This is to certify that the

thesis entitled

APCI1307K AND THE RISK OF
COLORECTAL CANCER IN ISRAEL

presented by

Joseph Bonner

has been accepted towards fulfillment
of the requirements for

Master's degree in Epidemiology

Dorothy Pathak
Major professor

Date December 12, 2000

APC I1307K AND THE RISK OF COLO-RECTAL CANCER IN ISRAEL

By

Joseph Donald Bonner

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

2000

ABSTRACT

APC I1307K AND THE RISK OF COLO-RECTAL CANCER IN ISRAEL

By

Joseph Donald Bonner

Colo-rectal cancer (CRC) is the leading cause of cancer mortality in Israel Most CRC patients have no known risk factors other than advanced age. In 1997, APC I1307K, a single nucleotide polymorphism, was discovered and identified as conferring nearly a two-fold excess risk of CRC in carriers and six-fold excess risk in carriers with a family history of CRC. In 1998, the Molecular Epidemiology of Colorectal Cancer (MECC) Study was launched in Israel and the United States to investigate the genetic and environmental risk factors for CRC. This thesis project analyzes data from the ongoing MECC study. The preliminary MECC data show an odds ratio of 1.875 (95%CI 0.795-4.422) that is consistent with published relative risk estimates. This small and likely underpowered analysis found no evidence for interactions or confounding between APCI1307K and other known CRC risk factors.

ACKNOWLEDGEMENTS

To my bride Tracie, for her patience, consideration and gentle reminding, I am indebted. Without which I would never have finished.

To my boss and friend Steve Gruber, I extend my gratitude and appreciation.

To the faculty and staff of the MSU Department of Epidemiology, I am very grateful for their dedication and excellence.

TABLE OF CONTENTS

TABLE OF CONTENTS cont'd

1. LIST OF TABLES

LIST OF TABLES cont'd

2. LIST OF FIGURES

3. LIST OF SYMBOLS AND ABBREVIATIONS

APC = Adenomatous Polyposis Coli protein and gene

APCI1307K = a substitution of isoleucine for lysine at position 1307 of the APC gene

CRC = Colo-rectal Cancer

PHREG = SAS™ Proportional Hazards Regression procedure

FAP = Familial Adenomatous Polyposis

HNPCC = Hereditary NonPolyposis Colorectal Cancer

BMI = Body Mass Index

MECC = Molecular Epidemiology of Colorectal Cancer

KHC = Klalit Health Services

COX2 = cyclooxygenase-2

4. INTRODUCTION

In Israel, cancer of the colon and rectum is the leading cause of cancer mortality. In the United States it is the second cause of cancer mortality. In Israel, there are an estimated 2,000 new CRC cases annually (Israel Statistical Abstract, 1996). Across the globe, CRC is third-most incident behind lung and breast cancers. Nearly 9% of new cancer cases are CRC. (Landis, 1998)

In the United States there are estimated 150,000 new cases of CRC and 130,000 deaths from CRC annually. (Landis, 1998) This background section will summarize the global patterns in CRC epidemiology, CRC risk factors, and CRC molecular pathways.

4.1. Epidemiology of CRC

4.1.1. Geographic Differences in Incidence - The western lifestyle

Internationally, the age adjusted incidence rates of colon cancer vary from 34.1 per 100,000 Japanese men in Hawaii to 2.9 per 100,000 women in Bombay India. (Cancer Incidence on Five Continents, 1992) Within this range between Hawaii and India, the countries with the highest incidence rates are often considered "westernized" (Table 1). Increased CRC incidence follows a "western lifestyle" around the globe. This "western lifestyle" has the hallmarks of low physical activity, low vegetable/low fiber and high meat/high fat consumption. Among the 24 global geographic regions with colon cancer incidence data, Israel ranks 16th.

4.1.2. CRC Risk changes in Migrants

Migrant studies show that immigrants often adopt the health risks of a destination population. The risk changes among migrants are evident in the 1985 work of King et al. (King, 1985) They demonstrated that the age-adjusted mortality rate of large bowel cancers among first-generation Chinese-Americans in the US from Guangzhou China were 5.6 and 2.7 times the age-adjusted mortality rates of men and women remaining in Guangzhou China. (King, 1985; Schottenfeld,1996) A 1991 survey of CRC mortality rates showed that following 20 years of residency in the US, Japanese migrants to the United States have a CRC mortality rate similar to US whites; while Japanese in Japan have mortality and incidence rates significantly lower than in the US (Wynder, 1991; Schottenfeld, 1996). Puerto Rican-born residents of New York City exhibit 2.5 times the mortality and incidence rates of CRC than in Puerto Rico. At the same time this group also exhibits 0.5 - 0.67 the CRC mortality and incidence rates of whites in New York City. (Schottenfeld 1996; Warshauer, 1986). The picture of risk change in migrants is not always as clear as the Chinese, Japanese and Puerto Ricans in the United States. In Australia, the CRC incidence rate among Caucasians is 24.9 per 100,000 men and 25.6 per 100,000 women. (McMichael, 1980) Immigrants to Australia from Poland, Yugoslavia, Italy and Greece, after 16 years of residence, show differing patterns of risk changes and CRC mortality rate. The risk among Polish immigrants increased to the risk of other Australian whites. The risks among Greek, Italian and Yugoslavian immigrants were 0.7 times that of Australian whites. (McMichael, 1980, Schottenfeld 1996).

While the evidence of CRC risk changes in immigrants is not consistent, in general, migrants from low-risk areas to high-risk areas acquire the risk of destination areas. (Schottenfeld, 1996) These patterns of risk change indicate that CRC is a complex disease involving environmental and lifestyle exposures.

4.1.3. Ethnic Risk Differences for CRC

CRC incidence rates vary within populations sharing a common geographic area. Between 1986 and 1988, age-adjusted CRC incidence rates among the United States white population was of 27.7 and 19.8 per 100,000 males and females respectively. In the same time period, the age-adjusted CRC incidence rate among Blacks was 30.4 and 23.3 per 100,000 men and women respectively (Schottenfeld, 1996, MMWR, 1992). Among the Hispanic population in the US, CRC incidence rates show marked sub-population heterogeneity. Overall, Hispanics had an age adjusted CRC incidence rate of 13.6 and 8.9 per 100,000 men and women. Within the Puerto Ricans Hispanic sub-population, the age-adjusted mortality rate is 18.9 and 13.8 per 100,000 men and women. Within the Mexican sub-population, the age adjusted incidence rate is less than one-half that of Puerto Ricans; one-third that of Whites and nearly one-quarter that of Blacks. (Schottenfeld, 1996; Warshauer, 1986) (Table 2)

In Israel, the colonic and rectal cancer incidence rates vary by six-fold among ethnic groups. The population of Israel is 81% Jewish and 19% non-Jewish (Arab, Christian and other non-Jewish religions). (Statistical Abstract of Israel, 1996). The highest incidence rate is 21.1 per 100,000 Jews born in Europe or America and the lowest

incident rate is 3.4 per 100,000 non-Jews. (Cancer Incidence on Five Continents 1992; Schottenfeld,1996). (Table 3).

This striking degree of ethnic variation suggests that ethnic specific differences may account for some of the variability in rates of CRC. Well-documented evidence of founder mutations in tumor-suppressor genes is consistent with the hypothesis that genetic susceptibility contributes to ethnic variation for some cancers (e.g. Breast Cancer and BRCA1 and BRCA2) (Strewing, 1997). However other ethnic-specific lifestyle factors are equally plausible as explanations for these observed differences.

4.1.4. Family history as a CRC risk factor

Individuals who have a first degree relative with CRC appear to be at two to three times background risk for CRC. (Potter, 1999; Schottenfeld, 1996, St. John 1993). About 15-20% of all CRC cases occur in families where another relative is affected. (Cannon-Albright, 1988; Gryfe, 1999). There are two highly penetrant autosomal dominant familial CRC syndromes: hereditary non-polyposis colorectal cancer (HNPCC) and familial adenomatous polyposis (FAP), which account for about 7% and 1% respectively of all CRC burden. (Potter, 1999; Schottenfeld, 1996). It has been well established for some time that a family history of CRC is a risk factor. With this common knowledge, individuals with a family history might seek screening more rigorously than individuals with a negative family history. Also physicians probably recommend more intensive screening in individuals with a positive family history. Intensive screening efforts in the

population with a positive family history, probably augments the effect of family history as a CRC risk factor.

4.1.5. Hereditary NonPolyposis Colorectal Cancer

HNPCC is characterized by early onset CRC in individuals of families where cancers of other body systems are also present. CRC tumors in HNPCC patients are histopathologically distinct from sporadic CRC. These tumors typically demonstrate poor differentiation, a mucinous appearance, lymphocytic infiltration, histologic heterogeneity, and signet ring features. Tumors in HNPCC patients also have a distinct molecular fingerprint. The somatic mutations within HNPCC tumors demonstrate a loss of DNA replication error detection and correction capacity, termed microsatellite instability. (Lynch, 1985). Microsatellite instability is the phenotypic expression in cells arising as a consequence of mutations in five genes responsible for mismatch repair: MSH2, MHL1, MSH6, PMS1, PMS2. (Kolodner, 1995). HNPCC families also have individuals with cancers in other body systems: endometrium, stomach, upper urinary tract, small bowel, ovary, and bile ducts (Lynch, 1985). HNPCC is classically recognized by "Amsterdam Criteria" of the International Collaborative Group on HPNCC (Vassen, 1991). (Table 4). A recent report suggests that the microsatellite instability negative, HNPCC-suspected cases might be caused by mutations in the TGF-Beta type II receptor gene (Lu, 1998). It is thought that the TGF-Beta type II receptors work with APC, B-catenin and E-cadherin to facilitate inter-cellular communication and cellular adhesion. (Lu, 1998)

4.1.6. Familial Adenomatous Polyposis (FAP)

Familial adenomatous polyposis (FAP) is characterized by hundreds or thousands of adenomas that blanket the large intestine. This form of polyposis usually becomes evident in patients before age 35 and is usually identified in adolescence. In the absence of a colectomy, most patients with FAP will develop CRC by the age of 50. FAP patients can also present with pigmented ocular fundus lesions, super-numerary teeth, ostemoas, odontomas, dermoid tumors, epidermoid cysts, and follicular thyroid cancer. FAP is found in 1 out of every 8000 persons with CRC. (Foulkes, 1995) In 1987, the locus for FAP was mapped to chromosome 5q21 (Bodmer, 1987;Leppert, 1987). In 1991, the APC (adenomatous polyposis coli) gene was cloned. Studies of this protein have demonstrated that inactivating germline mutations of APC are responsible for FAP. (Kinzler, 1987;Fodde,1991;Potter, 1997). Attenuated FAP is clinically similar to FAP but is characterized by fewer polyps. Attenuated FAP is associated with an inactivating mutation within the first 158 codons of the APC gene. The multiple clinical signs of FAP and attenuated FAP appear to be associated with mutations at different loci within the APC gene. (Giardello, 1997).

