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Iron as a Risk Factor for Cancers of the Gastrointestinal Tract

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Iron as a Risk Factor for Cancers of the Gastrointestinal Tract

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Abstract

Background: Iron is a pro-oxidant and involved in inflammatory response and endogenous nitrosation. It is a candidate modifiable risk factor for cancers of the gastrointestinal (GI) tract.

Methods: A gene-disease systematic review was conducted to investigate associations between polymorphisms in iron metabolism genes, including *HFE*, and GI tract cancers. Data from a case-control study of dietary risk factors and esophageal adenocarcinoma (EAC) were analysed to investigate the association between *HFE* variants and EAC and reported intakes of red meat, iron (total and heme-bound) and EAC.

Results: The results of the systematic review did not support an association between *HFE* variants and colorectal neoplasia. No studies were identified that investigated genetic variants in iron metabolism genes and EAC. The secondary case-control analyses found no association between *HFE* variants and EAC. The analyses provided evidence suggestive of an association between intake of heme iron and EAC.

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List of Abbreviations & Definitions

ATNCs	apparent total N-nitroso compounds
BE	Barrett's Esophagus
BMI	body mass index
CLE	columnar-lined esophagus
CO	carbon monoxide
CP	ceruloplasmin gene
Cu	copper
DMSO	dimethyl sulphoxide
DMT1	divalent metal transporter-1
DOM	Diagnotisch Onderzoek Mammacarcinoom
EAC	esophageal adenocarcinoma
ESCC	esophageal squamous cell carcinoma
FAP	familial adenomatous polyposis
Fe	iron
FFQ	food frequency questionnaire
FPN1	ferroportin disease, HH Type 4
GERD	gastroesophageal reflux disease
GI	gastrointestinal
HAMP	Hepcidin gene (juvenile hemochromatosis, HH Type 2B)
HEPH	Hephaestin
HFE	hereditary hemochromatosis Type 1
HH	hereditary hemochromatosis
HJV	Hemojuvelin gene (juvenile hemochromatosis, HH Type 2A
HMOX1	Heme-oxygenase-1
HNPCC	hereditary non-polyposis colon cancer

List of Abbreviations and Definitions (continued)

MMPs	Matrixmetalloproteinases
MQ	main questionnaire
NOCs	N-nitroso compounds
NSAIDs	non-steroidal anti-inflammatories
OR	odds ratio
OR_{joint}	odds ratio from joint effects parameterization
RE	reticuloendothelial
RERI	relative excess risk due to interaction
ROS	reactive oxygen species
RR	relative risk
s.d.	standard deviation
SAQ	self-administered questionnaire
SCCE	squamous cell carcinoma of the esophagus
SF	serum ferritin
SNP	single nucleotide polymorphism
TCAG	The Centre for Applied Genomics
TF	transferrin gene
TfR2	transferrin receptor-2 gene
TFRC	transferrin receptor-1
Tfsat	transferrin saturation = (serum iron/TIBC)*100
TIBC	total iron binding capacity (a measure of the sites available for iron binding on transferrin)
UIBC	unsaturated iron binding capacity – another name for TIBC
VEGF	vascular endothelial growth factor
Wt	"wild-type" – major allele for a given polymorphism

Chapter 1: Rationale & Objectives

1.1 Rationale

Interest in the role of iron in cancer pathogenesis (reviewed by Huang, 2003) was initially stimulated by the observation in the late 1950's that malignancies developed in rats injected with an iron oxyhydroxide complex. Iron can react with H_2O_2 by a Fenton-type reaction to produce reactive intermediates and then the hydroxyl radical ($OH^{\cdot}$). Iron supplementation with oral ferrous sulphate has been observed to increase free radical production in the colon of healthy volunteers (Lund et al., 1999). Reactive oxygen species, in particular the hydroxyl radical, can directly produce oxidative damage to DNA (Meneghini, 1997). Recent studies have suggested that heme iron in particular may be involved in endogenous nitrosation, increasing the formation of carcinogenic N-nitroso compounds (NOCs) in the gastrointestinal tract (Kuhnle et al., 2007; Lunn et al., 2007; Lewin et al., 2006; Bingham et al., 2002; Cross et al., 2003; Sesink et al., 2001). Studies in rats have also provided evidence that iron overnutrition in combination with gastroesophageal reflux may contribute to the development of adenocarcinoma of the esophagus (Chen et al., 1999, 2000).

Consistent inverse associations between dietary intakes of vegetables rich in antioxidants and cancers also stimulated interest in the role of oxidative stress in cancer etiology. Bonassi et al. (2007) recently reported an association between oxidative damage to DNA and risk of cancers of the gastrointestinal tract. Intracellular and intraluminal levels of

oxygen-containing radicals are determined to a large extent by the balance between antioxidants, such as carotenoids, and pro-oxidants, such as free iron (Fe), "labile" iron (loosely bound with citrate or other low molecular weight ligands) and heme iron. The α-Tocopherol and β-Carotene (ATBC) trial was a randomized, primary prevention trial conducted among white male smokers in Finland to determine whether daily supplementation with the antioxidants β-carotene and vitamin E reduced the incidence of lung cancer. Contrary to expectations, assignment to take supplements was associated with an increased risk of lung cancer. The trial results also did not support the use of these supplements to reduce the risk of cancers of the GI tract, a secondary outcome of the trial (Wright et al., 2007; Malila et al., 2002; Albanes et al., 2000). In addition, another antioxidant supplement trial among smokers and asbestos workers, the Beta-Carotene and Retinal Efficacy Trial (CARET), was stopped due to evidence for an increase in risk of lung cancer and cardiovascular adverse events in the intervention arm (Bjelakovic et al., 2008; Omenn et al., 1996a,b). Considerable debate has followed the findings of these trials and a rekindling of interest in other micronutrients such as iron, as potentially modifiable determinants of oxidative stress and cancer development.

Cancers of the gastrointestinal (GI) tract represent a major burden of disease. Colorectal cancer, the most common of these cancers, is the third leading cause of death among men and women globally (WCRF/AICR, 2007). Approximately 80% of cancers of the colorectum are sporadic and arise through the adenoma-dysplasia-carcinoma sequence (Cappell, 2008; Dionigi et al., 2007). Some of these sporadic cancers may arise from serrated polyps, a type of hyperplastic polyp (Jass, 2007). Known genetic syndromes

such as familial adenomatous polyposis (FAP) and hereditary non-polyposis colon cancer (HNPCC) constitute about 5-6% of all colorectal cancer cases. FAP results from a germline mutation in the *APC* gene and HNPCC from mutations in DNA mismatch repair genes. The remaining 15% or so of colorectal cancer cases have a clear familial component of unknown origin (Dionigi et al., 2007). Earlier diagnosis, improved staging and advances in treatment techniques have led to a more favourable prognosis for colorectal cancer in recent years (Wang and Wasan, 2008) with a five year survival rate of about 55% in industrialized countries (Little and Sharp, 2008).

The incidence of stomach cancer has been decreasing for 40 years but it is still the fourth most common type of cancer worldwide, the second most common cause of cancer mortality worldwide (WCRF/AICR, 2007). The decrease in recent years is due in part to the treatment of *Helicobacter pylori* infection (Tsuji et al., 2006; Danesh, 1999). The advent of home refrigeration reducing the need to consume foods preserved by pickling or salting may have contributed to the decrease over the longer-term (Howson et al., 1986). Incidence rates of gastric cardia cancer have shown an increase in recent years while non-cardia cancer has decreased suggesting a different etiology for the two sites (Corley and Kubo, 2004; Devesa et al., 1998).

Esophageal cancer is the eighth most common cancer but the sixth most common cause of cancer mortality (WCRF/AICR, 2007). The prognosis for esophageal cancer is poor with 5-year survival rates <15 % (Baquet et al., 2005). While the overall incidence of esophageal cancer is steady, esophageal adenocarcinoma (EAC) is the most rapidly

increasing type of cancer in industrialized countries with a 300 – 400% increase in incidence since 1974, predominantly in white men (Bosetti et al., 2008; Devesa et al., 1998). The reason for this increase is not clear. The squamous cell (ESCC) form is more common in blacks and white females and, overall, is decreasing in incidence (Baquet et al., 2005; Trivers et al., 2008) possibly due to a decrease in tobacco use (Trivers et al, 2008). In the early 1980's, ESCC represented more than 85% of esophageal cancer cases. By 2000, it accounted for less than 40%.

Epidemiological studies investigating associations between body iron levels and gastrointestinal tract cancers are hindered by uncertainties in exposure assessment (Milman, 2006). For example, serum ferritin, the most commonly used index for iron stores, is influenced by acute-phase processes (Hallberg and Hulthén, 2003; Gabay and Kushner, 1999), chronic inflammatory conditions (Ganz, 2005; Andrews, 2004) and progression of the disease process itself. It also exhibits intraindividual variations on short time scales (Cooper and Zlotkin, 1996). The investigation of germline genetic variants that are associated with elevated levels of iron, using a Mendelian Randomisation approach (Nitsch et al., 2006; Davey-Smith and Ebrahim, 2003; Little and Khoury, 2003; Katan, 1986), may represent a means to circumvent the measurement issues encountered with biochemical measures of iron. The best characterized genetic variants associated with high body iron are polymorphisms in the *HFE*-gene (6p21.3) (Beutler and Beutler, 2004). Several studies in recent years have investigated the association between *HFE* variants and cancers at different sites in the body. However,

there has been no attempt to assess the overall evidence for an association in relation to GI tract cancers.

Men typically consume larger amounts of heme-containing red meat than women and access to iron-containing vitamin and mineral supplements is largely uncontrolled in North America. More than 15% of men in the United States report use of iron-containing supplements, predominantly in the multivitamin form (Blanck et al., 2005). In Canada and the United States, the current recommended daily allowance of iron is 8 mg Fe/d for men over the age of 19 and women over the age of 50. In the early 1970's, the typical Canadian diet was estimated to contribute between 15 and 20 mg Fe/d (Méranger and Smith, 1972; Kirkpatrick, 1977). In a large population-based study of men in North America, 21.4%, 26.0% and 19.4% of white, black and Native American participants, respectively, had serum ferritin levels consistent with iron overload (HEIRS Study; Barton et al., 2006; Barton et al., 2005). Food-sufficient middle-aged and older men represent a sub-population potentially at risk of iron over-nutrition.

Esophageal adenocarcinoma (EAC) has the most rapidly increasing incidence of all types of cancer among men in industrialized countries. By the time EAC is diagnosed, prognosis is poor. EAC has recognized pre-cursor conditions, namely gastroesophageal reflux disease (GERD) and Barrett's Esophagus (BE), presenting the opportunity for prevention. Results from studies of an animal model suggest that iron over-nutrition may play a role in the development of this disease (Chen et al., 1999, 2000). Iron is important in determining levels of oxidative stress, may influence chronic inflammatory

processes and, in the heme-bound form, may affect levels of endogenous nitrosation. There is a strong biological rationale for investigating the role of iron stores and iron intakes in the development of EAC to attempt to identify modifiable environmental risk factors for progression to disease.

1.2 Objectives

The goal of this thesis was to examine the hypotheses that increased iron intake and elevated body iron are associated with the risk of developing EAC. This was addressed by means of a systematic literature review of genetic variants putatively associated with body iron levels and types of GI tract cancer and analysis of a case-control study concerning the etiology of EAC.

More specifically, the objectives were:

1. To investigate the associations between polymorphisms in iron metabolism genes, including the *HFE*-gene (Type I hereditary hemochromatosis), and cancers of the gastrointestinal tract through a gene-disease systematic review with meta-analysis, if appropriate. The systematic review included the complete gastrointestinal tract as it was considered unlikely that many studies would be available for EAC alone.

2. To investigate the association between iron and esophageal adenocarcinoma (EAC) through analysis of data from a multisite case-control study of dietary risk factors and esophageal adenocarcinoma (EAC) addressing the research questions:
    a. Is there an association between reported dietary intakes of red meat, iron (total and heme-bound) and EAC?
    b. Is there an association between *HFE*-gene variants and EAC?

1.3 Guide to the Thesis

As this thesis differs from a traditional thesis structure, a brief guide is included here. Chapter 2 of this thesis provides background information on iron metabolism, more detail on the biological rationale for the involvement of iron in cancer pathogenesis and a literature review of epidemiological studies of the association between iron and cancer. Chapter 2 also includes a section on genes involved in iron homeostasis - background information gathered to identify genes and variants for inclusion in the systematic review in Chapter 3 and to identify variants of most use for applying a Mendelian randomisation approach for iron stores in the EAC study. Chapter 3 includes the methods, results, evidence synthesis and summary of the systematic review. Chapter 4 includes the methods, results, discussion and conclusions for the secondary case-control analyses. Chapter 5 is a summative of the conclusions and main points of discussion from Chapters 3 and 4 leading into a discussion of the public health implications of the research and future directions.

Chapter 2: Background

2.1 Introduction

Iron is a transition metal essential for cellular processes due to its ability to accept and donate electrons. It is critical for energy production as an electron acceptor/donor in cytochromes and also as a cofactor or component of cellular enzymes such as catalase and hydrogenases (Chua et al., 2007; Nemeth and Ganz, 2006). Ribonucleotide reductase, an enzyme that regulates the availability of DNA precursor deoxyribonucleotides (Herrick and Sclavi, 2007; Le and Richardson, 2002), is iron dependent. Iron is required for a cell to move from the G1 to the S-phase of cell division (Bohnsack and Hisrchi, 2004). Intracellular levels of iron regulate the expression of molecules such as p53, HIF-1α, p21 and c-myc that are critical in the cell cycle and cellular proliferation (Le and Richardson, 2002). Iron also plays a role in immune response and host-pathogen interaction (Maggini et al., 2007; Wintergerst et al., 2007; Doherty, 2007). As a central component of hemoglobin, heme-bound iron plays a key role in the transport of oxygen to body tissues.

Epidemiological studies have found positive associations between red and processed meat and cancers of the gastrointestinal (GI) tract (Gonzalez et al., 2006; Norat et al., 2005; Sandhu et al., 2001; Mayne et al., 2001). For the colorectal region there are differences in the magnitude of some diet-disease associations by anatomical subsite (Little and Sharp, 2008). For example, meta-analysis of 15 prospective studies showed a somewhat higher association between reported red meat intake and cancer of the rectum

than cancer of the colon (Larsson and Wolk, 2006). The WCRF/AICR (2007) systematic review also found limited evidence for an association between cancer of the stomach and reported intakes of processed, smoked, grilled or barbecued meats. Limited evidence, mainly from retrospective studies, exists for positive associations between reported red and processed meat intakes and esophageal adenocarcinoma (WCRF/AICR 2007; Gonzalez et al., 2006; Jakszyn and Gonzalez, 2006; Mayne et al., 2001). Meat is the source of a number of compounds that have exhibited carcinogenic potential in animal studies including heterocyclic amines (Ito et al., 1999), polycyclic aromatic hydrocarbons (IARC, 1998) and nitrosamines (Lijinsky, 1999), but it is also the main dietary source of the more bioavailable form of iron – heme-Fe.

This chapter provides a brief overview of iron homeostasis, the mechanisms through which iron may play a role in cancer pathogenesis and a narrative review of the current epidemiological evidence for associations between dietary iron intakes, body iron levels and cancers of the GI tract. Since variants in genes involved in iron metabolism and uptake are possible proxies for body iron levels, an overview of genes involved in iron uptake and homeostasis is also included.

2.2 Iron Homeostasis

There are several excellent and recent reviews on the topic of iron homeostasis and metabolism (Nemeth and Ganz, 2006; Chua et al., 2007; Nemeth, 2008; Mackenzie et al., 2008). Although the body load of iron varies with age, gender, health status and dietary habits, the adult body contains on average 2 – 4 g of iron (Nemeth and Ganz, 2006). Diet is the major source of iron. Iron consumed in drinking water represents a minor contribution (M. Deveau, Health Canada, *personal communication*). Figure 2.1 is a compartmental diagram of iron uptake, distribution and losses in a healthy adult. Approximately two-thirds of body iron resides within erythrocyte hemoglobin of which about 1% is recycled daily (Ganz, 2008; Griffiths, 2007). The remainder is in the form of myoglobin in muscles or enzymes (0.3-0.4g), stored in the liver hepatocytes as ferritin or hemosiderin or found within macrophages of the reticuloendothelial system. Only about 3mg resides as transferrin-bound iron (TBI) in the serum. Transferrin (Tf) is the main plasma protein responsible for the transport of iron within the body. Transferrin saturation levels normally range from 25-35% (Kontoghiorghes and Kolnagou, 2005). A very small amount of iron resides in a labile, low-molecular weight iron pool. In adult men, uptake of 1-2mg per day is required to balance minor losses due to cell shedding. Women of childbearing age have higher losses due to menstruation and require higher daily intake to maintain iron balance as do pregnant and breast-feeding women. The recommended daily intake of iron is 8mg/day for men and women over 50 and 11 mg/day for women of child-bearing age.

Figure 2.1. Iron homeostasis in a healthy adult male

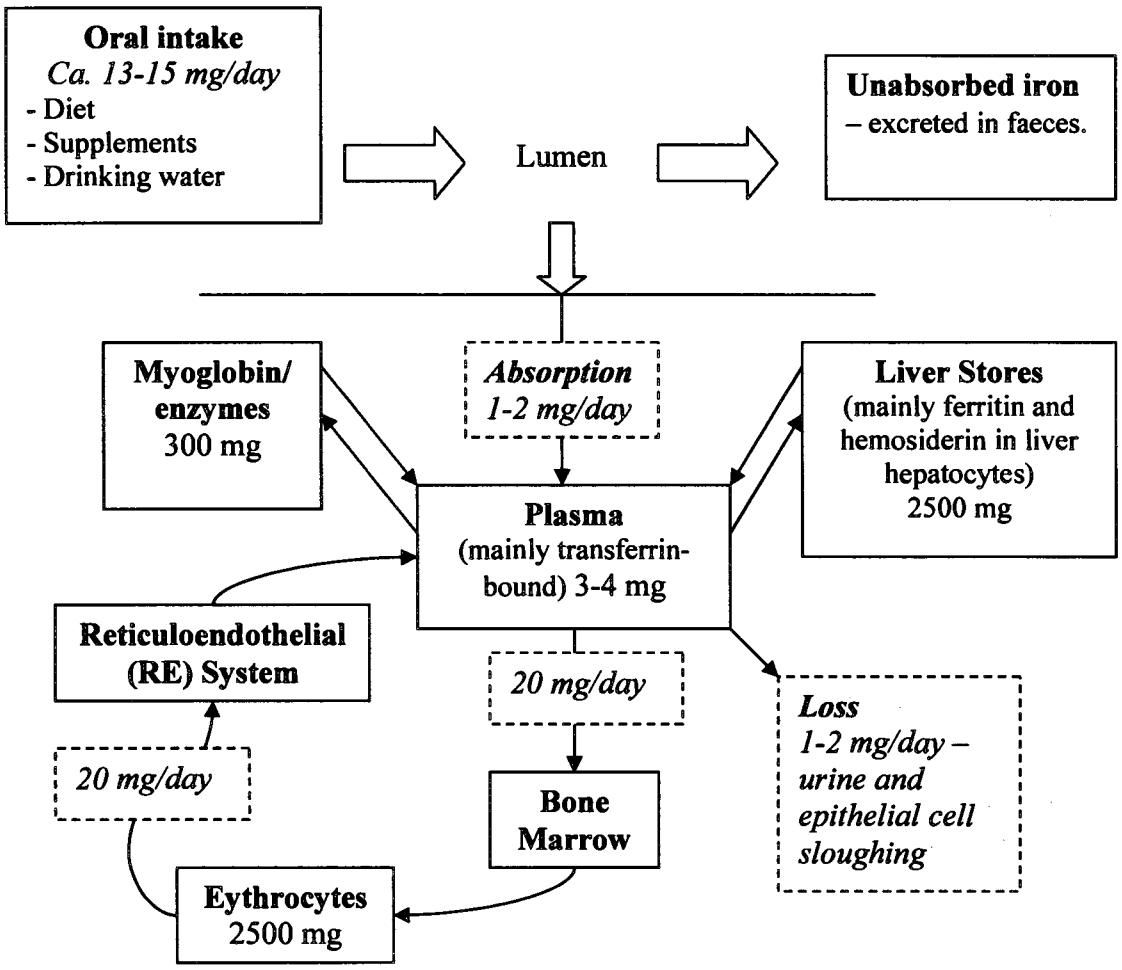
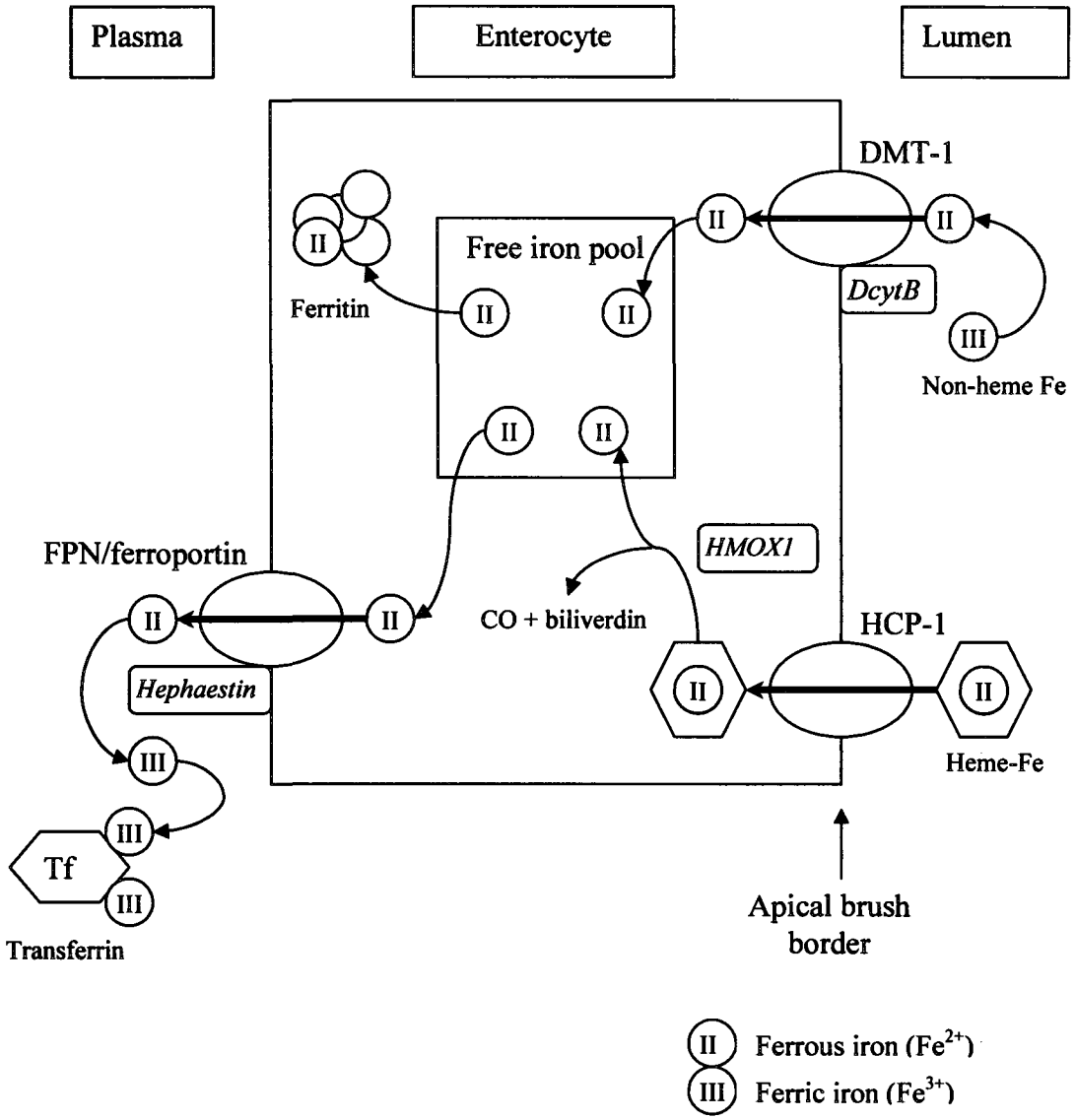


Figure 2.2 Uptake of heme and non-heme iron by duodenal enterocytes



2.2.1 Iron Uptake

Iron uptake in the duodenum is the main mechanism by which total body levels of iron are regulated. As shown in Figure 2.2, iron can be taken up in both the non-heme and heme-bound forms. Ferric iron ion (Fe (III)) is reduced to ferrous iron ion (Fe (II)) by duodenal cytochrome b (DcytB), a reductase, at the apical brush border. It is then transferred into the enterocyte through divalent metal transporter-1 (DMT-1), a transmembrane ion pump. The bioavailability of non-heme iron in the GI tract depends on other dietary components such as phytate, calcium and tannins (Monsen and Balintfy, 1982). Uptake is usually about 2% in iron replete individuals but can be as high as 20% in iron-deficient individuals consuming other dietary factors that enhance iron absorption such as vitamin C and meat protein. Despite the increasing understanding of factors influencing non-heme iron bioavailability, predicting iron absorption from mixed meals remains a challenge (Beard et al., 2007). Heme iron exhibits higher bioavailability (15-35%) and tends to be the primary source of dietary iron. Its bioavailability is less dependent on other dietary components (Monsen and Balintfy, 1982; Carpenter and Mahoney, 1992; Reddy et al., 2006). Heme uptake may occur through the recently discovered heme transporter (HCP1; Shayeghi et al., 2005) and/or through heme receptor-mediated endocytosis (West and Oates, 2008). Once taken up, heme is catabolised in the enterocyte through the action of heme-oxygenase-1. Whether heme degradation within the enterocyte is the rate-limiting step for heme iron absorption is not clear (West and Oates, 2008).

Recent studies indicate that hepcidin, an antimicrobial polypeptide produced predominantly in the liver (Park et al., 2001), plays a pivotal role in regulation of iron uptake in the duodenum (Nicolas et al., 2001, 2002; Oates and Ahmed, 2007; Nemeth and Ganz, 2006; Vyoral and Petrak, 2005). Higher hepcidin levels reduce the uptake of iron in the duodenum while lower hepcidin levels lead to an increase in iron uptake. Hepcidin binds with ferroportin, the only known iron exporter (Figure 2.2), leading to the migration of ferroportin away from the cell membrane and its subsequent degradation. Iron is thus retained within the enterocytes as it can no longer be transferred to the plasma. The higher level of iron within these cells leads to a decrease in iron uptake from the duodenum. Results from animal studies and *in vitro* experiments suggest that alcohol may suppress the production of hepcidin leading to an increase in uptake of iron in the duodenum (Flanagan et al., 2007; Harrison-Findik et al., 2006) – a possible explanation for the positive association between indices of body iron levels and consumption of alcohol (Harrison-Findik, 2007; Kowdley, 2007; Ioannou et al., 2004).

2.2.2 Iron Distribution

Hepcidin is also important in determining the distribution of iron throughout the different body compartments through its regulation of iron efflux from hepatocytes and macrophages. Although considerable evidence now exists for the central role of hepcidin in iron homeostasis, the details of the signaling circuitry regulating its production are not well-characterised (Beutler, 2006; Nemeth and Ganz, 2006; Nemeth, 2008; Babitt at el. 2006). HFE, TFRC (transferrin receptor-1), TfR2 (transferrin receptor-2) and HJV (hemojuvelin) have been implicated, potentially involving bone-morphogenetic proteins

and SMAD4 (Nemeth, 2008; Milward et al., 2007; Babitt et al., 2006). The HFE/TFRC complex and TfR2 are thought to be iron sensors and diferric transferrin as the signal regulating hepcidin production, although hypoxia may also be involved. Erythropoietic activity may also regulate hepcidin expression (Oates and Ahmed, 2007; Pak et al., 2006).

Hepcidin also appears to be a mediator of the hypoferremic inflammatory response, potentially a host defense mechanism to limit the amount of iron available to invading pathogens (Vyoral and Petrak, 2005; Andrews, 2004). Hepcidin production increases in response to elevated levels of interleukin-6 (IL-6) associated with the inflammatory response (Nemeth et al., 2004). The increase in hepcidin leads to sequestration of iron into the reticuloendothelial (RE) system and liver and results in a lack of iron available for hemoglobin and erythrocyte production. The biomarker potential of hepcidin in urine and other body fluids is receiving attention as a means to distinguish between iron deficiency anemia and the anemia resulting from chronic disease ("anemia of chronic disease"; Andrews, 2004).

2.2.3 Iron Losses

In healthy men iron losses are small and occur only through sloughing of epithelial cells from the gut. There is some evidence that excretion of iron in the urine may occur under conditions of iron overload (Kontoghiorghes and Kolnagou, 2005). Blood loss for other reasons, such as occult bleeding in the gastrointestinal tract or blood donation, alters the

amount of iron required from the diet. In women of child-bearing age, iron losses due to menstruation and childbirth greatly increase the amount of iron required.

2.3 Iron and Cancer: Possible Mechanisms

There are several plausible and inter-related mechanisms through which iron may contribute to the carcinogenic process (Huang, 2003; Valko et al., 2006): contributing to oxidative stress, influencing response to inflammation and hypoxia and, for heme iron, enhancing endogenous nitrosation. These mechanisms are described in more detail below.

2.3.1 Iron and Oxidative Stress

Iron is essential to many cellular processes due to its redox properties. If loosely bound to citrate or other low molecular weight ligands ("labile" iron) or in the free ionic form, it can influence ambient levels of reactive oxygen radicals. Respiratory cellular processes in the mitochondria, activated macrophages, cytochrome P450 metabolism and peroxisomes lead to the endogenous production of reactive oxygen species (ROS) such as the superoxide anion ($O_2^{\cdot -}$) (Valko et al., 2006). The superoxide anion is quickly converted to hydrogen peroxide (H_2O_2) by superoxide dismutase enzymes (Galaris et al., 2008). "Labile" or free iron can then react with H_2O_2 by a Fenton-type reaction (Equation 1) to produce reactive intermediates and then the hydroxyl radical ($OH^{\cdot}$) as follows:

$$Fe^{2+} + H_2O_2 \rightarrow \text{reactive intermediates} \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-}$$

[Equation 1: Fenton Reaction]

Hydrogen peroxide can also interact with the heme-bound iron in heme-containing proteins such as hemoglobin and myoglobin (Galaris et al., 2008). Exogenous exposures such as other transition metals, radiation and xenobiotics can also lead to the production of ROS. ROS, in particular the hydroxyl radical, can directly produce oxidative damage to DNA (Meneghini, 1997). Recent research has also focused on the involvement of ROS and H_2O_2 in cell signaling. Iron may influence signal transduction by H_2O_2 and modulate the activation of redox-sensitive transcription factors such as NF-κB (nuclear factor-kappaB; Ornstein and Zacharski, 2008).

High intake of non-heme iron may also be a risk factor for cancers of the GI tract due to the production of free radicals within the lumen. Iron supplementation increases the free radical content of feces in healthy individuals (Lund et al., 1999), an effect reduced by high phytate levels. Concern has been raised over the appropriateness of oral iron supplementation and the encouragement of high iron diets in those suffering from ulcerative-colitis associated anemia (Seril et al., 2006), as ulcerative colitis is associated with an increased risk for colorectal cancer (Little and Sharp, 2008).

2.3.2 Iron, Inflammation and Cellular Proliferation

Chronic inflammation leads to the production of low but sustained levels of H_2O_2. *In vitro* experiments have shown that this leads to increased uptake of iron in various cell

types (HepG2 – human hepatoma, HeLa – cervical cancer, HT29 – colon adenocarcinoma, HCT116 – colonic carcinoma) through translational induction of *TFRC* (Andriopoulos et al., 2008). In addition, up-regulation of hepcidin under inflammatory conditions has been shown to decrease the efflux of iron from cells (Nemeth et al., 2004). With a prolonged inflammatory response, levels of intracellular iron could become cytotoxic by surpassing the capacity of homeostatic control mechanisms. This effect has been studied in a rat model for EAC. Studies by Chen et al. (1999) showed increased occurrence of esophagitis, columnar-lined esophagus (CLE) and EAC in rats with anastomosis between the gastroesophageal junction and the duodenum. Rats that had also received iron supplementation by injection of iron dextran (an iron oxyhydroxyide compound in a glucose polymer - 50 mg Fe/kg/month) demonstrated a higher grade of esophagitis, greater frequency of CLE and EAC and larger EAC tumor volume. In a subsequent study, Chen et al. (2000) reported increased iron deposition within macrophages in areas of esophagitis and also higher oxidative damage to DNA measured by 8-hydroxy-2'-deoxyguanosine (8-OH-dG). *HMOX1* and *TFRC* expression were also higher in CLE and EAC cells than normal esophageal mucosa.

Iron interacts with NF-κB, a proinflammatory transcription factor that controls the expression of numerous genes that are involved in cellular proliferation, survival, metastasis, invasion and angiogenesis (Maeda et al., 2008; Sethi et al., 2008). NF-κB activation has shown antiapoptotic effects *in vitro* but the effects *in vivo* remain to be clarified (Maeda et al., 2008). Iron has been shown to play a role in the activation of NF-κB in Kupffer cells in the liver (Xiong et al., 2003) but evidence for an effect in non-

macrophage cells is less apparent (Xiong et al., 2003b). NF-κB have been implicated in the upregulation of *TFRC* in response to inflammation leading to an increase in cellular uptake of transferrin bound iron (Tacchini et al., 2008).

2.3.3 Iron and Hypoxic Response

HIF-1α (hypoxia-inducible factor 1α) is critical in the regulation of cellular responses to decreased levels of oxygen (Weidemann and Johnson, 2008; Galanis et al., 2008). When oxygen levels are normal, HIF-1α is present at only very low concentrations due to effective degradation by a mechanism involving the von Hippel-Lindau tumor suppressor protein (pVHL) (Postovit et al., 2005). Recognition of HIF-1α by pVHL requires hydroxylation of prolyl residues in an oxygen-dependent degradation domain of HIF-1α (Cockman et al., 2000). Iron is required as a co-factor for the enzymes that catalyze the hydroxylation process, possibly as an oxygen sensor (Maxwell et al., 1999). Under conditions of rapid cell growth and proliferation during the promotion phase of carcinogenesis, oxygen levels can be depleted within a tumor. Hypoxia interferes with the hydroxylation process and the degradation of HIF-1α, allowing levels of HIF-1α to rise activating the signaling pathway that upregulates the expression of genes such as VEGF (vascular endothelial growth factor) and MMPs (matrixmetalloproteinases). VEGF and MMPs can promote angiogenesis (Weidemann and Johnson, 2008; Ushio-Fukai and Nakamura, 2008), a key process in the growth, progression and metastasis of tumors (Eichhorn et al., 2007). HIF-1α (hypoxia inducible factor – α), along with NF-κB, has also been implicated in the upregulation of *TFRC* in response to inflammation (Tacchini et al., 2008).

2.3.4 Heme-Iron, Heme-Iron Metabolism and Cancer

Heme may play a role in the endogenous formation of N-nitroso compounds (NOCs) (Kuhnle et al., 2007; Lunn et al., 2007; Lewin et al., 2006; Bingham et al., 2002; Cross et al., 2003; Sesink et al., 2001), the majority of which are known carcinogens (Mirvish, 1995). Although some human exposure to NOCs comes from pre-formed NOCs in cigarette smoke, nitrates in drinking water and preformed NOCs in cured and smoked meats, a large proportion of exposure comes from NOCs formed by endogenous nitrosation (Lunn et al., 2007) and by the activity of intestinal microflora (Jaksyn and Gonzalez, 2006) on precursor chemicals in foods. Heme is rapidly nitrosated and can subsequently act as a nitrosating agent similar to the nitrosonium ion, thus enhancing endogenous nitrosation. Bingham et al. (2002) reported a dose response association between red meat intake (beef and pork) and apparent total N-nitroso compounds in fecal samples from healthy volunteers but no such association with white meat intake (chicken, turkey or white cod). Fecal outputs from healthy volunteers and ileal outputs from ileostomy volunteers who were fed red meat containing diets had significantly higher amounts of nitrosothiols and nitrosylated heme than outputs following consumption of a vegetarian diet (Kuhnle et al., 2007; Lunn et al., 2007). *In vitro* studies have shown that the acidic conditions in the stomach favour the formation of nitrosothiols in the presence of heme and nitrite and that accessible thiol groups on the heme molecule may be important active sites promoting endogenous nitrosation in the upper digestive tract in (Kuhnle et al., 2007).

Heme-bound iron has pro-oxidant activity and may have direct genotoxic effects. Glei et al. (2006) reported evidence of DNA damage in HT29 colon carcinoma cells and primary colon cells obtained from surgical resections when they were exposed to hemoglobin at sub-toxic concentrations (10μM), although a dose-response relationship was not apparent. DNA damage, measured using a microgel electrophoresis comet assay, was reduced in the presence of DMSO (dimethyl sulphoxide, a free radical quencher) providing evidence for free radical involvement. Studies by Sesink et al. in rats (Sesink et al., 2000; 2001; de Vogel et al., 2008) have shown that exposure to heme leads to increased cytolytic activity of fecal water (a measure of irritants in the lumen), hyperproliferation of colonic epithelial cells (measured through ^{3}H-thymidine incorporation into DNA), development of hyperplastic lesions in crypts and reduced cell death (measures through caspase-3 levels in mucosal cells).

Heme metabolism is involved in the complex circuitry associated with inflammatory response and hypoxic response. A wide range of stimuli are capable of inducing expression of heme oxygenase-1 as is evidenced by the variety of positive regulatory elements in the *HMOX1* proximal promoter region, for example AP-1 (activating protein-1) and NF-κB which are both important for inflammatory signaling. Positive regulatory elements for heme, cadmium, and H_2O_2, among others, can be found in the distal promoter region of *HMOX1* (Loboda et al., 2008). Bilirubin, one of the by-products of heme catabolism, has demonstrated antioxidant and anti-inflammatory effects (Courtney and Maxwell, 2008). Heme catabolism (Equation 2) leads to the production of free Fe(II)

ion, carbon monoxide (CO), biliverdin and bilirubin (Equation 2; Fredenburgh et al., 2007).

$$\text{Heme (oxidant)} \xrightarrow{\textit{Heme oxygenase}} \text{Fe (II)} + \text{CO} + \text{biliverdin} \xrightarrow{\textit{Biliverdin reductase}} \text{bilirubin}$$

[Equation 2: Heme catabolism]

The Fe (II) ion produced by heme catabolism can have a net antioxidant effect due to the induction of ferritin, but a pro-oxidative effect if produced at rates too high to be efficiently sequestered (Suttner and Dennery, 1999). Suttner and Dennery (1999) propose a beneficial threshold of heme oxygenase-1 up-regulation (x5) above which the accumulation of reactive iron leads to a net cytotoxic effect. CO, the other byproduct of heme catabolism, exhibits antiapoptotic, anti-inflammatory and anti-proliferative effects in endothelial cells. Whether an increase in heme oxygenase-1 activity has a net protective or detrimental effect may depend on the degree of upregulation under the specific conditions, the cell type and possibly the stage of disease.

2.4 Iron and Cancer of the Gastrointestinal Tract: Observational Epidemiological Studies

There are at least two routes through which iron might affect the risk of gastrointestinal cancer. First, as discussed above, body iron levels may affect inflammatory response. Second, the GI tract is directly exposed to the different forms of iron passing through the

GI tract itself leading to a local effect mediated by the concentrations of iron in food. The epidemiological evidence for both exposure routes is reviewed below. Studies are described for the body iron level exposure looking firstly at the different sites in the gastrointestinal tract followed by tables with summaries of the studies for this exposure. The next section includes descriptions of the studies that examined associations between different measures of iron intake and cancers at the different sites, again followed by the relevant tables.

2.4.1 Body Iron Levels and Rectal Cancer (Table 2.1)

Three studies have examined the association between serum iron levels and rectal cancer (Knekt et al., 1994; Wurzelmann et al., 1996; Cross et al., 2006). Cross et al. (2006) conducted a nested case-control within the α-Tocopherol and β-Carotene Cancer Prevention study (ATBC Study) in Finland. All participants were white men who smoked more than 5 cigarettes per day. The odds ratio for serum iron was not significantly elevated (OR=1.7, 95% CI 0.5-6.1) after adjustment for age, education, BMI, smoking history, physical activity, total caloric intake, alcohol intake, ever daily aspirin use and serum levels of C-reactive protein.

Wurzelmann et al. (1996) found a positive association with serum iron (Quartile 4 v 1: RR=3.63, 95% CI 1.05-12.5) among participants in NHANES I (National Health and Nutrition Examination Survey) and NHEFS (National Health Evaluation Follow-up Survey) although the sample size was small (41 cases in the 15 years of follow-up). The overall association was driven by the magnitude of the relationship among women

(RR=7.31, 95% CI 1.13-47.2). Knekt et al. (1994) did not find a significant association in either men or women in a population-based cohort study they conducted in Finland. The relative risks were 1.29 among men (49 cases) and 0.99 among women (40 cases) for the comparison of the highest with the lowest quartile of intake after adjustment for age and smoking.

Cross et al. (2006) also examined baseline levels of serum ferritin in the ATBC study. The OR comparing the highest with the lowest quartile was 0.8 (95% CI 0.2-2.9) and the trend across quartiles was not significant (p=0.79).

Transferrin saturation (TfSat) is a commonly used index of iron stores. None of the three studies that have looked at this index in relation to rectal cancer reported significant associations (Cross et al., 2006; Knekt et al., 1994; Herrinton et al., 1995). Cross et al. (2006) reported an odds ratio of 2.3 (95% CI 0.6-7.9). The odds ratios for men and women in the Finnish population-based cohort study (Knekt et al., 1994) were close to null (OR=1.04 and 0.94 for men and women, respectively). Seventy rectal cancer cases were identified among women during 17.8 years of follow-up of a Californian cohort (Herrinton et al., 1995). The RR comparing the highest category of TfSat (≥34.5%) with the lowest (≤20.3%) was 0.9 (95% CI 0.5-1.7). The trend across categories was not significant (p=0.50). There were fewer cases of rectal cancer identified among men (n=31) than among women in this cohort. The RR comparing the highest with lowest TfSat category was less than 1.0 (OR = 0.3, 95% CI 0.1-1.1) and the trend was also not statistically significant (p=0.16).

Knekt et al. (1994) reported results suggestive of a positive association between total iron binding capacity (TIBC) and rectal cancer among women (Quartile 4 v 1: RR=2.47, p-trend=0.02). There was no evidence of a positive association among men. However, these results were based on only a small number of cases; 40 cases among women and 49 among men. Cross et al. (2006) did not observe a significant association between TIBC and rectal cancer among white, male smokers (quartile 4 v 1: OR=0.8, 95% CI 0.2-2.8) in the ATBC Study. There was also no significant association between UIBC (Unsaturated Iron Binding Capacity, an index of iron deficiency) and rectal cancer (OR=0.4, 95% CI 0.1-1.7).

2.4.2 Body Iron Levels and Colon Cancer (Table 2.2)

A significant inverse association between baseline serum iron levels and colon cancer was reported by Cross et al. (2006) based on 73 white, male cases included in their case-control study nested in the ATBC Study. Cross et al. (2006) determined iron parameters in fasting serum samples collected in 1993. Incident cases of cancer of the colon or rectum were identified through the Finnish Cancer Registry until April 2002. Those that occurred during the first five years of follow-up were excluded from the analyses. The odds ratio was 0.2 (95% CI 0.1-0.9; p-trend=0.05 across quartiles) after adjustment for lifestyle factors, smoking, ever daily aspirin use and serum C-reactive protein.

Wurzelmann et al. (1996) also observed an inverse, although not statistically significant, association for cancer in the proximal colon (RR=0.63, 95% CI 0.24-1.65; 52 cases) in

the NHANES I and NHEFS studies but not for those with disease in the distal colon (RR=2.04, 95% CI 0.85-4.89; 57 cases). Knekt et al. (1994) reported non-significant results stratified by gender. The age and smoking adjusted RR comparing the highest with the lowest quartile of serum iron was 1.64 among men (27 cases) and 0.94 among women (57 cases). The trend across quartiles was not significant for either sex.

There was an inverse association between baseline serum ferritin levels and colon cancer in the nested case-control study of Cross et al. (2006). The odds ratio was 0.2 (95% CI 0.1-0.7; p-trend=0.02) among these male smokers. Transferrin saturation was also inversely associated with colon cancer (OR=0.1, 95% CI 0.02-0.5) with a significant trend across quartiles (p=0.003). The results in the selected subpopulation in the Cross et al. study were in contrast to those found in the population-based cohort study of Knekt et al. (1994) where no significant effects were noted. Among men, the association between TfSat and colon cancer was not significant (OR=1.7, p-trend=0.35). Among women, the odds ratio was 0.97 (p-trend=0.82). In a larger study among health plan members in the United States, Herrinton et al. (1995) found an inverse association between TfSat and colon cancer approaching statistical significance among men. Based on the 81 male cases identified during 17.6 years of follow-up, the age-adjusted RR was 0.6 (95% CI 0.4-1.1) comparing the highest with the lowest quartile ($\geq$40.7% v $\leq$26.0%). The test for trend gave a p-value of 0.08. Two hundred and fifty-nine women developed colon cancer in this cohort. The age-adjusted RR comparing the highest ($\geq$34.5%) with the lowest quartile ($\leq$20.3%) was close to 1.0 (RR=0.98, 95% CI 0.7-1.4) for women in this cohort.

Neither Cross et al. (2006) or Knekt et al. (1994) found significant associations between total iron binding capacity (TIBC) and colon cancer. TIBC is inversely associated with iron levels. Consistent with the results for TfSat, the association measure was greater than 1.0 in the Cross et al. study in white, male smokers suggesting an inverse association between body iron levels and outcome but less than 1.0 in the male subgroup of Knekt et al. (1994), suggesting a positive association with body iron levels. The small number of male cases is a limitation in the Knekt et al. study.

Consistent with the other results in their study, Cross et al. (2006) observed a positive association between UIBC and colon cancer. The odds ratio comparing the highest with the lowest quartile was 4.7 (95% CI 1.4-15.1) and the trend across quartiles was significant (p-trend=0.009).

2.4.3 Body Iron Levels and Cancer of the Colon or Rectum Combined (Table 2.3)

Kishida et al. (1994) and Kato et al. (1999) examined the relationship between serum iron in cancer-free controls and individuals with cancer of the colon or rectum. Kato et al. (1999) found no significant association in a case-control study (105 cases, 523 controls) nested within the New York University Women's Health cohort. The OR comparing the highest with the lower quartile of intakes was 0.85 (95% CI 0.5-1.6). The trend was not significant (p=0.52). Results were adjusted for family history of colorectal cancer, beer intake, prior occult blood testing, and number of hours spent in sports activities in their early thirties. Cases and controls were individually matched on age, menopausal status and dates of subsequent blood sampling. Kishida et al. (1994) examined serum Fe levels

in a hospital-based case-control study. Controls were age-matched to cases and were being investigated for other gastrointestinal disease. Kishida et al. (1994) reported serum iron levels significantly lower in an advanced disease group (n=23) than in controls.

The nested case-control study of Kato et al. (1999) found an inverse association between baseline serum ferritin levels and colorectal cancer (OR=0.40, 95% CI 0.2-0.8; p-trend <0.01) comparing the highest with the lowest quartile. Serum ferritin levels were also significantly lower in the advanced disease group in the Kishida et al. (1994) study. In contrast, Scholefield et al. (1998) conducted similar analyses and found no significant different between cases and controls with respect to serum ferritin levels. Although cases and controls were selected on the basis of disease status in both of these studies, exposure was determined on a cross-sectional basis at the time of the study. Given the effect of disease progression on iron indices due to occult bleeding and the anemia of chronic disease, the results are not useful for determining the role of iron stores as a risk factor for the initial development or promotion of the disease.

Kato et al. (1999) also examined the association between TfSat and colorectal cancer. The association with TfSat was less than 1.0 although it did not reach statistical significance (OR=0.6, 95% CI 0.3-1.2; p-trend=0.11). TIBC was not significantly associated with cancer of the colon or rectum (OR=1.2, 95% CI 0.6-2.3; p-trend=0.44) among the women in this study.

2.4.4 Body Iron Levels and Colorectal Adenoma (Table 2.4)

Kishida et al. (1997) conducted a cross-sectional study of serum iron levels among participants in a colorectal cancer screening programme. All participants included in their study had undergone endoscopic screening and were male. Individuals with large adenoma (≥1cm) had lower serum iron levels than those with normal colonic pathology. Serum ferritin levels were also significantly lower among those with large adenomas when compared with the control group. Nelson et al. (1994) conducted a case-control study among individuals undergoing colonoscopy at a single institution in the United States. The study sample was 73% male and controls were undergoing colonoscopy due to occult blood in stool. With 145 cases and 159 controls, the OR was 2.1 (95% CI 0.8-5.3) comparing the highest with the lowest quintile of serum ferritin when adjusted for age, sex, BMI, race, ever smoking, a family history of polyps or cancer and alcohol consumption. Bird et al. (1996), however, did not observe a positive association between serum ferritin and adenoma risk among men in their case-control study in Southern California. The OR comparing quartile 4 with quartile 2 was 1.3 (95% CI 0.8-2.0) after adjustment for age, sex, date of sigmoidoscopy and centre based on 300 male cases and 331 male controls. Among female participants (167 cases and 167 controls), the corresponding OR was 1.1 (95% CI 0.5-2.3).

Chan et al.(2005) conducted a case-control study nested within the Nurses' Health Study and included 527 controls individually matched to 527 cases with respect to date of endoscopy, birth year, indication for endoscopy, month and year of blood draw, time period of any prior endoscopy and fasting status at times of blood draw. These authors

observed no significant differences between cases of distal adenoma and the control group with respect to several biochemical iron indices (serum iron, transferrin, transferrin saturation, soluble transferrin receptors, serum ferritin, transferrin receptor/ferritin ratio) measured at baseline (1989-1990) in the 380 cases and 379 matched control subjects for which these measurements were available.

2.4.5 Body Iron Levels and Colorectal Adenoma Recurrence (Table 2.4)

Tseng et al. (2000) found no significant association between serum ferritin levels and recurrence of adenoma in a case-control study nested within the multicentre Antioxidant Polyp Prevention Trial in the U.S. Based on 251 cases and 436 controls, the odds ratio was 1.32 (95% CI 0.73-2.38; p-trend 0.48) comparing recurrence in individuals with serum ferritin levels >160μg/L with recurrence in the 0 to 30 μg/L group. These analyses were adjusted for age, sex, centre, intake of energy, alcohol, fibre, folate and total fat, the number of months between follow-up colonoscopies, smoking status and aspirin use.

2.4.6 Body Iron Levels and Stomach Cancer (Table 2.5)

Knekt et al. (1994) reported results for the association between serum iron levels and stomach cancer in their population-based cohort study in Finland. There were 120 cases of stomach cancer among men and 76 cases among women. There was a statistically significant inverse association between serum iron and stomach cancer among men (quartile 4 v 1: age and smoking adjusted RR=0.60; p-trend <0.01). The association was also less than 1.0 among women (age and smoking adjusted RR=0.59; p-trend=0.17) but

did not reach statistical significance. Nomura et al. (1992) found an inverse association between serum ferritin levels and stomach cancer among men of Japanese-American descent in Oahu. This case-control study was nested within a cohort of men originally identified as part of the Honolulu Heart Program in 1965. Serum ferritin was measured in samples taken 20 years prior to diagnosis or inclusion in the gastric cancer study. The authors reported an OR of 0.2 (95% CI 0.1-0.8) among individuals with serum ferritin levels higher than 403 μg/L compared with those with levels <221 μg/L. However, Nomura et al. (1992) did not find a significant association between TfSat (>403 μg/L v <221 μg/L) and stomach cancer in their study (OR=0.7, 95% CI 0.3-1.9). An earlier case-control study nested within a cohort of survivors of the Nagasaki and Hiroshima atomic bombs (Akiba et al. 1991) also reported a negative association between serum ferritin and gastric cancer. The odds ratio comparing the highest quintile with the lowest was 0.28 and the test for trend significant ($p<0.0001$). The case and control groups were frequency-matched on city, sex, age at examination and year and month of blood collection. Serum transferrin levels on the other hand showed a positive association with gastric cancer (quintile 5 v 1: OR=0.5; p-trend = 0.03).

The cohort study of Herrinton et al. (1995) also examined the association between TfSat and stomach cancer risk. However, the number of people who developed gastric cancer during follow-up was small (35 among women, 32 among men) leading to low statistical power. Among women, the trend across quartiles approached significance ($p=0.06$) with an odds ratio of 3.5 (95% CI 1.0-1.2) comparing the highest (≥34.5%) with the lowest quartile (≤20.3%) of TfSat. Quartile cut-offs were defined separately for men and

women in this study. Among men the odds ratio was 0.6 (95% CI 0.2-1.9) comparing the highest (≥40.7%) versus the lowest quartile (≤26.0%) of TfSat. In contrast, Knekt et al. (1994) found interquartile relative risks that were less than 1.0 for both men and women in their population-based cohort study: 0.55 in men (120 cases) and 0.60 in women (76 cases). The inverse trend between TfSat and stomach cancer was statistically significant ($p<0.001$) among men but did not reach statistical significance among women ($p=0.10$).

2.4.7 Body Iron Levels and Esophageal Cancer

Only one study was identified that looked at measures of iron stores and cancer of the esophagus. Herrinton et al. (1995) had only 14 cases of esophageal cancer among women on which to base their analyses of the association between this outcome and TfSat. The age-adjusted odds ratio was 1.4 (95% CI 0.3-6.0) comparing those with a TfSat ≥34.5% with those exhibiting a TfSat ≤20.3%. Corley et al. (2008a) reported that individuals with Barrett's Esophagus had lower iron stores than population controls but did not report quantitative differences in their paper.

Table 2.1 Summaries of studies that have examined the association between biochemical iron measures and rectal cancer.

Citation	Outcome	Study Location	Study Design/Participant Characteristics	Study Size	Fe - measure	Comparison	RR or OR (95% CI)	P-trend	Adjustment factors/ Remarks
Cross 2006	Rectal cancer	Finland	Nested case-control α-tocopherol , β-carotene cancer prevention study; All participants were smokers, male and white. Individual matching in age, study area, intervention group and month of baseline blood draw. Cases diagnosed in the first 5 years excluded.	57 cases, 260 controls	serum Fe	Quartile 4 v 1	1.7 (0.5-6.1)	0.35	Age, education, BMI, number of years smoking, total number of cigarettes per day, physical activity (active = moderate or heavy occupational or recreational activity versus inactive), total caloric intake, alcohol (g/day), ever daily aspirin use, serum C-reactive protein. Median follow-up=14.2 yrs
					serum ferritin	Quartile 4 v 1	0.8 (0.2-2.9)	0.79	
					TfSat	Quartile 4 v 1	2.3 (0.6-7.9)	0.12	
					TIBC	Quartile 4 v 1	0.8 (0.2-2.8)	0.92	
					UIBC	Quartile 4 v 1	0.4 (0.1-1.7)	0.22	
Wurzel-mann 1996	Rectal cancer	U.S.	Cohort study (NHANES I and NHEFS) Cases diagnosed in the first 3 years excluded.	N=14407, 38 cases	Serum Fe	Quartile 4 v 1	3.63 (1.05-12.5)	0.06	Energy by the residuals method. Authors reported that age, sex, body mass index, fat, aspirin use, vegetable and meat consumption, physical activity were not confounders. Follow-up: 15 years
Knekt 1994	Rectal cancer	Finland	Population-based cohort study. Sensitivity analysis excluded cases from first 5 years.	N=41276, women: 49 cases, men: 40 cases	Serum Fe	Quartile 4 v 1			Age and smoking
						women:	0.99	0.22	
						men:	1.29	0.82	Mean follow-up=14 years
					TfSat	Quartile 4 v 1			
						women:	0.94	0.66	
						men:	1.04	0.71	
					TIBC	Quartile 4 v 1			
						women:	2.47	0.02	
						men:	0.62	0.68	

Citation	Outcome	Study Location	Study Design/Participant Characteristics	Study Size	Fe - measure	Comparison	RR or OR (95% CI)	P-trend	Adjustment factors/ Remarks
Herrinton 1995	Rectal cancer	California US	Cohort study in health care plan members. Excluded cases from the first 5 years of follow-up	N=38538, women: 70 cases men: 31 cases	TfSat	Women: ≤20.3	1.0	0.50	Age. Stratified by gender. Average follow-up period 17.6 years in men and 17.8 years in women
						20.4-26.8	0.8 (0.4-1.6)		
						26.9—34.4	0.5 (0.3-1.1)		
						≥34.5	0.9 (0.5-1.7)		
						Men: ≤26.0	1.0	0.16	
						26.1-32.0	1.1 (0.4-2.6)		
						32.1-40.6	0.8 (0.3-2.0)		
						≥40.7	0.3 (0.1-1.1)		

Table 2.2 Summaries of studies that have examined the association between biochemical measures of iron and colon cancer.

