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An investigation of the progression from Barrett's esophagus to adenocarcinoma

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AN INVESTIGATION OF THE PROGRESSION FROM BARRETT’S ESOPHAGUS TO ADENOCARCINOMA

by

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AN INVESTIGATION OF THE PROGRESSION FROM BARRETT'S ESOPHAGUS TO ADENOCARCINOMA

SCOTT PALMESE

ABSTRACT

Barrett’s esophagus is a metaplasia of the epithelium of the lower esophagus from a normal squamous appearance to a columnar appearance more typically found in the stomach. It is normally caused by prolonged gastric reflux. While Barrett’s esophagus is not usually the direct cause of adverse symptoms, it does put a person at greater risk for developing esophageal adenocarcinoma, one of the least treatable cancers currently known.

While the progression from gastric reflux to Barrett’s esophagus is fairly clear, the relationship between Barrett’s esophagus and esophageal adenocarcinoma is not as well understood. Not all patients diagnosed with Barrett’s esophagus will go on to develop esophageal adenocarcinoma. There are several factors that may have some impact on this progression, including obesity, lifestyle, and genetic predisposition. The purpose of this study was to evaluate the literature to determine the potential impacts of each of these factors on development of esophageal adenocarcinoma.

While obesity and lifestyle clearly have some impact on development of esophageal adenocarcinoma, it was found that the exact nature of that impact is still unclear. Obesity leads to several consequences, including increased gastroesophageal
reflux, hormonal changes, and reduction in the bacterium *H. pylori*, all of which have been shown to have some impact on metaplasia in the esophagus. Lifestyle choices, including alcohol or tobacco use, also have been shown to have at least some effect on development of esophageal adenocarcinoma.

The literature also reveals that inherited risk factors, namely genetic predisposition, may play a role in development of esophageal adenocarcinoma. Genetic predisposition to obesity may have some impact, but other studies have identified genetic variations that seem to directly influence development of esophageal adenocarcinoma.

While it is clear that there are several factors that influence development of esophageal adenocarcinoma, we do not yet understand the complete etiology. By continuing to study these risk factors, we will be able to develop new treatments to combat the rising incidence of Barrett’s esophagus and esophageal adenocarcinoma.
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The rs3088440 SNP disrupts the binding site of miRNA during transcription

Endoscopic images of the lower esophagus in a 52-year old male before and after treatment with cryotherapy
LIST OF ABBREVIATIONS

GERD.................................................................Gastroesophageal Reflux Disease
INTRODUCTION

What is Barrett’s Esophagus?

Barrett’s esophagus is a disease state that arises primarily as a complication of gastroesophageal reflux (Stein and Siewert, 1993). It is characterized by a metaplasia in the distal esophagus, a change in cell lining from the normal stratified squamous epithelium to a columnar epithelium more common to the stomach (Clemons, 2014). The disease state is more common in men than women and is strongly associated with central obesity (Reid, 2010). While Barrett’s esophagus does not necessarily constitute a cause for immediate concern, it must be carefully monitored because it increases a person’s predisposition for the development of esophageal cancer (Kumar, 2013).

Previous studies have shown that obesity and lifestyle are two of the most significant risk factors for the development of gastroesophageal reflux, and, consequently, the progression to Barrett’s esophagus. The link between Barrett’s esophagus and adenocarcinoma is a little less clear.

This paper will first describe the history of the condition, before delving into the normal physiology of the esophagus and stomach. Next the etiology of gastroesophageal reflux and, subsequently, Barrett’s esophagus will be described. While this progression is mostly understood, it is less clear how Barrett’s esophagus leads to esophageal cancer. This literature review will attempt to elucidate the connection between Barrett’s esophagus and development of adenocarcinoma. Specifically:
1) How do diet, lifestyle, and other potentially modifiable factors contribute to the development of adenocarcinoma once a patient has been diagnosed with Barrett’s esophagus? Is there any evidence to suggest that diet and lifestyle changes can improve the prognosis of a patient already diagnosed with Barrett’s esophagus by preventing the development of esophageal cancer?

2) Are there any specific genetic markers that can identify patients predisposed to the development of cancer from an existing diagnosis of Barrett’s esophagus? How does genetics play a role in the development of adenocarcinoma?

3) Is there a way to reverse the metaplasia or dysplasia associated with Barrett’s esophagus? Can current knowledge about the mechanism of development of adenocarcinoma help shed light on possible new treatments?

By clarifying the relationship between Barrett’s and development of cancer, this review will hopefully serve as a resource for medical professionals with patients suffering from these conditions.
BACKGROUND

The History of Barrett’s Esophagus

Barrett’s esophagus was first described by thoracic surgeon Norman Barrett in 1950 and it wasn’t until 1953 that a link was made between gastroesophageal reflux and the development of the metaplasia (Barrett, 1950; Allison and Johnstone, 1953). For several years, physicians could not determine any negative effects arising from the development of this condition, as there were usually no additional symptoms other than those traditionally associated with acid reflux. Endoscopic procedures, however, clearly showed a visually distinct pattern in the lower esophagus that was not present in all patients suffering from gastroesophageal reflux [Figure 1]. It wasn’t until 1975 that Barrett’s esophagus was described as a premalignant state, a condition that puts patients at a greater risk for developing adenocarcinoma of the esophagus (Naef et al, 1975).

Normal Physiology of the Esophagus and Stomach

The main function of the esophagus, along with the upper and lower esophageal sphincters, is to propel food from the mouth to the stomach and to prevent reflux from the stomach. In other words, the esophagus is designed to make sure anything entering the mouth only travels in one direction – towards the stomach for the start of the digestive
The esophagus is lined by stratified squamous epithelium, a thick type of epithelium that is ideal for areas of the body subject to abrasive forces. In the esophagus, the epithelium is regenerated as the outer layers are sloughed off as food travels toward the stomach (Marieb et al, 2013).

There are three major structures that make up the esophagus: the upper esophageal sphincter, the body, and the lower esophageal sphincter (Yazaki and Sifrim, 2011). The lower esophageal sphincter is of most interest to this investigation, as it is the junction between the esophagus and the stomach [Figure 2].
The lower esophageal sphincter is surrounded by smooth muscle, and is normally innervated by both excitatory and inhibitory neurons. While the full functionality of the lower esophageal sphincter is not yet understood, it is clear that there is a myogenic property of the smooth muscle associated with this region. This property keeps the

**Figure 2. Anatomical depiction of the lower esophageal sphincter.** This image depicts the lower esophageal sphincter (LES), the junction between the esophagus and the stomach. Myogenic (muscle-originating) input from the surrounding smooth muscle, depicted here in two layers (a longitudinal layer and a circular layer), keeps the LES closed. Contraction and relaxation of the diaphragm also is involved in the normal functioning of the LES. The squamocolumnar junction is the anatomical “line” between the stratified squamous epithelium of the esophagus and the columnar epithelium of the stomach. If Barrett’s esophagus develops, this “line” will move proximally into the body of the esophagus. (Figure taken from Yazaki and Sifrim, 2011).
sphincter tonically closed and the excitatory and inhibitory neurons appear to modulate this activity (Goyal and Rattan, 1975; Goyal and Chaudhury, 2008). Malfunction in this neural input could account for development of esophageal reflux, disrupting the normal tonic closure and allowing acids from the stomach to enter the esophagus (Powley et al, 2013).

The stomach, unlike the esophagus, is lined by a simple columnar epithelium. This type of epithelium lines most of the gastrointestinal tract, and is responsible for the secretion of digestive enzymes. Most importantly, columnar epithelium is much more resistant than squamous epithelium to hydrochloric acid and pepsin, two enzymes regularly secreted in the stomach. It is believed that the metaplasia observed in Barrett’s esophagus is a defense mechanism by the body in reaction to the abnormal presence of stomach acid in the lower esophagus. Columnar epithelium is better equipped to prevent stomach acids from penetrating the deeper layers of the inner esophageal lining (International Foundation for Functional Gastrointestinal Disorders, 2014).