4.2. APC structure and function

The APC protein contains 2843 amino acids encoded across 15 exons. (Cunningham, 1996) The APC protein is expressed in colonic epithelium (Cunningham, 1996). Germline mutations in various positions of the gene are associated with different FAP manifestations and different types of CRC. A recent study identified the region

between codon 1300 and 1500 as a mutational hot spot for the germline mutations of FAP and the somatic mutations identified in sporadic CRC. (Bodmer, 1999). Figure 1

APC has regions that correspond to B-catenin binding sites. B-catenin interacts functionally with E-cadherin. Together this triad (APC, B-catenin, E-cadherin and possibly TGF-Beta type receptors) acts as a gatekeeper for cell division and cell death. (Kinzler, 1997) Mutations in APC result in an inability to bind and degrade B-catenin, causing intracellular B-catenin levels rise. High B-catenin levels turn on transcription through the TCF-LEF system and a cascade of downstream proteins, including *myc*, are expressed. These downstream proteins, in turn are responsible for the unregulated cellular growth of polyps. Colonic epithelial cells with APC mutations fail to differentiate and fail to migrate from the base of the crypt thereby clustering as polyps. It is hypothesized that as polyps grow, APC mutated cells are exposed to the mutagenic environment of the lumen where they are likely to become a cancerous lesion. (Rubinfeld, 1987; Su, 1993; Bodmer, 1999; Potter, 1999).

4.2.1. APCI1307K

In 1997, the work of Laken et al. identified a germline T to A tranversion of codon 1307 of the APC gene that was transmitted in an autosomal dominant manner in a family with an increased susceptibility to colonic neoplasia. (Laken, 1997) This polymorphism is a functional change from isoleucine to lysine in the APC protein. APCI1307K appears to make cells highly susceptible to somatic mutations within the APC gene. (Laken, 1997)

The initial clinical case-control study of the polymorphism showed a relative risk for CRC of 1.8 among Ashkenazim CRC cases and Ashkenazim and non-Ashkenazim controls. This initial study did not find the polymorphism in the 243 cancer-free, non-Ashkenazim that they examined. (Laken, 1997) The published studies of APCI1307K and CRC show odds ratio estimates of 1.78, 1.887 and 3.4 (Laken 1997;Woodage1998; Drucker,2000). Of the studies that publish ethnicity values of subjects they show a carrier rate among Ashkenazim of 4.5%, 10%, 10%, 13% and 13.5%. (Petrukhin, 1997;Gryfe, 1999;Woodage, 1998; Drucker,2000). (Table 5) Since the initial publication in 1997, the polymorphism has also been discovered in Sephardim. (Drucker, 2000) One study of APCI1307K and familial CRC and breast cancer in 393 Norwegian patients found the single Jew in the study to be the single carrier. This individual was of Ashkenazi decent, affected with colon cancer and reported no first or second-degree relatives with either colon or breast cancer. (Lothe, 1998).

4.3. Other CRC Risk Factors

All CRC is the result of either somatic or germline genetic changes. The known inherited contributions to CRC genetics represent a small fraction of the CRC burden. (Kinzler, 1987) Other known risk factors for CRC include: advanced age, inflammatory bowel disease, a diet low in fiber and high in fat, and a sedentary lifestyle. There are other risk factors that are significant yet less consistent. The other risk factors include: excessive alcohol, smoking, changes in body weight, reproductive history, exogenous estrogens, and specific dietary components. (Potter, 1999). Aspirin use and non-steroidal

anti-inflammatory drug use have been identified as a protective factor against CRC. (Potter 1999) However, most patients with CRC exhibit no known or suspected risk factors other than age.

Most CRC patients are diagnosed when they are above 60 years old (Cancer Facts and Figures, 2000) . Very few individuals die of a competing cause of death with latent CRC. A recent autopsy series predicts the fraction of death of all causes with latent CRC to be 2.5% (6 / 231) (Kawaharada, 1998). The population in this series was all Japanese where the incidence of CRC is low but increasing. (Schottenfeld, 1996)

4.3.1. Aspirin and Non-Steroidal Anti-inflammatory Drugs

Individuals who use non-steroidal anti-inflammatory drugs (NSAIDS) appear to have less risk of developing CRC. (Potter, 1999) In rats, aspirin and other NSAIDS inhibit the production of COX2 and decrease the incidence rate of gastrointestinal neoplasia. COX2 promotes carcinogenic processes and specific COX2 inhibitors reduce both the number of and size of polyps in both animal and human studies.(Potter, 1999) The evidence generally shows a strong protective effect of aspirin but not all of the evidence is consistent. As of mid-1998, seven case control studies of aspirin and 4 cohort studies of aspirin have shown a significant effect on reducing CRC mortality rates. (Potter, 1999). However, an intervention trial of aspirin analyzed as a randomized trial that showed a strong protective effect, when analyzed as a follow-up study showed no association between aspirin and CRC mortality rates. (Paganinni-Hill, 1989;Gann, 1999; Sturmer, 1998).

4.3.2. Body Mass Index (BMI)

Body Mass Index (BMI) measures obesity. Epidemiologic studies have demonstrated weak and inconsistent associations between elevated BMI and elevated risk for CRC. Data from the Health Professionals Follow-up study was examined to address the issue. (Giovanucci, 1995) In 1995 Giovanucci et al. reported on a large (2000 cases and 2400 controls) multi-site study of CRC in the US, men with the largest body mass, lowest physical activity and highest energy intake demonstrated an odds ratio of 7.3 (95%CI 3.4-5.2) for CRC when compared with the opposite extremes of these variables. In the Giovanucci study there was little association between these individual variables (exercise, BMI and energy intake) and CRC, however the highest level of each variable, versus the lowest of each variable, demonstrated a strong association. (Giovanucci, 1995)

4.3.3. Inflammatory Bowel Disease

Inflammatory Bowel Disease is a clinical condition consisting of ulcerative colitis and/or Crohn's disease. Individuals with either one of these conditions are at 10-20 times elevated risk for CRC. In these conditions colonic epithelial cells are in a continual state of inflammation. It is thought that these cells are at greater risk for genetic changes to occur and be replicated. In the mutagenic environment of intestinal lumen, these cells are likely to acquire a cancerous mutation. (Potter, 1999)

4.3.4. Meat consumption

A diet high in meat consumption appears to increase the risk of CRC. Specifically, eating red meats prepared at high temperatures or otherwise processed

shows elevated risks of CRC (Potter, 1997). Red meats prepared at lower temperatures show somewhat elevated risks, however between studies, the evidence is inconsistent (Potter, 1997). White meats shows inconsistent relative risks, some studies show modest risk elevations; others show risk reductions (Potter, 1997). According the World Cancer Research Fund, there were seven cohort studies of meat consumption published by 1997. (Potter, 1997) A study of Seventh-day Adventists, a population of mostly vegetarians, found no association with meat consumption and CRC. (Phillips, 1985) In the Nurses Health Study, frequent vs. rare red-meat consumption showed a relative risk of 2.5 (1.2-5.0). (Willett, 1990) When consumption of all types of meat are examined collectively there is little or no elevated risk of CRC; however when individual types of meat and different types of preparation are examined the evidence points toward an association with CRC. Commercially prepared meat products like sausage show elevated risks similar to high-temperature meat preparation. (Potter, 1997)

The biological mechanisms by which meats promote cancerous growth are unclear. There have been two mechanisms postulated, 1) while meat is prepared at higher temperatures, carcinogens are formed (Potter, 1997; Potter, 1999) and 2) the consumption of meats causes the body to produce bile which has a promoting effect of CRC on colonic cells (Potter, 1997; Potter, 1999)

According to the World Cancer Research Fund, by 1997, of the 86 studies on the relationship between meat consumption and CRC, 47 show relative risks above unity, 31 show risks consistent with unity and eight report a protective effect. This group makes the following statement regarding meats and cancer of the colon and rectum:

> "It is unclear whether the specific mechanisms of increased risk associated with meat intake involve animal fat, processing and cooking methods or other factors. The evidence shows that red meat probably increases risks and processed meat possibly increases risk of colorectal cancer." World Cancer Research Fund (Potter, 1997) Page 246.
>
> "Cooking meat at high temperatures possibly increases the risk of colorectal cancer." World Cancer Research Fund (Potter, 1997) Page 251.