Study	Outcome	Study Location	Study Design/Participant Characteristics	Study Size	Fe-measure	Comparison	RR or OR (95% CI)	P-trend	Adjustment factors/ Remarks
Cross 2006	Colon cancer	Finland	Nested case-control α-tocopherol , β-carotene cancer prevention study; All participants were smokers, male, white. Individual matching on age, study area, intervention group, month of baseline blood draw. Exclusion of cases diagnosed in first 5 yrs.	73 cases, 260 controls	Serum Fe	Quartile 4 v 1	0.2 (0.1-0.9)	0.05	Age, education, BMI, number of years smoking, total number of cigarettes per day, physical activity (active = moderate or heavy occupational or recreational activity versus inactive), total caloric intake, alcohol (g/day), ever daily aspirin use, serum C-reactive protein.
					Serum Ferritin	Quartile 4 v 1	0.2 (0.1-0.7)	0.02	
					TfSat	Quartile 4 v 1	0.1 (0.02-0.5)	0.003	
					TIBC	Quartile 4 v 1	1.9 (0.7-5.8)	0.19	
					UIBC	Quartile 4 v 1	0.4 (0.1-1.7)	0.009	
Wurzel-mann 1996	Colon cancer	U.S.	Cohort study (NHANES I and NHEFS) Cases diagnosed in the first 3 years excluded.	N=14407, Proximal: 52 cases Distal: 57 cases	Serum Fe	Quartile 4 v 1 (Proximal)	0.63 (0.24-1.65)	0.70	Energy by the residuals method. Authors reported that age, sex, body mass index, fat, aspirin use, vegetable and meat consumption, physical activity were not confounders.
						Quartile 4 v 1 (Distal)	2.04 (0.85-4.89)	0.10	

Study	Outcome	Study Location	Study Design/Participant Characteristics	Study Size	Fe-measure	Comparison	RR or OR (95% CI)	P-trend	Adjustment factors/ Remarks
Herrin-ton 1995	Colon cancer	California, US	Cohort study.	N=38538, women: 259 cases men: 81 cases	TfSat	Women: ≤20.3 20.4-26.8 26.9—34.4 ≥34.5 Men: ≤26.0 26.1-32.0 32.1-40.6 ≥40.7	1.0 1.2 (0.8-1.7) 1.1 (0.8-1.6) 1.0 (0.7-1.4) 1.0 0.5 (0.3-0.9) 0.5 (0.3-1.0) 0.6 (0.4-1.1)	NR* 0.08	Age. Stratified by gender. Average follow-up period 17.6 years in men and 17.8 years in women
Knekt 1994	Colon cancer	Finland	Population-based cohort study. Sensitivity analysis excluded cases from first 5 years.	N=41276, Women: 57 cases Men: 27 cases	Serum Fe TfSat TIBC	Quartile 4 v 1 Quartile 4 v 1 Quartile 4 v 1 Quartile 4 v 1 Quartile 4 v 1 Quartile 4 v 1	0.94 (women) 1.64 (men) 0.97 (women) 1.73 (men) 1.18 (women) 0.69 (men)	0.75 0.63 0.82 0.35 0.72 0.13	Age and smoking

* NR, not reported

Table 2.3 Summaries of studies that have examined the association between biochemical measures of iron and colorectal cancer.

Citation	Outcome	Study Location	Study Design/ Participant Characteristics	Study Size	Fe-measure	Comparison	RR or OR (95% CI)	P-trend	Adjustment factors/Remarks
Kato 1999	Colorectal cancer	New York, US	Nested case-control. Individually matched on age, menopausal status at enrolment, dates of subsequent blood donations. 100% female.	105 cases, 523 controls	Serum Fe	Quartile 4 v 1	0.85 (0.5-1.6)	0.52	Family history of colorectal cancer, beer intake, prior occult blood testing, number of hours spent in sport activities in their early thirties.
					Serum ferritin	Quartile 4 v 1	0.40 (0.2-0.8)	<0.01	
					TfSat	Quartile 4 v 1	0.63 (0.3-1.2)	0.11	
					TIBC	Quartile 4 v 1	1.17 (0.6-2.3)	0.44	
Schol-efield 1998	Colorectal cancer	Nottingham, UK	Hospital-based case-control. Controls were patients with no colonic pathology. 100% male.	50 cases, 49 controls	Serum ferritin	Serum ferritin level central tendency	NR	NR	*Note:* There was no significant difference between the colorectal cancer cases and the patients with no colonic pathology with respect to serum ferritin.
Kishida 1994	Colorectal cancer	Tokyo, Japan	Hospital-based case-control; age-matched controls who were being investigated for gastrointestinal diseases. 100% male.	23 cases (advanced disease), 18 cases (early disease), 23 controls	Serum Fe	Serum iron level central tendency	NR	NR	*Note*: Serum iron levels were significantly lower in the advanced disease group.
					Serum ferritin	Serum ferritin level central tendency	NR	NR	*Note*: Serum ferritin levels were significantly lower in the advanced disease group.

Table 2.4 Summaries of studies examining the association between biochemical measures of iron and colorectal adenoma.

Citation	Outcome	Study Location	Study Design/ Participants	Ca/Co	Fe-measure	Comparison	OR (95% CI)	P-trend	Adjustment factors
Tseng 2000	Recurrence of colorectal adenoma	Multi-centre, U.S.	Nested in the Antioxidant Polyp Prevention Trial	251 cases, 436 controls	Serum ferritin	>160 µg/L v 0-30 µg/L	1.32 (0.73-2.38)	0.48	Age, sex, center, intake of energy, alcohol, fibre, folate, total fat, number of months between follow-up colonoscopies, smoking status and aspirin use.
Kishida 1997	Colorectal adenoma	Tokyo, Japan	Cross-sectional study within a screening program; all participants had endoscopic screening; 100% male.	184 cases, 92 with large adenoma, 92 with smaller adenoma, 92 controls	Serum iron	Central tendency of serum iron			None.
						Adenoma (≥1cm)	p<0.05	NR	
						Adenoma (<1 cm	p>0.05		
						Controls	comparator		
					Serum ferritin	Central tendency of serum ferritin			
						Adenoma (≥1cm)	p<0.01		
						Adenoma (<1 cm	p<0.05	NR	
						Controls	comparator		
Bird 1996	Colorectal adenoma	Southern California, US	Case-control study; individual-matching on age, sex, date of sigmoidoscopy, centre. Cases and controls underwent sigmoidoscopy only.	Men: 300 cases, 331 controls	Serum ferritin	Quartile 4 v 2	1.3 (0.8-2.0)	NR	Adjusted for matching variables.
						Quartile 1 v 2	1.2 (0.7-1.9)		
				Women: 167 cases, 167 controls		Quartile 4 v 2	1.1 (0.5-2.3)	NR	
						Quartile 1 v 2	1.1 (0.6-1.8)		
Nelson 1994	Colorectal adenoma	Illinois, US	Single institution, unmatched case-control. Controls were undergoing colonoscopy due to occult blood in the stools. Cases: 19.6% female Controls: 35% female	145 cases, 159 controls	Serum ferritin	Quintile 5 v 1	2.1 (0.8-5.3)	NR	Age, sex, BMI, race (white/non-white), ever smoked, family history of polyps or cancer, alcohol consumption.

Table 2.5 Summaries of studies examining the association between biochemical measures of iron and stomach cancer.

Citation	Outcome	Study Location	Study Design/Participant characteristics	Study size	Fe-measures	Comparison	RR or OR (95% CI)	P-trend	Adjustment factors
Herrinton 1995	Stomach cancer	California, US	Cohort study. Average follow-up period 17.6 years in men and 17.8 years in women	Women: 35 cases Men: 32 cases	TfSat	Women: ≤20.3 20.4-26.8 26.9—34.4 ≥34.5	1.0 2.7 (0.7-9.8) 2.5 (0.7-9.1) 3.5 (1.0-12)	0.06	Age and race (white, black, other). Stratified by gender.
						Men: ≤26.0 26.1-32.0 32.1-40.6 ≥40.7	1.0 0.7 (0.2-2.2) 1.6 (0.7-3.8) 0.6 (0.2-1.9)	NR	
Knekt 1994	Stomach cancer	Helsinki, Finland	Population-based cohort study	N=41276, Women: 76 cases Men: 120 cases	Serum Fe TfSat	Quartile 4 v 1 Quartile 4 v 1	0.59 (women) 0.60 (men) 0.60 (women) 0.55 (men)	0.17 <0.01 0.10 <0.001	Age and smoking.
Nomura 1992	Stomach cancer	Oahu	Nested case-control. Individual matching on age and date of serum collection. Japanese-American descent. 91 intestinal cases, 25 diffuse cases and 5 of unknown type/	121 cases/ 121 controls	Trans-ferrin (mg/dl)	<235 235-269 270+	1.0 1.0 (0.4-2.3) 0.7 (0.3-1.9)	0.55	None.

2.4.8 Dietary Iron Intake and Rectal cancer (Table 2.6)

Kabat et al. (2007) found no association between dietary iron intake and rectal cancer in a prospective cohort study among Canadian women. Dietary intakes were assessed at study entry using a food frequency questionnaire with 86 items and portion sizes. The average follow-up of individuals included in the analyses was 16.4 years. One hundred and ninety-five incident cases of rectal cancer were identified through linkage with the Canadian Cancer Database and the National Mortality Database. The multivariate adjusted odds ratio comparing the highest with the lowest quintile of dietary iron intake was 1.12 (95% CI 0.67-1.88; Table 2.7). Balder et al. (2006) investigated the relationship between baseline dietary iron intake and rectal cancer using a case-cohort design within the Netherlands Cohort Study of on Diet and Cancer. Dietary information was collected using a self-administered food frequency questionnaire. The RR comparing the highest with the lowest quintiles of intake, adjusted for age, family history of colorectal cancer, smoking status, no-occupational physical activity, total energy intake, alcohol consumption and total vegetable consumption were not significant (1.44, 95% CI 0.85-2.45, p-trend=0.08 among men; and 1.11, 95% CI 0.53-2.30, p-trend=0.63 among women). Cross et al. (2006) also observed no association between rectal cancer and dietary iron intake (Quartile 4 v 1; OR=0.5, 95% CI 0.1-2.8; p-trend=0.38) based on 57 cases among white, male smokers. Ullen et al. (1997) and Wurzelmann et al. (1996) also observed null results in population-based studies. Ullen et al. reported an age and gender-adjusted OR of 0.8 (95% CI 0.5-1.4) comparing the highest with the lowest quintiles of dietary iron intake in a study conducted in Sweden. The interquartile RR from the NHANES I/NHEFS cohort examined by Wurzelmann et al. (1996) was 1.01

(95% CI 0.42-2.42) with no evidence of a trend. Both Freudenheim et al. (1990) and Deneo-Pellgrini et al. (1999), however, reported positive associations between iron intake and rectal cancer. Deneo-Pellegrini et al. (1999) conducted a hospital-based case-control study in Montevideo, Uruguay, and recruited 216 cases and 433 controls. Intakes were divided into three categories: ≤14.7 mg/day; 14.8-18.3 mg/day and >18.3 mg/day. In the sample with men and women combined, the OR comparing the highest with the lowest level was 3.2 (95% CI 1.9-5.3) with a significant positive trend ($p<0.001$) after adjustment for age, sex, residence, urban/rural status, family history of rectal cancer, and intakes of total energy, folate and dietary fibre. Among women, this OR was 4.0 (95% 2.1-7.7). It was also significantly greater than 1.0 among men (OR=2.6, 95% CI 1.1-6.1). Although the authors indicated that the control group had conditions "not related to smoking, alcohol consumption and diet", it is hard to assess the generalisability of these results. The case-control study of Freudenheim et al. (1990) included 277 male case-control pairs and 145 female case-control pairs. Controls were matched to cases on age, gender and neighbourhood of residence. They reported a positive association between dietary iron intake and rectal cancer among men (quartile 4 v 1; OR=2.15, 95% CI 1.29-3.57) but not among women (quartile 4 v 1: OR=0.84, 0.47-1.50). The authors reported that the case and control groups did not differ with respect to BMI and that associations with iron intake were not affected by smoking history or consumption of alcohol. With energy adjustment using the residuals method, however, iron intake was not associated with risk.

2.4.9 Dietary Iron Intake and Colon Cancer (Table 2.7)

A large, prospective study among Canadian women did not find an association between dietary iron intake and colon cancer (Kabat et al., 2007). The multivariate odds ratio based on 428 women was 1.07 (95% CI 0.75-1.53) comparing the highest with the lowest quintile of energy-adjusted intake. Balder et al. (2006) in a study of case-cohort design did not find a significant association among 484 women (adjusted RR=1.14, 95% CI 0.73-1.80, p-trend=0.91). Among 539 men, the adjusted RR was 1.30 (95% CI 0.84-2.01, p-trend=0.43). Shaheen et al. (2003) reported a colon cancer odds ratio of 1.05 (95% CI 0.75-1.49) comparing the highest with the lowest quartiles of total iron intake (dietary plus supplement) in a population-based case-control study conducted in North Carolina, U.S. Covariates included in the logistic regression were sex, age, race, red meat consumption and NSAID use. A study in Sweden found an odds ratio of 0.6 (95% CI 0.4-1.0) comparing the highest quintile with the lowest adjusting for age and gender (Ullen et al., 1997). Cross et al. (2006) also found an inverse association in the ATBC cohort in Finland although the results did not reach statistical significance (quartile 4 v 1; OR=0.3, 95% CI 0.1-1.6; p-trend=0.09). In contrast, analysis of the association between dietary iron intake at baseline in the NHANES I/NHEFS cohort and subsequent development of colon cancer (Wurzelmann et al., 1996) found a positive association between dietary iron intake and colon cancer in the proximal regions (quartile 4 v 1; age and gender-adjusted RR = 1.44, 95% CI 1.23-1.69) but not the distal (RR = 1.03, 95% CI 0.8-1.32).

2.4.10 Dietary Iron Intake and Colorectal Cancer (Table 2.8)

Two case-control studies have looked at the association between cancer in the colon or rectum and iron intake, one in the U.S. (Kato et al., 1999) and one in France (Senesse et al., 2004). Neither of these studies presented results separately by site. The Kato et al. study was nested within a cohort of women. The authors found no significant association between total iron intake (dietary plus supplements) and colorectal cancer (quartile 4 v 1: OR=1.2, 95% CI 0.6-2.3; p-trend=0.44). Senesse et al. (2004) however, reported a positive association between dietary iron intake and colorectal cancer (quartile 4 v 1: OR=2.2, 95% CI 1.1-4.7; p-trend=0.02). The Senesse et al. study recruited 309 population controls. Of the 171 cases, 38% had rectal cancer. Analyses were adjusted for age, sex, energy intake, body mass index and physical activity. Two prospective studies of diet and colorectal cancer estimated dietary iron intake but did not provide quantitative results for the associations with disease in the published articles (Glynn et al., 1996; Konings et al.,2002).

2.4.11 Dietary Iron Intake and Colorectal Adenoma (Table 2.9)

Chan et al. (2005) assessed the association between dietary iron intake and colorectal adenoma in a case-control study nested within the Nurses' Health Study. The adjusted odds ratio was not significantly different from 1.0 (quartile 4 v 1: OR=1.04, 95% CI 0.68-1.57; p-trend=0.94). It was not clear from the publication whether the iron intake measure used in the analyses was dietary iron or total iron including iron from supplements. McGlynn et al. (2005) did not report odds ratios for iron intakes but indicated that there was no significant difference in total iron intake between cases and

controls and that the controls tended to consume more iron (p=0.07). Of three studies that used total iron intake as the exposure (Almendingen et al., 2001; Bird et al., 1996; Tseng et al., 1996), one reported an odds ratios >1.0 (Bird et al., 1996) but only in men. The Bird et al. case-control study was conducted in Southern California. Cases and controls were recruited at two medical centres from among individuals who underwent sigmoidoscopy. The 2nd quintile of intake was used as the referent in this study due to the possibility of a 'u'-shaped risk curve. The odds ratio comparing the highest with the second quintile of total iron intake was 1.6 (95% CI 0.9-2.8) and the fourth with the second quintile was 1.9 (95% CI 1.2-3.2) among the male participants. Among women these odds ratios were 1.0 (95% CI 0.5-2.0) and 0.9 (95% CI 0.4-1.8). There was also a positive association between very low iron intake (<11.6 mg/day) and colorectal adenoma among men but not among women. Neither dietary nor total iron intakes were significantly associated with colorectal adenoma in the case-control study reported by Almendingen et al. (2001). Odds ratios were lower for dietary iron intake than for total iron intake with an inverse trend approaching statistical significance (p=0.08). Tseng et al. (1996) observed odds ratios less than 1.0 for both men and women in a hospital-based case-control study when comparing the highest with the lowest quartiles of intake. Trend tests were not significant for either sex. Four earlier case-control studies reported odds ratios less than 1.0 that did not reach statistical significance for the dietary iron exposure (Benito et al., 1993; Little et al., 1993; Macquart-Moulin et al., 1987; Hoff et al., 1986).

Tseng et al. (1997, 2000) reported results of associations between dietary iron intake and recurrence of colorectal adenoma within the Antioxidant Polyp Prevention Study, a

multicentre clinical trial. The multivariate adjusted odds ratio comparing the highest quartile of intake (median = 20.9 mg/L) with the lowest (median=11.0 mg/L) was 0.37 (95% CI 0.19-0.73) and the trend across quartiles was significant (p=0.005). The authors included adjustment for age, sex, centre, treatment group, caloric intake, and energy adjusted intakes of alcohol, fibre, folate and total fat. In the more recent report, Tseng et al. reported an inverse association between dietary iron and recurrence of colorectal polyps, particularly in individuals with serum ferritin levels >70μg/L (OR=0.44, 95% CI 0.22-0.84).

2.4.12 Dietary Iron Intake and Stomach Cancer

Chen et al. (2002) did not observe an association between dietary iron intake and adenocarcinoma of the distal stomach in a population-based case-control study in the U.S. The odds ratio comparing the highest with the lowest quartile of energy adjusted intake was 0.9 (95% CI 0.5-1.7; p-trend=0.8) with adjustment for age, gender, alcohol use, smoking, education level, family history of gastric cancer, vitamin supplement use, BMI and age-squared. Results from another population-based case-control study in the U.S. (Mayne et al., 2001) also did not show a significant association. The odds ratio for noncardia gastric cancer was 1.13 (95% CI 0.85-1.50) and for gastric cardia adenocarcinoma 1.03 (95% CI 0.75-1.40). The analyses included adjustment for sex, site, age, race, proxy status, income, education, usual body mass index, smoking, alcohol consumption and energy intake. Harrison et al. (1997) reported an odds ratio of 0.6 (95% CI 0.4-0.98) for the intestinal subtype of gastric cancer and 0.7 (95% CI 0.4-1.1) for the diffuse type. The authors adjusted for energy intake, age, gender, race, education level,

pack-years of smoking, alcohol drinking and body mass index. This study was hospital-based. Sixty individuals had the intestinal type of tumor and 31 the diffuse histological type. Controls had inflammatory conditions of the stomach or esophagus (n=94) or normal examinations (n=38). The recent WCRF/AICR review of nutrition and cancer (WCRF/AICR, 2007) considered the results from studies between iron and stomach cancer too limited to make any assessment of the association.

2.4.13 Dietary Iron Intake and Esophageal Cancer (Table 2.10)

Several studies have reported results for iron intake and adenocarcinoma of the esophagus (Mayne et al., 2001; Chen et al., 2002; Zhang et al., 1997; Brown et al., 1995; Wolfgarten et al. 2001). Chen et al. (2002) conducted a population-based case-control study in eastern Nebraska. Dietary intake information was collected by telephone interviews during 1992-1994 using a short version of the HHHQ (Block et al., 1990). Respondents were asked to recollect dietary intake and lifestyle behaviours for the period before 1985. Next of kin were the source of information for 76% of the EAC cases and 61% of the controls. It is unclear whether supplemental iron was included in the iron intake measure in this study. Based on the sample of 124 cases of EAC and 449 controls, the odds ratio comparing the highest with the lowest quartile of iron intake was 0.6 (95% CI 0.3-1.1) with adjustment for age, age-squared, gender, respondent type, BMI, alcohol use, tobacco use, education level, family history and vitamin supplement use. Mayne et al. (2001) also reported an odds ratio less than 1.0 from a population-based case-control study conducted in the U.S. Information was collected from proxy respondents for 29.6% of the 282 cases and 3.4% of the 687 controls in this study. Controls were

identified through random-digit-dialing and frequency-matched to cases on 5-year age group, sex and study site. Cases and controls were asked to recall their dietary and lifestyle habits for the period 3 to 5 years prior to diagnosis or interview. After adjustment for sex, site, age, race, proxy status, income, education, usual body mass, number of cigarettes smoked per day, years of consuming beer, wine and liquor and energy intake, the OR for EAC was 0.79 (95% CI 0.57-1.09) comparing the 75th percentile of iron intake with the 25th percentile. Wolfgarten et al. (2001) reported results from an unmatched, case-control study conducted in Cologne, Germany. Cases of esophageal adenocarcinoma incident between January 1, 1997, and December 31, 1998, were included although the participation rates were not provided. A computer program was used to collect dietary information. It is unclear whether the study investigators specified a particular time period for participants to consider when reporting their dietary intakes. The unadjusted odds ratio comparing the highest with the lowest quartile of intake was 0.2 (95% CI 0.0-0.7). The authors do not appear to have adjusted for differences between the case and control groups with respect to sex, BMI, smoking or alcohol consumption. An earlier hospital-based study by Zhang et al. (1997) recruited cases and controls from a consecutive series of patients undergoing an upper GI endoscopic examination at a single gastroenterology endoscopy unit in the U.S. Cases had histopathologically confirmed adenocarcinoma of the esophagus or gastric cardia (n=95). Almost 29% of controls were free of any gastrointestinal disease but 51% had chronic gastritis and the remainder another inflammatory condition of the esophagus, stomach or duodenum. The time referent for the recall of dietary intakes and lifestyle habits is unclear. The OR comparing the highest with the lowest quartile of dietary

intake of iron was 0.6 (95% CI 0.3-0.9) with adjustment for age, sex, race, education, total dietary intake of calories, pack-years of smoking, alcohol use and body mass index. The trend across quartiles reached significance (p=0.03). The study reported by Brown et al. (1995) was of population-based design and recruited 174 incident cases of adenocarcinoma of the esophagus or esophogastric junction. Cases were identified through rapid reporting systems of three cancer registries in Georgia, the Detroit region and the state of New Jersey. Medicare rolls were the source for the control group who were recruited by random-digit dialing, frequency-matching to the age and race distribution of cases within each geographic region. Participants were asked to recall their dietary and lifestyle habits prior to 5 years before the study. The multivariate adjusted odds ratio for white men was 0.5 and the trend across quartiles not significant (p=0.51).

Wolfgarten et al. (2001) reported a significant inverse association between dietary iron intake and squamous cell carcinoma of the esophagus (SCCE) (Quartile 4 v 1: OR=0.2, 95% CI 0.1-0.8; no adjustment). However, Mayne et al. (2001) did not find a significant association (Quartile 4 v 1: OR=0.75, 95% CI 0.49-1.16; adjustment for sex, site, age, race, proxy status, income, education, usual body mass, cigarettes/day, years of consuming beer, wine and liquor, and energy intake). Current evidence is insufficient on the SCCE endpoint.

Table 2.6 Summaries of studies that have examined the association between iron intake and cancer of the rectum.

Citation	Study Location	Study Design/Participant Characteristics	Supp. Fe*	Ca/Co†	Comparison	OR /RR (95% CI)	P-trend	Adjustment factors/Remarks
Kabat 2007	Canada	Cohort study (Canadian National Breast Screening Study – randomized controlled trial of breast cancer screening). 100% female.	No	N=49,654 195 cases	Quintile 5 v 1	1.12 (0.67-1.88)	0.62	Energy adjustment using residuals method. Age, BMI, menopausal status, oral contraceptive use, hormone replacement use, dietary intakes of fat, fibre, folic acid, total calories, pack-years of smoking, alcohol intake, education and physical activity.
Balder 2006	Netherlands	Case-cohort within the Netherlands Cohort Study.	No	Subchort: 2215 women, 2156 men. Women: 185 cases Men: 333 cases	Quintile 5 v 1 Women: Men:	1.11 (0.53-2.30) 1.44 (0.85-2.45)	0.63 0.08	Age at baseline, BMI, family history of colorectal cancer, smoking status, non-occupational physical activity, total energy intake, alcohol consumption, total vegetable consumption.
Cross 2006	Finland	Nested case-control α-tocopherol , β-carotene cancer prevention study; All participants were smokers, male and white.	No	57 cases, 260 controls	Quartile 4 v 1	0.5 (0.1-2.8)	0.38	Age, education, BMI, number of years smoking, total number of cigarettes per day, physical activity (active = moderate or heavy occupational or recreational activity versus inactive), total caloric intake, alcohol (g/day), ever daily aspirin use, serum C-reactive protein.
Deneo-Pellegrini 1999	Montevideo Uruguay	Hospital-based case-control. Controls had conditions not related to smoking, alcohol consumption or	No	216 cases, 433 controls	mg/day ≤14.7 14.8-18.2 18.3+	1.0 1.4 (0.9-2.2) 3.2 (1.9-5.3)	<0.001	Age, sex, residence, urban/rural status, family history of colorectal cancer in first-degree relative, total energy, folate and dietary fibre intake.

* Supp Fe, were contributions to iron intake from supplements included in the exposure used by the authors? Yes/No

† Ca/Co – number of cases and number of controls for a case-control study; cohort size and number of cases for a cohort study.

Citation	Study Location	Study Design/Participant Characteristics	Supp. Fe*	Ca/Co†	Comparison	OR /RR (95% CI)	P-trend	Adjustment factors/Remarks
		diet. Frequency matched to cases on age (10 year age group), sex, residence (Montevideo/other) and urban/rural status. Cases: 34.7% female Controls: 34.4% female			18.3+ v ≤14.7 (women) 18.3+ ≤14.7 (men)	4.0 (2.1-7.7) 2.6 (1.1-6.1)		
Ullen 1997		Population-based case-control study	Fe supp. results report-ed sep-arately.	NR cases, 549 controls	Quintile 5 v 1	0.8 (0.5-1.4)	NR	Age, gender. *Note:* This odds ratio is for dietary iron intake.
Wurzel-mann 1996	U.S.	Cohort study (NHANES I and NHEFS) Follow-up: 15 years	No	N=14407, 38 cases	Quartile 4 v 1	1.01 (0.42-2.42)	0.98	Energy by the residuals method. Authors reported that age, sex, body mass index, fat, aspirin use, vegetable and meat consumption, physical activity were not confounders.
Freuden-heim 1990	New York, U.S.	Case-control study; individual matching on age, gender and neighbourhood of residence.	No	Men: 277 case-control pairs Women: 145 case-control pairs	Men: Quartile 4 v 1 Women: Tertile 3 v 1	2.15 (1.29-3.57) 0.84 (0.47-1.50)	NR NR	None. *Note*: Authors indicate that case and control groups did not differ with respect to BMI and that associations were not affected by smoking history and alcohol intake.

Table 2.7 Summaries of studies that have examined the association between iron intake and cancer of the colon.

Citation	Study Location	Study Design/Participant Characteristics	Supp. Fe*	Ca/Co	Comparison	OR (95% CI)	P-trend	Adjustment factors/Remarks
Kabat 2007	Canada	Cohort study (Canadian National Breast Screening Study). 100% female.	No	N=49,654 428 cases	Quintile 5 v 1	1.07 (0.75-1.53)	0.96	Age, BMI, menopausal status, oral contraceptive use, hormone replacement use, dietary intakes of fat, fibre, folic acid, total calories, pack-years of smoking, alcohol intake, education and physical activity.
Balder 2006	Netherlands	Case-cohort within the Netherlands Cohort Study.	No	Subchort: 2215 women, 2156 men. Women: 484 cases Men: 539 cases	Quintile 5 v 1 Women: Men:	1.14 (0.73-1.80) 1.30 (0.84-2.01)	0.91 0.43	Age at baseline, BMI, family history of colorectal cancer, smoking status, non-occupational physical activity, total energy intake, alcohol consumption, total vegetable consumption.
Cross 2006	Finland	Nested case-control α-tocopherol , β-carotene cancer prevention study; All participants were smokers, male and white.	No	73 cases, 260 controls	Quartile 4 v 1	0.3 (0.1-1.6)	0.09	Age, education, BMI, number of years smoking, total number of cigarettes per day, physical activity (active = moderate or heavy occupational or recreational activity versus inactive), total caloric intake, alcohol (g/day), ever daily aspirin use, serum CRP.
Shaheen 2003	North Carolina, U.S.	Population-based case-control study	Yes	475 cases, 833 controls	Quartile 4 v 1	1.05 (0.75-1.49)	NR	Sex, age, race, iron intake, red meat consumption, NSAID use.
Ullen 1997	Stockholm, Sweden	Population-based case-control study	Fe supp. results report-	NR cases, 549 controls	Quintile 5 v 1	0.6 (0.4-1.0)	NR	Age, gender *Note:* This odds ratio is for dietary iron intake.

* Supp Fe, were contributions to iron intake from supplements included in the exposure used by the authors? Yes/No

Citation	Study Location	Study Design/Participant Characteristics	Supp. Fe*	Ca/Co	Comparison	OR (95% CI)	P-trend	Adjustment factors/Remarks
			ed separately.					
Wurzelmann 1996	U.S.	Cohort study (NHANES I and NHEFS)	No	N=14407, Proximal: 52 cases	Quartile 4 v 1	3.15 (1.41-7.04)	0.007	*Note*: RR(proximal)=1.44, 1.23-1.69 95% CI, when adjusted for age and gender.
				Distal: 57 cases	Quartile 4 v 1	1.47 (0.73-2.97)	0.61	RR(distal)=1.03, 0.80-1.32 95% CI, when adjusted for age and gender. Energy adjustment was done using the residuals method. Authors reported that age, sex, body mass index, fat, aspirin use, vegetable and meat consumption, physical activity were not confounders.

Table 2.8 Summaries of studies that have examined the association between dietary iron intake and cancer of the colon or rectum.

Citation	Study Location	Study Design/Participant Characteristics	Ca/Co	Supp. Fe*	Comparison	OR (95% CI)	P-trend	Adjustment factors
Kato 1999	New York, US	Nested case-control. Individually matched on age, menopausal status at enrollment, dates of subsequent blood donations. 100% female.	105 cases, 523 controls	Yes	quartile 4 v 1	1.17 (0.6-2.3)	0.44	Family history of colorectal cancer, beer intake, prior occult blood testing, number of hours spent in sport activities in their early thirties.
Senesse 2004	Burgundy, France	Case-control study with population controls; 65 cases of rectal cancer (38%). Exclusions: familial colorectal cancer syndromes, inflammatory bowel disease, other types of cancer or colectomy. Cases: 36% female	171 cases, 309 controls	No	Quartile 4 v 1	2.2 (1.1-4.7)	0.02	Age, sex, energy intake, body mass index and physical activity.

* Supp Fe, were contributions to iron intake from supplements included in the exposure used by the authors? Yes/No

Table 2.9 Summaries of studies that have examined the association between dietary iron intake and colorectal adenoma.

Citation	Outcome	Study Location	Study Design/ Participants	Supp. Fe*	Ca/Co	Comparison	OR (95% CI)	P-trend	Adjustment factors/Remarks
Chan 2005	Colorectal adenoma	Nurses' Health Study, U.S.	Nested case-control; individual matching on date of endosocopy, age, indication for endoscopy, fasting status, month and year of blood draw, time period of any prior endosocopy. 100% female.	No	527 case-control pairs	Quartile 4 v 1	1.04 (0.68-1.57)	0.94	Adjusted for age, fasting status, date of blood draw, time period of endoscopy, symptoms at endoscopy, time period of any prior endoscopy, body mass index, family history of colorectal cancer, pack-years of smoking, physical activity, energy-adjusted intake of calcium and folate, servings of red meat, alcohol consumption, regular use of multivitamins, regular use of aspirin, menopause status, postmenopausal hormone use, age at first menarche, age at last menstrual period.
McGlynn 2005	Colorectal adenoma (advanced distal)	Multi-site, U.S.	Nested case-control. Frequency-matching on sex.	Yes	679 cases, 697 controls	Distribution across quartiles of intake (χ^2-test for independence)	NR	NR	None. *Note*: No significant difference in dietary iron intake between cases and controls (p=0.07) although controls tended to consume more.
Almendingen 2001	Colorectal adenoma	Oslo, Norway	Case-control within the 3-year follow-up of a double-blind antioxidant trial of colorectal polyps, calcium and antioxidants. Frequency-matching on	Both	87 cases, 35 hospital controls, 35 healthy controls	Dietary Iron ≤12 mg/L 13-18 mg/L 19+ mg/L, Total iron (including supp.)	1.00 0.3 (0.1-1.1) 0.2 (0.1-2.3)	0.08	Body mass index, colorectal cancer among first degree relatives, energy, fat, fibre and smoking status.

* Supp Fe, were contributions to iron intake from supplements included in the exposure used by the authors? Yes/No

Citation	Outcome	Study Location	Study Design/ Participants	Supp. Fe*	Ca/Co	Comparison	OR (95% CI)	P-trend	Adjustment factors/Remarks
			sex and age.			≤12 mg/L 13-18 mg/L 19+ mg/L	1.00 0.5 (0.1-1.6) 0.9 (0.2-2.3)	0.9	
Tseng 2000	Colorectal adenoma recurrence	US, multicentre	Nested case-control study within the Antioxidant Polyp Prevention Study	No	72 cases, 150 controls	Tertile 3 v 1 (serum ferritin ≤70µg/L)	0.44 (0.22-0.84)	0.02	Age, sex, centre, energy intake, alcohol, fibre, folate, total fate, number of months between follow-up colonoscopies, smoking status, aspirin use (exposure = energy adjusted iron).
					179 cases, 186 controls	Tertile 3 v 1 (serum ferritin >70µg/L)	0.65 (0.22-1.91)	0.29	
Tseng 1997	Colorectal adenoma recurrence	US, multicentre	Nested case-control study within the Antioxidant Polyp Prevention Study	No	247 cases, 419 controls	Quartile 4 v 1	0.37 (0.19-0.73)	0.005	Age, sex, center, treatment group, caloric intake, and energy adjusted intakes of alcohol, fibre, folate and total fat.
Bird 1996	Colorectal adenoma	Southern California, US	Case-control study; individual-matching on age, sex, date of sigmoidoscopy, centre. Cases and controls underwent sigmoidoscopy only.	Yes (see *Note*)	Men: 300 cases, 331 controls	<11.6 mg/day 11.6-13.6 13.7-17.2 17.3-27.3 >27.3	2.1 (1.3-3.5) 1.00 1.4 (0.9-2.4) 1.9 (1.2-3.2) 1.6 (0.9-2.8)	NR	Adjusted for matching variables and smoking. *Note*: Total iron intake includes supplements. 15% of participants who reported use of a supplement but were not able to describe it were assumed to consume a supplement containing the recommended daily allowance of iron.
					Women: 167 cases, 167 controls	<11.6 mg/day 11.6-13.6 13.7-17.2 17.3-27.3 >27.3	0.9 (0.4-1.9) 1.00 0.6 (0.3-1.4) 0.9 (0.4-1.8) 1.0 (0.5-2.0)	NR	
Tseng 1996	Colorectal adenoma	North Carolina, US	Hospital-based case-control study.	Yes	Men: 105 cases, 165 controls	Quartile 4 v 1	0.41 (0.12-1.36)	0.60	Adjusted for age, BMI, calorie intake, current smoking status, regular use of supplements, family history of colon cancer, intakes of fat, fibre, and alcohol.
					Women: 131 cases, 244 controls	Quartile 4 v 1	0.71 (0.27-1.86)	0.13	*Note*: Among men: Quartile 4: median=27.6 mg/day Quartile 1: median=8.9 mg/day

Citation	Outcome	Study Location	Study Design/ Participants	Supp. Fe	Ca/Co	Comparison	OR (95% CI)	P-trend	Adjustment factors/Remarks
									Among women: Quartile 4: median=25.2 mg/day Quartile 1: median=6.5 mg/day
Benito 1993	Colorectal adenoma (adenomatous and villous)	Mallorca, Spain	Population-based case-control study; individual matching on age; cases 33.7% female, controls 40.5% female.	No(?)	101 cases, 242 controls	Quartile 4 v 1	0.64 (n.s.)	n.s.	Total calories, age, sex, physical activity in longest job held, rural residence.
Little 1993	Colorectal adenoma	Nottingham, England	Case-control nested within a faecal occult blood screening programme. Cases: 38% female Controls: 39% female	No	147 cases, 153 controls		n.s.		*Note*: Authors reported qualitatively that there was no association. Odds ratios were not presented. Dietary iron.
Macquart-Moulin 1987	Colorectal adenoma (adenomatous and villous)	Marseilles, France	Hospital-based case-control; individual matching on sex and age. Controls were patients undergoing rehabilitation for injuries or trauma and had no gastrointestinal diseases or alcoholism.	No	238 cases, 252 controls	Quartile 4 v 1	0.60 (n.s.)		Age, sex, caloric intake, weight.
Hoff 1986	Colorectal adenoma	Norway	Case-control study within a population screening study	No	55 cases <5mm, 23 cases >5mm, 77 controls	Mean dietary iron intake in >5mm polyp group v controls	p=0.10	nr	Energy.

Table 2.10 Summaries of studies that have examined the association between dietary iron intake and esophageal adenocarcinoma.

Citation	Outcome	Study Location	Study Design/Participant Characteristics	Supp. Fe*	Ca/Co	Comparison	OR (95% CI)	P-trend	Adjustment factors/ Remarks
Chen 2002	Esophageal cancer (adenocarcinoma)	Eastern Nebraska, US	Population-based, case-control. Frequency-matching on age, gender and vital status	Unclear	124 cases/ 449 controls	quartile 4 v 1	0.6 (0.3-1.1)	0.1	Energy adjustment by residuals method. Age, gender, alcohol use (never, past, current), tobacco use (nonsmokers, <30/day, ≥30/day), education level, family history of esophageal adenocarcinoma, vitamin supplement use, body mass index, age-squared.
Mayne 2001	Esophageal cancer (adenocarcinoma)	CT, NJ, WA - US	Multisite, population-based, case-control. Frequency-matching on age, gender, geographic area	No	282 cases/ 687 controls	75^{th} v 25^{th} percentile	0.79 (0.57-1.09)	NR	Energy adjustment by standard multivariate method. Sex, site, age, race, proxy status, income, education, usual BMI, cigarettes/day, years of consumption of beer, wine, liquor, energy intake. *Note*: results were similar using residuals method.
Wolfgarten 2001	Esophageal cancer (adenocarcinoma)	Cologne, Germany	Single site, population-based, case-control.	No	40 cases/ 100 controls	mg/day 0.1-14.4 14.2-16.1 16.1-17.9 >18	 1.0 0.4 (0.1-1.3) 0.6 (0.2-1.7) 0.2 (0.0-0.7)	<0.05	None.
Zhang 1997	Adenocarcinoma of esophagus	New York - US	Hospital-based, case-control.	No	95 cases/ 132 controls	Quartile 4 v 1	0.6 (0.3-0.9)	0.03	Energy adjustment by the residuals method. Age, sex, race, years of education, pack-years

* Supp Fe, were contributions to iron intake from supplements included in the exposure used by the authors? Yes/No

	or gastric cardia								smoking, frequency of alcohol use, BMI, total dietary intake of calories.
Brown 1995	Adeno-carcinoma of the esophagus or esophog-astric junction	GA, MI, NJ – US	Population-based case-control. White men included only.	No	174 cases/ 750 controls	Quartile 4 v 1	0.5	0.51	Energy adjustment by standard multivariate method. Age, geographic area, cigarettes, liquor, recent annual income, BMI, total calories from food, *Note*: case group included 113 with adenocarcinoma of the esophogastric junction and 61 with adenocarcinoma of the esophagus.

2.4.14 Heme-iron Intake and Rectal Cancer

Kabat et al. (2007) investigated the association between heme iron intake and rectal cancer in a cohort of 49,654 Canadian women. During a mean follow-up of 16.4 years, 195 cases of rectal carcinoma were identified. The authors reported hazard ratios comparing quintiles of energy-adjusted (residuals method) heme iron intake. The multivariate odds ratio comparing the lowest (<1.58 mg/day) with the highest quintile (>2.95 mg/day) of heme iron intake was 1.27 (95% CI 0.74-2.19) and the test for trend was not significant (p=0.71). There was no evidence of effect modification by alcohol intake.

The Netherlands Cohort Study on Diet and Cancer included both men and women (Balder et al., 2006). With an average follow-up of 9.3 years, there were 518 incident cases of rectal cancer, 333 among men and 185 among women. Using a case-cohort design, Balder et al (2006) observed no evidence of an association between heme iron intake and cancer of the rectum among women or men. The RRs for rectal cancer among men were 1.04 (95% CI 0.80-1.35) for the continuous measure of exposure and 0.98 (95% CI 0.64-1.50) for the comparison between highest and lowest quintiles. Among women the corresponding odds ratios were 1.12 (95% CI 0.86-1.46; continuous exposure measure) and 1.23 (95% CI 0.73-2.07; quintile comparison). The analysis included adjustment for age, BMI, family history of colorectal cancer, smoking history, leisure

physical activity, total energy intake, alcohol consumption, and total vegetable consumption.

2.4.15 Heme-iron Intake and Colon Cancer

Lee et al. (2004) did not observe a significant trend in colon cancer risk with increasing heme iron intake in the Iowa Women's Health Study until analyses were further adjusted for zinc intake. Before adjustment for zinc intake, the multivariable RR for proximal colon cancer was 1.41 (95% CI 0.90-2.21) comparing the highest with the lowest quintile of intake. The test for trend gave a *p*-value of 0.24. After adjustment for zinc intake, the corresponding RR was 2.18 (95% CI 1.24-3.86) and the test for trend attained formal statistical significance (p=0.01). Subgroup analyses indicated that the risk was restricted to proximal colon cancer (438 cases) and to women who consumed alcohol. Larsson et al. (2005) observed a relative risk of 1.26 (95% CI 0.97-1.64) in the highest quintile of heme iron intake in the population-based Swedish Mammography Cohort. Five hundred and forty-seven incident cases of colon cancer were identified during 14.8 years of follow-up. The risk was again higher among women who consumed more alcohol ($\geq$ 20 g/week) and the authors reported a statistically significant interaction between heme iron intake and alcohol (p=0.01).

In the Netherlands Cohort Study on Diet and Cancer (Balder et al., 2006), there were 1023 incident cases of colon cancer, 539 among men and 484 among women. Balder et al (2006) reported a multivariate-adjusted RR of 1.29 (95% CI 0.92-1.81) comparing the highest with the lowest quintile of intake and an insignificant test for trend (p=0.10)

among men. Exclusion of cases diagnosed during the first 2 years of follow-up increased the RR to 1.50 (95% CI 1.03-2.17; p-trend=0.02). Among women, the RR was 1.20 (95% CI 0.83-1.74) including all cases and 1.19 (95% CI 0.80-1.77) excluding those found during the first 2 years of follow-up. These relative risks were adjusted for age, BMI, family history of colorectal cancer, smoking history, leisure physical activity, total energy intake, alcohol consumption, and total vegetable consumption.

2.4.16 Heme-iron Intake and Colorectal Adenoma

Chan et al. (2005) found no difference in heme–iron intake between cases of colorectal adenoma (n=527) and controls (n=527) among women in the Nurses' Health Study (Wilcoxon signed rank test, p=0.24).

2.4.17 Heme-iron Intake and Esophageal Cancer

One prospective epidemiological study has investigated the association between heme iron and cancer of the upper digestive tract (Lee et al., 2005). Among 34,708 women aged 55 to 69 years at baseline in the Iowa Women's Health Study 52 gastric cancer and 23 esophageal cancer cases were identified in 16 years of follow-up. The relative risk (RR) comparing the highest with the lowest quintile of heme iron intake was 1.07 (95% CI 0.41-2.82) with adjustment for age, total energy intake, smoking and alcohol consumption (*p*-trend=0.97). The authors then adjusted further for zinc intake and observed a marked change in the odds ratios. The RR comparing the highest and lowest quintiles was 2.83 (95% CI 0.83-9.54) and the trend approached formal significance

(p=0.06). Given the small number of cases in the study, the possibility of a false positive finding is high.

2.5 Iron and Cancer of the Gastrointestinal Tract: Intervention Study Results

A recent publication reported results for cancer outcomes in the FeAST study (Zacharski et al., 2008). The FeAST study was a multicentre, controlled single-blind clinical trial among individuals with stable peripheral artery disease that was designed to test whether reducing iron levels through periodic phlebotomy might improve vascular outcomes. Cancer was a secondary outcome in the study (Edgren et al, 2008). Participants were required to have no history of a clinically apparent malignancy in the 5 years preceding the study. Patients randomized to the intervention arm received phlebotomy at 6-monthly intervals to keep serum ferritin levels within the range 25 – 60 ng/mL. Nearly all of the participants were male (98.8%), the mean age was 67 years and the groups did not exhibit significant differences in serum ferritin values at baseline. Compliance with phlebotomy was 88% and analyses were intent-to-treat. The mean follow-up was 4.5 years. During this time, 98 cases of cancer occurred, 60 in the control group and 38 in the iron reduction arm. Nine of the cancers in the control group were colorectal and 6 upper aerodigestive compared with 4 colorectal and only 1 upper aerodigestive in the intervention arm. Bladder cancer was the only site which showed a higher number of cases in the intervention arm (1 in the control group and 7 in the intervention arm). Mean ferritin levels were statistically significantly lower in the intervention arm compared with the control arm at all time points. The intervention arm exhibited a lower cancer

incidence (HR=0.65, 95% CI 0.43-0.97, p=0.036) and a lower cancer mortality (HR = 0.39, 95% CI 0.21-0.72, p=0.003). As pointed out in the editorial by Edgren et al. (2008), the generalisability of the results of this study is limited and cancer was not the primary outcome. Edgren et al. (2008) also question whether blinding was sufficient allowing the possibility of earlier diagnosis in the control group. However, the results are provocative due to the clear improvement in cancer outcomes in the phlebotomy intervention arm. Although too few on which to base a Kaplan-Meier analysis, the number of colorectal and aerodigestive cancers was clearly lower in the intervention arm.

2.6 Iron and Cancer of the Gastrointestinal Tract: Summary

There is considerable biological plausibility and evidence from *in vitro* and animal studies for the involvement of iron in the tumorigenic process. However, evidence from observational epidemiological studies for an association between body iron levels or iron intakes and cancers of the gastrointestinal tract is limited and inconsistent.

All four prospective studies that examined the association between rectal cancer and biochemical iron indices had a small number of cases. Results were conflicting (Table 2.2). Cross et al. (2006) reported a significant inverse association between body iron levels and colon cancer (73 cases) but not between body iron levels and rectal cancer (57 cases). This study, although of restricted generalisability due to the selected nature of the participants, was the only study to address the possible influence of inflammation on levels of serum ferritin by adjusting for serum C-reactive protein. Results were also

adjusted for aspirin use. Relatively fewer cases than controls reported ever daily use of aspirin ($p=0.002$). Cases exhibited marginally higher baseline levels of serum C-reactive protein than the controls ($p=0.04$) suggesting higher levels of systemic inflammation. Baseline iron parameters were lower in the case group, although not significantly so, in univariate analyses. As discussed by the authors, an inverse association between body iron levels and colon cancer might result from reduced absorption leaving greater amounts of iron in the digestive tract capable of interacting directly with the gastrointestinal mucosa. However, reduced body iron levels may also result from occult disease. Although cases diagnosed during the first five years of follow-up were excluded, it is possible that baseline serum measurements in the case group were influenced by the presence occult disease. Individuals with gastrointestinal symptoms are generally less likely to use aspirin. The difference in ever daily use of aspirin between the case and control groups, although potentially interpretable as a protective effect, may also reflect the presence of gastrointestinal symptoms at baseline.

An inverse association was also observed between iron status and colon cancer among the male participants in a cohort study in California (Herrinton et al., 1995) and a weak inverse association ($p<0.01$) among the women in a nested case-control study in New York (Kato et al., 1999). In the Kato et al. study, the mean time period between blood sample collection and cancer diagnosis was only 4.7 years. Thus, the inverse association between colon cancer and iron levels at baseline may be due to pre-clinical disease in the case group. Overall, the colon cancer studies were of low power and controlled for different factors. Some of the studies reported results stratified by gender while others

did not. There were also differences in the disease location subgroups examined making it difficult to compare the results. Although there is some consistency between studies with respect to an inverse association between colon cancer and body iron levels, the studies are few, small and with potentially significant sources of bias.

The nested case-control study conducted by Tseng et al. (2000) did not find a significant association between serum ferritin and recurrence of colorectal adenoma. Bird et al. (1996) also observed no significant association between serum ferritin and incidence of colorectal adenoma in a case-control study with 467 cases and 498 controls. Nelson et al. (1994) reported a positive, although not statistically significant, association. However, the control group in their study was undergoing colonscopy due to occult blood in their stool, a factor likely to affect iron status.

The studies of stomach cancer and body iron levels were small. Herrinton et al. (1995) and Knekt et al. (1994) both observed an inverse association beween TfSat and stomach cancer among men. The associations among women from these studies were conflicting, however. Overall, the evidence for an association between stomach cancer and body iron levels is limited in amount and quality.

The available evidence does not provide support for a positive association between body iron levels and neoplasms of the rectum, colon or stomach. However, most of the studies were small, differed with respect to protocols for taking blood samples (fasting versus non-fasting) and did not address the possibility of acute effects on iron measures.

Most of the prospective studies excluded cases diagnosed within the first 3 to 5 years after follow-up to attempt to minimize the possibility of confounding by occult disease. Functional variants in genes involved in iron uptake and metabolism may represent a means to better define the role that iron plays in the carcinogenic process avoiding the uncertainties in measurement encountered with biochemical iron indices.

Of four studies that provided results for the association between dietary iron intake and rectal cancer among women, one study in Uruguay found a significant positive association (Deneo-Pellegrini et al., 1999) and three reported no significant association (Kabat et al., 2007; Balder et al., 2006; Freudenheim et al., 1990). The generalisability of the results from the Deneo-Pellegrini et al. hospital-based case-control study is unclear. Results among men provided some suggestion of a positive association between iron intake and rectal cancer (Freudenheim et al., 1990; Balder et al., 2006) but this was not observed in all studies (Cross et al., 2006). Results for colon cancer were also inconsistent but did not suggest a positive association among either men or women.

All five studies of dietary iron intake and EAC reported inverse associations, although not all associations were statistically significant. Given the retrospective nature of the studies, it is not possible to assess whether gastrointestinal symptoms preceding EAC influenced reported dietary intake among the cases. Also, proxy respondents constituted 76% of the cases and 61% of the controls in the Chen et al. study and about 30% of cases and 3.5% of controls in the Mayne et al. (2001) study. White men have been the focus of most studies to date, targeting the population apparently most at-risk, but restricting the

generalisability of the results. Current evidence, although limited due to the retrospective nature of the available studies, is not supportive of a positive association between total dietary iron intake and EAC. On the contrary, it suggests an inverse association.

Overall, the existing evidence, although limited in amount, is not supportive of an association between heme iron intake and cancer of the colon or rectum among women. Only one study has investigated this relationship among men (Balder et al., 2006) and reported a significant association with colon cancer on exclusion of the cases diagnosed in the first 2 years of follow-up.

In a randomised trial setting, Zacharski et al. (2008) reported a markedly lower cancer incidence and mortality among a group of men with peripheral artery disease who underwent phlebotomy to maintain their ferritin levels in an optimal range (25 to 60 ng/mL) when compared with the group not receiving the intervention. The number of cases was small, precluding risk estimates by cancer site. However, the number of colorectal and upper aerodigestive cancers was clearly lower in the intervention group. Although these results may not be transferable to other populations and need to be replicated, to date they are the most supportive piece of epidemiological evidence for an association between elevated iron levels and cancer.

2.7 Genetics of Iron Homeostasis

The study of hereditary hemochromatosis (HH) has been central to the rapid progress in understanding iron homeostasis during the last 5 to 10 years. There are five recognized clinical forms of HH that are associated with mutations in *HFE* (hereditary hemochromatosis Type 1), *HJV* (juvenile hemochromatosis, HH Type 2A), *HAMP* (juvenile hemochromatosis, HH Type 2B), *FPN1* (ferroportin disease, HH Type 4), and *TfR2* (TfR2 hemochromatosis, HH Type 3) (Beutler, 2006; Le Gac and Ferec, 2005; Griffiths, 2007). Type 1 HH is the dominant form in white, northern European populations. It exhibits an autosomal recessive mode of inheritance and is characterized by elevated serum ferritin and transferrin saturation, iron accumulation in hepatocytes but a sparing of the macrophages in the RE system (Wallace and Subramanian, 2007). Disease onset is usually in the 40's for men and 50's for women. Types 2A, 2B and 3 generally follow a similar path to Type 1 although with a younger age of onset and more severe iron loading. They exhibit an autosomal recessive mode of inheritance. HH Type 4 (ferroportin disease) is an adult onset condition and exhibits considerable heterogeneity. It differs from Type 1 hereditary hemochromatosis with respect to the location of iron accumulation and mode of inheritance. Type 4 HH is inherited in an autosomal dominant fashion. At early stages, serum ferritin levels increase but transferrin saturation remains unchanged. Iron accumulates first in the Kupffer cells and only later in hepatocytes. Liver damage is less common than is the case for Type 1 HH (Griffiths, 2007; Wallace and Subramanian, 2007). The inability to regulate iron levels observed in these conditions probably results from impaired hepcidin response or impaired engagement of

hepcidin with ferroportin (Beutler, 2006; Papanikolaou et al., 2005; DeDomenico et al., 2006).

Key genes in the homeostasis of iron are shown in Table 2.11 with a brief description of the gene product function. This list is not all-inconclusive but contains those genes identified in a literature review as important in the regulation of iron levels and iron transport. Further details on variants and population frequencies are provided in the paragraphs following the table.

Table 2.11 Genes involved in Iron Homeostasis.

Gene	Location	Gene product & function	References
HFE (Type 1 HH*)	6p21.3	Involved in the regulation of iron uptake through formation of complexes with other iron transfer proteins including transferrin receptor-1, transferrin receptor-2, and hemojuvelin; involved in hepcidin regulation and possibly in iron sensing	Feder et al. (1996), Beutler (2006), Nemeth (2008), Schmidt et al. (2008), Ganz (2008)
HAMP (hepcidin, juvenile or Type 2B HH)	19q13.1	Key regulator of iron homeostasis, an antimicrobial peptide produced in the liver, implicated in the development of the anemia due to inflammation	Park et al. (2001), Nicolas et al. (2000), Nemeth and Ganz (2006)
TFRC (transferrin receptor-1)	3q29	Receptor for uptake of transferrin-bound iron (TBI) by endocytosis, ubiquitous expression except on mature erythroctes, involved in regulation of hepcidin expression	Chua et al. (2007), Sargent et al. (2005), McCLelland et al. (1984), Evans and Kemp (1997), Schmidt et al. (2008).
TFR2 (transferrin receptor-2)	7q22	Receptor for uptake of transferrin-bound iron (TBI) by endocytosis, expressed mainly in liver and erythroid cells	Kawabata et al., (1999), Chua et al. (2007)
SLC40A1 (ferroportin-1 *FPN1*, *HFE4*) ("ferroportin disease")	2q32	The only iron export protein currently known, key for efflux of iron from enterocytes, macrophages, hepatocytes and parenchymal cells.	Nemeth and Ganz (2006), Liu et al. (2005)
HFE2/HJV (hemojuvelin, juvenile or Type 2A HH)	1q21.1	Involved in iron sensing and regulation of iron uptake	Nemeth (2008), Papanikolau et al. 2004), Lanzara et al. (2004), Pietrangelo et al. (2004)
TF (transferrin)	3q22.1	Iron transport protein synthesized mainly in the liver, binds two Fe(III) ions and supplies iron to tissues	Yang et al. (1984), Lee et al. (1999), Chua et al. (2007)
DMT1 (*NRAMP2*/divalent metal transporter-1, *SLC11A2*)	12q13	Iron import protein for uptake of transferrin bound and non-transferrin bound iron (NTBI) by enterocytes and from endosomes	Andrews (1999), Chua et al. (2007)

* HH, hereditary hemochromatosis

Table 2.11 (Contd.) Genes involved in Iron Homeostasis.