**Development of Gastroesophageal Reflux**

In order to understand the transition from Barrett’s esophagus to adenocarcinoma, it is important to first understand the development of gastroesophageal reflux (GERD), the first stage in the progression to Barrett’s esophagus. GERD affects 10-20% of the western world and is not always cause for concern (Hershcovici and Fass, 2011). Many people experience reflux occasionally, however, reflux occurring more than twice a week
may be an indication of a chronic problem (Mayo Clinic, 2014). A current official
definition of GERD explains it as “a condition which develops when the reflux of
stomach contents causes troublesome symptoms (i.e. at least two heartburn episodes per
week) and/or complications” (Vakil et al, 2006). While regurgitation and heartburn are
the most common symptoms of GERD, other less universal symptoms include sore
throat, nausea, chest pain, and coughing (Kahrilas et al, 2000).

As discussed previously, GERD is usually caused by impairment of the lower
esophageal sphincter, the part of the gastrointestinal tract that separates the lower
esophagus from the upper portion of the stomach. The angle at which the esophagus
enters the stomach is called the Angle of His. In healthy individuals, this angle creates a
narrowing in this part of the esophagus, essentially creating a valve. This structure, in
conjunction with the lower esophageal sphincter, helps to prevent stomach acid from
entering the esophagus. The Angle of His develops as an individual ages. This angle
usually is not fully developed during infancy, a primary reason why gastric reflux is
relatively common in infants (Sircar, 2008).

Many times GERD is caused partially by a hiatal hernia. A hiatal hernia is a
protrusion of the stomach through the esophageal opening in the diaphragm (Van
Weyenberg, 2006). This increases the pressure observed in vivo in the stomach relative
to the pressure observed in the esophagus, causing the lower esophageal sphincter to be
open at times when it should be closed (Pettersson et al, 1981; de Vries et al, 2008;
Figure 3. Relationship of gastric and esophageal pressure in the presence and absence of a hiatal hernia. “A” represents the relationship of pressure (in cm H$_2$O) with time after a gastric infusion of both the esophagus and stomach in a healthy apparatus in vivo. “B” represents the relationship of pressure with time after a gastric infusion in the presence of a hiatal hernia. In “B”, there is a greater disparity between gastric pressure and esophageal pressure, causing a large pressure gradient between the two regions of the gastrointestinal tract. Due to this larger gradient, the gastric infusion (and consequently other gastric juices) are more inclined to be pushed from the stomach to the esophagus. This illustrates the impact of a hiatal hernia on the development of gastroesophageal reflux (Figure taken from Pettersson et al, 1981).

Kahrilas et al, 2000) [Figure 3]. When the lower esophageal sphincter is open at the wrong time, gastroesophageal reflux results.
Diagnosis and Treatment of Gastroesophageal Reflux

While reflux typically causes the symptoms outlined above, it is not always symptomatic. A proper diagnosis of gastroesophageal reflux disease requires the patient to be symptomatic and to have reflux of stomach contents into the esophagus (Kahrilas et al, 2008). The current gold standard for diagnosis is esophageal pH monitoring. In many cases, pH monitoring is used to track pH changes in a subject’s esophagus during a period of short-term proton pump inhibitor treatment. The pH within the esophagus is measured before, during, and after treatment in order to determine the effect of proton pump inhibitors. To do this, a thin tube with an attached monitor is passed through the mouth or nose into the subject’s stomach. Once the tube is in the stomach, it is pulled back into the lower esophagus. The monitor records the pH of the acid content in the lower esophagus for 24 hours before the tube is removed. The concentration of protons (H\(^+\)), by definition, determines the acid content of a solution. Proton pump inhibitors decrease gastric acid content by decreasing the concentration of protons being pumped into the lumen of the gastrointestinal tract – in this case the stomach. By treating a patient with proton pump inhibitors and decreasing the amount of gastric acid available for reflux (tracked by the aforementioned pH monitoring), a determination of the presence or absence of gastroesophageal reflux could be determined by a change in symptoms. An improvement of symptoms would suggest that GERD is indeed present (Numans, M.E. et al, 2004; Medline Plus, 2012).
Proton pump inhibitors are usually the first line of treatment for gastroesophageal reflux for the reasons described above. If proton pump inhibitors do not lead to a significant improvement of symptoms, surgical intervention may be required to improve the patient’s quality of life and decrease the likelihood that the reflux will lead to Barrett’s esophagus or esophageal adenocarcinoma. The most common surgery is called the Nissen fundoplication, and it is only recommended for patients that have seen at least some benefit from the use of proton pump inhibitors (Katz et al, 2013).

The Nissen fundoplication works to correct two potential problems that may have initially led to the development of gastroesophageal reflux: a weak lower esophageal sphincter and a hiatal hernia [Figure 4]. The upper portion of the stomach is called the fundus, hence the name of the surgical procedure. During the surgery, the fundus of the stomach is wrapped around the lower esophagus, reinforcing the sphincter to improve closing functionality and preventing the stomach from protruding through the diaphragm. When a patient who has had this procedure is attempting to digest food, the stomach will contract and, since it would now be wrapped around the esophagus, the lower esophagus would be forced to contract as well. This would prevent gastric acids from entering the esophagus. Additionally, the opening of the lower esophagus is tightened so that a hiatal hernia can be improved or, ideally, completely eliminated (Nissen, 1961; Abbas et al, 2004).
Figure 4. Diagram illustrating a Nissen Fundoplication. The goal of a Nissen Fundoplication is to treat a patient suffering from gastroesophageal reflux. The upper portion of the stomach (the fundus) is wrapped around the lower esophagus, strengthening the lower esophageal sphincter. In addition, any contraction of the stomach during food digestion will also contract the lower esophagus, preventing acid reflux from occurring. Figure taken from http://www.illustratedverdict.com/projectreview/IllustratedVerdict_2011v3.asp).
The Progression from Gastroesophageal Reflux to Barrett’s Esophagus

Gastroesophageal reflux, as described in the previous sections, sometimes leads to Barrett’s esophagus. Barrett’s esophagus and its complications (namely its link to adenocarcinoma) will be the focus of the remainder of this investigation. Barrett’s esophagus is not a condition that occurs spontaneously and it is relatively rare in the population. While it is strongly linked to gastroesophageal reflux, a majority of patients suffering from gastroesophageal reflux do not develop Barrett’s esophagus. Only about 10-20% of patients suffering from gastroesophageal reflux actually exhibit the metaplasia associated with Barrett’s esophagus (Modiano and Gerson, 2007).

Unfortunately, the rising incidence of obesity in the population, especially in parts of the Western world, is contributing to a rise in Barrett’s esophagus. Obesity leads to an increase in intra-abdominal pressure, which distorts the junction between the esophagus and the stomach. Ultimately, this may lead to a hiatal hernia or other disruptions of the lower esophageal sphincter. This contributes greatly to gastric reflux, which in turn leads to Barrett’s esophagus (Lee and McColl, 2014). Strangely, Barrett’s esophagus has also been present in patients who do not exhibit gastroesophageal reflux, though this is less understood (Shaheen and Richter, 2009).

Both macroscopic and histological features must be present in order to make a definitive diagnosis of Barrett’s esophagus. An endoscopic investigation of the lower esophagus will clearly show a difference in cell type if Barrett’s esophagus is present because the lumen walls will be of a different color [Figure 1]. Most importantly, the
altered histology must be observed as well with a tissue sample. As discussed previously, a positive result for Barrett’s esophagus would show a columnar epithelium usually native to the stomach rather than a stratified squamous epithelium typically found in the esophagus (Flejou, 2005).

