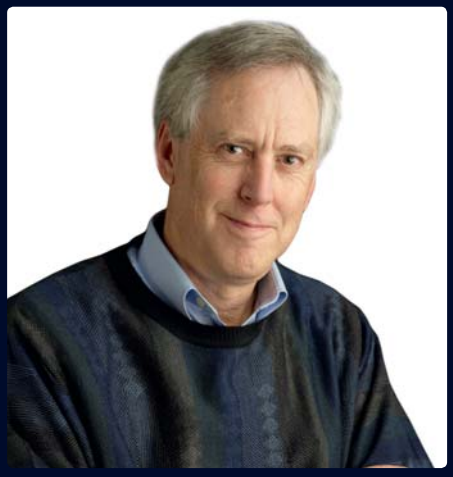
PROFESSORS THOMAS VAUGHAN & DAVID WHITEMAN

Barrett's and Esophageal Adenocarcinoma Genetic Susceptibility Study

Two epidemiologists co-leading the BEAGESS project, **Professor Thomas Vaughan** and **Professor David Whiteman**, give an insight into how they look for risk factors associated with Barrett's esophagus and esophageal adenocarcinoma, and the technology that has helped them



Could you begin by detailing the main aims and objectives of this research?

The Barrett's and Esophageal Adenocarcinoma Genetic Susceptibility Study (BEAGESS) is a large-scale genome-wide association study using pooled data and DNA from 15 epidemiologic studies to study esophageal adenocarcinoma (EA), a rapidly fatal disease. Specifically, we are evaluating the influence of genetic susceptibility on the risk of this cancer and its main precancerous condition, Barrett's esophagus (BE), and exploring the extent to which susceptibility factors vary according to key environmental and host risk factors for these conditions.

Why is so little known about the underlying reasons for the variability of EA development?

Until the mid-1980s, EA was considered a very rare cancer and not often the subject of studies of etiology, prevention and treatment. Within a very short time-frame it became the most common type of esophageal cancer in the U.S., eclipsing esophageal squamous cell carcinomas in the late 1990s. Thus it has been on the 'radar screen' of epidemiologists and basic scientists for only a short while compared with more common cancers such as lung, breast, prostate and colon cancer.

Can you offer an insight into the cofactors that may contribute to the chances of contracting EA?

In most Western countries, the incidence of EA is up to eightfold higher in men than women, suggesting either that men are more highly exposed or more susceptible to known risk factors than women, or that women are in some way 'protected' from these cancers. Most studies indicate that men and women are exposed to known risk factors with similar frequencies, and so interest is now turning to sex-specific susceptibility factors which might modify the effects of other causal factors.

Obvious candidates of interest include reproductive or hormonal factors, although to date evidence is lacking that any of these factors play a significant role. There is growing evidence that established risk factors may interact in unexpected ways to increase EA risk. For example, there is preliminary evidence that people who are both substantially overweight and who suffer from frequent or severe gastroesophageal reflux may develop EA much more frequently than expected.

How are you investigating the influence and impact of these cofactors on EA?

The formation of the Barrett's and Esophageal Adenocarcinoma Consortium (BEACON) has been a huge step forward in facilitating research into causes and potential prevention of this highly-fatal cancer, as members represent much of the epidemiologic expertise and experience in the etiology of BE and EA in the world at present. BEACON made our current genome-wide study possible.

Participants eligible for this investigation were those who had taken part in one of 15 studies of EA or BE conducted at 12 sites around the world. In total, there were 2,736 patients with BE, 1,585 patients with EA and 2,268 controls. Geographic areas represented include Western Europe, Australia and North America.

For the primary analyses, we will be restricting the sample to non-Hispanic Caucasians to minimise possible confounding influences of ethnicity, but no other restrictions will be applied. Supplemental analyses will investigate whether genotypes linked with increased risks of BE or EA are modified in their associations by exposure to environmental (eg. smoking, obesity) or demographic (eg. sex, age) factors known or suspected to be related to these conditions.

How have recent advances in technology played a role in the development of this study?

When the seeds for this study were first planted in 2006, affordable methods for genotyping would allow for the investigation of several thousand variants in or near a few hundred selected genes. When the grant application was initially submitted to the U.S. National Institutes of Health in 2007, we proposed studying approximately 600,000 variants, and by the time we began the study in 2009, technologic advances allowed a study of over 1 million markers. Thus we are clearly able to perform a much broader and more powerful scan of potential genetic susceptibility markers than we would have just a few years earlier. Once our initial analyses are complete, we anticipate being able to use powerful next-generation sequencing to follow-up on the most interesting findings.

