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A Meta-Analysis of Association Between One-Carbon Metabolism Gene Polymorphisms and Risk of Prostate Cancer

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This is to certify that the doctoral dissertation by

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Abstract

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MSc, Carleton University, 2010

MSc, University of Guelph, 2006

BSc, Azad University, 1992

Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

Public Health

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Abstract

Prostate cancer is the most common cancer among men. The purpose of this quantitative, meta-analysis study was to examine one-carbon metabolism gene polymorphisms in a group of genes to determine their association with prostate cancer risk. The genetic epidemiology theory provided the framework for the study. The data collected were from published articles. From over 2,800 individual studies, 20 articles were retained for results and data abstraction, following the title, abstract screen, and full text screening in the second phase. The data were analyzed by a meta-analysis statistical method, combining the results from selected studies to estimate the overall association. According to study results by the adjusted p-values of fixed model, there was a significant association between decreased risk of prostate cancer and the variant of Allele T, Genotype TT, and the recessive model of C667T polymorphism. In the random model, the adjusted p-values show a significant association between decreased risk of prostate cancer, the variant of Genotype TT, and recessive model. There was an increased risk of prostate cancer in A1298C polymorphism by adjusted p-value on the variant of Genotype AC, in the fixed model. This study leads to positive social change by providing information on an optimization surveillance strategy to ensure valid screening test for prostate disease reporting. Future studies with a greater number of samples are needed, including gene-gene and gene-environment interaction to verify study results.

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Dedication

I do dedicate this study to the people who are involved in research to make a positive social change in our community, especially those who are in health sciences and work hard to make our world healthy. I do respect everyone who helped me to make this study happen and will help me to bring the results to the practice.

Acknowledgments

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Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction to the Study

Introduction

Researchers have pointed out the association between genetic factors and diseases, especially cancer (Gibson et al., 2011). Many scholars have identified gene-disease association at the first level of study (Ponder, 2001). However, there is not an appropriate sample size in an individual study to analyze this genetic disease association. In this study, I used a meta-analysis statistical method to increase the power of the test by the summary of effect size estimation. The selected genes, including their polymorphisms, were MTHFR (C667T, A1298C), MTR (A2756G), and MTRR (A66G). In this chapter, I will highlight the background of gene-disease association, including the problem statement on prostate cancer risk plus research questions. This chapter will include the theoretical framework with the assumptions, limitation, and scope. In this chapter, I will indicate the purpose of this quantitative meta-analysis on the combination of multiple studies' results to determine the association of one-carbon metabolism gene polymorphisms and prostate cancer risk.

Background of the Study

Prostate cancer should be a health concern for all males. Prostate cancer was the second leading cause of death among men in the United States in 2009 ("CDC - Prostate Cancer," n.d.). In the United States in 2009, 206,640 males were diagnosed with prostate cancer and 28,088 males died from prostate cancer ("CDC - Prostate Cancer," n.d.). The mean age of males diagnosed with prostate cancer decreased from late 1990s, and the disparities between ethnic groups diagnosed with cancer decreased as well (Crawford,

2003). The rate of new prostate cancer among males in the United States, by race or ethnicity, is the highest among African American males. The incidence rate per 100,000 males for all races is 156.9; in a subcategories by race, it is 226.0 for African Americans, 145.1 for European Americans, 121.6 for Hispanic Americans, 78.2 for Asian Americans, and 71.7 for Native Americans ("CDC - Prostate Cancer," n.d.). The heritability of prostate cancer is high, as the first-degree relative of males with prostate cancer have two to three times the chance of developing the disease (Evans, Metcalfe, Ibrahim, Persad, & Ben-Shlomo, 2008).

More than 1,000 researchers have reported an association between prostate cancer, with single nucleotide polymorphism (SNPs), and other genetic variants, in which the genome-wide association (GWA) studies identified replicated the association (Gudmundsson et al., 2009). One-carbon metabolism gene polymorphisms are involved in the folate metabolic pathway (Bailey & Gregory, 1999). There is a growing body of evidence that one-carbon metabolism gene polymorphisms are related to cancers such as colorectal cancer, breast cancer, and lung cancer (Suzuki et al., 2008; Suzuki et al., 2007; Theodoratou et al., 2012). Theodoratou et al. (2012) reported the risk factors of one-carbon metabolism gene polymorphisms and colorectal cancer. Suzuki et al. (2008) determined the effect of one-carbon metabolism related gene polymorphisms on breast cancer, and Suzuki et al. (2007) studied the effect of one-carbon metabolism related gene polymorphisms in lung cancer. Collin et al. (2009) examined the folate pathway of gene polymorphisms with the risk of prostate cancer and meta-analysis methodology. Eussen et al. (2010) researched vitamins and genes as risk factors for gastric adenocarcinoma.

Zhang et al. (2012) analyzed MTHFR polymorphism and prostate cancer risk.

Researchers found that polymorphisms in the gene-encoding methylenetetrahydrofolate

reductase (MTHFR) may impede homocysteine remethylation (Wojcieszynska, Hupert-

Kocurek, & Guzik, 2012). Wojcieszynska et al. (2012) also found a mutation of C677T

polymorphism has a frequency of 0.32 in the European American population, which the

homozygous (T677T) of this mutation has about 30%-35% of the normal MTHFR

activity. There is a gap in the literatures regarding a potential association between

MTHFR, MTR, and MTRR genes with prostate cancer risk. The gap is due to small

sample size in prior studies that reduced the power of the tests. The interrelationship

between MTHFR genotype, riboflavin, and folic acid showed in Figure 1. The riboflavin

and folate can protect individuals against loss of function of MTHFR and can reduce the

risk of cancer (Heijmans et al., 2003).
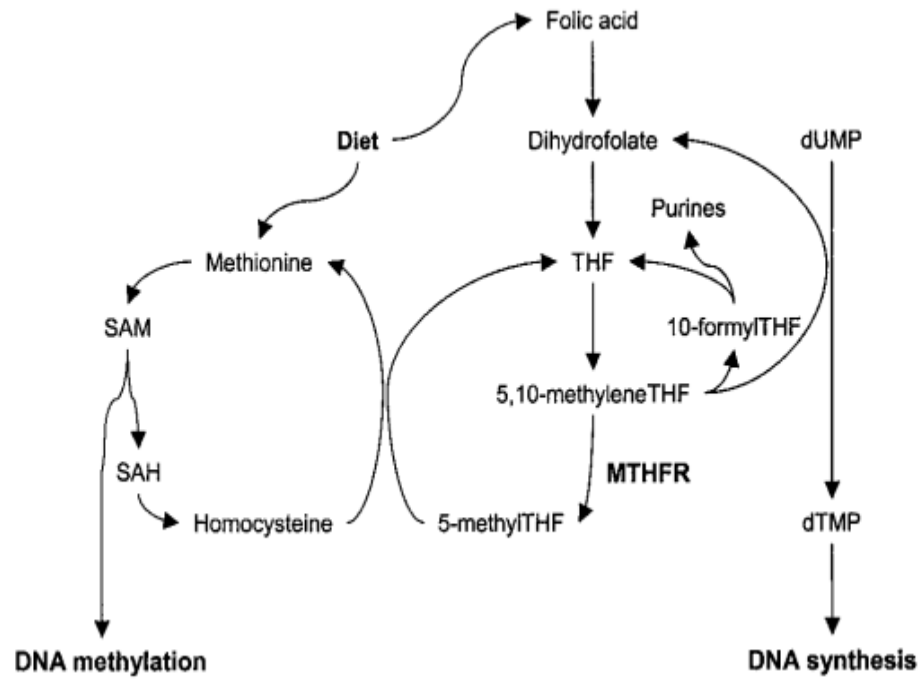
Figure 1. The interrelationship between MTHFR genotype, riboflavin, and folic acid

The relationship between riboflavin status and plasma total homocysteine (tHcy) concentration is confined mainly to subjects with the T-allele, but not in subjects with the C677C genotype. Jackson et al. (2013), one of selected subjects for this systematic review, reported an interaction between levels of folate and prostate cancer but found no interactions between genotype and folate concentration. In general, Jackson et al. reported a weak MTHFR (A1298C) effect on low-grade prostate cancer.

Descriptive Epidemiology

There are different methods of preventing cancer risk, such as screening, which serves to prevent cancer by detecting precancerous lesions. Cancer is defined as abnormal cells that divide uncontrollably that are able to invade other tissues. There are risk factors related to cell abnormality such as age, ethnic group, and family history, that may increase the likelihood of genetic mutation due to gene-environment and gene-gene interaction (Grönberg, 2003). In the etiology of prostate cancer, researchers have suggested that the Vitamin E and beta-carotene may affect the development of prostate cancer (Heinonen et al., 1998). In a study of Alpha-Tocopherol intake by male smokers in Finland, Heinonen et al. (1998) showed a 32 % decrease in the incidence of prostate cancer and a 41% lower mortality among males with prostate cancer than those taking the placebo and not receiving alpha tocopherol. Heinonen et al. found a reduction in clinical prostate cancer, but not in latent cancer. Klein et al. (2011) examined the long-term effects of Vitamin E in a defined population in the United States, Canada, and Puerto Rico and concluded that dietary supplementation with Vitamin E increased the risk of prostate cancer among healthy males.

The genetic epidemiology of prostate cancer is related to endogenous hormones, including both androgens and estrogen hormones. In studying prostate cancer risk, Ross et al. (1998) indicated the role of genetic variation in androgen biosynthesis and metabolism. The androgen receptor (AR), amino acid named CAG, which repeat length in Exon 1, may relate to prostate cancer risk. There are prostate cancer-associated genes in the length of the polymorphic CAG repeat in androgen receptor gene (AR) greater than or equal to 20 repeats (Gu, Dong, Zhang, & Niu, 2012). The purpose of this systemic review was to evaluate the risk of prostate cancer on the evidence of SNP-based genotyping panels of three one-carbon metabolism gene polymorphisms (MTHFR, MTR, and MTRR) in the quantitative literature.

A polymorphic in an allele is a variation in DNA if it occurs in at least 1% of the population (Feero, Guttmacher, & Collins, 2010). Feero et al. (2010) also indicated that SNP are variations in the DNA sequence that occur about once in every 800 base pairs. In this study, I examined a group of genes named one-carbon metabolism gene polymorphisms: MTR (5-methyltetrahydrofolate-homocysteine methyltransferase), MTRR (5-methyltetrahydrofolate-homocysteine methyltransferase reductase), and MTHFR (methylenetetrahydrofolate reductase).

The MTR gene is located on the long (q) arm of Chromosome 1 at position 43. The MTR gene is in the structure of an enzyme called methionine synthase. This enzyme plays a role in processing amino acids in a particular methionine synthase, which perform a chemical activity to convert the amino acid homocysteine to another amino acid called

methionine (López-Cortés et al., 2013). The variant of MTR (A2756G) may increase the risk of cancer (Jackson et al., 2013).

The MTRR gene is located on the short (p) arm of Chromosome 5 at location 15.31. The MTTR gene provides instructions for making methionine synthase reductase, which it is for the proper function of the methionine synthase enzyme (Watkins et al., 2013). This enzyme can continue to produce methionine. Without methionine synthase reductase, the cycle of synthesis cannot convert homocysteine to methionine (National Center for Biotechnology Information [NCBI], 2014). The variant of the MTRR (A66G) gene may be associated with an increased risk of cancer (Jackson et al., 2013).

The MTHFR gene is located on the short (p) arm of Chromosome 1 at location 36.3. The MTHFR gene making methylenetetrahydrofolate reductase enzymes, which involves a chemical reaction of the B-vitamin folate, also called folic acid or vitamin B9 (NCBI, 2014). In the description of the enzyme, methylenetetrahydrofolate reductase, it converts 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The mutation of changes in single amino acids (677C>T) in methylenetetrahydrofolate reductase causes inactivated (turned off) the enzyme (NCBI, 2014). The mutation can increase the risk of cancer (López-Cortés, 2013).

Prostate Cancer Screening

The aim of this meta-analysis was to summarize the results of previous studies and evaluate the evidence of an association between one-carbon metabolism gene polymorphism and prostate cancer risk, due to prostate cancer genetic screening. It is the value of SNP's in the detection of and prediction of prostate cancer.

Cancer screening program is used to test cancer in individuals, to evaluate asymptomatic people in the community, and to detect an unsuspected disease or risk due to improved health outcomes. Prostate cancer screening occurs in two ways: by prostate-specific antigen test (PSA) of the blood sample and the digital rectal exam (DRE). The prostate cancer screening is a program for monitoring progression in patients who are involved in prostate cancer risk, the process approved by the U.S. Food and Drug Administration (FDA) in 1986 (Rao, Motiwala, & Karim, 2008). According to Ilic, O'Connor, Green, and Wilt (2011), prostate cancer screening does not have a statistically significant mortality difference between those males who are randomized to screening and the control group. In addition, there is no an indication that age is a risk factor for participants who are screened for prostate cancer mortality. Early prostate cancer screening is beneficial as males have a life expectancy of less than 10-15 years once diagnosed (Ilic et al., 2011).