The key to dealing with gastroesophageal reflux is keeping it under control through both lifestyle changes (diet, exercise, etc.) and medical intervention, as discussed previously. While gastroesophageal reflux does cause damage to the luminal walls of the lower esophagus, it does not do so all at once. Instead, the metaplasia associated with Barrett’s esophagus happens gradually, and it may not even happen at all [Figure 5]. Even for those who do develop Barrett’s esophagus, the time it takes to develop the condition once diagnosed with GERD varies. The progressive etiology leading to cancer can be halted and even reversed if it is caught before any metaplasia takes place through the normal regeneration of the esophagus’ squamous epithelium.

**Barrett’s Esophagus to Cancer: an Overview**

While the exact pathogenesis from Barrett’s esophagus to cancer is not understood, it seems that cancer arising from Barrett’s esophagus is due to a change from metaplasia to dysplasia. Dysplasia is the proliferation of immature cells that would not normally be found in large numbers in an adult. The cancer most commonly associated with Barrett’s esophagus, adenocarcinoma, is characterized by several changes at the genomic level. Many genes commonly associated with a variety of cancers, including
Figure 5. Progression of gastroesophageal reflux to Barrett’s esophagus. Prolonged gastroesophageal reflux will cause a metaplasia (change in cell type) in the esophagus from a squamous epithelium to a columnar epithelium, characteristic of Barrett’s esophagus. If treated early enough, reflux may not lead to Barrett’s esophagus and affected cells will heal and assume the normal esophageal appearance (Figure taken from Stein and Siewert, 1993).
tumor suppressor genes p53 and p16, have been shown to be defective in patients with adenocarcinomas (Koppert et al, 2005).

For a patient that has developed Barrett’s esophagus, the standard procedure is to have routine endoscopies to monitor for development of cancerous tissue. Depending on the degree of cellular abnormality upon diagnosis, the patient may be advised to see a doctor anywhere from once every few months to once every five years (American Gastroenterological Association, 2014). While it is well documented that diet, exercise, and other lifestyle interventions can improve reflux, it is unclear whether or not those changes alter the progression or risk of an adenocarcinoma. Currently, researchers are attempting to determine why some patients with Barrett’s esophagus develop cancer while others do not. Presumably, if a direct link is found between Barrett’s and cancer, treatment can be better directed towards specific patients (Muthusamy and Sharma, 2011; Lee and McColl, 2014)
DISCUSSION OF PUBLISHED STUDIES

Since it was first discovered that there is a link between development of Barrett’s esophagus and esophageal cancer, major risk factors have been identified that may provide some clarity on the etiology of adenocarcinoma of the esophagus. A few of these risk factors, obesity and lifestyle, are attributes that are modifiable, while another, genetic predisposition, is not something that can be altered. These risk factors will now be discussed in detail, with a focus on previously published studies in the field. Finally, this investigation will explore current cutting-edge treatments and discuss how our knowledge of the risk factors may help decrease the rising incidence of esophageal adenocarcinoma.

Obesity: General Implications

The incidence of esophageal adenocarcinoma and the incidence of obesity have both increased markedly since the 1970s, trends that first caused researchers to look into a possible correlation. Even today, the relationship between obesity and adenocarcinoma is not fully understood, and many recent studies have published widely varying conclusions on the exact nature of the impact of obesity on development of esophageal cancer. Researchers have, however, identified several consequences of obesity that may have some impact on the development of esophageal adenocarcinoma [Figure 6]. Each of these factors will be explored in this investigation.
In 2014, Long and Beales published a review in which they conducted a meta-analysis of 22 studies looking at the relationship between body mass index (a standard measure of obesity) and risk for development of esophageal adenocarcinoma. They found that relative risk of developing adenocarcinoma was almost three times higher in individuals with body mass indices greater than 30 kg/m². In addition, they found that

![Figure 6. A summary of the interrelated factors between obesity and the progressive etiology leading to development of esophageal adenocarcinoma.](image)

While it is not understood exactly how obesity affects the development of esophageal adenocarcinoma, there are several factors that may play a role. Several consequences of obesity, including increased gastroesophageal reflux, hormonal changes, and decreased *H. pylori* may lead to an increased risk of developing adenocarcinoma. Lifestyle and dietary habits may reverse or strengthen the trend towards development of cancer as well. (Figure taken from Alexandre et al, 2014).
the relative risk increased (RR = 1.11) for every 5 kg/m² increase in body mass index among subjects in this study. These relationships held true for both men and women.

The results compiled by Long and Beales were also stratified to separate those also experiencing gastroesophageal reflux. They found that the same relationships held true, even when reflux was not taken into account. This implies that obesity has an impact on development of esophageal adenocarcinoma independent of the impact of gastric reflux. Since individuals with Barrett’s esophagus usually are suffering from gastroesophageal reflux, it can be concluded that obese individuals have a higher relative risk for adenocarcinoma than those who have Barrett’s but are not obese.

**Obesity: Associated Hormonal Changes**

In an obese individual, levels of certain hormones have been found to be, in many cases, much different than levels of the same hormones in non-obese individuals. Two of the primary hormones that are affected are adiponectin and leptin, two substances normally secreted from the body’s adipose tissue. These two hormones function to regulate appetite and body mass. When an individual is obese, that person usually secretes a greater concentration of leptin and conversely a lower concentration of adiponectin. Insulin, the hormone usually most closely associated with obesity, also increases in obese patients. Obesity is often said to be caused by either a leptin or insulin resistance, since levels of these hormones usually rise while appetite and nutrient regulation fail.
Duggan et al. (2013) investigated the association between leptin, adiponectin, and insulin levels and the development of adenocarcinoma in a cohort of patients already diagnosed with Barrett’s esophagus. To do this, they measured fasting levels of glucose, insulin, leptin, and adiponectin in 392 patients previously diagnosed with Barrett’s esophagus. Fasting levels of glucose were measured because it is affected by body levels of leptin, adiponectin, and insulin. Duggan et al. used homeostatic model assessment scores (a measure of insulin sensitivity) to compare their subjects. The results are tabulated in Table 1.

Duggan et al. found that risk of developing adenocarcinoma increased as leptin levels increased. Examining the data in Table 1, one can see that the relative risk of developing adenocarcinoma is highest in the tertile representing the greatest concentration of leptin (12.6-63.6 ng/mL) at all three time points measured and lowest in the tertile representing the least concentration of leptin (1.90-6.30ng/mL). This suggests an association between obesity and development of esophageal cancer since leptin levels are higher in obese subjects.

When other functions of leptin are taken into account, these findings are not surprising. Leptin is also known to have an anti-apoptotic and proliferative effect on certain intracellular signaling pathways (Ando and Catalano, 2011). Ogunwobi et al. (2006) found that leptin specifically inhibits apoptosis and stimulates proliferation in esophageal adenocarcinoma cells,
Table 1. Relationship between serum concentration of leptin and adiponectin and relative risk for development of esophageal adenocarcinoma. (Table taken from Duggan et al, 2013).

<table>
<thead>
<tr>
<th>Leptin Tertile (ng/mL)</th>
<th>3 Years (23 events)</th>
<th>6 years (32 events)</th>
<th>Full follow-up (43 events)</th>
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<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
<td>HR</td>
</tr>
<tr>
<td>1.90-6.50</td>
<td>5/133</td>
<td>1.00</td>
<td>Ref.</td>
</tr>
<tr>
<td>6.40-12.4</td>
<td>9/120</td>
<td>1.96</td>
<td>0.60-6.46</td>
</tr>
<tr>
<td>12.6-63.6</td>
<td>9/130</td>
<td>3.68</td>
<td>0.94-14.45</td>
</tr>
<tr>
<td>ln(Leptin)</td>
<td>23.392</td>
<td>2.51</td>
<td>1.09-5.81</td>
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<tr>
<td>ln(Leptin) – Males only</td>
<td>22.321</td>
<td>2.80</td>
<td>1.19-6.54</td>
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<tr>
<td>ln(Leptin) – Males only</td>
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<tr>
<td>HMW Adiponectin</td>
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<td>10/131</td>
<td>1.00</td>
</tr>
<tr>
<td>1.26-2.45</td>
<td>4/131</td>
<td>0.40</td>
<td>0.12-1.29</td>
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<tr>
<td>2.46-11.46</td>
<td>9/130</td>
<td>0.84</td>
<td>0.30-2.35</td>
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<td>Total Adiponectin µg/mL</td>
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<td>1.17-3.84</td>
<td>10/1314</td>
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<td>0.41</td>
<td>0.13-1.35</td>
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<tr>
<td>5.95-17.54</td>
<td>9/130</td>
<td>0.87</td>
<td>0.31-2.41</td>
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giving further support to the idea that increased leptin puts a person at a greater disposition for developing esophageal cancer. A later study by

Howard et al. (2010) illustrated that leptin receptors were indeed upregulated in esophageal adenocarcinoma cells. The anti-apoptotic nature of leptin therefore enables the cancer to take hold and proliferate by its own mechanism of action.

