sole explanation.

Smoking and alcohol consumption are the main risk factors for ESCC and high BMI and gastroesophageal reflux are the main risk factors for EAC. The observed association between achalasia and EAC could not be due to confounding by BMI because these patients are naturally underweight because of their disease (study II). Moreover, a high pressure at the lower esophageal sphincter makes confounding by reflux unlikely in these patients. Because there is no reason for a higher prevalence of smoking and alcohol use among achalasia patients, the strong association with ESCC was assumed to be causal. Null associations between achalasia and lung cancer – a highly tobacco-related disease – and also with oral and liver cancer that are alcohol dependent diseases, further excluded the possibility of confounding by these risk factors.

With regards to the association between snus use and esophageal cancer we made detailed analyses to shed light on possible confounding by smoking on the observed risks associated with snus use (study III). Since snus use is introduced as a risk reducing factor, smokers who have taken up snus might decrease the amount of smoking over time and thus decrease the risk of cancer. Therefore, in our analyses we performed analyses with and without adjustment for smoking variables. If the reduced smoking intensity is a causal component of association between snus use and cancer risk, the relative risk estimates without adjustment would provide a net effect of snus use. We also adjusted for smoking to provide a direct biological effect of snus. Confounding by alcohol consumption and nutritional factors for association between snus use and ESCC might still be a concern, though.

The concept of confounding in genetic association studies needs more careful evaluation. Usually there is no association between a polymorphism and environmental exposure, confounding by environmental risk factors could not be an issue. Moreover, unnecessary adjustment could only decrease the precision and widen the confidence

intervals. If the risk factors are linked to the genetic polymorphism, they would be an intermediate factor between the polymorphism and the outcome of interest. Therefore, adjustment would still be inappropriate. Population stratification is the only factor that needs to be taken into account in the analysis of genetic association studies (151). The confounding occurs when individuals are selected from two genetically different populations in different proportions in cases and controls. Thus, the cases and controls are not matched for their genetic background. This may cause spurious associations, or it may mask true associations like any other unknown confounder. Restriction of the cases and controls to native Swedes allayed the concern about confounding by population admixture in our study. Chi-square method revealed that the *GST* polymorphisms were not associated with any of the established risk factors (study IV). Therefore, we don't think that confounding by any of the major risk factor could explain the observed associations.

6.2.4 Misclassification

Misclassification, also called measurement error, is probably the most common form of bias in epidemiological studies. Misclassification refers to an error in the classification of exposure or disease under study. There are two types of misclassification, i.e., differential or nondifferential. Differential misclassification refers to error on one axis (e.g., exposure) that are related to the other axis (e.g., outcome). This type of misclassification can bias the results of a study either upward or downward. A nondifferential misclassification exists when the errors occur for instance in exposure measurement but without any relation to the outcome, or vise versa. A nondifferential misclassification biases the results towards the null and leads to underestimation of the findings.

The outcome misclassification was not a notable concern in this thesis. First, the Swedish Cancer Registry which is more than 98% complete (133) has provided the cancer diagnosis in studies I-III. Although EAC might be misclassified with gastric cardia adenocarcinoma, it has been shown that such misclassification leads to underestimation rather than overestimation of EAC (152). Moreover, in the SECC study (study IV) great efforts were made to standardize the tumor classification and, therefore, ensured a proper classification.

Peptic ulcer is classified into duodenal and gastric ulcers based on the location of the involvement (study I). The disease status was defined based on the discharge diagnosis codes (ICDs) which is usually assigned after clinical and endoscopic evaluation. However, patients may have both duodenal and gastric ulcers and the location of ulcers change between stomach and duodenum over time. To remedy such a problem, we used the data from repeat visits and excluded all such patients who were diagnosed for both gastric and duodenal ulcer at different points in time.