The hunt for cancer risk factors

Esophageal adenocarcinoma is a dangerous cancer with a high mortality rate. New research looks at different risk factors associated with this cancer and how to classify susceptibility among populations

CANCER OF THE esophagus is relatively rare compared to some of the better known cancers, but one particular form of esophageal cancer, esophageal adeoncarcinoma (EA), is on the rise. Over the past four decades EA has been increasing at an alarming rate in many regions of the Western world, including the US, UK, Western Europe and Australia. There is even evidence that indicates an increasing occurrence of EA in some Asian countries, such as Singapore and Japan, where the disease has traditionally been rare. In the past 35 years, EA incidence has increased sixfold overall and tenfold among men of Caucasian ancestry in the U.S.

EA is particularly lethal with a mortality rate greater than 85 per cent and a typical survival time of less than a year from diagnosis. The high mortality of EA can largely be attributed to the fact that patients remain asymptomatic until tumours have grown to a substantial size. Unfortunately at this point the cancer has usually spread beyond the esophagus. If patients are diagnosed early, surgical treatment (esophagectomy) is usually successful, but other treatments, such as radiotherapy or chemotherapy, in the large majority of people diagnosed with more advanced cancer, are generally ineffectual.

Professor Thomas Vaughan, co-head of Epidemiology at the Fred Hutchinson Cancer Research Center in Seattle, describes the known risk factors associated with developing EA and the precursor condition Barrett's esophagus (BE): "Epidemiologic studies from around the world have shown convincingly that the principal risk factors for EA and BE are frequent symptoms of gastroesophageal acid reflux (GER), obesity and smoking". While smoking is in decline (especially in men), GER is a common condition and obesity has become a prime public health concern in many developed nations.

RISK FACTORS

Gastroesophageal reflux (GER), widely known as acid reflux, is common and causes heartburn, happening when acid rises up from the stomach into the esophagus. Studies have shown that people suffering frequently (at least weekly) with GER are five times more likely to contract EA than those who never or rarely experience GER. Vaughan explains how this can cause EA: "The prevailing mechanistic explanation is that the repeated exposure of the esophageal lining to stomach acid results in a cycle of inflammation, necrosis and repair, during which mutations arise in the cells of the esophageal epithelium, thereby initiating carcinogenesis". Reflux appears to be particularly crucial to the neoplastic process, with associated chronic inflammation and cellular proliferation providing a fertile ground for carcinogenesis.

The importance of obesity with respect to EA has been well-documented in many developed countries, but the mechanisms by which excess weight increases an individual's chances of contracting EA are still a mystery. One possible explanation is that obesity often increases reflux, suggesting the two risk factors might be closely linked. Smoking is thought to increase risk through a combination of direct genotoxic and local inflammatory mechanisms.

While current trends point to the importance of recent changes in the prevalence and severity of these factors, EA development is not a foregone conclusion in all obese smokers who experience GER. Only about 10-15 per cent of individuals with longstanding reflux symptoms develop the precursor to EA, BE, in their lifetimes and among those who do, the vast majority never develop EA. The underlying reasons for this variability are not known; clearly EA development is a complex process.

These results will aid in the identification of biological pathways important in the etiology of this cancer, and help target people at highest risk so that screening, prevention and surveillance efforts can be directed most effectively

BARRETT'S AND ESOPHAGEAL ADENOCARCINOMA GENETIC SUSCEPTIBILITY STUDY (BEAGESS)

OBJECTIVES

By using DNA samples from more than 7,000 participants recruited from three continents, BEAGESS aims to identify the genetic variants that confer susceptibility to esophageal adenocarcinoma and its only known precursor, Barrett's esophagus.

KEY COLLABORATORS

Leslie Bernstein, City of Hope, San Diego • **Brian Reid**, Fred Hutchinson Cancer Research Center, Seattle • **Douglas Corley**, Kaiser Permanente Research Division, San Francisco • **Olof Nyrén; Weimin Ye**, Karolinska Institute, Stockholm, Sweden • **Yvonne Romero**, Mayo Clinic, Rochester • **Xifeng Wu**, MD Anderson, Houston • **Wong-Ho Chow**, National Cancer Institute, Bethesda • **Nick Hayward**, Queensland Institute of Medical Research, Brisbane, Australia • **Liam Murray**, Queens University, Belfast, Northern Ireland • **Alan Casson**, University of Saskatchewan, Manitoba, Canada • **Geoffrey Liu**, University of Toronto, Toronto, Canada • **Marilie Gammon**, University of North Carolina, Chapel Hill • **Rebecca Fitzgerald**, University of Cambridge, Cambridge, UK • **Laura Hardie**, University of Leeds, Leeds, UK • **Anna Wu**, University of Southern California, Los Angeles • **Harvey Risch**, Yale University, New Haven • **David Levine**, University of Washington, Seattle • **Nigel Bird**, University of Sheffield, Sheffield, UK

FUNDING

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THOMAS VAUGHAN studied biomedical engineering (BS) at Cornell University, medicine (MD) at The University of Illinois and epidemiology (MPH) at The University of Washington before joining the faculty of the Fred Hutchinson Cancer Research Center. He investigates environmental and host factors that underlie uppergastrointestinal and respiratory cancers.