In the study conducted by Duggan et al., adiponectin was shown to have the opposite effect to that shown by leptin. The second tertile (1.26-2.45 ng/mL), not the third (2.46-11.46 ng/mL), was associated with the greatest risk to development of
adenocarcinoma in patients with preexisting Barrett’s esophagus. This represents an inverse relationship: as adiponectin levels decreased, incidence of adenocarcinoma increased. This again supports the positive correlation between obesity and development of esophageal cancer since obesity is usually associated with a decrease in adiponectin level.

The effects of adiponectin on esophageal adenocarcinoma cells have been shown to counteract the effects of leptin. Naturally, this antagonism has been the subject of a large amount of research, as a greater understanding of these processes could lead to therapeutic advances. Two simultaneous studies (Konturek et al, 2008; Ogunwobi and Beales, 2008) illustrated this opposition of adiponectin to leptin, showing that adiponectin increases cell apoptosis and specifically inhibits leptin-induced proliferation in esophageal adenocarcinoma cells. In this case, therefore, adiponectin can be thought of as an “anti-cancer” hormone and the fact that obesity is associated with a decreased adiponectin level further supports the conclusion that obesity increases the risk for development of esophageal cancer from Barrett’s esophagus.

The Howard et al. (2010) study also measured the change in levels of adiponectin receptors in the same manner for which they measured the leptin receptors. They found that adiponectin receptors were downregulated in a majority of tumor cells. The result is that the “anti-cancer” signaling that adiponectin is able to perform is decreased, allowing esophageal adenocarcinoma cell proliferation to take place.

This relationship between the level of adiponectin and development of adenocarcinoma may not be as straightforward as it seems. A recent study by Almers et
al. (2015) found what seem to be contradictory results to those of the studies previously discussed. In this study, the investigators sampled three groups of individuals: population controls, patients with Barrett’s esophagus, and patients with gastroesophageal reflux disease but no diagnosis of Barrett’s esophagus. The levels of adiponectin were sampled in all three subject groups, and it was found that greater hormone levels were associated with a greater risk of developing Barrett’s esophagus in patients with gastroesophageal reflux disease. This association was strongest in those reporting more frequent reflux symptoms. No such association was found in the population control group.

Based on the majority of the research that has been published, the results of the Almers et al. study do not seem to make much sense. If obesity puts a person at a greater risk of developing Barrett’s esophagus, and Barrett’s esophagus puts a person at a greater risk for developing esophageal cancer, it follows that obesity (and all factors associated with obesity) should put a person at a greater risk for adenocarcinoma by the transitive property. There are a few potential explanations for this supposed paradox. One hypothesis is that this is an issue of timing. It is possible that greater levels of adiponectin contribute to the development of Barrett’s esophagus, and that Barrett’s esophagus subsequently leads to a decreased level of adiponectin. The decreased adiponectin then leads to development of adenocarcinoma. This is supported by the finding that the association between adiponectin levels and development of Barrett’s esophagus is greatest when patients report more frequent reflux symptoms. If Barrett’s esophagus is usually the result of prolonged reflux, it makes sense that this relationship would be strongest in patients that are “closest” to the Barrett’s state. Unfortunately, this
explanation does not account for the positive correlation between obesity and Barrett’s esophagus since obesity is usually associated with a lower level of adiponectin.

It is possible that the Almers et al. study simply illustrates that the progressive etiology to adenocarcinoma is not as simple as obesity → gastroesophageal reflux → Barrett’s esophagus → adenocarcinoma. Adiponectin may only be protective if close to an ideal concentration. If the Almers et al. study is correct, a concentration of adiponectin that is too high may put someone at a greater risk of developing Barrett’s esophagus and therefore esophageal cancer. Lower concentrations of adiponectin, usually associated with obesity, may also lead to development of Barrett’s esophagus and consequently adenocarcinoma. Only a moderate amount of adiponectin may truly be protective.

**Effects of the H. pylori Bacterium**

Another factor associated with obesity that may explain the link to adenocarcinoma is the *Helicobacter pylori* (*H. pylori*) bacterium. This bacterium, which is associated with non-obese individuals has been shown to be protective against esophageal adenocarcinoma (Macadam et al., 2004). Several studies have investigated this relationship to determine how this process works. Understanding this bacterium’s protective function may be another avenue for potentially paving the way for therapeutic advance.

Rubenstein et al. (2013) investigated the relationship between infection with *H.*
*H. pylori* and development of Barrett’s esophagus, erosive esophagitis, and gastroesophageal reflux symptoms. To do this, they analyzed 613 men. 533 of them had undergone screening for colorectal cancer over a period of three years and volunteered to undergo the upper endoscopy required of this study. The remaining 80 subjects were already diagnosed with Barrett’s esophagus in a previous examination by upper endoscopy. This study was particularly interested in the cagA+ strain of the bacterium, as this was the strain suspected to have protective properties against reflux and its associated symptoms.

Logistic regression was used to find an association between presence of serum antibodies against *H. pylori* or cagA and gastroesophageal reflux symptoms, Barrett’s esophagus, and esophagitis. Since the production of serum antibodies represents interaction with foreign particles within the body, antibodies would have been produced to *H. pylori* and cagA if they had indeed entered the body at any point in the past. Evaluating the serum for antibodies, therefore, gives the researchers a way to track any previous infection. The results of the experiment are listed in **Table 2**.

The most apparent result is that the hypothesis posed by Rubenstein et al. appears to be correct: subjects seropositive for the antibodies against *H. pylori*, particularly antibodies against cagA, had fewer outcomes of Barrett’s esophagus and erosive esophagitis. To further support this conclusion, the 80 men previously diagnosed with Barrett’s esophagus showed significantly fewer antibodies for *H. pylori*, indicating that they did not benefit from the apparent protective function of the bacterium.

Of note, no association was discovered between those reporting gastroesophageal reflux symptoms and seropositivity of *H. pylori* or cagA. This could be explained,
however, by the complex etiology of gastroesophageal reflux disease. As discussed previously in this investigation, there are many factors that may lead to gastroesophageal reflux disease. The lack of association between presence of antibodies to the bacterium and appearance of gastroesophageal reflux may be due to other nonrelated factors. In other words, confounding variables may be at play. Rubenstein et al. realized this and they stratified their subjects further. They found that those suffering from gastroesophageal reflux symptoms were more likely to be obese, more likely to smoke, and were more likely to have hiatal hernias, all risk factors for gastroesophageal reflux disease. It is likely then, that these findings do not disprove the findings of the rest of the experiment. Instead, it shows that while lack of *H. pylori* infection may be one possible cause of gastroesophageal reflux disease (and therefore increase risk for adenocarcinoma), it is not required for the disease to take hold.