Gene	Location	Gene product & function	References
CYBRD1 (Dcytb, duodenal cytochrome reductase – b)	2q31.1	Ferrireductase, highly expressed at the gut apical brush border, transforms Fe(III) to Fe(II) prior to import by DMT1	Chua et al. (2007), Sargent et al. (2005)
HEPH (hephaestin)	Xq11-q12	Ferroxidase, oxidizes Fe(II) to Fe(III) for subsequent binding with transferrin following the efflux of iron from enterocytes	Chua et al. (2007)
CP (ceruloplasmin)	3q23-q25	Ferroxidase, oxidizes Fe(II) to Fe(III) for subsequent binding following efflux of iron from macrophages and hepatocytes	Chua et al. (2007), Hellman and Gitlin (2002)
HMOX1 (heme-oxygenase 1)	22q12-q13.1	Inducible form of heme-oxygenase, the rate limiting enzyme in the catabolism of heme to CO and biliverdin, protective through anti-inflammatory and antiproliferative effects, upregulated by several stimuli including heme, hydrogen peroxide, cytokines, nitric oxide, heavy metals, UV radiation, hyperoxia, hypoxia and growth factors.	Exner et al. (2004), Otterbein et al. (2003), Shibahara et al. (1989)

2.7.1 HFE

The *HFE*-gene, located on chromosome 6 (6p21.3), was discovered by Feder et al. (1996). DbSNP (Build 129) lists six coding polymorphisms in this gene; three non-synonymous SNPs (single nucleotide polymorphisms) in exon 2, two non-synonymous SNPs in exon 4 and a non-synonymous SNP in exon 6. Two polymorphisms in *HFE* are associated with Type 1 hereditary hemochromatosis (Feder et al., 1996; Burke et al., 1998; Hanson et al., 2001), namely C282Y (rs1800562, exon 4) and H63D (rs1799945, exon 2). A third variant (S65C, rs1800730, exon 2) has also been studied in relation to

iron homeostasis (Barton et al., 1999) but is rare and not associated with the hemochromatosis phenotype (Beutler, 2006).

C282Y is a loss of function polymorphism. A cysteine is replaced with a tyrosine at a binding site for the *HFE* gene product with B2 microglobulin. B2 microglobulin chaperones HFE to the cell surface. In the presence of the C282Y variant, this does not occur and HFE remains in the endoplasmic reticulum (Beutler, 2006). The C282Y variant has highest carrier frequencies (10-15 %) in those of Northern European, likely Celtic, descent (Dorak et al., 2005; Lucotte and Dieterlen, 2003) and is rare in those of Asian or African descent (0 to 0.5%; Dorak et al., 2005).

The H63D polymorphism replaces a histidine with aspartic acid at codon 63. The minor allele (D63) carrier frequency ranges from 3 to 15% (Adams et al., 2005). The functional effects of the H63D variant remain to be characterized.

The C282Y and H63D variants are associated with elevated transferrin saturation and serum ferritin levels (Barton et al., 2005), although less strongly in the case of H63D. However, the biochemical penetrance of the variants is variable and may be mediated by alcohol intake (Waalen et al., 2005), age, gender (Allen et al., 2008), ethnic group (Cogswell et al., 2003) and other genes (Milet et al., 2007; Biasiotto et al., 2004).

2.7.2 HAMP

HAMP, also known as *HEPC* and *LEAP1*, is located on chromosome 19 (19q13.1; Park et al. 2001) and encodes the key iron regulatory protein, hepcidin. Several genetic variants have been identified in *HAMP* (Wallace and Subramanian, 2007; Roetto et al., 2003; Roetto et al., 2004; Majore et al., 2004; Delatycki et al., 2004; Matthes et al., 2004). Homozygosity for a rare frameshift mutation was identified in a family with juvenile HH (93delG) (Roetto et al., 2003). A second variant (C70R; Roetto et al., 2004) is located within a region of conserved cysteines important for the stability of the protein. It has been identified among individuals with the juvenile hemochromatosis phenotype but was absent in a sample of healthy controls. The G71D variant identified by Biasiotto et al. (2003) is located between two pairs of cysteines and is of uncertain functional significance. The *HAMP* variants that have been identified to date in association with abnormal iron parameters are rare.

2.7.3 TFRC

Transferrin receptor-1 (*TFRC*) is located on chromosome 3 (3q29) and has 19 exons (Evans and Kemp, 1997). The gene product is essential for the uptake of iron required for cellular metabolism and growth and knockout studies have shown embryo lethality (Daniels et al., 2006). *TFRC* exhibits increased expression in cells with a high proliferation rate, such as the intestinal epithelium, and also cells with high iron needs such as the placenta and erythrocyte precursors (Daniels et al. 2006). Expression levels in normal cells are sensitive to iron concentrations. Malignant and pre-malignant cells exhibit increased expression (Daniels et al., 2006; Boult et al., 2008; Brookes et al., 2006;

Prutki et al., 2006) probably reflecting the increased iron needs for DNA replication. Of the non-synonymous SNPs identified in this gene to date only one occurs at a frequency greater than 5% and has been investigated in relation to disease (S142G; rs3817672, exon 4; dbSNP Build 129). The functional significance of S142G is unclear.

2.7.4 TfR2

The *TfR2 (Transferrin-receptor 2)* gene is located on chromosome 7, contains 18 exons (Kawabata et al., 1999) and is linked with type 3 hereditary hemochromatosis (Type 3 HH). It has two splice-forms, α and β. The α-form is believed to be the second transferrin-receptor (Chua et al., 2007) since the β form is missing exons 1 and 3 that code for the transmembrane domain. TfR2 is expressed mainly in the liver and erythroid cells (Chua et al., 2007) and is involved in the regulation of hepcidin possibly through iron sensing. Camaschella et al. (2000) first identified a nonsense variant (Y250X) in this gene in two Sicilian families exhibiting the hemochromatosis phenotype (Type 3 HH). Since then at least coding 8 variants have been identified in individuals with Type 3 HH in different populations (Le Gac and Ferec, 2005; Wallace and Subramanian, 2006; Beutler and Beutler, 2004; Biasiotto et al., 2003). From a population perspective, however, the frequencies of the variants are low (Camaschella, 2005).

2.7.5 FPN1

The *FPN1 (Ferroportin-1)* gene, also known as *SLC40A1, IREG1* and *HFE4*, is located on chromosome 2 and contains 8 exons. The gene product, ferroportin, is the only

identified iron export protein in mammals. Variants in this gene have been associated with "ferroportin disease" – a disorder which, unlike the other recognized forms of hereditary hemochromatosis, is inherited in an autosomal dominant fashion (Wallace and Subramanian, 2007). Three variants in *FPN1* have evidence for an association with reduced iron transport (Drakesmith et al., 2005; Schimanski et al., 2005; Liu et al., 2005) - A77D (rs28939076, exon 3), V162del (rs28939076, exon3; Devalia et al., 2002; Cazzola et al., 2002) and G490D. Of these, the A77D and V162del variants are the most widespread (Wallace and Subramanian, 2007). The Q248H variant (rs11568350, exon 6; Gordeuk et al., 2003) is observed in individuals of sub-Saharan African descent and shows a weak association with serum ferritin levels (Barton et al., 2007; Rivers et al., 2007). Most of the other variants in *FPN1* have been found in single families and are rare.

2.7.6 HJV

HJV (*Hemojuvelin*), also known as *HFE2A*, is located on chromosome 1 and linked with the rare juvenile hemochromatosis phenotype (JH Type 2A; Papanikolau et al., 2004). Evidence suggest that the gene product is involved in the regulation of hepcidin (Nemeth, 2008). *HJV* is expressed in the liver, heart and skeletal muscle (Chua et al., 2007). Wallace and Subramanian (2007) list 37 variants in this gene identified in families with the JH Type 2A phenotype. Most variants have been observed only in single families with the exception of G320V (exon 4) that has been observed in Greek, French-Canadian and Italian families (Lanzara et al., 2004; Wallace and Subramanian, 2007). From a population perspective, all the iron-dysregulation associated variants are rare.

2.7.7 TF

The *TF* (*Transferrin*) gene is located on chromosome 3 (3q22.1; Yang et al., 1984) and is expressed mainly in the liver (Chua et al., 2007). It is highly polymorphic with more than 30 different genetic variants (Lee et al., 1999). The most common polymorphic form (TF C) has three recognized variants – C1, C2 and C3. Rarer forms have also been identified, including B, D and D-chi. The genetic variants resulting in the C1, C2 and C3 phenotypes have been identified. A SNP in exon 15 (rs1049296) of the *TF* gene (3q22.1) leads to the replacement of a proline at codon 570 with a serine. This distinguishes the C1 and C2 forms (Namekata et al., 1997). The C3 variant is characterized by an amino acid substitution glycine→serine in exon 7 (3q22.1) due to a G→A single base change (rs1799899) (Beckman et al., 1998). The C2-allele is more prevalent in populations of Han ancestry (ca. 30%) than in populations of European or African ancestry (16-17% and 10%, respectively). The C3-allele, however, is very rare in Asian populations and African populations but reaches frequencies of 4% in individuals of European ancestry.

2.7.8 DMT1

DMT1 (*Divalent metal transporter-1*) is located on chromosome 12 (12q13) and contains 17 exons but has four splice variants some with and some without iron-responsive elements (Lee et al., 1998; Hubert and Henze, 2002; Mackenzie et al., 2007). The gene product is involved in the uptake of divalent metals, including iron, zinc, cadmium and lead in the gut and into endosomes within cells (Chua et al., 2007; Andrews, 1999).

DMT1 expression is high in the apical brush border of duodenal enterocytes (Canonne-Hergaux et al. 1999) and has also been observed in macrophages and in the brain and kidneys (Mims and Prchal, 2005). Boult et al. (2008) reported increased expression in association with higher iron deposition in samples of Barrett's metaplasia and esophageal adenocarcinoma compared with healthy control samples. An earlier study found similar results for colorectal adenoma (Brookes et al., 2006). Lee et al. (1998) identified five variants in *DMT1* – one coding, four intronic and one a polymorphic microsatellite. A further variant was identified in intron 15 by Stokkers et al. (2000) in an investigation of the relationship between *DMT1* variants and inflammatory bowel disease. Mims et al. (2005) reported a variant in a splice donor site of DMT1 that enhanced the skipping of exon 12 in a patient with microcytic anemia and was associated with reduced DMT1 production (Priwitzerova et al., 2005). The functional significance of the other identified variants is unclear.

2.7.9 CYBRD1

CYBRD1, also known as *DcytB*, is located on chromosome 2 (2q31.1) and is expressed in the brush border of enterocytes in the duodenum (McKie et al., 2001). The gene product is a ferric reductase, possibly only one of several (Mackenzie and Garrick, 2005) involved in the reduction of ferric iron to the ferrous state prior to import through DMT1. Increased *CYBRD1* expression has been observed in esophageal dysplasia and adenocarcinoma compared with normal tissue (Boult et al., 2008). McKie et al. (2001) reported a variant in exon 4 (S266N, rs10455). Based on limited data, the G-allele (serine) seems to be more prevalent in individuals of east Asian descent (*ca.* 70%) than

either European, white individuals (20-30%) or individuals of African-American ethnicity (*ca.* 10%) or sub-Saharan origins (<5%) (NCBI, dbSNP – Build 36.3). Zaahl et al. (2004) reported four other variants in individuals of white or black ancestry in South Africa – one non-synonymous SNP in exon 4 (R226H) and synonymous SNPs in each of exons 3, 2 and 1. The variant in exon 1 (IVS1-4C→G) was observed only in white individuals with the HH phenotype but with a low frequency (3%).

2.7.10 HEPH

HEPH (Hephaestin), located on the X chromosome (Xq11-12), encodes hephaestin, a ferroxidase, which is found on the basolateral membrane of duodenal enterocytes collocated with ferroportin (Han and Kim, 2007). Hephaestin acts to oxidize the ferrous iron to the ferric state before complexation of the iron with transferrin in the portal circulation (Griffiths et al., 2005). Hephaestin expression has also been observed in the placenta, colon, spleen, kidney and brain (Petrak and Vyoral, 2005). There is currently little information on associations between variants of this gene and disease or dysregulation of iron homeostasis. Brookes et al. (2006) observed reduced *HEPH* expression in colorectal cancer samples compared with normal controls. More extensive vascular invasion was also associated with reduced *HEPH* expression. Boult et al. (2008) reported higher hephaestin expression measured as mRNAs in Barrett's metaplasia compared with normal squamous esophageal mucosa but no significant difference in expression between the normal mucosa and esophageal adenocarcinoma.

2.7.11 CP

CP (*Ceruloplasmin*), is located on chromosome 3 (3q13-25), contains 19 exons (Koschinsky et al., 1986; Yang et al., 1986; Daimon et al., 1995) and encodes another ferroxidase. The gene product, ceruloplasmin, is a homolog of hephaestin but contains 6 copper (Cu) atoms and carries about 95% of the Cu present in the plasma. *CP* is predominantly expressed in the liver but has also been observed in astrocytes in the nervous system, retinal neurons, testis, lung and spleen (Hellman and Gitlin, 2002; Gonzalez-Cuyar et al., 2008). It is not expressed in the intestine (Mackenzie et al., 2008). Evidence that ceruloplasmin plays a role in iron homeostasis came originally from animal studies but was confirmed with the discovery of aceruloplasminemia (Hellman and Gitlin, 2002). Ceruloplasmin associates with ferroportin and, similar to hephaestin in intestinal enterocyotes, oxidizes the ferrous iron to the ferric state prior to its binding with transferrin. The absence of functional ceruloplasmin prevents the efflux and mobilization of iron from cells of the reticuloendothelial system, hepatocytes and other tissues. This leads to a deficit in the amount of iron that can be mobilized from stores or recycled from erythrocyte turnover for use in hematopoiesis. Aceruloplasminemia results from loss-of-function mutations in *CP* and is typified by low serum iron, high ferritin, diabetes and a buildup of iron in the brain resulting in neurological symptoms (Hellman and Gitlin, 2002; Gonzalez-Cuyar et al., 2008). A number of variants have been identified in *CP* in individuals with the aceruloplasminemia phenotype (Hellman and Gitlin, 2002), however, whether other more common variants in this gene impact iron metabolism in a clinically or biologically significant way is not known.

2.7.12 HMOX1

HMOX1 (*Heme-oxygenase-1*) is located on chromosome 22 (22q12-q13.1) and contains 5 exons (Shibahara et al., 1989). The gene product heme-oxygenase-1 is induced in response to a variety of stimuli including iron deficiency, heme, oxidative stress (H_2O_2), nitric oxide, hypoxia, hyperoxia, oxidized lipids, inflammation and prostaglandins (Loboda et al., 2008; Oates and West, 2006; Exner et al., 2004). The highest expression of *HMOX1* is observed in the duodenum (Rosenberg and Kappas, 1989) where it plays a central role in the catabolism of heme iron, a pro-oxidant, taken up from the lumen. Heme catabolism by this route produces free iron but also carbon monoxide (CO) and bilirubin which may exhibit anti-inflammatory, anti-apoptotic and anti-proliferative effects (Otterbein et al., 2003). Carbon dioxide, however, does have the potential to produce pro-oxidant effects also due to its affinity for heme proteins (Piantadosi et al., 2008). *HMOX1* expression has also been reported in *in vitro* studies with lung cells (Lee et al., 1996; Fredenburgh et al., 2007), endothelial cells (Kim et al., 2007) and Kupffer cells from the liver (Bauer et al., 2003). A (GT)n repeat microsatellite polymorphism in the promoter region (rs30784372 – Hong et al., 2007; or rs1805173 – dbSNP, Build 129) was identified by Kimpara et al. (1997). Studies in cell lines have shown an inverse relationship between the number of repeats and *HMOX1* promoter activity and expression (Chen et al., 2002; Okamoto et al., 2006; Yamada et al., 2000). Two other proximal promoter region polymorphisms, both SNPs (G(-1135)A and T(-413)A), have also been identified although their functional significance is less clear (Exner et al., 2004). Given the central role that heme-oxygenase-1 plays in cellular response to oxidative and inflammatory insults, several epidemiological studies have been undertaken to investigate

the relationship between the number of (GT) repeats in the promoter polymorphism, a possible index of the ability of an individual to mount an heme-oxygenase-1 response, and different disease outcomes including pulmonary disease (Fredenburgh et al., 2007), cardiovascular diseases, renal transplantation, neurological disease, obstetrics, hematological disorders (Exner et al., 2004) and, more recently, cancer.

Chapter 3: A Systematic Review - Associations of Polymorphisms in Iron Metabolism Genes with Neoplasia of the Gastrointestinal Tract

This chapter describes a systematic review of studies of associations between variants of several genes implicated in iron metabolism or storage (Griffiths, 2007; Beutler, 2006; Dorak et al, 2005; Camaschella, 2005; Beutler and Beutler, 2004) and neoplasia of the gastrointestinal tract. The majority of iron gene-cancer association studies to date have investigated the C282Y and H63D polymorphisms of the *HFE*-gene (6p21.3).

Strong evidence exists for the Y282 variant as a risk factor for the development of the iron storage disease Type 1 hereditary haemochromatosis (HH). Fifty-two to 100% of HH probands of white, European origin are homozygous for the Y282 variant (Hanson et al., 2001). Evidence from laboratory studies implicates the Y282 variant in the dysregulation of iron-uptake through a deficiency in hepcidin although the details of the mechanism are not yet fully understood (Feder et al., 1996; Chua et al., 2007; Oates and Ahmed, 2007). Numerous studies have observed associations between Y282 homozygosity and iron status (Adams and Barton, 2007; Adams et al., 2005; Adams et al., 2000; Sanchez et al., 2003; Merryweather-Clarke et al., 1998) with evidence of variations in penetrance with gender and age (Cogswell et al., 2003). The relationship is less strong for H63D, but higher transferrin saturation and serum ferritin levels have been observed in C282Y/H63D combined heterozygotes and D63-homozygotes compared

with wild-type (C282 homozygote/ H63 homozygote) individuals (Barton et al., 2005, 2006). Overall among healthy individuals, those possessing an *HFE* mutation are more likely to have serum ferritin and transferrin saturation levels higher than those without. With chronic disease onset or inflammation, however, the body distribution of iron shifts to produce the anemia of chronic disease (Ganz, 2005; Andrews, 2004), with an increase of iron stores within cells, particularly macrophages and enterocytes, at the expense of plasma iron.

Genetic variants with demonstrated relationships with iron status and distribution may represent a means to study the role of iron in the etiology of gastrointestinal cancers, avoiding the measurement and exposure assessment problems that affect biochemical iron measures.

3.1 Methods

3.1.1 Inclusion Criteria

For this review, the gastrointestinal tract was defined as the esophagus, stomach, small and large intestines, rectum and anus. Twelve genes were included: *HFE* (Type 1 HH, 6p21.3); *HAMP* (hepcidin, 19q13.1); *TFRC* (Transferrin receptor-1, 3q29); *TFR2* (Transferrin-receptor-2, 7q22); *FPN1* (Ferroportin, 2q32); *HJV* (Hemojuvelin, 1q21.1); *TF* (Transferrin, 3q22.1); *DMT1* (divalent metal transport-1, 12q13); *CYBRD1* (duodenal cytochrome-b, 2q31.1); *HEPH* (Hephaestin, Xq11-q12); *CP* (Caeruloplasmin, 3q23-q25); *HMOX1* (heme-oxygenase-1, 22q12-q13.1). These genes are described in more detail in Table 2.1 (Chapter 2). Association studies of cross-sectional, case-control, cohort, case-only or family-based design met the inclusion criteria. No exclusions were made on the basis of language. The eligibility checklist used in the screening of abstracts and eligibility assessment of studies is in Table 3.1.

3.1.2 Identification of Studies

Published studies were identified from searches of the MEDLINE (1950 – 2007, October, week 1) and EMBASE (1980 – 2007 week 42) bibliographic databases using the OVID interface, a search of BIOSIS previews (1990 – 2007, October, week 1), manual searching of reference lists of retrieved articles and a search of the HuGE Published Literature database (Lin et al., 2006). Dissertations and Theses (ProQuest, 1957- October 7, 2007) was also searched using the relevant gene and polymorphism names and aliases, iron or h(a)emochromatosis, broad-based cancer-related outcome terms and terms

specific to the individual cancer sites. Further detail on the search terms can be found in the MEDLINE search strategy in appendix Ai.

Table 3.1 Fe metabolism genes and GI cancers systematic review: Eligibility Checklist

Criterion	*Inclusion Criteria*	*Detail*
Study Design	□ Cross-sectional □ Case series (n>5) □ Case-control □ Cohort □ Case-cohort □ Nested case-control □ Case-only □ Case-parent trios □ Intervention study □ Other. *Specify:*	N.B. Treatment for cancer affects iron homeostasis. Level 1 to 2: Include systematic reviews with relevant exposures **and/or** outcomes and narrative reviews that have **both** relevant exposures **and** relevant outcomes. Reference lists of reviews will be screened at Level 2. Level 2 to 3: Screen reference lists of reviews. If review itself has no primary data or is not systematic with/without meta-analysis, then exclude.
Population	**Adults or biosamples from adults (>20 years of age)**	*Exclude mechanistic studies with cell lines. Exclude studies where participants have undergone treatment (chemo, radiation or surgery) for cancer.*
Exposures	***Genetic*** **Polymorphism in iron metabolism gene**	*Include: HFE, TFRC, TfR2, SLC40A1 (ferroportin), HFE2 (HJV), TF, SLC11A2 (DMT-1), CBYRD (DcytB), HAMP (hepcidin), HEPH (hephaestin), CP(ceruloplasmin), HMOX1(Heme-oxygenase 1)*
Outcomes	***Cancer outcomes*** *Primary* **Cancerous or pre-cancerous states of GI tract** *Malignant and pre-malignant states, adenoma, polyps.*	*Exclude hematologic cancers and pre-cancers.*
Secondary	**Other non-hematologic malignant neoplasms**	
Language	□ English □ Other. *Specify:*	
Publication type & status	□ Published peer-reviewed article □ Dissertation □ Unpublished results □ Book chapter □ Response or comment: **EXCLUDE** if no primary data primary data. □ Editorial: **EXCLUDE** □ In press □ Meeting abstract □ Technical report	

3.1.3 Article Processing and Eligibility Assessment

Removal of Duplicates

The citations identified from searches of the bibliographic databases were uploaded to Reference Manager 9.0 and duplicates removed manually by examining authors, titles and abstracts. All citations were then output to MS Excel which was used for tracking article flow through the review and reasons for exclusions.

Abstract Screening

Abstracts were screened with reference to the eligibility checklist. All studies considered potentially relevant were retrieved.

Eligibility Assessment of Retrieved Articles

All potentially relevant studies that were identified in reference lists of retrieved articles were retrieved to increase the sensitivity of the search. Twenty-five percent of the retrieved articles were assessed for eligibility by an additional reviewer (CJ) (kappa=0.56, n=49). Differences of opinion were resolved by consensus.

3.1.4 Data Extraction

Data was extracted on study design, methods and results of the included studies using a structured Excel spreadsheet that contained drop-down lists to constrain responses and comments columns for providing further detail where necessary. A list of the fields in the Data Extraction Excel file is provided in Appendix Aii. One reviewer (VT) did the primary data extraction on all included studies. A second reviewer (CJ) did the duplicate extraction on the nine studies with colorectal neoplasia outcomes.

3.1.5 Assessing Study Generalisability and Sources of Bias

A number of scales and checklists have been used to assess the bias and generalisability of observational studies (Mallen et al., 2006) including one for studies of gastrointestinal diseases (Saito et al., 2006). For the current project, the Newcastle-Ottawa Scale (Wells et al., 2000) was used as the basis for assessing the methodological strength of the identified papers. The scale was modified for application to genetic association studies with reference to the criteria suggested by Little et al. (2002) and the HuGE Review handbook v1.0 (Little and Higgins, 2006). The checklist was revised based on feedback from the reviewers. Two reviewers independently applied the checklist items to the colorectal neoplasia studies. Disagreements were resolved by consensus. The modified checklist was used to explore the internal validity and generalisability of the included studies. It is provided in Appendix Aiii.

3.1.6 Statistical Analyses and Software

Crude odds ratios and ninety-five percent confidence intervals were calculated using STATA version 9.2 (Statacorp, College Station, TX). Meta-analyses were carried out using the meta function in STATA using random effects models. The heterogeneity measure I^2 (Higgins and Thompson, 2002) was calculated using Excel.

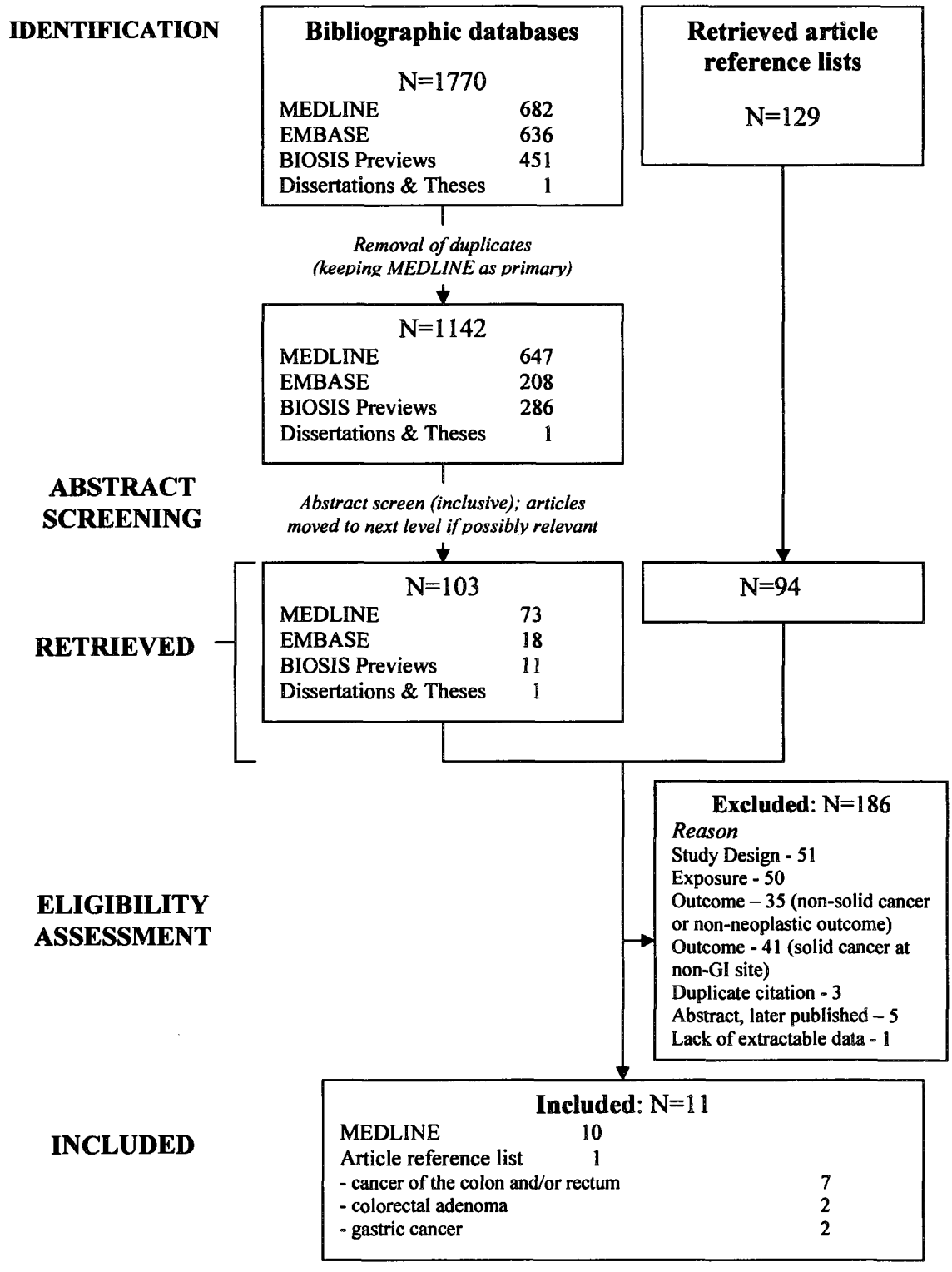
3.2 Results

Searches of the bibliographic databases identified 1770 articles, 628 of which were duplicate articles identified in more than one database (Figure 3.1). After removal of the duplicates, 1142 abstracts remained. One hundred and three of these articles were considered relevant to the systematic review question and retrieved. Manual searching of the reference lists of the retrieved articles identified a further 129 potentially relevant articles, 94 of which were retrieved. One hundred and forty-four studies were excluded when assessed against the eligibility criteria checklist, 51 on the basis of study design or publication type, 50 on the basis of exposure (variants in genes not included in the review, non-genetic exposures), 35 due to an ineligible disease outcome, 3 which were duplicate citations and 5 which were abstracts later published as full articles. Fifty-three articles had polymorphisms in iron genes as their exposure and a solid cancer or cancers as their outcome. One article (Geier et al., 2002) was excluded subsequently due to the absence of extractable data for cancer at different sites in the body. The study described in the article by Geier et al. included only 3 cases of colorectal cancer and 2 cases of gastric cancer. The author was not contacted for more information due to the small numbers of cases.

Of the 52 articles with a solid cancer outcome, 11 examined cancer of the GI tract, the primary outcome of this review, while 41 examined solid tumors at non-GI sites: 15 with liver cancer as their outcome; 2 with pancreatic cancer; 7 with breast cancer; 1 with breast cancer, endometrial cancer and ovarian cancer; 1 cervical cancer; 3 lung cancer; 1

lung cancer and head and neck cancer; 1 prostate cancer; 1 prostate cancer and breast cancer in males; 1 brain cancer; 3 skin cancer; 2 oral cancer and 3 kidney cancer.

Figure 3.1 Diagram of the flow of articles through the identification, selection and inclusion process of the systematic review.



3.2.1 Included Studies

Eleven studies had neoplasia of the gastrointestinal tract as their outcome or included within their outcome definition (Figure 3.1). Seven of these studies focused on cancer of the colon and/or rectum (Altes et al., 1999; Beckman et al., 1999; Macdonald et al., 1999; Shaheen et al., 2003; Van der A et al., 2003; Barton et al., 2004; Robinson et al., 2005), two investigated colorectal adenoma (Chan et al., 2005; McGlynn et al., 2005) and two gastric cancer (Lo et al., 2007; Lu et al., 1992).

All the colorectal neoplasia studies reported results for the relatively well-characterized C282Y (rs1800562/HFE-02) and H63D (rs1799945/HFE-01) polymorphisms in the Type 1 hereditary hemochromatosis gene (*HFE*). Two colorectal cancer studies (Beckman et al., 1999; Robinson et al., 2005) and one adenoma study (McGlynn et al., 2005) also investigated the S142G polymorphism (rs3817672/TFRC-01) in the transferrin receptor gene (*TFRC*) as a potential modifier of *HFE*-gene C282Y/H63D polymorphism associations. We identified no studies that investigated associations between polymorphisms in other iron metabolism genes and cancer or adenoma of the colon or rectum.

No studies were identified that compared genetic polymorphisms in *TF* between cancer cases and controls. However, a number of studies, mostly prior to 1997, used gel electrophoresis on serum samples to compare the polymorphic transferrin protein forms between cancer cases and controls including one study examining gastric cancer (Lu et al., 1992). As the SNPs associated with the major polymorphic forms have been

identified (Namekata et al., 1997; Beckman et al., 1998; Chapter 2 *ibid*), the Lu et al. study was included in this review.

The literature search identified seven articles that investigated associations between polymorphisms in the *HMOX1* gene (22q13.1) and cancer, one of which looked at gastric cancer (Lo et al., 2007). This study compared alleles defined with respect to the number of (GT)n repeats at microsatellite polymorphism (rs3074372) (Kimpara et al., 1997) in the promoter region of the gene. Studies in cell lines have shown an inverse relationship between number of repeats and *HMOX1* promoter activity and expression (Chen et al., 2002b; Okamoto et al., 2006; Yamada et al., 2000).

The literature review to October 2007 did not identify any studies that looked at esophageal cancer or Barrett's Esophagus and polymorphisms in iron metabolism genes. Since that time, one study has been published comparing *HFE* polymorphisms between patients with clinically diagnosed gastroesophageal reflux disease, patients with Barrett's Esophagus and a healthy control group (Corley et al., 2008a) and a second looking at the *HMOX1* promoter (GT)n repeat polymorphism and gastric cancer (Sawa et al, 2008). These studies have been included in this chapter for completeness.

3.2.2 Descriptions of Colorectal and Colon Cancer Studies

The primary characteristics of these studies are provided in Table 3.2 with further detail of study methods provided in the following paragraphs. The results from these studies are described in more detail in section 3.3. All the included colorectal/colon cancer

studies were case-control designs among unrelated individuals. Most studies identified included only "white" or "Caucasian" participants (terms used by study authors). Only one study (Shaheen et al., 2003) included sufficient participants of African ancestry to calculate and present results subdivided by ethnicity.

In a Spanish study, Altes et al. (1999) compared banked colorectal cancer DNA samples (n=116) with DNA samples from 108 healthy blood donors. Cases were older than controls (mean age 66.9 years among cases and 40 years among controls) but had a similar proportion of females (46% and 43% for cases and controls, respectively). The ethnicity of the subjects was not reported nor their family or personal history of cancer, hemochromatosis or other medical conditions. Both the C282Y and H63D variants were measured and genotype and allele frequencies provided in the publication. None of the subjects were homozygous for the Y282 variant and only 14 were heterozygous. One case and 2 controls were compound heterozygotes. The authors reported no significant differences between cases and controls with respect to frequency of *HFE* mutations and combined C282Y-H63D genotypes.

Table 3.2 Descriptions of the included studies that investigated associations between polymorphisms of the *HFE* gene and gastrointestinal neoplasias.*

First Author/ Year	Outcome	Location of Study	Design	Size, Ca/ Co	Description of Cases	Description of Controls	Genetic Variants Examined	Reported Comp-arison	Comment HWE†
Altes 1999	Colorectal cancer	Spain	Unmatched case-control	116/ 108	*Source*: Colorectal cancer DNA bank samples *Selection*: NR *Ethnicity*: NR, presumably Spanish. *Age*‡: mean, 66.9 years *Gender*: 46% female *CRC family history*: NR	*Source*: Healthy blood donors *Selection*: "Healthy", no further details provided *Ethnicity*: NR, presumably Spanish *Age*: mean, 40 years *Gender*: 43% female *CRC family history*: NR	*HFE*-C282Y *HFE*-H63D	Combined HFE genotype distributions	The result was reported qualitatively - no significant differences between the distributions of combined genotypes between case and control groups (Fisher's exact test). HWE: Assessment not reported.
Beckman 1999	Colorectal cancer	Sweden	Single site unmatched case-control	173/ 294	*Source:* Patients diagnosed at Umea University Hospital *Selection:* Unclear if cases were incident; no further details were provided on selection. *Ethnicity*: White Swedish *Age:* NR *Gender:*48% female *CRC family history:* NR	*Source*: Aggregate of placental samples (n=201) and controls (n=93) from earlier study of lung cancer in central Sweden. *Selection*: Convenience, otherwise NR *Ethnicity*: White Swedish *Age:* NR *Gender:* NR *CRC family history:* NR	*HFE*-C282Y *HFE*-H63D *TFRC*-S142G	Combined *HFE-TFRC* genotype groups	No odds ratios were reported for *HFE* polymorphisms alone. H63D genotype frequencies cannot be extracted from the publication for the colorectal cancer subgroup. HWE: Assessed using chi-sq. No significant deviations reported.
Macdonald 1999	Colorectal cancer	Brisbane, Australia	Single site unmatched case-control	229/ 228	*Source:* Cases who underwent resection at the Royal Brisbane Hospital *Selection*: Unselected series of sporadic cases *Ethnicity:* White *Age*: mean, 68.0 years *Gender*: 45% female *CRC family history:* NR	*Source:* Same geographical region as cases *Selection:* "Healthy", otherwise NR. *Ethnicity:* White *Age:* mean, 69.7 years *Gender:* 36% female *CRC family history:* NR	*HFE*-C282Y	CY v CC	No adjustment. H63D was not measured. HWE: Assessment not reported.
Shaheen 2003	Colon adeno-	N. Carolina,	Population-based,	475/ 833	*Source:* North Carolina Cancer Registry	*Source:* Medicare rolls, drivers' licenses or	*HFE*-C282Y *HFE*-H63D	(YY+CY) v CC	Adjusted for gender, age, race, total iron intake, red

* Ca, cases. Co, controls. MAF, minor allele frequency. Freq., frequency. NR, not reported in publication. OR, odds ratio. HH, hereditary hemochromatosis. CRC, colon, rectal or colorectal cancer.

† HWE, whether testing for deviations from Hardy-Weinberg Equilibrium (HWE) in the controls was undertaken (NR=not reported) and if so, the method (Chi-sq/Exact) and result (ns=not significant, if significant – the p-value).

‡ Age is expressed as the range, the mean or the mean±standard deviation as provided in each article.

First Author/ Year	Outcome	Location of Study	Design	Size, Ca/ Co	Description of Cases	Description of Controls	Genetic Variants Examined	Reported Comp-arison	Comment HWE†
	carcinoma	USA.	frequency-matched (age, race, gender)		*Selection*: Incident, all African-American patients and a random subsample of white patients diagnosed between Oct 1, 1996, and Oct 1, 2000. *Ethnicity*: 43% African-American *Age*: 63.0±10 years *Gender*: 48% female *CRC family history**: 21%	identification cards, resident in the same geographic region *Selection:*2-stage randomized recruitment strategy to match on race, age and gender *Ethnicity:* 38% African-American *Age*: 65.2±9.4 years *Gender*: 48% female *CRC family history:* 9% *Other*: No personal history of colorectal adenocarcinoma.		(DD+HD) v HH Any *HFE* minor variant v none	meat consumption, NSAID use. HWE: Assessment not reported.
Van der A 2003	Colorectal cancer death or diagnosis	Utrecht, Nether-lands	Retrospective, case-control nested within breast screening cohort	191/ 574	*Source*: Participants in the second screening in a breast cancer screening cohort (n=12,242) initially ascertained between Dec. 1974 and Oct. 1980 and followed-up until Jan 1, 1996. *Selection*: Unselected; incident cases post-1987 were identified through the regional cancer registry; deaths and mobility were tracked using municipal registries. *Ethnicity*: NR, but likely white of Dutch origin *Age*: 59.4±4.1 years *Gender*: 100% female *CRC family history*: NR *Other*: 175 colon cancer cases (ICD-9 153) and 65 rectal cancer cases (ICD-9 154).	*Source:* As for cases. *Selection*: Chosen randomly from 12,002 women free of CRC at the end of follow-up. *Ethnicity*: NR, but likely white of Dutch origin *Age*: 58.2±4.1 years *Gender*: 100% female *CRC family history*: NR	*HFE*-C282Y	CY v CC	Adjusted for age. This was the only study to use stored urine samples as the source of DNA. A high failure rate for DNA extraction and/or genotyping was reported with a difference between cases and controls. HWE: Deviations assessed using chi-sq, p=0.02 for C282Y 100% follow-up of cohort.
Barton 2004	Colorectal adeno-carcinoma	Central Alabama USA	Unmatched case-control, single site, population controls	12/ 318	*Source*: Single community oncology/hematology practice, referred for chemotherapy adjuvant to surgery or management of advanced disease *Selection*: Incident, consecutive (12 from 100), exclusion of cases with primary non-melanoma skin cancer or radiation-induced malignancies and	*Source:* Same geographic region, recruited for two earlier studies of the genetics of hemochromatosis in Alabama *Selection:* convenience, apparently healthy *Ethnicity:* White *Age:*18 to 86, 52±15 years	*HFE*-C282Y *HFE*-H63D	Y allele frequency D allele frequency	No adjustment. Odds ratios compared case and control allelic frequencies. HWE: Assessment not reported.

* Shaheen et al. (2005) defined a family history of colon cancer as having one or more first degree relatives diagnosed with colon cancer at any age.

First Author/ Year	Outcome	Location of Study	Design	Size, Ca/ Co	Description of Cases	Description of Controls	Genetic Variants Examined	Reported Comp-arison	Comment HWE†
					cancers inherited in Mendelian fashion. *Ethnicity*: White *Age*: NR *Gender*: NR *CRC family history*: NR *Other*: No diagnosis or family history of HH.	*Gender:* 50% female *CRC family history*: NR			
Robinson 2005	Colorectal cancer	Southern England	Unmatched case-control, cases with family history of CRC compared with population controls	327/ 322	*Source*: Regional Genetic Centres *Selection*: On the basis of a positive family history of colorectal cancer *Ethnicity*: White *Age*: 30 to 70 years *Gender*: 58% female *CRC family history**: 100% *Other*: Negative for hemochromatosis, mismatch repair, APC and MYH germline mutations.	*Source*: Households in catchment areas *Selection:* Random digit dialing *Ethnicity*: White *Age*: 50 to 75 years *Gender:* 52% female *CRC family history*: 0% *Other*: No previous diagnosis of colorectal neoplasia, and no investigation for colorectal disease within the 2 years prior to recruitment.	*HFE*-C282Y *HFE*-H63D *TFRC*-S142G	Y allele frequency D allele frequency	No adjustment. HWE: Assessed, chi-sq. No significant deviations found.
Chan 2005	Colorectal adenoma	USA	Case-control nested within Nurses' Health Study, individually matched (incl. age and indication for endoscopy)	527/ 527	*Source:* Nurses' Health Study participants who provided a blood specimen 1989-1990 and reported having at least one sigmoidoscopy or colonoscopy between 1990 and 1998 (n=14,019) *Selection:* Incident, self-report, negative for inflammatory bowel disease, negative for a familial polyposis syndrome, and no diagnosis of adenoma or cancer prior to blood draw. *Ethnicity:* 99% white *Age:*58.1±6.5 years *Gender:* 100% female *CRC family history*†: 27% *Other*: 5 matched pairs were excluded due to missing data.	*Source:* As for cases. *Selection:* Individually matched to cases on date of endoscopy (2 years), birth year, indications for endoscopy, time period of prior endoscopies, month and year of blood draw and fasting status at blood draw. *Ethnicity:* 98% white *Age:*58.0±6.5 years *Gender:* 100% female *CRC family history*: 17%	*HFE*-C282Y *HFE*-H63D	Combined genotype distributions Any HFE minor variant v none	Unadjusted. Exposure groups were defined using both the C282Y and H63D polymorphisms; no analyses were reported based on C282Y or H63D genotypes alone. Multivariate analyses were also reported with adjustment for: (1) age (2) a variety of demographic, operational and lifestyle factors. * HWE: Assessed using chi-sq. No significant deviations reported.

* Robinson et al. (2005) defined a positive family history as the existence of at least two affected family members.
† Chan et al. (2005) defined a positive family history of CRC as the occurrence of CRC in a parent or sibling.

First Author/ Year	Outcome	Location of Study	Design	Size, Ca/ Co	Description of Cases	Description of Controls	Genetic Variants Examined	Reported Comp-arison	Comment HWE[†]
McGlynn 2005	Advanced distal colorectal adenoma-tous polyps/	USA	Case-control nested within the screening arm of a randomized trial, frequency-matched (age, gender)	635/ 650	*Source:* Participants in the screening arm of the PLCO Cancer Screening trial who had a successful sigmoidoscopy between Sep. 1993 and Sep. 1999 (n=42,037). *Selection:* Random selection of 720 from the 1234 patients with positive sigmoidoscopy who were also negative for a self-reported history of cancer (except basal cell carcinoma), negative for ulcerative colitis and Crohn's disease, negative for familial polyposis, colorectal adenoma or Gardner's syndrome. *Ethnicity:* White *Age:* 33% < 60 years old. *Gender:* 30% female *CRC family history*[†]*:* 12.1%	*Source:* As for cases. *Selection:* Negative sigmoidoscopy (n=26,651) then frequency-matching to cases on gender and age. *Ethnicity:* White *Age:* 46% < 60 years old. *Gender:* 31% female *CRC family history:* 9.2%	*HFE*-C282Y *HFE*-H63D *HFE*-IVS2+4 *TFRC*-S142G	YY v CC CY v CC DD v HH HD v HH	Adjusted for sex, age, smoking, intakes of total iron, total fibre, total energy, family history of colorectal cancer. HWE: Assessed using chi-sq. No significant deviations reported. *Other:* Non-white participants and those with unsuccessful genetic assays were excluded from the analyses (n=85 cases, n=83 controls)
Corley 2008	Barrett's Esophagus	Northern California, US	Nested case-control study with frequency matching on age, gender and geographic region	317/ 308	*Source:* Members of health services delivery organization with more than 2 years of enrollment, 18 to 79 years of age *Selection:* Endoscopically confirmed incident cases identified through ICD-9 code 530.2 (Barrett's esophagitis). *Ethnicity:* 87% White, 8% Hispanic, 1% Black, 1% Pacific Islander, 3% Other. *Age:* 41% < 60 years *Gender:* 27% female *EAC family history:* NR *Other:* Excluded if the biopsies were only from an irregular squamocolumnar junction.	*Source:* Members of health services delivery organization with more than 2 years of enrollment, 18 to 79 years of age. *Selection:* Randomly selected from members at risk using risk-set sampling. *Ethnicity:* 84% White, 4% Hispanic, 5% Black, 4% Pacific Islander, 2% Other *Age:* 38% < 60 years *Gender:* 31% female *EAC family history:* NR *Other:* No prior BE but there is no method to confirm the absence.	*HFE*-C282Y *HFE*-H63D	Any *HFE* minor variant v none Mild (wt/D63) moderate (wt/Y282) D63/D63) and Severe (Y282/Y282, Y282/D63) genotype groupings	Adjusted for age, gender, ethnicity and smoking status. HWE: assessment not reported.

[*] Chan et al. (2005) adjusted for age alone and also for the following list of factors: Age, fasting status, date of blood draw, time of blood draw, time period of endoscopy, symptoms at endoscopy, time period of any prior endoscopy, family history of colorectal cancer, body mass index, pack-years smoking, physical activity, energy-adjusted intake of calcium and folate (including supplements), servings of red meat , alcohol intake, regular use of multivitamins, regular use of aspirin, menopausal status, postmenopausal hormone use, age at first menarche, age at last menstrual period.

[†] McGlynn et al. (2005) defined a positive family history as colorectal cancer in any first-degree relatives.

Subjects in the study reported by Beckman et al. (1999) were predominantly Swedish. Cases (n=173) were identified at a single institution (Umeå University Hospital) in northern Sweden. Forty-eight percent were female. Information on age, personal and family cancer history was not provided. A composite control group was used: placental samples from 201 consecutive deliveries at the institution were pooled with samples from 93 individuals (laboratory staff, welders or chimney sweeps) who had been controls in an earlier genetic study of lung cancer (Birgander et al., 1995; Alexandrie et al., 1994). Case and control groups were not compared with respect to demographic and lifestyle factors. Beckman et al. (1999) reported no significant differences between cases and controls with respect to C282Y and H63D allele frequencies and genotypes. Exposure groups were defined using combined *TFRC*-S142G/HFE-C282Y/H63D genotypes to test whether, consistent with a previous study of multiple myeloma published by the same research group (Van Landeghem et al., 1998), individuals homozygous for the S142 (serine) variant but also carrying the Y282 variant were at increased risk of other cancers. When the exposed group was *TFRC*-S142S/*HFE*-Y282 carrier, the odds ratio was 1.6 (95% CI 0.7-3.7). For the *TFRC*-S142S/*HFE* C282Y/H63D compound heterozygotes, the investigators reported an odds ratio of 8.7 (95% CI 1.0-75.2).

Macdonald et al. (1999) recruited 229 otherwise unselected patients with sporadic colorectal cancer who underwent surgery at the Royal Brisbane Hospital in Australia as their case group. Controls (n=228) were selected from the same geographical region and were "healthy" but the methods used to select them or verify their disease-free status were not provided in the article. Whether there were exclusions on the basis of family

history of colorectal cancer or hemochromatosis is not clear. The mean age in the case-group was 68.0 years and was similar to that in the control group (69.7 years). Women constituted 45% of the case group and 36% of the control group. All cases and controls were white. Only the C282Y variant was genotyped for this study. No Y282 homozygous participants were found and the number of heterozygous subjects in the study was small (21 cases and 23 controls). The reported unadjusted colorectal cancer odds ratio was 0.90 (95% CI 0.48-1.69) when comparing C282Y heterozygotes with wild-type homozygous individuals. The authors reported qualitatively that stratification by gender did not affect the results and that heterozygosity for C282Y was not associated with site (right or left colon) or Duke's stage of cancer.

Shaheen et al. (2003) identified their colon adenocarcinoma cases from the North Carolina Colon Cancer Study. Ascertained through the North Carolina Cancer Registry, disease status was confirmed through hospital pathology reports. Cases were incident between Oct. 1, 1996, and Oct. 1, 2000 and selected to give approximately equal proportions of white and African-American individuals in the sample. Patients with rectal cancer were not included. Controls were selected from Medicare rolls and driver license and identification card registries. The investigators used a two-stage randomized recruitment strategy in order to produce the equivalent of frequency-matching on race, age and sex. Controls were required to have no reported history of invasive colorectal adenocarcinoma. Lifestyle, diet and demographic information were assessed using NCI questionnaires in face-to-face interviews administered by trained nurse interviewers. Cases were asked to recall dietary and vitamin/mineral supplement usage for the year

prior to their diagnosis. Controls were asked to report their habits for the previous year. The final analysis sample of cases had a mean age of 63.0±10 years, was 48% female and 43% African-American. Twenty-one percent of cases had a family history of colon cancer (one or more first degree relatives diagnosed). Controls had a mean age of 65.2±9.4 years, 48% of them were female, 38% were African-American and only 9% had a family history of colon cancer. Shaheen et al. reported comparisons of C282Y and H63D allele frequencies, carrier status for each and both mutations and compound genotypes between the case and control groups. P-values (two-sided Fisher's exact tests) did not reach statistical significance for any of these comparisons. A large difference in the frequency of mutations between white and African-American participants (37.0% of white participants carried one or both mutations compared with 9.9% of African-Americans, $p<0.001$) was found in this study. After adjustment for sex, age, race, iron intake, red meat consumption and NSAID use, possession of any *HFE* mutation was positively associated with colon cancer (OR=1.40, 95% CI 1.07-1.87). Planned interaction analyses showed no statistically significant interactions between *HFE* mutation status and either age ($p=0.15$) or total iron intake ($p=0.40$). The investigators also reported adjusted odds ratios for possession of any *HFE* mutation in different age strata (<50, 50-69 and ≥70 years), for different quartiles of iron intake, separately for the white and African-American ancestry groups and for those with and without a family history of colon cancer.

Van der A et al. (2003) carried out their case-control study among second examination participants (n=12,242) of the breast cancer screening DOM (Diagnotisch Onderzoek

Mammacarcinoom) project in Utrecht, the Netherlands. The DOM cohort was originally recruited between December 1974 and October 1980. Participants were women born between 1911 and 1924 in Utrecht, still resident there and, although this was not reported explicitly, were thus likely of Dutch, white origin. Mortalities within the DOM cohort were identified through municipal registries. Cause of death was also ascertained through general practitioners. From 1987 until the end of follow-up (1 January, 1996), incident cases were identified through linkage with the regional cancer registry using ICD-9 codes. Van der A et al. identified 240 people who either developed or died from colorectal cancer; 175 cancers of the colon and 65 cancers of the rectum. The proportion of cases identified at death versus clinical diagnosis is unclear. Controls were chosen randomly from the 12,002 women who were still alive and free of colorectal cancer at the end of follow-up. The mean age of cases was 59.4 years and of controls 58.2 years. Only three participants, all controls, were found to be homozygous for the Y282 allele in this study. The age-adjusted colorectal cancer odds ratio comparing Y282 heterozygotes with wild-type/wild-type women was 1.2 (95% CI 0.6-2.2). Van der A et al. also reported odds ratios for joint smoking (ever/never)-C282Y exposure categories and tested the significance of a multiplicative smoking-C282Y interaction term in their logistic regression analyses.

Barton et al. (2004) identified 12 cases of colorectal adenocarcinoma in a series of 100 consecutive patients treated in a community medical oncology practice in Alabama. These patients had been referred for chemotherapy adjuvant to surgery or management of advanced malignancy and were thus more representative of individuals with relatively

advanced disease. Cases had neither personal nor family history of hemochromatosis and did not have chemotherapy or radiation-induced malignancies or cancer inherited in a Mendelian fashion. The comparison group (n=318) consisted of "apparently healthy" controls from two earlier studies in the same geographic area and was 50% female with a mean age of 52±15 years. Verification of the absence of colorectal disease in these individuals was not reported. All cases and controls were identified as white. Interpretation of association analyses of the subgroup of colorectal cancer cases with the control group was limited due to the small numbers. Unadjusted odds ratios comparing allelic frequencies were 0.4 (p=0.6445) for C282Y and 0.8 (p=0.9725) for H63D.

Robinson et al. (2005) recruited their cases through Regional Genetic Centres in southern England requiring that all subjects had to have a family history of colorectal cancer (at least two affected family members), that either they or a relative were diagnosed before age 70 and that they were currently between 30 and 70 years old. Eligible cases also had no reported hemochromatosis and were negative for mismatch repair, *APC* and *MYH* germline mutations. Controls were recruited by random-digit-dialing of households in the south of England, did not have family or personal history of colorectal cancer, were between 50 and 75 years of age and had not undergone investigation for colorectal disease in the preceding two years. Participation rates among cases and controls were not reported. Fifty-eight percent of participating cases and 52% of controls were female and all were white. Cases and controls did not differ with respect to C282Y or H63D minor allele or genotype frequencies or possession of any *HFE* polymorphism in this study, despite selection of cases on the basis of family history. Based on the observation of a

higher frequency of C282Y/H63D compound heterozygotes in cases compared with controls (p=0.038; Fisher's exact test), Robinson et al. defined three exposure groups for *post hoc* logistic regression analyses: compound heterozygotes, carriers of either Y282 or D63 (i.e. Y282/Y282, Y282/wt, D63/D63, D63/wt – "single mutation carriers") and the wild-type/wild-type (wt/wt) individuals who carried neither minor variant. Single mutation carriers were not at increased risk of colorectal cancer compared with wild-type individuals (OR=1.01, 95% CI 0.73-1.40). Although compound heterozygotes showed an increased risk (OR=3.03, 95% CI 1.06-8.61), the investigators stated that the p-value of 0.038 did not reach significance given the *post hoc* nature of the analyses (Bonferroni correction for two *post-hoc* tests). Allele and genotype frequencies of the S142G variant in the *TFRC* gene were very similar in cases and controls (p=0.940; Fisher's exact test). The investigators qualitatively reported no significant interaction between the *HFE* genotypes and *TFRC*-S142G although the analyses were not described further.