4.3.5. Sedentary Lifestyle

In many studies men reporting high levels of physical activity are at a lower risk of colon cancer. There is no evidence for an association between rectal cancer and physical activity. The evidence in women is inconsistent but tends to lean on the side of no association. Upon examination of physical activity at various periods of one's lifetime, vigorous activity throughout one's lifetime is associated with lower risks than physical activity in young adulthood or older adulthood. It is thought that rigorous physical activity stimulates or assists peristalsis and thus decreasing transit time. As transit time is decreased, mutagens within the lumen are expelled faster and susceptible cells have less exposure. (Giovanucci,1995; Potter, 1997; Potter, 1999)

4.3.6. Vegetable consumption

According to the World Cancer Research Fund, by 1997 there were four published cohort studies and 21 published case-control studies examining the association between vegetable consumption and risk of CRC. Of these studies, 17 show some reduced risk of CRC with increased vegetable consumption. (Potter, 1997) When specific vegetable items are examined, most studies show the protective effect of broccoli to be greater than all vegetables combined. (Potter, 1997) Most studies also show raw vegetables to have a

greater protective effect than cooked vegetables. (Potter, 1997) A postulated biologic mechanism for the protective effect of vegetables involves roughage in the lumen forcing cells to be removed rather than waiting for cells to slough. Thus cells have less time exposed to the mutagenic environment and are less likely to become a cancerous lesion. (Potter, 1997) It is also postulated that vegetables contain a vast array of chemicals that the body needs to bolster natural biological protection against cancer (Potter, 1997). The World Cancer Research Fund makes the following statement regarding vegetable consumption and risks of CRC:

> "Evidence that a diet rich in vegetables protect against cancers of the colon and rectum is convincing." World Cancer Research Fund (Potter, 1997) Page 239.

4.4. Measurement of diet in epidemiologic studies

In the study of human chronic disease etiology, measurement of diet is of paramount importance, yet is highly difficult. In modern epidemiology, there are three basic instruments used to measure diet, namely: 24-hour food recall, food diary and food frequency. Each instrument has both positive and negative aspects.

4.4.1. The 24-hour food recall instrument

The 24-hour food recall instrument asks subjects to recall all food items and quantities consumed in the past 24 hours on a randomly chosen day, and can be self or interviewer-administered. When interviewer administered, research subjects are queried for all items consumed over the previous 24-hour period. This instrument can only gain information about the most current dietary patterns. Some obscure, yet meaningful, items can be missed. For example a research subject might eat a food item of particular interest

regularly but just happened to not eat it in the reference period. When using the 24-hour recall instrument, users estimate the portion sizes and preparation methods of foods eaten. This instrument is often used when the period of time of interest is short and when the interview time from subjects is short and the degree of commitment of research subjects is limited. This instrument might work better in some cultures than in others. (Willett, 1990) This instrument has an underlying assumption that food consumption patterns are similar in day to day comparisons.

4.4.2. The food diary instrument

The food dietary instrument asks subjects to document all food consumed in accurate portion sizes. Research subjects typically have a printed form with them at each meal and document every item that they consume during a given time period. Research subjects must have a high degree of commitment to the project since the labor required by subjects using a diary type of instrument to be cumbersome. In the measurement of diet in chronic disease etiology, the dietary diary is considered the gold standard. This instrument is also subject to the same criticisms as the 24-hour recall in that items consumed with frequency of less than 1 per day are seldom found. (Willett, 1990)

4.4.3. The food frequency instrument.

The food frequency instrument queries subjects on their typical consumption patterns of a given list of items over a given period of time. It is commonly employed in chronic disease research. It can be self- or interviewer- administered. It takes comparatively little time compared with the dietary diary instrument but more time than a

24-hour recall. Food frequencies are recorded as number of times per month, per week or per day a specific food in a given portion item is consumed. This instrument is frequently used because it is easy to administer and can cover a broader period of time than other instruments. However this instrument is subject to recall bias in that users are often unable to accurately recall consumption frequency of some items. (Willett, 1990) In studies of chronic diseases, like cancer, the period of exposure is often lengthy. Constructing or administering a food frequency instrument to query on the biologically relevant period of time is very difficult and often of questionable validity. (Willett, 1990)

When a food frequency instrument is used it is often subjected to a measure of its reliability. Subsets of subjects are asked to complete a diary instrument as well as the food frequency instrument. Results of macronutrients consumed as measured by each instrument are correlated. If the macronutrient values consumed as measured by a food frequency instrument correlates with those measured by diary method then the food frequency method is then considered reliable. Often a food frequency instrument will be subjected to validity tests as well as reliability tests. When validity is being tested, a measure of a micronutrient is measured from a subject's blood. The results are then correlated with the micronutrient values calculated from the food frequency instrument. When the instrument and the blood levels correlate, then the food frequency instrument is considered valid. (Willett, 1990)

4.4.4. Nutrient Databases

When any dietary instrument is used in epidemiologic research, the macronutrients (calories, carbohydrates, fat and protein) and micronutrients (vitamins and minerals) can be referenced with every item consumed. Typically researchers use a commercially available database of common food items and their individual components to estimate subject's consumption of macro and micronutrients. However, when the research population is from a different or broad geographic area, nutrient databases prepared for one group or area might not be comparable to another group or area. Frequently when unique populations are being researched, the general nutrient databases are not appropriate and specific nutrient databases must be developed and used. (Willett, 1990)

5. HYPOTHESIS AND OBJECTIVES OF THIS STUDY

The objectives of this thesis project are: 1) an examination the relative risk estimate of APCI1307K and CRC in the MECC study. 2) an evaluation of the relative risk estimate in the presence of other risk factors for CRC. Each individual risk factor (aspirin, body mass index (BMI) ethnicity, inflammatory bowel disease (IBD), meat consumption, NSAIDS, relatives with cancer, sports participation and vegetable consumption) will be tested individually for association with CRC. The variables presenting significant odds ratio estimates will be tested in an adjusted model with APCI1307K.

This thesis project will test these null hypotheses 1) there is no association of APCI1307K and CRC and 2) an association of APCI1307K and CRC is independent of the relative risks of other risk factors and CRC.

6. METHODS

6.1. The MECC Study

The Molecular Epidemiology of Colorectal Cancer (MECC) study is an ongoing population-based case control study aimed at measuring the cancer risks associated with APCI1307K.

The sampling frame for the MECC study is Northern Israel (Figure 2), which includes the Northern and Haifa governmental districts. All men, women and children who are newly diagnosed with CRC from five major hospitals in northern Israel are eligible cases. The five major hospitals are Carmel, Rambam, Nahariya, Ben Zion and Afula. More than 80% of all CRC among patients who live in northern Israel are diagnosed at these five hospitals. Controls are gleaned from the membership list of Klalit Health Services (KHS), the largest health maintenance organization in Israel covering 65% of the population of the country.

Newly diagnosed patients with CRC are invited to participate. After obtaining informed consent they are invited to donate blood samples and answer interview questions. Following a case interview, a list of 10 controls is generated for each case from the database of KHS subscribers. Controls are individually matched to cases based on exact year of birth, gender and clinic code. The clinic code offers a match for geographic region of residence of the case. Each individual from the list is contacted until one agrees to participate. Controls are invited to participate, asked to give informed consented, asked to donate blood and interviewed at a scheduled appointment.

When cases are not members of KHS, they are still invited to participate. The KHS clinic near the residence of the non-KHC cases is used to match for a control. Geographic regions and even neighborhoods within Israel are ethnically homogenous. Individuals live near other individuals of their religion and ethnicity. Matching on clinic code and thus geographic area potentially matches on ethnicity.

The MECC questionnaire contains 1071 questions. It includes questions on demographics, in-depth health history, medication health history, nutrition history, four-generation pedigree with specific cancer questions and a 171-item food frequency questionnaire. Trained interviewers administer the questionnaire instrument in person.

6.2. Laboratory Methods

In Israel, DNA is extracted from subject's blood and shipped to Ann Arbor, Michigan. Molecular assays for APCI1307K are preformed in the laboratory of Dr. Stephen Gruber at the University of Michigan Medical School following the protocol of Laken et al. 1997. (Laken, 1997)

6.3. The MECC food frequency instrument.

The MECC food frequency instrument follows the prototype of Willet's 189-item food frequency questionnaire. It queries subjects on 171 food items common in the Israeli diet. Each item is presented within groups of like items and with common serving sizes. Table 6 lists the items within the meat and vegetable sections. This instrument is a close adaptation of Willet's instrument but its reliability and validity are currently being

studied. Currently a data source of micro- and macronutrients corresponding to this instrument is under development and unavailable for this analysis.

The MECC food frequency instrument was delivered in two forms. The first form allowed users to select a frequency as one of nine coded classifications; a second form offered seven coded classifications. The form with seven frequency categories was administered to 91/374 (24%) of subjects. The instrument with nine frequency categories was administered to 283/374 (76%) of subjects. For this analysis the scores for the individual FFQ items are harmonized into servings per week following the logic in Table 7.

6.4. Statistical Methods

All analyses are presented in matched and unmatched contingency tables. The unmatched tables are used to illustrate the frequency distribution of specific factors among cases and controls. The matched tables are used to display the matching within pairs and calculate the matched odds ratio. Contingency tables, matched and unmatched are both produced by SAS Proc FREQ. Given the matched design of the study, conditional logistic regression was required for multivariate analysis. Conditional logistic regression modeling was performed using SAS PHREG. This procedure is typically used to calculate proportional hazards in a survival analysis; however, it can be used to calculate the conditional logistic regression models required for a matched analysis. In survival analysis, a time variable and a censoring value are used. When the PHREG procedure is used for conditional logistic regression, a one-unit change in the time

variable is used. Cases have the value of 1 and controls have a value of 2 for the dummy time variable. The model building process starts with the dummy time and censoring variables and APCI1307K and following a forward selection strategy introduces the statistically significant variables found in the bi-variate analyses.

This analysis includes three continuous variables (Body mass index , weekly servings of meat and weekly servings of vegetables). Any value equal to or greater than the mean of these variables plus or minus three standard deviations of the value in the controls is considered an outlier.