Some people fear mandatory genetic tests or prostate cancer genetic screening, which may reduce the right of privacy and potentially leading to discrimination (Fulda & Lykens, 2006). In addition, some people fear genetic testing of predictive diseases, like prostate cancer, because employers or the insurance company may absolve their financial responsibility for treating prostate cancer. The employer would not wish to hire a person with prostate cancer because they do not wish to pay increased medical or sick day benefits. The insurance company for individuals with prostate cancer may reduce benefits, refuse health care coverage, or increase premiums (Fulda & Lykens, 2006).

Ethically, some family members may not wish to be informed of the positive test's result, as the disease would be an inherited disease.

The primary disadvantage of screening is the high rate of false-positive results for a PSA test. There is also the risk of infection, bleeding, and pain in trans rectal ultrasonography guide biopsies ("CDC - Prostate Cancer," (n.d.). The rate of the PSA test reducing mortality rates remains unclear, and Canada no longer recommends the PSA test as a population-based screening test ("Canadian Cancer Statistics Publication," n.d.). The purpose of this study was to better understand genetics and its relationship to diseases, including how to able to prevent the disease by establishing a policy and treatment procedures. The results of this study may help health professionals to determine one-carbon metabolism gene polymorphisms as a risk for prostate cancer. This study will lead to positive social change by providing health care workers information on how to better diagnose prostate cancer at the early stages and how to follow a sufficient treatment approach. Treatment of prostate cancer at an early stage will increase the chance of surviving and life expectancy in the community (Woods, Montgomery, Herring, Gardner, & Stokols, 2006).

Policy Implications

Public health policies are used to promote health and prevent diseases and to reduce the morbidity and mortality in the population. Policymakers need to make policies to address public health outcomes. Policymakers may use the results of this study to improve health care delivery for patients with prostate cancer. Policymakers must create occupational health and safety, environmental quality, and drug safety to support patients

with prostate cancer disease control. In epidemiologic studies, researchers have indicated the association between occupational exposure to pesticides to an increased risk of prostate cancer (Maele-Fabry & Willems, 2003). Maele-Fabry and Willems (2003) found a significant increase in the prevalence of prostate cancer for both private (farmer) and commercial applicators. Legislative policies need to improve the prevention and control of prostate cancer through efficient genetic cancer screening.

Key Stakeholders

Cancer of the prostate is the second leading cause of cancer death in males in the United States and Canada ("Canadian Cancer Statistics Publication," n.d.). One out of seven Canadian males will develop prostate cancer in their life ("Canadian Cancer Statistics Publication," n.d.). In this systematic review, I increased awareness of the symptoms of prostate cancer and provided insight into the potential barriers to treatment for males who are at an increased risk for prostate cancer. A genetic test is an effective way to decrease the risk of prostate cancer, including the prognosis of prostate cancer at an early stage to an effective treatment strategy. This study could lead to improved genetic screening for prostate cancer and provide information on how males at risk of developing prostate cancer can adopt a healthy lifestyle. Health professionals can use the concept of genetic analysis to find ways to protect men from prostate cancer by using surveillance techniques and increased accurate genetic tests. Policymakers may establish a policy to develop genetic screening regarding developing a healthy lifestyle for individuals and communities.

Problem Statement

Prostate cancer includes abnormal cells that grow out of control in a male's body; prostate cancer is the most common cancer. Prostate cancer is the second leading cause of cancer death among males (Muslumanoglu et al., 2009). Gene mutation can cause prostate cancer. Researchers have found that one-carbon metabolism-related gene polymorphisms are related to cancers, such as colorectal cancer (Theodoratou et al., 2012), breast cancer (Suzuki et al., 2008), and lung cancer (Suzuki et al., 2007). One-carbon, metabolism-related gene polymorphisms are a group of genes, but, in this study, I was interested in following three genes MTHFR, MTR, and MTRR (Gibson et al., 2011). The one carbon metabolism gene polymorphisms are involved in the folate metabolic pathway (Bailey & Gregory, 1999).

In this study, I examined the association between genes and prostate cancer risk. The purpose of my study was to fill the gap in the literature in identifying the association between prostate cancer and one-carbon metabolism gene polymorphisms, MTHFR (C667T, A1298C), MTR (A2756G), and MTRR (A66G). There were three previous studies on this subject with smaller samples of the test than I had in this study. A unit in a meta-analysis test is an individual study, which will increase the number of individual studies in a meta-analysis (samples), and the power of the test will increase (Borenstein, Hedges, Higgins, & Rothstein, 2009). In my study, the number of individual studies included in the analysis will be almost twice the number of individual studies than previous meta-analysis studies.

One assumption for a meta-analysis is the independence between included studies in the analysis. One of a previous meta-analysis study (Zhang et al., 2012) included the results from a previous meta-analysis as an individual study, which departed from this assumption. I used a meta-analysis statistical process regarding the weighting strategy by combining the results of individual independent studies from previous researches from the year 2000 to the year 2014 (Borenstein et al., 2009).

I used two concepts of epidemiology in this study to examine cancer genetic epidemiology and epidemiological methods. In cancer genetic epidemiology, I studied the cause of cancer and its related risk factors to prostate cancer, including gene variants of one-carbon metabolism gene polymorphisms in prostate cancer (López-Cortés et al., 2013). In this epidemiological method, I used a statistical method of analysis to combine the results of multiple studies with a common hypothesis to determine a significant association. The goal of this study was to identify genetic risk factors related to prostate cancer risk in the early stage of the disease and to determine an appropriate treatment method. The results of this study may be used to inform treatment methods to treat prostate cancer in the early stage of the disease.

Purpose of the Study

The aim of this meta-analysis, a quantitative study, was to summarize the results of previous studies and evaluate the evidence of an association between one-carbon metabolism gene polymorphism and prostate cancer risk. It is the value of mutant genes (SNPs) in the detection and prediction of prostate cancer risk. The independent variables were the one-carbon metabolism gene polymorphisms MTHFR, MTR, and MTRR. The

dependent variable was prostate cancer risk among men. The covariates were regions and ethnicity groups.

Research Questions and Hypotheses

The research question regarded the examination of the relationship between one-carbon metabolism gene polymorphisms and prostate cancer risk.

1. Is there any significant association between one-carbon, metabolism gene polymorphisms and prostate cancer risk?

$H1_0$*:* There is no association between one-carbon, metabolism gene polymorphisms and prostate cancer risk.

$H1_A$*:* There is an association between one-carbon, metabolism gene polymorphisms and prostate cancer risk.

2. Are there any significant differences between regions of the globe due to gene-disease association?

$H2_0$*:* There are no differences between regions due to gene-disease association.

$H2_A$*:* There is a difference between regions due to gene-disease association.

3. Are there any significant differences between ethnic groups due to gene-disease association?

Null Hypothesis $H3_o$: There are no differences between ethnic groups due to gene-disease association.

Alternative Hypothesis $H3_A$: There is a difference between ethnic groups due to gene-disease association.

The region in this study was an independent variable to determine its association with prostate cancer risk. People in different regions have different ancestry, which is important in a population genetics study. The ancestry carries a set of genes, which are common for those groups of the people. Those specific genotypes in the ethnic groups make a different phenotype for them, which may affect their lifestyle and risk factors for prostate cancer, such as nutrient intake and healthy activity. The regions in this study were in three groups of continents: the Americas, Europe, and Asia. The ethnic groups were in four groups of Caucasian, Caucasian-African American, Caucasian-Spanish, and Asian.

Theoretical Framework

Theories and models are used to improve the interventions targeted to improve health behavior and create a positive change in behavior. The theories are a set of interrelated concepts used to explain and predict phenomena by specifying relations among variables (Coreil, 2010). The most successful public health programs are those that include theories of health behaviors. In social epidemiology, the basis of the theory is on society and biology. Social epidemiology is a connection of sociological frameworks that provide a basis for epidemiological inquiry by defining the role of societal factors in the etiology of disease (Krieger, 2001).

The social determinant of health theory includes the genes that are related to health outcomes, such as genes and biology, physical environment, social environment or social characteristics, health behaviors, and health services or medical care ("CDC - Prostate Cancer," n.d.), and were the theoretical construct used in my research. Heijmans

et al. (2003) incorporated health theories to improve public health by assessing the relationship between prostate cancer and one-carbon, metabolism gene polymorphisms, and the causation and association of genes and cancer. Some researchers examined the association between genetics, disease, and associated risk factors such as diet (Cai et al., 2010). Some of these scholars looked at the relationship between prostate cancer and risk factors like age, race, alcohol consumption, and environment (Kobayashi et al., 2012). In some of those studies, there was no improvement in the creation of theoretical frameworks to refine the association between genes and prostate cancer.

In this study, a genetic epidemiology with a multiple level approach was the major theoretical framework, in which human genomics and mutated gene determination are the way to promote health benefits and improve population health.

The quantitative method was used to determine the measure of variables, as well examine the statistical relationships between them. The quantitative tradition was used to help me examine how genes may affect prostate cancer. The results of this study could help to understand the relationship between genes and prostate cancer with other variables such as age, race, education, family history, and geographical situation (Creswell, 2009, pp. 57-61). Ancestral region may also be related to an increased risk of prostate cancer risk due to genetic variation among regions of people (Grönberg, 2003).

Genetic Epidemiology

In order to understand disease prevention, public health workers must know human genomics (NCBI, 2014; Figure 2). Human genomics can be used to determine ways to promote health benefits and improve population health. Public health workers

may use genetic epidemiology to examine a gene's role in the health of a population by

analyzing how a healthy lifestyle affects gene-environment interaction (Khoury et al.,

2011).
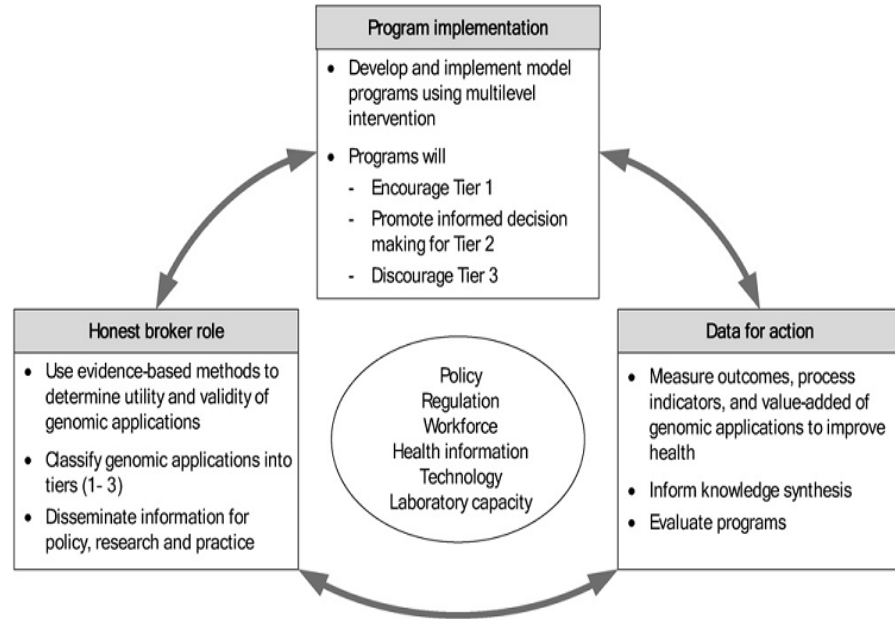
Figure 2. Synergy of public health actions in addressing the role of genomics in population health (Khoury et al., 2011).

According to genetic epidemiology, a gene disease is associated with a multiple level approach. The multilevel approach includes individual behavior changes by improving the knowledge of prostate cancer genetic screening; the interpersonal level as friends and familily levels are affected by prostate cancer. At the organization level, males may benefit from an improved health status by improving the affordability and accessibility of genetic screening. At the community level, the health in the community could be improved by promoting cancer genetic screening, and at the public policy level, recommendations could be made to establish genetic screening regimins and policies (Coreil, 2010).