Although achalasia is a disease with clear clinical presentation and the diagnosis is made after a series of clinical and radiographic examinations, a few diseases like post-reflux stricture could be misclassified as achalasia. As the gastroesophageal reflux is a strong risk factor for EAC, a spurious association could occur if a considerable number of reflux patients were included in this cohort. However, our validation study allayed the concern about such a bias as only a few of the misclassified patients had a disease related to esophageal cancer.

In the study of snus and esophageal cancer (study III), we were concerned if never-smoking snus users were more inclined to take up smoking during the follow-up compared to never-user of any tobacco and, thus, the observed association was biased due to confounding by smoking. A differential misclassification of smoking in these two groups at the entry to the cohort could have distorted our results. Using the available information from the repeat visits, we found that this was a valid concern in our data. We showed that never-smoking snus users had a higher smoking prevalence (13.2%) compared to never-user of any tobacco (6.7%) in their subsequent records. However, because of low prevalence of unmeasured smoking among these individuals, smoking appeared not to be an important confounding factor for the observed association between snus use and cancer. We estimated that adjustment for this confounding could change the relative risk of ESCC among never-smoking snus users by 50% from 3.5 into 3.0. We figured out that more than 60% of these snus users needed to be smokers to elevate the relative risk from unity to the observed 3.5.

Our quality control measures for genotyping data (study IV) confirmed that exposure (genotyping) classifications are satisfactory. Moreover, the quality of interview information, including smoking, in the SECC study was also convincing (20).

7 Interpretation of Findings

7.1 *H. pylori* Infection and Risk of Esophageal Cancer

Duodenal ulcer which is linked to an antrum-predominant *H. pylori* infection leads to hyperchlorhydria. Gastric ulcer, on the other hand, is associated with a more proximal distribution of *H. pylori* infection and leads to atrophy and hypochlorhydria. A strong inverse association between *H. pylori* seropositivity, especially with cagA positive strains, and risk of EAC (36, 38, 41, 153-155) was originally assumed to be due to *H. pylori*-induced atrophic gastritis, hypochlorhydria, and reduction of acid reflux into the esophagus, the strong risk factor for EAC. However, as further investigation showed no association between gastric atrophy and EAC risk (36, 156), the postulated mechanism was challenged.

If *H. pylori* infection *per se* was responsible for the observed inverse association with EAC, both duodenal and gastric ulcer patients should have a decreased EAC risk. Contrary to this hypothesis, our study (study I) revealed a 70% excess risk for EAC among duodenal ulcer patients, while the relative risk among gastric ulcer patients was close to unity. It appears that our findings among duodenal and gastric ulcer patients are, again, more in line with the original explanation that *H. pylori* infection protects against EAC via atrophic gastritis and hypoacidity. The esophageal mucosa in individuals with duodenal ulcer is, on average, more exposed to gastric acid than that in healthy individuals (157), whereas it is likely to be less exposed among those with gastric ulcer, because of corpus gastritis and hypoacidity in these patients.

According to the old hypoacidity hypothesis, gastric ulcer patients, and those who manifest with atrophic gastritis, should be the ones mostly protected against EAC. Although we could not confirm this hypothesis in this study, the *H. pylori*-associated protection may not be faithfully reflected in the comparison with the general population who are also infected with *H. pylori* up to 60%-70%. The remaining protective effect due to 30% higher exposure prevalence in the gastric ulcer cohort might have been cancelled by confounding from smoking. An alternative explanation is that the non-surgical treatment offered to these patients may modify the inverse *H. pylori* – EAC relationship (158, 159).

Our finding on excess risk of ESCC among gastric ulcer patients are in line with the recent reports on positive associations between *H. pylori* infection (cagA seropositivity) and ESCC risk as well as the relation between gastric atrophy and risk of ESCC (36). Although confounding by smoking could explain part of the observed risk, a differential association for gastric ulcer and duodenal ulcer somewhat disagree with such confounding as the sole explanation. A postulated mechanism is that atrophic, hypoacidic stomach may provide suitable intragastric environment for bacterial overgrowth leading to generation of N-nitroso compounds (160) – a suspected key risk factor for ESCC (60).