7. RESULTS

7.1. Univariate, Unmatched frequencies and Unadjusted Analyses

7.1.1. Demographic variables

The MECC project as of March 1, 2000 had 610 CRC cases and 260 Controls. This analysis is on the 219 case and control pairs for which data were available. Given the matched design of the study, cases and controls are not different in age and gender. In this analysis there 112/219 (51%) male matched pairs and 95/219 (43.4%) female matched pairs. Gender as a variable in these data was missing for 12/219 (5.5%) of the matched pairs. (Table 8)

The age of the matched pairs ranged from 22-98. The mean values 71.8 and the standard deviation were 10.5. The inner quartile range was 67-78. (Table 9)
Cases and controls were also not different between each group in country of birth, religion or ethnicity. However, a statistically significant difference between each group was evident in years of education. Controls were more educated than cases. p=0.0346. (Table 10)

In this study population of 438 subjects 369 (96.6%) were Jews, 6 (1.6%) were Christian Arab, 1 was a Moslem Arab and 2 were Christian non-Arabs. (Table 11)
Among the Jews, 296/369 (80%) were of the Ashkenazi ethnic group, 71/369 (19%) were Sephardi and 2/369 (1%) were of mixed ethnicity. (Table 12)

7.1.2. APCI1307K

Of the 438 subjects in the 219 matched pairs, APCI1307K results were available for 183/219 (84%) of cases and 195/219 (89%) of controls. (Table 13) The APCI1307K polymorphism was found in 28/378 (7.4%) of all subjects 18/183 (9.8%) of all cases and 10/195 (5.1%) of all controls. (Table 14) Among the Ashkenazim, APCI1307K was found in 13/127 (10%) of cases and 7/126 (5.5%) of controls. (Table 15). The relative risk of CRC and APCI1307K in this matched analysis was 1.875 (95%CI 0.795 - 4.422). (Table 15) This value, although not statistically significant, is consistent with published relative risk estimates as outlined in Table 5. In the unmatched analysis the odds ratio of APCI1307K and CRC among all ethnicities was 2.0 (0.9-4.5). (Table 15) Among Ashkenazim the unmatched odds ratio was 1.9386 (0.7467 – 5.331). (Table 16) Each of these odds ratio values is consistent with the published odds ratio estimates. There were two discordant pairs among Ashkenazim matched pairs for a matched analysis. (Table 17). Thus a matched relative risk estimate among the Ashkenazim is not available.

Of the reported countries of birth reported, Lithuania had the highest carrier rate of 1/3 (33.3%), followed by Germany with 5/17 (29.4%). The remaining countries reported in the study had carrier rates of less than 10%. (Table 18).

7.1.3. Aspirin

The MECC questionnaire includes the following four questions "Have you taken aspirin regularly within the past year?" and "Have you ever taken aspirin at least once a week during your life?". If a subject answers yes to either of these aspirin questions, in

this analysis they were considered positive for aspirin. If individual answers no to both questions they were coded as negative. The variables were missing for 49/219 (22.4%) of cases and 43/219 (19.6%) of controls and in 33/219 (15%) of cases. Among cases 77/176 (44%) and among controls 62/186 (33%) reported positive for aspirin. (Table 19).

7.1.4. Body Mass Index

Body Mass Index was calculated as weight*height^{-2}. The mean BMI among cases was 26.4 kg*cm^{-2} (+\-3.7). The mean BMI among controls was 25.8 kg*cm^{-2} (+\-4.3). The cutoff point for outliers was above 38.7. Within both groups the mean BMI was 26.1 (+/- 4.0). One case reporting BMI of 39.5, and three controls reporting BMI of 43.4, 39.2 and 40.1 were removed as outliers. (Table 20)

7.1.5. Ethnicity

In this analysis, the ethnicity was analyzed as Ashkenazi and non-Ashkenazi. Among cases 153/219 (70%) were Ashkenazi and 62/219 (28%) where non-Ashkenazi cases; among controls 143/219 (63%) were Ashkenazi and 76/219(35%) non-Ashkenazi controls. Ethnicity was not associated with CRC risk in this analysis (OR=1.39 95%CI=0.89-2.18). Table 21

7.1.6. Inflammatory Bowel Disease

The MECC questionnaire asks: "Have you ever been diagnosed with Ulcerative Colitis?' and "Have you ever been diagnosed with Crohn's Disease?". The allowed responses are: Yes, No, Don't Know. In this analysis a yes to either is a positive for

inflammatory bowel disease. No to both is negative. Among cases 1/188 (<1%) reported positive and 1/186 (<1%) controls reported positive. Table 22

7.1.7. Meat consumption

Table 6 lists the meat and vegetable items on the MECC food frequency instrument. For this analysis, the sum of weekly servings was calculated for both meat items and vegetable items. For each item the coded frequency response was converted to a weekly serving amount following the logic in Table 7. The mean weekly sum of meat consumption was 10.6 (+/-7.8)weekly servings among cases and 10.7 (+\- 7.1) weekly servings among controls. (Table 23). The inner quartile range of meat consumption for cases was 6 to 13.5. Among controls the average reported value was 10.7 (+/- 7.2). Among both cases and controls the mean reported meat servings per week was 10.6 (+/- 7.5). Any reported value above 32.3 was considered an outlier. Three cases reporting 35, 37 and 42.5 servings per week were removed. Two controls reporting 38.5 and 55 servings per week were removed as outliers.

7.1.8. NSAIDS

As with aspirin use, the MECC questionnaire asks subjects "Have you taken non-steroidal anti-inflammatory drugs regularly within the past year?"; "Have you ever taken non-steroidal anti-inflammatory drugs at least once a week during your life?". Yes to either is coded as positive for NSAIDS. No to both is coded as negative for NSAIDS. In this analysis, 57/219 (26%) of cases and 40/219 (18%) of controls were missing NSAIDS

responses. Among cases 16/162(9%) reported positive for NSAID use and among controls, 14/179 (8%) reported positive for NSAID use. (Table 24)

7.1.9. Relatives with CRC

The MECC instrument asks users about their relatives. It queries subjects on individuals in four generations of their pedigree. Subjects are asked to report any cancer in any relative as they answer general questions about the relative. Table 25 outlines the relatives about whom cancer history information is queried from subjects. In this analysis, if a subject reports cancer of the colon or rectum in a Mother, Father, Son, Daughter, Sister or Brother then the subject was determined to have a positive family history of CRC among first-degree relatives. Family history data was available for191/219 (87%) of cases and 190/219 (87%) or controls. In this analysis 23/191 (12.4%) cases and 14/190 (7.4%) of controls had a positive family history of CRC. (Table 26). No APCI1307K carrier reported a positive family history of CRC among first-degree relatives. (Table 27).

7.1.10. Sports Participation

The MECC questionnaire queries subjects as to whether they participate in a sport activity. For this analysis, an answer of YES to this question is used as a proxy of physical activity beyond activities of daily life. In this analysis 91/187 (49%) of controls reported sports participation and 80/187 (43%) of cases. Table 28.

7.1.11. Vegetable Consumption

Vegetable items were calculated as outlined above in the meat section. The mean of weekly vegetable servings was 44 (+/-26.6) among cases and 51 (+/- 25.9) among controls. Among cases and controls the mean reported value was 48.0 (+/- 26.5). Among the controls the mean value reported was 51.4 (+/- 25.9). Any value above 129.1 was removed as an outlier. Three cases who reporting vegetable servings per week of 142.5, 151 and 162 were removed as outliers. Two controls reporting 143.5 and 148.5 servings per week were also removed as outliers. (Table 29)

7.2. Unadjusted Analyses

7.2.1. Aspirin

In the matched analysis, taking aspirin once a week ever in ones lifetime was significantly associated with an elevated risk of CRC OR=1.696 (95%CI=1.013-2.839). (Table 30). This significant odds ratio shows aspirin as a risk factor, which is not consistent with the literature, which generally reports this exposure as a protective effect.

7.2.2. Body Mass Index

In this analysis, body mass was bifurcated at the median value of controls. The relative risk for CRC and BMI was 1.750 (1.100-2.784). This significant relative risk estimate is stronger that appears in most literature as mentioned above. (Table 31)

7.2.3. Vegetable Consumption

In this analysis, a weekly vegetable serving score for all subjects was bifurcated at the median value reported among controls. The relative risk estimates of CRC and vegetable consumption were 0.460 (0.281-0.754). This significant protective effect of vegetables is consistent with the literature as mentioned above. (Table 32)

7.2.4. Other variables of interest.

No other variable measured in this analysis demonstrated a statistically significant relative risk estimate in a matched bi-variate analysis. Education, which was significant in the unmatched analysis, retained no significance in matched analysis. (Table 33)

7.3. Adjusted Analyses

Aspirin use, body mass index and vegetable consumption demonstrated statistically significant unadjusted odds ratios. Using a forward selection strategy, an initial model was evaluated using these three variables and APCI1307K and all possible interaction terms. The interaction terms were not statistically significant. A second model was built using APCI1307K, Aspirin, body mass index and vegetables, in which body mass index was not statistically significant. A third model with APCI1307K, Aspirin and Vegetables was built, in which aspirin was not significant. The final model was one of APCI1307K and vegetable consumption on a continuous scale. The matched odds ratio estimate of APCI1307K was 1.875 (0.795-4.422). (Table 34) In the final model the odds ratio estimate of APCI1307K was 1.320. At first glance, using the rule of a +/- 20% change to identify possible confounding, it would appear that the association between APCI1307K and CRC is confounded by vegetable consumption. To examine this potential confounding further a model was built of APCI1307K among the cases and controls where vegetable consumption was available for both the case and the control. In this interim, model the APCI1307K odds ratio estimate changed to 1.199. In the final model the ACPI1307K OR term was 1.320. The odds ratio difference for ACPI1307K between these two models is 10% and does not suggest substantial confounding. (Table 34 and Table 35).