Nature of the Study

There is a reported relationship betweeen one-carbon metabolism gene polymorphisms and cancers such as colorectal cancer, breast cancer, and lung cancer (Suzuki et al., 2008; Suzuki et al., 2007; Theodoratou et al., 2012). The purpose of this meta-analysis study design was to increase the power of the test in gene-disease association by increasing the sample size. The purpose of this quantitative, meta-analysis study was to test whether there was a relationship between genetic factors and prostate cancer risk (Creswell, 2009, p.132). I used a deductive approach to improve the study of prostate cancer. The data were collected from published articles. The collected data combined other researchers' study results who examined the relationship between one-carbon metabolism gene polymorphisms and prostate cancer. Specifically, the plan of this study was to estimate the relationship between risk factors of genes associated with a risk of prostate cancer among males in national and international outcomes. The statistical

analysis in this study was based on a meta-analysis method with combining the odds ratios of selected studies to make an overall effect size (odds ratio). This meta-analysis helps to make a higher statistical power to measure the gene and prostate cancer association than selected individual studies. The independent variables included three genes (MTHFR, MTR, and MTRR) and their four polymorphisms of the one-carbon metabolism gene polymorphisms. The dependent variable was the risk of prostate cancer. The covariates of analysis were geographical areas as regions and ethnic groups. The regions were coded in three groups of continents: Americas, Europe, and Asia. The ethnic groups were in four groups of Caucasian, Caucasian-African American, Caucasian-Spanish, and Asian.

Definitions

Meta-analysis: A statistical method used to combine the results of different studies to make an overview of the effect sizes, resulting in a higher statistical power of the test than an individual study.

MTHFR Gene: The methylenetetrahydrofolate reductase (MTHFR) with two polymorphisms of 677C>T (rs1801133) and 1298A>C (rs1801131; NCBI, 2014).

MTR Gene: The 5-methyltetrahydrofolate-homocysteine methyltransferase gene (MTR) with polymorphism of 2756 A>G (rs1805087; NCBI, 2014).

MTRR Gene: 5-methyltetrahydrofolate-homocysteine methyltransferase reductase gene (MTRR) with polymorphism of 66A>G (rs1801394; NCBI, 2014).

Prostate cancer: A cancer that occurs in a male organ (prostate). The prostate is a reproductive organ, which adds fluid and nutrients to the sperm. It is about the size of a walnut in front of the rectum below the bladder ("CDC - Prostate Cancer," n.d.).

Assumptions

In this meta-analysis, the data collection process was based on identifying, selecting, appraising, and extracting results from relevant research (Yach, 1990). In this epidemiological method, a quantitative analysis method was based on study gene variants and one-carbon metabolism gene polymorphisms that related to prostate cancer risk. In all of the selected studies, the researchers employed a valid statistical analysis to determine the effect size of the association between genes and prostate cancer risk. In this study, I amassed large amounts of data from different studies with a common hypothesis. In the combined studies' statistical analysis (meta-analysis), there was a higher power of the test than what was included in the individual study, considering the growth of the sample size. The assumptions that I made in this study were similar to the assumptions made in the individual studies with the same criteria, which I selected. The assumption of this meta-analysis was independence between selected studies, and each study had the same inclusion and exclusion criteria with other selected studies. The selected studies should be independent because the result of the individual study added to the combination of studies' results. As an example, if a study included a result from a combination of four studies' results, which each one of those studies already involved combined results, it would be a departure from accurate results because each study result

would be included in the final result twice, one as an individual study and one as combined with other studies.

Scope and Delimitations

The data gathered were based on published individual studies from the year 2000, which was the year that the genetic clinical tests improved, to the year of 2014. The intent of this study was to estimate the association between one-carbon metabolism gene polymorphisms and prostate cancer risk. The gene disease association is an important subject in recent clinical studies. The focus of this study was on prostate cancer risk among men because prostate cancer is the second leading cause of death among men in the United States ("CDC - Prostate Cancer," n.d.). I also examined the effect of regions and ethnic groups on the gene-disease association. The included populations were those men who were at risk of prostate cancer as a case group, and they were selected by researchers who conducted individual studies. The case group was compared to those healthy men as a control group. The delimiting factors come from environmental factors, and the study-by-study included variables, such as a folate intake variable. The collected studies were from across the globe. The delimitation of the analysis method is on the range of variables that included an individual study to measure the gene-prostate cancer association. The theory was on genetic epidemiology based on a gene-disease association with a multiple level approach. The multilevel includes individual behavior, interpersonal level, organization level, community level, and the public policy level recommendations (Coreil, 2010).

Validity is indicated as follows: the ability to generalize the results in the real world using a measurement strategy, the ability to generalize the results from the measurement and the study design, and the ability to determine that the results are accurate. The validity of the study refers to the researcher being able to answer the questions with the variables included to the studies (Reis & Judd, 2000). In this meta-analysis, the validity increased by including comparative studies that met the inclusion criteria with high quality and similar methods and same variables.

Internal validity relates to causal relationships (Hogg & Cooper, 2007; Reis & Judd, 2000). Internal validity was the ability to measure observed prostate cancer risk, which should be void of systematic error and biases. The internal validity included the lab results for the gene validation (genotyping) of the three involved genes (MTHFR, MTR, and MTRR) in this study. To meet external validity, a study's result should be generalizable to other related populations (Reis & Judd, 2000). This study met external validity by identifying those men who were at risk of prostate cancer by the genotyping method. Although this study selected the samples from men who were in the population boundaries by researchers, the samples were from men around the globe. In this study, the validity of the statistical conclusions will improve by examining both the fixed and random effects models.

The reliability of a study is related to the quality of measurement: its repeatability and consistency of the measurement (Hogg & Cooper, 2007). In this study, the quality of measurement related to the laboratory methods of gene mapping for the three gene polymorphisms in cases and controls. In addition, the measures of cases, which related to

the diagnoses of prostate cancer, were affected by the quality of measurement. The measure of prostate cancer risk would be based on two methods: chemicals and physical exams. The relationship of validity and reliability is based on the consistency and repeatability of the measurements. The consistency of the same or a close result in all repeated measurements will make it a reliable and valid measure.

Limitations

This investigation was limited to the information included in the studies in the meta-analysis. This information lacked a systematic study review. In the identification of individual studies, I developed research questions to examine the association between prostate cancer and the identified genes. This study was limited to a human genome study and an SNP analysis of the human population. The language of reviewing articles was limited to English, unless there was a translation of non-English article to the English language. A limitation to internal validity was the way variables were measured in individual selected studies that may have varied slightly. The type of variables measured were identical for all of the selected studies. External validity may have been limited by the inability to generalize the results because of the lack of an adequate sample size for the cases of study (men with prostate cancer) in the globe.

There also were three limitations on selected articles: search bias, publication bias, and selection bias. The search bias may have occurred in identifying relevant issues, as some researchers may have missed some of the related research. A study with a positive result, which is usually in favor of a new treatment or against a well-constituted one, is more likely to be printed, which could lead to publication bias. Selection bias may

have occurred because principals such as drug manufacturers are not interested (mostly)

in publishing negative studies (Walker, Hernandez, & Kattan, 2008). There may have

beeen a limitation of a Type I error if there was correlation among the four

polymorphisms. In the future studies, the gene-gene and gene-environment interaction

related to the cancers with common genes' risk factors should be distinguished.

Significance of the Study

Through this study, I could help public health workers provide an early diagnosis

method for prostate cancer. This study can help clarify the role of genetic factors in

determining the likelihood of developing prostate cancer. The results of this study may be

used to better understand genetics and its relationship to diseases, as well as how to be

able to prevent the disease by providing more effective treatment strategies. This study

could aid health professionals to recognize one-carbon metabolism gene polymorphisms

as a risk factor for prostate cancer risk. This study could lead to positive social change by

providing health workers with more information on the diagnosis of prostate cancer in the

early stages, including a sufficient treatment approach using gene therapy, which could

increase the chance of surviving by adopting a healthy lifestyle.

Summary

In this chapter, I highlighted the concept of this study including the nature of

study and problem statement based on a multilevel theoretical model. I provided the

overview of gene and prostate cancer association. The research question of this study was

based on an evaluation of the association between one-carbon metabolism gene

polymorphisms and risk of prostate cancer. The statistical method was a meta-analysis

method, which will be explained in detail in Chapter 3. In this chapter, I explained and addressed the epidemiology of prostate cancer and cancer genetic screening benefits. Genetic screening for breast cancer and colorectal cancer do help to diagnose the disease at an early stage and reduce the morbidity and mortality (Hugosson et al., 2010). In summary, prostate cancer genetic screening (genotyping) could help health care workers to diagnose this disease at an early stage of the disease. Prostate cancer genetic screening could be used to diagnose the disease at an early stage and could help to establish effective treatment strategies to prevent prostate cancer development. Chapter 2 is the literature review, which includes an expanded explanation of independent and dependent variables.

Chapter 2: Literature Review

Introduction

In Chapter 2, I review the literatures related to the subject of prostate cancer, one-carbon metabolism gene polymorphisms, and the association between them.

Literature Search Strategy

In this section, I review how I found the literature for the meta-analysis. I explain my search strategy and inclusion and exclusion criteria. I describe how I looked for o one-carbon metabolism gene polymorphism where there is an explanation of three involved genes: MTHFR, MTR, and MTRR (Gibson et al., 2011). To find pertinent articles on my topic, I used the following databases: EMBASE, MEDLINE, Index Medicus, and WaldenU database. The key words used for the search were *cancer*, *prostate cancer*, *one-carbon metabolism*, *MTHFR*, *MTR*, *MTRR*, *genetics*, *genomics*, *gene*, *one-carbon*, *epidemiology*, *genetic epidemiology*, *systematic review*, *cohort*, *case control*, and *cross section*. I started the review of scientific papers based on search keys, from the year of 2000 to 2014, including full texts and abstracts.

There was different criteria for selecting studies due to inclusion criteria (Walker et al., 2008). The criteria listed was as follows:

- There should be enough information for analysis, such as point estimate and standard deviation

- The study design should be the same

- The year of study and collected data should be related to the target population reduce the selection bias

- The minimum sample size must be met

- The age of the study group must be appropriate

I excluded studies on different genes than the selected one-carbon metabolism gene polymorphisms in this study. The other exclusion criteria included independent studies or statistical methods and study designs that were different from the goal of this study.

Inclusion and Exclusion Criteria

The eligibility criteria for the studies were the following:

- All collected data were from males

- All types of publications

- All published articles in all languages

- Studies with a case control and cohort design

- Studies with results of odds ratios, related risks, and *p*-values

- Reliable measures

The study collection was limited to a publication date of 2000 or after.

In this study, I planned to limit the research on the human genome,and SNP analysis on the human population. Some studies were excluded if they included an analysis of several genes and did not include the selected one-carbon metabolism gene polymorphisms (MTHFR, MTR, and MTRR). I also excluded research where the statistical methods and study designs were different from the goal of this study.

Prostate Cancer

Researchers have examined the association between one-carbon metabolism gene polymorphisms and prostate cancer with differing results. Not only is prostate cancer a health concern for many males, the health costs associated with treating this cancer are also significant. The cost of treating prostate cancer for diagnosis and follow-up treatment in the United States in 2006 was 9.862 billion (Roehrborn & Black, 2011), and the cost of treatment in Canada was 9.76 billion dollars (Fradet, Klotz, Trachtenberg, & Zlotta, 2009). Prostate cancer affects all social, physical, and psychological aspects of a patient's life.

Epidemiology of Prostate

Prostate cancer is the most common diagnosed cancer among Canadian males. According to statistics, 23,600 Canadian males will be diagnosed with prostate cancer, of which 3,900 of them will die from the disease ("About Prostate Cancer - Prostate Cancer Canada," n.d.). One in 7 males will develop prostate cancer during their lifetime; the rate for death from prostate cancer is 1 in 27 or 1 of 4 diagnosed males (Fradet et al., 2009). Fradet et al. (2009) claimed that the diagnosing and mortality from the disease of prostate cancer occurs as often in males as breast cancer occurs among females. However, the death rate from prostate cancer decreased significantly (almost 4%) from 2001 to 2009 due to improved testing procedures for prostate cancer and more efficient treatment strategies to reduce the risk.

The prostate is a male organ. The prostate organ is located below the bladder, in front of the rectum, and is about the size of a walnut ("CDC - Prostate Cancer," n.d.). The

prostate is the male reproductive organ that adds nutrients and fluid to the sperm.