3.2.3 Descriptions of Colorectal Adenoma Studies

Chan et al. (2005) identified their case and control subjects from the 14,019 female participants in the Nurses' Health Study who provided a blood sample in 1989-1990 and had had at least one sigmoidoscopy or colonoscopy between 1990 and 1998. The case definition applied by Chan et al. (2005) was based on the results of the endoscopic investigations. Incident cases were identified through subject self-report of a polyp on a biennial questionnaire and verified through review of medical records and pathology reports. Lesions $\geq$ 1 cm in diameter or any size with tubulovillous, villous or severely dysplastic histology were classified as advanced and lesions less than 1 cm in size and

with tubular histological features were considered "early". The reasons or indications for the endoscopic investigations were not described. Subjects with inflammatory bowel disease, familial polyposis syndrome or prior cancer (other than non-melanoma skin cancer) were excluded. Controls were selected from the remaining polyp-free subjects matching individually to cases on date of endoscopy (2 year period), birth year, indication for endoscopy, month and year of blood sample, fasting status at time of blood sample and time period of any endoscopy prior to the study. Dietary and lifestyle information was available from a validated questionnaire administered in 1990. Of the 557 matched pairs identified, 25 were excluded due to lack of DNA from one member and 5 due to lack of dietary data in one member leaving 527 individually-matched pairs in the final analysis. According to responses to the baseline questionnaire, cases were more likely to have a family history of colorectal cancer (cases 27%, controls 17%; $p<0.001$), be smokers ($p<0.01$), be less active ($p<0.01$), not use aspirin ($p<0.01$), and not use postmenopausal hormones ($p=0.03$). The p-values approached statistical significance for univariate comparisons between case and controls groups for calcium intake (intake in cases < controls, $p=0.06$) and red meat (intake in cases > controls, $p=0.09$). Thus overall, at baseline, cases tended to report less healthy lifestyles and fewer disease preventive behaviours. Cases and controls did not differ significantly with respect to biochemical measures of iron (serum iron, $p=0.83$; transferrin saturation, $p=0.89$; ferritin, $p=0.83$; soluble transferrin receptors, $p=0.93$; transferrin receptor/ferritin ratio, $p=0.80$) at baseline. Ninety-nine percent of cases and 98% of controls were white and the average age of cases was 58.1±6.5 years, essentially the same as that in controls (58.0±6.5 years). Similar results were obtained using conditional and unconditional logistic regression.

The authors therefore presented the results from the unconditional analyses with adjustment for matching as well as other potentially important factors (see Table 3.2). Cases and controls did not differ with respect to combined C282Y/H63D *HFE* genotype frequencies (p=0.52). No significant difference in risk of adenoma was observed between women who carried an *HFE* mutation compared with those who did not. The odds ratio was 1.08 (95% CI 0.83-1.39) after adjustment for age, fasting status at blood draw, date of blood draw, time of blood draw, time period of endoscopy, symptoms at endoscopy (screening, bleeding or abdominal pain), time period of any prior endoscopy, family history of colorectal cancer, body mass index, pack-years of smoking, physical activity, energy-adjusted intake of calcium and folate, servings of red meat, alcohol consumption, regular use of multivitamins, regular use of aspirin, menopausal status, postmenopausal hormone usage, age at first menarche, and age at last menstrual period. The crude odds ratio for this association was similar (OR=1.02, 95% CI 0.80-1.32). Subgroup analyses by adenoma severity (early/advanced) and size (small,<1cm/large,≥1cm) did not show any evidence of increased risk. The multivariate-adjusted OR was 1.10 (95% CI 0.81-1.50) among those with early adenomas and 1.11 (95% CI 0.78-1.57) among advanced adenoma cases. The investigators assessment of the joint-effect and possible interaction of iron intake (total iron and also, separately, heme iron) and *HFE* mutation carrier status provided no evidence of significant effect. The joint-effects of total body iron and *HFE* mutation carrier status were also assessed. Results of these analyses did not approach formal statistical significance.

McGlynn et al. (2005) reported results from a case-control study nested within the 42,037 participants with a successful sigmoidoscopic exam in the screening arm in the PLCO (Prostate, Lung, Colorectal and Ovarian) Cancer Screening Trial. This study recruited at 10 centres in the United States. Individuals with a self-reported history of cancer (other than non-melanoma skin cancer), ulcerative colitis, Crohn's disease, familial polyposis, previous colorectal adenoma or Gardner's syndrome had been excluded from the case-control pool. In contrast to Chan et al. (2005), these authors included only advanced, distal adenomatous polyps in the case group. Seven hundred and twenty cases were randomly selected from the 1234 participants with advanced, distal polyps. Seven hundred and thirty-three controls subjects were then selected from the pool of 26,651 participants without any polyps or suspect lesions, frequency-matching to cases on gender and ethnicity. To assess the possible influence of population stratification on any association measures resulting from their study, McGlynn et al. (2005) carried out genetic fingerprinting on study participants using a nine tetranucleotide single tandem repeat loci panel and a sex-specific locus. The analysis sample was restricted to white participants due to the small number of non-white subjects. This led to the exclusion of 85 cases (44 non-white, 24 genotype failures, 17 genetic fingerprint failures) and 83 controls (47 non-white, 22 genotype failures, 14 genetic fingerprint failures). Lifestyle and diet during the 12 months preceding the study was assessed using a self-administered questionnaire. The case and control groups in the final analysis sample did not differ with respect to the proportion of women (30.2% of cases, 30.9% of men; p=0.79), the proportion using NSAIDs (42.0% of cases, 40.0% of controls; p=0.46) or total fibre intake (p=0.16). Cases showed a tendency towards a positive family history of colorectal cancer (12.1% of

cases, 9.2% of controls; p=0.09), to consume more iron (diet plus supplements; p=0.07) and to take in more energy in the form of dietary foodstuffs (p=0.09) than controls although none of these differences met formal criteria of statistical significance. The Y282 allele frequency was identical in the case and control group in this study (0.063). The authors reported no significant differences between cases and controls for genotype frequencies of either *HFE*-C282Y or *HFE*-H63D in crude or adjusted analyses (Tables 3.4 and 3.5). The multivariate analyses controlled for sex, age, smoking, intakes of total iron, total fibre, total energy and family history of colorectal cancer. A third *HFE* variant (IVS2+4/rs2071303/ HFE-04*) also showed no difference between cases and controls. This variant was combined with the C282Y and H63D variants to construct haplotypes using the SNPHAP software package. Four haplotypes for H63D/IVS2+4/C282Y were found: wt/wt/wt; wt/C-allele/wt, wt/wt/A -allele (Y282), and G-allele (D63)/C-allele/wt. Odds ratios comparing minor variant containing haplotypes with the wt-wt-wt group in adenoma cases and controls were close to 1.00 in both adjusted and unadjusted analyses. McGlynn et al. (2005) also conducted analyses of *HFE* combined genotypes stratified by the *TFRC* S142G polymorphism and found no evidence for an increased risk of colorectal cancer in the S142 homozygous group.

3.2.4 Description of Barrett's Esophagus Study

Corley et al. (2008a) conducted a nested case-control study within a health services delivery organization in Northern California. Three hundred and seventeen confirmed incident cases of Barrett's Esophagus (BE) were recruited among the organization's

* The correct designation is HFE-04 corresponding to rs2071303.

members between October, 2002, and September, 2005. Population controls (n=308) were selected using risk set sampling, frequency matching to cases on age, gender and geographic region. A second comparison group of individuals (n=306) with clinically diagnosed, pharmacologically treated GERD (gastroesophageal reflux disease) were also recruited from among the organization's members. The absence of BE among these individuals was confirmed endoscopically. Information on dietary intakes (Block FFQ - 1998), lifestyle factors and medical history was collected in a face-to-face interview. Germline DNA was extracted from whole blood. The authors did not provide details of the genotyping method or results from genotyping quality control procedures. All three study groups were predominantly white (87%, 80% and 84% for the BE, GERD and healthy population controls, respectively) and predominantly male (73%, 69% and 69% for the BE, GERD and control groups, respectively). The mean BMI (kg/m^2) was 30 (s.d. 6) in the BE group, 29 (s.d. 5) in the GERD group and 20 (s.d. 6) in the control group. Corley et al. (2008a) constructed categories of *HFE* mutations according to their association with iron indices: mild mutation (wt/D63), moderate mutation (wt/Y282, D63/D63) and severe mutation (Y282/Y282, Y282/D63). The odds ratios for BE adjusted for gender, age, ethnicity (white/non-white) and smoking status for mild, moderate and severe mutations, respectively, were 1.21 (95% CI 0.83-1.77), 1.75 (95% CI 1.03-2.97) and 0.77 (95% CI 0.24-2.48) when compared with the population controls. The frequency of D63-homozygotes was 3.5% in the case group and only 1.3% in the population control group contributing to the elevated odds ratio in the moderate mutation category. The BE odds ratios using the GERD group for comparison were 1.39 (95% CI 0.94-2.05), 1.40 (95% CI 0.85-2.31) and 1.90 (95% CI 0.45-8.12) for mild, moderate and

severe mutations. The odds ratio for BE was 1.41 (95% CI 1.01-1.97) for any *HFE* mutation relative to the GERD comparison group. The authors did not find evidence of confounding of the relationship between the genetic variants and BE by total energy intake, BMI, waist circumference, smoking status, alcohol use, aspirin or NSAID consumption, *H. pylori* antibody status, education level, income level or comorbidity.

3.2.5 Descriptions of Gastric Cancer Studies

Two studies identified in the original search, one looking at polymorphic forms of transferrin (Lu et al., 1992) and one at the (GT)n repeat polymorphism in the promoter region of the *HMOX1* gene (Lo et al., 2007), had gastric cancer as their outcome. A third study, published since October 2007, was conducted in Japan and also looked at the *HMOX1* (GT)n repeat polymorphism and gastric cancer (Sawa et al., 2008).

Lu et al. (1992) conducted their study in Shanghai, China, among individuals of Han nationality. Cases were recruited from three hospitals and were between 29 and 78 years of age. The control group was recruited from among employees at the hospitals with individual matching to cases on geographic region of residence and age (±5 years). Controls were healthy and free of gastric cancer. Information was not provided on the gender of the case and control groups. The C1, C2 and C3 allele frequencies were 67.8%, 29.7% and 0.7% among the controls and 57.2%, 41.1% and 0.7% among the cases - a significant ($p<0.01$) over-representation of the C2-allele frequency among cases. A small percentage of participants possessed the rare D-chi allele.

Lo et al. (2007) included 183 consecutive patients who underwent surgery for gastric adenocarcinoma between May 2003 and May 2005 as their case group. All patients were treated at one institution in Taiwan. The mean age of the patients was 67.5±12.75 years and 29% were female. Two hundred and fifty consecutive individuals attending physical check-ups constituted the control group. Eligibility for the control group in the study required the absence of autoimmune disorders, blood diseases and no personal history of cancer. The mean age in the control group was 51.1±16.5 years and 30% were female. The definitions for short (S), medium (M) and long (L) *HMOX1* alleles were ≤25 (GT) repeats, 26 to 30 repeats and ≥31 repeats, respectively, in this publication. The authors reported no significant association between age and the *HMOX1* allele frequencies in the controls. Among controls, the number of repeats varied from 16 to 37 with peaks at 23 and 30. Short, medium and long allele frequencies were 42%, 43% and 15%, respectively, among controls and 46.2%, 32.8% and 21% among the gastric adenocarcinoma case group. Examination of the genotypes showed a significant deficit of MM heterozygotes among cases relative to controls (8.7% versus 19.2%; p=0.002). The SL genotype on the other hand was over-represented among cases (20.8% versus 12.8%; p=0.026). Lo et al. (2007) reported a gastric adenocarcinoma odds ratio of 0.65 (95% CI 0.44-0.97; unadjusted) for those carrying the M-allele compared with those without. L-allele and S-allele carriers were at marginal increased risk. Among cases, a lower lymphovascular invasion frequency was also inversely associated with the MM genotype.

Sawa et al. (2008) recruited 154 cases of gastric cancer and 163 disease-free controls in a case-control study in Japan. Cases (95 intestinal gastric cancer type, 56 diffuse type and 3 indeterminant) were recruited from one hospital in Kitakyushu. Controls were selected from Japanese individuals who attended the same institution for general health check-ups frequency-matching to cases on age (±5 years) and gender. Genomic DNA was isolated from peripheral leucocytes. Short, medium and long alleles were defined as <27 repeats, 27-32 repeats, ≥33 repeats, respectively. The mean age of the case group was 62.5±10.3 years and 30% were female. The controls had a mean age of 66.2±17.1 years and 51% were female. The authors defined their exposure groups according to possession of the short (S) and long (L) alleles. The HO-1-L group included LL, LM and LS genotypes and the HO-1-S group included MM, MS and SS genotypes. A lower percentage of cases were in the HO-1-L group (higher promoter activity) - 25.3% of cases and 41.7% of controls ($p<0.01$). Age-adjusted odds ratios comparing the HO-1-L group with HO-1-S were 1.7 (95% CI 0.9-3.1) among men and 3.6 (95% CI 1.5-8.6) among women.

3.2.6 Assessment of Generalisability and Possible Bias

Results from the bias and generalisability checklist are provided in Tables 3.3a-d. Overall, earlier studies were smaller and provided less information on origins and selection of the control groups and less of the information needed to determine the comparability of the case and control groups. Sporadic colorectal cancer is a complex, multi-factorial disease and the risk attributable to any of the minor alleles under investigation is likely to be small. Confounding due to population stratification requires differences in allele frequencies between the underlying populations that track

appreciable differences in disease rates (Wacholder et al., 2000, 2002). Most studies included individuals of predominantly white, European ancestry in whom disease rates were likely to be relatively uniform. Although population stratification cannot be ruled out, it is unlikely to be an important source of bias in most of these studies. Due to the over-selection of subjects of African-American descent in the Shaheen et al. study, the authors adjusted for ethnicity and also presented results stratified by ethnicity. Only one study (McGlynn et al. 2005) provided information on genotyping success rates in the cases and controls and only four studies reported the use of genotyping quality assessment procedures (Shaheen et al., 2003; Robinson et al., 2005; Chan et al., 2005; McGlynn et al., 2005). The C282Y and H63D polymorphisms now have validated assays. It is unlikely that genotyping error was an important source of bias in more recent studies. However, Van der A et al. (2003) reported a difference in overall extraction and genotyping success rates between cases and controls with a 20% failure rate in cases and 8% in controls. Van der A et al. extracted DNA from stored urine samples. The amount of DNA that can be isolated from urine is limited but the quality is often sufficient for successful amplification (Van der Hel et al., 2002). There is no reason to believe that the different failure rates between cases and controls were a source of bias. Only one study provided information on completeness of case and control recruitment (Shaheen et al., 2003).

Table 3.3a Results from the Bias and Generalisability Checklist for the Included Gastrointestinal Neoplasia Studies: Study Purpose and Selection

Item Study	Study Purpose	Adequate case definition	Representat-ive cases	Completeness of case recruitment	Source of controls	Verification of control status	Control selection methods	Completeness of control recruitment	Assessment of HWE* in controls
Altes 1999	Detect an association	Yes, with independent validation	Unclear	Not stated	Blood donors	Not verified	Not stated	Not stated	Not assessed or not stated
Beckman 1999	Detect an association	Yes, diagnosed	Unclear	Not stated	Aggregate	Not verified	Convenience	Not stated	Assessed, no HWE deviation
Macdonald 1999	Detect an association	Yes, with independent validation	Unclear	Not stated	Not stated	Unclear or not stated	Not stated	Not stated	Not assessed or not stated
Shaheen 2003	Detect an association	Yes, with independent validation	Selected, with oversampling of African-American cases and random sample of white cases	Stated, 84%	General population	Not verified	Random, population-based	Stated, 62%	Not assessed or not stated
Van der A 2003	Detect and estimate the magnitude of an association	Yes, record linkage	Consecutive	Not stated	Other, community Breast Screening cohort	Unclear or not stated	Random, population-based	Not stated	Assessed, HWE deviation (p=0.02; chi-square test but small numbers)
Barton 2004	Detect an association	Yes, with independent validation	Selected, referred for adjuvant chemotherapy/ management of advanced disease	Not stated	Community	Not verified	Not stated	Not stated	Not assessed or not stated

* HWE, Hardy-Weinberg Equilibrium.

Item Study	Study Purpose	Adequate case definition	Representat-ive cases	Completeness of case recruitment	Source of controls	Verification of control status	Control selection methods	Completeness of control recruitment	Assessment of HWE* in controls
Robinson 2005	Detect an association	Unclear or not stated	Selected, family history of disease	Not stated	General population	Not verified	Random, population-based	Not stated	Assessed, no HWE deviation
Chan 2005	Detect an association	Yes, with independent validation	Unclear, all had indications for endoscopy and were self-reported	Not stated	Other, Nurses' Health Study	Unclear or not stated	Selected, individually matched on date of endoscopy, birth year, indication for endoscopy, time of previous endoscopy, month and year of blood draw, fasting status.	Not stated	Assessed, no HWE deviation
McGlynn 2005	Detect and estimate an association	Yes, with independent validation	Selected, advanced distal adenoma	Not stated	Other, screening arm of randomized trial	No history of disease or outcome	Random, population-based	Not stated	Assessed, no HWE deviation
Corley 2008	Detect and estimate an association	Yes, with independent validation	Consecutive cases	Unclear	General population or community	Not verified	Random, population-based	Not stated	Not assessed or not stated

Table 3.3b Results from the Bias and Generalisability Checklist for the Included Gastrointestinal Neoplasia Studies: Comparability of Case and Control Groups

Item Study	Same geographic region	Same time period	Control for population stratification	Comment	Control for other important confounders	Comment
Altes 1999	Unclear or not stated	Unclear or not stated	Not stated	The ethnicity of subjects was not reported.	Not applicable	There is little evidence for the existence of other confounders of the relationship between *HFE* allele frequencies and cancer.
Beckman 1999	No	Unclear or not stated	Not stated	Authors state that the two control groups were combined due to there being no difference between the groups. It is unlikely that population stratification is of importance in this study.	Not applicable	
Macdonald 1999	Yes	Unclear or not stated	Not stated	All subjects were "Caucasian".	Not applicable	
Shaheen 2003	Yes	Unclear or not stated	Through design, frequency matching on race	Race was categorized into African-American and white – groups with known differences in C282Y and H63D allele frequencies. Analyses were also carried out stratified by race.	Not applicable	
Van der A 2003	Yes	Yes	Not stated	Population stratification is unlikely to be a significant problem in this study as all participants were women born in Utrecht between 1911 and 1925 and are thus likely to be predominantly of Dutch, white origin.	Not applicable	
Barton 2004	Unclear or not stated	Unclear or not stated	Through design, restricting to whites	All subjects were white. Population stratification is unlikely to be important in this study.	Not applicable	
Robinson	Yes	Unclear or	Not stated	All subjects were of "Caucasian origin". Population	Not applicable	

Item Study	Same geographic region	Same time period	Control for population stratification	Comment	Control for other important confounders	Comment
2005		not stated		stratification is unlikely to be important in this study. It does not appear that non-white participants were actively excluded.		
Chan 2005	Unclear or not stated	Yes	Not stated	Over 98% of participants were "Caucasian". In the multivariate model, white included Southern European/ Mediterranean/ Scandinavian and "other Caucasian" and non-white included African-American, Hispanic and Asian. Population stratification is unlikely to be important, but lack of information on proportions of people from different regions of Europe increases the uncertainty.	Not applicable	
McGlynn 2005	Unclear or not stated, participants from 10 sites in the U.S. (including Hawaii)	Yes	Through design, frequency matching on ethnicity	Only white were included in the reported association analyses. Authors also stated that genetic fingerprint analysis using 10 loci showed that population stratification was not important in their sample. Unclear how ethnicity was defined.	Not applicable	
Corley 2008	Yes	Yes	Through analysis, adjustment for ethnicity		Not applicable	

Table 3.3c Results from the Bias and Generalisability Checklist for the Included Gastrointestinal Neoplasia Studies: Genetic Variant Assessment and Potential Confounder/Effect Modifier Exposure Assessment

Item	Genetic Variant Assessment						Other Exposure Assessment			
Study	Same sampling, handling and storage in cases and controls	Same DNA extraction & genotyping methods	Validated assays	Quality assessment procedures	Same success rates in cases and controls	Analysts blind to case-control status	Ascertainment of exposure	Validated measure-ment tool or method	Same tool or method was used in cases and controls	Non-response rates
Altes 1999	Unclear or not stated	Unclear or not stated	Unclear or not stated	Not reported	Rates not stated	Unclear or not stated	No description, age and gender only	Not applicable	Not applicable	Not applicable
Beckman 1999	Unclear or not stated	Unclear or not stated	Unclear or not stated	Not reported	Rates not stated	Unclear or not stated	No description, gender only	Not applicable	Not applicable	Not applicable
Macdonald 1999	No, colonic tissue for some cases, blood for controls	Yes	Unclear or not stated	Not reported	Rates not stated	Unclear or not stated	No description; age and gender only	Not applicable	Not applicable	Not applicable
Shaheen 2003	Yes	Yes	Unclear or not stated	Yes	Rates not stated	Yes	Structured interview, blinding not reported	Yes, NCI questionaire	Yes	Rates not stated
Van der A 2003	Yes	Yes	Unclear or not stated	Not reported	No, rates different and reasons not described*	Unclear or not stated	Self-administered questionnaire	Unclear or not stated	Yes	Rates not stated
Barton 2004	Unclear or not stated	Unclear or not stated	Unclear or not stated	Not reported	Rates not stated	Unclear or not stated	No description, age and gender only	Not applicable	Not applicable	Not applicable
Robinson 2005	Unclear or not stated	Yes	Yes	Yes	Rates not stated	Unclear or not stated	No description, gender only	Not applicable	Not applicable	Not applicable
Chan 2005	Yes	Yes	Yes	Yes	Rates not stated	Yes	Self-administered questionnaire	Yes	Yes	Rates not stated

* *20% of cases (n=49) and 8% of controls (n=51) were not successfully extracted or genotyped.*

Item	Genetic Variant Assessment						Other Exposure Assessment			
Study	Same sampling, handling and storage in cases and controls	Same DNA extraction & genotyping methods	Validated assays	Quality assessment procedures	Same success rates in cases and controls	Analysts blind to case-control status	Ascertainment of exposure	Validated measure-ment tool or method	Same tool or method was used in cases and controls	Non-response rates
McGlynn 2005	Yes	Yes	Yes	Yes	Yes, same rate for both groups	Unclear or not stated	Self-administered questionnaire	Unclear or not stated	Yes	Rates not stated
Corley 2008	Unclear or not stated	Yes	Yes	Not reported	Rates not stated	Unclear or not state	Structured interview, blinding not reported	Yes	Yes	Rates not stated

Table 3.3d Results from the Bias and Generalisability Checklist for the Included Gastrointestinal Neoplasia Studies: Probability of Robust Results and Overall Assessment

Item	Probability of Robust Results					Overall		
Study	**Planned, *ad hoc* or subgroup**	**First report or replica-tion**	**Multiple testing considered**	**Study Size: Ca/Co**	**Study Size: MAF* in controls**	**Internal Validity**	**Probability of Robust Results**	**Generalis-ability**
Altes1999	Planned *a priori*	First report	Not applicable (few analyses)	116/108	*C282Y* 0.037 *H63D* 0.157	Low	Low	Low
Beckman 1999	Planned *a priori*	First report	Not applicable (only a few planned analyses)	173/294	*C282Y* 0.073 *S142G* 0.395 - 5 cases, 1 control in combined *TFRC/HFE* group	Low	Low	Low
Macdonald 1999	Planned *a priori*	First report	Not applicable (only one analysis)	229/228	*C282Y* 0.050	Low	Low/Unclear	Low
Shaheen 2003	Planned *a priori*, *ad hoc*	Replication	No	475/833	*C282Y* 0.044 *H63D* 0.09	Medium (Medium-High)	Medium	Medium (Medium-High)
Van der A 2003	Planned *a priori*, subgroup	Replication	No	191/574	*C282Y* 0.039	Medium (Low-Medium)	Low/Unclear	Low/ Unclear
Barton 2004	Subgroup analyses	Replication	Not applicable (Few analyses)	12/318	*C282Y* 0.09 *H63D* 0.145	Low	Low	Low
Robinson 2005	Planned *a priori*	Replication	Yes, Bonferroni	327/322	*C282Y* 0.073 *H63D* 0.138 *S142G* 0.495	Low	Low	Low
Chan 2005	Planned *a priori*	Unclear	No	527/527	*C282Y* 0.059 *H63D* 0.146	Medium	Medium	Medium
McGlynn 2005	Planned *a priori*	Replication	No (mentioned in multiple analyses of combined *HFE/TFRC* genotypes)	635/650	*C282Y* 0.063 *H63D* 0.135 *S142G* 0.444	Medium (Medium-High)	Medium	Medium

* MAF, minor allele frequency

Item	Probability of Robust Results					Overall		
Study	Planned, *ad hoc* or subgroup	First report or replica-tion	Multiple testing considered	Study Size: Ca/Co	Study Size: MAF* in controls	Internal Validity	Probability of Robust Results	Generalis-ability
Corley 2008	Planned *a priori*	First report	Not applicable (few analyses)	317/308	*C282Y* 0.050 *H63D* 0.140	Medium	Medium (Medium-High)	Med (Medium-High)

3.3 Evidence Synthesis

3.3.1 Main Effects

HFE

Tables 3.4 and 3.5 give the reported disease-variant/genotype association measures for the C282Y and H63D polymorphisms in the *HFE*-gene for the gastrointestinal outcomes.

HFE-C282Y/*Cancer of the Colon or Rectum (Table 3.4)*

Seven colorectal cancer studies with a total of 1523 cases and 2677 controls provided sufficient information to extract genotype frequencies for the C282Y polymorphism. Only 163 cases and 282 controls in the combined samples carried the rare Y282 variant. Y282-allele frequencies among the controls in the 7 studies ranged from 0.039 to 0.09, within the range expected for predominantly white populations (Merryweather-Clarke et al., 2000; Hanson et al., 2001; Lucotte, 2001). The highest frequencies were observed in the sample of 318 healthy white people from central Alabama (Barton et al., 2004). Due to the small sample size and relative rarity of the Y282 variant, comparisons based on this exposure were limited. Shaheen et al. (2003) compared cases and controls with respect to Y282 carrier status and found an insignificantly elevated odds ratio (OR = 1.39, 95% CI 0.88-2.19, when adjusted for gender, age, race, total iron intake, red meat consumption and NSAID use.

Table 3.4 Associations between the *HFE*-C282Y polymorphism and gastrointestinal neoplasia*.

First Author/ Year	MAF in Ca/Co	Genotype count and frequency			Reported Comparison	Reported Result, OR (95% CI), p	Adjustment	Calculated crude ORs (95% CI) for different inheritance models			Comment
			Cases n(%)	Controls n(%)				*Recessive*	*Co-dominant*	*Dominant*	
Altes 1999 (Colorectal)	0.026/ 0.037	YY CY CC	0 (0.0) 6 (5.2) 110 (94.8)	0 (0.0) 8 (7.4) 100 (92.6)	Compound genotype distributions	-	None	-	YY v CC - CY v CC 0.68 (0.18-2.33)	0.68 (0.18-2.33)	
Beckman 1999 (Colorectal)	0.072/ 0.073	YY CY CC	2 (1.2) 21 (12.1) 150 (86.7)	4 (1.4) 35 (11.9) 255 (86.7)	-	-	-	0.85 (0.08-5.99)	YY v CC 0.85 (0.08-6.01) CY v CC 1.02 (0.54-1.88)	1.00 (0.55-1.80)	No odds ratios were reported for *HFE* polymorphisms alone.
Macdonald 1999 (Colorectal)	0.046/ 0.050/	YY CY CC	0 (0.0) 21 (9.2) 208 (90.8)	0 (0.0) 23 (10.1) 205 (89.9)	CY v CC	0.90 (0.48-1.69)	None	-	YY v CC - CY v CC 0.90 (0.46-1.76)	0.90 (0.46-1.76)	
Shaheen 2003 (Colon only)	0.046/ 0.044	YY CY CC	0 (0.0) 44 (9.3) 431 (90.7)	3 (0.4) 68 (8.2) 762 (91.5)	(YY+CY) v CC	1.39 (0.88-2.19)	Gender, age, race, total iron and red meat intakes, NSAID use.	-	YY v CC - CY v CC 1.14 (0.75-1.73)	1.10 (0.72-1.65)	
Van der A 2003 (Colorectal)	0.042/ 0.039/	YY CY CC	0 (0.0) 16 (8.4) 175 (91.6)	3 (0.5) 39 (6.8) 532 (92.7)	CY v CC	1.2 (0.6-2.2)	Adjusted for age	-	YY v CC - CY v CC 1.25 (0.63-2.35)	1.16 (0.59-2.17)	HWE: Deviations assessed using chi-sq, p=0.02 for C282Y
Barton 2004 (Colorectal)	0.042/ 0.090	YY CY CC	0 (0.0) 1 (8.3) 11 (91.7)	1 (0.3) 55 (17.3) 262 (82.4)	Y allele freq.	OR=0.4, p=0.6445	None	-	YY v CC - CY v CC 0.43 (0.01-3.10)	0.43 (0.01-3.04)	Odds ratios in article compares case and control allelic freqs.
Robinson 2005 (Colorectal)	0.083/ 0.073	YY CY CC	2 (0.6) 50 (15.3) 275 (84.1)	4 (1.2) 39 (12.1) 279 (86.6)	Y allele freq.	p=0.536	None	0.49 (0.04-3.44)	YY v CC 0.51 (0.05-3.58) CY v CC 1.30 (0.81-2.10)	1.23 (0.78-1.95)	
Chan 2005 (Adenoma)	0.061/ 0.059	YY CY CC	1 (0.2) 62 (11.8) 464 (88.0)	4 (0.8) 54 (10.2) 469 (89.0)	Compound genotype distributions	-	-	0.25 (0.01-2.53)	YY v CC 0.25 (0.01-2.57) CY v CC 1.16 (0.77-1.74)	1.10 (0.74-1.64)	No analyses were reported based on C282Y or H63D genotypes alone.
McGlynn 2005 (Adenoma)	0.063/ 0.063	YY CY CC	5 (0.8) 70 (11.0) 560 (88.2)	3 (0.5) 76 (11.7) 571 (87.8)	YY v CC CY v CC	1.86 (0.43-7.96) 0.90 (0.63-1.28)	Sex, age, smoking, intakes of total iron, total fibre, total energy, family history of colorectal cancer.	1.71 (0.33-11.06)	YY v CC 1.70 (0.33-11.0) CY v CC 0.94 (0.66-1.35)	0.97 (0.68-1.38)	
Corley 2008 (Barrett's)	0.063/ 0.050	YY CY CC	2 (0.6) 36 (11.4) 279 (88.0)	2 (0.7) 27 (8.8) 279 (90.6)	-	-	-	0.97 (0.14-6.94)	YY v CC 1.00 (0.14-7.15) CY v CC 1.33 (0.79-2.26)	1.31 (0.79-2.18)	No analyses were reported based on C282Y or H63D genotypes alone.

* Ca, cases. Co, controls. MAF, minor allele frequency. Freq., frequency. NR, not reported in publication. OR, odds ratio.

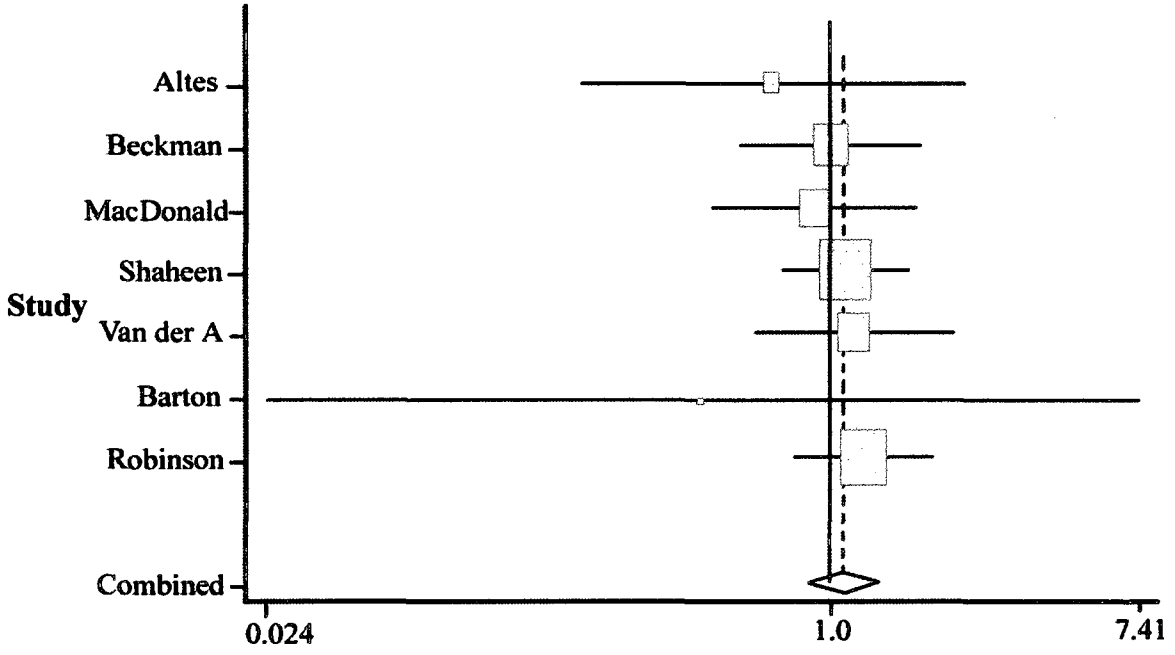
Table 3.5 Associations between the *HFE*-H63D polymorphism and gastrointestinal neoplasia*.

First Author/ Year	MAF in Ca/Co	Genotype count and freq			Reported Comparison	Reported Result, OR (95% CI) or p	Adjustment	Calculated crude ORs (95% CI) for different inheritance models			Comment
			Cases n(%)	Controls n(%)				*Recessive Model*	*Co-dominant*	*Dominant*	
Altes1999 (Colorectal)	0.211/ 0.157	DD HD HH	6 (5.2) 37 (31.9) 73 (62.9)	2 (1.9) 30 (27.8) 76 (70.4)	Compound genotype distributions	-	-	2.89 (0.50-29.77)	DD v HH 3.12 (0.53-32.39) HD v HH 1.28 (0.69-2.39)	1.40 (0.77-2.55)	
Shaheen 2003 (Colon only)	0.11/ 0.09/	DD HD HH	10 (2.1) 88 (18.5) 377 (79.4)	12 (1.4) 135 (16.2) 686 (82.4)	DD+HD v HH (dominant model)	1.44 (1.04-1.98)	Adjusted for gender, age, race, total iron intake, red meat consumption, NSAID use.	1.47 (0.57-3.75)	DD v HH 1.52 (0.58-3.87) HD v HH 1.19 (0.87-1.61)	1.21 (0.91-1.61)	
Barton 2004 (Colorectal)	0.125/ 0.145	DD HD HH	0 (0.0) 3 (25.0) 9 (75.0)	10 (3.1) 72 (22.6) 236 (74.2)	D allele freq.	OR=0.8, p=0.9725	None	-	DD v HH - HD v HH 1.09 (0.19-4.54)	0.96 (0.16-3.97)	
Robinson 2005 (Colorectal)	0.151/ 0.138/	DD HD HH	8 (2.4) 83 (25.4) 236 (72.2)	8 (2.5) 73 (22.7) 241 (74.8)	D allele freq.	p=0.528	None	0.98 (0.32-3.05)	DD v HH 1.02 (0.33-3.18) HD v HH 1.16 (0.80-1.70)	1.15 (0.80-1.65)	
Chan 2005 (Adenoma)	0.150/ 0.146	DD HD HH	15 (2.8) 128 (24.3) 384 (72.9)	14 (2.7) 126 (23.9) 387 (73.4)	Compound genotype distributions	-	-	1.07 (0.48-2.43)	DD v HH 1.08 (0.48-2.45) HD v HH 1.02 (0.76-1.37)	1.03 (0.78-1.37)	
McGlynn 2005 (Adenoma)	0.150/ 0.135	DD HD HH	13 (2.0) 164 (25.8) 458 (72.1)	15 (2.3) 146 (22.5) 489 (72.5)	DD v HH HD v HH	0.84 (0.38-1.85) 1.26 (0.97-1.64)	Adjusted for sex, age, smoking, intakes of total iron, total fibre, total energy, family history of colorectal cancer.	0.88 (0.38-2.01)	DD v HH 0.93 (0.40-2.11) HD v HH 1.20 (0.92-1.56)	1.17 (0.91-1.52)	
Corley 2008 (Barrett's)	0.167/ 0.140	DD HD HH	11 (3.5) 84 (26.5) 222 (70.0)	4 (1.3) 78 (25.3) 226 (73.4)	-	-	-	2.73 (0.86-8.67)	DD v HH 2.80 (0.88-8.92) HD v HH 1.10 (0.77-1.57)	1.18 (0.83-1.67)	

* Ca, cases. Co, controls. MAF, minor allele frequency. Freq., frequency. NR, not reported in publication. OR, odds ratio.

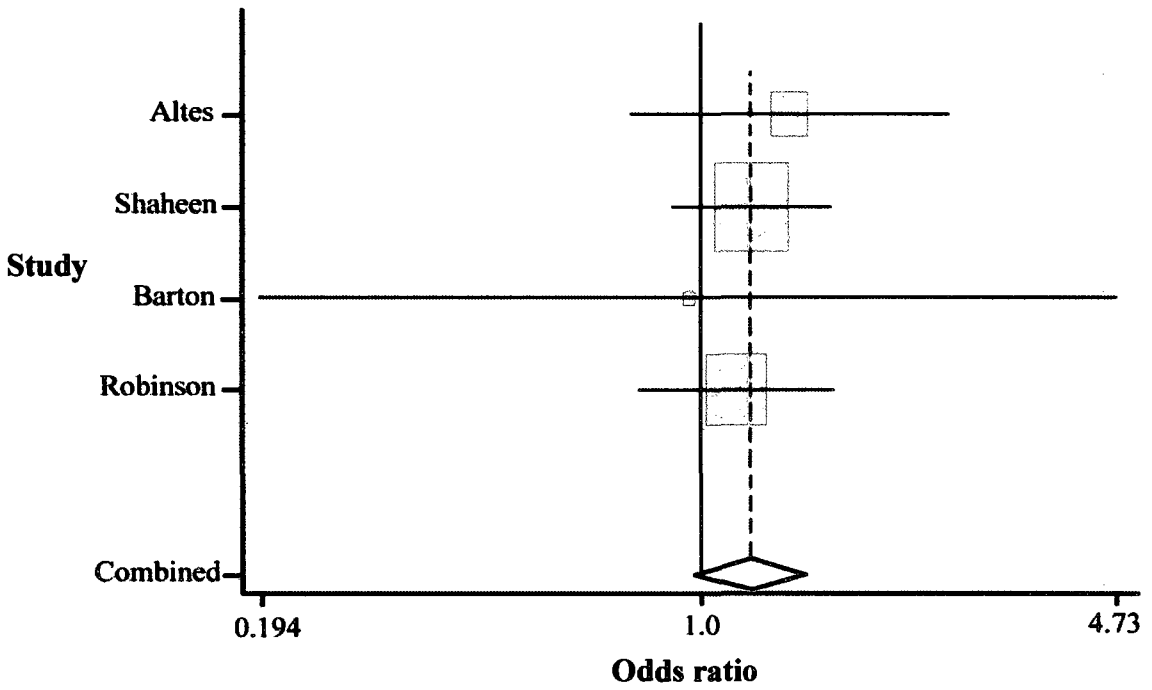
Combining the dominant mode of inheritance results from these studies using a random effects model gives an odds ratio of 1.07 (95% CI 0.85-1.35) with no evidence of heterogeneity (Figure 3.2, $Q_{df=6}=1.60$, $p=0.953$, $I^2=0\%$). A dominant mode of inheritance was used due to the rarity of the Y282 homozygous genotype.

Figure 3.2. Meta-analysis of colorectal cancer odds ratios comparing Y282-Allele carriers with non-carriers.



Study	OR (95% CI)	Weight
Altes et al. (1999)	0.68 (0.18-2.33)	2.42
Beckman et al. (1999)	1.00 (0.55-1.80)	10.92
Macdonald et al. (1999)	0.90 (0.46-1.76)	8.49
Shaheen et al. (2003)	1.10 (0.72-1.76)	22.39
Van der A et al. (2003)	1.16 (0.59-2.17)	9.13
Barton et al. (2004)	0.43 (0.01-3.04)	0.47
Robinson et al. (2005)	1.23 (0.78-1.95)	18.07
Combined	1.07 (0.85-1.35)	

Figure 3.3 Meta-analysis of colorectal cancer odds ratios comparing D63-Allele carriers with non-carriers.



Study	OR (95% CI)	Weight
Altes et al. (1999)	1.40 (0.77-2.55)	10.76
Shaheen et al. (2003)	1.21 (0.91-1.61)	44.16
Barton et al. (2004)	0.96 (0.16-3.97)	1.51
Robinson et al. (2005)	1.15 (0.80-1.65)	28.92
Combined	1.21 (0.98-1.49)	

HFE-H63D/*Cancer of the Colon or Rectum (Table 3.5)*

D63 allele frequencies among the controls ranged from 0.09 to 0.157, within the range expected from estimates of population frequencies (Adams et al., 2005; Cade et al., 2005; Rossi et al., 2004; Jackson et al., 2001; Phatak et al, 2000; Beutler et al., 2000). Shaheen et al. (2003) reported the lowest D63-allele frequency reflecting the high percentage of African-Americans in their sample. It was possible to extract H63D genotype frequencies from four colorectal cancer studies (Robinson et al., 2005; Barton et al., 2004; Shaheen et al., 2003; Altes et al., 1999). The total number of cases was 930 and the total number of controls 1581. Robinson et al. (2005) and Barton et al. (2004) observed no difference in D63-allele frequencies between colorectal cancer cases and controls (p=0.528 and 0.9725, respectively). With D63 carriers as the exposed group, Shaheen et al. (2003) observed a positive association between possession of the D63 polymorphism and colon adenocarcinoma (OR=1.44; 95% CI 1.04-1.98 – adjusted for gender, age, race, total iron intake, red meat consumption and NSAID use). Combining the dominant mode of inheritance crude odds ratios from the four studies (Altes et al., 1999;, Shaheen et al., 2003; Barton et al., 2004; Robinson et al., 2005) using a random effects model gives an odds ratio of 1.21 (95% CI 0.98-1.49) with no evidence of heterogeneity (Figure 3.3, $Q_{df=3}$=0.390, p=0.953, I^2=0%).

HFE-C282Y/*Adenoma of the Colon or Rectum (Table 3.4)*

A total of 138 of 1162 cases and 137 of 1177 controls in the two adenoma studies (McGlynn et al., 2005; Chan et al., 2005) were Y282 carriers. Y282 allele frequencies were similar in both studies (0.063 and 0.059 in McGlynn et al. (2005) and Chan et al.

(2005), respectively). Only McGlynn et al. (2005) reported an odds ratio (OR) for the association between Y282 homozygosity and disease. The OR was not significantly different from 1.00 for the crude (OR = 1.70, 95% CI 0.40-7.14) or adjusted analyses (OR = 1.86, 95% CI 0.43-7.96). Combining the crude odds ratios from these studies for the dominant mode of inheritance gives an odds ratio of 1.02 (95% CI 0.79-1.33).

HFE-H63D/*Adenoma of the Colon or Rectum (Table 3.5)*

D63 allele frequencies in controls in the two adenoma studies in the United States (Chan et al., 2005; McGlynn et al., 2005) were 0.146 and 0.136. Chan et al. (2005) did not report results for comparisons between D63-allele frequencies or genotypes in adenoma cases and controls. McGlynn et al. (2005) reported odds ratios not significantly different from 1.00 in comparisons of D63 homozygotes and heterozygotes with wild type subjects.

Combining the crude odds ratios from these studies for the dominant mode of inheritance gives an odds ratio of 1.11 (95% CI 0.92-1.34).

Combined HFE-C282Y/*HFE*-H63D/*Cancer of the Colon or Rectum (Tables 3.6-3.8)*

Table 3.6 presents study results for combined *HFE*-C282Y/*HFE*-H63D genotypes, Table 3.7 brings together comparisons of carriers of either of the minor variants and Table 3.8 looks at odds ratios with genotypes grouped according to their relationship with iron stores.

Two studies reported comparisons of combined genotypes between cases and controls (Altes et al., 1999; Robinson et al., 2005). Altes et al. (1999) reported qualitatively that the observed differences were not statistically significant. Robinson et al. (2005) noted a significantly elevated odds ratio comparing Y282/D63 heterozygotes with wild-type subjects (OR = 3.06, 95% CI 1.09-8.58). All other odds ratios comparing combined genotype frequencies were not significantly different from 1.00 in this study.

Shaheen et al. (2003) found an elevated risk of colon cancer among carriers of any *HFE* variant (Y282 or D63 - OR = 1.40, 95% CI 1.07-1.87). These analyses were adjusted for gender, age, race, quartiles of total iron intake, quartiles of red meat consumption and NSAID use.

When crude odds ratios from these four studies comparing *HFE*-genotypes grouped according to their putative associations with biochemical measures of iron status were combined, the "hi-iron" genotype group was not at increased risk (OR=1.15, 95% CI 0.89-1.49).

Combined HFE-C282Y/HFE-H63D/Adenoma of the Colon or Rectum (Tables 3.6-3.8)

Chan et al. (2005) compared the distributions of adenoma case and control subjects across combined C282Y/H63D genotypes and reported no significant difference overall (p=0.52) (Table 3.6). The frequency of carriers of either Y282 or D63 also did not differ between cases and controls (OR=1.02, 95% CI 0.80-1.32; with adjustment for age).

Further adjustment for family history of colorectal cancer, smoking, BMI, dietary and other lifestyle factors did not appreciably change the odds ratio.

Table 3.6 Associations between combined C282Y-H63D genotypes and gastrointestinal neoplasia*.

First Author/ Year	Combined C282Y/H63D category	Cases n (%)	Controls n(%)	Reported Result, OR (95% CI) or p-value	Adjustment	Calculated Crude ORs (95% CI)	Comment
Altes1999 (Colorectal)	CC-HH	68 (58.6)	70 (64.8)	Comparison across all categories; ns (Fisher's exact test)		1.00	
	CY-HH	5 (4.3)	6 (5.6)			0.86 (0.26-2.78)	
	CC-HD	36 (31.0)	28 (25.9)			1.32 (0.73-2.39)	
	CY-DH	1 (0.9)	2 (1.9)			-	
	CC-DD	6 (5.2)	2 (1.9)			-	
	YY-HH	0	0			-	
Shaheen 2003 (Colon only)	CC-HH	338 (71.2)	626 (75.2)	-	Adjusted for gender, age, race, total iron intake, red meat consumption, NSAID use.	1.00	Numbers extractable for total sample (white and African-American participants) only.
	CY-HH	39 (8.2)	57 (6.8)			1.27 (0.83-1.94)	
	CC-HD	83 (17.5)	124 (14.9)			1.24 (0.91-1.69)	
	CY-DH	5 (1.1)	11 (1.3)			0.84 (0.29-8.44)	
	CC-DD	10 (2.1)	12 (1.4)			1.54 (0.66-3.61)	
	YY-HH	0	3 (0.4)			-	
Barton 2004 (Colorectal)	CC-HH	9 (75.0)	197 (62.0)	-		-	
	CY-HH	0	38 (12.0)			-	
	CC-HD	2 (16.7)	55 (17.3)			-	
	CY-DH	1 (8.3)	17 (5.4)			-	
	CC-DD	0	10 (3.2)			-	
	YY-HH	0	1 (0.3)			-	
Robinson 2005 (Colorectal)	CC-HH	199 (60.9)	203 (63.0)	1.00	None	1.00	
	CY-HH	35 (10.7)	34 (10.6)	-		1.05 (0.63-1.75)	
	CC-HD	68 (20.8)	68 (21.1)	-		1.02 (0.69-1.50)	
	CY-DH	15 (4.6)	5 (1.6)	3.06 (1.09-8.58)		3.06 (1.09-8.58)	
	CC-DD	8 (2.4)	8 (2.5)			1.02 (0.38-2.77)	
	YY-HH	2 (0.6)	4 (1.2)	-		-	
				-			
Chan 2005 (Adenoma)	CC-HH	329 (62.4)	332 (63.0)	Comparison across all categories; p=0.52	None	1.00	
	CY-HH	54 (10.2)	51 (9.7)			1.07 (0.71-1.61)	
	CC-HD	120 (22.8)	123 (23.3)			0.99 (0.73-1.32)	
	CY-DH	8 (1.5)	3 (0.6)			-	
	CC-DD	15 (2.8)	14 (2.7)			1.08 (0.51-2.28)	
	YY-HH	1 (0.2)	4 (0.8)			-	
McGlynn 2005 (Adenoma)	CC-HH	404 (63.6)	430 (66.2)	-	-	1.00	Calculated from frequencies and odds ratios in the article.
	CY-HH	49 (7.7)	56 (8.6)			0.93 (0.62-1.40)	
	CC-HD	143 (22.5)	126 (19.4)			1.21 (0.92-1.59)	
	CY-DH	21 (3.3)	20 (3.1)			1.12 (0.60-2.09)	
	CC-DD	13 (2.0)	15 (2.3)			0.92 (0.43-1.96)	
	YY-HH	5 (0.8)	3 (0.5)			-	
Corley 2008 (Barrett's)	CC-HH	187 (59.0)	202 (65.6)	Comparison between mild, moderate and severe iron status mutation† categories	Age, gender, ethnicity and smoking status.	1.00	
	CY-HH	33 (10.4)	22 (7.1)			1.62 (0.91-2.88)	
	CC-HD	81 (25.6)	73 (23.7)			1.20 (0.82-1.74)	
	CY-DH	3 (0.9)	5 (1.6)			-	
	CC-DD	11 (3.5)	4 91.3)			2.97 (0.93-9.49)	
	YY-HH	2 (0.6)	2 (0.6)			-	

* Ca, cases. Co, controls. MAF, minor allele frequency. Freq., frequency. NR, not reported in publication. OR, odds ratio. Odds ratios were not calculated where cell counts were less than 5.

† Mild mutation=wt/D63, moderate=wt/Y282 or D63/D63, severe=Y282/Y282 or Y282/D63.

Table 3.7 Associations between grouped *HFE*-C282Y/H63D genotypes and gastrointestinal neoplasia*.

First Author/ Year	Combined C282Y/H63D category	Cases n (%)	Controls n(%)	Reported Result, OR (95% CI) or p-value	Adjustment	Calculated Crude ORs (95% CI)	Comment
Altes 1999 (Colorectal)	Any *HFE* variant	48 (41.4)	38 (35.2)	-	-	1.30 (0.76-2.23)	
	No *HFE* variant	68 (58.6)	70 (64.8)				
Shaheen 2003 (white subjects) (Colon only)	Any *HFE* variant	108 (39.6)	184 (35.7)	1.2 (0.8-1.6)	None	1.20 (0.88-1.62)	Reported OR for all subjects combined: 1.40 (1.07-1.87), adjusted for gender, age, race, total iron intake, red meat consumption, NSAID use.
	No *HFE* variant	163 (60.4)	332 (64.3)				
Shaheen 2003 (African-American subjects) (Colon only)	Any *HFE* variant	29 (14.2)	23 (7.3)	2.1 (1.1-3.9)	None	2.1 (1.19-3.78)	
	No *HFE* variant	175 (85.8)	294 (92.7)				
Barton 2004 (Colorectal)	Any *HFE* variant	3 (25.0)	121 (38.1)	-	-	0.54 (0.14-2.04)	
	No *HFE* variant	9 (75.0)	197 (61.9)				
Robinson 2005 (Colorectal)	Any *HFE* variant	128 (39.1)	119 (37.0)	-	-	1.10 (0.80-1.51)	
	No *HFE* variant	199 (60.9)	203 (63.0)				
Chan 2005 (Adenoma)	Any *HFE* variant	198 (36.8)	195 (37.0)	(1) 1.02 (0.80-1.32) (2) 1.08 (0.83-1.39)	(1) Age (2) Age and several study design-related, demographic and lifestyle factors.†	1.02 (0.80-1.32)	
	No *HFE* variant	329 (63.2)	332 (63.0)				
McGlynn 2005 (Adenoma)	Any *HFE* variant	231(36.4)	220 (33.8)	-	-	1.12 (0.89-1.41)	
	No *HFE* variant	404 (63.6)	430 (62.2)				
Corley 2008 (Barrett's)	Any *HFE* variant	130 (41.0)	106 (34.4)	1.32 (0.95-1.84)	Age, gender, ethnicity and smoking status.	1.32 (0.96-1.83)	
	No *HFE* variant	187 (59.0)	202 (65.6)				

* Ca, cases. Co, controls. MAF, minor allele frequency. Freq., frequency. NR, not reported in publication. OR, odds ratio. Odds ratios were not calculated where cell counts were less than 5.

† Adjusted for fasting status at time of blood draw, date of blood draw, time of blood draw, time period of endoscopy, symptoms at endoscopy, time period of any prior endoscopy, family history of colorectal cancer, body mass index, pack-years smoking, physical activity, energy-adjusted intake of calcium and folate (including supplements), servings of red meat, alcohol intake, regular use of multivitamins, regular use of aspirin, menopausal status, postmenopausal hormone use, age at first menarche, age at last menstrual period.

Table 3.8 Associations between gastrointestinal neoplasia and *HFE*-C282Y/H63D genotypes grouped according to their putative relationship with iron stores*.

First Author/ Year	C282Y/H63D category	Cases n (%)	Controls n(%)	Reported Result, OR (95% CI) or p-value	Adjustment	Calculated Crude ORs (95% CI)	Comment
Altes1999 (Colorectal)	*HFE*-hi iron†	12 (10.3)	10 (9.3)	-	-	1.13 (0.47-2.74)	
	Not	104 (89.7)	98 (90.7)				
Shaheen 2003 (Colon only)	*HFE*-hi iron	54 (11.4)	83 (10.0)	-	-	1.16 (0.81-1.67)	Counts could not be extracted for white and African-American participants separately for these categories.
	Not	421 (88.6)	750 (90.0)				
Barton 2004 (Colorectal)	*HFE*-hi iron	1 (8.3)	66 (20.8)	-	-	0.35 (0.04-2.74)	
	Not	11 (91.7)	252 (79.2)				
Robinson 2005 (Colorectal)	*HFE*-hi iron	60 (18.3)	51 (15.8)	-	-	1.19 (0.79-1.80)	
	Not	267 (81.7)	271 (84.2)				
Chan 2005 (Adenoma)	*HFE*-hi iron	78 (14.8)	72 (13.7)	-	-	1.10 (0.78-1.55)	
	Not	449 (85.2)	455 (86.3)				
McGlynn 2005 (Adenoma)	*HFE*-hi iron	88 (13.9)	94 (14.5)	-	-	0.95 (0.70-1.30)	
	Not	547 (86.1)	556 (85.5)				
Corley 2008 (Barrett's)	*HFE*-hi iron	49 (15.5)	33 (10.7)			1.52 (0.95-2.44)	
	Not	268 (84.5)	275 (89.3)				
Corley 2008 (Barrett's)	*Severe*	5 (1.6)	7 (2.3)	Severe 0.77 (0.24-2.48)	Age, gender, ethnicity and smoking status.	0.77 (0.24-2.47)	Severe mutation: Y282/Y282, Y282/D63
	Moderate	44 (13.9)	26 (8.4)	Moderate 1.75 (1.03-2.97)		1.83 (1.08-3.09)	Moderate mutation : wt/Y282, D63/D63
	Mild	81 (25.6)	73 (23.7)	Mild 1.21 (0.83-1.77)		1.20 (0.82-1.74)	Mild mutation : wt/D63
	No HFE mutation	187 (59.0)	202 (65.6)				

* Ca, cases. Co, controls. MAF, minor allele frequency. Freq., frequency. NR, not reported in publication. OR, odds ratio. Odds ratios were not calculated where cell counts were less than 5.