8. CONCLUSION

The first null hypothesis of this study was that there was no association between ACPI1307K and CRC. In these data this null hypothesis cannot be rejected as the confidence interval of 0.795 to 4.422 around the odds ratio point estimate of 1.875 includes unity. The second null hypothesis of this study is that the association between APCI1307K and CRC is independent of other known risk factors for CRC. Since the first null hypothesis cannot be rejected, the second null hypothesis can only be examined and not thoroughly tested. In these data the non-significant point estimate odds ratios indicate that the association could be independent of other factors however this null hypothesis cannot be rejected.

The final sample size of the MECC study will be 2100 matched pairs. This analysis on the first 219 or 10% of these pairs is likely to be quite different from the analyses to be performed on the final complete dataset. This analysis is underpowered to find the effects in the direction and magnitude of the final sample size. As stated earlier, MECC is a population-based 1:1 matched case-control study. It is beyond the scope of this project to estimate power of the given sample size to find effects anticipated from the literature review, given this complex study design.

9. DISCUSSION AND LIMITATIONS OF THIS STUDY

This study examined measures of association between many known risk factors for CRC. In this analysis, only vegetable consumption and body mass index were found to have the anticipated magnitude and direction of odds ratio.

Ethnicity did not show the anticipated risk factor association, however the study design effectively matches for ethnicity as thus predetermines the inability to measure an effect. Inflammatory bowel disease, meat consumption, relatives with cancer and sports participation did not show the anticipated effects however the study is quite definitely underpowered to measure these effects.

The matching scheme for the MECC study includes a clinic code that might serve as a geographic indicator. Also stated previously within given areas of Israel individuals of a given ethnic group generally live close to others of their ethnic group. It is infrequent to find heterogeneity within a given geographic area or neighborhood. This with absence of ethnic heterogeneity in a geographic area, matching on geographic area probably functionally matches on ethnicity. In the literature, being Ashkenazi is associated with increased risk of CRC. The absence of an association in this study is due in part to lack of power, and in part to the functional match on ethnicity. The case population for this project, as stated earlier will include 80% of the CRC cases diagnosed in the region of Northern Israel. The control population for this project is limited to the 65% of the population who are members of the participating health maintenance organization. If the 20% of cases missed or the 35% of controls ineligible for the study have a different ethnic

frequency distribution, then the study design might also preclude finding an effect of ethnicity based on eligibility criteria.

The population carrier rate in this study was 7.9% (Table 14). Among the Ashkenazim in this study the carrier rate was 8.5%. Other studies among Ashkenazim in the United States (Petrukhin, 1997), 6.7% among Ashkenazim and Sephardim in Israel, (Drucker, 2000), 7.0% among Ashkenazim and non-Jews the United States (Laken, 1997), 7.2% among Ashkenazim in the United States (Woodage, 1998) and 10% among Ashkenazim in Toronto Canada (Gryfe, 1999). The carrier rate results of this analysis are consistent with other published studies.

Among the Ashkenazim the polymorphism was found in 13/127 (10.2%) of Ashkenazim cases and 7/126 (5.6%) of Ashkenazim controls. (Table 15) The studies, which publish ethnicity and APCI1307K results, show 4.5% (Petrukhin, 1997), 10% (Gryfe,1999;Laken,1997), 13% (Woodage,1998) and 13.5 (Drucker,2000) in Ashkenazim cases and 5.7% (Drucker,2000), 6%(Laken,1997), 7%(Woodage,1998) among Ashkenazim controls (Table 5). The results of this analysis are clearly consistent with these published estimates.

The lack of positive family history among carriers is not consistent with the work of Laken et al. They found a 6-fold elevated risk among carriers with a family history. Their conclusion was based on a set of 25 individuals with family history data (Laken, 1997). However, this thesis analysis is underpowered to conclude the lack of association.

It is unclear as to why the early analysis of data in this project, estimates aspirin exposure as a risk factor and not a projective effect. One possible explanation might be that aspirin consumption is associated with gastrointestinal side effects. It is feasible that the side effects cause cases to seek medical attention, where a CRC is found; but yet the aspirin has little to do with the genesis of the CRC. Another possible explanation is that individuals experiencing early symptoms of CRC often self-medicate with aspirin-like medications. But if the self-medication does not alleviate the symptoms they seek medical attention. A third possible explanation is that the questions regarding aspirin are somehow not measuring the anticipated exposure patterns.

The validity MECC food frequency instrument has yet to be tested. However, to make the results of this thesis and the MECC project more scientifically rigorous the instruments validity and reliability should be tested and the results published.

It has long been supposed that specific types of vegetables, namely broccoli, confer greater protective effect than vegetables in general. This analysis did not test for an effect of broccoli separate from all vegetables. One could surmise that the relationship between APCI1307K and vegetables might change with broccoli tested separately from other vegetables. This lack of vegetable-specific analysis is a source of unmeasured confounding in this analysis.

In cancer epidemiology, histopathologic confirmation of cancer cases is of paramount importance, generally when cancer cases are collected from a many institutions, a pathologist will review slides from the many institutions, and confirm or

refute the presence of the diagnosed cancer in the case. The MECC study followed this standard of cancer epidemiology, however this analysis did not incorporate the pathology data when including cases for analysis. The resultant set of subjects might include some misclassified cases. The net result of this misclassification is difficult to anticipate and further analysis will fully incorporate the pathology data being collected.

10. Further Study Needs

Future studies of APCI1307K and CRC should include both a measurement of vegetable consumption and history of polyps and pathology data in a larger dataset in order to fully evaluate the genetic and environmental contributions to CRC.

11. REFERENCES

Aaltonen LA, Salovaara R, Kristo P. Canzian F, Hemminki A, Peltomaki P. et.al. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screen for the disease. N Engl J Med 1998;338:1481-7.

Benito E, Cabeza E, Moreno V, Obrador A, Bosh FX. Diet and colorectal adenomas: a case-control study in Majorca – I dietary factors. Intl J. Cancer 1990;55:166-213.

Bingham SA, Nelson M. Assessment of food composition and nutrient intake in :BM Margetts , M. Nelson (eds) Design and concepts in Nutritional Epidemiology (pp153-191) Oxford: Oxford Medical Publications.

Bingham SA, Williams DDR, Cole TJ, James WPT Dietary fibre and retional large bowel cancer mortality in Britian. Br J Cancer 1979:40;456-63.

Bodmer WF, Bailery CH, Bodmer J, Bussey HJR, Ellis A, Gorman P et.al. Localization of the gene for familial adenomatous polyposis on chromosome 5. Nature 1987; 328:614-16

Bodmer W. Familial adenomatous polyposis (FAP) and its gene, APC. Cytogenet Cell Genet 1999 ; 86:99-104.

Cancer Incidence on 5 Continents 1992, Volume VI

Cannon-Albright LA, Skolnick MH, Bishop DT. Le RG, Burt RW Common inheritance of susceptibility to colonic adenomatous polyps and associated colorectal cancers. N Engl J Med 1988;391 : 533-537

Central Burreau of Statistics. Statistical Abstract of Israel 1996.

Cunningham C, Dunlop MG. Molecular genetic basis of colorectal cancer susceptibility [Reviews] The British Journal of Surgery 1996; 83(3):321-329.

Drucker L, Shpillberg O, Neumann A, Shapira J, Stackievcz R, Beyth Y, Yarkoni S. Adenomatous Polyposis Coli I1307K Mutation in Jewish patients with different ethnicities: prevalence and phenotype. Cancer 2000: 88#4; 755-760

Ekbom A, Helmick CG, Zack M, Holmberg L, and Adami HO Gastroenterology 1992;103:954-960.

Evans DG, Walsh S, Jeacock J, Robinson C, Hadfield L. Davies DR et.al. Incidence of hereditary non-polyposis colorectal cancer. Br J Surg 1997; 84:1281-5.

Fodde R, Smits R, Hofland N, Kielman M, Khan PM. Mechanisms of APC-driven tumorigenesis: lessons from mouse models. Cytogenet Cell Genet 1991 86:105-111.

Food, Nutrition and the prevention of cancer: a global perspective. World Cancer Research Fund in association with the American Institute for Cancer Research. 1997 John D Potter (Editor) Washington DC.

Foulkes WD. A tale of four syndromes: familial adenomatous polyposis, Gardner Syndrome and attenuated APC and turcot syndrome. QJM 1995 88:12 p853-863.

Gann PH, Manson JE, Glynn RJ, Burning JE, Hennekens CH. Low-dose aspirin and incidence of colorectal tumors in a randomized trial. J Natl Cancer Inst

1999;85:1220-4.

Giardello FM, Peterson GM, Piantadosi S, Gruber SB, Traboulsi EI, Offerhaus GJ et.al. APC gene mutations and extraintestinal phenotype of familial Adenomatous Polyposis. Gut 1997; 40:521-525

Giovanucci E, Ascherio A, Rimm EB, Colditz GA, Stamper MJ, Willett WC. Physical Activity, Obesity and Risk for Colon cancer and adenoma in men. Annals of internal medicine 1995:122;327-334.

Giovanucci E. Stampfer MG, Colditz GA, Rimm EB, Willett WC. Relationship of diet to colorectal adenoma in men. JNCI 1992;84;91-98.

Gryfe R, Nando DN, Geeta L, Gallinger S, Redston M. Inherited colorectal polyposis and cancer risk of the APCI1307K polymorphism Am J Hum Genet 1999 64:378-384.

Gryfe R, Nicola ND, Lal G, Gallinger S, Redston M. Inherited colorectal polyposis and the risk of the APCI1307 polymorphism. Am J Hum Genet 1999: 64;378-384.

Harpaz N, Talbot IC Semin Diagn. Pathol. 1996 :13;339-357.

Hoff G, Moen IE, Mowinckel P, Rosef O. Nordbo E, Sauar J, Vatn MG, Torgrimsen T. Drinking water and the prevalence of colorectal adenomas: an epidemiologic study in Telemark Norway. Eur J Cancer Prev 1992;423-428.