There are a few hypotheses as to why the association found by Rubenstein et al. appears to be true. The cagA+ strain of *H. pylori*, the focus of the Rubenstein study, has been shown to provoke more intense gastric mucosal inflammation. This inflammation, in turn, may lead to less gastric acid secretion and therefore less opportunity for gastroesophageal reflux. The mechanism for how inflammation leads to less gastric acid production is not entirely clear. It could be due to local cytokine production or it could
Table 2. Relationship between presence or absence of *H. pylori* and development of gastroesophageal reflux, erosive esophagitis, or Barrett’s esophagus. (Table taken from Rubenstein et al, 2013).

<table>
<thead>
<tr>
<th><em>H. pylori</em> / cagA Status</th>
<th># No GERD, EE, or BE</th>
<th># GERD</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)&lt;sup&gt;a&lt;/sup&gt;</th>
<th># EE</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
<th># BE&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. pylori</em> –</td>
<td>131</td>
<td>115</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
<td>182</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
<td>125</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
</tr>
<tr>
<td><em>H. pylori</em> +</td>
<td>46</td>
<td>38</td>
<td>0.94 (0.57, 1.55)</td>
<td>0.95 (0.55, 1.64)</td>
<td>40</td>
<td>0.63 (0.39, 1.01)</td>
<td>0.63 (0.37, 1.08)</td>
<td>25</td>
<td>0.57 (0.33, 0.98)</td>
<td>0.53 (0.29, 0.97)</td>
</tr>
<tr>
<td><em>H. pylori</em> +, cagA –</td>
<td>22</td>
<td>20</td>
<td>1.04 (0.54, 1.99)</td>
<td>0.93 (0.46, 1.88)</td>
<td>25</td>
<td>0.82 (0.44, 1.51)</td>
<td>0.78 (0.40, 1.54)</td>
<td>16</td>
<td>0.76 (0.36, 1.52)</td>
<td>0.64 (0.30, 1.36)</td>
</tr>
<tr>
<td><em>H. pylori</em> +, cagA +</td>
<td>23</td>
<td>18</td>
<td>0.89 (0.46, 1.74)</td>
<td>0.97 (0.46, 2.03)</td>
<td>15</td>
<td>0.47 (0.24, 0.93)</td>
<td>0.47 (0.21, 1.03)</td>
<td>8</td>
<td>0.385 (0.16, 0.85)</td>
<td>0.36 (0.14, 0.90)</td>
</tr>
</tbody>
</table>
be due to irreversible gastric atrophy (Beales and Calam, 1998; El-Omar et al, 1997).

More research will need to be done in order to determine how this mechanism works. A greater understanding of this process could potentially illustrate ways that \textit{H. pylori} could be used as treatment for gastroesophageal reflux disease and its complications.

\textbf{H. pylori and Ghrelin}

It is clear that the lack of \textit{H. pylori} infection and eventual development of esophageal adenocarcinoma are potentially linked, but there must be several other factors involved. One of these confounding factors is the presence of ghrelin, another hormone normally involved in the digestive process. Ghrelin is a peptide hormone normally secreted from cells in the stomach itself. In short, this hormone is the “hunger hormone” – its main function is to stimulate appetite.

The most straightforward hypothesis that could explain this stimulation of appetite is that lower levels of \textit{H. pylori} lead to an increased presence of ghrelin. The increased body ghrelin would increase appetite, and this increase in appetite would potentially lead to obesity. Obesity, as has been discussed extensively, puts one more at risk for gastroesophageal reflux disease, Barrett’s esophagus, and adenocarcinoma.

This relationship was found by Nwokolo et al. for the first time in 2003. In this experiment, Nwokolo et al. measured plasma ghrelin, leptin, and gastrin for six hours following an overnight fast in 10 subjects. The results are illustrated in \textbf{Figure 7}. 

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Of the three hormones tested by Nwokolo et al, ghrelin was the only one to show significant change after *H. pylori* infection was eradicated. Levels of gastrin and leptin did not change significantly. Ghrelin, therefore, must be most closely linked to infection by this bacterium. Significant increases in ghrelin level would, as previously mentioned, increase a subject’s appetite and potentially lead to obesity. If this mechanism holds true, *H. pylori* may not be the sole cause of adenocarcinoma – it may only be a contributing factor for development of this cancer. It is also worth noting that Nwokolo et al. found that curing their subjects of *H. pylori* infection also led to a significant increase in gastric acid secretion. Again, this may not be simply a cause and effect relationship. If
increased ghrelin leads to increased appetite, then the extra food needing to be digested would ultimately signal the body to produce more gastric acid. This could be the mechanism by which ghrelin leads to increased risk of gastroesophageal reflux and therefore Barrett’s esophagus and adenocarcinoma.

A simultaneous study conducted in 2003 by Gokcel et al. found results completely contradictory to those published by Nwokolo et al. In this study, Gokcel et al. took biopsy specimens during gastric endoscopy from 39 women, matched for potential confounding variables such as age and body mass index. Fifteen of the subjects were found to be *H. pylori* negative and the remaining 24 women were positive for *H. pylori*. Their results, including the variables for which their subjects were matched, are tabulated in Table 3.

No significant differences were found between any of the variables measured in the Gokcel experiment. These results clearly are at opposition with the results found by Nwokolo et al. While Gokcel is not saying that *H. pylori* does not have an impact on development of esophagitis or Barrett’s esophagus, it does raise questions about whether or not ghrelin is involved. It is also worth pointing out that this study included a larger subject pool than the study conducted by Nwokolo. Gokcel’s study, therefore, has more
Table 3. No significant difference was found by Gokcel et al. in serum ghrelin level between \textit{H. pylori} negative and \textit{H. pylori} positive subjects (Table taken from Gokcel et al, 2003).

statistical power. While this does not prove that one study is superior to the other, it does certainly encourage further research on the relationship between ghrelin and \textit{H. pylori}.

The relationship between ghrelin and development of Barrett’s esophagus and adenocarcinoma remains unclear. In 2013, Rubenstein et al.’s study also investigated the impact of ghrelin on Barrett’s esophagus and esophageal adenocarcinoma. What they found confirms that the exact mechanism of action is still unknown to us. In their study, ghrelin appeared to \textit{protect} against gastroesophageal reflux. Based on the results of the previously discussed studies, the opposite would be expected. If ghrelin increases appetite, which increases gastric acid production, it does not make sense for ghrelin to decrease reflux symptoms. Rubenstein et al. hypothesized that ghrelin’s ability to facilitate gastric emptying may override the tendency for ghrelin to increase gastric acid production. They note that ghrelin may antagonize the development of gastroesophageal reflux disease, but through the production of growth hormone may still promote the development of Barrett’s esophagus.
In 2007, de Martel et al. investigated the direct link between serum ghrelin levels and the risk of esophageal adenocarcinoma. The results of the de Martel study were consistent with the findings of the Rubenstein study. The de Martel investigation originally hypothesized that decreased serum ghrelin during *H. pylori* infection would protect against cancer of the esophagus. What they found was that high serum ghrelin was protective against adenocarcinoma, not the opposite. Interestingly, they found that this relationship only held true for subjects with body mass index greater than or equal to $25\text{kg/m}^2$. They also found that the effects of *H. pylori* and ghrelin were independent, supporting the work of Gokcel et al.

A potential explanation for ghrelin’s complicated relationship with development of adenocarcinoma could again be one of timing. The relationship between *H. pylori* and ghrelin remains unclear, but it seems that there is at least some correlation between ghrelin and development of esophageal cancer. It is likely that ghrelin, which is known to increase appetite, has some impact on obesity. Obesity may cause reflux independent of an increase in gastric acid production. Therefore, Barrett’s esophagus may result over time due to a number of these factors. This is supported by the Rubenstein study, which still illustrates a positive correlation between increased ghrelin production and increased risk for Barrett’s esophagus. Once Barrett’s esophagus has developed, obesity may still be present and ghrelin levels may remain high. If ghrelin does protect against adenocarcinoma in subjects with high body mass indices, it is possible that this is the time at which this protective effect occurs. As stated previously, most patients with Barrett’s esophagus do not go on to develop esophageal cancer. It is possible, therefore,
that ghrelin could be another treatment avenue for preventing the onset of adenocarcinoma in Barrett’s patients.