Testosterone has a role in the growth of cells in the prostate organ in males (see Figure

3).
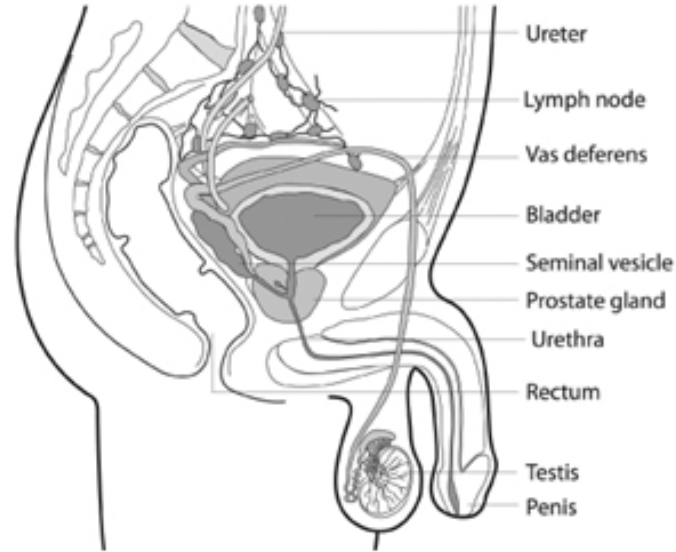
Figure 3. The anatomy of the prostate organ in the human body, male. From "About Prostate Cancer - Prostate Cancer Canada," n.d. .

The incidence rate and mortality of prostate cancer with adjusted age-standardized rates are shown in Figure 4 (Dorr, Schlesinger-Raab, & Engel, 2013).

Region	Incidence absolute	Incidence ASR (W)	Mortality absolute	Mortality ASR (W)
World	899	27.9	258	7.4
More developed regions	644	61.7	136	10.5
Less developed regions	255	11.9	121	5.6
Asia	133.2	7.2	59.6	3.2
North America	213.7	85.7	32.6	9.9
Central America	20.5	34.8	8.1	12.6
South America	84.1	50.2	29.2	16.2
Australia and New Zealand	21.0	104.2	4.0	15.4
Central and Eastern Europe	58.4	29.1	23.1	10.9
Northern Europe	64.9	73.1	17.4	15.4
Southern Europe	79.5	50.0	20.4	10.4
Western Europe	167.9	93.1	28.7	12.4
Germany	70.8	82.7	12.2	11.7
Japan	38.7	22.7	10.0	5.0
USA	186.3	83.8	28.6	9.7
Brazil	41.6	50.3	14.4	16.3
China	33.8	4.3	14.3	1.8
India	14.6	3.7	10.4	2.5
Russian Federation	22.1	26.1	9.5	10.8
South African Republic	7.5	59.7	2.5	20.8

Absolute numbers in thousands; ASR (W): age standardised rate per 100,000 by world standard

Figure 4. Incidence rate and mortality of prostate cancer

Dorr et al. (2013) indicated that prostate cancer is the second most diagnosed cancer in males after lung cancer. Dorr et al. pointed out that the highest incidence rates of prostate cancer occur in Australia and New Zealand. The lowest incidence rate for prostate cancer is in South-Central Asia. Dorr et al. showed that the highest mortality rate for prostate cancer occurs in the South African Republic. Almost three quarters of these diagnosed males are from developing countries.

Grönberg (2003) explained the differences between ethnic populations and diagnosis of prostate cancer as related to their genetic background and the environment effects. Grönberg found that, when Japanese people moved from Japan to the United States, the incidence rate of prostate cancer increased among the males in this population. The increase in prostate cancer was associated with adoption of the Western lifestyle, including dietary intake and habits. Specifically, Grönberg indicated that there was a relationship between dietary factors and prostate cancer risk. The dietary factors related to prostate cancer include a large consumption of red meat, as well as the way of cooking and preparing such as high temperature cooking that may cause diseases such as colorectal, bladder, and kidney cancer. Phyto-estrogens, in soybean products, is also linked to reducing prostate cancer (Grönberg, 2003). Grönberg explained that frequent intake of tomato-based products might cause a reduction in prostate cancer because of lycopene.

The Economic Burden of Prostate Cancer

There are many costs associated with prostate cancer, specifically in diagnosis and death. Prostate cancer leads to an increased cost of health care in Canada. The cost of

prostate cancer is evaluated in five phases per 100 days (2004, $Canadian): (a) before diagnosis (6 months before, Phase I) $1,297, (b) initial care (12 months after diagnosis, Phase II) $3,289, (c) continuing care (Phase III) $1,495, (d) preterminal care (from 18 to 6 months before death, Phase IV) $5,629, and(e) terminal care (6 months before death, Phase V) $16,020 (Krahn et al., 2010).

Risk Factors

There are many risk factors related to prostate cancer. In the United States, age is a factor relating to prostate cancer; more than 70% of all cases of prostate cancer are diagnosed in males aged over 65-years-old (Crawford, 2003). Crawford (2003) added that there is some evidence of diagnoses of prostate cancer among males age less than 50-years-old. Ethnicity, another risk factor, is related to the diagnosis of prostate cancer as African Americans have the highest rate of the disease in the world. Crawford indicated that males who have a family history of the prostate cancer are also at an increased risk of prostate cancer. The androgen hormone level in the blood is related to the incidence of prostate cancer. Males with a high body mass index (BMI) of 35 to 39.9 have a 34% greater risk of dying of prostate cancer than those with a lower BMI (Crawford, 2003). Smoking may correlate to prostate cancer tumor growth, although the direct effect is not evident (Grönberg, 2003). Chemical exposure to pesticides and herbicides may affect a male's likelihood of developing prostate cancer (Fradet et al., 2009). Fradet (2009) indicated that Selenium and Vitamin E might affect prostate cancer risk.

One-Carbon Metabolism Gene Polymorphisms

One-carbon metabolism has a role in DNA synthesis and methylation; it plays a

role in processing amino acids for building proteins. The genomic DNA is at the root cause of certain diseases, including prostate cancer, by mutating the genes and their protein structure. Some materials involved in one-carbon metabolism, due to DNA synthesis and methylation modification, are Vitamin B families, in particular folate or Vitamin B9 (Donkena, Yuan, & Young, 2010). There are different genes involved in one-carbon metabolism reaction. I studied three one-carbon metabolism genes: MTHR, MTR, and MTRR. The other genes involved in the one-carbon metabolism reaction are CBS, FOLR1, TYMS, BHMT, SHMT, SLC19A1, GGH, and ALDH1L1.

MTHFR (C667T, A1298C)

The function of the MTHFR gene is to produce the methylenetetrahydrofolate reductase enzyme (NCBI, 2014). This enzyme is important to the reaction of Vitamin B folate, folate metabolism (Figure 6). This enzyme transforms the amino acid homocysteine to amino acid methionine (NCBI, 2014). Without functional MTHFR, the body will not transform homocysteine to methionine, which leads to a reduction of methionine amount. Wojcieszynska et al. (2012) believed

> The concentration of homocysteine in plasma is associated with several metabolic disorders such as; triglyceride level in plasma, body mass index (BMI), hypertension, and abnormal oxidation of low-density lipoprotein, which may lead to the development of a wide variety of cancers, such as breast, ovarian, and pancreatic cancers. (p.16755)

There were two polymorphisms of the MTHFR gene analyzed in this study: 677C>T and 1298A>C. The replacement of cytosine by nucleotide thymine at position

677 (677C>T, rs1801133) at Chromosome 1 location p36.3, Exon 4 in humans genome (Figure 5) leads to the substitution of alanine amino acid to valine amino acid, which may cause reduced activity of the enzyme (Wojcieszynska et al., 2012). The wild type of this gene is nucleotides C, and the mutated gene in MTHFR may choose to turn off the enzyme activity by changing to Nucleotides T. The second most frequently occurring polymorphism is changing nucleotide adenine to cytosine (1298A>C, rs1801131) at Exon 7, Chromosome 1 Location p36. 3 (see Figure 5).
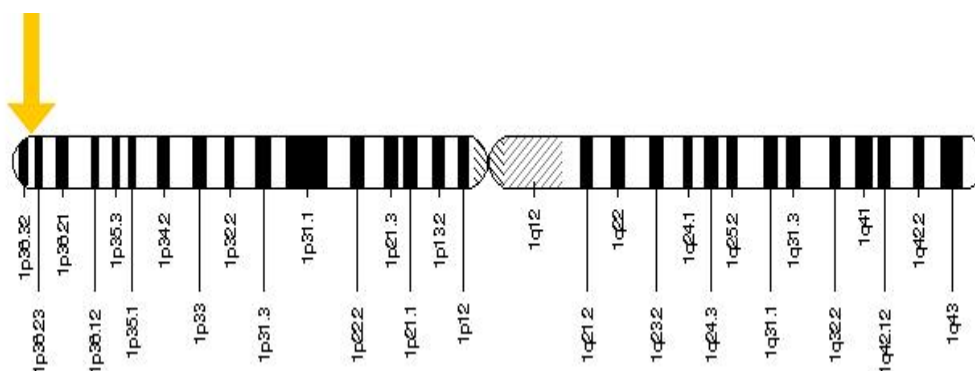
Figure 5. The location of *MTHFR* gene on the short arm (*p) of chromosome 1 at* position 36.3. The *MTHFR* gene is located from base pair 11,845,786 to base pair 11,866,159 on chromosome 1 (Source: NCBI, 2014).

The wild allele is A, against mutate one T. This transforming nucleotide results in the changing of glutamate amino acid to alanine amino acid at Codon 429 (NCBI, 2014). Those two folate and riboflavin protect against the loss of function of MTHFR, which can reduce the risk of cancer (Figure 6).

MTR (A2756G)

The MTR is located on long (q) arm of Chromosome 1 at Position 43, Exon 33

(Figure 6). This gene interacts at an enzyme called methionine synthase (NCBI, 2014).

This gene affects the synthesis of amino acid methionine from converting amino acid to

homocysteine (Figure 7).
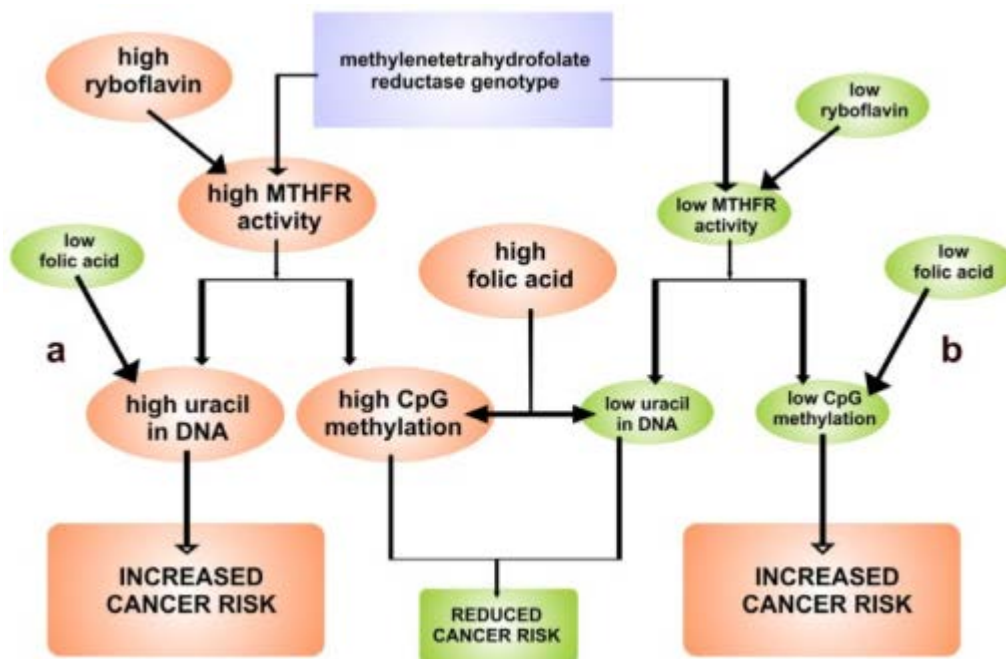
Figure 6. The relation between MTHFR genotype, riboflavin, and folic acid with respect to (a) cytosine-phosphate-guanosine dinucleotide (CpG) methylation and uracil in DNA and (b) initiation of cancer caused by CpG hypomethylation. The level of riboflavin and folic acid depends on supplementation modified (Wojcieszynska et al., 2012).