In conclusion, our study suggested that the repeatedly confirmed strong inverse relationship between *H. pylori* seropositivity and risk of EAC does not pertain to all infections. Association between *H. pylori* and esophageal cancer follow a complex pathway, probably through mechanisms linked to the pattern of the change in gastric acid secretion. It appears as if the pattern of gastric colonization and/or the clinical

consequences in the stomach plays a crucial role. Therefore, the effect of *H. pylori* eradication on cancer risk could be positive, negative, or even null depending on biological interaction of infection with stomach function (42). Variation of *H. pylori* strains in different populations as well as host genetic factors should also be entertained.

7.2 Achalasia and Risk of Esophageal Cancer

Barrett's esophagus, a presumably obligatory precursor lesion in the development of EAC, typically develops following longstanding gastroesophageal reflux. The hypertensive LES in achalasia may lessen the probability of reflux, but the possibility of iatrogenic reflux after esophagomyotomy has been a matter of concern among surgeons, and routine prophylactic fundoplication has been advocated (161, 162). In our study only 1 out of 6 EAC patients (17%) were from the operated patient, while operated patients accounted for 33% of the total person-time observed in the cohort. Therefore, our findings do not support the surgically induced reflux as a quantitatively important mechanism of EAC occurrence in achalasia patients. Development of EAC in achalasia could be attributed to other mechanisms including:

a) Concomitant gastroesophageal reflux, existing already before the diagnosis of achalasia, may be one alternative mechanism. Several reports have suggested that esophageal motility disorders may progress from one type to another (163-165).
b) Notwithstanding the hypertensive LES in achalasia, complete and prolonged relaxation of the LES was demonstrated in the majority of 11 such patients when monitored for 24 hours (166). It was shown that reflux symptoms exist among patients with a lower pressure in the sphincter. Therefore, less severe achalasia patients are at higher risk of gastroesophageal reflux and, thus, EAC. This hypothesis is in line with our findings as less severe patients are managed with more conservative procedures than surgery and the risk of EAC was higher among unoperated patients.
c) Given the importance of acid clearance in the development of EAC (167-169), it may be hypothesized that even a minor reflux in a dysmotility-stricken esophagus increases the risk of Barrett's esophagus and EAC.
d) It has been hypothesized that chronic inflammation caused by fermentation of retained food to lactic acid within the esophagus (46, 170) may lead to metaplastic transformation of the squamous mucosa to Barrett's esophagus.
e) Adenocarcinogenetic factors may act without metaplastic transformation (20), conceivably proceeding from the submucosal glands or from islands of heterotopic columnar epithelium.

Because of a dilated esophagus, achalasia patients are highly prone to esophageal cancer. Surgical myotomy of the sphincter to remove the pressure from LES may modify the risk of ESCC. However, it may also lead to gastroesophageal reflux and, thus, increase the risk of EAC. Surgically treated patients had a higher SIR for esophageal cancer than the unoperated ones, but it is reasonable to assume that operated patients had more severe and/or long-standing disease. Moreover the difference between operated and unoperated patients did not attain statistical significance. Stratification by time since surgery demonstrated a tendency towards falling SIRs with time, contrary to the tendency for increasing SIRs in the unoperated group. Thus, our overall data give us little reason to suspect that the surgical manipulation *per se* may contribute to cancer development in achalasia patients. If

anything, it appears that esophagomyotomy might somewhat reduce the risk of ESCC in the long term.

In conclusion, while the risk assessment among women with achalasia was inconclusive due to small numbers, our study provided evidence that men with achalasia are at increased risk not only of ESCC but also of EAC through mechanisms that are yet to be determined. Investigations into the nature of these mechanisms may offer some additional general clues to the etiology of this rapidly increasing cancer. The information from this study can be used to inform patients about the risk of esophageal cancer. It also provides statistics to be used in decision making process for endoscopic surveillance. Our estimation on number needed to screen showed that 658 and 5975 is required to detect one esophageal cancer among men and women, respectively. With increasing the screening intervals to 2-3 years, the estimated numbers would decrease to 329 and 165 among men and to 3988 and 1494 among women. Therefore, if active surveillance on achalasia is recommended, our data speaks in favor of the screening program only among male achalasia patients.