† *HFE*-hi iron refers to Y282/Y282, Y282/D63, D63/D63, and wt/Y282 genotypes. Not : wt/D63, wt/wt

The crude odds ratios for possession of any *HFE* variant (Table 3.7) were close to 1.00 in both studies of colorectal adenoma - 1.02 (95% CI 0.80-1.32) in the Chan et al. (2005) among women in the United States and 1.12 (95% CI 0.89-1.41) in the McGlynn et al. (2005) study which had 30% of subjects who were female. Odds ratios comparing "*HFE*-hi iron" genotypes with others were also close to 1.00 in both studies (Table 3.8).

HFE-C282Y *and HFE*-H63D*/Barrett's Esophagus*

The odds ratios for BE adjusted for gender, age, ethnicity (white/non-white) and smoking status for mild, moderate and severe mutations, respectively, were 1.21 (95% CI 0.83-1.77), 1.75 (95% CI 1.03-2.97) and 0.77 (95% CI 0.24-2.48) when compared with population controls in the study by Corley et al. (2008a). No significant associations were observed between BE and the individual C282Y and H63D variants (Table 3.4 and 3.5). The odds ratio for "any *HFE*-variant" was 1.32 (95% CI 0.96-1.83), approaching statistical significance. However, the biological relevance of "any *HFE*-variant" is unclear. The association was driven by an excess of the D63-allele among cases.

3.3.2 Stratified Analyses and Interactions

HFE - Environment

Shaheen et al. (2003) performed analyses comparing subjects with any *HFE* variant with those who had none and stratified by total dietary iron intake (quartiles of dietary iron plus iron from supplements), age (<50, 50 to 69 and 70 years and over) and family history of colon cancer. The adjusted odds ratios increased with increasing iron intake. Among those who reported consuming more than 26.5 mg Fe/day, the odds ratio was

significantly greater than 1.00 (1.86, 95% CI 1.09-3.18). The odds ratios also increased with increasing age becoming significantly greater than 1.00 in the ≥70 years age group (1.90, 95% CI 1.34-2.84). The multiplicative interaction terms for *HFE* variant carrier status with age and total iron intake were not statistically significant however (p=0.15 and p=0.40 for age and total iron, respectively). The odds ratio among those with a positive family history (1.58, 95% CI 0.73-3.41) was not significantly different from the odds ratio among those without a family history (1.29, 95% CI 0.94-1.77). Oversampling of the African-American population allowed accrual of sufficient numbers to carry out analyses stratified by race. Possession of one or both of the variants was associated with a significantly increased risk among the African-American subjects in their study (OR = 2.1, 95% CI 1.1-3.9) while the association was not significant among white subjects (OR = 1.2, 95% CI 0.8-1.6). There were 52 *HFE* variant allele carriers among the African-American subjects; 29 cases and 23 controls. Significantly fewer African-American subjects than white subjects possessed either or both the Y282 and D63 alleles (9.9% vs. 37%; p<0.001), consistent with reported allele population frequencies.

In their study of colorectal cancer among US women, Chan et al. (2005) did not find significant multiplicative interaction terms between *HFE* polymorphism carrier status (possession of Y282 and/or D63) and total iron intake (p = 0.70) nor heme iron intake (p = 0.69). The adjusted odds ratio comparing polymorphism carriers in the highest quartile of total iron intake with a referent group of non-carriers in the lowest quartile of reported total iron intake was 1.14 (95% CI 0.64-2.03). The corresponding adjusted odds ratio for polymorphism carriers in the highest quartile of heme iron intake was 1.11 (95% CI 0.64-

1.93). In analyses restricted to cases with distal adenoma for whom iron status measurements were available, women with a transferrin saturation >45% who were carriers of Y282 or D63 were not at a significantly increased risk of distal colorectal adenoma when compared with those who were wild-type for both C282Y and H63D and exhibited transferrin saturations ≤45% (OR_{adj} = 1.06, 95% CI 0.63-1.80; p=0.82). When iron status was defined using the ratio of soluble transferrin receptors to ferritin, the odds ratio was 0.83 (95% CI 0.43-1.58).

Only one study reported association analyses stratified by smoking status (never/ever) (Van der A et al., 2003). The age-adjusted odds ratio comparing the C282Y heterozygotes who had ever smoked with never smoking wild-type C282 homozygotes was 2.3 (95% CI 0.7-7.4) based on only 5 cases and 8 controls in the highest joint-exposure category.

Corley et al. (2008a) reported no statistical evidence of effect modification of *HFE*-variant/Barrett's esophagus association measures by ethnicity (white/non-white; p=0.47 for the multiplicative interaction term). The interaction term for sex also did not reach statistical significance (p=0.17) but analyses stratified by sex provided some suggestion that the risk may be higher among men. Comparing subjects with moderate or severe *HFE*-genotypes (Y282/Y282, Y282/D63, D63/D63) with the group with neither minor variant gave an odds ratio of 1.87 (95% CI 1.03-3.37) among men and 0.93 (95% CI 0.37-2.35) among women.

Gene – Gene

Beckman et al. (1999) defined exposure groups using combined *TFRC* S142G and *HFE* C282Y/H63D genotypes. With the exposure group defined as S142S homozygotes carrying the Y282 variant (11 cases and 12 controls), the crude odds ratio was 1.6 (95% CI 0.7-3.7). Further restriction of the exposure group to S142S homozygote - C282Y/H63D compound heterozygotes increased the odds ratio to 8.7 (95% CI 1.0 – 75.2) although this analysis was based on 5 exposed cases and 1 exposed control. A second study looked at *HFE-TFRC* combined genotypes and colorectal cancer (Robinson et al., 2005) and included a large number of subjects. Their reported results did not confirm the suggestion of possible effect modification of *HFE*-variant associated colorectal cancer risk by *TFRC*-S142G genotype. McGlynn et al. (2005) also found no evidence of effect modification of *HFE*-variant adenoma association by *TFRC*-S142G genotype.

3.3.3 Variants in Other Iron Metabolism Genes

Transferrin Receptor-1 (TFRC) (Table 3.9)

TFRC-S142G/*Cancer of the Colon or Rectum*

Although two studies measured the frequency of the S142G polymorphism in the *TFRC* gene (Robinson et al., 2005; Beckman et al., 1999), neither reported results for this polymorphism alone. G-allele frequencies in the studies were 0.490 for Robinson et al. (2005) in population controls in southern England and 0.395 in the control group of the Swedish study of Beckman et al. (1999).

Table 3.9 Results from studies of the associations between the S142G polymorphism of the *TFRC* gene and colorectal carcinoma or adenoma and polyps*.

First Author/ Year	MAF in Ca/Co		Genotype counts, Ca/Co		Reported Comparison	Reported Result, OR (95% CI) or p-value	Adjustment	Calculated crude ORs (95% CI) for different inheritance models			Comment
			Cases n(%)	Controls n(%)				*Recessive*	*Co-dominant*	*Dominant*	
Beckman 1999	0.367/ 0.395	GG SG SS	23 (13.3) 81 (46.8) 69 (39.9)	51 (17.3) 130 (44.2) 113 (38.4)	Genotypes and G allele frequency	ns, reported qualitatively	None	0.73 (0.43-1.24)	GG v SS 0.74 (0.42-1.31) GS v SS 1.02 (0.68-1.53)	0.94 (0.64-1.38)	
Robinson 2005	0.491/ 0.495	GG SG SS	37 (21.6) 94 (55.0) 40 (23.4)	45 (24.5) 92 (50.0) 47 (25.5)	G allele frequency	p=0.940, Fisher's exact test	None	0.85 (0.52-1.40)	GG v SS 0.97 (0.53-1.77) GS v SS 1.20 (0.72-2.00)	1.12 (0.69-1.82)	
McGlynn 2005	0.448/ 0.444	GG SG SS	122 (19.2) 325 (51.2) 188 (29.6)	130 (20.0) 317 (48.8) 203 (31.2)	Genotypes	(1) SS v GG 0.99 (0.72-1.36) GS v GG 1.09 (0.81-1.46) (2) SS v GG 1.02 (0.74-1.42) GS v GG 1.13 (0.84-1.53)	(1) None (2) Sex, age, smoking, total intakes of iron, fibre and energy, and family history of colorectal cancer.	0.95 (0.72-1.25)	GG v SS 1.01 (0.74-1.39) GS v SS 1.11 (0.86-1.42)	1.08 (0.85-1.37)	The authors used the GG group as the reference category.

* Ca, cases. Co, controls. MAF, minor allele frequency. Freq., frequency. NR, not reported in publication. OR, odds ratio.

TFRC-S142G/*Adenoma of the Colon or Rectum*

McGlynn et al. (2005) reported results for exposures defined using this polymorphism alone. The adjusted (sex, age, total iron intake, total fibre, total energy, family history of colorectal cancer) and unadjusted associations comparing genotype groups were very close to 1.00 (S142/S142 vs. G142/G142 - OR_{adj} = 1.02, 95% CI 0.74-1.42 and S142/G142 vs. G142/G142 - OR_{adj} = 1.13, 95% CI 0.84-1.53). The G-allele frequency in this study was 0.444 among the controls. Overall, the odds ratios in Table 3.9 do not provide evidence supportive of an association between this variant and colorectal cancer or adenoma although the number of studies available is few.

Transferrin (*TF*) and *Heme-oxygenase-1(HMOX1)*

Lu et al. (1992) observed an over-representation of the *TF* C2-allele among cases of gastric cancer relative to a convenience sample of healthy controls in their study in Shanghai, China. Lo et al. (2007) reported a protective effect of the (GT)n *HMOX1* promoter polymorphism M-allele (medium length) (OR=0.65; 95% CI 0.44-0.97; unadjusted) for gastric adenocarcinoma. The frequencies of the short (S), medium (M) and long (L) alleles in their study were in good agreement with another case-control study in Taiwan (Chang et al., 2004). Sawa et al. (2008) defined their exposure groups differently from Lo e al. (2007) and did not provide sufficient information to extract frequencies of the short, medium and long alleles as defined in the Lo et al. study. Sawa et al. (2008) reported a positive association between shorter alleles (higher promoter activity) and gastric cancer. No other studies have investigated associations between the number of (GT)n repeats in the promoter region of *HMOX1* and neoplasia of the

gastrointestinal tract. Although several other studies have investigated the association between this variant and cancers at other sites in other populations (Okamoto et al., 2006; Kikuchi et al., 2005; Chang et al., 2004; Lin et al., 2006b) different definitions of lengths of alleles and the need for inter-comparisons of laboratory methods preclude comparing the results further at this point. There is currently insufficient evidence to draw conclusions concerning the association between the *TF* C-variants or the *HMOX1* (GT)n promoter polymorphism and different types of gastrointestinal neoplasia.

3.4 Summary

The *HFE*-C282Y minor variant is associated with measures of body iron and thus may have use as an index of chronically elevated iron levels. The rarity of the variant, however, limits interpretation of the GI tract cancer associations from existing studies. *HFE*-Y282 is most prevalent in individuals of Celtic and northern European origin but even in unselected samples from these populations the allele frequency is typically less than 0.10. Despite having 527 matched pairs in their study, Chan et al. (2005) found only 1 case and 4 controls homozygous for the Y282 variant. Based on the seven colorectal cancer studies identified in this review, there was little evidence to support an association between this variant and colorectal cancer in individuals of white, predominantly European ethnic origin. The results of the review are limited by the small size and low quality of most of the identified colorectal cancer studies. The generalisability of the results is also limited. Only one study (Shaheen et al., 2003) included enough individuals of African-American origin to calculate results for this population; there is a need for additional studies in ethnic/racial groups. Two studies that examined the association

between colorectal adenoma and *HFE*-Y282 were of reasonable size and quality. Neither reported a significant association.

Overall, possession of the D63 allele was positively associated with cancer of the colon or rectum (combined OR=1.21, 95% CI 0.98-1.49). This association was driven by the increased risk observed among African-American participants in the Shaheen et al. (2003) study. The Shaheen et al. study was population-based, the largest study to date and without serious threats to internal validity. Although Corley et al. (2008a) did not find formally significant associations between the C282Y and H63D variants and Barrett's Esophagus (BE), the results did provide some indication of an association between possession of the D63-allele and BE. However, the functional significance of the D63 variant is not well-characterized and although it is associated with biochemical iron indices (Samarasena et al., 2006) the relationship is less strong than for Y282. These results are interesting but have little bearing on the association between iron stores and neoplasia of the gastrointestinal tract. A recently published systematic review and meta-analysis (Ellervik et al., 2007) reported combined odds ratios for comparisons of the H63D/wt and H63D/H63D genotypes with the wt/wt group for the colorectal cancer outcome as well as 30 other disease endpoints. The odds ratios for the colorectal cancer outcome were 1.20 (95% CI 0.9-1.6) and 1.5 (95% CI 0.7-3.2) for the H63D/wt and H63D homozygotes, respectively, based the results from Altes et al. (1999), Shaheen et al. (2003) and Robinson et al. (2005).

Odds ratios comparing combined *HFE*-C282Y/H63D genotypes grouped according to the likelihood of elevated iron levels also did not provide evidence supporting an increased risk of colorectal cancer or colorectal adenoma with higher iron levels. Overall, the amount of evidence available to assess the magnitude of an association between the *HFE*-variants and neoplasia of the colon or rectum is limited. The studies carried out to date, however, provide little indication of a positive association.

This systematic review did not find any studies that examined the association between *HFE*-variants and cancers at other sites in the gastrointestinal tract, including the esophagus. Corley et al. (2008a) found some evidence of a higher odds ratio of BE in men with *HFE*-genotypes putatively associated with higher iron stores. There was no evidence of an association among women.

Chapter 4: Analysis of Data from a Canadian Esophageal Adenocarcinoma Case-Control Study

4.1 Introduction

As discussed in chapter 1, the incidence of esophageal adenocarcinoma (EAC) has shown a marked increase in the last two to three decades, mainly in white males (Devesa et al., 1998; Baquet et al., 2005), the reasons for which are unclear. Five-year survival rates for EAC are <15 % (Baquet et al., 2005) and have shown little improvement although some recent meta-analyses suggest that the use of chemotherapy adjuvant to surgery may increase survival relative to surgery alone (Malthaner et al., 2005, 2006).

There is strong evidence for the involvement of diet in EAC etiology. Higher intakes of fruit, vegetables and the antioxidant micronutrients vitamin C and beta-carotene are consistently associated with reduced risk (Mayne et al., 2001; WCRF/AICR 2007; Bollschweiler et al., 2002; Kubo and Corley, 2007). Limited evidence exists for positive associations between red and processed meat intakes, major sources of dietary iron, and esophageal adenocarcinoma, mainly from retrospective studies (WCRF/AICR 2007; Gonzalez et al., 2006; Jakszyn and Gonzalez, 2006; Mayne et al., 2001). Results from cohort studies are currently limited due to the long follow-up required to accrue sufficient cases. Heme-bound iron is one component of meat with evidence of carcinogenic potential from laboratory studies (Lund et al., 1999; Cross et al., 2003). Only one study has investigated the association between heme iron intake and EAC (Lee et al. 2005). Lee et al. (2005) reported a positive but statistically insignificant trend (p=0.06) with

increasing baseline heme iron intake among participants in the Iowa Women's Health Study when the analysis was adjusted for zinc intake as well as age, total energy intake, smoking and alcohol consumption. Without adjustment for zinc intake, however, the trend was not close to statistically significant and the relative risk comparing the 5th with the 1st quintiles of intake was 1.07 (95% CI: 0.41-2.82). The few epidemiological studies carried out to date that looked at total dietary iron intake and EAC (Chapter 2, Table 2.10) do not support the existence of a positive association. The three retrospective studies reported significant inverse associations between dietary iron intake and EAC (Brown et al., 1995; Wolfgarten et al., 2001; Zhang et al., 1997) and the two larger population-based case-control studies reported non-significant inverse associations (Mayne et al., 2001; Chen et al., 2002).

Elevated iron levels have been postulated as a possible risk factor for EAC based on laboratory studies in rats (Chen et al., 1999; Chen et al., 2000; Chen and Yang, 2001) and the role of iron in oxidative stress and inflammatory processes (Meneghini, 1997; Petersen, 2005) (Chapter 2; section 2.3). No epidemiological studies have been published to date investigating the association between iron stores and development of EAC (Chapter 2).

Both iron levels and dietary iron intakes present measurement challenges (Milman, 2006; Hallberg and Hulthén, 2003; Gabay and Kushner, 1999; Ganz, 2005; Natarajan et al, 2001; Kristal et al., 2005; Kipnis et al., 2003; Lombardi-Boccia et al., 2004; Lopez and Martos, 2004; Purchas et al., 2004). The investigation of associations between

polymorphisms in genes known to be involved in iron uptake and metabolism is one means through which the role of iron in EAC pathogenesis may be clarified. The literature review in Chapter 2 (section 2.7) and the systematic review in Chapter 3 confirmed that the most commonly studied variants in iron metabolism genes are the C282Y (rs1800562) and H63D (rs1799945) variants in the *HFE*-gene (Type I hereditary hemochromatosis, 6p21.3) (Feder et al., 1996; Burke et al., 1998; Hanson et al., 2001; Dorak et al., 2005; Barton et al., 2005; Barton et al., 2006). The C282Y variant has clear evidence for involvement in dysregulation of iron uptake and has highest carrier frequencies in people of Northern European, most likely of Celtic descent (Dorak et al., 2005; Lucotte et al., 2003). An increased cancer risk has been observed among parents and siblings of individuals with clinically diagnosed hereditary hemochromatosis who are likely to be heterozygous for the C282Y variant (Elmberg et al., 2003; Nelson et al., 2001; Nelson et al., 1995). The functional effects of the H63D polymorphism, however, are less clear. Transferrin receptors on tissue cell surfaces are key for the uptake of transferrin-bound iron from the circulating serum (Ponka and Lok, 1999; Chua et al., 2007) and are highly expressed by malignant cells to meet the high iron needs of rapidly proliferating cells (Le and Richardson, 2002; Boult et al., 2008). A SNP (single nucleotide polymorphism) in the transferrin receptor gene (*TFRC*, 3q29), S142G (rs3817672), has received attention due to preliminary evidence suggesting an increased risk of colorectal, breast, myeloma and liver cancer risk in combined *HFE*-C282Y/H63D/*TFRC*-S142G genotype subgroups (Beckman et al., 1999; 2001; see Chapter 3). Another non-synonymous SNP in *TFRC* (C363W, rs9852079) is predicted to be "probably damaging" by PolyPhen (Ramensky et al., 2002) due to the structural and

physicochemical changes to the gene product resulting from the cysteine (polar, hydrophilic) to tryptophan (non-polar, hydrophobic) amino acid change.

This study focuses on the role of iron in EAC pathogenesis. It uses analysis of dietary questionnaire data from a Canadian case-control study to investigate the associations between meat intake and dietary iron intake (total and heme) and the risk of EAC and banked germline DNA samples from the same study to investigate associations between polymorphisms of the *HFE* and *TFRC*-genes and the risk of EAC.

4.2 Methods

4.2.1 Study Design and Subjects

The original study was of population-based design with the goal of recruiting all incident cases of EAC in 2000 and 2001 from geographically-defined regions in southern Ontario. The boundaries of the geographic regions were defined using approximate one hour driving distances from tertiary care medical centres (London, Hamilton, Toronto-West, Toronto-East and Ottawa). Residential postal codes were used to assess case geographic eligibility. Cases also had to be between ages 30 and 79 inclusive, able to complete the interview in English or French and considered able to participate by their attending surgeon or physician. Of 31 thoracic surgeons identified at the tertiary care centres, 16 referred incident cases and, of these, seven provided 86% of the final sample. Very low recruitment at one centre early in the study time frame led to inclusion of an additional

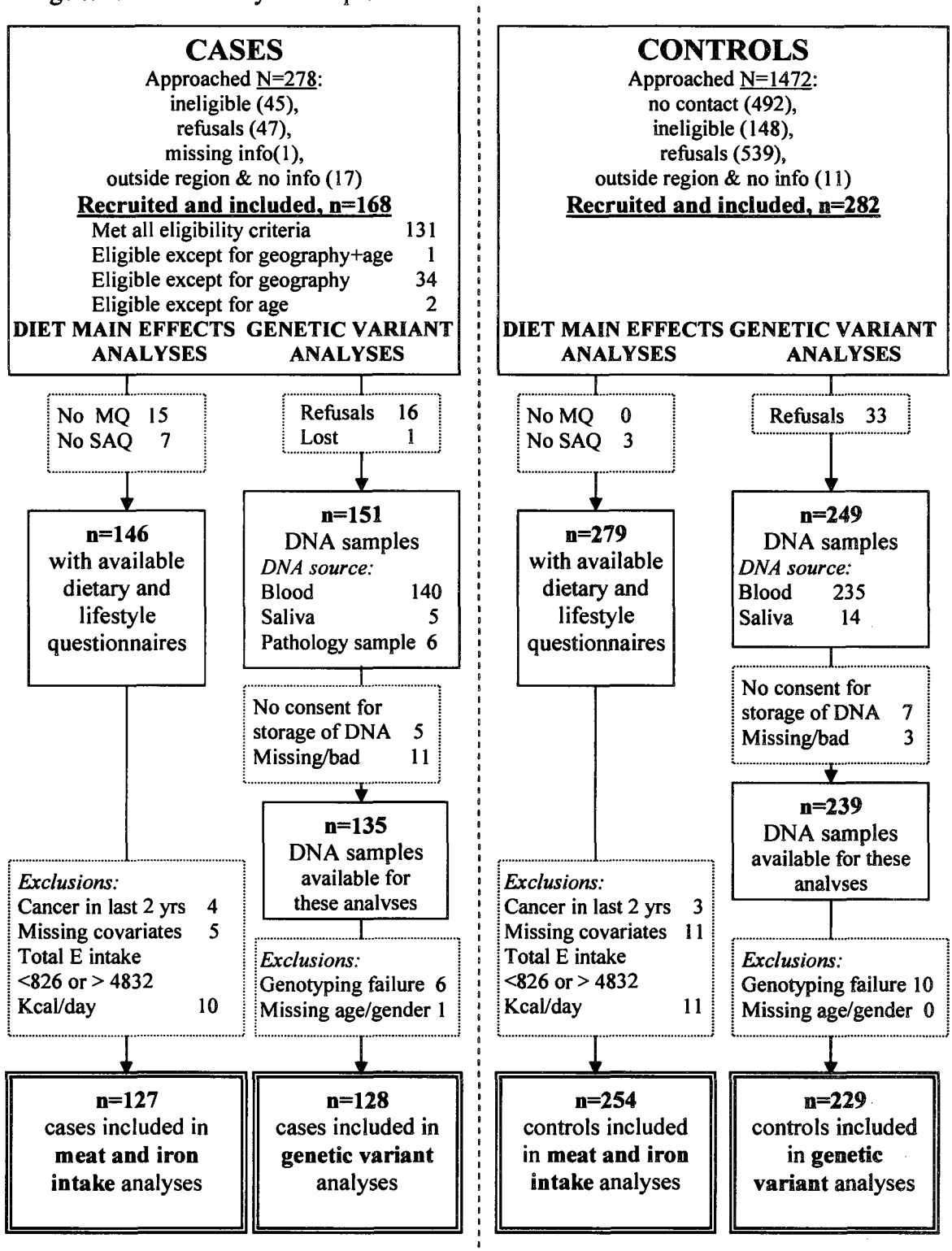
centre (Montreal). Figure 4.1 is a flow diagram of subject recruitment and exclusions applied prior to statistical analysis.

Some cases who were referred did not strictly meet the geographic eligibility criteria (34 of 168 cases). These "out of area" individuals lived within rural areas of the counties overlapping the geographically-defined regions, except two individuals from Sudbury and one from out of province. Although the potential for rural/urban differences in lifestyle and diet exists, these cases were included in the analysis.

Controls were obtained from a pool of geographically eligible individuals identified through municipal property assessment rolls obtained from the Municipal Property Assessment Corporation (MPAC). Recruitment then proceeded by phone using lists of randomly selected phone numbers from this pool. Controls were required to be competent to complete the questionnaires and have no prior diagnosis of cancer of the esophagus or stomach. In Montreal, controls were recruited through random digit dialing. Controls were frequency matched to cases on age and gender within each region.

Ethics approval for the original study was obtained from the ethics boards at each recruiting institution. Signed informed consent for study participation and use of collected information and material was obtained from all subjects. A copy of the primary consent form is provided in Appendix Bi. Approval for these analyses was obtained from the Ottawa Hospital Research Ethics Board (OHREB: #2006803-01H).

Figure 4.1 Flow diagram of subject recruitment and exclusions for the main effects diet and genetic variant analysis samples.*



* SAQ, self-administered questionnaire; MQ, main questionnaire.

4.2.2 Measurement of Lifestyle and Dietary Exposures

Participants completed two questionnaires to report their habitual dietary intake from the time period two years prior to the interview. The first questionnaire ("SAQ, self-administered questionnaire") was mailed to participants and contained items from a self-administered food frequency questionnaire validated in a US population (Block FFQ - NCI/Block, 1992; Block et al., 1986). The second questionnaire ("MQ, main questionnaire") included more detailed questions concerning meat intake. It was administered in a face-to-face interview setting and included the meat items from the Block FFQ, the tool developed by Sinha et al. (the "HCA" tool) for the estimation of heterocyclic amines from cooked meat products (Sinha et al., 2005; Sinha et al., 1998) and additional items for assessment of nitrite and nitrate intake as per Choi (1985). The MQ also collected information on demographic variables, self-reported height and weight, alcohol intake, smoking history, exercise, medical history, medication usage and family history of cancer. Copies of the SAQ and MQ are included in Appendix Bii.

4.2.3 Estimation of Dietary Intakes

Summation of responses for relevant questionnaire items allowed the estimation of total daily meat intake and daily intakes of different types of meat. In this study, "red meat" included hamburgers, steak, roast beef, liver, and pork; "white meat" included chicken and turkey; "fish" included both white fish and tuna; "shellfish" included oysters, other shellfish and crustacea; and "processed meats" included lunch/sandwich meats (bologna, ham), sausages, hot dogs and bacon.

Total dietary Fe intake, total energy intake and selected macro- and micronutrient measures were estimated using the Block FFQ items and its accompanying software - DIETSYS version 4.0 (NCI, 2005). The analyses reported here used the NHANES-II based food composition tables provided as part of DIETSYS version 4.0.

The Block FFQ items were distributed across the two questionnaires in order to reduce respondent burden. Differences in response options in the two questionnaires meant that some recoding of answers was necessary to produce the input file necessary for DIETSYS. The consumption frequency categories "Never" and "Less than once per month" were separate in the MQ but combined in the SAQ. The items on the MQ asked for serving sizes in absolute amounts (e.g. ozs, bacon strips or sausage links) while the SAQ requested serving size as small, medium or large, with an absolute quantity provided for a medium serving as a guide. Numerical serving sizes from the MQ items were converted to small, medium or large using the portion sizes file used by the DIETSYS software.

DIETSYS was used to calculate intakes of macronutrients (fat, carbohydrate, protein), total energy, dietary iron and iron from supplements.

Red meat (hamburger, steak, roast beef, liver, pork), white meat (chicken, turkey), fish (white fish, tuna), shellfish, and processed meats (lunch meats, sausages, hot dogs, bacon) all contain heme iron. The total iron from meat products was estimated by multiplying the weight of each meat product consumed by the iron content per unit weight obtained

from the DIETSYS food composition tables. Total meat iron was multiplied by a conversion factor of 0.4 (Monsen and Balintfy, 1982) to estimate the heme iron component.

4.2.4 Biosample Collection and Storage

Participants were asked to permit the collection of four tubes (10mL, EDTA-containing purple-top) of venous blood for extraction of germline DNA. Venepuncture was carried out either at a community blood-sample collection service or during routine clinical care. After collection, two tubes were sent by courier (24-48 hours, ambient temperature) to Montreal (St. Justine Hospital), the primary site for DNA extraction and genotyping during the original study. The other two tubes were couriered to the Ottawa Hospital for use as back-up in case of lost tubes and for the preparation of DNA samples for banking if consent for this process had been given by the participant. Participants who did not wish to provide blood samples were subsequently asked if they would consent to provide a saliva sample instead (Genotek™). DNA was extracted from tumor pathology samples for a small number of case participants who died prior to providing a DNA sample.

Eighty-nine percent of the original study participants, 151 cases and 249 controls, provided biosamples for extraction and genotyping of germline DNA (Figure 4.1), 93.8% in the form of venous blood.

4.2.5 DNA Extraction

During the original study, the buffy coat was separated from whole blood and stored at -20°C until extraction of DNA. DNA was extracted using a Tris/EDTA (AE) buffer (QIAGEN DNA isolation kits), quantified using absorbance at wavelength A620 and then stored in single aliquots at -20°C. DNA Genotek kits were used for extraction of DNA from saliva.

4.2.6 Genotype Measurement

DNA samples stored in Ottawa (n=374; 135 cases, 239 controls. See Figure 4.1) were used. Laboratory personnel thawed the DNA samples, aliquoted them into 96-well plates and adjusted the concentrations to 40ng/μL DNA. The samples were then refrozen and shipped overnight to The Centre for Applied Genomics (TCAG) for genotyping (R. Cole, laboratory technician, *personal communication*).

Four non-synonymous SNPs in iron metabolism genes were determined by TCAG using TaqMan assays and real-time PCR: *HFE*-C282Y (rs1800562), *HFE*-H63D (rs1799945), *TFRC*-S142G (rs3817672) and *TFRC*-C363W (rs9852079). Seventeen replicate samples and five negative controls were included with the DNA samples for quality control purposes. Laboratory analyses and interpretation of results were carried out by personnel blinded to sample case-control status and quality control sample locations.

4.2.7 Definition of Analysis Samples

In order to maximize the number of subjects available for estimation of the main effects association measures, exclusions were applied separately for the diet-disease and genetic variant-disease analyses (Figure 4.1). For meat and iron intake association analyses, 7 individuals (4 cases and 3 controls) were excluded due to a diagnosis of another cancer during the two years prior to the study; 8 (5 cases and 3 controls) were excluded due to missing values for some of the covariates (alcohol intake, smoking, BMI or severe heartburn); and 21 individuals (10 cases and 11 controls) were excluded due to total daily energy intakes which deviated by more than two standard deviations from the mean energy intake (<826 KCal/day or >4832 KCal/day). The analysis sample consisted of 381 individuals; 127 cases and 254 controls. The analysis sample for estimation of genetic variant-disease main effects excluded samples with a genotyping failure (undetermined or bad sample) for any of the genetic variants (6 cases, 10 controls) or missing values for matching variables (1 case). The final genetic-variant disease analysis sample had 357 individuals; 128 cases and 229 controls.

For the exploratory interaction analyses, subjects with missing genotype data were excluded from the dietary main effects sample leaving 309 subjects; 103 cases and 206 controls.

4.3 Statistical Analysis

4.3.1 Descriptive Analyses and Univariate Case-Control Comparisons

Case and control groups were compared with respect to lifestyle, demographic factors and selected dietary variables using Wilcoxon two-sample tests for continuous forms and χ^2 or Fisher exact tests for categorical forms. Diets were contrasted between the case and control groups with respect to consumption of meat, different types of meat, fruit and vegetables. Case-control groups were also compared with respect to factors with consistent evidence in the literature for either a positive or negative association with EAC (severe reflux symptoms, diagnosis of Barrett's esophagus, NSAID use and physician diagnosis of *Helicobacter pylori*/stomach ulcers).

For the genetic variants, minor allele and genotype frequencies were compared between cases and controls (χ^2 – test; Fisher exact probability test). Deviations from Hardy-Weinberg Equilibrium (HWE) among controls were assessed using the exact goodness of fit test (Fisher, 1935; Salanti et al., 2005).

4.3.2 Main Effects Association Analyses

Unconditional logistic regression models were constructed for four primary exposures: red meat intake; processed meat intake; total dietary iron intake and heme iron intake. Quintile cut-offs were defined using the whole sample (Hsieh et al., 1991) and tests for trend and deviations from linearity used quintile median values. In the main analysis, odds ratios were adjusted for the matching factors - age (as a categorical variable; 40 to

49 years/50 to 59 years/60 to 69 years/ 70 years and over), gender and geographical region (categorical - Ontario/Quebec). The model also adjusted for body mass index (BMI, usual adult, continuous), smoking (pack-years, continuous), frequency of alcohol consumption (drinks per day, quintiles), vegetable intake (quintiles), total energy intake (KCal/day, continuous) and self-report of severe heartburn. There are advantages and disadvantages to using continuous or categorized exposure measures. Categorization leads to the loss of information and also reduces the power to detect an association. Also, in small studies, results can be sensitive to the category cut-offs applied. Use of a continuous exposure variable, however, makes an assumption that the exposure-risk relationship is linear. In this study, there is no statistical evidence of a deviation from linearity for any of the main dietary exposures or covariates using quintile medians with the exception of vegetable intake ($p=0.006$). The odds ratios comparing quintiles of vegetable intake indicate little protective effect except in the lowest quintile of intake suggesting a threshold effect. The variable for frequency of alcohol intake was estimated by summing the contributions for beer, wine and liquor. Although no statistically significant deviation from linearity was evident based on the median quintile values for frequency of alcohol intake ($p=0.151$), examination of the individual reported summed intakes showed several extreme high values that were suspect. The alcohol variable was included in the model in the categorized form. Severe heartburn was categorized as ever/never based on responses to the following questionnaire item: "Before (2 years prior to interview), did you ever have severe heartburn, that is heartburn so painful that it awoke you or prevented you from sleeping?". Adjustment for energy intake in the main

analysis employed the standard multivariate method by including total daily caloric intake (obtained from DIETSYS v4.0) in the models.

Unadjusted and adjusted (age, gender, geographic region) odds ratios for the associations between *HFE*-C282Y, *HFE*-H63D and *TFRC*-S142G genotypes and the risk of EAC were estimated using unconditional logistic regression. A planned analysis compared disease odds in participants with *HFE*-genotypes generally associated with higher biochemical indices of iron (C282Y/C282Y, C282Y/H63D, C282Y/wild type, H63D/H63D) with the disease odds in those without these genotypes. Combined *HFE-TFRC* genotypes were examined as per the genotype groups used by Beckman et al. (1999, 2001).

With 130 cases and an approximate 1:2 case to control ratio, the power to detect an odds ratio of 2.0 when comparing the highest with the lowest quartiles of Fe intake is 66% assuming a disease prevalence of 2 per 100,000 (Devesa et al., 1998; Baquet et al., 2005) (Type I error level=0.05, two-sided test). The power to detect an odds ratio of 1.2 is only 9% (Calculated using Power v3.3. Lubin et al., 1990; Garcia-Closas and Lubin, 1999). The frequency of C282Y homozygotes was considered unlikely to exceed 0.5% in the sample. An "*HFE*- hi-iron" group was defined *a priori* based on a preliminary literature review: higher risk=C282Y/C282Y, C282Y/H63D, C282Y/wild type, H63D/H63D; lower risk= H63D/wild type, wild type/wild type. The likely prevalence of the higher risk group was 15% based on the literature review. The power to detect an odds ratio of

1.6 for 10%, 15% and 20% prevalence of the "*HFE*-hi-iron" group was 0.306, 0.398 and 0.467, respectively (calculated using Quanto v1.1.1; Gauderman and Morrison, 2006.).

4.3.3 Main Effects Sensitivity Analyses

Association analyses were also carried out using nutrient measures energy-adjusted using the residuals method (Willett et al., 1997, 1998; Willett and Stampfer, 1986). In this method, the nutrient intake is regressed against total dietary energy intake. The residuals from the regression are then approximations of the nutrient values with the effect of the association with energy intake removed. The nutrient value expected for the mean caloric intake is then added to each residual to bring the values back up to realistic levels.

Further sensitivity analyses: (a) excluded those cases who were outside the original geographical and age eligibility criteria (25 participants); (b) excluded those with any previous cancer diagnosis (except basal cell skin cancer) (28 participants); and (c) restricted the analysis sample to white males (excluding 35 participants).

4.3.4 Exploratory Interaction Analyses

The statistical interactions analyses are exploratory in nature although specified *a priori* based on results from epidemiological studies or evidence from laboratory investigations. For these analyses, observations in the lower three quintiles of dietary intake were classified as "lower" and those in the upper two quintiles as "higher". Interactions were assessed on the additive scale as the relative excess risk due to interaction (RERI)

(Rothman, 1974) with 95% confidence intervals for these measures estimated using the multivariate delta method (Hosmer and Lemeshow, 1992). Cross-product terms were added to the logistic regression models to assess interaction on a multiplicative scale (Likelihood ratio test; Greenland, 1983). Two ExE interactions were investigated: Fe intakes and alcohol intake (Petersen, 2005); and Fe intakes and reflux symptoms (Chen and Yang, 2001). Analyses were also carried out stratified by the matching factor age (Beard et al., 2002; Fleming et al., 2002). Two planned GxE interactions were investigated: *HFE*-variant and Fe intakes (Cogswell et al., 2003) and *HFE*-variant and alcohol intake (Scotet et al., 2003).

4.3.5 Statistical Analysis Software

All analyses were carried out using SAS v9.1 (SAS Institute, Cary, NC.) except assessment of the deviation from Hardy-Weinberg Equilibrium of genetic variants using an exact probability test. This was carried out using STATA version 9.2 (Statacorp, College Station, TX).

4.4 Results

4.4.1 Descriptive Analyses and Univariate Case-Control Comparisons

Demographic, lifestyle and non-dietary factors

Table 4.1 shows comparisons of the case and control groups with respect to demographic, lifestyle and other non-dietary risk factors for EAC. The majority of participants in the study were male (93.4%) and self-identified as "White or Caucasian" (96.9%). The

sample was well-educated with 28% having attained a university degree. Overall, cases reported lower levels of educational attainment than controls (p=0.006). Cases also reported heavier smoking than controls. Although a higher percentage of cases than controls were in the highest quintile of alcohol consumption, the difference in distribution between cases and controls was only marginally significant (p=0.048). Body mass index (BMI), calculated from self-reported usual adult height and weight, was significantly higher among cases than controls. Cases also tended to report higher energy intakes although the difference did not reach formal statistical significance in the categorical analysis (p=0.105).

Table 4.1 Demographic, lifestyle factors and non-dietary factors in the case and control groups in the esophageal adenocarcinoma study.

Characteristic	Metric or Category	Cases	Controls	p*
Gender†		n(%)	n(%)	
	male	120 (94.5)	236 (92.9)	
	female	7 (5.5)	18 (7.1)	0.558
Age (years)†	age 40 to 49	17 (13.4)	33 (13.0)	
	age 50 to 59	39 (30.7)	69 (27.2)	
	age 60 to 69	30 (23.6)	74 (29.1)	
	age ≥ 70 years	41 (32.3)	78 (30.7)	0.707
Ethnicity (self-report)	white	123 (96.9)	246 (96.9)	
	not white	4 (3.1)	8 (3.1)	1
Formal education level	Less than high school	26 (20.5)	40 (15.7)	
	High school/vocational training	50 (39.4)	68 (26.8)	
	Some college/college grad/some university	28 (22.0)	61 (24.0)	
	Undergraduate or graduate degree	23 (18.1)	85 (33.5)	0.006
Smoking (pack-years)	median (range)	25.0 (0-149.6)	8.5 (0-220.0)	0.002
		n(%)	n(%)	
	never	32 (25.2)	104 (40.9)	
	tertile 1 (median=13.0)	25 (19.7)	56 (22.0)	
	tertile 2 (median=31.8)	33 (26.0)	51 (20.1)	
	tertile 3 (median=65.0)	37 (29.1)	43 (16.9)	0.004
Alcohol consumption (drinks/day)	median (range)	1.3 (0-19.7)	1.1 (0-32.0)	0.370
		n(%)	n(%)	
	Q1 (median=0)	28 (22.0)	46 (18.1)	
	Q2 (median=0.43)	20 (15.7)	58 (22.8)	
	Q3 (median=1.14)	26 (20.5)	51 (20.1)	
	Q4 (median=2.14)	19 (15.0)	57 (22.4)	
	Q5 (median=6.02)	34 (26.8)	42 (16.5)	0.048
BMI (usual adult, kg/m^2)	median (range)	28.0 (21.0-55.0)	26.7 (18.1-42.1)	0.0004
		n(%)	n(%)	
	Q1 (median=23.6)	16 (12.6)	62 (24.4)	
	Q2 (median=25.9)	23 (18.1)	53 (20.9)	
	Q3 (median=27.5)	26 (20.5)	50 (19.7)	
	Q4 (median=29.7)	28 (22.0)	46 (18.1)	
	Q5 (median=33.2)	34 (26.8)	43 (16.9)	0.028
Total E intake (KCal/day)	median (range)	2270 (844-4615)	2021 (832-4445)	0.016
		n(%)	n(%)	
	Q1 (median=1283)	22 (17.3)	54 (21.3)	
	Q2 (median=1788)	20 (15.7)	56 (22.0)	
	Q3 (median=2076)	22 (17.3)	55 (21.7)	
	Q4 (median=2541)	31 (24.4)	45 (17.7)	
	Q5 (median=3237)	32 (25.2)	44 (17.3)	0.105

* P-value for comparison of the cases and controls (Chi-square test for categorical variables, Wilcoxon two-sample test for continuous variables).

† Frequency-matching factor.

Table 4.1 (continued). Demographic, lifestyle factors and non-dietary factors in the case and control groups in the esophageal adenocarcinoma study.

Characteristic	Metric or Category	Cases	Controls	p*
		n(%)	n(%)	
Severe heartburn†	Yes	66 (52.0)	71 (28.0)	
	No	61 (48.0)	183 (72.0)	<0.0001
Among those who replied Yes:				
Frequency (tertiles)‡				
	< once/week	12 (19.0)	33 (46.5)	
	1 to 4/week	23 (36.5)	23 (32.4)	
	≥5 times/week	28 (44.4)	15 (21.1)	0.0013
Duration (tertiles)	1 mo. to 5 yrs	15 (23.8)	27 (40.9)	
	6 to 19 yrs	24 (38.1)	18 (27.3)	
	20 to 65 yrs	24 (38.1)	21 (31.8)	0.110
Acid regurgitation§	Yes	67 (52.8)	103 (40.6)	
	No	60 (47.2)	151 (59.4)	0.024
Among those who replied Yes:				
Frequency (tertiles)	≥1 to 4x/yr	10 (15.9)	44 (44.0)	
	5x/yr to once/wk	25 (39.7)	37 (37.0)	
	1.5x/wk to 6x/day	28 (44.4)	19 (19.0)	0.0001
Duration (tertiles)	1 to 3 yrs	20 (33.9)	31 (35.6)	
	4 to 10 yrs	22 (37.3)	24 (27.6)	
	>10 years	17 (28.8)	32 (36.8)	0.418
Barrett's Esophagus**				
	Yes	7 (5.6)	2 (0.8)	
	No	119 (94.4)	250 (99.2)	0.008
Regular NSAID/aspirin use††	Ever	36 (28.8)	84 (33.1)	
	Never	89 (71.2)	170 (66.9)	0.401
H. pylori infection or stomach ulcer‡‡	Yes	21 (17.2)	33 (13.0)	

* P-value from Chi-square or Fisher's exact test comparing case and control groups.

† Before (2 years prior to interview), did you ever have severe heartburn, that is heartburn so painful that it awoke you or prevented you from sleeping?

‡ Missing data, n=3 cases

§ Before (2 years prior to interview), did you ever have acid regurgitation, that is, a sour taste in your mouth from the contents backing up into your mouth or throat?

** Before (2 years prior), did a doctor ever tell you that you had Barrett's esophagus?

†† Missing data, n=2. Regular use was defined as taking a medication two or more times a week for one month or longer.

‡‡ Missing data, n=6. 3 cases (2.4%) and 11 controls (4.4%) reported a diagnosis of *H. pylori* (p=0.346); 20 cases (16.1%) and 29 controls (11.4%) reported a diagnosis of stomach ulcers (p=0.200).

More cases than controls recalled that they had suffered from severe heartburn, painful enough to wake them up or prevent them from sleeping, at some time in their lifetime more than two years prior to the interview (52% versus 28%, $p<0.0001$). Among those who had experienced severe heartburn, those who reported higher frequencies were more likely to be cases. The relationship was less strong for the duration of symptoms, however. Acid regurgitation was reported much less frequently than severe heartburn but, similar to heartburn, cases reported a higher frequency. The reported duration of acid regurgitation was the same in cases and controls. A physician diagnosis of Barrett's esophagus (BE), a recognized risk factor for esophageal adenocarcinoma, was more frequent among cases than controls ($p=0.0076$) although the number of diagnosed BE cases was small. There was no significant difference in proportions of individuals who reported ever regularly taking "Motrin, Advil or similar non-steroidal anti-inflammatories" (11% of cases and 13% of controls). "Regularly" was defined as at least two times per week for one month or longer. A question pertaining specifically to aspirin was added to the questionnaire part way through the study on recognition that not all respondents were including aspirin with NSAIDs. Thirty-seven percent of cases (26 of 70) and 38% of controls (58 of 152) who responded to this question reported that they had regularly taken aspirin. There were also no differences between the case and control groups with respect to the proportions of individuals who reported a physician diagnosis of *H. pylori* and/or stomach ulcer or either of these conditions.

Dietary Intakes

Table 4.2 shows comparisons of meat, meat types and vegetable intakes between the case and control groups. Cases reported higher intakes of total meat and red meat and lower consumption of vegetables. Cases also reported higher intakes of processed meat although the difference between the case and control groups did not reach formal statistical significance when analyzed in the categorical form. Cases did not differ from controls with respect to reported intakes of white meat (chicken and turkey, $p=0.52$), fish ($p=0.25$) or fruit ($p=0.78$).

As shown in Table 4.3, dietary intakes of iron estimated by DIETSYS ranged from 5.1 to 27.7 mg/day (median 13.5) among cases and 3.0 to 26.7 mg/day (median 13.3) among controls. In Canada and the United States, the current recommended daily allowance of iron is 8 mg Fe/d for men over the age of 19 and women over the age of 50 (WHO, 2004a). About 20% of participants reported consuming vitamin supplements containing iron and 40% reported using multivitamins. There were no differences between cases and controls for the dietary supplement items. With supplemental iron included, only 3 individuals were estimated to have exceeded the tolerable upper limit of total iron intake associated with gastrointestinal distress (45 mg/day) (FNB/IoM, 2000).

Reported heme iron intake was significantly greater in cases than controls ($p<0.003$). Intakes ranged from 0.38 to 5.32 mg/day in the cases and from 0.08 to 4.60 mg/day in the controls. On average, 50 to 60 percent of heme Fe in this index came from red meat sources although proportions showed considerable variability ranging from 9 to 88% in

cases and zero to 97% in controls. The Pearson correlation coefficient between heme iron and red meat intake in the entire sample was 0.86 and between heme iron and total meat intake was 0.96. Iron from meat products (heme and non-heme) comprised on average 25 to 30% of the total dietary iron intake (range 2 to 68%).

Table 4.2 Dietary intakes of meat, meat types, fruit and vegetables in the case and control groups in the esophageal adenocarcinoma study.

Dietary component	Metric or quintile	Cases	Controls	p*
Total meat intake (g/day)	median (range)	46.1 (14.0-169.0)	41.0 (2.3-164.6)	0.012
		n(%)	n(%)	
	Q1 (median=23.8)	22 (17.3)	54 (21.3)	
	Q2 (median=32.6)	24 (18.9)	52 (20.5)	
	Q3 (median=42.3)	22 (17.3)	55 (21.7)	
	Q4 (median=53.8)	22 (17.3)	54 (21.3)	
	Q5 (median=77.7)	37 (29.1)	39 (15.4)	0.036
Red meat intake (g/day)	median (range)	22.7 (2.0-93.9)	17.5 (0-82.6)	0.0009
		n(%)	n(%)	
	Q1 (median=7.1)	22 (17.3)	54 (21.3)	
	Q2 (median=13.3)	18 (14.2)	58 (22.8)	
	Q3 (median=18.5)	22 (17.3)	55 (21.7)	
	Q4 (median=26.8)	29 (22.8)	47 (18.5)	
	Q5 (median=42.1)	36 (28.3)	40 (15.7)	0.016
Processed meat intake (g/day)	median (range)	7.1 (0-53.8)	5.1 (0-44.7)	0.002
		n(%)	n(%)	
	Q1 (median=1.33)	17 (13.4)	59 (23.2)	
	Q2 (median=3.35)	25 (19.7)	51 (20.1)	
	Q3 (median=5.85)	24 (18.9)	53 (20.9)	
	Q4 (median=9.19)	29 (22.8)	47 (18.5)	
	Q5 (median=15.15)	32 (25.2)	44 (17.3)	0.103
White meat intake (g/day)	median (range)	6.6 (0-64.0)	7.0 (0-52.5)	0.373
		n(%)	n(%)	
	Q1 (median=1.60)	30 (23.6)	46 (18.1)	
	Q2 (median=4.40)	21 (16.5)	49 (19.3)	
	Q3 (median=6.84)	26 (20.5)	56 (22.0)	
	Q4 (median=12.00)	30 (23.6)	51 (20.1)	
	Q5 (median=21.00)	20 (15.7)	52 (20.5)	0.519
Vegetables (medium servings/week)	median (range)	11.3 (1.2-42.1)	13.5 (0.3-50.5)	0.004
		n(%)	n(%)	
	Q1 (median=5.59)	41 (32.3)	35 (13.8)	
	Q2 (median=8.72)	19 (15.0)	57 (22.4)	
	Q3 (median=12.50)	24 (18.9)	53 (20.9)	
	Q4 (median=17.44)	22 (17.3)	54 (21.3)	
	Q5 (median=24.81)	21 (16.5)	55 (21.7)	0.0008

* P-value from Chi-square test or Wilcoxon two-sample test comparing case and control groups.

Table 4.3 Oral intakes of iron (Fe) in the case and control groups of the esophageal adenocarcinoma study

Iron Intake (mg/day)	Category or Metric	Cases	Controls	p*
Dietary iron intake	median (range)	13.5 (5.1-27.7)	13.3 (3.0-26.7)	0.402
		n(%)	n(%)	
	Q1 (median=8.0)	25 (19.7)	51 (20.1)	
	Q2 (median=11.0)	21 (16.5)	55 (21.7)	
	Q3 (median=13.4)	27 (21.3)	50 (19.7)	
	Q4 (median=16.0)	24 (18.9)	52 (20.5)	
	Q5 (median=20.2)	30 (23.6)	46 (18.1)	0.624
Heme iron intake	median (range)	1.49 (0.38-5.32)	1.25 (0.08-4.60)	0.0018
		n(%)	n(%)	
	Q1 (median=0.70)	20 (15.7)	56 (22.0)	
	Q2 (median=1.00)	22 (17.3)	54 (21.3)	
	Q3 (median=1.29)	22 (17.3)	55 (21.7)	
	Q4 (median=1.71)	23 (18.1)	53 (20.9)	
	Q5 (median=2.51)	40 (31.5)	36 (14.2)	0.0028
Iron intake as supplements				
		n(%)	n(%)	
	Yes	25 (19.7)	57 (22.4)	
	No	102 (80.3)	197 (77.6)	0.537
Total iron intake *(supplement plus dietary)*	median (range)	15.4 (5.1-68.0)	15.0 (3.0-106.0)	0.817
Use of Multivitamins				
		n(%)	n(%)	
	Yes	52 (41.3)	108 (42.7)	
	No	74 (58.7)	145 (57.3)	0.792

* P-value for the Wilcoxon two-sample test for comparing continuous measures and chi-square test for comparing categorical measures.

Genetic Variants

Table 4.4 provides the genotyping success rates and quality control results. Genotypes were called for 97.5% of the samples for C282Y (393 of 403), 97.8% (394) for H63D and 96.5% (389) for S142G. Agreement between replicates was 94% (16 out of 17) for C282Y and S142G and 100% for H63D. The discordant C282Y and S142G replicates were not observed in the same samples. Re-interpretation of all the sample results by the laboratory analyst blind to the identities of the problem samples did not resolve the discrepancies in the replicates. The C282Y and S142G results were omitted for these specific samples in the subsequent analyses. However, given the high replicate agreement and in the absence of any evidence for a systematic problem, the remaining samples were all included in the analyses.

Table 4.4 Genotyping quality control results.

Variant	Total analyzed*	Blanks correctly called	Failures	Call rate	Agreement between replicates	Geno-types of replicates
HFE-C282Y (rs1800562)	408	5	10	97.5%	16 of 17	**CY/CC, n=1** CY/CY, n=1 CC/CC, n=15
HFE-H63D (rs1799945)	408	5	9	97.8%	17 of 17	HD/HD, n=4 HH/HH, n=13
TFRC-S142G (rs3817672)	408	5	14	96.5%	16 of 17	**SG/GG, n=1** GG/GG, n=3 SG/SG, n=8 SS/SS, n=5

* There were 410 analyses but 2 had visibly bad samples for which calls were not attempted.

Tables 4.5a and b show the genotype and allele frequencies for the *HFE*-C282Y, *HFE*-H63D and *TFRC*-S142G polymorphisms. The minor W-variant of the *TFRC*-C363W polymorphism was not present in any of the samples. The Y282-allele frequency was 0.069 overall, in good agreement with population frequencies for white, western Europeans (Merryweather-Clarke et al., 2000; Hanson et al., 2001; Lucotte, 2001). No subjects were homozygous for the Y282-allele and there was no evidence of a deviation from Hardy-Weinberg Equilibrium (HWE; χ^2 goodness of fit test, p=0.613) in the controls. D63-allele frequency was 0.133 in cases and controls, in the range expected for the ethnic mix of the study population (Merryweather-Clarke et al., 2000; Girouard et al. 2002; Campbell et al., 2003). Only 2 subjects, 1 case and 1 control, were D63-homozygotes, but there was no evidence of a deviation from HWE in the controls (p=0.144). There were 11 compound heterozygotes in the sample, 3 cases and 8 controls (Table 4.5c). *TFRC*-G142 allele frequencies were similar between cases and controls. The G142-allele frequency was 0.424 and heterozygosity 48.9% among controls, in general agreement with results for white, European populations (McGlynn et al., 2005; Beckman et al., 1999). The *TFRC*-S142G polymorphism also did not show evidence of a deviation from HWE in the controls.

None of the variants showed significant differences between the case and control groups with respect to allele or genotype frequencies. There were also no significant differences in "*HFE*-hi-iron" genotype frequencies or combined *HFE/TFRC* genotype frequencies between the case and control groups (Table 4.5c).

Table 4.5a C282Y, H63D and S1412G genotype frequencies for cases and controls in the esophageal adenocarcinoma study.

Polymorphism	Genotype	Cases, n (%)	Controls, n (%)	p*
HFE-C282Y	Y282/Y282	0 (0.0)	0 (0.0)	
	C282/Y282	15 (11.7)	33 (14.4)	
	C282/C282	113 (88.3)	196 (85.6)	0.475
HFE-H63D	D63/D63	1 (0.8)	1 (0.4)	
	H63/D63	32 (25.0)	59 (25.8)	
	H63/H63	95 (74.2)	169 (73.8)	1.000
TFRC-S142G	G142/G142	19 (14.8)	41 (17.9)	
	S142/G142	70 (54.7)	112 (48.9)	
	S142/S142	39 (30.5)	76 (33.2)	0.553

Table 4.5b C282Y, H63D and S1412G minor allele frequencies for cases and controls in the esophageal adenocarcinoma study.

Polymorphism	Allele	Cases	Controls
HFE-C282Y	Y-allele frequency	0.059	0.072
HFE-H63D	D-allele frequency	0.133	0.133
TFRC-S142G	G-allele frequency	0.422	0.424

* P-value for chi-square test for independence or Fisher's exact test, as appropriate.

Table 4.5c Combined C282Y, H63D and S1412G genotype frequencies for cases and controls in the esophageal adenocarcinoma study.

Polymorphism	Genotype	Cases, n (%)	Controls, n (%)	p*
HFE-C282Y/*HFE*-H63D	Y282/Y282	0 (0.0)	0 (0.0)	
	H63/Y282†	12 (9.4)	25 (10.9)	
	D63/Y282	3 (2.3)	8 (3.5)	
	D63/D63	1 (0.8)	1 (0.4)	
	C282/D63	29 (22.7)	51 (22.3)	
	C282/H63	83 (64.8)	144 (62.9)	0.964
HFE-any‡	Yes	45 (35.2)	85 (37.1)	
	No	83 (64.8)	144 (62.9)	0.712
HFE-hi iron§	Yes	16 (12.5)	34 (14.8)	
	No	112 (87.5)	195 (85.2)	0.540
HFE/TFRC combined genotypes (as per Beckman et al., 1999)	*TFRC*-S142/S142: *HFE*-Y282 carrier	5 (3.9)	18 (7.9)	
	Other	123 (96.1)	211 (92.1)	0.180
	TFRC-S142/S142: *HFE*-D63/Y282	1 (0.8)	4 (1.7)	
	Other	127 (99.2)	225 (98.3)	0.658

* P-value for chi-square test for independence or Fisher's exact test, as appropriate.