The transformation of adenine to guanine is a mutation of this gene at 2756 position (2756 A>G, rs1805087). A few scholars have examined MTR and prostate cancer risk (Watkins et al., 2002; Yu et al., 2010). The wild and normal version of allele at base pair is A, which is the mutated version of G. The mutation in the MTR gene causes the production of an unusually small, nonfunctional version of methionine synthase. The 2756GG is significantly related to cancer risk (Yu et al., 2010).
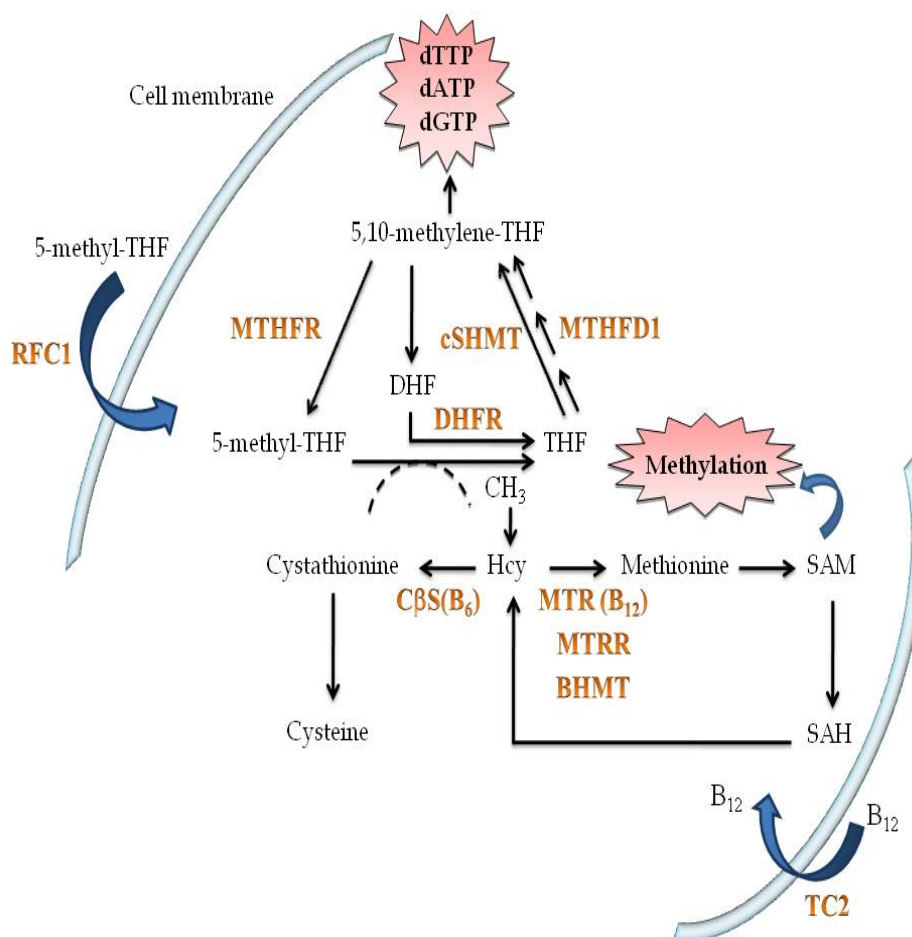
Figure 7. Folate metabolism. BHMT = Betaine-homocysteine methyltransferase; B_6 = vitamin B6; B_{12} = vitamin B12; CβS = Cystathionine β- synthase; CH_3 = Methyl; dATP = Deoxyadenosine 5'-triphosphate; dGTP = Deoxyguanosine 5'-triphosphate; dTTP = Deoxythymidine 5'-triphosphate; DHF = Dihydrofolate; DHFR = Dihydrofolate reductase; Hcy = Homocysteine; MTHFD1 = Methylenetetrahydrofolate dehydrogenase 1; MTHFR = Methylenetetrahydrofolate reductase; MTR = Methionine synthase; MTRR = Methionine synthase reductase; RFC1 = Reduced folate carrier 1; SAH = S-adenosylhomocysteine; SAM = S- adenosylmethionine; cSHMT= Serine hydroxymethyltransferase; TC2 = Transcobalamin 2; THF = Tetrahydrofolate (source; Cristina, Lancia, Matos, & Goloni Bertollo, 2011).

The *MTR* gene is located from base pair 236,958,580 to base pair 237,067,280 on Chromosome 1 (see Figure 8).
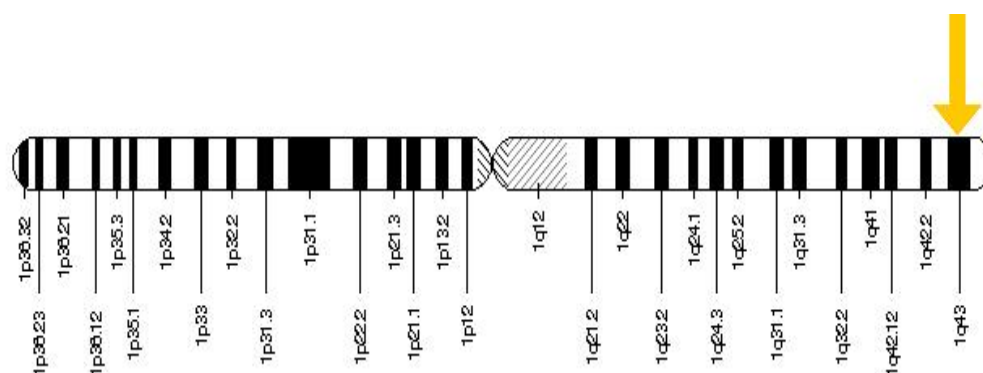
Figure 8. The MTR gene is located on the long arm (q) of chromosome 1 at position 43. (Source: NCBI, 2014).

MTRR (A66G)

The MTRR instructs an enzyme to methionine synthase reductase name. This enzyme helps to process amino acids used to build proteins, which the MTRR enzyme causes to produce an active or inactive methionine synthases (Watkins et al., 2002). The wild version of allele in this gene is A, and the mutated version is G, which may happen at Position 66 of the MTRR gene (66A>G). The mutated version may cause abnormalities and a nonfunctional version of the enzyme, which changes the single amino acids in the process of methionine synthase reduction (Rai, Yadav, Kumar, & Yadav, 2013). This prevents functionality of the enzyme, which may lead to a reduction for (the amount) methionine associated with homocystinurin process (Figure 7). The job of the MTRR is to activate or inactivate MTR (Rai et al., 2013). This gene is in the short arm (p) of Chromosome 5, at Position 15.31 (rs1801394, base pairs 7,851,298 to 7,901,236. The gene is in Exon 2 (Figure 9).
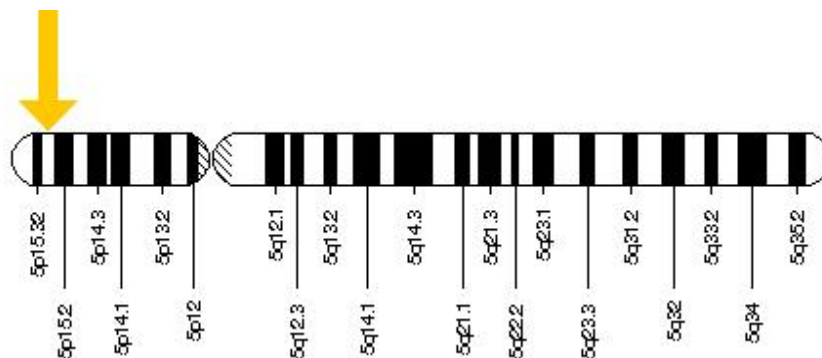
Figure 9. The MTRR gene is located on the short arm (p) of Chromosome 5, at Position 15.31. The MTRR gene is located from base pair 7,851,298 to base pair 7,901,236 on Chromosome 5 (Source: NCBI, 2014).

Gene Interactions

Public health workers lead health care policy and disease prevention. Genetic study is involved in the study of gene and environment interaction. The ancestry and ethnicity of people play a role in the location of genes in their genomic model (Feero, Guttmacher, & Collins, 2010). Feero et al. (2012) indicated that the difference in gene location in different people leads to specific gene variations. The definition of a normal or wild gene refers to the most common variant of a specific gene location in a given population group. These genetic variations affect human health. The normal frequency for a minor allele is 99.9%, and if there is more than a 1% variant in the same population group, it is called a polymorphism (Feero et al., 2010).

Gene-Environment Interaction

The association between common diseases and environmental factors such as diet and lifestyle also relate to genes. The interaction between genes and the environment interaction leads to variations between populations. The combination of genetics and the

environment is in the etiology of most diseases (Feero et al., 2010). The genotype affects the phenotype; with gene mapping (DNA analysis), the phenotype of the diseases can be seen. In epidemiological methods and analysis, researchers analyze environmental exposure and lifestyle and how it relates to disease (Hunter, 2005). Rhee and Waldman (2002) explained the etiology of antisocial behavior as a combination of genetic and environmental influences. There is a significant association (P=0.04) as reported by Lindstrom et al. (2011) on gene –environment interaction between diabetes and JAZF1gene in addition to a significant association (P=0.03) between JAZF1 and BMI. Kobayashi et al. (2012) indicated the effect of nutrient intake, Vitamin B groups, and folate intake to prostate cancer risk.

Gene-Gene Interaction

In genetic study, scholars look at the association between genotype and phenotype at the individual level. The gene-gene association includes looking at different loci (gene) association in the chromosome, which is measured by a linkage disequilibrium value (Cordell, 2009). The linkage disequilibrium is a nonrandom association between two or more loci in the chromosome (Cordell, 2009). Yeager et al. (2009) showed a gene-gene association of prostate cancer on Chromosome 8 (q-arm). There is an association between rs62086 and rs13281615 with linkage disequilibrium (LD) of 0.38 (r^2=0.30), which is a modest LD (Yeager et al., 2009).

Diet Including Folate Intake, and Prostate Cancer

Mazhar and Waxman (2004) and Shirai et al. (2002) suggested that the incidence of prostate cancer can be reduced by diet by studying immigrant people who migrated

from Asia to the United States. Diet as a risk factor for prostate cancer includes the intake of food rich in fat and as well as the consumption of red meat. To prevent prostate cancer, a male should consume soy protein and nutritional supplements such as Vitamin E, selenium, zinc, isoflavones, and lycopenes (Mazhar & Waxman, 2004). According to Shannon et al. (2009), folate is a micronutrient that involves metabolism; one-carbon metabolism is associated with changes in the methylation status of genes that involve carcinogenesis. Although there is not a strong association between folate intake and prostate cancer, Pelucchi et al. (2005) showed this association in an Italian population. More study on this interaction needs to be done.

Cancer Genetics

Cancer genetics includes studying genes that relate to cancers. The genes controlling the cell maintain the function of the cell. In cancer, the genes show a mutation that causes abnormalities in cellular activities, such as an uncontrolled growth (oncogenes), which may cause tumors like BRCA1 and BRCA2 that cause breast cancer (Ponder, 2001). Cancer is caused by mutation in DNA (deoxyribonucleic acid) segments, which leads to abnormal growth in a cell. The genotype will map mutated genes, which can be inherited and transferred from parents to the offspring; in epidemiology, a gene by environmental influence causes an individual's phenotype (Feero & Guttmacher, 2010). A small portion of cancers can be inherited (Ponder, 2001). Prostate cancer occurs due to metastatic disease; there are some environmental risk factors that affect the incidence of this disease (Dean & Lou, 2013). Dean et al. (2013) concluded that there is a high risk of prostate cancer for males who have a father or brother who has had prostate cancer; in

Sweden, males with a family history of PC is 3 to 10 folds higher than regular males in the same community group.

Theoretical Foundation

In health and health behavior research, the theories and models help scholars to understand the concepts of the study and design interventions through its approaches. Theories and models are used to answer the health problems and find the effective ways to change a person's behavior (Coreil, 2010). In this study, the outcomes were based on genetic epidemiology at multilevel models of health behavior,w hich could be used to help change health behavior in individuals. The gene-prostate cancer association, if found, could help facilitate genetic screening to diagnose the disease at the early stage of prostate cancer. At the individual level, the men who partake in prostate cancer screening could detect the disease at an early stage, which would benefit him with an early treatment strategy to increase his life expectancy. The early diagnosis of prostate cancer could effect to the treatment cost (less cost for patients) and effective treatment strategies. At the community level, the prostate cancer genetic screening could help community members to understand the health conditions that lead to prostate cancer and plan programs at different private and public health sectors to alleviate these conditions (Coreil, 2010).

One-Carbon Metabolism Gene Polymorphisms and Prostate Cancer Risk

In genetic association studies, researchers test the relationship between genetic variations and health outcomes; health outcomes are mainly diseases. In recent studies, the association between genetic factors and cancers has been reported (Khoury et al.,

2011). Khoury (2011) believed that public health authorities are responsible for coordinating health care and disease prevention, which involves the examination of human genomes to improve preventative health measures. Genomic information not only helps to improve population health, but also can be used to develop health protection strategies for the future (Khoury et al., 2011). A genetic test would improve the detection of genetic diseases at an early stage.