7.3 Snus Use and Tobacco Smoking and Esophageal Cancer Risk

Analysis of the large cohort of the Swedish construction workers with a long and essentially complete follow-up confirms the well-established link between smoking and all major types of gastroesophageal cancer (study III). This study also provided new data suggestive of snus-associated carcinogenic risks.

The observed association between tobacco smoking and both major histological types of esophageal cancer, in particular ESCC, is in good accordance with a fairly consistent previous reports (171-177). It appeared as if pipe smoking was more strongly related to the risk of ESCC, but not EAC, than were other types of smoking habits. We noted that after smoking cessation; the risk of both major histological types of esophageal cancer fell to the unexposed level within 5 years of quitting, suggesting late stage carcinogenic effects of smoking, but this finding is at odds with the conclusion of a recent review stating that the risk remains elevated for at least 10 years (178). It must be emphasized that our analyses of time since cessation were based on small numbers of both ESCC and EAC; hence, we cannot confidently rule out the role of chance.

Never-smoking snus users had a substantially increased risk of ESCC when compared to never-users of any tobacco. On the other hand, these snus users had a reduced risk of EAC. There was a non-significant tendency for a lower risk of ESCC among smokers who also used snus, but the purported harm reduction by snus use (79, 179, 180) did not impress overall. A randomized intervention trial is required to confidently refute the hypothesis of important harm reduction by snus use. Generally, adjustment for smoking variables in analyses that also included smokers changed the relative risk estimates surprisingly little. The main reason is that the proportions who reported being or having been smokers at entry were almost identical among users (56.8%) and non-users (57.8%) of snus. Hence, based on the smoking information obtained at entry, the scope for confounding was limited to the observed variation among smokers with regards to smoking dose, smoking status (current or ex-smoker), and type of smoking tobacco. If this information did not reflect the relevant smoking exposure status, either because of erroneous reporting at entry or due to subsequent changes in habits (differential or non-differential), residual confounding by smoking might be a concern.

The observed departure from the proportional hazards assumption in our analyses forced us to stratify our analyses by attained age (below and above age 70). This suggests effect modification by age. The relative risks tended to be higher among workers who were older than 70, compared to those who were younger, consistent with a very long induction time. The oldest were also most exposed to snus from earlier parts of the 20th century. Such snus contained higher levels of carcinogenic tobacco-specific nitrosamines compared to the snus sold today (181).

If it is assumed that the relative risks observed among never-smokers constitute best available estimate of the true effects of snus, and that the observed associations are causal, population attributable risk (182) for esophageal squamous cell carcinoma, i.e., the proportion of all such tumors that is attributable to snus use in this highly exposed population of Swedish construction workers, could be as high as 15%.

Although some uncertainty remains regarding the causality and the strength of the association as well as the generalizability to other populations than Swedish men, we conclude that Scandinavian snus cannot be considered a safer alternative to smoking and should not be marked as a means of harm reduction until strong evidence is able to refute its carcinogenicity.

7.4 *GST* Polymorphisms and Risk of Esophageal Cancer

Neither the *GSTM1* or *GSTT1* deletion polymorphisms, nor the *GSTP1* Ile105Val SNP was associated with risk of EAC (study IV). On the other hand, our data suggested a positive association between the presence of the variant *GSTP1* Val^{105} allele and the risk of ESCC, with the highest relative risks seen among homozygous carriers and among smokers. To our knowledge, this was so far the largest study that evaluated the associations of *GST* polymorphisms and EAC. Strengths of this study include the population based design in an ethnically homogenous native Swedish population, comprehensive face-to-face interviews with all cases and controls, rapid case ascertainment designed to capture all incident cases – not only surgically treated ones – and prospective careful classification of every incident case.