Kato I, Tominaga S, Ikari A. A case-control study of male colorectal cancer in Aichi Prefecture, Japan: with special reference to occupational activity level, drinking habits and family history. Jpn J Cancer Res 1990 : 81;115-121.

Kawaharada M. Satoh Y. Nippon Ronen Igakkai Zasshi - Japanese Journal of Geriatrics. 35(12):891-7,1998 Dec.

King H, et al 1985, Patterns of site-specific displacement in cancer mortality among migrants, American Journal of Public Health 1985 75:237-242.

Kinzler KW, Vogelstein B, Gatekeepers and caretakers, Nature 1997; 386:762-3

Kinzler KW, Vogelstein B. Landscaping the Cancer Terrain. Science 1998: 280;1036-1037.

Kinzler KW, Vogelstein B: Lessons from hereditary colorectal cancer. Cell 1987:159-170.

Kolodner RD Mismatch repair: mechanisms and relationships to cancer susceptibility. Trends Biochem Sci 1995;20:397-401.

Kune GA, Kune S, Read A, MacGowan K, Penfold C, Watson LF. Colorectal polyps, diet, alcohol and family history of colorectal cancer: a case-control study. Nutr Cancer 1991;16:25-30.

Laken SG, Peterson GM, Gruber SB, Oddous C, Ostrer H, Giardiello FM, Hamilton SR, Hampel H, Markowitz A, Klimstra D, Jhanwar S et al. Familial colorectal cancer in Ashkenazim due to a hypermutable trace in APC. Nature Genetics 1997; 17:79-83.

Landis SH, Murray T, Bolden S, Wingo PA. Cancer Statistics 1998. Ca: A Cancer Journal for Clinicians 1998; 48:6-29.

Leppert M, Dobbs M, Scambler P. O'Connell P, Nakamura Y, Stauffer D. et.al. The

gene for familial polyposis coli maps to the long arm of chromosome 5. Science 1987; 238: 1411-13

Lothe RA, Hektoen M, Johnsen H, Meling GI, Ikdahl TI, Rognumm TO, Linblom, Borresen-Dale AL. The **APC** gene I1307K variant is rare in Norwegian patients with familial and sporadic **colorectal** or breast cancer. Cancer Research 1998. 58(14):2923-*4*.

Lu S-L, Kawabata T, Imamura T, Yoshimitsu A, Nomizu T, Miyazono K, et.al. HNPCC associated with germline mutation in the TGF-Beta type II receptor gene. Nature Genetics 1998: 19:17-18

Lynch HT, de la Chapelle A. Genetic susceptibility to non-polyposis colorectal cancer. Genetic susceptibility to non-polyposis colorectal cancer [Review] Journal of Medical Genetics. 36(Potter, 1999):801-19,1999 Nov.

Lynch HT, Lynch JF, Cristofaro G. Genetic epidemiology of colon cancer. In Lynch HT Hirayama T. editors Genetic Epidemiology of cancer. Boca Raton (FL) CRC Press; 1989 p. 251-77.

Lynch PM, Lynch HT, Colon cancer genetics, New York, NY: Van Nostrand Reinhold; 1985.

MacQuart-Moulin G, Riboli E, CornJe J et al. Case-control study in Marseilles. Int J Cancer 1987; 40:179-188.

McKeownEyssen G. Epidmeiology of colorectal cancer revisited: are serum triglycerides and/or plasma glucose associated with risk? Cancer Epidemiol Biomark Prev 1994: 3; 687-695.

McMichael AJ, McCall MG, Hartshorne JM et.al. 1980 Patterns of gastrointestinal change in European migrants to Australia: The role of dietary change. Int J Cancer (Suppl) 25:431-437.

Morbidity and mortality Weekly Report (MMWR), 1992.

Nugent FW, Haggitt RC, Gilpin PA Gastroenterology 1991;100:1241-1248.

Paganini-Hill A, Chao A, Ross RK, Henderson BE. Aspirin use and chronic diseases: a cohort study of the elderly. BMJ 1989:299:1247-50.

Petrukhin L, Dangel J, Vanderveer L, Costalas J, Bellacosa A, Grana G, Daly M, Godwin AK. The I1307K APC mutation does not predispose to colorectal cancer in jewish Ashkenazi breast and breast-ovarian kindred. Cancer Research 1997:57;5480-5484.

Phillips RL, Snowdon DA. Dietary habits and risk of cancer among Seventh-day Adventists. JNCI 1985;74:307-317.

Potter JD. Colorectal Cancer: Molecules and Populations. JNCI 1999 91:11 pp 919-932

Rose DB, Boyar AP, Wynder EL. International comparisons of mortality rates for cancer of the breast, ovary, prostate and colon and per capita food consumption Cancer 1986:58;2363-71.

Rubinfeld B, Souza B, Albert I, Muller O, Chamberlain HS, Masiarz FR, Mumenmitsu S, Polaskis P. Association of APC gene prouct with beta-catenin. Science 1987; 262: 1734-1737.

Sandler RS, Lyles CM, Peipins LA, McAuliffe CA, Woosley JT, Kupper LL. Diet and risk of colorectal adenomas: macronutrients, cholesterol and fiber JNCI 1993:85;884-891.

Schottenfeld D, Winawer S. Cancers of the Large Intestine. In Cancer Epidemiology and Prevention.1996 Schottenfeld and Fraumeni (eds) Oxford University Press, Oxford.

St John DJB, McDermott FT, Hopper JL, et.al. Cancer risk in relatives of patients with common colorectal cancer. Ann Intern Med 1993 118:785-790.

Strewing JP, Hartage P, Wacholder S, Baker SM, Berlin M, McAdams M, et.al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews [see comments]. New Engl J Med. 1997; 336:1401-1408.

Sturmer T. Glynn RJ, Lee IM, Manson JE, Burning JE, Hennekins CH, Aspirin use and colorectal cancer: post-trial followup data from the physicians health study. Annals of Internal Medicine 1998;128:713-20.

Su L-K, Vogelstein B, Kinzler KW. Association of the APC tumor suppressor protein with catenins. Science 1993 ; 262:1734-7

Vassen HF, Mecklin JP, Khan PM, Lynch HT. The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). Dis Colon Rectum 1991;34:424-5.

Vassen HF, Mecklin JP, Khan PM, Lynch HT. The International Collaborative Group on HNPCC. Anticancer Res 1994; 14:1661-1664.

Warshauer ME, Sliverman DT, Shottenfeld D. et.al. 1986. Stomach and colorectal cancer incidence and mortality in Puerto Rican-born residents in New York City. J Natl Cancer Institute 1986 76:591-595

Willet WC, Nutritional Epidemiology. Oxford University Press, New York 1990.

Willett, Stampfer MG, Colditz GA et al. Relation of meat fat and fiber intake to the risk of colon cancer in a prospective study among women. N Engl J Med 1990;323:1664-1672.

Woodage T, King SM, Wacholder S, Hartage P, Streuwing JP, McAdams M, Laken SJ, Tucker MA, Brody LC. The APCI1307K allele and cancer risk in a community-based study of Ashkenazi Jews. Nature Genetics 1998: 20;62-65.

Wynder EL, Fujita Y, Harris ER et.al. 1991 Comparative epidemiology of cancer between the United States and Japan. Cancer 1991 67:746-763.

Yin J, Harpaz N, Souza RF, Zou T, Kong D, Wang S, Leytin A, Medalie NS, Smolinski KN, Abraham JM, Fleisher AS, Meltzer SJ. Low prevalence of APCI107K sequence in Jewish and non-Jewish patients with inflammatory bowel disease. Oncogene 1999 18:3902-3904.

Zeigler RC, Devesa SS, Fraumeni JF Jr. Epidemiologic patterns of colorectal cancer. In DeVita VT, Jr. Hellman S. Rosenberg SD (eds): Important Advance in Oncology. Philadelphia, J.B. Lippincott, pp. 209-232.

Table 1 Geographic difference in Age-Adjusted colonic cancer incidence rates

Per 100,000 Males	**Region/Population**	**Per 100,000 Females**
34.1	Hawaii, Japanese	22.0
34.1	Connecticut, Whites	26.1
33.1	California, Alameda, Blacks	28.5
32.0	Detroit, Blacks	29.0
24.9	Australia, Queensland	25.6
24.1	Canada	22.5
22.6	Hawaii, Chinese	20.4
20.8	Italy, Varese	16.5
19.4	Norway, Urban	18.9
18.9	Denmark	18.9
16.8	Sweden	15.8
16.6	UK England and Wales	14.7
15.9	Norway, Rural	16.5
15.8	Hong Kong	12.5
14.7	France, Calvados (urban)	10.5
13.0	**Israel, Jews born in Israel**	**14.2**
10.3	Puerto Rico	9.7
9.6	Japan, Miygai	9.4
8.5	Shanghai	7.6
7.8	Hungary, Szaboica	7.1
5.3	Costa Rica	5.3
5.2	Columbia, Cali	6.3
4.7	**Israel, Non-Jews**	**4.5**
3.4	India, Bombay	2.9
	Adjusted to 1970 world population. Source: Cancer Incidence on Five Continents, Vol VI, 1992	

Table 2 Ethnic differences in CRC in the US. Per 100,000

Race	**Males**	**Females**
Total	27.7	19.8
Whites	27.9	19.4
Blacks	30.4	23.3
Hispanics	13.6	8.9
Puerto Ricans	18.9	13.8
Mexicans	8.3	6.7
Native Americans/Alaskan Americans	12.5	11.5