The studies on genetic-prostate cancer association help to identify gene effect to the risk of prostate cancer due to an individual's genotype. The genetic polymorphisms frequencies present differentially among men in different regions and ethnic groups. There is an association between genetic polymorphisms among diverse populations and risk of prostate cancer (Li, Mercer, Gou, & Lu, 2013). Males who immigrate from Asia to The United States respond to genetic polymorphisms related to the incidence rate of prostate cancer, and have a higher risk of prostate cancer (Grönberg, 2003; Li et al., 2013). The highest incidence rate of prostate cancer is among African men, with intermediate rates among Caucasian men, and the lowest rates among Asian men (Ntais, Polycarpou, & Tsatsoulis, 2003). In this this meta-analysis study, I examined the association between ethnic groups and risk of prostate cancer due to mutated genes on MTHFR, MTR, and MTRR genetic polymorphisms.

The association between one-carbon metabolism gene polymorphisms and cancers are reported in different articles (Gibson et al., 2011; Theodoratou et al., 2012; Xu et al., 2008). In this meta-analysis, I combined the results of 20 selected studies on the association between one-carbon metabolism gene polymorphisms and prostate cancer

risk. The 20 selected studies were from 2800 studies review (Figure 10). Researchers have reported differing relationships between this association, but the studies have a variety of regions and ethnicity of participants studied. The results may be used to better understand how the association for each group could relate to common ancestry, environmental risk factors, and genotype. The gene-environment interaction is an important subject in genetic studies.
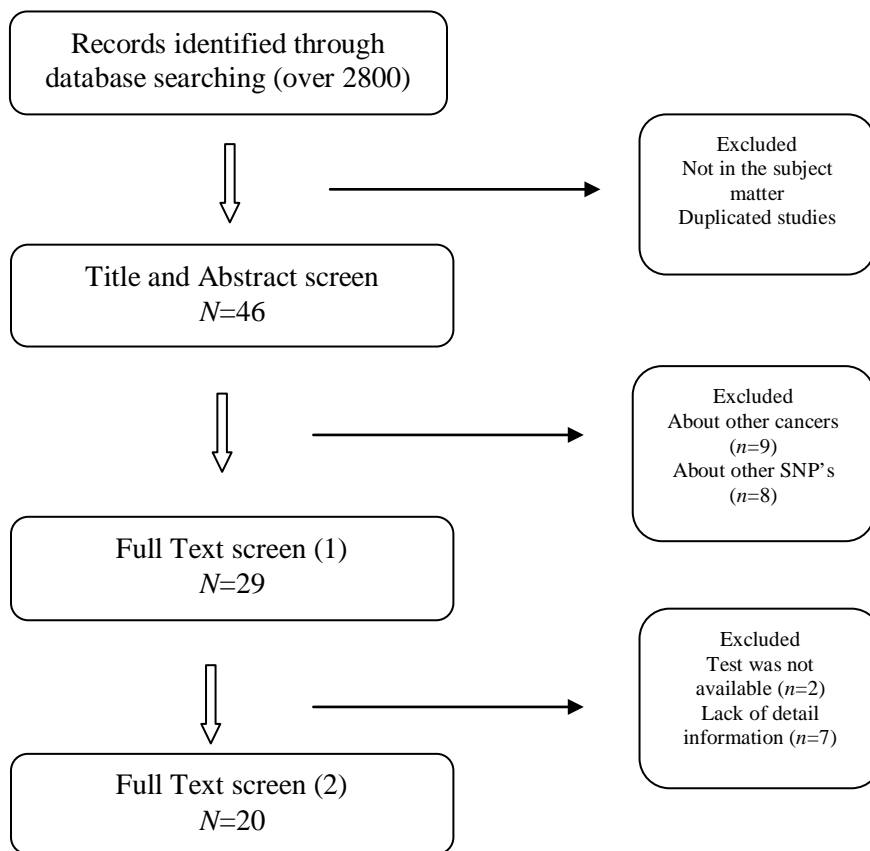
Figure 10. The flow diagram of study selection process.

In this study, I reviewed 20 articles with similar boundaries. In this study, I provided a better understanding of the results because of the increased sample size in the analysis, which increases the power of the test. Although a meta-analysis would improve the power of the test, there may are some missing technical performance that can make the study more accurate; this is the advantage of research. This meta-analysis involved 20 primary studies on the association between one-carbon metabolism gene polymorphisms and prostate cancer. In this study, three genes MTHFR, MTR, and MTRR were identified. An abstract on the 20 included studies is located in Appendix A.

Kimura et al. (2000) conducted a hospital-based case control study and reported a slight association between MTHFR and prostate carcinoma, but this association was not statistically significant at the 5% level of the test. Kimura et al. sampled participants from a German population (Caucasian); there were 132 prostate carcinoma patients, 66 of whom were involved in this study and 150 participants who were PC free as a control group. Although Kimura et al. found no significant association between MTHFR and prostate cancer; there was an association between MTHFR Val allele (T) and higher tumor grade. Kimura et al. suggested further study with a higher sample size. The results of the genotype and allele frequencies were used for this meta-analysis study.

Heijmans et al. (2003) reported different results than those of Kimura et al. (2000). Heijmans et al. suggested that at Polymorphism C677T, Genotype CC is a risk factor for cancer in elderly males. In a cohort study design, Heijmans et al. examined 149 new cases of cancer among 793 males without cancer. The population of the study was Caucasian from Netherland (Dutch). Heijmans et al. found an increased risk of prostate

cancer among males who consumed large amounts of alcohol and those with less folate intake.

Cicek et al. (2004) found a slight association between prostate cancer and A1298C. There was a positive association between the C677T variant and prostate cancer risk among males who had a less advanced disease. In this case-control study, there were with 439 cases and 479 sibling controls. The study was a population-based study among Caucasian ethnic groups. The sample study was from sibling groups. The brothers with cancer were aged less than 73 years, and the control group included brothers who were <8 years younger than the older brother diagnosed with the disease. Van Guelpen et al. (2006) found different results than Cicek et al. Van Guelpen et al. showed that there was no significant association between prostate cancer and C677T polymorphism. Van Guelpen et al.'s population included Swedish males.

Singal et al. (2004) showed a significant association between polymorphisms (C677T, A1298C) and the reduction of the risk of developing prostate cancer. The study was a case-control study with 81 patients and 42 controls, including Caucasians and African American ethnic groups, in the United States. There was a significant association between polymorphism C677T and risk of prostate cancer among males under 65 years of age in Johansson et al.'s (2007) study, but, overall, Johansson et al. found no significant association. Johansson et al.'s study was a case-control, population-based study of Swedish people.

Reljic et al. (2007) studied MTHFR polymorphisms and cancer risks. Reljic et al. studied 95 prostate cancer cases and 37 healthy controls of the Croatian people, a case-

control study. This study was a population-based study that resulted in no significant association between the C677T polymorphism and prostate cancer. Marchal et al. (2008) indicated a significant association between MTHFR C677T and prostatic carcinogenesis among Caucasian people in Spain. Marchal et al.'s study was a hospital-based, case-control study with 182 cases and 205 controls.

Stevens et al. (2008) showed no association between one-carbon, metabolism gene polymorphisms (MTHFR, MTR, MTRR) and prostate cancer risk. The study was a population based case-control study. Stevens et al. studied the association between nine one-carbon, metabolism genes and the risk of prostate cancer in 1,144 cases and 1,144 controls. Muslumanoglu et al. (2009) studied the Turkish population (Caucasian) in a hospital-based case-control study. The study involved 93 prostate cancer patients and 166 individuals in the control group; participants were aged between 5- to 89-years-old. Muslumanoglu et al. did not find a significant difference of T allele frequency between patients and control groups on A677T polymorphism, but there was a difference in C allele frequency of A1298C polymorphisms between patients and control groups.

Cai et al. (2010) pointed to a significant association in genotypes and alleles between cases and controls for C677T polymorphisms, but there was not any association for C677T polymorphism and MTR and MTRR genes with prostate cancer. The study was a hospital-based case-control study involving 217 cases and 220 benign prostatic hyperplasia (BPH) controls. Safarinejad et al. (2010) believed that there was an inverse association risk of prostate cancer with C677T and A1298C polymorphisms. The study

was from an Iranian population (Caucasian) as a population-based case-control study. The study involved dietary Vitamin B12 and folate intake.

Wu et al. (2010) conducted a Taiwanian, hospital-based case-control study on 218 cases and 436 controls. Wu et al. showed that C677T polymorphism had a significant association with the decrease in the risk of prostate cancer, but there were no frequency differences between patients and controls for A1298C polymorphism. Wu et al. provided the first evidence of an association on C allele frequency of C677T polymorphism and developing prostate cancer. Küçükhüseyin et al. (2011) found no significant association between the C677T polymorphism and prostate cancer risk, even though there was a decreased risk of prostate cancer. The study was a hospital-based case-control study of Turkish people. There were 55 cases and 50 healthy controls involve to this study.

Fard-Esfahani et al. (2012) studied an Iranian population and found no association between the C677T polymorphism and the risk of prostatic carcinoma. The study involved a hospital-based case-control study with 67 cases and 75 controls. There was a slight effect of homozygote (TT vs. CC) on the carcinogenesis. Jackson et al. (2013) indicated no association between MTHFR, MTR, and MTRR genes and prostate cancer. Jackson et al. reported a gene- prostate cancer association for those male with high folate concentrations. The study involved a population-based case-control study among males aged 40-80 years old.

López-Cortés et al. (2013) found an association between C677T polymorphism and the risk of prostate cancer, plus a slight association between A66G polymorphism and the risk of prostate cancer. It was a hospital-based case-control study of Ecuadorian

individuals. The study involved 104 cases and 110 participants in a healthy control group; the participants were of Spanish ethnicity. Kobayashi et al. (2012) showed a significant association between alcohol consumption and folate intake related to prostate cancer by C677T variants. The study was a hospital-based case-control study with 80 cases and 334 controls. There was no association between the C677T variant and prostate cancer. This study was on gene-environment interactions subject.

De Vogel et al. (2014) indicated that, in the gene-environment interaction, there was an interaction between MTHFR C677T polymorphism and Serum sarcosine and glycine concentrations. De Vogel et al. showed that high glycine concentration or serum sarcosine had a moderate effect on reducing prostate cancer risk. De Vogel et al. found a significant association between variant TT relation to prostate cancer (*p*-value=0.004). The study was a population-based nested case-control study with 2,522 cases and 2,607 controls. Ebrahimi et al. (2013) found that there was no significant association between the C677T variant and prostate cancer risk. It was a hospital-based case-control study with 30 cases and 40 controls. Ebrahimi et al. also studied prothrombin (PTH, G20210A), and Venous thromboembolism (VTE, G1691A) genes, in which Ebrahimi et al. could not find a significant association.

Limitations of these studies were in sample size; some of the studies had a small sample size. The age group was another limitation; some studies involved a varied group of males (ages 40-85); the studies should have included younger people who are at risk of prostate cancer. The other limitation was dietary intake; some studies involved folate and Vitamin B12 intake, but some of them did not. Although the studies were from different

regions and different ethnic groups, in the final meta-analysis, this disparity may affect the analysis of differences between regional groups.

Review of Relevant Methodology, Meta-Analysis

This study's methodology was based on a multiple study analysis or meta-analysis. A meta-analysis is a study that combines the results from different studies to identify the common patterns among studies. The main goal of a meta-analysis is to develop the power of the test by increasing the sample size. The other objectives of a meta-analysis are to analyze the differences among the studies' results, to evaluate the effect of subsets in disease exposure, and to determine the gap for future studies (Walker et al., 2008).

The advantages of meta-analysis are

- To improve the power of test to estimate the effect size

- Generalize the results of study in the wider area

- To estimate the variation between studies (heterogeneity)

- To evaluate the publication bias

The disadvantages of meta-analysis are

- The way that data are collected at each study and the process of inclusion and exclusion

- The statistical analysis and adjustments for cofactors in individual studies

- The publication bias

- The search bias

- The selection bias

- A lack of uniformity of outcome measurement

In a meta-analysis, there are different models used to conduct statistical analysis: fixed effects, random effects, heterogeneity of results, and sensitivity analysis. In addition, a funnel plot and forest plot are used. In the fixed effects model, the treatment effect assumes the same in all of the studies, and the analysis will estimate this unknown effect, which would be more precise than in an individual study. In the random effect model, the treatment effect is not the same across studies, and a meta-analysis researcher estimates the average effect of the studies. The random effect model has larger variances and confidence interval compared to a fixed effect model. The heterogeneity analysis will be used to show the variation among the studies. The heterogeneity analysis determines homogeneity of effect size in all of the studies, which involves the meta-analysis. I was interested in finding the differences among studies by heterogeneity analysis. If there was a high value of heterogeneous, greater than 0.55, I conducted a meta-regression analysis. The meta-regression analysis may be used to show the cause of variation among study variability (Bartolucci & Hillegass, 2010).