Although the previous meta-analysis concluded that there is no association between *GST* polymorphisms and the risk of esophageal cancer (112), the difference between EAC and ESCC as well as the heterogeneity between different populations should be acknowledged. Table 14 present the results of the pooled estimates (random effect) of the all previously publish reports on associations between *GST* genes for EAC and ESCC exclusively (see section 2.4. for the individual risk estimates).

Five previous studies that analyzed EAC included 158 patients (range 9 to 56 cases), excepting one Indian study the rest were based on Western Caucasian populations. The only significant findings were a 4.6-fold risk of EAC among Dutch carriers of the variant *GSTP1* Val^{105} allele, and a 90% risk reduction among French individuals who had the *GSTT1* null genotype (128, 129). Although all studies of the *GSTP1* Ile105Val polymorphism and EAC risk showed relative risk estimates above one, the Dutch result appears to be an outlier. As the relative risk was high also for ESCC in the Dutch study, albeit not statistically significant, it is conceivable that the blood donors who served as controls may not have been entirely representative of the population that generated the cases. In the French study, there was no indication that the *GSTP1* Val^{105} allele is associated with an increased risk of ESCC. The risk reduction for EAC observed

Table 14. Pooled estimates of the published studies on GST polymorphisms in relation to esophageal cancer, including our study.

Polymorphism	Squamous cell carcinoma	Adenocarcinoma
GSTM1 null genotype*	1.1(0.8-1.6.)	1.0 (0.7-1.3)
GSTT1 null genotype*	0.9 (0.8-1.1)	0.7 (0.4-1.3)
GSTP1 Ile/Val Val/Val*	0.9 (0.6-1.3)	1.4 (1.0-2.0)
Ile/Val	0.9 (0.7-1.3)	1.3 (0.7-1.7)
Val/Val	1.0 (0.5-2.1)	1.3(0.6-2.7)

*Null genotypes of *GSTM1* and *GSTT1* are compared with the corresponding active genotypes and wild genotype (Ile/Ile) was the reference group for analyses of *GSTP1*.

among French people with the *GSTT1* null genotype was driven by an unusually low allele frequency among the cases, and since the number of cases was no more than 26, chance could have played a role, despite the statistical significance. Had the 27th case, whose genotyping failed, had the null genotype, the 95% Confidence Interval would have been 0.05-1.02 and statistically non-significant. The other studies did not provide support for a protective effect of *GSTT1* null genotype.

Hence, the overall results of previous studies on *GST* polymorphisms and EAC risk, with the exceptions noted above, are well in line with our negative results. However, our and previous studies alike were insufficiently powered to detect moderately increased relative risks. As opposed to our own data, a weak overall tendency among Caucasians towards an increased risk for EAC – not ESCC – was noted, though. In fact, when pooling the raw data from the present study and the previous four studies on the association between the *GSTP1* Val^{105} genotype in Caucasian populations, the odds ratio is statistically significant for both EAC (OR=1.5; 95% CI 1.1-2.0) (115, 128-130) and ESCC (OR=1.6; 95% CI 1.1-2.4) (128, 129). This is to be compared to the OR: 1.01 in the published meta-analysis encompassing seven mainly Asian studies (112) and also our pooled estimate from the all previously published reports (Table 14). Thus – with the reservations voiced above – it appears that among Caucasians, carriage of the *GSTP1* Val^{105} allele might be associated with increased risks for both ESCC and EAC, despite our negative result regarding the former.

Notwithstanding that the positive association between *GSTP1* Val^{105} and EAC was not supported by the combined Asian experiences, and the data from the few and small individual studies in Caucasian populations are seemingly inconsistent, the significantly elevated pooled relative risk estimates for both histological types of esophageal cancer may be biologically plausible. *GSTP1* is expressed in the esophagus, and the *GSTP1* Ile105Val polymorphism is functional so that the catalytic efficiency of the variant enzyme for 1-chloro-2,4-dinitrobenzene is about 3- to 4-fold lower than the wild type protein (183). On the other hand, the variant enzyme more effectively metabolizes the diol epoxides of polycyclic aromatic hydrocarbons.