Source Morbidity and Mortality Weekly Report, 1992

Table 3 Ethnic differences in Age-adjusted CRC Incidence in Israel. Per 100,000

	Males		Females		Overall
Population	**Colon**	**Rectum**	**Colon**	**Rectum**	**Colon**
All Jews	19.7	16.3	16.9	13.6	18.3
Jews born in Europe or America	22.5	19.0	19.7	16.1	21.1
Jews born in Africa or Asia	13.2	11.0	11.1	9.2	12.2
Jews born in Israel	18.1	12.8	20.5	11.0	19.3
Non-Jews	4.6	3.6	4.6	2.9	3.4

Source: Cancer Incidence in Five Continents, Vol VI, 1992

Table 4 Amsterdam Criteria

1. Three cases of familial CRC, in which two of the affected individuals are first-degree relatives of the third.
2. Colorectal cancer occurring across two generations
3. One colorectal cancer occurring before 50.
No evidence of FAP

Table 5 Studies of CRC and I1307K

Reference	Ethnicity	APCI1307 K Positive rate in cases (%)	APCI1307K Positive rate in controls (%)	Odds Ratio (95% CI)	Comment
Laken 1997	Ashkenazim Carriers = 69 N= 977 Prevalence = 7% Population	22/211 (10%)	47/766 (6%) 0/243	1.78 (1.08-2.96)	Initial Publication
Woodage, 1998	Ashkenazim Carriers = 362 N = 5003 +Rate = 7.2%	7/55 (13%)	355/4948 (7%)	1.887 (0.84-4.2)	
Petrukhin, 1998	Ashkenazim, Breast/Ovarian Kindred Carriers = 12 N = 264 Prevalence=4.5%	12/264 (4.5%)	- no published controls-		Reported no elevated risk in CRC patients, Reported 0/2 positive CRC patients. Would have expected fewer than 1 CRC case in person years reported.
Gryfe, 1999		48/476 (10%)	- no published controls		
Drucker, 2000	Jewish, Ashkenazi and Sephardi. Hospital Controls Carriers = 34 N= 502 Prevalence = 6.7%	Among Ashkenazim 5/111 (13.5%) Among Sephardim 3 / 4 (75%)	Among Ashkenazim 17/298 (5.7%) Among Sephardim 9/189 (4.8%)	Among Ashkenazim. 3.4 (1.2-7.2) Population 2.7 (2.6-2.9)	- Very high positive rate among cases. - Note positive rate among sephardim. -Sephardim from Yemen

Table 6 - FFQ Items on the MECC Questionnaire.

Category	Item
Vegetables	Artichoke (1 medium)
	Beet leaves cooked (100 g)
	Beet root cooked red beet (1)
	Cooked Broccoli (1/2 cup)
	Cooked Cabbage (1/2 cup)
	Cooked Carrots (1/2 cup)
	Cooked Okra (1/2 cup)
	Cooked asparagus (1/2 cup)
	Cooked cauliflower (1/2 cup)
	Cooked celery (1/2)
	Cooked pumpkin (1/2 cup)
	Cooked/baked sweet potato (1/2)
	Coriander
	Corn 1 ear or 1/2 cup canned
	Cucumber (1)
	Egg plant (1/2 cup)
	Fresh Broccoli (1/2 cup)
	Fresh Cabbage (1/2 cup)
	Fresh Celery (33g/stalk)
	Fresh carrot (1)
	Fresh cauliflower (1/2 cup)
	Garlic (fresh or powdered) 1 CLOVE
	Green Beans (1/2 cup)
	Lettuce (30 grams)
	Mushrooms (1/2 cup)
	Onion (1/4)
	Other vegetable
	Parsley (10g 1 stalk)
	Peas (1 cup)
	Pepper (1)
	Pickled cucumbers/eggplant/peppers
	Radish (1)
	Spinach (1/2 cup cooked)
	Sprouts (1/2 cup)
	Tomato (1)
	Zuchini (1/2 cup)

Table 6 cont'd

Meats and Eggs	Beef-steak,roast (100-150g)
	Canned Tuna
	Chicken/Turkey w/Skin (100-150g)
	Cooked Fish (100-150g)
	Deli Meat (Sliced Salami)
	Egg (1)
	Hamburg/meatballs/Kabob (100-150g)
	Hot Dogs (3)
	Internal Organs
	Lamb
	Liver (50-100 g)
	Mackerel, Salmon, Sardines (canned)
	Other meat or fish
	Pork
	Schnitzel (1)
	Shrimp or Crab
	Skinless Chicken/Turkey (100-150g)
	Smoked Turkey/Sliced Pastrami
	Soy shnitzel

Table 7 – Logic to harmonize FFQ frequency codes into weekly servings

FFQ Reported Frequency	**Weekly Harmonized Frequency**
0 per month	0 / week
1-3 per month	0.5 / week
1 per week	1 / week
2-4 per week	3 / week
1 per day	7 / week
2-3 per day	17.5 / week
4-5 per day	31.5 / week
4+ per day	28 / week
6+ per day	42 / week

Table 8 – Gender among pairs

Male Pairs	112 (51%)
Female Pairs	95 (42%)
Missing	12 (7%)

Table 9 Age Univariate statistics of matched pairs

Missing	1
Min	22
25th Percentile	67
Median	73
75th Percentile	78
Max	98
Mean (SD)	71.8 (10.5)

Table 10 Education between groups

Education	Cases (%)	Controls (%)	p-value
			0.0346
Never Studied	5 (2.2)	6 (2.7)	
Primary Grade 1-6	29 (13.2)	29 (17.4)	
Partial High School Grades 7-11	46 (21)	38 (17.4)	
Completed High School	52 (23.7)	38 (17.4)	
Post Secondary Vocational	19 (8.6)	12 (5.5)	
Post Secondary Academic	34 (15.5)	58 (26.5)	
Missing	24 (10.9)	29 (13.2)	

Table 11 Religion between groups

Religion	**Cases (%)**	**Controls (%)**
Jew	187 (85.4)	185 (84.5)
Christian Arab	3 (1.4)	3 (1.4)
Moslem Arab	0	1 (<1)
Christian – Non Arab	2 (<1)	0
Missing	27 (12.3)	30 (13.7)

Table 12 Ethnicity between groups

Ethnicity	Cases (%)	Controls (%)
Ashkenazi	153 (83.61)	143 (76.88)
Sephardi	29 (15.85)	42 (22.58)
Mixed	1 (0.55)	1 (0.54)
Arab	1 (0.55)	0

Table 13 Frequency of Cases and controls missing APCI1307K results

Missing APCI1307K Results		
	Cases	Controls
	36	24

Table 14 Unmatched analyses of APCI1307K

Unmatched Analysis - All subjects		
APCI1307K Carrier Status	**Cases**	**Controls**
Positive	18	10
Negative	166	185
Odds Ratio 2.006 (1.20 – 4.468)		

Table 15 Matched analysis of APCI1307K

Matched Analysis - All Matched Subjects		
	APCI1307k Controls Positive	APCI1307k Controls Negative
APCI1307k Cases Positive	0	15
APCI1307k Cases Negative	8	141
Odds Ratio 1.875 (95% CI 0.795 - 4.422)		

Table 16 Unmatched analysis of APCI1307K among Ashkenazim

Unmatched Analysis - Among Ashenazim		
APCI1307K Carrier Status	**Cases**	**Controls**
Positive	13	7
Negative	114	119
Odds Ratio 1.938 (95% CI 0.9845 – 5.0331)		

Table 17 Matched analysis of APCI1307K among Ashkenazim

Matched Analysis – Both Case and Control Askenazi		
	APCI1307k Controls Positive	APCI1307k Controls Negative
APCI1307k Cases Positive	0	0
APCI1307k Cases Negative	2	26

Both case and control = Ashkenazi

Table 18 - Frequency of birth country among APCI1307K Carriers

Country of Birth	**Number in Study (%)**	**Number of Carriers** (% of positives)	**Carrier Rate**	**Cases** (% of positive cases)	**Controls** (% of positive controls)
Lithuania	3 (0.7)	1 (5)	33.3%	1 (7)	0
Germany	17 (3.9)	5 (24)	29.4%	1 (7)	4 (57)
Romania	63 (14.4)	6 (29)	9.5%	5 (36)	1 (14)
Moldova	13 (3.0)	1 (5)	7.7%	1 (7)	0
Poland	70 (16.0)	5 (24)	7.1%	4 (29)	1 (14)
Ukraine	39 (8.9)	2 (10)	5.1%	2 (14)	0
Israel	55 (12.6)	1 (5)	1.8%	0	1 (14)
Other	120 (27.4)				
Missing	58 (13.2)	7			

Table 19 Unmatched analyses of study variables - Aspirin

Aspirin	Cases	Controls	Statistic
			OR=1.56 (95%CI = 1.016-2.382)
Yes	77	62	
No	99	124	
Missing	43	33	

Table 20 Unmatched analyses of study variables Body Mass Index

Body Mass Index	Cases	Controls	Statistic
Missing	50	42	
Min	17	14.8	
25th Percentile	24.1	22.9	
Median	25.9	25.1	
75th Percentile	28.4	28.1	
Max	39.6	43.4	
Mean (SD)	26.4 (3.7)	25.8 (4.3)	
	38.7	38.7	
Number of Outliers	1	3	
Binary at control Median			OR = 1.5 (95%CI = 0.98 2.31)
Above 25.1	103	90	
Below 25.1	66	87	

Table 21 Unmatched analyses of study variables - Ethnicity

Ethnicity	**Cases**	**Controls**	**Statistic**
			OR = 1.3 (95%CI=0.87-1.96)
Ashkenazi	153	143	
Non Ashkenazi	62	76	
Missing	4	0	

Table 22 Unmatched analyses of study variables Inflammatory Bowel Disease

Inflammatory Bowel Disease	Cases	Controls	Statistic
			OR=0.98 (95%CI = 0.06 15.9)
Yes	1	1	
No	187	185	
Missing	31	33	