The sensitivity analysis was used to determine the strength of the findings and to show how the results may differ if there is more inclusion or exclusion studies in the meta-analysis. The sensitivity analysis in a meta-analysis involves an investigation with a high number of studies. A funnel plot is used to show the biases in the meta-analysis. The funnel plot has a funnel symmetric shape with an average effect size in the center of the shape and a departure from standard deviation; any bias may lead to asymmetry. A

forest plot is a summary of individual studies' point estimate, which is bounded by its confidence interval including an overall effect. The forest plot is used to display the information easily (Borenstein et al., 2009).

Summary

In this chapter, I explained the literature related to one-carbon metabolism gene polymorphisms association and prostate cancer risk (Appendix A). The included genes were MTHFR gene with two polymorphisms C677T and A1298C, the MTR gene with A2756G polymorphism, and MTRR gene with A66G polymorphism. In this chapter, I discussed the prostate cancer epidemiology and its relation to public health care and the economic burden of prostate cancer regarding the diagnosis and death. The search strategy, including inclusion and exclusion criteria, were described in this chapter.

The number of included eligible studies for MTHFR gene C667T polymorphism was 20 studies with 8,675 cases and 9,207 control participants in each group. The total number of eligible studies for A1298C polymorphism was 11 studies with the total number of 2,922 cases and 3,644 controls. The gene of MTR, A2756G polymorphism, had four eligible studies included this meta-analysis with the total number of 701 cases and 739 controls included in the analysis. The MTRR gene, A66G polymorphism, included four eligible studies with the total number of 698 cases and 737 control participants.

This study covered the gap of gene-prostate cancer associated with a higher power of test than previous studies by conducting a meta-analysis study. The risk factors, including genetic variants related to developing prostate cancer, were explained in this

chapter. I explained the morphology and selected gene's cytogenetic location. I explained how these genes would influence folate balance. The gene-gene and gene-environment interaction is a subject for future research. Policy-makers must develop policies to diagnose prostate cancer at an early stage to reduce the development of the disease. In Chapter 3, I present the methods of research for statistical analysis.

Chapter 3: Research Method

Introduction

The aim of this meta-analysis study was to increase the power of the test for gene disease association. In this study, I planned to conduct an analysis of the association between one-carbon metabolism gene polymorphisms and prostate cancer risk. One-carbon metabolism gene polymorphisms are the genes related to diseases, such as breast cancer, colorectal cancer, and lung cancer (Suzuki et al., 2008; Suzuki et al., 2007; Theodoratou et al., 2012). In this study, I combined the results of each selected study (effect size) with a common concept to estimate the combined effect size with a more accurate estimation than in an individual study. In a meta-analysis, each study will bring an effect size with a defined sample size to the analysis. With an increased power of the test, a more accurate result of the association will be provided. Each individual study has a method to calculate the odds ratio (effect size), including different independent variables, such as age, family history, ethnic groups, region, and folate consumption. The meta-analysis research design is based on the association between the dependent and independent variables common to each study. The published articles had to meet inclusion criteria to be involved in this study; the data collection was based on study subject and common concepts.

In this chapter, I describe the analysis of the data in different processes: fixed effect model, random effects model, and heterogeneity. I used a funnel plot and forest plot to show a summary of the individual studies' point of estimate and their boundaries, as well as the summary effect size and bounded confidence interval.

Research Design and Rationale

Each included individual study of this meta-analysis had its own study design, but all of the studies included in this meta-analysis looked at the association between one-carbon, metabolism gene polymorphisms as independent variables and prostate cancer as a dependent variable. Each study had its own statistical methods to estimate the effect size, but the most common results were an odds ratio (Szumilas, 2010). The odds ratio is the ratio of two odds, odds=probability (D: disease) /probability (H: no disease), which show the probability of the disease in the target population. The study had the following genotype tabulation (Table 1).

Table 1

Genotype Association with Cases (D: Disease) and Controls (H: Healthy)

Genotype	Cases	Controls	Case: Control Ratio
A/A	$D_{A/A}$	$H_{A/A}$	$D_{A/A}/H_{A/A}$
A/a	$D_{A/a}$	$H_{A/a}$	$D_{A/a}/H_{A/a}$
a/a	$D_{a/a}$	$H_{a/a}$	$D_{a/a}/H_{a/a}$

The estimation of odds with a/a as a reference are

$$\frac{D_{A/A}/H_{A/A}}{D_{a/a}/H_{a/a}} \quad , \text{ and } \quad \frac{D_{A/a}/H_{A/a}}{D_{a/a}/H_{a/a}}$$

The calculation of confidence interval (CI), usually at 95% level, was based on the value of *OR*. The CI is the width used to estimate the precision of *OR*; the larger value of CI shows a low level of precision of *OR*, and a lower value of *OR* indicates a higher precision of the calculated *OR*. The formula for calculating the CI is exponentiated endpoints of

$$\ln (OR) \mp z_{\alpha/2}(SD)$$

Research Questions and Hypothesis

The genes and disease association were reported from many studies, including gene-cancer association (Taylor, Najafi, & Dobson, 2007; Theodoratou et al., 2012; Xu et al., 2008;). This study involved three genes and their polymorphisms in the risk of

prostate cancer. The purpose of this study was to examine the relationship of prostate cancer risk and one-carbon metabolism gene polymorphisms. Although other researchers have analyzed this association, there were different findings: some scholars reported a positive association; some reported that there was no association, and some asked for an increased sample size to make an accurate study result. The purpose of this meta-analysis was to estimate the overall effect size by increasing the statistical power of the test, compared to a primary study, based on an increase the sample size of the test. In this meta-analysis, I combined multiple studies to achieve a quantitative estimation of the overall effect size at alpha (0.05) level of the test.

Research Questions

The research question regarded the examination of the relationship between one-carbon metabolism gene polymorphisms and prostate cancer risk.

1. Is there any significant association between one-carbon, metabolism gene polymorphisms and prostate cancer risk?

$H1_o$: There is no association between one-carbon, metabolism gene polymorphisms and prostate cancer risk.

$H1_A$: There is an association between one-carbon, metabolism gene polymorphisms and prostate cancer risk.

2. Are there any significant differences between regions of the globe due to gene-disease association?

$H2_o$: There are no differences between regions due to gene-disease association.

$H2_A$: There is a difference between regions due to gene-disease association.

3.   Are there any significant differences between ethnic groups due to gene-

disease association?

H3$_o$: There are no differences between ethnic groups due to gene-disease

association.

H3$_A$: There is a difference between ethnic groups due to gene-disease association.

The region in this study was the independent variable to determine its association

with prostate cancer risk. People in different regions have different ancestry, which is

important in a population genetics study. The ancestry carries a set of genes, which are

common for those groups of the people. Those specific genotypes in the group make a

different phenotype for them, which may affect their lifestyle and risk factors for prostate

cancer such as nutrient intake and healthy activity. The regions in this study were in three

groups of continents: the Americas, Europe, and Asia. Those studies in the Americas

continent were added as the American region, those countries in Europe counted as the

Europe anregion, and those countries in Asia were count as Asian region. In this study, I

included research on four different ethnic groups: Caucasians, African American, Asian,

and Spanish. I adjusted for those covariates at the first level of study.

Methodology

The target population was those men who were at risk of prostate cancer.

Data Collection

The data collected from selected studies were based on inclusion and exclusion

criteria. I found over 2,800 studies involving genetic association with cancers. I narrowed

down the meta-analysis to 20 selected studies that involved one-carbon metabolism gene

polymorphisms (MTHFR, MTR, MTRR) and prostate cancer risk. All of these selected studies used the same statistical model to determine the effect sizes (odds ratios); only one of the studies had calculated the effect size as related risk. In the case of rare diseases, the odds ratio value was close to relative risk value (Borenstein et al., 2009). The data collection included the data available from the 20 selected individual studies, which allowed me to calculate the size effect value (OR) and its confidence interval value of the combined data.

Dominant Model

The gene has two alleles; in the dominant model, one allele trumps the other allele (Lewis & Knight, 2012). The dominant model in the gene MTHFR, polymorphism C677T, is (CT+TT) vs. CC. (CC, reference), and in the polymorphism A1298C, it is (AC +CC) vs. AA (AA, reference). The dominant model in the gene MTR, polymorphism A2756G is ((AG +GG) vs. AA (AA, reference), and in the gene MTRR, polymorphism A66G, it is ((AG +GG) vs. AA (AA, reference).

Codominant Model

The codominant model in the gene MTHFR, polymorphism C677T, is TT vs. CC or CT vs. CC (CC, reference) and, in the polymorphism A1298C, it is CC vs. AA or AC vs. AA (AA reference). The codominant model in the gene MTR, polymorphism A2756G, is GG vs. AA or AG vs. AA (AA, reference) and, in the gene MTRR, polymorphism A66G, it is GG vs. AA or AG vs. AA (AA, reference).

Recessive Model

The recessive model in the gene MTHFR, polymorphism C677T, is TT vs. (CT+CC; CT+CC, reference) and, in the polymorphism A1298C, it is CC vs. (AC+AA; AC+AA, reference). The recessive model in the gene MTR, polymorphism A2756G, is GG vs. (AG +AA; AG+AA, reference) and, in the gene MTRR, polymorphism A66G, it is GG vs. (AG +AA; AG+AA, reference).

In some articles, the allele count was not included; I calculated it based on the genotype. The formula for allele count was

Allele A= 2 [AA+ (AB/2)]

AA was when both alleles in a genotype were the same; AB was while one of those two alleles that was counted.

Data Analysis

In a meta-analysis, the researcher is looking for two variations in outcome: within-study variation and between-study variation. The within-study variation is due to the individual of number of cases and the response to the treatment (genes) effect. The between-study variation is due to the mean of the outcome in study-to-study (Laird & Mosteller, 1990). The variation estimation is important because of its role to the weight of the study (Borenstein et al., 2009). The weighting strategy was based on the method inverse of variance ($W_i = \frac{1}{v_{Y_i}}$), in which the variance is based on combined, within, and between variances.

In the fixed-effect analysis, I used the within-study variance in weight of the study (weight=1/within-study variance) and, in the random-effects analysis, I used both variances to weight the study (weight=1/within plus between-study variances).

In this study, the effect size for each individual study was *OR*, which was the ratio of cases in the control group. With the combination of the size effects (overall *OR*), the statistical method included log *OR*s:

LogOddsRatio= Ln (Odds Ratio)

The confidence interval for lower and upper limits respectively was

$LL_{OddsRatio}= \exp(LL_{LogOddsRatio})$, $UL_{OddsRatio}= \exp(UL_{LogOddsRatio})$

The formula of converting relative risk to odds ratio is (Zhang & Yu, 1998):

$$OR = \frac{RR\left[1 - \frac{s}{pRR+1-p}\right]}{1 - \frac{RR_s}{pRR+1-p}}$$

In the case of rare diseases, the odds are valued the same as related risk (Borenstein et al., 2009; Zhang & Yu, 1998). The statistical analysis software for this study was Statistical Analysis System (SAS 9.3).

Heterogeneity

In the heterogeneity analysis, the null hypothesis was no heterogeneity, which means the genes have the same effect of prostate cancer risk in all studies (*k*=20). The alternative hypothesis is the gene's effects varied over studies included in the meta-analysis.

H_0: No heterogeneity (all genes have the same effect of the prostate cancer)

H_a: There is variation between studies regarding gene-prostate cancer association

I used this test to estimate the consistency among the effect size across the included studies in the meta-analysis. If I rejected the null hypothesis, it means that there was heterogeneity among studies. To test the heterogeneity among studies, I used a formal test called Q statistics (quantifying heterogeneity). In the estimation of a Q-statistic, if the value is close to 0, it means there is no heterogeneity. If each study has an effect size close to the mean effect size, there are no differences in effect size. The Q-statistics has a chi-square distribution with all studies minus one degree of freedom (K-1= df).

H_0: Q=0

H_a: Q≠0

The computation formula to estimate heterogeneity is (Borenstein et al., 2009)

$$Q = \sum_{i=1}^{k} W_i Y_i^2 - \frac{\left(\sum_{i=1}^{k} W_i Y_i\right)^2}{\sum_{i=1}^{k} W_i}$$

Where W_i is the study weight calculated by the inverse of a variance, Yi is the study effect size (OR), and k is the study numbers.

The I^2 index explained the extent of heterogeneity across studies by comparison to the expected value. The value of I^2 has a value of 0% to 100%, which was used to interpret the percentage of heterogeneity across studies (variances between studies). It is not sensitive to the number of studies or the metric of OR. If the value of I^2 is 0 or close

to 0, it means the variation in effect size is due to sampling error within studies

(Borenstein et al., 2009);

$$I^2 = \left(\frac{Q - df}{Q}\right) \times 100\%$$

The value of I^2 by Higgins, Thompson, Deeks, and Altman (2003) is considered

to be equal to 25%, 50%, and 75%, which is called small, moderate, and large degrees of

heterogeneity among effect sizes.