‡ Carriers of either or both of the Y282 and D63 alleles.

§ Yes=Y282 homozygotes, D63 homozygotes, Y282 heterozygotes. No = other.

4.4.2 Association Analyses

Dietary Intake: Main Effects

Table 4.6 shows the odds ratios for the meat and iron intake main exposures after adjustment for matching variables and other putative risk factors for esophageal adenocarcinoma. Red meat intake analyzed in continuous form was significantly associated with esophageal adenocarcinoma after adjustment for age, gender, region (Ontario/Quebec), usual adult BMI, smoking, frequency of alcohol intake, self-report of severe heartburn and vegetable intake. The test for trend approached statistical significance (p=0.063). Processed meat intake was not associated with EAC after adjustment for lifestyle factors, reported severe heartburn and vegetable intake. Mutual adjustment by the other meat type did not affect the results for either red or processed meat appreciably.

Total dietary iron intake showed no association with EAC before or after adjustment for lifestyle variables, heartburn and vegetable intake. Intake of heme iron showed a significant association with EAC in the multivariate analysis when analyzed in the continuous form. For an increase of 1mg heme iron intake per day, the odds ratio was 1.50 (95% CI 1.08-2.08). Analysis of the heme iron intake categorized into quintiles showed that the association was driven mainly by subjects in the highest quintile. However, there was no statistical evidence of a deviation from linearity of the relationship (p=0.659). The test for trend was significant (p=0.012) and the odds ratio comparing the highest with the lowest quintile of intake was 2.84 (95% CI 1.25-6.47).

Table 4.6 Multivariate adjusted odds ratios for the associations between red meat, processed meat, iron intakes and esophageal adenocarcinoma.

Main Exposure	Category or Metric	OR-1 (95% CI)*	OR-2 (95% CI)†
Red meat intake	continuous, +25 g/day	2.10 (1.45-3.03)	1.77 (1.17-2.69)
	Q1 (median=7.1)	1.00	1.00
	Q2 (median=13.3)	0.82 (0.40-1.71)	0.73 (0.32-1.66)
	Q3 (median=18.5)	1.09 (0.53-2.21)	0.96 (0.43-2.13)
	Q4 (median=26.8)	1.62 (0.82-3.22)	1.21 (0.56-2.63)
	Q5 (median=42.1)	2.43 (1.22-4.83)	1.68 (0.76-3.72)
	P-trend	0.001	0.063
	P-heterogeneity	0.020	0.332
Processed meat intake	continuous, +25 g/day	3.74 (1.58-8.85)	2.40 (0.90-6.41)
	Q1 (median=1.3)	1.00	1.00
	Q2 (median=3.4)	1.78 (0.86-3.70)	2.06 (0.90-4.74)
	Q3 (median=5.8)	1.61 (0.77-3.38)	1.46 (0.64-3.32)
	Q4 (median=9.2)	2.18 (1.06-4.50)	1.75 (0.78-3.93)
	Q5 (median=15.2)	2.47 (1.19-5.14)	2.05 (0.88-4.78)
	P-trend	0.023	0.223
	P-heterogeneity	0.148	0.417
Dietary Fe intake	continuous, +5 mg/day	1.08 (0.84-1.39)	1.30 (0.96-1.76)
	Q1 (median=8.0)	1.00	1.00
	Q2 (median=11.0)	0.70 (0.34-1.45)	0.87 (0.38-2.00)
	Q3 (median=13.4)	1.01 (0.50-2.05)	1.59 (0.67-3.76)
	Q4 (median=16.0)	0.81 (0.40-1.66)	1.44 (0.61-3.40)
	Q5 (median=20.1)	1.27 (0.62-2.59)	2.00 (0.82-4.91)
	P-trend	0.392	0.070
	P-heterogeneity	0.534	0.337
Heme Fe intake	continuous, +1 mg/day	1.67 (1.26-2.21)	1.50 (1.08-2.08)
	Q1 (median=0.70)	1.00	1.00
	Q2 (median=1.00)	1.21 (0.58-2.50)	1.40 (0.63-3.15)
	Q3 (median=1.29)	1.18 (0.57-2.44)	1.19 (0.53-2.68)
	Q4 (median=1.71)	1.25 (0.60-2.61)	1.32 (0.58-2.99)
	Q5 (median=2.51)	3.24 (1.59-6.60)	2.84 (1.25-6.47)
	P-trend	<0.001	0.012
	P-heterogeneity	0.005	0.096

* OR-1: Odds ratios adjusted for original matching factors - age (40-49, 50-59, 60-69, ≥70 years), gender, and geographical region (Quebec/Ontario).

† OR-2: Odds ratios adjusted for age (40-49, 50-59, 60-69, ≥70 years), gender and geographical region (Quebec/Ontario), and also smoking (pack-years, continuous), frequency of alcohol intake (quintiles), usual adult BMI (continuous), self-report of severe heartburn (ever/never) and vegetable intake (quintiles).

Odds ratios with adjustment for energy using the standard multivariate method (the main analysis) and the residuals method are shown in Table 4.7. Adjustment for total energy intake further attenuated the association between red meat and EAC to 1.58 for an increase of 25g red meat per day (95% CI 1.00-2.51). The association with heme iron intake was also attenuated (OR=1.34 for +1 mg heme iron per day, 95% CI 0.91-1.98). With main exposure variables in continuous form, energy adjustment by both methods gave similar results. Including total energy intake as an independent covariate in the residuals model did not have an appreciable effect on the associations.

Genetic Variants: Main Effects

Neither *HFE*-C282Y nor *HFE*-H63D genotypes were associated with EAC (Table 4.8). Individuals with combined *HFE*-genotypes associated with higher biochemical indices of iron (*HFE*-hi-iron) did not show higher odds of EAC compared with those with other *HFE*-genotypes (OR=0.77, 95% CI 0.40-1.48; with adjustment for age, gender and region). Due to the small numbers of D63-homozygotes, the results for the *HFE*-hi-iron group were essentially the same as for the C282Y polymorphism alone. *TFRC*-S142G genotypes were not associated with EAC and odds ratios comparing combined *HFE-TFRC* genotype groups defined as per Beckman et al. (1999) showed no formally significant associations.

Table 4.7 Odds ratios for the associations between main dietary exposures and esophageal adenocarcinoma with energy adjustment using the standard multivariate and residuals methods.

	Standard Multivariate Method				Residuals Method			
Main Exposure	**Variable**	**OR-main (95% CI)***	**LR, p†**	**H-L, p‡**	**Variable**	**OR-3 (95% CI)§**	**LR, p**	**H-L, p**
Red meat intake, g/day	continuous, +25 g/day	1.58 (1.00-2.51)	0.051	0.154	continuous, +25 g/day	1.59 (0.99-2.55)	0.058	0.197
	Q1 (median=7.1)	1.00			Q1 (median=8.2)	1.00		
	Q2 (median=13.3)	0.70 (0.31-1.59)			Q2 (median=15.3)	1.80 (0.80-4.04)		
	Q3 (median=18.5)	0.90 (0.41-2.01)			Q3 (median=20.3)	1.32 (0.58-3.01)		
	Q4 (median=26.8)	1.04 (0.47-2.32)			Q4 (median=26.2)	1.41 (0.61-3.24)		
	Q5 (median=42.1)	1.30 (0.55-3.08)	0.694	0.105	Q5 (median=38.6)	2.12 (0.94-4.77)	0.391	0.325
	P-*trend*	0.293			P-*trend*	0.128		
Processed meat intake, g/day	continuous, +25 g/day	1.62 (0.54-4.84)	0.385	0.394	continuous, +25 g/day	1.53 (0.51-4.61)	0.447	0.235
	Q1 (median=1.3)	1.00			Q1 (median=1.9)	1.00		
	Q2 (median=3.4)	1.85 (0.80-4.28)			Q2 (median=4.6)	0.66 (0.29-1.49)		
	Q3 (median=5.8)	1.20 (0.51-2.83)			Q3 (median=6.4)	0.96 (0.43-2.14)		
	Q4 (median=9.2)	1.35 (0.57-3.18)			Q4 (median=9.3)	0.96 (0.43-2.14)		
	Q5 (median=15.2)	1.49 (0.59-3.74)	0.666	0.559	Q5 (median=14.2)	0.97 (0.44-2.12)	0.848	0.319
	P-*trend*	0.722			P-*trend*	0.751		

* OR-main: Odds ratios adjusted for matching factors -age (40-49, 50-59, 60-69, >=70 years), gender, region (Quebec/Ontario) – and also smoking (pack-years, continuous), alcohol intake (quintiles), usual adult BMI (continuous), self-report of severe heartburn (ever/never), vegetable intake (quintiles) and total energy intake (continuous), the standard multivariate method.

† LR, p: Likelihood Ratio test, p-value.

‡ H-L, p: p-value from Hosmer-Lemeshow goodness-of-fit test.

§ OR-3: Odds ratios from energy-adjusted residuals with further adjustment as for OR-main with the exception of total energy intake.

Table 4.7 (continued). Odds ratios for the associations between main dietary exposures and esophageal adenocarcinoma with energy adjustment using the standard multivariate and residuals methods.

	Standard Multivariate Method				Residuals Method			
Primary Exposure	**Variable**	**OR-main (95% CI)***	**LR, p†**	**H-L, p‡**	**Variable**	**OR-3 (95% CI)§**	**LR, p**	**H-L,p**
Dietary Fe intake (mg/day)	continuous, +5 mg/day	1.01 (0.63-1.62)	0.975	0.143	continuous, +5 mg/day	0.93 (0.56-1.53)	0.762	0.261
	Q1 (median=8.0)	1.00			Q1 (median=10.8)	1.00		
	Q2 (median=11.0)	0.74 (0.31-1.76)			Q2 (median=12.2)	1.07 (0.50-2.30)		
	Q3 (median=13.4)	0.23 (0.48-3.16)			Q3 (median=13.5)	0.83 (0.38-1.78)		
	Q4 (median=16.0)	1.02 (0.37-2.79)			Q4 (median=14.8)	1.10 (0.50-2.43)		
	Q5 (median=20.1)	1.15 (0.33-3.95)	0.819	0.375	Q5 (median=17.6)	0.78 (0.34-1.79)	0.869	0.342
	P-*trend*	0.702			P-*trend*	0.546		
Heme Fe intake (mg/day)	continuous, +1 mg/day	1.34 (0.91-1.98)	0.137	0.121	continuous, +1 mg/day	1.33 (0.89-1.99)	0.161	0.133
	Q1 (median=0.70)	1.00			Q1 (median=0.78)	1.00		
	Q2 (median=1.00)	1.32 (0.59-2.99)			Q2 (median=1.18)	1.76 (0.78-3.97)		
	Q3 (median=1.29)	1.09 (0.48-2.47)			Q3 (median=1.41)	1.92 (0.86-4.29)		
	Q4 (median=1.71)	1.12 (0.48-2.66)			Q4 (median=1.69)	1.35 (0.59-3.06)		
	Q5 (median=2.51)	2.23 (0.89-5.58)	0.341	0.283	Q5 (median=2.33)	2.37 (1.05-5.36)	0.263	0.059
	P-*trend*	0.092			P-*trend*	0.085		

* OR-main: Odds ratios adjusted for matching factors -age (40-49, 50-59, 60-69, >=70 years), gender, region (Quebec/Ontario), smoking (pack-years, continuous), alcohol intake (quintiles), BMI (continuous), vegetable intake (quintiles) and total energy intake (continuous - standard multivariate method).

† LR, p: Likelihood Ratio Test, p-value.

‡ H-L, p: p-value from Hosmer-Lemeshow goodness-of-fit test.

§ OR-3: Odds ratios from energy-adjusted residuals with further adjustment as for OR-main with the exception of total energy intake.

Table 4.8 Odds ratios for esophageal adenocarcinoma comparing genotype groups.

Polymorphism	Category	Crude OR (95% CI)	Adjusted OR (95% CI)*
HFE-C282Y	Y282/Y282	-	-
	C282/Y282	0.79 (0.41-1.51)	0.74 (0.38-1.44)
	C282/C282	1.00	1.00
HFE-H63D	D63/D63	-	-
	H63/D63	0.97 (0.59-1.59)	1.00 (0.60-1.67)
	H63/H63	1.00	1.00
TFRC-S142G	G142/G142	0.90 (0.46-1.76)	0.95 (0.48-1.88)
	S142/G142	1.22 (0.75-1.98)	1.31 (0.80-2.16)
	S142/S142	1.00	1.00
HFE-any†	Yes	0.92 (0.59-1.44)	0.94 (0.59-1.49)
	No	1.00	1.00
HFE-hi iron‡	Yes	0.82 (0.43-1.55)	0.77 (0.40-1.48)
	No	1.00	1.00
HFE/TFRC combined genotypes (as per Beckman et al., 1999)	*TFRC*-S142/S142: *HFE*-Y282 carrier	0.48 (0.17-1.32)	0.41 (0.15-1.15)
	Other	1.00	1.00
	TFRC-S142/S142: *HFE*-D63/Y282	0.44 (0.05-4.01)	0.35 (0.04-3.17)
	Other	1.00	1.00

* Adjusted for age (40-49, 50-59, 60-69, ≥70 years), gender, and geographical region (Quebec/Ontario). Further adjustment for broad ethnic group (white/non-white) did not affect the results.
† Carriers of either or both of the Y282 and D63 alleles.
‡ Yes=Y282 homozygotes, D63 homozygotes, Y282 heterozygotes. No = other.

4.4.3 Main Effects Sensitivity Analyses

Excluding those individuals outside the population base with respect to geographic location or age did not appreciably change the results for any of the four primary dietary outcomes. Restricting the sample to only white males or excluding individuals with any previous diagnosis of cancer other than basal cell skin cancer also did not change the results such that their interpretation would differ (Appendix Biii; Table 1). Applying the same exclusions did not appreciably influence the genetic variant-EAC association results (Appendix Biii; Table 2).

4.4.4 Exploratory Interaction and Joint-Effects Analyses

As shown in Table 4.9, there was no clear evidence of statistical interaction between heme iron or dietary iron intake and alcohol intake or self-report of severe heartburn with the categorizations applied. The adjusted odds ratio for higher versus lower heme in the no severe heartburn group was 1.36 (95% CI 0.68-2.74) very similar to that in the severe heartburn group: 1.27 (95% CI 0.55-2.94). The odds ratio for heme iron intake (upper two quintiles versus lower three) in those aged less than 60 years (OR=1.64 95% CI 0.72-3.72) was not statistically different from the odds ratio in those over age 60 (OR=1.08 95% CI 0.53-2.22). Total dietary iron intake also showed no indication of effect modification by age (age<60 years: OR=1.26 95% CI 0.53-2.97, age≥60 years: OR=0.96 95% CI 0.44-2.08).

Table 4.9 Environment/iron intake interaction analyses

Category* Exposure 1 (E1)	Exposure 2 (E2)	Cases (n)	Controls (n)	OR-joint E1,E2 (95% CI)	RERI† (95% CI)	Multiplicative interaction, p‡	OR-E1 (95% CI)§
Lower heme Fe	Lower alcohol	43	108	1.00			1.00
Higher heme Fe	Lower alcohol	31	47	1.33 (0.66-2.67)			1.33 (0.66-2.67)
Lower heme Fe	Higher alcohol	21	57	0.76 (0.36-1.62)			1.00
Higher heme Fe	Higher alcohol	32	42	1.05 (0.45-2.43)	-0.04 (-1.10-1.01)	0.948	1.37 (0.61-3.11)
Lower dietary Fe	Lower alcohol	46	96	1.00			1.00
Higher dietary Fe	Lower alcohol	28	59	0.80 (0.38-1.71)			0.80 (0.38-1.71)
Lower dietary Fe	Higher alcohol	27	60	0.65 (0.31-1.36)			1.00
Higher dietary Fe	Higher alcohol	26	39	0.73 (0.28-1.95)	0.28 (-0.34-0.89)	0.515	1.13 (0.48-2.64)
Lower heme Fe	No severe heartburn**	34	120	1.00			1.00
Higher heme Fe	No severe heartburn	27	63	1.36 (0.68-2.74)			1.36 (0.68-2.74)
Lower heme Fe	Severe heartburn	30	45	3.48 (1.77-6.85)			1.00
Higher heme Fe	Severe heartburn	36	26	4.42 (2.03-9.65)	0.58 (-2.77-3.93)	0.892	1.27 (0.55-2.94)
Lower dietary Fe	No severe heartburn	40	109	1.00			1.00
Higher dietary Fe	No severe heartburn	21	74	0.76 (0.35-1.68)			0.76 (0.35-1.68)
Lower dietary Fe	Severe heartburn	33	47	2.53 (1.32-4.86)			1.00
Higher dietary Fe	Severe heartburn	33	24	3.93 (1.72-2.63)	1.63 (-1.30-4.56)	0.17	1.55 (0.65-3.67)

* Unless otherwise indicated, "lower" refers to the lower three quintiles of intake and "higher" the upper two quintiles.

† RERI, relative excess risk due to interaction.

‡ P-value for significance of independent contribution of multiplicative interaction term to the fully adjusted model.

§ OR-E1: Full adjustment model (energy adjustment by standard multivariate method): Odds ratios adjusted for age, gender, region, smoking (pack years, continuous), alcohol intake (quintiles) (*if applicable*) , BMI (continuous), total energy intake (continuous), severe heartburn (ever - Yes/No) (*if applicable*), and vegetable intake (quintiles).

** Severe heartburn – self report in answer to the question: Before (2 years prior to interview), did you ever have severe heartburn, that is heartburn so painful that it awoke you or prevented you from sleeping?

Table 4.10 Genetic variant/iron and genetic variant/alcohol intake interaction analyses

Category*/ Exposure 1 (E1)	Exposure 2 (E2)	Cases (n)	Controls (n)	OR-joint (95% CI)	RERI† (95% CI)	Multiplicative interaction, p‡	OR-E1 (95% CI)§
lower heme Fe	C282/C282	45	121	1.00			1.00
higher heme Fe	C282/C282	45	54	1.47 (0.74-2.92)			1.47 (0.74-2.92)
lower heme Fe	Y282 carrier	4	20	0.41 (0.12-1.42)			1.00
higher heme Fe	Y282 carrier	9	11	1.35 (0.42-4.34)	0.47 (-1.19-2.13)	0.345	3.33 (0.67-16.50)
lower dietary Fe	C282/C282	55	111	1.00			1.00
higher dietary Fe	C282/C282	35	64	0.85 (0.38-1.88)			0.85 (0.38-1.88)
lower dietary Fe	Y282 carrier	6	17	0.62 (0.20-1.91)			1.00
higher dietary Fe	Y282 carrier	7	14	0.55 (0.17-1.86)	0.09 (-0.99-1.17)	0.952	0.89 (0.19-4.19)
lower alcohol	C282/C282	55	110	1.00			1.00
higher alcohol	C282/C282	35	65	0.76 (0.38-1.50)			0.76 (0.38-1.49)
lower alcohol	Y282 carrier	5	15	0.59 (0.17-1.99)			1.00
higher alcohol	Y282 carrier	8	16	0.50 (0.16-1.58)	0.16 (-0.81-1.12)	0.887	0.85 (0.17-4.18)
lower heme Fe	H63/H63	32	105	1.00			1.00
higher heme Fe	H63/H63	44	46	2.18 (1.06-4.48)			2.18 (1.06-4.48)
lower heme Fe	D63 carrier	17	36	1.86 (0.83-4.15)			1.00
higher heme Fe	D63 carrier	10	19	1.31 (0.46-3.74)	-1.73 (-4.03-0.57)	0.080	0.70 (0.22-2.22)
lower dietary Fe	H63/H63	43	92	1.00			1.00
higher dietary Fe	H63/H63	33	59	0.84 (0.38-1.87)			0.84 (0.38-1.87)
lower dietary Fe	D63 carrier	18	36	1.22 (0.54-2.73)			1.00
higher dietary Fe	D63 carrier	9	19	0.96 (0.32-2.96)	-0.09 (-1.46-1.27)	0.929	0.79 (0.23-2.74)
lower alcohol	H63/H63	44	94	1.00			1.00
higher alcohol	H63/H63	32	57	0.75 (0.35-1.56)			0.75 (0.36-1.56)
lower alcohol	D63 carrier	16	31	1.23 (0.53-2.85)			1.00
higher alcohol	D63 carrier	11	24	0.83 (0.33-2.08)	-0.15 (-1.39-1.10)	0.867	0.67 (0.22-2.10)

* Unless otherwise indicated, "lower" refers to the lower three quintiles of intake and "higher" the upper two quintiles.
† RERI, relative excess risk due to interaction.
‡ P-value for significance of independent contribution of multiplicative interaction term to the adjusted model.
§ OR-E1: Full-adjustment model: Odds ratios adjusted for age, gender, region, smoking (pack years, continuous), alcohol intake (quintiles) (if applicable), BMI (continuous), total energy intake (continuous), severe heartburn (ever - Yes/No), and vegetable intake (quintiles).

Results of the planned *HFE*-variant/iron intake and *HFE*-variant/alcohol intake interaction analyses are shown in Table 4.10. These exploratory analyses do not provide any indication of effect modification of iron or alcohol intake/EAC association measures by *HFE*-C282Y although interpretation of these results is severely hindered due to the small number of Y282 carriers in the sample. The odds ratio comparing higher with lower heme intakes in the H63-homozygous group (OR=2.18, 95% CI: 1.06-4.48) was higher than in the group of individuals carrying the D63 allele (OR=0.70, 95% CI: 0.22-2.22) suggesting a protective effect of the D63 allele among those consuming higher levels of heme iron. The RERI was negative (-1.73, 95% CI -4.03-0.57) consistent with antagonism on the additive scale although it was not statistically significant. The multiplicative interaction term also did not reach formal statistical significance (p=0.080).

4.5 Discussion

These analyses found no evidence of increased risk of esophageal adenocarcinoma associated with intake of dietary iron or total iron (dietary + supplements). There was some evidence of a positive association between heme iron intake and EAC with an odds ratio of 2.8 (95% CI 1.2-6.5) comparing the highest quintile of reported intake with the lowest quintile. The association did not remain statistically significant after adjustment for energy intake, however (OR=2.2; 95% CI 0.9-5.6). Red meat intake also showed some indication of a positive association but was not significant after adjustment for lifestyle factors, self-report of reflux symptoms and energy intake. There was no evidence supporting an association between EAC and *HFE* variants C282Y and H63D individually or grouped according to the likelihood of elevated iron stores. The small

number of Y282 carriers limited the usefulness of the analyses. There was also no suggestion of increased risk of EAC associated with possession of the G142-variant of *TFRC*.

4.5.1 EAC and Dietary Iron Intake

Several other studies have reported results for dietary iron intake and adenocarcinoma of the esophagus (Mayne et al., 2001; Chen et al., 2002; Zhang et al., 1997; Brown et al., 1995; Wolfgarten et al. 2001). Results consistently show an inverse association between reported dietary iron intake and EAC (Chapter 2, Table 2.11). In the present study, while the effect of dietary iron intake was not statistically significant, it did show an inverse association: comparing the 75th percentile of intake with the 25th percentile of intake (a difference of 6.25 mg Fe/day) gives an odds ratio of 0.79 (95% CI: 0.45-1.40) when adjusted for age, gender, province, smoking, usual adult BMI, severe heartburn, frequency of alcohol intake, total energy, and also educational attainment and race (white/non-white), the adjustment factors employed by Mayne et al. (2001). The odds ratio reported by Mayne et al. (2001) was 0.79 (95% CI 0.57-1.09). With vegetable intake also included in the model, the odds ratio increased to 1.01 (95% CI 0.56-1.82). Corley et al. (2008a) compared dietary iron intake between population controls (n=308) and cases of Barrett's esophagus (BE; n=317), a known pre-cursor of esophageal adenocarcinoma, in a study in Northern California. The authors qualitatively reported that BE cases had lower dietary iron intake than the controls also suggesting an inverse association with the condition.

Total dietary iron represents a composite index from several dietary components - some that have shown a positive association with disease (i.e. meat components) and others that have shown an inverse association (i.e. dietary fibre – Wu et al., 2007). In the EAC study the majority of iron in the EAC participants' diets (73±12%; mean±s.d.) came from non-meat sources. There was a high correlation between dietary iron and phytate intakes (r=0.80 among the controls) reflecting the cereal source for the major part of the dietary iron. Overall, the evidence, including that from this study, is not supportive of a positive association between total dietary iron intake and EAC suggesting that elevated risk of EAC is not associated with higher total levels of iron passing through the upper gastrointestinal tract.

Both Mayne et al. (2001) and Chen et al. (2002) found no association between iron consumed as supplements and EAC. Individuals in the present study who took iron-containing supplements were also not at an increased risk of EAC compared with those who did not (OR=0.98; 95% CI 0.54-1.80).

4.5.2 EAC and Intakes of Meat and Heme Iron

The epidemiological evidence for meat intake as a risk factor for EAC is limited at present (Brown et al., 1995; Ward et al., 1997; Mayne et al., 2001; Bahmanyar and Ye, 2006; Jaksyn and Gonzalez, 2006; WCRF/AICR, 2007). Whether increased consumption of a particular type of meat is associated with a higher risk is also not clear, at least in part due to the variable definitions of "red" and "processed" meats employed in different studies. Gonzalez et al. (2006) reported positive but not formally significant associations

between total meat and red meat intakes and EAC in the on-going, prospective EPIC study (European Prospective Investigation Into Cancer and Nutrition) based on 65 accrued cases and a mean follow-up of 6.5 years. The odds ratio for an additional 50 g of processed meat per day was 1.16 (95% CI 0.82-1.65) (Gonzalez et al., 2006). The definitions of 'red' and 'processed' meat in the EPIC study were different from those used in this study. Gonzalez et al. (2006) included hamburger(s) in their processed meat index. If we re-allocate hamburger from the red meat group to the processed meat group in our study, the OR for processed meat intake and EAC becomes significant (continuous, +25 g/day OR=2.55; 95% CI 1.29-5.03) and the odds ratio for red meat becomes very close to 1.00 (continuous, +25g/day OR=1.04; 95% CI 0.57-1.93).

The results from our study also differed from Gonzalez et al. (2006) with respect to the association between EAC and poultry intake. Gonzalez et al. (2006) reported a marginally positive association between EAC and poultry intake (chicken, turkey and duck). The RR was 1.93 (95% CI 0.99-3.76) comparing the highest with the lowest tertile and 1.12 (95% CI 1.06-1.20) for a difference in intake of 10g/day. The results of our study, however, indicated a marginally inverse association between intake of chicken and turkey ("white meat") and EAC. The white meat intake odds ratio, after adjustment for the other meat types, was 0.68 (95% CI 0.35-1.32) comparing the highest with the lowest tertiles of intake and 0.73 (95% CI 0.52-1.02) for an increase in intake of 10g/day.

The EPIC study was prospective, avoiding the potential for recall bias. Symptoms of EAC itself and the gastroesophageal reflux that often precedes it may influence dietary

intakes and reporting - another source of uncertainty in the retrospective assessment of diet in case-control studies of esophageal adenocarcinoma ("pathological bias" or "reverse causation"). The EPIC study investigators adjusted their association measures for work and leisure physical activity, citrus fruit intake, non-citrus fruit intake and education level in addition to the adjustment factors employed in this study, but did not adjust for symptoms of severe reflux. It is possible that the inverse association with white meat intake observed in our study may reflect residual confounding by consumption of vegetables and refraining from tobacco use. It may also be due to other lifestyle and dietary choices such as increased physical activity that may reduce disease risk. Both studies are limited by the small number of cases.

Heme is a pro-oxidant with evidence for involvement in the endogenous formation of carcinogenic N-nitroso compounds (NOCs) (Kuhnle et al., 2007; Lunn et al., 2007; Lewin et al., 2006; Bingham et al., 2002; Cross et al., 2003; Sesink et al., 2001; Chapter 2). Bingham et al. (2002) found a dose response between heme-rich red meat intake (beef and pork) and apparent total N-nitroso compounds in fecal samples but no dose response with white meat intake (chicken, turkey or white cod). Results from *in vitro* studies suggest that the heme molecule may also be important for promoting endogenous nitrosation in the upper digestive tract (Kuhnle et al., 2007; Lunn et al., 2007). The only study that has previously examined the association between heme iron intake and cancer of the upper digestive tract (Iowa Women's Health Study; Lee et al., 2005) reported a significant association when results were further adjusted for zinc (RR=2.83, 95% CI 0.83-9.54). When adjusted for only age, total energy intake, smoking and alcohol

consumption, the relative risk (RR) comparing the highest with the lowest quintile of heme iron intake was 1.07 (95% CI 0.41-2.82; p-trend=0.97). The analyses of Lee et al. were based on a small sample of 52 gastric cancer and 23 esophageal cancer cases combined. In our study of esophageal adenocarcinoma in a sample dominated by men, further adjustment for zinc intake did not affect the magnitude of the odds ratios for heme iron intake of esophageal adenocarcinoma (OR=2.23, 95% CI 0.89-5.58, without adjustment for zinc intake and 2.35, 95% CI 0.79-6.97, with adjustment). Zinc is an essential element found in a more bioavailable form in products of animal origin (Ho, 2004). It is involved in immune function and has evidence of antioxidant and antiproliferative properties and involvement in tumor development and progression (Murakami and Hirano, 2008; Ho, 2004). There is little consistent evidence from epidemiological studies of an inverse association between zinc intake and cancer, however (Navarro Silvera and Rohan, 2007). It is not immediately clear why adjustment for zinc intake would lead to a significant association between heme iron and cancers of the upper digestive tract among women as observed by Lee et al.

4.5.3 EAC and Genetic Variants

There were no significant associations between genotypes associated with higher iron levels and EAC in our study. Interpretation of the results is limited, however, by the small numbers of cases and controls with "*HFE*- hi iron" genotypes and the variable penetrance of the C282Y variant. One recent study (Corley et al., 2008a) looked at the association between *HFE*-genotypes and Barrett's esophagus among 317 BE cases, 306 gastroesophageal reflux disease (GERD) patients and 308 population controls. The odds

ratio of Barrett's esophagus comparing individuals carrying any *HFE* mutation with those carrying none was 1.32 (95% CI 0.95-1.84) when adjusted for gender, age, ethnicity and smoking status. The inclusion of the adjustment factors did not change the magnitude of the odds ratio. Corley et al. (2008a) also classified *HFE*-mutations as "severe" (C282Y/C282Y, C282Y/H63D), moderate (H63D/H63D, C282Y/wt) or mild (H63D/wt) based on their associations with elevated iron indices. The odds ratio of BE comparing those with a moderate or severe mutation with those with none was 1.54 (95% CI 0.94-2.52). Among men, this odds ratio was 1.87 (95% CI 1.03-3.37) while among women it was 0.93 (95% CI 0.37-2.35) raising the possibility of gender differences in risk. Using the numbers in the Corley et al. paper, the crude odds ratio of EAC for "*HFE* hi-iron" genotypes as defined in the present study was 1.52 (95% CI 0.95-2.44). As stated by the authors, the odds ratios in their study approached statistical significance due to a slightly higher frequency of the D63-allele among cases. 3.5% of cases in the Corley et al. study were D63-homozygotes. The D63-allele, however, does not show a strong association with elevated iron levels. In the present study, D63-homozygote frequencies in the cases (0.4%) and controls (0.8%) were lower than typically observed in large, unselected studies of white individuals of European origin (French-Canadians - 2.4% (Girouard et al., 2002), Scots - 2.0% (Campbell et al., 2003) non-Hispanic whites in the United States - 2.15 to 2.4% (Adams et al., 2005; Steinberg et al., 2001)). Overall, neither this study nor that reported by Corley et al. (2008a) has found evidence supporting a role for iron levels in the pathogenesis of EAC through examination of the associations between common *HFE* mutations and the intermediate BE and terminal EAC end-points.

Beckman et al. (1999) reported increased risks associated with *TFRC*-S142 homozygote/*HFE*-Y282 combined genotypes for breast and colon cancer. In contrast, the odds ratios for the same comparison in this study were less than 1.00 (Table 4.8) but, similar to Beckman et al. (1999), were based on small numbers in the "exposed" group. There is a biological basis for an interaction between *HFE* and *TFRC* as their gene products form a complex important for the uptake of transferrin bound iron and potentially for the sensing of iron levels and regulation of hepcidin production (Ganz, 2008; Schmidt et al., 2008; Nemeth, 2008; Chua et al., 2008). Based on changes in structural and physicochemical properties resulting from the amino acid substitution, PolyPhen (Ramensky et al., 2002) predicts that the *TFRC*-S142G polymorphism is likely not functional, however.

4.5.4 EAC and Symptoms of Reflux/GERD

The presence of severe reflux symptoms or GERD is a condition often preceding EAC (Holmes and Vaughan, 2006; Layke and Lopez, 2006; Farrow et al., 2000). The prevalence of GERD is 10-20% in North America (Dent et al., 2005) with some evidence for increasing rates over the last two decades (El-Serag, 2007). Eighteen percent of controls in the EAC study reported experiencing severe heartburn at least once per week. The odds ratio of EAC comparing those who reported ever experiencing severe heartburn with those who did not was 3.37 (95% CI 2.02-5.59, with full adjustment).

4.5.5 EAC and BMI

There is consistent evidence from epidemiological studies for a positive association between BMI and EAC (Abnet et al., 2008; Whiteman et al., 2008; Merry et al., 2007; Kubo and Corley, 2006; MacInnis et al., 2006; Lagergren et al., 1999; Brown et al., 1995). Several mechanisms have been postulated to explain the relationship, including an increase in intragastric pressure promoting reflux of stomach contents into the lower esophagus (Pandolfino, 2008) and differing levels of endogenous hormones associated with body fat composition, insulin and glucose metabolism (Pischon et al., 2008; Renehan et al., 2008a,b; Calle et al., 2003). In the EAC study, the odds ratio for an increase in BMI of 5 kg/m^2 was 1.59 (95% CI 1.18-2.14). The trend was significant across quintiles (p=0.018) and there was no statistical evidence of a deviation from linearity in the risk-BMI relationship (p=0.786 using quintile medians). When categorized according to WHO definitions of underweight or healthy (<25 kg/m^2), overweight (25 to 30 kg/m^2) and obese (≥30 kg/m^2) (WHO, 2004), the adjusted odds ratios were 1.40 (95% CI 0.70-2.79) and 2.52 (95% CI 1.21-5.26) for the overweight and obese categories, respectively. In the EAC study, BMI was calculated from self-reported height and weight and therefore subject to some uncertainty. Evidence suggests that those who are overweight tend to under-report their weight (Shields et al., 2008). Given that cases have significantly higher self-reported BMI than controls, it is possible that there is a differential misclassification with respect to actual BMI between the case and control groups that would bias the odds ratio for BMI towards the null.

4.5.6 EAC and Helicobacter pylori infection

A recent meta-analysis (Rokkas et al., 2007) reported a significant inverse association between esophageal adenocarcinoma and *H. pylori* infection based on 10 included studies. A protective effect is thought to result perhaps from gastric atrophy and reduced acid secretion in the presence of *H. pylori* infection (Anderson et al., 2008; McColl et al., 2008; Corley et al., 2008b). Participants in the EAC study were asked if they had ever been diagnosed with *H. pylori.* Laboratory tests were not carried out to determine *H. pylori* status. Only 3 cases (2.4%) and 11 controls (4.4%; Chi-square test, p=0.346) reported a diagnosis of *H. pylori.* A previous diagnosis of stomach ulcers was more common in cases but did not achieve statistical significant: 20 (16.1%) and 29 (11.4%), respectively; Chi-square test, p=0.200).

4.5.7 EAC and NSAIDs

There is consistent evidence for an inverse association between use of aspirin or NSAIDs and esophageal adenocarcinoma. The use of anti-inflammatory drugs as a chemopreventive measure for EAC is an area of active research (Duan et al., 2008; Vaughan et al., 2005; Hur et al., 2004; Gammon et al, 2004; Corley et al., 2003; Farrow et al., 1998). Interference with prostaglandin synthesis represents a plausible biological mechanism for a protective effect (Thun et al., 2002). However, the observational nature of the epidemiological studies cannot preclude the possibility of reverse causation or confounding by indication (Lagergren, 2006; Mehta et al., 2005; Lindblad et al., 2005). Individuals who experience severe reflux symptoms or have other upper gastrointestinal tract disorders are less likely to take or be prescribed aspirin or NSAIDs (non-steroidal

anti-inflammatories) due to the known gastrointestinal side-effects. Regular users of aspirin may also differ from others with respect to other health behaviours or conditions.

In contrast to the literature results, in this study there was no significant difference in proportions of cases and controls who reported ever regularly taking "Motrin, Advil or similar NSAIDs". Aspirin use was also not related to EAC risk, as measured in a sub-set of the sample: 63% of cases (44 of 70) and 62% of controls (94 of 152) had never regularly taken aspirin. Individuals who reported taking either aspirin or NSAIDs regularly were not at a significantly lower risk of having EAC in this study (OR=0.79; 95% CI 0.45-1.39). The lack of significance may be a type 2 error due to the small sample size. Inclusion of this variable (Ever/Never) in the multivariate model did not affect the odds ratios for the main dietary exposures or genetic variants.

4.5.7 Analysis Issues: Energy Adjustment

Energy adjustment had a major impact on the results, eliminating the statistically significant trend for heme iron intake and reducing the OR for a 1 mg/day higher heme iron intake from 1.50 to 1.33. Adjustment for energy intake is controversial, with strong arguments having been made for and against adjustment and in favour of different methods of adjustment (Willett et al., 1997; Willett and Stampfer, 1996; Freedman et al., 1997). Willett is one of the strongest proponents for energy adjustment. Perhaps the strongest reason for energy adjustment is to control for confounding, particularly important for nutrients such as fat that are strongly correlated with total energy intake. The case is less strong for nutrients that are found in only a subset of food items

(Freedman et al., 1997). Willett et al. (1997) argue that risk as a function of dietary composition is of more use than risk as a function of absolute intake as dietary interventions and advice needs to be transferrable to individuals with a range of body sizes, physical activities and metabolic efficiencies. It is well-known that nutrient and energy intakes derived from food frequency questionnaires (FFQs) are subject to considerable measurement error (Subar et al., 2001; Kipnis et al., 2003) and that those who underreport tend to do so for all food groups. Under these conditions, energy-adjustment may reduce measurement error and give more accurate nutrient values (Subar et al., 2001). However, under-reporting does not necessarily occur to the same extent for each food group or type (Subar et al., 2001). For conditions of the gastrointestinal tract where the amount of an active compound coming in contact with the surface of the gut itself is of more interest than the dietary composition, the argument can be made that energy adjustment is not necessary unless total energy intake is associated with the disease itself. In this study, there was evidence of an association between total energy intake and EAC and also, not surprisingly, between total energy intake and red meat, processed meat, total dietary iron and heme iron intakes in the controls, supporting its inclusion in the final dietary exposure models for the purposes of confounder control.

Kushi et al. (1992) and Brown et al. (1994) recommend caution when interpreting the results from energy adjusted analyses when exposure variables are categorized. The standard multivariate and residuals energy adjustment methods are no longer comparable when exposures are quantized. As seen in Table 4.8, the overall impression of the

strength and of the relationship between red meat or heme iron intake and EAC might be interpreted differently depending on the method of energy adjustment used.

4.5.8 Analysis Issues: Adjustment of Genetic Variant-Disease Association Analyses

There is little evidence in the literature for confounders of the relationship between the *HFE* and *TFRC* genetic variants studied and EAC. Associations between genetic variants and the exposures and covariates were examined in the controls to determine whether any adjustment for confounding was necessary or whether variants might be associated with severe reflux symptoms, an intermediate state on the pathway to disease. The association between *TFRC*-S142G G-allele carrier status and self-report of symptomatic severe heartburn in the controls was unexpected and may well have occurred by chance. The odds ratio of severe heartburn associated with carrying the *TFRC*-G142 allele was 2.75 (95% CI 1.32-5.74) among the controls, adjusted for age, gender and province. Among those who reported severe heartburn, the odds ratio of EAC associated with possession of the *TFRC*-G142-allele was 0.48 (95% CI 0.12-1.21) while among those who did not, the odds ratio was 2.42 (95% CI 1.13-5.21). There is evidence for a genetic component to GERD from twin studies (Mohammed et al,. 2003; Cameron et al., 2002; Zheng et al., 2007) and familial clustering of severe reflux symptoms and Barrett's esophagus (Sappati et al., 2007; Chak et al., 2006; Trudgill et al. 1999; Eng et al., 1993). *TFRC* expression appears to be regulated in response to inflammatory stimuli at both the transcription (Tacchini et al., 2008) and translation (Andriopoulos et al., 2007) levels and recent laboratory investigations have found the overexpression of *TFRC* and other iron transport proteins associated with increased intracellular iron deposition and progression of

Barrett's esophagus to adenocarcinoma (Boult et al., 2008). Since the serine to glycine amino acid substitution of *TFRC*-S142G is not predicted to impact the structure or functionality of the transferrin receptor gene product appreciably (PolyPhen, Ramensky et al, 2002), any association between severe heartburn or EAC and the G142 allele is likely due to its linkage disequilibrium with a variant of functional significance. Replication of this result in a larger study would be required.

4.5.9 Challenges of Exposure Assessment

This study has several limitations related to exposure assessment. Measurement error and potential for bias are known problems with dietary micronutrient intakes assessed using self-report food frequency questionnaires, 24-hour records and food diaries (Natarajan et al, 2001; Kristal et al., 2005; Kipnis et al., 2003). The Block FFQ/DIETSYS-derived estimates of dietary iron intake have not been independently validated in the Canadian population. It may also be questioned whether the U.S. food composition database included with DIETSYS v4.0 accurately reflects the micronutrient composition of foods in Canada, particularly those such as iron that are affected by fortification practices. Csizmadi et al. (2007) reported DHQ (Dietary History Questionnaire; NCI, 2007) estimates of dietary iron intake 5 to 6% higher with the use of the U.S. food composition database in place of nutrient values from the CNF 2001b (Canadian Nutrient File, 2001b). Ready-to-eat cereals and commercially prepared baked goods were the main food items that required modification (Csizmadi et al., 2007). Dietary intakes of iron in the present study (Table 4.3) were slightly lower but in reasonable agreement with 1990 intakes reported for men aged 51 to 70 in Nova Scotia and Quebec (FNB/IoM, 2000).

The median intake in that survey was 14.5 mg/day and the 99th percentile was 29.5 mg/day. Csizmadi et al. (2007) reported a median intake of 13.8 mg Fe/day among 2373 men aged 51 to 69 years participating in the Alberta Cohort Study baseline assessment (2001-2003) close to the 13.3 mg Fe/day (3.0-26.7) observed in this study. As reviewed by Cooper et al. (2006), recent data on the iron intakes of the Canadian population is lacking but numbers based on 24-hour dietary recall will become available with the synthesis and publication of the Canadian Community Health Survey (CCHS) Cycle 2.2 (2004). Based on the results from the present study, 10% of subjects consume less than the DRI (Dietary Reference Intake; 8mg Fe/day for men. FNB/IoM, 2000) and 10% consume more than 20 mg/day. Due to the lack of validation of the questionnaire in the target population, however, these proportions should be interpreted only broadly.

There are several sources of uncertainty in the estimation of the amount of heme iron consumed. Meat-component iron values in the DIETSYS database were compared with those in the Canadian Nutrient File (2007b; CNF, 2007) to determine whether the use of the U.S. nutrient values might influence the heme-iron association results. However, in only one instance was there arguably any justification for using a different iron concentration. The iron content for hamburger in the DIETSYS food composition database was 2.11 mg/100g. The range of values in the Canadian Nutrient File (2007b) for cooked hamburger was 2.09-2.99 with an average of 2.57 mg/100g. For all other meat components for which items were available in the EAC study, the value in the DIETSYS database lay within the mid-range of similar items in the Canadian Nutrient File (2007b). Employing the CNF 2007b derived value for hamburger in the analyses did

not change the odds ratio for heme iron intake (OR(+1mg/day)=1.34, 95% CI 0.92-1.95). The proportion of iron in the heme form varies with the type of animal, location of meat on the animal, feeding and slaughter practices and the cooking temperature and duration (Lombardi-Boccia et al., 2004; Lopez and Martos, 2004; Purchas et al., 2004; Lombardi-Boccia et al., 2002). Balder et al. (2006) conducted a literature search to determine the best estimates of the heme iron proportions in different cooked meat products. They reported an average of 0.65 for beef, 0.39 for pork, and 0.26 for chicken and fish based on results from five published studies. Kongkachuichau et al. (2002) gives a conversion factor of 0.21 for liver. When these meat-type specific conversion factors were applied to the meat items in the Block FFQ, the odds ratio for an increase of 1 mg/heme iron per day was 1.27 (95% CI 0.95-1.69) similar to the 1.34 (95% CI 0.91-1.98) obtained using a the 0.4 bulk conversion factor applied. To increase the certainty in the estimation of dietary heme-iron intake in the Canadian population would require validation of a heme-iron database and cooking method questionnaire such as that under development by Sinha and others at the NCI (Sinha et al., 2005). At this point, the use of a 0.4 bulk conversion factor provided in Monsen and Balintfy (1982) is the most justified method.

The disagreements between one genotyping duplicate for each C282Y and S142G are of concern, particularly for C282Y variant due to the rarity of the Y282-allele. However, the minor allele and genotype frequencies were within ranges expected for white individuals of European descent. All duplicates were in agreement for H63D.

4.5.10 Confounders

Given the likelihood of an influence of diet on BMI, BMI was considered a possible confounder of the diet-disease relationships examined. Inclusion of BMI in the model reduced the heme Fe and red meat odds ratios by 11%. There was evidence of associations between heme iron intake, red meat intake and processed meat intakes and usual adult BMI in the controls. None of the genetic variants showed a significant association with usual adult BMI in the controls, however.

Inclusion of vegetable intake in the model led to a 29% increase in the odds ratio between dietary iron and EAC. There was also evidence of association between processed meat and vegetable intake and between heme iron intake and vegetable intake in the controls. Removing vegetable intake from the model altered the odds ratios for these exposures by less than 10%, however.

4.5.11 Other Biases

In common with other case-control studies, retrospective self-report of dietary and lifestyle variables may be subject to recall bias, a threat to the internal validity of the association results. Moreover, EAC is a condition of the gastrointestinal tract therefore the potential for recall bias of self-reported dietary intakes is particularly high. Cases may be much more aware of what they consume contributing to more complete reporting, or sometimes over-reporting, of their diet than controls particularly for food items that are perceived to be associated with risk. However, a higher proportion of cases had missing questionnaires than controls. It is possible that disease-status may have influenced the

ability to complete the questionnaire with implications for completeness and quality of the responses.

For EAC the situation is further complicated. Severe reflux symptoms may lead to actual changes in diet that are independent of progression to EAC. The appropriate time frame for exposure assessment is difficult to define. In this study, participants were asked to recall their dietary intakes 2 years prior to either their diagnosis (cases) or their interview (controls) to avoid dietary changes that may have resulted as a consequence of symptoms of EAC onset. However, the relationship between dietary and lifestyle exposures and EAC obtained in this case-control setting may reflect a combination of an association with reflux symptoms independent of disease and an association with disease in the presence of reflux. Associations between the main exposures, genetic and dietary, and self-report of severe heartburn were examined in the controls (Table 4.11). There were no significant differences with respect to red meat, processed meat, total dietary iron or heme iron intakes between the heartburn groups. Surprisingly, possession of the *TFRC*-G142 allele was associated with self-report of more frequent severe heartburn among the controls. Neither of the *HFE*-C282Y nor *HFE*-H63D minor alleles showed an association. There were also no significant differences with respect to dietary fibre, white meat or vegetable intakes between heartburn groups. Although severe heartburn may lead to changes in dietary habits, this was not evident among the controls for the dietary variables assessed.

Table 4.11 Associations between the main exposures, the genetic variants, selected covariates and self-report of severe heartburn in the control group.

Severe heartburn frequency/ Exposure or covariate		Never, n(row%)	<1/week, n(row%)	≥1/week, n(row%)	p*
Red meat intake					
	Q1 to Q3	116 (69.5)	22 (13.2)	29 (17.4)	
	Q4+Q5	67 (77.0)	11 (12.6)	9 (10.3)	0.309
Processed meat intake					
	Q1 to Q3	122 (74.9)	21 (12.9)	20 (12.3)	
	Q4+Q5	61 (67.0)	12 (13.2)	18 (19.8)	0.259
Dietary Fe intake					
	Q1 to Q3	109 (69.9)	20 (12.8)	17 (17.3)	
	Q4+Q5	74 (75.5)	13 (13.3)	11 (11.2)	0.414
Heme Fe intake					
	Q1 to Q3	120 (72.7)	21 (12.7)	24 (14.6)	
	Q4+Q5	63 (70.8)	12 (13.5)	14 (15.7)	0.947
HFE-C282Y					
	No Y282	126 (72.0)	23 (13.1)	26 (14.9)	
	Y282 carrier	22 (71.0)	2 (6.5)	7 (22.6)	0.419†
HFE-H63D					
	No D63	108 (71.5)	16 (10.6)	27 (17.9)	
	D63 carrier	40 (72.7)	9 (16.4)	6 (10.9)	0.313†
TFRC-S142G					
	No G142	58 (84.1)	6 (8.7)	5 (7.3)	
	G142 carrier	90 (65.7)	19 (13.9)	28 (20.4)	0.017†
White meat intake					
	Q1 to Q3	111 (73.5)	18 (11.9)	22 (14.6)	
	Q4+Q5	72 (69.9)	15 (14.6)	16 (15.5)	0.788
Vegetable intake					
	Q1+Q2	122 (75.3)	18 (11.1)	22 (13.6)	
	Q3 to Q5	61 (66.3)	15 (16.3)	16 (17.4)	0.294
Fibre intake					
	Q1+Q2	119 (73.9)	20 (12.4)	22 (13.7)	
	Q3 to Q5	64 (68.8)	13 (14.0)	16 (17.2)	0.665

* P-value from Chi-square test for independence, unless otherwise indicated.
† P-value from Fisher's exact test.

Several acid suppressive medications can be prescribed to ameliorate reflux and its associated symptoms, for example, Proton Pump Inhibitors (Losec or Pantaloc) and H2-antagonists (Zantac, Tagamet, Pepcid). A greater proportion of cases had been prescribed one of these medications consistent with the greater severity of reflux symptoms reported. Recent studies have shown a three-fold increased in risk of esophageal adenocarcinoma associated with prolonged use (>3 years) of an acid suppressive drug (Garcia-Rodriguez et al., 2006; Langman and Logan, 2007). However, the use of the drug shows a relationship with treatment indications that are known risk factors for EAC which can lead to a bias called confounding by indication (Weiss, 2008). With the retrospective observational nature of the current study, it is not possible to separate an association between the drug and disease and confounding by indication.

The potential for bias from population stratification requires consideration in gene-disease association studies (Thomas and Witte, 2002; Wacholder et al., 2002). More than 96% of participants in the study reported "white" ethnic backgrounds and European ancestry. Although the C282Y variant shows a strong latitudinal gradient among "white" European individuals, with highest Y282-allele frequencies among those of north-western European, specifically Celtic, descent (Lucotte et al., 2003), it is unlikely that population stratification is of major concern in the interpretation of the results in this study. Genetic variants were not associated with dietary and lifestyle factors in the controls. Restricting the analysis sample to white males did not appreciably influence the results.

4.5.12 Other Limitations

The small study size overall and the low numbers of exposed in some analyses restricts the interpretation of some of the main effects results and the interaction analyses. The interaction analyses were hypothesis-generating and not hypothesis testing. It was decided *a priori* to categorize exposures as "lower" for the lower three quintiles and "higher" for the upper two quintiles for these analyses. With the small numbers, results can be sensitive to the choice of category cut-offs. There was some suggestion that the association between heme iron intake and EAC was lower among carriers of the *HFE* D63-allele. This result would require confirmation in larger studies.

Although designed as a population-based case-control study, lower recruitment rates at some sites led to an uneven distribution of cases and controls by geographic region and the addition of another site, Montreal, from outside the original sampling frame. There is an excess of controls relative to cases from the Montreal site. This brings into question whether the cases and controls were recruited from the same underlying population. This was adjusted for in the multivariate analyses by including a variable for region and also smoking, a factor known to differ between Ontario and Quebec (Health Canada, 2003) and which has some evidence for an association with EAC. About one-third of the control group had an undergraduate or graduate university degree which is higher than expected based on the Ontario average in 2001 (25.3%; Statistics Canada, 2001). This suggests a possible self-selection effect operating on the controls. Although lifestyle factors were included as adjustment factors in the analyses, residual confounding cannot be ruled out. Thirty-four cases who lived in rural areas of counties overlapping the

geographically-defined regions were included in the main effects analyses. A sensitivity analysis conducted excluding these individuals showed that their inclusion did not lead to appreciably different results for the main effects.

Heme has two properties that may lead it to increase cancer risk in the gastrointestinal tract – it is a pro-oxidant and a nitrosating agent. However, several other components of meat may be carcinogenic, notably heterocyclic amines (HCAs; Ito et al., 1991) and polycyclic aromatic hydrocarbons (IARC, 1998). Separating the effects of different meat-associated carcinogens would require a level of accuracy of exposure assessment beyond food frequency questionnaires.

4.5.13 Generalisability

The cases considered "capable" to participate in the study by their attending physician were the healthiest of the patients diagnosed with EAC (D. Maziak, *personal communication*) and may have differed with respect to factors associated with disease severity from those not considered capable. Those considered capable but who declined may also have differed from those who consented. Interestingly, a significantly higher proportion of cases than controls were not married or living as married.

4.5.14 Strengths

The EAC study was of population-based design. Although all identified physicians did not refer patients, there is no reason to believe that any eligible cases that would have

been referred by the missing physicians would have differed from the patients who were referred. All data was collected directly from patients with no need to use proxy respondents, unlike in earlier studies. Comprehensive data on lifestyle, medical and dietary risk factors was collected in face-to-face interviews.

4.6 Conclusions

Esophageal adenocarcinoma, a disease with very poor outcome, has shown a marked increase in the past two decades mainly in white men. Animal studies have provided evidence supporting a role of iron in the pathogenesis of this disease. This study in a sample of predominantly white men is the first to look at associations between genetic variants in the iron metabolism genes *HFE* and *TFRC* and esophageal adenocarcinoma. There were no significant associations between the genetic variants assessed and EAC. Interpretation of the results for the variant most strongly associated with iron stores (C282Y) and EAC were limited by the rarity of the minor variant. This study is also the first to investigate the association between heme iron intake and esophageal adenocarcinoma. Results provided some support for an association between heme iron intake and EAC.

Chapter 5: Concluding Discussion & Future Directions

The goal of this thesis was to examine the hypotheses that elevated body iron levels and increased iron intake are associated with the risk of developing cancer of the gastrointestinal tract with a focus on esophageal adenocarcinoma. Due to its pro-oxidant activity, its involvement in the inflammatory response and its potential for enhancing endogenous nitrosation, there is a strong biological rationale for investigating iron stores and intakes as modifiable risk factors for these cancers.

5.1 Iron Stores and Cancer of the Gastrointestinal Tract

Most research to date has focused on cancers of the lower gastrointestinal tract. Results from observational studies are inconsistent but, overall, have not provided evidence supporting a positive association between iron stores and cancers of the colon and rectum. One intervention study has provided evidence for an improvement in cancer outcomes associated with a reduction in serum ferritin by phlebotomy (Zacharski et al., 2008). The results of this intervention study are of limited generalisability, require replication, gastrointestinal tract cancer cases were few and, due to incomplete blinding, a detection bias cannot be ruled out.