Table 23 Unmatched analyses of study variables Meat Consumption

Meat (Servings per week)	**Cases**	**Controls**	**Statistic**
Missing	44	29	
Min	0	0	
25th Percentile	6.0	6	
Median	9.5	10	
75th Percentile	13.5	13	
Max	72.5	55	
Mean (SD)	9.6 (7.9)	10.7 (7.2)	
	32.3	32.3	
Number of Outliers	4	3	
Binary at control Median			OR=0.71 (95%CI = 0.47-1.07)
Above 10	71	93	
Below 10	104	97	

Table 24 Unmatched analyses of study variables - NSAIDS

NSAIDS	Cases	Controls	Statistic
			OR=1.29 (95%CI = 0.61 2.73)
Yes	16	14	
No	146	165	
Missing	57	40	

Table 25 - Relatives queried on the MECC questionnaire

Relationship	**Number reported per case ****	**Number reported per control ****
* Father	0.98	0.99
* Mother	0.98	0.99
* Sons	1.3	1.3
* Daughters	1.0	1.2
* Brothers	1.5	1.3
*Sisters	1.4	1.4
Paternal Grandfather	0.4	0.4
Paternal Grandmother	0.5	0.4
Maternal Grandfather	0.5	0.4
Maternal Grandmother	0.5	0.5
Paternal Uncles	1.1	1.0
Paternal Aunts	0.9	0.9
Maternal Uncles	1.1	1.0
Maternal Aunts	0.9	1.0
Cousins with cancer	0.2	0.3

* First Degree Relatives

** Numbers less than 1 indicate a subject's lack of information about a given person.

Table 26 Unmatched analyses of study variables Relatives with cancer/Positive Family History

	Cases	Controls	Statistic
Relatives with Cancer			OR = 1.7 (95%CI=0.9 - 3.4)
Yes	23	14	
No	168	176	
Missing	28	29	

Table 27 Family history of CRC in first degree relatives among APCI1307K carriers.

	First Degree relative with CRC	No First Degree relative with CRC
APCI1307K Positive	0	20
APCI1307K Negative	29	278

Table 28 Unmatched analyses of study variables Sports Participation

	Cases	Controls	Statistic
Sports			OR = 0.8 (95%CI=0.5 1.2)
Yes	80	91	
No	107	96	
Missing	32	32	

Table 29 Unmatched analyses of study variables Vegetables

	Cases	Controls	Statistic
Vegetables (servings per week)			
Missing	42	29	
Min	1.5	0.5	
25th Percentile	28.5	32.5	
Median	40	47.5	
75th Percentile	55	64.5	
Max	162	148.5	
Mean (SD)	41 (26.6)	51.4 (25.9)	
	129.1	129.1	
Number of Outliers	3	2	
Binary at control Median			OR=0.5 (95%CI=0.33-0.78)
Above 47.5	60	95	
Below 47.5	117	95	

Table 30 Matched analyses of study variables Aspirin

Aspirin	Controls Positive	Controls Negative	Odds Ratio (95%CI)
			1.69 (1.013-2.84)
Cases Positive	69	39	
Cases Negative	23	27	

Table 31 Matched analyses of study variables – Body Mass Index

Body Mass Index	**Controls Positive**	**Controls Negative**	**Odds Ratio (95%CI)**
Continuous			1.063 (0.995-1.136)
Positive/Negative *(Above and belowControl Median of 25.0 kg*cm^{-2})*			1.68 (1.05-2.68)
Cases Positive	42	47	
Cases Negative	28	26	
Second quartile of Controls (22.8 kg*cm^{-2} – 25.0 kg*cm^{-2}) Vs First Quartile of Controls (< 22.8 kg*cm^{-2})			1.20 (0.36-3.9)
Cases Positive	10	6	
Cases Negative	5	5	
Third quartile of Controls (25.0 kg*cm^{-2} – 28.0 kg*cm^{-2}) Vs First Quartile of Controls (< 22.8 kg*cm^{-2})			N/A
Cases Positive	0	0	
Cases Negative	0	5	
Fourth quartile of Controls (>28.0 kg*cm^{-2}) Vs First Quartile of Controls (< 25.0 kg*cm^{-2})			2.40 (1.15 – 5.02)
Cases Positive	44	24	
Cases Negative	10	5	

Table 32 Matched analyses of study variables – Vegetables

Vegetable Consumption	**Controls Positive**	**Controls Negative**	**Odds Ratio (95% CI)**
Continuous			0.984 (0.974-0.994)
Positive/Negative *(Above and belowControl Median of 47.5 servings/week)*			0.472 (0.293 - 0.759)
Cases Positive	56	53	
Cases Negative	25	27	
Second quartile of Controls (32.5 servings/week – 47.5 servings/week) Vs First Quartile of Controls (<32.5 Servings /week)			1.5 (0.534 – 4.214)
Cases Positive	10	9	
Cases Negative	6	9	
Third quartile of Controls (47.5 servings/week – 64.5 servings/week) Vs First Quartile of Controls (<32.5 Servings /week)			0.4 (0.125 – 1.275)
Cases Positive	8	4	
Cases Negative	10	9	
Fourth quartile of Controls (>64.5 servings/week) Vs First Quartile of Controls (<32.5 Servings /week)			0.353 (0.139 – 0.895)
Cases Positive	16	6	
Cases Negative	17	9	

Table 33 Matched analysis of other study variables

Ethnicity Ashkenazi vs. Not-Ashkenazi	**Controls Positive**	**Controls Negative**	**Odds Ratio (95% CI)**
			1.39 (0.89 – 2.18)
Cases Positive	107	46	
Cases Negative	33	29	

Inflammatory Bowel Disease Ever vs. Never	**Controls Positive**	**Controls Negative**	**Odds Ratio (95% CI)**
			1.0 (0.63 – 15.9)
Cases Positive	0	1	
Cases Negative	1	169	

Meat Servings Per Week			**Odds Ratio (95% CI)**
Continuous			0.979 (0.939-1.020)

NSAIDS	**Controls Positive**	**Controls Negative**	**Odds Ratio (95% CI)**
			1.56 (0.67-3.59)
Cases Positive	119	14	
Cases Negative	9	1	

Relatives with CRC	**Controls Positive**	**Controls Negative**	**Odds Ratio (95% CI)**
			1.818 (0.871 – 3.79)
Cases Positive	1	20	
Cases Negative	11	142	

Table 33 cont'd

Sports Participation	Controls Positive	Controls Negative	Odds Ratio (95% CI)
			0.805 (0.509-1.273)
Cases Positive	39	33	
Cases Negative	41	55	

Table 34 Multivariate analyses of APCI1307K and significant study variables

Model	Variables	Parameter Estimate	Hazard Ratio	95%CI
1. APCI1307K alone	I1307K	0.43	1.875	0.795 4.422
-2LogL=227.352				
2. Vegetables alone	VEG Servings/Week Continuous	-0.01620	0.984	0.974 0.994
-2LogL = 217.648				
3. Vegetables Binary	VEGBin <47.5 vs >=47.5	-0.75122	0.434	0.266-0.708
-2LogL = 205.478				
4. BMI Alone	BMIQ4 weight*height^{-2} <22.9 vs >28.0	0.87547	2.400	1.148 5.019
-2LogL=111.186				
5. Aspirin Alone	ASP	0.52805	1.696	1.013-2.839
-2LogL=220.029				
4. Fully Saturated	I1307K	-15.65	0	0-.
-2LogL=89.241	VEGBin	0.408	1.504	0.1-22.663
	BMIQ4			
	ASP	3.800	44.719	1.49-1338.32
	VEGBin*BMIQ4	-2.657	0.1265	0.002-2.119
	VEGBin*I1307K	-33.781	0	0-.
	BMIQ4*I1307K	32.1170	$1.78*10^{-15}$	0-.
	VEGBin*BMIQ4*ASP	24.34	$3.75*10^{-10}$	0-.
	ASP*I1307K	-3.455	0.021	0-.
	ASP*VEGBin	-20.57	0.032	0-.
	ASP*BMIQ4 *p=0.021*	-3.84	0.021	0.001-0.774

Table 34 cont d

Model	Variables	Parameter Estimate	Hazard Ratio	95%CI
5. Higher Order Terms Removed	I1307K	-0.19384	1.214	0.192-7.657
-2LogL= 72.288	Veg continuous	-0.027	0.973	0.949-0.997
	BMIQ4	0.5817	1.789	0.685-4.668
	ASP	1.154	3.171	0.995-10.104

Table 35 – Final Adjusted Analysis

Model	Variable	Matched Pairs	Parameter Estimate	Hazard Ratio	95% Confidence Interval
1. APCI1307K alone		164			
-2LogL=227.352	I1307K		0.43	1.875	0.795 – 4.422
2. Vegetables alone		148			
-2LogL = 217.648	VEG Servings/Week Continuous		-0.01620	0.984	0.974 – 0.994
3. I1307K in subjects with vegetable scores					
	I1307K		0.605	1.199	0.366-3.930
6. Final Model		139			
-2LogL=155.265	I1307K		0.27797	1.320	0.380-4.587
	Veg-continuous		-0.02232	0.978	0.965-0.991

Figure 1 Mutation Frequency in APC gene. From Bodmer et. Al

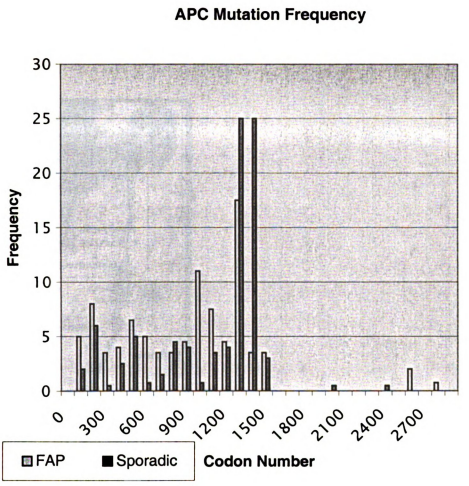

Figure 2 – Map of Israel.