This proposed treatment would present some problems. Most people who are suffering from obesity are looking forward to advances in the field of dietary medicine. They yearn for a day when they can take a drug that will lead to dramatic weight loss. Since ghrelin has been linked with appetite, it is naturally one of the major avenues of study for finding a treatment for obesity (Chollet et al, 2009). If ghrelin does indeed turn out to be the key to developing a drug for adenocarcinoma, patients may have to sacrifice weight loss for prevention of esophageal cancer. The problem is that obesity comes with its own related health problems and both obesity and adenocarcinoma come with high mortality rates. Finding a way to target the proposed ghrelin drug for adenocarcinoma may be the answer. It is clear, however, that a greater understanding of ghrelin’s role in the etiology of esophageal adenocarcinoma must be achieved before a large amount of work can be warranted in this arena.

Other Lifestyle Factors

In general, it is widely accepted that smoking is a risk factor for both development of Barrett’s esophagus and esophageal adenocarcinoma. The exact relationship between smoking, obesity, and development of these diseases, however, is still not yet completely understood.
In 2012, Coleman et al. investigated whether or not there is a correlation between smoking habits and risk of developing esophageal adenocarcinoma. They analyzed hospital case notes of 3,167 patients in Northern Ireland who had been previously diagnosed with Barrett’s esophagus. They noted the dates of diagnosis, and paid special attention to reported lifestyle choices, including tobacco use. Their results are compiled in Figure 8.

Of those who had a history of smoking, the patients that previously quit smoking were at lower risk of developing adenocarcinoma than those who were current smokers. This suggests a few conclusions. First, it implies that while smoking does put a person at greater risk of developing adenocarcinoma, it must do so at a gradual rate. A one-time smoker is, if this data is correct, less likely to contract adenocarcinoma than a long term smoker.

![Figure 8](image-url)

**Figure 8.** Plot comparing time since diagnosis of Barrett’s esophagus and progression towards adenocarcinoma based on smoking habits. It was found that smokers were at greater risk of developing adenocarcinoma among those previously diagnosed with Barrett’s esophagus. Current smokers were at greater risk than former smokers. (Figure taken from Coleman et al, 2012).
smoker. Second, it also suggests that cessation of smoking may halt, or potentially reverse, damage to the lower esophagus. In order to determine if this assumption is correct, duration and intensity of smoking habits would have to be studied.

Coleman et al. attempted to collect this information, but duration (number of years smoked) and intensity (number of cigarettes smoked per day) of smoking habits were not shown to have a significant impact on progression to adenocarcinoma. It must be noted, however, that this information was not widely reported among the subjects who participated in this experiment. Number of years smoked was only known for 17% of individuals, and intensity of smoking habits was only known for slightly more than half of the participants in the study.

Other lifestyle factors and variables were also looked at in the Coleman study. Alcohol intake was reported for about 70% of the study participants, but it was not shown to have a significant bearing on the progression from Barrett’s esophagus to adenocarcinoma. Few patients in the study had both height and weight recorded. For these subjects, body mass indices were calculated, and it was found that 75% of those who progressed to adenocarcinoma and 68% of those who did not progress could be classified as obese. This did not represent a statistically significant difference. This may have been due to the limited sample size, but it also may shed light on the impact of smoking on development of esophageal cancer. Smoking may put someone at a greater risk of developing adenocarcinoma than obesity, though further research would need to be conducted in order to determine if this is truly the case.
Although smoking is usually regarded as a risk factor for Barrett’s esophagus as well as adenocarcinoma, this relationship appears to be much more complicated. Similar to the research being done on *H. pylori* and ghrelin, the results of studies looking into this potential correlation are somewhat contradictory.

In 2013, Balasubramanian et al. analyzed 1,056 patients suffering from gastroesophageal reflux. All subjects completed a questionnaire which included questions on demographic information, cigarette smoking (amount, current/past, and duration), clinical data, and endoscopic findings (Barrett’s esophagus, hiatal hernia, etc.). The study looked at two main relationships. The first was whether duration of smoking had any impact on the relative risk of developing Barrett’s esophagus. The second was whether time since cessation of smoking had any correlation with the relative risk of developing Barrett’s esophagus. The results are compiled in Figure 9.

The results of the Balasubramanian experiment were not surprising. The longer the duration of a person’s smoking habits, the greater the risk of developing Barrett’s esophagus. The length of time since a subject stopped smoking had an inverse relationship with risk of developing Barrett’s esophagus. These results support the findings of the Coleman study. Since Barrett’s esophagus is the precursor to adenocarcinoma, it makes sense that smoking would be a risk factor for both the cancer and its precursor.

Unfortunately, these results have not been consistently replicated. In two 2014 studies, it was shown that there is no association between smoking habits and development of Barrett’s esophagus.
Yates et al. (2014) conducted a study of 104 participants and found that obesity had a significant association with development of Barrett’s esophagus, but found no significant association between risk for Barrett’s esophagus and lifestyle habits such as smoking and alcohol consumption. The data collected in the Yates study is reproduced in Table 4.

The Yates study did have one additional finding of interest. When stratifying subjects by type of alcohol consumed (wine vs. beer vs. spirits), borderline significance was found (p = 0.06) for a relationship between development of esophageal adenocarcinoma and wine consumption. An inverse relationship was found between the two factors. In other words, it was suggested that wine may have a protective effect on the development of adenocarcinoma. No association, however, was shown between wine consumption and risk for Barrett’s esophagus. It is possible then, that wine helps to prevent the dysplasia associated with development of cancer, but not the initial metaplasia needed for a diagnosis of Barrett’s esophagus. More research will have to be done in order to determine if wine truly does have a protective effect on the development of adenocarcinoma.

The second 2014 study, conducted by Thrift et al, also determined that overall smoking and alcohol consumption have no significant bearing on the risk for developing Barrett’s esophagus. Like the Yates study, the Thrift study also stratified alcohol consumption by type of alcohol.
Figure 9. Smoking duration and years since cessation has a direct impact on risk for Barrett’s esophagus. It was found that years of cigarette smoking had a direct correlation with the odds of contracting Barrett’s esophagus. Similarly, it was found that years since quitting smoking had an inverse relationship with risk of developing Barrett’s esophagus. (Figure taken from Balasubramanian et al, 2013).
Table 4. Relationship between lifestyle factors and risk of developing Barrett’s esophagus. Body mass index was shown to have a significant relationship with risk of developing Barrett’s esophagus (p<0.05). Smoking and alcohol use were not found to have significant relationships with risk of developing Barrett’s esophagus. (Table taken from Yates et al, 2014).

The Thrift study found that moderate (14-28 drinks per week) intake of beer corresponded with lower risk of developing Barrett’s esophagus. This contrasts with the Yates study, which found such an association only for intake of wine.

It can be concluded that the impact of lifestyle habits on the development of Barrett’s esophagus and adenocarcinoma are incredibly complex. Current research has not been able to determine a trend that is consistent. Further research will have to be conducted in order to further elucidate these connections.
Genetic Predisposition

While the majority of this investigation has dealt with more modifiable risk factors, the remainder will discuss inherited risk factors, namely those that can be analyzed through the use of genetics. The study of genetics, especially in relation to development of Barrett’s esophagus and adenocarcinoma is still very new. As research continues into the possibility of using genetic therapy to treat disease, research into the genetic causes of these diseases may shed some light on new targeted therapies.

It has been well-established that there is a genetic component to obesity. Thrift et al. (2014) conducted a “Mendelian randomization” to determine whether or not genetic risk for obesity can be associated with development of Barrett’s esophagus and esophageal adenocarcinoma. Mendelian randomization assumes that previously identified genetic markers for a certain trait (in this case, obesity) are analogous to phenotypes observed in the population. For instance, if a person has a very high genetic risk for obesity, then that person is considered to have a higher lifetime body mass index.