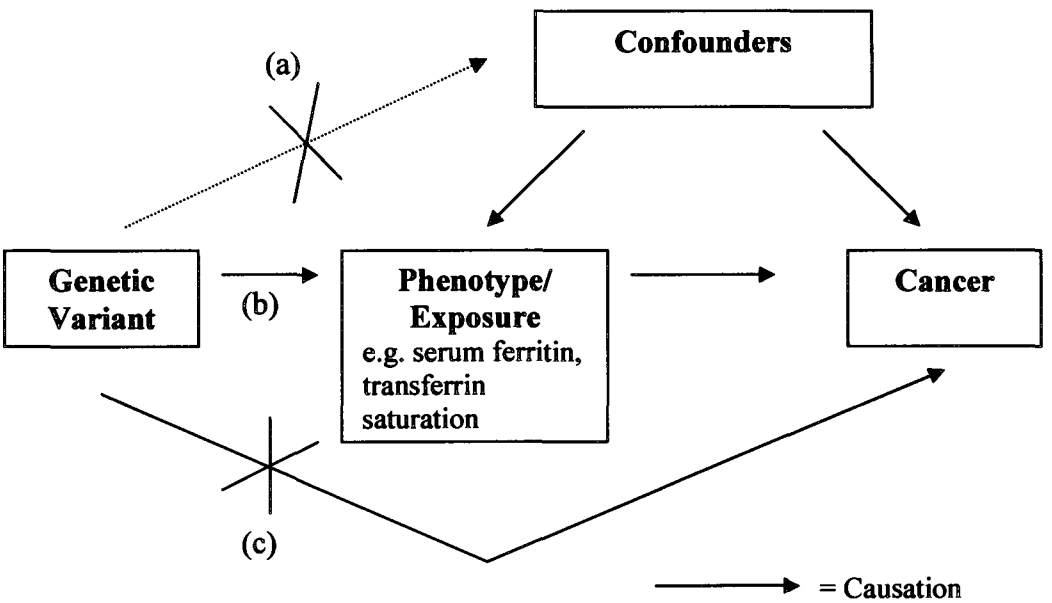
Genetic variants with clear relationships to iron status or distribution may offer a means to study the role of body iron levels in the etiology of gastrointestinal cancers avoiding the potential for reverse causation encountered with biochemical measures of iron. Based

on the systematic review in Chapter 3, *HFE* is the only iron metabolism gene that has been employed in this manner for gastrointestinal tract cancers to any extent. Nine association studies have looked at associations between the C282Y and H63D variants and neoplasia of the colorectal region. Odds ratios comparing combined *HFE*-C282Y/H63D genotypes grouped according to the likelihood of elevated iron levels did not provide evidence supporting an increased risk of colorectal cancer or colorectal adenoma with these genotypes.

Only one study had investigated associations between *HFE*-variants and neoplasia at another site in the GI tract. Corley et al. (2008a) reported some evidence of a higher odds ratio of Barrett's Esophagus (BE), a strong risk factor for esophageal adenocarcinoma, in men with *HFE*-genotypes associated with higher iron levels. The study reported in Chapter 4 is the first to examine associations between esophageal adenocarcinoma and *HFE* genetic variants. Participants in the case-control study were predominantly men of white European ancestry, a group in whom the Y282 variant shows its highest frequencies and in whom EAC has shown a marked increase in recent years. However, the *HFE* variants were not found to be associated with EAC in this study, either individually or combined as a possible marker of elevated iron levels. The interpretation of the results for both the Y282 and D63 variants was limited by their low frequencies and the small size of the study, similar to the majority of studies identified in the systematic review.

The use of the *HFE*-C282Y and *HFE*-H63D variants as indices of chronically elevated iron levels is based on the Mendelian Randomisation (MR) concept (Katan, 1986; Davey-Smith and Ebrahim, 2003). For an MR approach to be valid in assigning causality to a particular exposure requires that (a) the genetic variant be independent of confounding factors; (b) the genetic variant is consistently associated with the exposure; and (c) the genetic variant is not associated with the disease by other routes independent of the exposure of interest.

Figure 5.1 The Mendelian Randomisation Approach



If these requirements are met, the genetic variant can be used as a proxy for the exposure and, if the sample is sufficiently large, analyses do not require adjustment for lifestyle and other risk factors due to the random segregation of alleles at birth.

The Y282 variant shows variable penetrance with clear dependencies on gender, age (Allen et al., 2008), alcohol consumption (Waalen et al., 2005; Heath et al., 2008), blood donation (Heath et al., 2008) and possibly dietary intake of iron (Greenwood et al., 2005; Van der A et al., 2006). Other genes also appear to be modifiers of the phenotype (Biasiotto et al., 2004; Lee et al., 2002; Milet et al., 2007). The functional effect of the *HFE*-D63 variant independent of Y282 is unclear and it is not strongly associated with iron levels. Results from a recent study (Lawless et al., 2007) suggest that *HFE*-D63 may be positively associated with the expression of monocyte chemoattractant protein-1 (MCP-1), a small protein that is released at sites of inflammation to stimulate and attract white blood cells (Melgarejo et al., 2008). Since the discovery of the *HFE*-gene and the C282Y variant in 1996 (Feder et al., 1996), studies have led to a more detailed understanding of expression of the Type 1 hereditary hemochromatosis (HH) phenotype. It exhibits an autosomal recessive mode of inheritance characterized by elevated serum ferritin and transferrin saturation with accumulation of iron predominantly in the liver hepatocytes and not in the macrophages of the reticuloendothelial system (Wallace and Subramanian, 2007; Beutler, 2006; Griffiths, 2007). The studies conducted by Chen et al. (1999, 2000) in rats surgically-altered to cause reflux showed increased occurrence of esophagitis, columnar-lined esophagus (CLE) and EAC on injection with iron. Increased iron deposition was observed within macrophages in areas of esophagitis (Chen et al., 2000). Although *HFE*-Y282 is clearly associated with dysregulation of iron uptake and iron loading, evidence suggests that its iron loading effects are mostly found in the liver hepatocytes (Ellervik et al., 2007). The possibility exists that *HFE*-Y282 may exert a

protective effect during extra-hepatic inflammation and infection as the phenotype is associated with an inability of the macrophages to retain iron (Porto and De Sousa, 2007; Weinberg, 2008). Lawless et al. (2007) reported a significantly lower total peripheral white blood cell count in 87 patients homozygous for Y282 compared with 27 healthy individuals who carried neither the Y282 nor the D63 variant. It is clear that the "any *HFE* variant" grouping used in many of the gene-disease studies to date is of uncertain biological significance with respect to cancer pathogenesis and is not a simple index of iron levels. The Y282 variant shows variable penetrance, there is only a weak association between the D63 variant and biochemical iron indices and there is increasing evidence of more complex phenotypes and functions for both Y282 and D63. These variants are of questionable utility as unbiased proxies of body iron levels and do not currently meet the requirements for use in a Mendelian Randomisation approach.

5.2 Iron Intake and Cancers of the Gastrointestinal Tract

Studies of dietary iron intake and colorectal neoplasia were inconsistent for both the colon and rectal regions. The two rectal cancer studies of stronger design (larger, population-based with prospective exposure assessment) reported no significant association between dietary iron intake and rectal cancer among women (Kabat et al., 2007; Balder et al., 2006). A matched case-control study in New York also found no association among women (Freudenheim et al., 1990). Results were conflicting among men. Results from studies of colon cancer did not provide any consistent evidence of an association with iron intake among either men or women although again, the number of

studies was limited. Neither of the larger prospective studies reported significant associations for women (Kabat et al., 2007; Balder et al., 2006).

All five case-control studies of dietary iron intake and EAC identified in the narrative review reported inverse associations, although not all were significant. The results from the analyses presented in Chapter 4 showed no statistically significant association. As discussed briefly in Chapter 4, the total amount of iron in the diet has contributions from components that have demonstrated positive associations with EAC and others that have exhibited inverse associations. As such, it becomes difficult to interpret the biological significance of total "dietary iron". The majority of iron in North American diets comes from cereals and is in a form that is not readily bioavailable or "reactive" due to strong binding with molecules such as phytic acid.

Although confounding cannot be ruled out as an explanation, the results from these studies do raise the possibility that higher iron intakes could have a protective effect under some circumstances. Huang (2008) discussed the possibility of increased breast cancer risk associated with high iron levels in postmenopausal women due to increased oxidative stress and also the possibility of an increased risk of cancer recurrence in iron deficient younger women through hypoxia-induced up-regulation of HIF-1α and VEGF. Hypoxia has been observed in many tumor types and appears to be associated with invasion, progression and poorer prognosis (Vaupel, 2008). With respect to cancer risk, body iron levels could exhibit a 'U'-shaped risk curve.

5.3 Heme Iron Intake

To date, few studies have looked at heme iron intake and cancers of the gastrointestinal tract (Kabat et al., 2007; Balder et al., 2006; Larsson et al., 2005; Lee et al., 2004, 2005). The analyses in Chapter 4 are the first to investigate the association between heme iron intake and esophageal adenocarcinoma. After adjustment for age, gender, geographic region, smoking, frequency of alcohol intake, usual adult BMI, self-report of severe heartburn and vegetable intake, the odds ratio comparing the highest with the lowest quintile of intake was 2.8 (95% CI 1.25-6.47). The trend was significant across the quintile medians (p=0.01). After adjustment for energy by the standard multivariate method, however, the odds ratio and test for trend were no longer statistically significant (OR=2.23, 95% CI 0.89-5.58; p-trend=0.09). The analyses in this thesis were based on a relatively small case-control study (127 cases, 254 controls) with several possible biases of concern. However, the results for heme iron remained suggestive after adjustment for a range of other risk factors. BMI, self-report of severe heartburn and vegetable intake showed significant independent associations with EAC in the multivariate "main-effects" models consistent with other studies.

5.4 Public Health Importance

Cancers of the gastrointestinal tract represent a major public health burden and there is clear evidence for the involvement of diet in their etiology (WCRF/AICR, 2007). Cancers in the colorectal region and esophageal adenocarcinoma have recognized pre-cursor conditions that confer increased risk for progression to cancer – adenomatous

polyps for colorectal cancer and GERD and Barrett's Esophagus (metaplasia) for esophageal adenocarcinoma. Research into mechanisms and factors associated with progression from the pre-cursor conditions to cancer may allow intervention to prevent development of the disease. Inflammatory processes play an important role early in the disease process for both colorectal cancers and esophageal adenocarcinoma (Bax et al., 2005; Kim et al., 2008). There is considerable biological evidence for a role of iron in oxidative stress and inflammatory processes and a strong biological rationale for a role of iron deficiency in the development of hypoxia within tumors. Body iron levels and iron intakes are modifiable and therefore, if associated with a clinically significant risk of disease, a possible target for intervention. To date, there is no data available to determine whether iron levels are a risk factor for development of cancer of the esophagus in humans.

NSAIDs and aspirin are agents that are undergoing investigation for chemoprevention in both esophageal and colon cancer (Das et al., 2007; Peters and Fitzgerald, 2007; Corley et al., 2003). The AspECT trial (Aspirin Esomepraxole Chemoprevention Trial; Leedham and Jankowski, 2007), a randomized trial that plans to recruit more than 5000 men with Barrett's Esophagus, will compare the effects of PPI therapy in combination with low-dose aspirin on the progression of Barrett's Esophagus to carcinoma. It is interesting to note that PPIs, through reducing gastric acid production, actually decrease the absorption of non-heme iron in individuals with hereditary hemochromatosis (Hutchinson et al., 2007) and therefore may led to a reduction in body iron levels.

5.5 Future Directions

Laboratory studies with animals (Sasaki et al., 2007; Kumagai et al., 2004) and experimental studies with humans (Kuhnle et al., 2007; Lunn et al., 2007) have provided evidence supporting a role for endogenous nitrosation in the development of EAC. The case-control study analysed in this thesis has an extensive set of demographic, lifestyle and dietary information collected with widely-used validated tools. Several items in the main questionnaire in this study relate to intakes of nitrites and nitrates. Analyses testing whether high heme intake combined with high intakes of nitrosamine precursors leads to an increased risk may help to clarify the role of nitrosamines and endogenous nitrosation in EAC. Both the *HMOX1* and *iNOS* genes have promoter polymorphisms with *in vitro* evidence of changes in enzymatic activity (Warpeha et al., 1999; Shibahara et al., 1989). These genes could be considered interesting candidates to help study the effect of heme and endogenous nitrosation in EAC. However, the small size of the study limits the utility of this approach. Taking the candidate gene approach, as was also done in the present study, requires a very large sample size to detect what are likely to be small effects when looking at a complex disease (Lango and Weedon, 2007).

Iron involvement in the carcinogenic process is inevitable as iron is essential to cellular metabolism and growth but whether intervention to reduce iron will lead to improved outcomes is still uncertain. The FeAST study (Zacharski et al., 2008) is the first experimental study showing a clear reduction in cancer incidence and mortality due to an intervention that reduced body iron levels. However, it was conducted in a selected population and the short time period after which an effect was observed (2 years) have

led some (Edgren et al., 2008) to question whether there may be some bias, perhaps detection bias due to incomplete blinding, that contributed to the results. Similar trials in other "at-risk" populations are needed; perhaps among men and postmenopausal women with diabetes or metabolic syndrome. However, recruiting to such trials may be a challenge due to the invasiveness and unpleasant nature of the intervention.

Although *in vitro* and animal studies make invaluable contributions to our understanding of the biological processes underlying disease, choosing candidate genes and pathways based on results from these studies has not led to much success. Chronic disease phenotypes result from combinations of different genes interacting with the environment. Genes are single components within a complex system that employs multiple homeostatic mechanisms about which we have only limited understanding. Choosing the "correct" candidates for examination in a gene-disease association study is a challenge (Xavier and Rioux, 2008). A hypothesis-free genome-wide association (GWA) study in humans is an alternative possibly more efficient approach for identifying new candidates for further study (Hirschhorn and Daly, 2005). The GWA approach has had success in identifying, for example, the importance of the Wnt signaling pathway in Type 2 diabetes (Lango and Weedon, 2007) and autophagy in Crohn's disease (Rioux et al., 2007; Hampe et al., 2007). A GWA study may provide a better understanding of the determinants of measures of iron such as serum ferritin, transferrin and soluble transferrin receptors. Companion GWA studies of Barrett's Esophagus and EAC may also be more efficient ways to identify candidate pathways for further investigation and, in concert with the GWA study of determinants of iron measures, indicate how iron may play a role in the

disease. Whether a candidate gene approach or a genome-wide association approach is used, large sample sizes are required. Several thousand cases and controls are required to achieve reasonable power to detect a small effect (OR=1.2) when the minor allele frequency is low (less than 0.1) (Wang et al., 2005). Given the relative rarity of EAC, a collaborative consortium pooling samples and data from multiple studies would be the next step forward.

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Appendix A

Appendix Ai.

MEDLINE search strategy.

```
Database: Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and
Ovid MEDLINE(R) <1950 to Present>
Search Strategy:
--------------------------------------------------------------------------
---------
1      C282Y.tw.
2      H63D.tw.
3      cys282$.tw.
4      D63.tw.
5      HLA-H.tw.
6      HFE.tw.
7      HFE-gene.ab.
8      (HFE adj2 gene).ab.
9      (HH adj2 gene).ab.
10      rs1799945.tw.
11      rs1800562.tw.
12      hemochromatosis/
13      hemochromatosis.tw.
14      haemochromatosis.tw.
15      or/1-14
16      (HAMP or HEPC or HFE2B or LEAP1 or LEAP-1).tw.
17      hepcidin.tw.
18      (TFRC or TFR or CD71 or TFR1 or TRFR).tw.
19      (transferrin adj receptor).tw.
20      (TfR2 or HFE3 or TFRC2 or MGC126368).tw.
21      (SLC40A1 or FPN1 or HFE4 or MTP1 or IREG1 or MST079 or MSTP079
or SLC11A3).tw.
22      ferroportin$.tw.
23      (HJV or HFE2 or HFE2A or JH or RGMC or MGC23953).tw.
24      hemojuvelin.tw.
25      (TF or PRO1558 or PRO2086).tw.
26      transferrin.tw.
27      (SLC11A2 or DCT1 or DMT1 or NRAMP2 or FLJ37416).tw.
28      (divalent adj metal adj transporter).tw.
29      (CYBRD1 or DCYTB or FRRS3 or FLJ23462).tw.
30      (cytochrome adj2 reductase).ab.
31      (ALAS2 or ASB or ANH1 or XLSA).tw.
32      aminolevulinate.tw.
33      (HP or MGC111141 or hp2-alpha or haptoglobin).tw.
34      (HEPH or CPL or KIAA0698 or hephaestin).tw.
35      (HMOX1 or HO-1 or bK286B10).tw.
36      (heme adj oxygenase$).tw.
37      (FTL or MGC71996 or apoferritin or ferritin L-chain).tw.
38      (FTMT or MTF or (ferritin adj subunit)).tw.
39      (CP or CP-2 or ceruloplasmin or ferroxidase).tw.
40      or/16-39
41      mutation$.ab.
```

```
42      polymorphism$.ab.
43      variant$.ab.
44      allel$.ab.
45      or/41-44
46      cancer.mp.
47      cancer/
48      adenoma.mp.
49      dysplas$.ab.
50      metaplas$.ab.
51      polyp$.tw.
52      adenocarcinoma.mp.
53      Barrett$.ab.
54      leukemia.mp.
55      myeloma.mp.
56      leukem$.tw.
57      myelom$.tw.
58      neoplas$.tw.
59      colitis.tw. or colitis.mp.
60      Crohn's.tw.
61      (inflammatory adj bowel).tw.
62      IBD.tw.
63      (celiac or coeliac).tw.
64      sprue.ab.
65      gluten.ab.
66      or/46-65
67      ferritin.tw.
68      (iron adj binding).tw.
69      transferrin.tw.
70      TIBC.tw.
71      hemosiderin.tw.
72      (serum adj iron).ab.
73      iron.ab.
74      hemoglobin.ab.
75      haptoglobin.ab.
76      nutrient$.ab.
77      diet$.ab.
78      plasma$.ab.
79      serum$.ab.
80      biomarker$.ab.
81      76 or (77 and (78 or 79 or 80))
82      or/67-75,81
83      animal/
84      (15 or 40) and 45
85      84 and 66
86      84 and 82
87      82 and 66
88      or/85-87
89      88 not 83
```

Appendix Aii.

Fields in the MS Excel File Used for Data Extraction

Section	Field	Field Type	Remarks/Drop-Down List Options
Citation Details	Study ID	Numeric	
	First Author	Text	
	Year of Publication	Numeric	
Study Details	Design	Drop-down List	Case-control/cohort/nested case-control/cross-sectional/other/DK (don't know)
	Other Design? Specify	Text	
	Base	Drop-down	Population/Hospital/Other/DK
	If Other, specify	Text	
Case Group	Source of cases	Text	
	Case definition	Text	
	Case status validated?	Drop-down List	Yes/No/DK/NR (not reported)
	If so, how?	Text	
	Case selection	Drop-down	Consecutive/Selected/NR/unclear
	Specify selection criteria and method, if applicable	Text	
	Time period of case recruitment	Text	
	Other case eligibility criteria?	Text	
	Incident cases?	Drop-down	Incident only/Prevalent only/Incident and Prevalent/NR/DK
	Description of exclusions	Text	
	Participation rate (%)	Numeric	
	Comment on participation rate	Text	
Control Group	Source of controls	Text	
	Control type	Drop-down	Population/Hospital/Other/DK
	Control eligibility criteria	Text	
	Control status validated?	Drop-down	Yes/No/DK
	If so, how?	Text	
	Control selection	Drop-down	Matched/unmatched/NR/Unclear
	Matching criteria, if applicable	Text	
	Further description of control selection methods	Text	

Section	Field	Field Type	Remarks/Drop-Down List Options
	Time period of control recruitment	Text	
	Description of exclusions	Text	
	Participation rate (%)	Numeric	
	Participation comment	Text	
	Are cases and controls selected from the same underlying population?	Drop-down	Yes/No/Unclear
	Comment	Text	
Exposures: Genetic Variants Summary	HFE C282Y	Drop-down	Yes/No/DK
	HFE H63D	Drop-down	Yes/No/DK
	HFE other	Drop-down	Yes/No/DK
	TFRC S142G	Drop-down	Yes/No/DK
	TFRC other	Drop-down	Yes/No/DK
	TF (transferrin) – any	Drop-down	Yes/No/DK
	HMOX1 – any	Drop-down	Yes/No/DK
	Other gene	Text	
Genetic Exposure Assessment	Source of DNA (cases)	Drop-down	Peripheral leucocytes/whole blood/normal tissue in diseased organ/urine/other/NR
	Source of DNA (controls)	Drop-down	Peripheral leucocytes/whole blood/normal tissue in diseased organ/urine/other/NR
	Describe any differences between case and control DNA source, sampling, storage or extraction	Text	
	Genotyping method	Drop-down	PCR-RFLP/PCR-ASO/High-throughput array/ Sequencing/Other/NR
	Genotyping method – further detail	Text	
	Quality assessment methods described?	Drop-down	Yes/No/not applicable/NR
	Quality assessment results reported?	Drop-down	Yes/No/not applicable/NR
	Quality assessment results	Text	
Lifestyle and Demographic Factors	Collected?	Drop-down	Yes/No/DK
	If so, how?	Drop-down	Validated questionnaire, self-administered/Validated

Section	Field	Field Type	Remarks/Drop-Down List Options
			questionnaire, interview/Questionnaire, self-administered/Questionnaire, interview/Extracted from medical or health records/Method NR
	If so, provide list of factors for which information was provided.	Text	
	Provide further detail, if necessary.	Text	
Statistical Analyses	*A priori* power calculation?	Drop-down	Yes/No/DK
	Power comment	Text	
	Statistical techniques and tests described?	Drop-down	Yes/No/DK
	If so, describe briefly	Text	
	Multiple testing addressed?	Drop-down	Yes/No/Not applicable/NR
	HWE assessed in controls?	Drop-down	Yes/No/Not applicable/NR
	HWE method	Drop-down	Chi-sq/Exact/NR/Other
	HWE comment	Text	
Results Summary	List main effects analyses reported	Text	
	List stratified analyses done	Text	
	List joint-effects analyses done	Text	
	List interaction analyses done	Text	
Results: Sample Characteristics	Num. of cases	Numeric	
	Cases ancestry	Text	
	Cases age (mean, sd)	Text	
	Cases (% females)	Text	
	% cases with family history of outcome	Text	
	Num. of controls	Numeric	
	Controls ancestry	Text	
	Controls age (mean, sd)	Text	
	Controls (% female)	Text	
	% controls with family history of outcome	Text	
	Factors reported to differ between case and control groups	Text	

Section	Field	Field Type	Remarks/Drop-Down List Options
	Factors adjusted for in association analyses	Text	
Results: Minor allele frequencies in the controls	HFE Y282 freq.	Numeric	
	Y282 deviation from HWE?	Drop-down	Yes/No/Not applicable/Not reported
	HFE D63 freq.	Numeric	
	D63 deviation from HWE?	Drop-down	Yes/No/Not applicable/Not reported
	TFRC G142 freq.	Numeric	
	G142 deviation from HWE?	Drop-down	Yes/No/Not applicable/Not reported
	Other minor allele frequencies and deviations from HWE.	Text	
Results: Association Measures, Main Effects	Comparison 1	Text	
	Exposed cases (n)	Numeric	
	Exposed controls (n)	Numeric	
	Measure	Drop-down	OR/RR/p-value from allele frequency comparison/Other/DK
	Result1	Text	
	Adjustment Factors	Text	
	Comment	Text	
	Comparison 2	Text	
	Exposed cases (n)	Numeric	
	Exposed controls (n)	Numeric	
	Measure	Drop-down	OR/RR/p-value from allele frequency comparison/Other/DK
	Result2	Text	
	Adjustment Factors	Text	
	Comment	Text	
	Comparison 3	Text	
	Exposed cases (n)	Numeric	
	Exposed controls (n)	Numeric	
	Measure	Drop-down	OR/RR/p-value from allele frequency comparison/Other/DK
	Result3	Text	
	Adjustment Factors	Text	
	Comment	Text	
	Comparison 4	Text	
	Exposed cases (n)	Numeric	
	Exposed controls (n)	Numeric	

Section	Field	Field Type	Remarks/Drop-Down List Options
	Measure	Drop-down	OR/RR/p-value from allele frequency comparison/Other/DK
	Result4	Text	
	Adjustment Factors	Text	
	Comment	Text	
	Comparison 5	Text	
	Exposed cases (n)	Numeric	
	Exposed controls (n)	Numeric	
	Measure	Drop-down	OR/RR/p-value from allele frequency comparison/Other/DK
	Result5	Text	
	Adjustment Factors	Text	
	Comment	Text	
	Results from interaction/stratified analyses	Text	

Appendix Aiii.

Pilot Genetic Variant-Disease Association Study Bias and Generalisability Checklist
(Version 3, May, 2008)
(modification of Newcastle-Ottawa Scale items with reference to Little et al. 2002)

Review:_____ Study ID#:_____ First Author: _____ Pub.Year:_____

Study Purpose		**Assessment**	**Comment**
1. Purpose of Study	□ □ □	Detect an association Estimate magnitude of an association Unclear or not stated.	
Selection			
2. Is the case definition adequate?	□ □ □	Yes, with independent validation. *Specify method*: _____ _____ Yes, e.g. record linkage or based on self reports. Unclear or not stated.	
3. Are the cases representative?	□ □ □	Consecutive cases Selected, *specify:* _____ Unclear	
4. Case recruitment rate	□ □ □	Stated, *specify:* _____ Not stated Unclear	
5. Source of controls	□ □ □ □ □ □ □	General population or community Hospital Blood donors Family controls Aggregate, *specify:* _____ Other, *specify:* _____ Not stated	
6. Verification of control status	□ □ □	Controls have no history of disease/outcome Control status not verified Unclear or not stated	
7. Control selection methods	□ □ □ □	Random, population-based Selected, *specify:* _____ Convenience Not stated	
8. Control recruitment rate	□ □ □	Stated, *specify:* _____ Not stated Unclear	
9. Hardy-Weinberg Equilibrium in the control group	□ □ □ □ □	Assessed, no HWE deviation Assessed, HWE deviation; p = _____ Assessed, not reported Not assessed or not stated Not applicable, *reason*:_____	If assessed, specify method: _____ ____
Comparability			
10. Are cases and controls	□	Yes	

recruited from the same geographic region?	□ □	No Unclear or not stated	
11. Were cases and controls recruited during the same time period?	□ □ □	Yes No Unclear or not stated	
12. Control for potential confounding by population stratification?	□ □ □ □ □	Through design, *specify:*_____ Through analysis, *specify:* _____ Through other means, *specify:* _____ Not stated. Not applicable, *reason:* _____	
13. Study controls for other potentially important confounding factors (associated with genotype and disease), if applicable.	□ □ □ □ □	Through design, *specify:*_____ Through analysis, *specify:* _____ Through other means, *specify:* _____ Not stated. Not applicable.	*Specify factors:*
Exposure Assessment – Genetic			
14. Were sampling, handling, and storage of case and control samples the same?	□ □ □	Yes No, *specify:* _____ Unclear or not stated	
15. Were DNA extraction and genotyping methods the same for cases and control samples?	□ □ □	Yes No, *specify:* _____ Unclear or not stated	
16. Were the genotyping methods validated assays?	□ □ □	Yes No Unclear or not stated	
17. Were genotyping quality assessment procedures reported?	□ □	Yes, *specify*: _____ Not reported	Duplicates Positive controls
18. Were genotyping success rates the same in cases and controls?	□ □ □ □	Yes, same rate for both groups No, rates different and reasons described No, rates different and reasons not described Rates not stated	
19. Were analysts blinded to case-control status?	□ □ □	Yes No, *reason:* _____ Unclear or not stated	
Exposure Assessment – Environmental/Lifestyle			
20. Ascertainment of exposure	□ □ □ □ □ □ □	Secure record (e.g. surgical records) Structured interview, interviewer blind to case-control status Structured interview, interviewer not blinded Self-administered questionnaire Written self-report or medical record only Other, *specify:* _____ No description	
21. Was the measurement tool/questionnaire validated?	□ □ □ □	Yes, in the study population Yes, but in another population No Unclear or not stated	
22. Was the same method used for cases and controls?	□ □	Yes No, *specify:* _____	

	□	Unclear	
23. Non-response rate	□ □ □ □	Same rate in both groups Different rates, non-respondents described Different rates, no description of non-respondents Rates not stated	
Probability of Robust Results			
24. Were results relevant to this review from *a priori* planned analyses or *ad hoc* or subgroup analyses?	□ □ □ □	Planned *a priori* *Ad hoc* exploratory Subgroup analyses Unclear	
25. Was this the first report of an association or an attempt to replicate for this outcome?	□ □ □	First report Replication Unclear	
26. Did statistical significance assessment consider multiple comparisons, if undertaken?	□ □ □ □	Yes, *specify method*: _____ No Unclear Not applicable, *reason*: _____	
27. Study size. a. How large was the study and what was the frequency of the minor allele in the controls? b. What were the case and control cell counts for reported subgroup, joint effects or interaction analyses?		# cases: _____ # controls: _____ Minor allele frequency in the controls: _____ 1) Analysis: _____ Category: _____ # cases: _____ # controls: _____ 2) Analysis: _____ Category: _____ # cases: _____ # controls: _____	*Add other relevant variants and analyses here and overleaf.*
OVERALL:			
Internal Validity		Low Medium High	
Probability of Robust Results		Low Medium High	
Generalisability		Low Medium High	

Appendix B

Appendix Bi. EAC Study – Original Consent Form and Information Provided to Participants.

CONSENT FORM - ESOPHAGEAL CANCER PROJECT

Purpose and Description of the Study:

Cancer of the esophagus is an important and serious type of cancer. The purpose of this study is to understand some of the reasons why people get this type of cancer. We will be comparing the characteristics of people with this cancer to those without the cancer. We will be asking questions about your lifestyle, habits, diet and medical history. We will also be looking at some of the ways your body metabolizes foods in your diet.

Procedures:

In this study, you will be asked to do three things:

1) Complete a questionnaire on your own which will ask a number of questions about your usual diet. This questionnaire should take about 30 minutes to complete.
2) Participate in a face-to-face interview with a trained study interviewer. This interview will ask a series of questions about your past medical history, use of medication and personal habits such as smoking. We will also ask some further questions about your diet and the way you cook your food. This interview will take about 90 minutes to complete.
3) Provide a small sample of blood (30 cc or about 2 tablespoons). This sample will be collected by a qualified health professional at a convenient place (such as a local hospital or out-reach medical centre).

Risks:

There are no serious risks involved with this project. The blood sample will be collected using a small needle in your arm. There is a small risk of bruising at the place where the needle is put.

Compensation:

You will be reimbursed for reasonable travel expenses to come to the personal interview.

Confidentiality:

All of the information will be kept confidential. It will not be given to anyone without your written permission. Each person will be given a unique ID number. Any information linking you to this number and to your results will be discarded once the questionnaire information has been verified by the project staff. Any of the blood sample not used for the analyses will be discarded unless you give us permission to retain the sample for further analyses.

Voluntary Participation:

Your participation in this study is completely voluntary. You may withdraw from the study at any time. You can refuse to answer any question. Your participation or non-participation in this study will have no impact on the quality of medical care you receive.

Answers to Questions:

A detailed information sheet about this project is attached to this consent form. If you have any questions regarding this study, then you can contact:

Dr. Nicholas Birkett (Principal Investigator),
Room 3213D, Health Sciences Centre,
451 Smyth Rd., Ottawa, Ontario. K1H 5M8
613-562-5800 ext 8290.

If you have any questions regarding your rights as a participant in this project or any question concerning the way the project is being carried out, you can contact the chairperson of the Ottawa Hospital Research Ethics Board at: (613)-761-4902 (collect).

I agree to participate in this study (please check the appropriate boxes to indicate the aspects of the project which you are consenting to participate in):

		PLEASE ANSWER YES	NO
a)	I agree to complete a questionnaire about my usual diet.	☐	☐
b)	I agree to complete the face-to-face interview	☐	☐
c)	I agree to provide 30 cc (about 2 tablespoons) of blood	☐	☐
d)	I agree that my blood can be used as a source of DNA for this project	☐	☐
e)	I agree that any blood remaining after completing the primary analyses can be stored in special bank. This bank will be anonymous. I agree that my blood sample can be made available to the scientific community to study other genetic factors related to cancer risk. My blood will not be used in any new studies without the prior approval of the University Ethics Board.	☐	☐

Once we have completed the analyses to which you consent, your blood sample will be destroyed to prevent unauthorized use.

_____ _____

Date signed Signature of Participant

The above person has been given the opportunity to have questions answered about this project and their involvement in it.

_____

Signature of Witness

COMMON QUESTIONS ABOUT THIS PROJECT FOR PEOPLE WITH CANCER OF THE ESOPHAGUS

Who is funding the study?	The Cancer Research Society. This is a Montreal-based charity which funds cancer research in Canada. The money they give out comes from private donations from people such as you. It has no links to industries such as tobacco companies.
Why might my life style affect my risk of getting cancer?	We all know that smoking can cause lung cancer. However, your body is exposed to all sorts of other chemicals which can cause it to become ill. Some of these can damage the DNA (genetic material) in some of the cells of your body. Over time, this can sometimes lead to a cancer developing. If we can understand which features of your life style increase your risk of getting cancer, then we can find ways to stop people getting cancer and thus improve your health.
Who will be able to see my answers?	All of the information you give us will be kept confidential. The staff who collect your questionnaire will see your answers as will Dr. Birkett (the main research scientist on this project). No-one else will see your answers. Once your answers have been entered into our computer, we will delete your name from our records so no-one will be able to tell who gave us which information. Our reports will combine all of our data together and will never report on your individual answers.
What if you ask something I don't want to answer?	You only have to answer the questions you are comfortable with. Most of the questions we need to ask are not sensitive. However, if we do ask something you don't want to answer, just tell us or leave it blank and you can go on to the next question.
Will the blood sample hurt?	Most people have had a blood sample taken for medical reasons. For example, to have your cholesterol checked. Our blood sample will be exactly like those tests. You will feel a slight prick but there will be no real pain. Some people find that they have a small bruise around the part of the arm where the sample was collected. This will clear up in a few days.
I'd be happy to answer your questions but I don't want to give any blood. Is that OK?	Yes, that is your choice.
Is there any risk that I could get ill if I give you the blood	You can never be 100% certain that you won't get ill. However, the risk off getting an infection from giving a blood sample is very small. The laboratory will use sterile methods which will reduce this risk

sample?	almost to zero
.What will you do with the sample of blood you are taking?	People vary in the way their body handles the chemicals and toxins which we all are exposed to every day. However, doctors are not sure if these differences affect your risk of getting a serious disease ((like cancer). We are planning to look at differences in the way the body handles toxins. We want to learn if these differences change your risk of getting cancer of the esophagus. Your blood sample will give us the way to look at this. We will extract the DNA material from the white cells in your blood sample. We will then use sophisticated laboratory tests to look for differences in the way the body handles toxins. By comparing people with and without cancer, we will be able to learn if any of these differences are important.

COMMON QUESTIONS ABOUT THIS PROJECT FOR PEOPLE WITHOUT CANCER OF THE ESOPHAGUS

Who is funding the study?	The Cancer Research Society. This is a Montreal-based charity which funds cancer research in Canada. The money they give out comes from private donations from people such as you. It has no links to industries such as tobacco companies.
Where is the esophagus?	The esophagus is the tube which connects your mouth to your stomach.
What is cancer of the esophagus?	All organs of the body have a risk of developing cancer. Cancer refers to the situation when a part of the body grows out of control. Cancer of the esophagus refers to a cancer located in the esophagus. We are interested in one particular type of cancer (called adenocarcinoma). This cancer is located mainly in the bottom of the esophagus near where the esophagus joins the stomach. This cancer has become much more common over the last few years. But no one knows why this is happening.
Why might my life style affect my risk of getting cancer?	We all know that smoking can cause lung cancer. However, your body is exposed to all sorts of other chemicals which can cause it to become ill. Some of these can damage the DNA (genetic material) in some of the cells of your body. Over time, this can sometimes lead to a cancer developing. If we can understand which features of your life style increase your risk of getting cancer, then we can find ways to stop people getting cancer and thus improve your health.
Will you be able to tell if I have cancer?	No. This study is only asking questions about your past health. The best person to talk to in order to find out if you might have cancer is your family doctor.
Who will be able to see my answers?	All of the information you give us will be kept confidential. The staff who collect your questionnaire will see your answers as will Dr. Birkett (the main research scientist on this project). No-one else will see your answers. Once your answers have been entered into our computer, we will delete you name from our records so no-one will be able to tell who gave us the information. Our reports will combine all of the data together and will never report on your individual answers.
What if you ask something I don't	You only have to answer the questions you are comfortable with. Most of the questions we need to ask are not sensitive. However, if

want to answer? Will the blood sample hurt?	we do ask something you don't want to answer, just tell us or leave it blank and you can then go on to the next question. Most people have had a blood sample taken for medical reasons. For example, to have your cholesterol checked. Our blood sample will be exactly like those tests. You will feel a slight prick but there will be no real pain. Some people find that they have a small bruise around the part of the arm where the sample was collected. This will clear up in a few days.
I'd be happy to answer your questions but I don't want to give any blood. Is that OK?	Yes, that is your choice.
Is there any risk that I could get ill if I give you the blood sample?	You can never be 100% certain that you won't get ill. However, the risk off getting an infection from giving a blood sample is very small. The laboratory will use sterile methods which will reduce this risk almost to zero.
A close relative has cancer. Does that mean I will get it?	No. Some cancers do run in families. However, even then most people in the family will not get cancer. We would encourage you to visit your family doctor to discuss this question. He or she could arrange for you to talk with a specialist in genetics if this would be helpful.
What will you do with the sample of blood you are taking?	People vary in the way their body handles the chemicals and toxins which we all are exposed to every day. However, doctors are not sure if these differences affect your risk of getting a serious disease ((like cancer). We are planning to look at differences in the way the body handles toxins. We want to learn if these differences change your risk of getting cancer of the esophagus. Your blood sample will give us the way to look at this. We will extract the DNA material from the white cells in your blood sample. We will then use sophisticated laboratory tests to look for differences in the way the body handles toxins. By comparing people with and without cancer, we will be able to learn if any of these differences are important.

Appendix Bii. Self-Administered and Main Questionnaires

OFFICE USE ONLY

ID Number: _____-_____-_____-_____

Date Returned: _____|_____|_____
DD MM YY

OFFICE USE ONLY

Checked ☐

Data entry ☐

Verified ☐

FOOD QUESTIONNAIRE

INSTRUCTIONS

Thank you for your co-operation in taking part in this study. The first thing we are asking you to do is to fill-in these questions about your usual diet. All of these questions concern your diet **two years ago**. That is, if you are filling in these question in June 2000, we want you to try and remember back to your usual diet in 1998.

Most of the questions are about common foods or meals. Some concern vitamin and mineral supplements you may use. Please place a clear mark in the box which is your answer. It doesn't matter what mark you use as long as it is clear. Common marks would include a check (✓), or an 'X', or you might colour in the whole box.

Most of the questions have the same pattern of responses. You first say how often you eat the food item. Then, if you eat that food at least once per month, you fill in another box to show how big your usual serving size is. If you don't eat the food, then you don't need to fill in the size.

First, we need to ask about vitamin and mineral supplements you may have taken.

1. Two years ago, did you take any vitamins or minerals?

☐ no ☐ yes, but not regularly ☐ yes, fairly regularly

GO TO QUESTION #2

If YES, what do you take fairly regularly? Please complete this table.

	NUMBER OF TABLETS								FOR HOW MANY YEARS?				
VITAMIN TYPE	None	1-3 per week	4-6 per week	1 per day	2 per day	3 per day	4 per day	5+ per day	Less than 1 year	1-2 years	3-5 years	6-9 years	10+ years
Multiple Vitamins													
Stress-tabs type	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Therapeutic, Theragran type	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
One-a-day type	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other Vitamins													
Vitamin A	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vitamin E	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Calcium or Tums	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vitamin C	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

2. **If you took Vitamin E or Vitamin C two years:**

How many units per Vitamin E tablet? ☐100 ☐200 ☐400 ☐1000 ☐don't know

How many milligrams per Vitamin C tablet? ☐100 ☐250 ☐500 ☐1000 ☐don't know

3. **Two year ago, did you regularly take pills containing any of these nutrients?**

iron	☐ yes	☐no	☐don't know
beta-carotene	☐yes	☐no	☐don't know
zinc	☐yes	☐no	☐don't know
selenium	☐yes	☐no	☐don't know

Now, we want to ask about some of the ways you may have prepared your food two years ago.

4. **Two years ago, what kinds of fat did you *usually* use in cooking (to fry, stir-fry, or saute)? Mark only one to two.**

☐ Don't know or don't cook ☐ Lard, fatback, bacon fat ☐ Pam or no oil

☐ Crisco ☐ Stick margarine ☐ Butter

☐ Soft tub margarine ☐ Oil ☐ ½ butter, ½ margarine

☐ Low calorie margarine

5. **Two years ago, what kinds of fat did you *usually* add to vegetables, potatoes, etc.? Mark only one or two.**

☐ Don't know or don't cook ☐ Lard, fatback, bacon fat ☐ Low calorie margarine

☐ Stick margarine ☐ Soft tub margarine ☐ ½ butter, ½ margarine

☐ Butter ☐ Whipped butter ☐ Crisco

6. **When you ate the following foods, how often did you eat a low-fat or non-fat version of that food?**

Cheese	☐ Always low-fat	☐ Sometimes	☐ Rarely low-fat
Ice cream/yogurt	☐ Always low-fat	☐ Sometimes	☐ Rarely low-fat
Salad dressing	☐ Always low-fat	☐ Sometimes	☐ Rarely low-fat

7a.	**How often did you add salt to your food?**	☐ Seldom/never	☐ Sometimes	☐ Often/Always
7b.	**How often did you add pepper to your food?**	☐ Seldom/never	☐ Sometimes	☐ Often/Always
7c.	**How often did you eat the skin on chicken?**	☐ Seldom/never	☐ Sometimes	☐ Often/Always
7d.	**How often did you eat the fat on meat?**	☐ Seldom/never	☐ Sometimes	☐ Often/Always

8. **Two years ago, about how often did you eat the following food from restaurants or carry outs? Remember to think about all meals (breakfast, lunch dinner or snacks)**

	NUMBER OF VISITS TWO YEARS AGO						
RESTAURANT FOOD	Never in past year	1-4 times past year	5-11 times past year	1-3 times a month	Once a week	2-4 times a week	almost every day
Fried Chicken	☐	☐	☐	☐	☐	☐	☐
Burgers	☐	☐	☐	☐	☐	☐	☐
Pizza	☐	☐	☐	☐	☐	☐	☐
Chinese Food	☐	☐	☐	☐	☐	☐	☐
Mexican Food	☐	☐	☐	☐	☐	☐	☐
Fried Fish	☐	☐	☐	☐	☐	☐	☐

9. **Now, we are going to ask a series of question about your *usual* eating habits two years ago. Please follow these instructions to fill in this section.**

FIRST: Mark the column to show how often, on the average, you ate the food two years ago. Please BE CAREFUL which column you put your answer in.

SECOND: Mark whether your usual serving size was small, medium or large. Please DO NOT OMIT serving size.

ADDITIONAL COMMENTS:

- Please DO NOT SKIP any foods. If you never eat a food, mark "Never or less than once a month".
- A small serving is about one-half the medium serving size shown, or less.
- A large serving is about one-and-a-half times the medium serving size shown or more.

Here are some examples of how to complete these questions:

EXAMPLES.

o "Hank drinks whole milk once a day - about 1 ½ glasses each time."
This is how he would show that on the chart

	Never or less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium Serving	Your Serving Size		
											S	M	L
Whole milk and beverages made with whole milk (not. incl. on cereal)	☐	☐	☐	☐	☐	☐	☐	☒	☐	8 oz. glass	☐	☐	☒

o "Mary eats dark rye in a sandwich for lunch five times a week, two slices each time."
She would record her bread this way.

	Never or less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium Serving	Your Serving Size		
											S	M	L
Dark bread, such as wheat, rye, pumpernickel, (including sandwiches)	☐	☐	☐	☐	☐	☐	☒	☐	☐	2 slices	☐	☒	☐

TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	Never or less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium Serving	Your Serving Size S	M	L
FRUITS AND JUICES													
Apples, applesauce, pears	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium or ½ cup	☐	☐	☐
Bananas	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium	☐	☐	☐
Peaches, apricots (fresh or canned)	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium or ½ cup	☐	☐	☐
Cantaloupe	☐	☐	☐	☐	☐	☐	☐	☐	☐	1/4 medium	☐	☐	☐
Watermelon (in season)	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 slice	☐	☐	☐
Strawberries (in season)	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup	☐	☐	☐
Oranges	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium	☐	☐	☐
Grapefruit	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ medium	☐	☐	☐
Orange juice or grapefruit juice	☐	☐	☐	☐	☐	☐	☐	☐	☐	6 ounce glass	☐	☐	☐
Fruit drinks with added vitamin C, such as Hi-C	☐	☐	☐	☐	☐	☐	☐	☐	☐	6 ounce glass	☐	☐	☐
Any other fruit, including berries, fruit cocktail, grapes	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup	☐	☐	☐
BREAKFAST FOODS													
High fiber, bran or granola cereals, shredded wheat	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium bowl	☐	☐	☐
Highly fortified cereals, such as Total, Just Right or Product 19	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium bowl	☐	☐	☐
Other cold cereals, such as corn flakes, Rice Krispies	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium bowl	☐	☐	☐
Cooked cereal, or grits	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium bowl	☐	☐	☐
Milk on cereal	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup	☐	☐	☐
Sugar added to cereal	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 teasp	☐	☐	☐

TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	Never or less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium Serving	Your Serving Size S	M	L
VEGETABLES													
String Beans, green beans	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup	☐	☐	☐
Peas	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup	☐	☐	☐
Chili with beans	☐	☐	☐	☐	☐	☐	☐	☐	☐	3/4 cup	☐	☐	☐
Other beans such as baked beans, pintos, kidney, limas and lentils	☐	☐	☐	☐	☐	☐	☐	☐	☐	3/4 cup	☐	☐	☐
Corn	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup	☐	☐	☐
Winter squash/baked squash	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup	☐	☐	☐
Tomatoes, tomato juice	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium or 6 oz. glass	☐	☐	☐
Red chili sauce, taco sauce, salsa picante	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 tablesp	☐	☐	☐
Broccoli	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup	☐	☐	☐
Cauliflower	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup	☐	☐	☐
Brussels sprouts	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup	☐	☐	☐
Spinach (raw)	☐	☐	☐	☐	☐	☐	☐	☐	☐	3/4 cup	☐	☐	☐
Spinach (cooked)	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup	☐	☐	☐
Mustard greens, turnip greens, collards	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup	☐	☐	☐
Cole slaw, cabbage, sauerkraut	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup	☐	☐	☐
Carrots, or mixed vegetables containing carrots	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup	☐	☐	☐
Green salad	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium bowl	☐	☐	☐
French fries and fried potatoes	☐	☐	☐	☐	☐	☐	☐	☐	☐	3/4 cup	☐	☐	☐
Sweet potatoes, yams	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup	☐	☐	☐

TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	Never or less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium Serving	Your Serving Size S	M	L
Other potatoes, including boiled, baked, mashed & potato salad	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium or ½ cup	☐	☐	☐
Rice	☐	☐	☐	☐	☐	☐	☐	☐	☐	3/4 cup	☐	☐	☐
Any other cooked vegetable, including cooked onions, summer squash	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup	☐	☐	☐
Butter, margarine or other fat added to veg., potatoes, etc.	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 pats	☐	☐	☐
MEAT, FISH, POULTRY, LUNCH ITEMS													
Liver, including chicken livers	☐	☐	☐	☐	☐	☐	☐	☐	☐	4 ounces	☐	☐	☐
Tuna, tuna salad, tuna casserole	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup	☐	☐	☐
Oysters	☐	☐	☐	☐	☐	☐	☐	☐	☐	5 pieces, 1/4 cup or 3 oz.	☐	☐	☐
Shell fish, (shrimp, crab, lobster, etc.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	5 pieces, 1/4 cup or 3 oz.	☐	☐	☐
Spaghetti, lasagna, other pasta with tomato sauce.	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup	☐	☐	☐
Pizza	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 slices	☐	☐	☐
Mixed dished with cheese (such as macaroni and cheese)	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup	☐	☐	☐
Liverwurst	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 slices	☐	☐	☐
Ham, bologna, salami and other lunch meats	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 slices or 2 ounces	☐	☐	☐
Vegetable and tomato soups, including vegetable beef, minestrone	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium bowl	☐	☐	☐
Other soups	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium bowl	☐	☐	☐

TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	Never or less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium Serving	Your Serving Size		
											S	M	L
BREADS, SNACKS, SPREADS													
Biscuits, muffins, (including fast foods)	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium piece	☐	☐	☐
White bread (including sandwiches, bagels, burger rolls, French or Italian bread	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 slices	☐	☐	☐
Dark bread, such as wheat, rye, pumpernickel, (including sandwiches)	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 slices	☐	☐	☐
Corn bread, corn muffins, corn tortillas	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium piece	☐	☐	☐
Salty snacks, such as potato chips, corn chips, popcorn.	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 handfuls or 1 cup	☐	☐	☐
Peanuts, peanut butter	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 tablesp	☐	☐	☐
Margarine on bread or rolls	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 pats	☐	☐	☐
Butter on bread or rolls	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 pats	☐	☐	☐
DAIRY PRODUCTS (Excluding milk drinks)													
Cottage cheese	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup	☐	☐	☐
Other cheeses, and spreads	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 slices or 2 ounces	☐	☐	☐
Flavored yogurt, frozen yogurt	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup	☐	☐	☐

TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	Never or less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium Serving	Your Serving Size S	M	L
SWEETS													
Ice cream	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 scoop or ½ cup	☐	☐	☐
Doughnuts, cookies, cake, pastry	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 piece or 3 cookies	☐	☐	☐
Pumpkin pie, sweet potato pie	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 *medium* slice	☐	☐	☐
Other pies	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium slice	☐	☐	☐
Chocolate candy	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 small bar or 1 oz.	☐	☐	☐
Other candy, jelly, honey, brown sugar	☐	☐	☐	☐	☐	☐	☐	☐	☐	3 pieces or 1 tblsp.	☐	☐	☐
BEVERAGES													
Whole milk and beverages made with whole milk (not. incl. on cereal)	☐	☐	☐	☐	☐	☐	☐	☐	☐	8 oz. glass	☐	☐	☐
2% milk and beverages made with 2% milk (not including on cereal)	☐	☐	☐	☐	☐	☐	☐	☐	☐	8 oz. glass	☐	☐	☐
Skim milk, 1% milk or buttermilk (not including on cereal)	☐	☐	☐	☐	☐	☐	☐	☐	☐	8 oz. glass	☐	☐	☐
Regular soft drinks (not diet soda)	☐	☐	☐	☐	☐	☐	☐	☐	☐	12 oz can or bottle	☐	☐	☐
Coffee, regular or decaf	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 *medium* cup	☐	☐	☐
bq9h6a Tea (hot or iced)	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium cup	☐	☐	☐
bq9h6b Lemon in tea	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 teasp	☐	☐	☐
Non-dairy creamer in coffee or tea	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 tablesp	☐	☐	☐
Milk in coffee or tea	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 tablesp	☐	☐	☐
Sugar in coffee or tea	☐	☐	☐	☐	☐	☐	☐	☐	☐	2 teasp	☐	☐	☐
Glasses of water	☐	☐	☐	☐	☐	☐	☐	☐	☐	8 oz. glass	☐	☐	☐

SUMMARY QUESTIONS	AVERAGE USE TWO YEARS AGO								
	Less than once per week	1-2 per week	3-4 per week	5-6 per week	1 per day	1 ½ per day	2 per day	3 per day	4+ per day
10a. How often do you use fat or oil in cooking?	☐	☐	☐	☐	☐	☐	☐	☐	☐
10b. About how many servings of vegetables do you eat, not counting salad or potatoes?	☐	☐	☐	☐	☐	☐	☐	☐	☐
10c. About how many servings of fruit do you eat, not counting juices?	☐	☐	☐	☐	☐	☐	☐	☐	☐
10d. About how many servings of cold cereal do you eat?	☐	☐	☐	☐	☐	☐	☐	☐	☐

THANK YOU VERY MUCH FOR TAKING THE TIME TO FILL OUT THIS QUESTIONNAIRE. PLEASE TAKE A MOMENT TO CHECK OVER YOUR ANSWERS AND FILL IN ANY QUESTIONS YOU MAY HAVE SKIPPED OVER.

IN PARTICULAR, CHECK TO MAKE SURE THAT YOU HAVEN'T MISSED ANY PAGES.

PLEASE BRING THIS QUESTIONNAIRE WITH YOU TO YOUR MAIN INTERVIEW.

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STUDY CONTACTS:

Dr. Nicholas Birkett,
Department of Epidemiology and Community Medicine,
451 Smyth Rd.,
Ottawa, Ontario. K1H 8M5
613-562-5800 ext 8290

OFFICE USE ONLY

ID Number: _____

Date of Interview: _____

DD MM YY

Time Interview Began: _____ (hhmm)

Time Interview Ended: _____ (hhmm)

Interviewer ID: _____

Interview Outcome Code: _____

Interview Location: _____

OFFICE USE ONLY

Checked ☐

Data entry ☐

Verified ☐

ETIOLOGY OF ESOPHAGEAL CANCER STUDY:

MAIN QUESTIONNAIRE

Version for use from April, 2001 to March, 2002

June, 2000

Principal Investigator: Dr. Nicholas Birkett,
Department of Epidemiology and Community Medicine,
University of Ottawa,
451 Smyth Rd.,
Ottawa, Ontario. K1H 8M5
613-562-5410

General Introduction

In this interview, there are several areas we will be talking about. We will start off asking some questions about your past medical history. Then, we will talk about the food you eat and the way you prepare it. You may find these questions sound similar to some of the questions you answered in the form you filled in before this interview. This is intended since we now need to get some more detailed information about some of the things asked on that questionnaire. Finally, we will talk about your lifestyle and personal characteristics.

Most of the things I will be asking you about concern events that happened in the past. Most of the time we will be talking about things which happened about 12 months ago. That is, before 2000 or during the calendar year 1999. However, sometimes I may ask you to think back further in the past, perhaps as much as 10 or 15 years. I realize that it can be hard to remember details of your life from that long ago. I would only ask that you try as hard as you can.

Most of the questions will give you a series of choices for your answers. For example, if I asked you if you lived in Toronto, you would be given the choice of 'yes' or 'no'. For each question, I will read out the possible answers. If you are unsure of the answers, please ask me to read them out again. For some of the questions, I will show you a small card with the possible answers in order to help you out. In some cases, I will be showing some pictures and asking you to choose one. I will explain how this works when we get to that part of the interview.

If you do not hear a question clearly or do not understand what we are asking, please tell me and I can repeat the question. Take your time in answering. Remember that you can decline to answer any question. We would simply move on to the next question.

Do you have any questions about what is going to happen?

SUBJECT SHOULD BE PERMITTED TO ASK QUESTIONS AT THIS POINT. INTERVIEWER SHOULD PROVIDE APPROPRIATE ANSWERS BEFORE PROCEEDING.

SECTION A
MEDICAL FACTORS

To start off, I need to know how old you are since this will determine if some of my questions are relevant for you.

A1.	What is your date of birth?	___ ___ ___ D M Y
A2.	So that would make you _____ years old? Is that right?	Yes ❑ No ❑ *(If NO, correct the date and record it.)*

Now, let's move on to some questions about your height and weight.