DAVID WHITEMAN is a medical epidemiologist at the Queensland Institute of Medical Research in Brisbane, Australia. He received his medical degree and PhD from the University of Queensland, before pursuing postdoctoral studies at Oxford University. He returned to Australia in 2000 to lead studies of esophageal, ovarian and skin cancer.

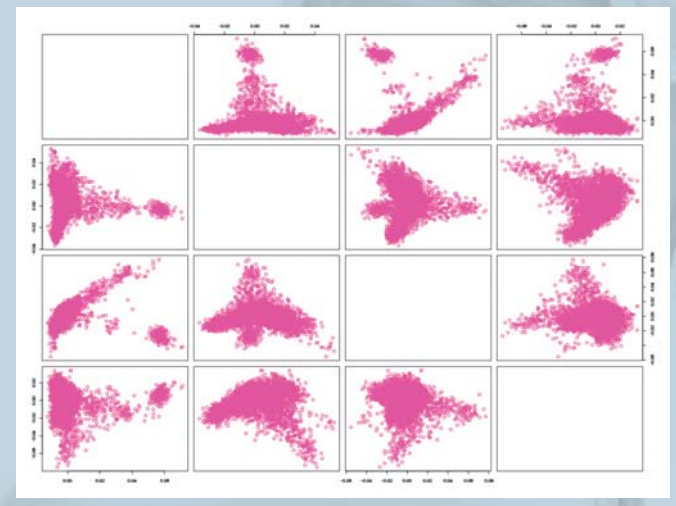

Figure 1. Extensive efforts are made to ensure that participants are homogeneous with respect to ethnic background. Here, analyses reveal discrete clusters of individuals for stratification.

BEAGESS

Exploring the underlying reasons as to why these factors affect people's susceptibility to BE and EA, the Barrett's and Esophageal Adenocarcinoma Genetic Susceptibility Study (BEAGESS), looks at genetic causes in association with host and environmental factors. Vaughan, who leads the study, is encouraged by the potential of BEAGESS: "Our results will aid in the identification of biological pathways important in the etiology of this cancer, and help target people at highest risk so that screening, prevention and surveillance efforts can be directed most effectively," he comments.

Researchers involved in BEAGESS hypothesise that cofactors contribute to the chance of contracting EA by modulating the chronic inflammatory effects of GER on the esophageal epithelium and the local and systemic consequences of being overweight and smoking cigarettes. Many of these cofactors are likely to be genetic. Essentially then, following this line of reasoning, obesity, GER and smoking combine to increase EA risk through a network of interconnected mechanisms: metabolic abnormalities associated with the insulin resistance syndrome, inflammation and oxidative stress and alterations in sex steroid hormone levels. These mechanisms act together to drive the neoplastic process. BEAGESS researchers believe that many of these steps might be mediated through genetic factors.

INVESTIGATING EA

Studies aimed at understanding and quantifying how the effect of risk factors might differ across subgroups of individuals (eg. women versus men, obese versus non-obese, etc.) require a much larger study size than normal. To meet these needs, BEAGESS takes advantage of the extensive data collected by investigators in the Barrett's and Esophageal Adenocarcinoma Consortium (BEACON), using these datasets to conduct a large-scale genome-wide association study. These studies comprise the majority of the well-designed, largely population-based studies of these diseases in the world; most have collected extensive information on key environmental and lifestyle risk factors along with DNA samples.

BEAGESS researchers use the Illumina HumanOmni1M Quad array which provides genotype and related information on over 1 million markers in a multi-phase design that includes both discovery and testing datasets. It is hoped that these results will aid in the identification of biological pathways important in the etiology of EA, and help target high-risk people.

SUSCEPTIBILITY

Vaughan anticipates considerable further research exploring whether known factors modify any of the genetic associations that might be observed: "We know that obesity, reflux and smoking are strong environmental determinants of EA risk, and it is therefore of interest to know whether some genotypes exert their influences only within subgroups of the population exposed to one or other of these factors". The genome-wide association studies will be disseminated widely so that the analyses can be scrutinised and replicated by others. Results will be published in peer-reviewed scientific literature and presented at clinical conferences, and raw data will be posted on publicly-available websites (dbGap).

Several novel approaches to analysis will be explored, including 'gene-based' and 'pathway-based' models. Instead of testing every single genetic polymorphism for an association with the outcome in isolation, these newer methods integrate information from groups of polymorphisms based on biology, such as whether they arise in the same gene, or in groups of genes that code for proteins in the same enzymatic pathway.

BEAGESS investigators are also in the process of developing comprehensive risk models from genetic and other BEACON-based studies. Methods to cost-effectively classify people into increasingly high-risk strata are being explored from various different angles so that patients can be offered programmes for prevention and early detection tailored to their absolute risk of developing EA.

The impact of these efforts should be huge: with new EA prevention and control interventions making optimal use of knowledge regarding inherited susceptibility, environmental and lifestyle risk factors, protective factors and biomarkers that predict neoplastic progression, response to therapy and survival.

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