The benefits of using such an approach are many. The most important benefit to conducting a study utilizing Mendelian randomization is the elimination of bias. Genetics cannot be confounded by environmental variables. In this instance, a predisposition to obesity should show a link to Barrett’s esophagus and adenocarcinoma if such a link exists. Other potential risk factors that have been previously discussed, including lifestyle habits, hormonal changes, and H. pylori infection, will not affect the results.
In order to increase the power of their study, Thrift et al. used multiple known genetic variants that have been previously linked to high body mass indices. They found that, as expected, higher genetic risk for obesity was associated with presence of esophageal adenocarcinoma, seemingly confirming that a link exists between the two states. Interestingly, the statistical significance disappeared among women when subjects were stratified based on gender. A similar trend was noted for the risk for Barrett’s esophagus. Thrift et al. found that there was a 12% increased risk for Barrett’s esophagus for every 1 kg/m$^2$ predicted increase in body mass index among the subjects in this study. This trend was statistically significant even when subjects were stratified by gender.

The Thrift study seems to confirm that people who have a higher genetic predisposition to obesity have a greater chance of contracting Barrett’s esophagus and esophageal adenocarcinoma than those who are not. Since this study was done via Mendelian randomization, it suggests that obesity has an impact on development of these disease states independently of other risk factors.

Of course, it cannot be that simple. Other investigators have noted that studies like the one carried out by Thrift et al. have primarily focused on a European population. As noted by Abrams and Chak in 2014, Barrett’s esophagus and esophageal adenocarcinoma seem to occur in those of European descent far more than in other populations. Obesity on its own, however, is more prevalent in African-American populations than it is in European populations. Based on the genetic research carried out by researchers like Thrift, it would make sense if African-Americans therefore suffered a higher incidence of adenocarcinoma and Barrett’s esophagus than those of European
descent, but this is not the case. Further studies will need to be done in order to
determine whether or not the same genetic risk scores are predictive of obesity, Barrett’s
esophagus, and adenocarcinoma in more heterogeneous populations.

Other researchers have tried to elucidate more direct genetic predispositions to
Barrett’s esophagus and adenocarcinoma. It has been found that two tumor suppressor
genes, CDKN2A and TP53, are frequently mutated in esophageal adenocarcinoma
tumors. Buas et al. (2014) conducted a genome-wide association study of 5,722 subjects,
2,515 of which were positive for esophageal adenocarcinoma. They analyzed 37
different single nucleotide polymorphisms at these loci for their study. Single nucleotide
polymorphisms, usually referred to as SNPs, occur when a single nucleotide in a DNA
sequence is variable in a population. SNPs are the most common type of genetic
variation in the population.

Of the 37 SNPs at the loci studied by Buas et al., three were shown to be
associated with risk of esophageal adenocarcinoma (Table 6). Two of the variants,
rs2518720 and rs3088440, were observed to be significantly associated with reduced risk
of developing esophageal cancer. The third SNP, rs4074785, was also shown to be
associated with reduced risk of adenocarcinoma, but at borderline significance.

Buas et al. decided to further investigate rs3088440, one of the SNPs shown to be
significantly associated with reduced risk of esophageal cancer. Specifically, they noted
that this polymorphism is characterized by a transition from guanine to alanine. They
found that this polymorphism may disrupt a binding site for a particular noncoding RNA
molecule (Figure 10). Since mRNA would need to bind to this site in order to complete
the transcription process, this could effectively slow down protein synthesis. In general, cancer cells are known to increase transcription and translation of growth factors in order to continue growing and proliferating. By blocking the transcription process, individuals with this SNP may not be able to host cancer cells as effectively because the machinery
Table 5. Three SNPs were shown to have an association with reduced risk of progression from Barrett’s esophagus to esophageal adenocarcinoma. (Table taken from Buas et al., 2014).

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>Pos</th>
<th>Alleles</th>
<th>No. minor alleles</th>
<th>No. Minor Alleles</th>
<th>HR^b</th>
<th>95% CI</th>
<th>P</th>
<th>q^c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Non-progressors (n = 353)</td>
<td>Progressors (n = 55)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0 (n)</td>
<td>1 (n)</td>
<td>2 (n)</td>
<td>0 (n)</td>
<td>1 (n)</td>
<td>2 (n)</td>
</tr>
<tr>
<td>rs2518720</td>
<td>9</td>
<td>21978979</td>
<td>T/C</td>
<td>149</td>
<td>152</td>
<td>52</td>
<td>31</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>rs3089440</td>
<td>9</td>
<td>21966159</td>
<td>A/G</td>
<td>283</td>
<td>64</td>
<td>2</td>
<td>51</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>rs4074785</td>
<td>9</td>
<td>21981583</td>
<td>T/C</td>
<td>286</td>
<td>64</td>
<td>3</td>
<td>50</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>
needed to nurture those cells is not functioning effectively.

It is not as clear how the other two identified SNPs, rs4074785 and rs2518720, may affect progression to esophageal adenocarcinoma. These two SNPs, unlike the previously discussed SNP, are located in intronic sections of the genome. Generally, intronic sections of the genome are less understood than those in the functional exons. It is known that certain intronic sections are enhancers to exonic sections. Depending on where the SNPs are located on the introns, they may also affect the splicing process during post-transcriptional modification.

![RNA structure](image)

**Figure 10. The rs3088440 SNP disrupts the binding site of miRNA during transcription.** By transition of a G to an A, the binding of miRNA has one less connection to the transcribed protein, causing a weaker process and potentially decreasing the viability of a potential cancerous cell. (Figure taken from Buas et al, 2014).
A Potential Reversal of Barrett’s Esophagus

When an individual is diagnosed with Barrett’s esophagus, they are not told that it is a condition that can be reversed. The patient is warned of the potential dangers of the disease, and its association with esophageal adenocarcinoma. They are given a list of lifestyle modifications that may stave off esophageal cancer, but the metaplasia that has already taken hold is considered irreversible.

By gaining a greater understanding of the risk factors and etiology of esophageal adenocarcinoma, we may be able to change this outlook. If Barrett’s esophagus is truly a pre-cancerous state, our focus should be on curing Barrett’s before the dysplasia associated with the cancer occurs.

The most common non-surveillance treatment for Barrett’s esophagus today is surgery. Caygill et al. (2011) noted that decreasing the gastroesophageal reflux may cause reversal of Barrett’s esophagus cells. A Nissen fundoplication, as described previously, could decrease symptoms of gastroesophageal reflux by tightening the opening of the lower esophageal spincter. Unfortunately, the results of antireflux surgery have been mixed. While some patients have reported a reversal of metaplasia, still others have gone on to develop adenocarcinoma.

For this reason, Caygill et al. recommends that patients who undergo antireflux surgery still go to regular appointments with their gastroenterologists for routine monitoring of the lower esophagus. Surgery, therefore, is not a surefire way to cure Barrett’s esophagus and it is clear that other avenues must be explored.
A few recent studies have begun looking into other methods of treating Barrett’s esophagus in order to prevent the onset of cancer. Inge et al. (2013) investigated the effects of Dasatinib, an inhibitor of the Src kinase, on Barrett’s esophagus cell lines. Src kinase is what is termed a “proto-oncogene”. A proto-oncogene is a normal gene that can become an oncogene (a pre-cancer gene) if it becomes dysfunctional. What they found was that Dasatinib reduced activation of the Src kinase, reducing phosphorylation of p27. p27 is a tumor suppressor (a molecule that normally functions to reduce genesis of tumors). When phosphorylated, p27 loses its ability to regulate cell proliferation.