A3.	How tall are you?	___ Ft. ___ in. **OR** _____ cms. DK ❑
A4.	What was your usual adult weight before 2000?	_____ Pounds **OR** _____ kgs. DK ❑
A5.	What was the most you ever weighed since the age of 20? *(Read for female subjects)*: Please exclude times that you were pregnant.	_____ Pounds **OR** _____ kgs. DK ❑
A6.	How old were when you first weighed *(weight in previous question)?*	_____ Age DK ❑

	Ask next two questions for each age through subject's current age:	
A6a.	What was your usual adult weight when you were in your:	
	i) 20's?.....	_____ Pounds **OR** _____ kgs. DK ☐
	ii) 40's?.....	_____ Pounds **OR** _____ kgs. DK ☐ NA ☐
	iii) 60's?....	_____ Pounds **OR** _____ kgs. DK ☐ NA ☐
A7.	During your (decade), how often did you engage in vigorous physical activity, that is, activity that caused you to work up a sweat?	
	i) 20's?.....	|___|___| TIMES PER Day Week Month Year DK
	ii) 40's?.....	|___|___| TIMES PER Day Week Month Year DK NA
	iii) 60's?.....	|___|___| TIMES PER Day Week Month Year DK NA

Thank you. Now we are going to move on to ask about some common symptoms which people experience.

A8.	Before 2000, did you ever have severe heartburn, that is, heartburn so painful that it awoke you or prevented you from sleeping?	YES ❑ NO ❑ DK ❑ *(Ask A8.1-8.6 ONLY if YES.)*
	A8.1 During periods when you had severe heartburn, did you typically use extra pillows or elevate the head of your bed to help relieve your symptoms?	YES ❑ NO ❑ DK ❑
	A8.2 Before 2000, how often did you have severe heartburn?	|___|___| TIMES PER Day Week Month Year DK
	(If study subject is female, ask question A8.3. Otherwise skip to question A8.4) A8.3 Did this occur only during pregnancy?	YES ❑ NO ❑ DK ❑
	A8.4 Before 2000, for how many months or years in total did you have severe heartburn?	|___|___| NUMBER OF Months Years DK
	A8.5 Before 2000, did you ever take any prescription medications for severe heartburn?	YES ❑ NO ❑ DK ❑
	A8.6 Before 2000, did you ever take any over-the-counter medications for severe heartburn? This includes things like Tums, Rolaids, antacids and so on.	YES ❑ NO ❑ DK ❑
A9.	Before 2000, did you ever have acid regurgitation, that is, a sour taste in your mouth from the contents of your stomach backing up into your mouth or throat?	YES ❑ NO ❑ DK ❑ *(Ask A9.1 & A9.2 ONLY if YES)*
	A9.1 Before 2000, how often did you have acid regurgitation?	|___|___| TIMES PER Day Week Month Year DK
	A9.2 Before 2000, for how many months or years in total did you experience acid regurgitation?	|___|___| NUMBER OF Months Years DK

A10.	Before 2000, did you ever have the feeling that solid food was sticking to your throat as it went down?	YES ☐ NO ☐ DK ☐ ***(Ask A10.1-10.3 ONLY if YES)***
	A10.1 Before 2000, how often did you have this feeling when you ate?	Every time ☐ Most of the time ☐ Some of the time ☐ Occasionally ☐ Rarely ☐ DK ☐
	A10.2 How long had this been going on before 2000?	|___|___| Months NUMBER OF Years DK
	A10.3 Which of the following types of food tended to stick:	
	i) Bread?	YES ☐ NO ☐ DK ☐
	ii) Meat?	YES ☐ NO ☐ DK ☐
	iii) Pasta?	YES ☐ NO ☐ DK ☐
	iv) Lettuce?	YES ☐ NO ☐ DK ☐
	v) Cereal?	YES ☐ NO ☐ DK ☐
	vi) Apples?	YES ☐ NO ☐ DK ☐
	vii) Liquids such as juice, milk or water?	YES ☐ NO ☐ DK ☐
	viii) Rice?	YES ☐ NO ☐ DK ☐

Now, I'm going to ask about a number of medical conditions which you may have experienced some time in the past. Please try your best to remember if you ever had any of these conditions.

IF YES TO A11, ASK A12-14, AS INDICATED.

A11. Before2000, did a doctor ever tell you that you had:	A12. When were you first diagnosed with:	A13. Did you ever take any prescription medications for this condition?	A14. Did you ever take any over the counter medications for this condition?
a. Esophagitis, that is, inflammation of the lining of the esophagus: ☐ Yes ☐ No ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__|	☐ Yes ☐ No ☐ DK	☐ Yes ☐ No ☐ DK
b. Barrett's esophagus: ☐ Yes ☐ No ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__|	☐ Yes ☐ No ☐ DK	☐ Yes ☐ No ☐ DK
c. Ulcers in the esophagus: ☐ Yes ☐ No ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__|	☐ Yes ☐ No ☐ DK	☐ Yes ☐ No ☐ DK
d. Ulcers in the stomach, often called peptic ulcers (*include unspecified ulcers*): ☐ Yes ☐ No ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__|	☐ Yes ☐ No ☐ DK	☐ Yes ☐ No ☐ DK
e. Ulcers in the small intestine or duodenal ulcers: ☐ Yes ☐ No ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__|	☐ Yes ☐ No ☐ DK	☐ Yes ☐ No ☐ DK

A11. Before2000, did a doctor ever tell you that you had:	A12. When were you first diagnosed with:	A13. Did you ever take any prescription medications for this condition?	A14. Did you ever take any over the counter medications for this condition?
f. A hiatal or diaphragmatic hernia, where the stomach slips through the diaphragm into the lower chest: ☐ Yes ☐ No ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__|	☐ Yes ☐ No ☐ DK	☐ Yes ☐ No ☐ DK
g. Polyps in the stomach: ☐ Yes ☐ No ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__|		
h. Benign, non-cancerous polyps in the colon or large intestine: ☐ Yes ☐ No ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__|		
i. Pancreatitis, that is, an inflammation or infection of the pancreas: ☐ Yes ☐ No ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__|		
j. Cirrhosis of the liver: ☐ Yes ☐ No ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__|		

A11. Before2000, did a doctor ever tell you that you had:	A12. When were you first diagnosed with:	A13. Did you ever take any prescription medications for this condition?	A14. Did you ever take any over the counter medications for this condition?
k. Hepatitis, that is, an inflammation or infection of the liver: ☐ Yes ☐ No ☐ DK	AGE |__|__| OR YEAR |__|__|__|__|		
l. Gallstones or gall bladder disease: ☐ Yes ☐ No ☐ DK	AGE |__|__| OR YEAR |__|__|__|__|		
m. Jaundice: ☐ Yes ☐ No ☐ DK	AGE |__|__| OR YEAR |__|__|__|__|		
n. Celiac disease or sprue: ☐ Yes ☐ No ☐ DK	AGE |__|__| OR YEAR |__|__|__|__|		
o. Hypochlorhydria or achlorhydria, that is, low stomach acid: ☐ Yes ☐ No ☐ DK	AGE |__|__| OR YEAR |__|__|__|__|	☐ Yes ☐ No ☐ DK	☐ Yes ☐ No ☐ DK
p. Pernicious anemia: ☐ Yes ☐ No ☐ DK	AGE |__|__| OR YEAR |__|__|__|__|	☐ Yes ☐ No ☐ DK	☐ Yes ☐ No ☐ DK

A11. Before2000, did a doctor ever tell you that you had:	A12. When were you first diagnosed with:	A13. Did you ever take any prescription medications for this condition?	A14. Did you ever take any over the counter medications for this condition?
q. *Helicobacter Pylori* infection (you might know this as the ulcer-causing bacterium): ☐ Yes ☐ No ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__|	☐ Yes ☐ No ☐ DK	☐ Yes ☐ No ☐ DK
r. Iron deficiency anemia: ☐ Yes ☐ No ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__|	☐ Yes ☐ No ☐ DK	☐ Yes ☐ No ☐ DK
s. Scleroderma: ☐ Yes ☐ No ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__|		
t. Parkinson's Disease: ☐ Yes ☐ No ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__|		
u. Genital Herpes or Genital warts: ☐ Yes ☐ No ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__|		

Now, I need to ask about a more common condition: canker sores. Most people have had canker sores at some time in their lives. However, people vary in how frequently they have this problem.

A15.	Before 2000, how often did you have canker sores?	All the time ☐ Most months during the year ☐ About 3-4 months during the year ☐ Once per year ☐ Less than once per year ☐ Never ☐ Don't know ☐
A15.1	Have you ever needed medical or surgical treatment for canker sores?	YES ☐ NO ☐ DK ☐ *IF YES: Record details verbatim below this chart:*

SPACE TO RECORD DETAILS OF MEDICAL/SURGICAL TREATMENT FOR CANKER SORES.

Finally, I am going to ask about any cancers which you may have had prior to 2000. ***Ask questions A17 and A18 for each type of cancer which the patient reports. After completing A18, prompt to see if there is any other cancer to be reported. If more than four cancers need reporting, continue on the blank pages at end of the questionnaire.***

A16. Before 2000, did a doctor ever tell you had a cancer or a tumour, including blood cancers such as leukemia, lymphoma, Hodgkin's Disease, or multiple myeloma?	A17. What type of cancer was this? ***(Record answer verbatim)***	A18. When was this first diagnosed?
a. Cancer #1 Yes ❑ No ❑ DK ❑	_____ _____ _____ _____	AGE |__|__| **OR** YEAR |__|__|__|__|
b. Cancer #2 Yes ❑ No ❑ DK ❑	_____ _____ _____ _____	AGE |__|__| **OR** YEAR |__|__|__|__|
c. Cancer #3 Yes ❑ No ❑ DK ❑	_____ _____ _____ _____	AGE |__|__| **OR** YEAR |__|__|__|__|
d. Cancer #4 Yes ❑ No ❑ DK ❑	_____ _____ _____ _____	AGE |__|__| **OR** YEAR |__|__|__|__|

Next, we are going to ask about a number of medical procedures which you might have undergone.

A19. Before 2000, had you ever undergone:	A20. When did you first undergo this procedure? *(If undergone more than once, record time of second procedure as well).*	A21. For what condition did you first undergo this procedure. *(If undergone more than once, record reason for second procedure as well).* ***(RECORD VERBATIM)***
a. An endoscopy, that is, a tube put down the throat to look at the esophagus or stomach: Yes ☐ No ☐ DK ☐	Age |__|__| **OR** Year |__|__|__|__| DK ☐	
	Age |__|__| **OR** Year |__|__|__|__| DK ☐	
b. A barium swallow to x-ray the esophagus or stomach: Yes ☐ No ☐ DK ☐	Age |__|__| **OR** Year |__|__|__|__| DK ☐	
	Age |__|__| **OR** Year |__|__|__|__| DK ☐	
c. Surgery on your esophagus: Yes ☐ No ☐ DK ☐	Age |__|__| **OR** Year |__|__|__|__| DK ☐	
	Age |__|__| **OR** Year |__|__|__|__| DK ☐	

A19. Before 2000, had you ever undergone:	A20. When did you first undergo this procedure? *(If undergone more than once, record time of second procedure as well).*	A21. For what condition did you first undergo this procedure. *(If undergone more than once, record reason for second procedure as well).* ***(RECORD VERBATIM)***
d. Surgery to remove all or part of your stomach: Yes ☐ No ☐ DK ☐	Age ___ **OR** Year ____ DK ☐	
	Age ___ **OR** Year ____ DK ☐	
e. Surgery to cut a nerve to the stomach, that is, a vagotomy: Yes ☐ No ☐ DK ☐	Age ___ **OR** Year ____ DK ☐	
	Age ___ **OR** Year ____ DK ☐	
f. Gallbladder surgery: Yes ☐ No ☐ DK ☐	Age ___ **OR** Year ____ DK ☐	
	Age ___ **OR** Year ____ DK ☐	

Thank you. Now, I have some questions about your family. We only need information about full blood relatives, that is your biological parents, biological children and siblings sharing the same mother and father. ***Note to interviewer: If respondent states that they were adopted, ask if they have any information about their biological family. If so, continue. If not, check here and skip these questions.***

SUBJECT ADOPTED ❑ → **SECTION B**

So, let's start with your parents. We'll discuss them one at a time.

QUESTION	FATHER	MOTHER
A22a. Any information available?	YES ❑ NO ❑	YES ❑ NO ❑
A22b. What year was ____ born?	|__|__|__|__| YEAR	|__|__|__|__| YEAR
A22c. Is _____ still living?	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑
A22d. In what year did ____ die?	|__|__|__|__| YEAR	|__|__|__|__| YEAR
A22e. Did ____ ever have cancer?	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑
A22f. What type(s) of cancer did ___ have? *(Use pages at end of questionnaire if more space needed)*	#1: _____ _____ #2: _____ _____	#1: _____ _____ #2: _____ _____
A22g. About how old was ____ when the cancer was diagnosed?	#1: |__|__| AGE D/K ❑ #2: |__|__| AGE D/K ❑	#1: |__|__| AGE D/K ❑ #2: |__|__| AGE D/K ❑
A22h. Was ____ ever diagnosed with Barrett's Esophagus?	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑
A22i. About how old was ____ when this was diagnosed?	|__|__| AGE D/K ❑	|__|__| AGE D/K ❑

Now, let's move on to your sisters.

How many full sisters do you have? |__|__|

I will now ask you questions about each sister, beginning with the oldest sister.

QUESTION	Sister #1	Sister #2	Sister #3	Sister #4
A23a. Any information available?	YES ❑ NO ❑	YES ❑ NO ❑	YES ❑ NO ❑	YES ❑ NO ❑
A23b. What year was she born?	|__|__|__|__| YEAR	|__|__|__|__| YEAR	|__|__|__|__| YEAR	|__|__|__|__| YEAR
A23c. Is she still living?	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑
A23d. In what year did she die?	|__|__|__|__| YEAR	|__|__|__|__| YEAR	|__|__|__|__| YEAR	|__|__|__|__| YEAR
A23e. Did she ever have cancer?	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑
A23f. What type(s) of cancer did she have? *(Use pages at end of questionnaire if more space needed)*	#1: _____ _____ #2: _____ _____	#1: _____ _____ #2: _____ _____	#1: _____ _____ #2: _____ _____	#1: _____ _____ #2: _____ _____
A23g. About how old was she when the cancer was diagnosed?	#1: |__|__| D/K ❑ AGE #2: |__|__| D/K ❑ AGE	#1: |__|__| D/K ❑ AGE #2: |__|__| D/K ❑ AGE	#1: |__|__| D/K ❑ AGE #2: |__|__| D/K ❑ AGE	#1: |__|__| D/K ❑ AGE #2: |__|__| D/K ❑ AGE
A23h. Has she ever been diagnosed with Barrett's Esophagus?	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑
A23i. About how old was she when this was diagnosed?	|__|__| D/K ❑ AGE	|__|__| D/K ❑ AGE	|__|__| D/K ❑ AGE	|__|__| D/K ❑ AGE

Now, let's move on to your brothers.

How many full brothers do you have? |__|__|

I will now ask you questions about each brother, beginning with the oldest brother.

QUESTION	Brother #1	Brother #2	Brother #3	Brother #4
A24a. Any information available?	YES ❑ NO ❑	YES ❑ NO ❑	YES ❑ NO ❑	YES ❑ NO ❑
A24b. What year was he born?	|__|__|__|__| YEAR	|__|__|__|__| YEAR	|__|__|__|__| YEAR	|__|__|__|__| YEAR
A24c. Is he still living?	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑
A24d. In what year did he die?	|__|__|__|__| YEAR	|__|__|__|__| YEAR	|__|__|__|__| YEAR	|__|__|__|__| YEAR
A24e. Did he ever have cancer?	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑
A24f. What type(s) of cancer did he have? *(Use pages at end of questionnaire if more space needed)*	#1: _____ _____ #2: _____ _____	#1: _____ _____ #2: _____ _____	#1: _____ _____ #2: _____ _____	#1: _____ _____ #2: _____ _____
A24g. About how old was he when the cancer was diagnosed?	#1: |__|__| D/K ❑ AGE #2: |__|__| D/K ❑ AGE	#1: |__|__| D/K ❑ AGE #2: |__|__| D/K ❑ AGE	#1: |__|__| D/K ❑ AGE #2: |__|__| D/K ❑ AGE	#1: |__|__| D/K ❑ AGE #2: |__|__| D/K ❑ AGE
A24h. Has he ever been diagnosed with Barrett's Esophagus?	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑
A24i. About how old was he when this was diagnosed?	|__|__| D/K ❑ AGE	|__|__| D/K ❑ AGE	|__|__| D/K ❑ AGE	|__|__| D/K ❑ AGE

Finally, let's talk about your biological children.

How many daughters do you have? |__|__|

I will now ask you questions about each daughter, beginning with the oldest child.

QUESTION	Daughter #1	Daughter #2	Daughter #3	Daughter #4
A25a. Any information available?	YES ❑ NO ❑	YES ❑ NO ❑	YES ❑ NO ❑	YES ❑ NO ❑
A25b. What year was she born?	|__|__|__|__| YEAR	|__|__|__|__| YEAR	|__|__|__|__| YEAR	|__|__|__|__| YEAR
A25c. Is she still living?	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑
A25d. In what year did she die?	|__|__|__|__| YEAR	|__|__|__|__| YEAR	|__|__|__|__| YEAR	|__|__|__|__| YEAR
A25e. Did she ever have cancer?	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑
A25f. What type(s) of cancer did she have? *(Use pages at end of questionnaire if more space needed)*	#1: _____ _____ #2: _____ _____	#1: _____ _____ #2: _____ _____	#1: _____ _____ #2: _____ _____	#1: _____ _____ #2: _____ _____
A25g. About how old was she when the cancer was diagnosed?	#1: |__|__| D/K ❑ AGE #2: |__|__| D/K ❑ AGE	#1: |__|__| D/K ❑ AGE #2: |__|__| D/K ❑ AGE	#1: |__|__| D/K ❑ AGE #2: |__|__| D/K ❑ AGE	#1: |__|__| D/K ❑ AGE #2: |__|__| D/K ❑ AGE
A25h. Has she ever been diagnosed with Barrett's Esophagus?	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑
A25i. About how old was she when this was diagnosed?	|__|__| D/K ❑ AGE	|__|__| D/K ❑ AGE	|__|__| D/K ❑ AGE	|__|__| D/K ❑ AGE

Lastly, we will talk about any biological sons you have.

How many sons do you have? |__|__|

I will now ask you questions about each son, beginning with the oldest child.

QUESTION	Son #1	Son #2	Son #3	Son #4
A26a. Any information available?	YES ❑ NO ❑	YES ❑ NO ❑	YES ❑ NO ❑	YES ❑ NO ❑
A26b. What year was he born?	|__|__|__|__| YEAR	|__|__|__|__| YEAR	|__|__|__|__| YEAR	|__|__|__|__| YEAR
A26c. Is he still living?	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑
A26d. In what year did he die?	|__|__|__|__| YEAR	|__|__|__|__| YEAR	|__|__|__|__| YEAR	|__|__|__|__| YEAR
A26e. Did he ever have cancer?	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑
A26f. What type(s) of cancer did he have? *(Use pages at end of questionnaire if more space needed)*	#1: _____ _____ #2: _____ _____	#1: _____ _____ #2: _____ _____	#1: _____ _____ #2: _____ _____	#1: _____ _____ #2: _____ _____
A26g. About how old was he when the cancer was diagnosed?	#1: |__|__| D/K ❑ AGE #2: |__|__| D/K ❑ AGE	#1: |__|__| D/K ❑ AGE #2: |__|__| D/K ❑ AGE	#1: |__|__| D/K ❑ AGE #2: |__|__| D/K ❑ AGE	#1: |__|__| D/K ❑ AGE #2: |__|__| D/K ❑ AGE
A26h. Has he ever been diagnosed with Barrett's Esophagus?	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑
A26i. About how old was he when this was diagnosed?	|__|__| D/K ❑ AGE	|__|__| D/K ❑ AGE	|__|__| D/K ❑ AGE	|__|__| D/K ❑ AGE

SECTION B
MEDICATIONS

Thank you. You are doing great. We are now going to ask about medications you might have taken. All of these questions refer to regular use of medications. That is, using it at least two or more times a week for one month or longer.

IF YES TO B1, ASK B2-4, AS INDICATED.

B1. Did you ever regularly take:	B2. When was the first time you regularly used _____	B3. When was the **last time** you regularly used _____	B4. On average, when you were using _____, how much did you normally take?
a. Tums, Rolaids or similar Antacids? ☐ Yes ☐ No ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__| ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__| ☐ DK ☐ Still Use	____ Pills ____ Day ____ Tsp ____ Wk ____ Tbsp ____ Mon ____ ml. ____ Yr.
b. Motrin, Advil or similar Non-steroidal anti-inflamatories. ☐ Yes ☐ No ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__| ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__| ☐ DK ☐ Still Use	____ Pills ____ Day ____ Tsp ____ Wk ____ Tbsp ____ Mon ____ ml. ____ Yr.
c. Losec or Pantaloc or similar medications (these drugs are often called Proton Pump Inhibitors) ☐ Yes ☐ No ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__| ☐ DK	AGE |__|__| **OR** YEAR |__|__|__|__| ☐ DK ☐ Still Use	____ Pills ____ Day ____ Tsp ____ Wk ____ Tbsp ____ Mon ____ ml. ____ Yr.

B1. Did you ever regularly take:	B2. When was the first time you regularly used _____	B3. When was the **last time** you regularly used _____	B4. On average, when you were using _____, how much did you normally take?
d. Theophyline Yes ☐ No ☐ DK ☐	AGE |__|__| **OR** YEAR |__|__|__|__| DK ☐	AGE |__|__| **OR** YEAR |__|__|__|__| DK ☐ Still Use ☐	____ Pills ____ Day ____ Tsp ____ Wk ____ Tbsp ____ Mon ____ ml. ____ Yr.
e. Zantac, Tagamet or Pepcid? Yes ☐ No ☐ DK ☐	AGE |__|__| **OR** YEAR |__|__|__|__| DK ☐	AGE |__|__| **OR** YEAR |__|__|__|__| DK ☐ Still Use ☐	____ Pills ____ Day ____ Tsp ____ Wk ____ Tbsp ____ Mon ____ ml. ____ Yr.
f. Motilium or Prepulsid? Yes ☐ No ☐ DK ☐	AGE |__|__| **OR** YEAR |__|__|__|__| DK ☐	AGE |__|__| **OR** YEAR |__|__|__|__| DK ☐ Still Use ☐	____ Pills ____ Day ____ Tsp ____ Wk ____ Tbsp ____ Mon ____ ml. ____ Yr.
g. Thiazide diuretics? Yes ☐ No ☐ DK ☐	AGE |__|__| **OR** YEAR |__|__|__|__| DK ☐	AGE |__|__| **OR** YEAR |__|__|__|__| DK ☐ Still Use ☐	____ Pills ____ Day ____ Tsp ____ Wk ____ Tbsp ____ Mon ____ ml. ____ Yr.

SECTION C
DIET

The next series of questions will be about your diet. I will be asking you about a number of common foods. For each of the food, I will ask the number of times you ate it as well as how they were prepared. Some of these foods were include on the other food questionnaire you completed. In this part of the interview, we are trying to get some more detail about some of these foods. Some of the information may be hard to remember. Please try to give your most accurate estimation. Some of the common methods of cooking we are looking at include: pan frying, deep frying, oven broiling, baking, grilling and microwaving.

We will start by asking about your use and preparation of meat and eggs during 1999.

C1. EGGS

	Never	Less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	What was your usual serving size?
C1.1 During 1999, how often did you eat eggs? *(If 'never', skip to C2)*	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____ eggs (1-80)
C1.2 During 1999, when you ate eggs, how often were they pan-fried and sunny side-up?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C1.3 During 1999, when you ate eggs, how often were they prepared in any other way?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	

C2. HAMBURGERS

	Never	Less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	What was your usual serving size?
C2.1 During 1999, how often did you eat hamburgers or cheeseburgers, including fast food? ***(If 'never', go to C3)***	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	____ oz. (1-80)
C2.2 During 1999, when you ate hamburgers and cheeseburgers, how often were they pan fried?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C2.3 During 1999, when you ate hamburgers and cheeseburgers, how often were they grilled or barbecued?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C2.4 During 1999, when you ate hamburgers and cheeseburgers, how often were they oven broiled?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C2.5 During 1999, when you ate hamburgers and cheeseburgers, how often were they prepared in a way I have not mentioned?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	

Now, I would like you to look at a photograph of hamburgers which have been cooked to different degrees. Please decide which photograph most closely resembles the way the hamburgers you usually eat are cooked. If you eat hamburgers that look to be between categories, you may indicate that. For example, you would select 2.5 to indicate that the hamburgers you eat look between pictures 2 and 3.

Please pay special attention to the way the hamburgers look inside as well as outside. For example, there is little difference in the internal appearances of the hamburgers shown in pictures 3 and 4 but there is more browning and charring on the external surface of picture 2 as compared to picture 3.

C2.6 During 1999, when you ate hamburgers and cheeseburgers, *which* picture most closely resembles the way they were usually cooked? *(SHOW PHOTOGRAPH OF HAMBURGERS)*

Don't eat	❑	2.5	❑
0	❑	3.0	❑
0.5	❑	3.5	❑
1.0	❑	4.0	❑
1.5	❑	4.5	❑
2.0	❑	DK	❑

C3. BEEF STEAKS

	Never	Less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	What was your usual serving size?
C3.1 During 1999, how often did you eat beef steaks? ***(If 'never', go to C4)***	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____ oz. (1-180)
C3.2 During 1999, when you ate beef steaks, how often were they *pan fried*?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C3.3 During 1999, when you ate beef steaks, how often were they grilled or barbecued?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C3.4 During 1999, when you ate beef steaks, how often were they oven broiled?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C3.5 During 1999, when you ate beef steaks, how often were they prepared in a way I have not mentioned?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	

Now, I would like you to look at a photograph of beef steaks which have been cooked to different degrees. Please decide which photograph most closely resembles the way the beef steaks you usually eat are cooked. If you eat beef steaks that look to be between categories, you may indicate that. For example, you would select 2.5 to indicate that the beef steaks you eat look between pictures 2 and 3.

Please pay special attention to the way the beef steaks look inside as well as outside. For example, there is little difference in the internal appearances of the beef steaks shown in pictures 3 and 4 but there is more browning and charring on the external surface of picture 2 as compared to picture 3.

C3.6 During 1999, when you ate beef steaks, which picture most closely resembles the way they were usually cooked? *(SHOW PHOTOGRAPH OF BEEF STEAKS)*

Don't eat	❑	2.5	❑
0	❑	3.0	❑
0.5	❑	3.5	❑
1.0	❑	4.0	❑
1.5	❑	4.5	❑
2.0	❑	DK	❑

C4. PORK CHOPS AND HAM

	Never	Less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	What was your usual serving size?
C4.1 During 1999, how often did you eat pork chops or ham? ***(If 'never', go to C5)***	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____ oz. (1-80)
C4.2 During 1999, when you ate pork chops or ham, how often were they pan fried?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C4.3 During 1999, when you ate pork chops or ham, how often were they oven broiled?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C4.4 During 1999, when you ate pork chops or ham, how often were they baked or roasted?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C4.5 During 1999, when you ate pork chops or ham, how often were they prepared in a way I have not mentioned?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	

Now, I would like you to look at a photograph of pork chops which have been cooked to different degrees. Please decide which photograph most closely resembles the way the pork chops you usually eat are cooked. If you eat pork chops that look to be between categories, you may indicate that. For example, you would select 2.5 to indicate that the pork chops you eat look between pictures 2 and 3.

C4.6 During 1999, when you ate pork chops, which picture most closely resembles the way they were usually cooked? *(SHOW PHOTOGRAPH OF PORK CHOPS)*

Don't eat	❑	2.0	❑
0	❑	2.5	❑
0.5	❑	3.0	❑
1.0	❑	3.5	❑
1.5	❑	DK	❑

We have talked about pork chops and ham in general. Now I would like to ask you about how often you eat different kinds of ham.

	Never	Less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	What was *your usual* serving size?
C4.7 During 1999, how often did you eat smoked ham?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	____ oz. (1-80)
C4.8 During 1999, how often did you eat cured ham?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	____ oz. (1-80)
C4.9 During 1999, how often did you eat cooked ham?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	____ oz. (1-80)
C4.10 During 1999, how often did you eat canned ham?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	____ oz. (1-80)
C4.11 During 1999, how often did you eat cottage roll?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	____ oz. (1-80)
C4.12 During 1999, how often did you eat semi-cured ham?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	____ oz. (1-80)

C5.BACON

	Never	Less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	What was your usual serving size?
C5.1 During 1999, how often did you eat bacon? ***(If 'never', go to C6)***	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	_____ # strips
C5.2 During 1999, when you ate bacon, how often was it pan fried?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
C5.3 During 1999, when you ate bacon, how often was it grilled or barbequed?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
C5.4 During 1999, when you ate bacon, how often was it oven broiled?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
C5.5 During 1999, when you ate bacon, how often was it micro-waved?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
C5.6 During 1999, when you ate bacon, how often was it prepared in a way I have not mentioned?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
C5.7 During 1999, how often did you use the fat from fried bacon in your cooking?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	

Now, I would like you to look at a photograph of bacon which has been cooked to different degrees. Please decide which photograph most closely resembles the way the bacon you usually eat is cooked. If you eat bacon that looks to be between categories, you may indicate that. For example, you would select 2.5 to indicate that the bacon you eat looks between pictures 2 and 3.

C5.8 During 1999, when you ate bacon, which picture most closely resembles the way it was usually cooked? *(SHOW PHOTOGRAPH OF BACON)*

Don't eat	☐	2.0	☐
0	☐	2.5	☐
0.5	☐	3.0	☐
1.0	☐	3.5	☐
1.5	☐	DK	☐

We have talked about bacon in general. Now I would like to ask you about how often you eat different kinds of bacon.

	Never	Less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	What was your usual serving size?
C5.9 During 1999, how often did you eat unsmoked side bacon?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	_____ # strips
C5.10 During 1999, how often did you eat unsmoked back bacon?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	_____ # strips
C5.11 During 1999, how often did you eat peameal bacon?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	_____ # strips
C5.12 During 1999, how often did you eat smoked bacon?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	_____ # strips
C5.13 During 1999, how often did you eat other kinds of bacon I ham not mentioned?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	_____ # strips

C6.SAUSAGE

	Never	Less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	What was your usual serving size?
C6.1 During 1999, how often did you eat sausage (including breakfast, Italian, Polish and Bratwurst)? ***(If 'never', go to C7)***	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	_____ servings
C6.2 During 1999, when you ate sausage, how often was it pan fried?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
C6.3 During 1999, when you ate sausage, how often was it grilled or barbequed?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
C6.4 During 1999, when you ate sausage, how often was it oven broiled?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
C6.5 During 1999, when you ate sausage, how often was it micro-waved?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
C6.6 During 1999, when you ate sausage, how often was it prepared in a way I have not mentioned?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
C6.7 During 1999, when you ate sausage, how well done did you usually cook it? Was it:	Don't eat ☐ Just until done ☐ Well-done/crisp ☐ Charred ☐ DK ☐										

We have talked about sausage in general. Now I would like to ask you about how often you eat different kinds of sausage.

	Never	Less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	What was your usual serving size?
C6.8 During 1999, how often did you eat unsmoked sausage?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____ servings
C6.9 During 1999, how often did you eat smoked sausage?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____ servings
C6.10 During 1999, how often did you eat German sausage?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____ servings
C6.11 During 1999, how often did you eat Polish sausage?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____ servings

C7. HOT DOGS AND FRANKS

	Never	Less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	What was your usual serving size?
C7.1 During 1999, how often did you eat hot dogs or franks? ***(If 'never', go to C8)***	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____ hot dogs
C7.2 During 1999, when you ate hot dogs or franks, how often were they pan fried?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C7.3 During 1999, when you ate hot dogs or franks, how often were they grilled or barbecued?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C7.4 During 1999, when you ate hot dogs or franks, how often were they oven broiled?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C7.5 During 1999, when you ate hot dogs or franks, how often were they prepared in a way I have not mentioned?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	

C8. CHICKEN/TURKEY

	Never	Less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	What was your usual serving size?
C8.1 During 1999, how often did you eat fried chicken? *(If 'never', go to C9)*	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____ oz
C8.2 During 1999, when you ate fried chicken, how often was it deep fat fried or fast food?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C8.3 During 1999, when you ate fried chicken, how often was it pan fried?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	

Now, I would like you to look at a photograph of fried chicken which has been cooked to different degrees. Please decide which photograph most closely resembles the way the fried chicken you usually eat is cooked. If you eat fried chicken that looks to be between categories, you may indicate that. For example, you would select 2.5 to indicate that the fried chicken you eat looks between pictures 2 and 3.

C8.4 During 1999, when you ate fried chicken, which picture most closely resembles the way it was usually cooked? *(SHOW PHOTOGRAPH OF PAN-FRIED CHICKEN)*

Don't eat	❑	2.0	❑
0	❑	2.5	❑
0.5	❑	3.0	❑
1.0	❑	3.5	❑
1.5	❑	DK	❑

Now, we are going to talk about ways you might eat chicken or turkey other than as fried chicken.

	Never	Less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	What was your usual serving size?
C8.5 Other than fried chicken, during 1999, how often did you eat chicken or turkey (including on sandwiches)?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____ oz
C8.6 During 1999, when you ate chicken or turkey, how often was it baked or roasted?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C8.7 During 1999, when you ate chicken or turkey, how often was it stewed?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C8.8 During 1999, when you ate chicken or turkey, how often was it oven broiled?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C8.9 During 1999, when you ate chicken or turkey, how often was it grilled or barbecued?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C8.10 During 1999, when you ate chicken or turkey, how often was it prepared in a way I have not mentioned?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	

Now, I would like you to look at a photograph of grilled chicken bacon which has been cooked to different degrees. Please decide which photograph most closely resembles the way the grilled chicken you usually eat is cooked. If you eat grilled chicken that looks to be between categories, you may indicate that. For example, you would select 2.5 to indicate that the grilled chicken you eat looks between pictures 2 and 3.

C8.11 During 1999, when you ate grilled chicken, which picture most closely resembles the way it was usually cooked? *(SHOW PHOTOGRAPH OF GRILLED CHICKEN)*

Don't eat	❑	2.0	❑
0	❑	2.5	❑
0.5	❑	3.0	❑
1.0	❑	3.5	❑
1.5	❑	DK	❑

C9. FISH

	Never	Less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	What was your usual serving size?
C9.1 During 1999, how often did you eat fried fish or fish sandwiches? ***(If 'never', go to C10)***	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____ oz
C9.2 During 1999, when you ate fried fish or fish sandwiches, how often were they pan fried?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C9.3 During 1999, when you ate fried fish or fish sandwiches, how often were they deep fried or fast food?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	

Now, we are going to talk about ways you might eat fish other than as fried fish or in fish sandwiches.

	Never	Less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	What was your usual serving size?
C9.4 During 1999, how often did you eat fish beside fried fish (including tuna)?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____ oz
C9.5 During 1999, when you ate fish other than fried fish, how often was it oven broiled?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C9.6 During 1999, when you ate fish other than fried fish, how often was it baked?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C9.7 During 1999, when you ate fish other than fried fish, how often was it in a casserole?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C9.8 During 1999, when you ate fish other than fried fish, how often was it in a salad?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C9.9 During 1999, when you ate fish other than fried fish, how often was it prepared in a way I have not mentioned?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	

C10. MISCELLANEOUS FOODS

	Never	Less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	What was your usual serving size?
C10.1 During 1999, how often did you eat roast beef (including sandwiches)?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	_____ oz
C10.2 During 1999, how often did you eat beef stew or pot pie with carrots or other vegetables?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	_____ oz
C10.3 During 1999, how often did you eat other ground beef (include meat loaf or taco)?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	_____ oz
C10.4 During 1999, how often did you eat meat gravies made with meat drippings?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	_____ tsp

C10.5 During 1999, when you ate meat gravies, how were they usually made?	Don't eat ☐ From meat drippings ☐ From can ☐ From packets ☐ DK ☐

	Never	Less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	What was your usual serving size?
C10.6 During 1999, how often did you eat toast at any meal?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	_____ slices

C10.7 During 1999, when you ate toast, how well done was it?	Don't eat ☐ Light ☐ Dark ☐ Very dark ☐ DK ☐

	Never	Less than once per month	1 per mon	2-3 per mon	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	What was your usual serving size?
C10.8 During 1999, how often did you eat fried bread?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____ slices
C10.9 During 1999, how often did you eat fats on bread which were saved after cooking. This means things such as bacon fat on bread.	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	_____ slices
C10.10 During 1999, in the summer, how often did you eat grilled or barbecued meats (including beef, pork, chicken or fish)?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C10.11 During 1999, in the remainder of the year, how often did you eat grilled or barbecued meats (including beef, port, chicken or fish)?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	
C10.12 During 1999, when you had grilled or barbecued meats, how often were they charred?	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	

SECTION D
SMOKING

Thank you. That's the end of the questions on diet. Now we have some questions on smoking.

D1	QUESTION	D1. Cigarettes
a.	Have you ever smoked at least 100 cigarettes in your entire life?	YES ❑ NO ❑ D/K ❑ *(IF 'NO', go to Question D2)*
b.	Has there ever been a period when you smoked cigarettes regularly (at least once a week)?	YES ❑ NO ❑ D/K ❑ *(IF 'NO' or 'D/K', go to Question D2)*
c.	How old were you when you first started smoking cigarettes regularly?	AGE |__|__| **OR** YEAR |__|__|__|__|
d.	Do you still smoke cigarettes now?	YES ❑ NO ❑ D/K ❑ *(If 'NO', ask part 'e')*
	e. When did you smoke your last cigarette?	_____ _____
f.	During all the years you smoked cigarettes, about how many did you smoke per day on average?	_____ cigarettes per day
g.	Were there periods when you gave up smoking for at least 12 months and then took it up again? *h. (If yes, record details about start and stop times for each period. Use back of page if more space needed.)*	YES ❑ NO ❑ D/K ❑ #1: _____ _____ #2: _____ _____

D1h. **Were you smoking regularly at:**	**D1i.** **How many cigarettes were you smoking each day (on average) at:**	**D1j.** **What brand of cigarette did you smoke most often at:**	**D1k.** **What type of cigarettes were these? Were they:**
I. Age 25 Yes ☐ No ☐ DK ☐	_____ Cigs/day	_____ _____ _____	Filter ☐ Non-filter ☐ Hand-rolled ☐ DK ☐
II. Age 40 Yes ☐ No ☐ DK ☐ NA ☐	_____ Cigs/day	_____ _____ _____	Filter ☐ Non-filter ☐ Hand-rolled ☐ DK ☐
III. Age 50 Yes ☐ No ☐ DK ☐ NA ☐	_____ Cigs/day	_____ _____ _____	Filter ☐ Non-filter ☐ Hand-rolled ☐ DK ☐
IV. Age 60 Yes ☐ No ☐ DK ☐ NA ☐	_____ Cigs/day	_____ _____ _____	Filter ☐ Non-filter ☐ Hand-rolled ☐ DK ☐

D2 to D5	QUESTION	D2. Cigars or Cigarillos	D3. Pipes	D4. Chewing Tobacco	D5. Snuff
a.	Has there ever been a period when you _____ regularly (at least once a week)?	YES ☐ NO ☐ D/K ☐	YES ☐ NO ☐ D/K ☐	YES ☐ NO ☐ D/K ☐	YES ☐ NO ☐ D/K ☐
b.	Have you _____ for longer than 6 months at a time?	YES ☐ NO ☐ D/K ☐	YES ☐ NO ☐ D/K ☐	YES ☐ NO ☐ D/K ☐	YES ☐ NO ☐ D/K ☐
c.	How old were you when you first started _____ regularly?	AGE |__|__| **OR** YEAR |__|__|__|__|	AGE |__|__| **OR** YEAR |__|__|__|__|	AGE |__|__| **OR** YEAR |__|__|__|__|	AGE |__|__| **OR** YEAR |__|__|__|__|
d.	Do you still _____ now?	YES ☐ NO ☐ D/K ☐ *(If 'NO', ask part 'e')*	YES ☐ NO ☐ D/K ☐ *(If 'NO', ask part 'e')*	YES ☐ NO ☐ D/K ☐ *(If 'NO', ask part 'e')*	YES ☐ NO ☐ D/K ☐ *(If 'NO', ask part 'e')*
	e. When did you last _____ ?	_____ _____	_____ _____	_____ _____	_____ _____
f.	During all the years you _____, about how many _____ did you _____ per day on average?	_____ cigars/cigarillos per day	_____ bowls per day	_____ times per day	_____ times per day
g.	Were there periods when you gave up _____ for at least 12 months and then took it up again? *(If yes, record details about start and stop times for each period. Use back of page if more space needed.)*	YES ☐ NO ☐ D/K ☐ #1: _____ _____ #2: _____ _____	YES ☐ NO ☐ D/K ☐ #1: _____ _____ #2: _____ _____	YES ☐ NO ☐ D/K ☐ #1: _____ _____ #2: _____ _____	YES ☐ NO ☐ D/K ☐ #1: _____ _____ #2: _____ _____

The next questions concern the residue left over from pipe smoking. We have found that some people use this residue in various ways such as mixing it with water and drinking it.

D6.	QUESTION	D6. Pipe Residue
a.	Has there ever been a period when you reused pipe residue regularly (at least once per week)?	YES ❑ NO ❑ D/K ❑ *(IF 'NO', go to Next Page)*
b.	Have you ever reused pipe residue for longer than 6 months at a time?	YES ❑ NO ❑ D/K ❑ *(IF 'NO' or 'D/K', go to Next Page)*
c.	How old were you when you first started reusing pipe residue regularly?	AGE |__|__| **OR** YEAR |__|__|__|__|
d.	Do you still reuse pipe residue now?	YES ❑ NO ❑ D/K ❑ *(If 'NO', ask part 'e')*
	e. When did you consume your last mixture of pipe residue?	_____ _____
f.	During all the years you reused pipe residue, about how many times did you reuse pipe residue per day on average?	_____ times per day
g.	Were there periods when you gave up reusing pipe residue for at least 12 months and then took it up again?	YES ❑ NO ❑ D/K ❑
	h. (If yes, record details about start and stop times for each period. Use back of page if more space needed.)	#1: _____ _____ #2: _____ _____

Finally, I need to find out if either of your parents were regular smokers.

		D7. FATHER	D8. MOTHER
a.	Was your _____ ever a regular smoker?	YES ❑ NO ❑ D/K ❑ *(IF 'NO' or 'D/K', go to Next Section)*	YES ❑ NO ❑ D/K ❑ *(IF 'NO' or 'D/K', go to Next Section)*
b.	For how many years was your _____ a regular smoker?	_____ Years	_____ Years
c.	Would you say that your _____was a light, medium or heavy smoker?	Light ❑ Medium ❑ Heavy ❑ DK ❑	Light ❑ Medium ❑ Heavy ❑ DK ❑

GO ON TO SECTION 'E'

SECTION E
ALCOHOL

Now I have some questions about your consumption of alcoholic beverages. Please think about your habits over your entire adult life.

E1. Have you ever had at least 12 drinks of any kind of alcoholic beverage in your entire life?

YES ❑ NO ❑ DON'T KNOW ❑

If subject answers 'YES' or 'DON'T KNOW', ask questions E1, E2 and E3. Otherwise, skip to SECTION F.

QUESTION	E1. BEER (12 oz bottle/can)	E2. WINE (4 oz glass)	E3. HARD LIQUOR (1 drink)
a. Has there ever been a period when you drank at least _____ per month for six months or longer?	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑	YES ❑ NO ❑ D/K ❑
b. How old were you when you first started drinking _____ regularly?	AGE |__|__| **OR** YEAR |__|__|__|__|	AGE |__|__| **OR** YEAR |__|__|__|__|	AGE |__|__| **OR** YEAR |__|__|__|__|
c When did you stop drinking _____ regularly?	_____ _____ STILL DRINK ❑	_____ _____ STILL DRINK ❑	_____ _____ STILL DRINK ❑

QUESTION	E1. BEER (12 oz bottle/can)	E2. WINE (4 oz glass)	E3. HARD LIQUOR (1 drink)
d. During all the years you drank ____ regularly, about how much did you usually drink per day, week or month? *(use units for each type of drink)*	____ bottles per day ____ bottles per wk ____ bottles per mon	____ glasses per day ____ glasses per wk ____ glasses per mon	_____ per day _____ per week _____ per mon Shot or mixed drinks ☐ Ounces ☐ Half-pints ☐ Fifths ☐ Quarts ☐ Half-gallons ☐ Gallons ☐ Other (specify) ☐ _____
e. Were there periods when you gave up drinking ____ for at least 12 months and then took it up again?	YES ☐ NO ☐ D/K ☐	YES ☐ NO ☐ D/K ☐	YES ☐ NO ☐ D/K ☐
(If yes, record details about start and stop times for each period. Use back of page if more space needed.)	#1: _____ _____ #2: _____ _____	#1: _____ _____ #2: _____ _____	#1: _____ _____ #2: _____ _____

QUESTION	E1. BEER (12 oz bottle/can)	E2. WINE (4 oz glass)	E3. HARD LIQUOR (1 drink)
g. What type of hard liquor did/do you drink most often?			☐ Gin ☐ Vodka ☐ Bourbon ☐ Scotch ☐ Whiskey ☐ Rum ☐ Tequila ☐ Brandy ☐ Other (specify) _____
h. Did/do you usually drink straight hard liquor, add hard liquor to a mixer or drink straight hard liquor and mixed drinks equally?			☐ Drink straight hard liquor ☐ Add hard liquor to mixer ☐ Drink straight hard liquor and mixed drinks equally
i. What kind of mixer did/do you usually add?			☐ NONE ☐ Water ☐ Juice ☐ Soda ☐ Tonic water ☐ Other (specify) _____

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SECTION F
GENERAL

Finally, the end is in sight. The last few questions are more general about you as a person and your background. We are going to start off by asking about your ethnic background and birthplace.

F1.	*(INTERVIEWER: Record but do not ask the gender of the participant.)*	Male ❑ Female ❑
F2.	What do you consider to be your race or ethnic group? If you belong to more than one group, please tell me all the groups you belong to.	
	A) White or Caucasian	❑ YES ❑ NO ❑ DK
	B) Black, Afro-American or African ancestry	❑ YES ❑ NO ❑ DK
	C) Native Canadian, Native Alaskan, or Indigenous	❑ YES ❑ NO ❑ DK
	D) Asian or Pacific Islander	❑ YES ❑ NO ❑ DK
	E) Other (specify) _____ _____	❑ YES ❑ NO ❑ DK

F3.	What is your father's race or ethnic group? If he belongs to more than one group, please tell me all the groups he belongs to. A) White or Caucasian B) Black, Afro-American or African ancestry C) Native Canadian, Native Alaskan, or Indigenous D) Asian or Pacific Islander E) Other (specify) _____ _____	☐ YES ☐ NO ☐ DK ☐ YES ☐ NO ☐ DK ☐ YES ☐ NO ☐ DK ☐ YES ☐ NO ☐ DK ☐ YES ☐ NO ☐ DK
F4.	What is your mother's race or ethnic group? If she belongs to more than one group, please tell me all the groups she belongs to. A) White or Caucasian B) Black, Afro-American or African ancestry C) Native Canadian, Native Alaskan, or Indigenous D) Asian or Pacific Islander E) Other (specify) _____ _____	☐ YES ☐ NO ☐ DK ☐ YES ☐ NO ☐ DK ☐ YES ☐ NO ☐ DK ☐ YES ☐ NO ☐ DK ☐ YES ☐ NO ☐ DK

F5.	In what country were you born?	_____ _____ _____
	(IF NOT BORN IN CANADA, ASK NEXT QUESTION; OTHERWISE SKIP TO F6.) F5a. How long have you lived in Canada? *(Record age at immigration or year of immigration).*	AGE |__|__| OR YEAR |__|__|__|__| DK ☐
F6.	What language did you first learn to speak?	_____ _____
F7.	What languages did your father speak?	1. _____ 2. _____ 3. _____ 4. _____
F8.	What languages did your mother speak?	1. _____ 2. _____ 3. _____ 4. _____

Now, we are going to move on to some more general questions.

F9.	What is your present marital status?	Married/living as married ☐ Widowed ☐ Divorced ☐ Separated ☐ Never married ☐ DK ☐
F10.	What is the highest grade or level of schooling you have completed?	Less than 8 years ☐ 8-11 years ☐ 12 years or completed high school ☐ Vocational, Technical or business training ☐ Some college ☐ Graduated from college ☐ Some university undergrad. courses ☐ Undergraduate degree ☐ Some university graduate courses ☐ Graduate degree ☐ DK ☐
F11.	Considering all of sources of income, please tell me which of the following represents your total household income during the last calendar year.	A) Less than $15,000 ☐ B) $15,000 - $29,999 ☐ C) $30,000 - $49,999 ☐ D) $50,000 - $74,999 ☐ E) $75,000 - $99,999 ☐ F) $100,000 or more ☐ Refused to answer ☐
F11a.	How many people were supported by this income?	_____ People

For the purpose of the study, we would also like to know what types of prescription medications you have taken in the past and at present. However, we realize that many people may not be able to tell us the name of the various medications which their doctor has prescribed. In addition, it would take a great deal of time to go through lists of all possible medications prescribed for various illnesses. Therefore, we were wondering if you would allow us to contact your doctor and get a list of the various prescriptions you have had from him/her?

(IF PARTICIPANT AGREES, HAVE PARTICIPANT PROVIDE DOCTOR NAMES AND SIGN CONSENT FORM.)

Thank you very much for taking part in this interview. I realize that it was very long and we appreciate the time that you have given us.

Do you have any questions about the study?
(Interviewer answers any questions the participant may have).

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Appendix Biii. Results from Sensitivity Analyses

Appendix Biii: Table 1. Adjusted* odds ratios for iron intakes, red meat and processed meat and EAC - sensitivity analyses.

			OR-main† (95% CI)			
Primary Exposure	**Variable/ Category**			**OR-elig (95% CI)**	**OR-mwh (95% CI)**	**OR-noC (95% CI)**
Red meat intake	continuous, +25		1.58 (1.00-2.51)	1.40 (0.83-2.38)	1.70 (1.05-2.76)	1.44 (0.89-2.33)
(g/day)		Q1	1.00	1.00	1.00	1.00
		Q2	0.70 (0.31-1.59)	0.64 (0.25-1.63)	0.57 (0.24-1.35)	0.79 (0.34-1.86)
		Q3	0.90 (0.41-2.01)	1.04 (0.44-2.48)	0.78 (0.34-1.77)	1.05 (0.46-2.40)
		Q4	1.04 (0.47-2.32)	1.09 (0.46-2.63)	0.93 (0.41-2.12)	1.05 (0.45-2.42)
		Q5	1.30 (0.55-3.08)	1.32 (0.52-3.31)	1.55 (0.65-3.70)	1.33 (0.55-3.22)
Processed meat intake	continuous, +25		1.62 (0.54-4.84)	2.06 (0.65-6.54)	1.76 (0.58-5.38)	2.03 (0.64-6.43)
(g/day)		Q1	1.00	1.00	1.00	1.00
		Q2	1.85 (0.80-4.28)	1.48 (0.59-3.73)	1.93 (0.81-4.61)	2.14 (0.88-5.22)
		Q3	1.20 (0.51-2.83)	1.03 (0.40-2.67)	1.13 (0.46-2.77)	1.55 (0.64-3.78)
		Q4	1.35 (0.57-3.18)	1.33 (0.52-3.37)	1.57 (0.65-3.81)	1.91 (0.78-4.69)
		Q5	1.49 (0.59-3.74)	1.50 (0.55-4.10)	1.74 (0.68-4.42)	1.88 (0.72-4.95)
Dietary Fe intake	continuous, +5		1.01 (0.63-1.62)	0.90 (0.54-1.52)	1.13 (0.69-1.86)	0.99 (0.61-1.61)
(mg/day)		Q1	1.00	1.00	1.00	1.00
		Q2	0.74 (0.31-1.76)	0.61 (0.23-1.64)	1.17 (0.45-3.02)	0.81 (0.34-1.97)
		Q3	0.23 (0.48-3.16)	1.38 (0.49-3.88)	1.95 (0.71-5.37)	1.51 (0.57-4.01)
		Q4	1.02 (0.37-2.79)	1.09 (0.36-3.30)	2.18 (0.75-6.33)	1.03 (0.36-2.93)
		Q5	1.15 (0.33-3.95)	1.95 (0.24-3.75)	2.00 (0.53-7.57)	1.44 (0.40-5.26)
Heme Fe intake	continuous, +1		1.34 (0.91-1.98)	1.33 (0.86-2.05)	1.47 (0.97-2.22)	1.21 (0.82-1.79)
(mg/day)		Q1	1.00	1.00	1.00	1.00
		Q2	1.32 (0.59-2.99)	2.16 (0.85-5.47)	2.08 (0.89-4.84)	1.51 (0.66-3.46)
		Q3	1.09 (0.48-2.47)	1.34 (0.51-3.49)	1.36 (0.56-3.29)	0.95 (0.40-2.22)
		Q4	1.12 (0.48-2.66)	1.91 (0.72-5.05)	1.74 (0.71-4.26)	1.34 (0.56-3.22)
		Q5	2.23 (0.89-5.58)	2.91 (1.03-8.22)	2.85 (1.09-7.47)	1.78 (0.70-4.55)

* Adjusted for age (40-49, 50-59, 60-69, ≥70 years), gender, geographical region (Ontario/Quebec), smoking (pack years, continuous), severe heartburn (ever - Yes/No), BMI (continuous), total energy intake (continuous), frequency of alcohol intake (quintiles) and vegetable intake (quintiles).

† OR-main, odds ratios from main analysis. OR-elig, odds ratios with exclusion of those outside the population base. OR-mwh, odds ratios in the sample when restricted to white males only. OR-no C, odds ratios excluding all subjects who had had any previous cancer except basal cell skin cancer.

Appendix Biii: Table 2. Adjusted* odds ratios comparing genotype groups – sensitivity analyses

Polymorphism		OR-main† (95% CI)	OR-elig (95% CI)	OR-mwh (95% CI)	OR-noC (95% CI)
HFE-C282Y					
Genotype	YY	-	-	-	-
	CY	0.74 (0.38-1.44)	0.72 (0.35-1.48)	0.81 (0.41-1.58)	0.88 (0.42-1.81)
	CC	1.00	1.00	1.00	1.00
HFE-H63D					
Genotype	DD	-	-	-	-
	HD	1.00 (0.60-1.67)	0.78 (0.44-1.39)	1.04 (0.60-1.80)	0.90 (0.53-1.55)
	HH	1.00	1.00	1.00	1.00
HFE-any‡					
	Yes	0.94 (0.59-1.49)	0.75 (0.45-1.24)	1.01 (0.62-1.64)	0.93 (0.57-1.51)
	No	1.00	1.00	1.00	1.00
HFE-hi iron§					
	Yes	0.77 (0.40-1.48)	0.77 (0.38-1.55)	0.84 (0.43-1.63)	0.92 (0.45-1.86)
	No	1.00	1.00	1.00	1.00
TFRC-S142G					
Genotype	GG	0.95 (0.48-1.88)	1.06 (0.52-2.17)	0.95 (0.46-1.97)	1.12 (0.54-2.33)
	SG	1.31 (0.80-2.16)	1.31 (0.77-2.24)	1.50 (0.88-2.55)	1.47 (0.86-2.49)
	SS	1.00	1.00	1.00	1.00
HFE/TFRC combined genotypes (as per Beckman et al., 1999)					
TFRC-SS/Y282 carrier		0.41 (0.15-1.15)	0.30 (0.09-1.04)	0.45 (0.16-1.26)	0.35 (0.10-1.28)
Other		1.00	1.00	1.00	1.00
TFRC-SS/*HFE*-DY		0.35 (0.04-3.17)	0.42 (0.05-3.89)	0.35 (0.04-3.23)	0.49 (0.05-4.87)
		1.00	1.00	1.00	1.00

* Adjusted for age (40-49, 50-59, 60-69, ≥70 years), gender and geographical region (Quebec/Ontario).
† OR-main, odds ratios from main analysis. OR-elig, odds ratios with exclusion of those outside the population base. OR-mwh, odds ratios in the sample when restricted to white males only. OR-no C, odds ratios excluding all subjects who had had any previous cancer except basal cell skin cancer.
‡ Carriers of either or both of the Y282 and D63 alleles.
§ Yes=Y282 homozygotes, D63 homozygotes, Y282 heterozygotes. No = other.