Since results in the published literature have been conflicting, more epidemiologically rigorous population based studies are warranted, particularly in the Western populations.

8 Conclusions

- The repeatedly confirmed strong inverse relationship between *H. pylori* seropositivity and risk of EAC does not pertain to all infections. Association of *H. pylori* and esophageal cancer follows a complex pathway, probably through mechanisms linked to the pattern of gastric acid secretion. It appears as if colonization of *H. pylori* and/or its clinical consequences in the stomach play crucial role.

- Men with achalasia are at increased risk not only of ESCC but also of EAC. We found little reason for suspecting that the surgical manipulation *per se* may contribute to cancer development in achalasia patients. If anything, it appears that esophagomyotomy might somewhat reduce the long term risk of ESCC.

- Smoking increases the risk of both ESCC and EAC. There is a positive association between snus use and the risk of ESCC. Although some uncertainty remains regarding the causality and the strength of the association as well as the generalizability to other populations than Swedish men, we conclude that Scandinavian snus cannot be considered as a safer alternative to smoking and should not be marked as a means of harm reduction until strong evidence is able to refute its carcinogenicity.

- Carriage of the variant *GSTP1* Val^{105} allele may be associated with the risks of both ESCC and EAC among Caucasian populations. There was a tendency for interaction between the variant *GSTP1* Val^{105} allele and tobacco smoking vis-à-vis the risk of ESCC.

9 Future Studies

Although numerous epidemiological studies have evaluated the etiology of esophageal cancer, several questions remain obscure. The results of our studies give rise to several hypotheses that need to be evaluated.

Further studies are needed to explain the reasons for the reported associations between *H. pylori* infection and the risk of esophageal cancer. In general, the reasons for the sharp geographic distribution of esophageal cancer as well as the large variation within the high risk areas remain mysterious. Investigations on the role of genetic factors, particularly their interaction with the environmental risk factors, the association between the risk of ESCC and *H. pylori* which is a highly prevalent infection in the high risk areas, may provide some opportunities to explore the major risk determinants for the ESCC risk.

Although several insights have been gained in the past 10 years with regard to the risk factors for EAC, the reasons for the current "epidemic" in Western populations is not explained yet. Has this epidemic taken on in the Asian esophageal cancer belt? And if not, why? It appears that the interplay between *H pylori* infection, other microbial or other environmental risk factors, and risk of EAC should be high on the agenda in the next 10 years to come. Future studies need to be large enough to allow studies on the interaction between the infection with other environmental factors, as well as with the host genetic factors. In addition to the consequence of *H pylori* on the stomach function, studies on different *H. pylori* strains and the host genetics could uncover the underlying mechanism of the *H. pylori*-esophageal cancer link.

The reason for the strong male predominance in the incidence of esophageal cancer has hardly been studied. The up to three time higher incidence of ESCC among male may be attributed to the higher prevalence of alcohol and smoking among males. However, due to the almost similar prevalence of the main risk factors of EAC including high BMI, gastroesophageal reflux and even Barrett's esophagus in both genders, the reason for the strikingly more than 6-fold higher incidence of EAC among males than among females remains to be answered. Although hormone-related factors did not appear as important determinants in the few published studies, almost all previous studies lack sufficient power. In order to uncover a moderate main effect of sex hormones as well as their interaction with other risk factors, much larger studies are needed.

A few studies have evaluated the health-related effects of snus use. Many more and larger studies are needed before marketing snus as a safer alternative to smoking. One also needs to bear in mind that snus has been a traditional habit among Swedes and introduction of snus in other nations may lead to quite different patterns of health hazards; people may respond differently to snus use which will be introduces as a new product with a prestigious popularity. Moreover, as the addiction to snus use is consistent – even more than smoking – clinical trials are needed to compare snus use with several other strategies, before any firm conclusion can be drawn about the risk reducing effect of snus among smokers.

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