Increasing the amount of active p27 led to decreased proliferation, arrest of the cell cycle, and activation of apoptosis (Table 6). The cell cycle is comprised of multiple phases. The two phases of interest to this experiment were the G phase and the S phase. The G phase is essentially a “rest” phase in which the cell is not actively undergoing the processes needed to replicate. The S phase is the phase in which the cell is replicating its DNA in preparation for proliferation. As treatment time increased, the Barrett’s

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>G1</th>
<th>S</th>
<th>G2</th>
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<tr>
<td>0</td>
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</tr>
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<td>24</td>
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<td>26.3%</td>
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<td>72</td>
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<td>24.94%</td>
<td>14.52%</td>
</tr>
<tr>
<td>96</td>
<td>73.27%</td>
<td>22.77%</td>
<td>14.36%</td>
</tr>
</tbody>
</table>

**Table 6.** Dasatinib induces cell cycle arrest at G1 in Barrett’s esophagus cells. As time of treatment in hours (top) increases, the percentage of cells arrested at G1 increases. (Table taken from Inge et al, 2013).
esophagus cells were arrested in the G1 phase, meaning that they were not allowed to progress to the stage in which DNA replication could take place. While more research needs to be done on the potential effects of Dasatinib, the results of this study are promising. This drug could be a pharmacological means of halting the progression of Barrett’s esophagus.

One of the newest potential approaches for treatment of Barrett’s esophagus is cryotherapy. In 2011, Xue et al. conducted a pilot study to determine the efficacy of cryotherapy by pressurized carbon dioxide gas in patients suffering from Barrett’s esophagus. They enrolled 22 patients into their study, 20 of which completed the treatment. The treatment constituted stepwise cryoablation with the pressurized gas. Most patients underwent two treatments in order to eradicate the Barrett’s esophagus metaplastic cells.

Once the cryogenic spray was applied, ice immediately formed on the esophageal mucosa. Once the cryoburn of the formed ice abated, the mucosa of the lower esophagus began to erode. Patients did not report any significant adverse events during treatment. After cryoablation treatment was complete, the Barrett’s esophagus cells were no longer present (Figure 11).

Of the patients that completed the treatment, only three showed recurrence of Barrett’s esophagus cells after six months. While this was only a pilot study that involved a limited number of participants, the implications are quite astounding. The rate of success of this treatment in this initial study was high (85%) and further improvements
to the technology may improve the outcome still. Cryotherapy seems to be a promising field that may help to turn the tide against rising incidence of adenocarcinoma.

Figure 11. Endoscopic images of the lower esophagus in a 52-year old male before and after treatment with cryotherapy. Before cryotherapy, this patient had a 2 cm. segment of Barrett’s esophagus (a). After three cryotherapy sessions, the lower esophagus resumed its normal appearance and the Barrett's esophagus cells were not present (b). (Figure taken from Xue et al, 2011).
CONCLUSIONS

After conducting this investigation, it is clear that we still do not understand the complex relationship that defines the etiology of esophageal adenocarcinoma. Several potential risk factors have been identified, many of which are modifiable. These modifiable risk factors include obesity, hormonal changes, *H. pylori* infection, and lifestyle habits such as smoking. Recently, it has also been found that there are genetic factors at work that may predispose individuals to certain risk factors for esophageal adenocarcinoma. It has also been postulated that genetics can predispose a person to Barrett’s esophagus or adenocarcinoma directly. Many studies of these risk factors are still producing conflicting results, so it is possible that there are other variables that are not yet being considered.

Researchers must continue to study these risk factors extensively in order to find potential new treatments for Barrett’s esophagus. While there have been some recent advances, including the advent of cryotherapy, researchers and physicians still have a long way to go before Barrett’s esophagus and adenocarcinoma are no longer a problem. Further research into the true mechanism that underlies the transition from metaplastic Barrett’s cells to dysplastic cancerous cells will be key in our understanding of the disease process and future treatment regimens.
REFERENCES


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Education: Master of Science in Medical Science, Expected May 2015
            Boston University School of Medicine, Boston, MA

            Bachelor of Engineering in Biomedical Engineering, May
            2013
            Vanderbilt University, Nashville, TN

Work & Research Experience

10/2014-Present Associate Director of Business Development
BlueTheory Clinical Trials, Boston, MA

- Managed the company’s business development department
- Designed training procedures for new employees and led professional development initiatives for existing employees
- Worked with company owners to develop company initiatives designed to increase company revenue
- Led the business development team at multiple conferences in the clinical research industry

06/2014-09/2014 Senior Business Development Coordinator
BlueTheory Clinical Trials, Boston, MA

- Worked with pharmaceutical companies and contract research organizations, connecting them with high-performing clinical research sites across the US and Canada
- Managed business development initiatives at a number of research sites, helping them to increase their clinical trial pipelines
- Served as main point of contact between research sponsors and clinical research sites for new trial opportunities, expediting the study start-up process
- Recruited new clinical research sites to the BlueTheory network

**07/2011-08/2013**  
Clinical Research Coordinator  
Compass Research, Orlando, FL  
- Conducted research trials as lead coordinator – indications were Alzheimer’s disease, Parkinson’s disease, and Multiple Sclerosis
- Saw patients, conducted lab work, and served as the main point of correspondence for the pharmaceutical companies and Compass Research
- Performed rating scales, including the Mini-Mental State Examination (MMSE), ADAS-Cog, and Clinical Dementia Rating (CDR)
- Assisted physicians with medical procedures, including lumbar punctures

**05/2011-08/2013**  
Business Development Assistant  
Compass Research, Orlando, FL  
- Gained experience with the business side of clinical research
- Submitted clinical protocols for review to IRB, served as contact for legal bodies regarding submissions of new clinical trials

**09/2012-05/2013**  
Research Assistant for Dr. Robert Galloway  
SNARL Laboratory, Vanderbilt University, Nashville, TN  
- Developed software utilized for image-guided surgery
- Shadowed ophthalmologist who utilizes SNARL technology in practice

**10/2012-04/2013**  
Project Leader, Coffee Ring Diagnostics  
- Worked with a team to develop a diagnostic test for malaria based on the principles of the microfluidics of an evaporating coffee drop to be used in resource-constrained environments
• One of 24 teams selected to present at Rice University’s Beyond Traditional Borders National Design Competition, Houston, TX (April 5, 2013)

05/2010-08/2010
Research Assistant
Compass Research, Orlando, FL
• Learned how to understand clinical protocols and the practice of clinical research
• Developed new procedures for the intake and analysis of data
• Trained staff members on new procedures

08/2009-05/2011
Reeve Front Desk Assistant
Vanderbilt University, Nashville, TN
• Served as a resource for residents, guests, and visitors to various dormitories at Vanderbilt
• Monitored traffic in the halls and assisted the Resident Advisors in keeping order in the dorms

Activities

• Mentor for Incoming Freshmen in Biomedical Engineering, Vanderbilt University (2012-2013)
• Studied abroad in Galway, Ireland at the National University of Ireland, Galway (Fall 2011)
  o Member of Biomedical Student Society
• Shadowed Dr. Ira Goodman, Neurologist, Orlando, FL (Summer 2011)
  o Saw patients with a variety of neurological complaints, primarily those with memory disorders
• Biomedical Engineering Society, Vanderbilt Chapter (2010-Present)
  o Member of Executive Board (2012-2013)
  o Historian (2012-2013)
• Dyer House President, Vanderbilt University (2010-2011)
  o Led programming initiatives for dormitory and quad, serving as representative for over 100 peers

Volunteer Experience

01/2013-Present
Seasons Hospice, Newton, MA
• Helped the needs of patients with a variety of terminal medical diseases in the greater Boston area
10/2012-04/2013 Alzheimer’s Association Mid-South Chapter, Nashville, TN
  • Helped run special events and led advocacy initiatives

Skills/Expertise

• Proficient in Salesforce, Microsoft Office Suite and MATLAB
• Working Knowledge of Wolfram Mathematica, C++, and Visualization Toolkit (VTK)