Meta-Regression

If I found a Q statistic with a large value of I^2 (>0.55), I used a meta-regression to

find the cause of variability among study. The dependent variable in this meta-regression

was the *OR* from individual studies. I assumed that those covariates had a major cause of

heterogeneity. The assumption in a meta-regression is due to the variables. In a meta-

regression, all interested variables are available in all studies as the source of

heterogeneity. These variables include the meta-regression analysis. As an example, age

may be a variable in one study as a continuous variable, but in the other study, age was as

a grouped variable; this difference may cause heterogeneity in meta-analysis with meta-

regression (van Houwelingen, Arends, & Stijnen, 2002). In this meta-regression analysis,

I planned to use regions and ethical groups as covariates. The linear model for regression

was as follows;

$$\theta_i = \beta_0 + \beta_1 x_{i1} + \ldots + \beta_p x_{ip}$$

The θ_i is the effect size in the study i, the $\beta_0, \cdots, \beta_p$ are the unknown regression coefficients, and $x_{i1}, \ldots, x_{ip}$ are the predictors value for study i (van Houwelingen et al., 2002).

Fixed Effects Model

In the fixed-effect analysis, the assumption was based on the same effect size in all studies, and the summary effect was an estimation of this common effect size (mean of the effect sizes). The variation between studies was used to define the sampling error, which was an error in estimation of effect size (Borenstein et al., 2009).

$$\theta_i \overset{iid}{\sim} N\left(\theta, \sigma_i^2\right)$$

The individual study effects were normally distributed with a mean of θ and, in the variance of σ_i^2, it is known for individual studies (Figure 11). The Shape 1 (Borenstein et al., 2009) explains a fixed effect model.
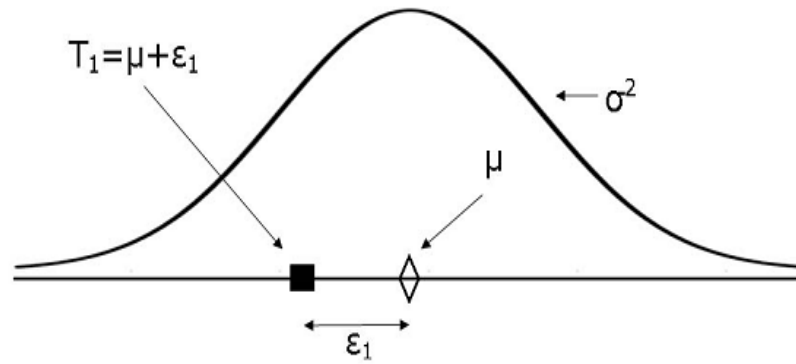
Figure 11. The sample of the observed effect, the fixed effect model. The fixed model is from a normal distribution with the true effect of μ, variance of σ^2 , ε_1 is the within-study error.

In the fixed-effect model, the hypothesis test is 0 effect in every study. The statistical formula is as follows:

Z-value to test the null hypothesis

$$Z = \frac{M}{SE_M}$$

The M value is weighted mean, which calculated as

$$M = \frac{\sum_{i=1}^{k} W_i Y_i}{\sum_{i=1}^{k} W_i}$$

The weight (W) for each study calculated as

$$W_i = \frac{1}{V_{Y_i}}$$

Where V_{Y_i} is the within-study variance for each study i.

The SE_M is the standard error of the summary effect, the square root of the variance, as follows:

$$SE_M = \sqrt{\frac{1}{\sum_{i=1}^{k} W_i}}$$

The confidence intervals (95%) calculated as

Lower limit, $LL_M = M - 1.96 \times SE_M$

Upper limit, $UL_M = M + 1.96 \times SE_M$

Random Effects Model

In the random-effects analysis, the assumption was based on effect size, which varies from study to study. This study-to-study effect size variation represents a random sample of the effect sizes. In the random-effects analysis, the weighting strategy makes more balance of weighting on studies than in a fixed-effect analysis. The effect size is similar, but not identical among studies. Figure 12 (Borenstein et al., 2009) shows a random effects model.

The hypothesis in the random-effects is zero for the mean effect (summary effect), as is listed in the following equation:

$$Z^* = \frac{M^*}{SE_{M^*}}$$
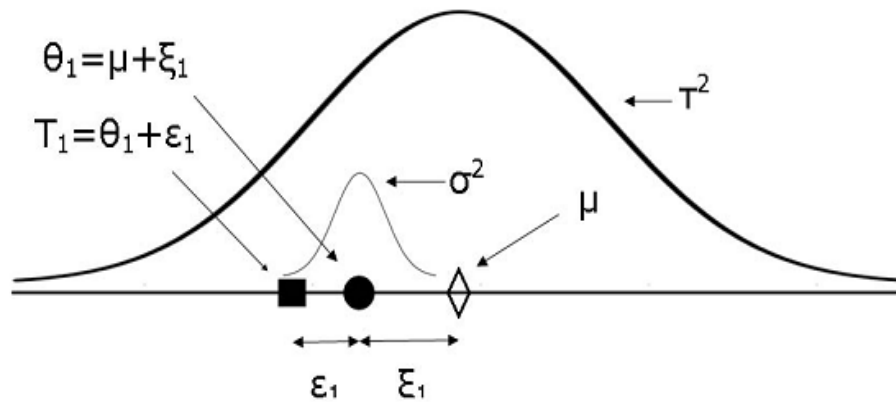
Figure 12. The sample of the observed effect, the random effect model. The true effect Θ_1 samples from a distribution of mean μ, and variance of T^2. The observed effect (T_1) is a sample from a distribution of true effect Θ_1, and variance of σ^2.

A test of the hypothesis is estimated at a *Z*-value with a value of 0 for mean effect

(μ). The M^* is weighted mean computed as

$$M^* = \frac{\sum_{i=1}^{k} W_i^* \, Y_i}{\sum_{i=1}^{k} W_i^*}$$

The W_i^* is the weighted assigned (variance inverse) to each study calculated as follows:

$$W_i^* = \frac{1}{V_{Y_i}^*}$$

The $V_{Y_i}^* = V_{Y_i} + T^2$, and the V_{Y_i} is within-study variances, and T^2 is the between-study

variance.

In a comparison of random-effects and fixed-effect meta-analysis, a fixed-effect is

used to estimate summary effect based on a common single effect to every study, but in

random-effects, the estimation of effect summary is based on the mean of the distribution

of effects in all studies. The results of random-effect analysis would generalize to the

same scenarios.

Multiple Testing Adjustments (Type I Error)

This study involved multiple testing. Each gene's polymorphism has five related

genetic model analysis that, in each analysis, commonly involves a mutated allele

(Conneely & Boehnke, 2010). There is not a routine multiple testing adjustment in

systematic reviews, but there are only some single studies that would be subject to

multiple comparison (Fellow & Director, 2008). There is not a multiple testing

adjustment for combined three genes (MTHFR, MTR, and MTRR). In this study, each gene polymorphism had five related analyses, which would be subject to multiple testing adjustments. Through this multiple testing adjustment, for each polymorphism with five related analysis, I used the Holm (Step-down Bonferroni) method to adjust the multiple testing analysis (Aickin & Gensler, 1996).

Sensitivity Analysis

Sensitivity analysis is used to determine the robustness of the findings. It is important to know how the results will change if I changed the aspects of data or analysis, such as criteria of including studies, changes in assumptions, or type of effect size (from *OR* to relate risk or *p*-value). In this study, the sensitivity analysis was used to remove individual study (repeatedly) on each pass to show how removing it affected the results (Borenstein et al., 2009).

Forest Plot

A forest plot is used to interpret statistics in context. In a meta-analysis, a forest plot shows the effect size of individual studies, including its confidence interval as well as the summary effect. In this study, the forest plot showed the conceptual issues as the *OR* for each study with its boundaries of confidence interval plus the summary effect and its boundaries at the end, fixed, and random model (Borenstein et al., 2009). The value of summary effects and fixed and random models in a forest plot are unadjusted values. The blue box in a forest plot is a computed width of box proportional to weight in the log scale.

Funnel Plot

A funnel plot is a diagnostic tool used for examining bias in the meta-analysis. It is an estimation of effect size versus the standard error, which in some studies is the effect size versus the sample size. In this study, the horizontal axis was the log of effect size (the log of *OR*), and the vertical axis was its standard error, in which the solid vertical line is the summary of effect sizes (fixed model) derived from a meta-analysis. This was a tool used to validate publication bias in a meta-analysis. The shape of the funnel, in the absence of bias, was symmetrical; in case of bias, the funnel plot would be skewed (Sterne & Harbord, 2004).

<center>**Threats of Validity**</center>

The validity of the study refers to the researcher being able to answer the questions within the variables of the study (Reis & Judd, 2000). The validity is indicated as follows: the ability to generalize the results in the real world using the measurement strategy, the ability to generalize the results from the measurement and the study design, and the ability to determine that the results are accurate. In this study, the validity was based on answering the following questions:

- Are the selected studies relevant to the inclusion criteria?

- How is the quality of the selected studies?

- What is the similarity of results from study to study?

The internal validity relates to causal relationships (Hogg & Cooper, 2007; Reis & Judd, 2000). To ensure internal validity, I looked at the measurements for variables. To meet external validity, the study's result should be generalizable to other related

populations (Reis & Judd, 2000). To determine the validity of a statistical conclusion, the validity of the procedures were examined in fixed and random effects models.

Reliability

The reliability of a study is related to the quality of measurement: its repeatability and consistency of the measurement (Hogg & Cooper, 2007). In this study, the quality of measurement was related to the laboratory methods of gene mapping for those three gene polymorphisms in cases and controls. In addition, the measures of cases that related to the diagnoses of prostate cancer were affected by the quality of measurement. The measure of prostate cancer risk was based on two methods: chemicals and physical exams. The relationship of validity and reliability was based on the consistency and repeatability of the measurement. The consistency of the same or a close result in all repeated measurements made it a reliable and valid measure.

Ethical Considerations

This meta-analysis was based on the results of the individual studies that have been done before. Ethical considerations were important in the primary study. The selected studies in this meta-analysis were from different regions around the globe with different ethnic groups. In this meta-analysis, I planned to combine the results from each individual study; I assumed that each researcher obtained the permission of the people who participated in the research; I also assumed that each researcher obtained the appropriate institutional review board (IRB) approval before conducting the study. In this study, I worked directly with Walden IRB to ensure the correct ethical standards for research at Walden University. As the researcher, I was responsible for ensuring that the

research was based on ethical standards with Walden University and U.S. federal regulations.

Summary

In this chapter, I explained the study design of this study and the statistical methods that were used to estimate the overall effect size, including its bounded confidence interval (*CI*). In a meta-analysis, the performance of regression depends on the individual number of studies that are involved in the analysis. In this meta-analysis, a unit of analysis was the individual study. As more studies were involved to the combine results, the power of the test becomes a more accurate result. The effect size of each study was *OR*, which was calculated from the genotype and the allele frequencies in each study. The definitions of dominant, codominant, and recessive models were explained, as well as how I represented them in the analysis. I explained the heterogeneity analysis to estimate the variation between studies and the fixed and random effects model to estimate the overall size effect and its *CI* boundaries. The validity and reliability concepts were explained in this chapter, including a sensitive analysis regarding the robustness of the outcome. I explained the concepts of forest and funnel plot in this meta-analysis study.

In the next chapter, I present the results of estimations. I focus on the outcomes. I describe the results and the interpretation of the outcomes, including tables and graphs.

Chapter 4: Results

Introduction

In this chapter, I present the results of the statistical analysis of the data from 20 collected studies via charts, graphs, and tables. The number of individual studies was different in each genetic polymorphism. Prostate cancer is the second leading cause of cancer death among men after lung cancer. The purpose of this study was to examine gene-prostate cancer association. I examined the association of prostate cancer with three one-carbon metabolism gene polymorphisms (MTHFR, MTR, and MTRR). The association in this study was examined by a meta-analysis method.

The research questions regarded the association between selected genotypes and prostate cancer risk.

1. Is there any significant association between one-carbon, metabolism gene polymorphisms and prostate cancer risk?

$H1_o$: There is no association between one-carbon, metabolism gene polymorphisms and prostate cancer risk.

$H1_a$: There is an association between one-carbon, metabolism gene polymorphisms and prostate cancer risk.

2. Are there any significant differences between regions of the globe due to gene-disease association?

$H2_o$: There are no differences between regions due to gene-disease association.

$H2_a$: There is a difference between regions due to gene-disease association.

3.   Are there any significant differences between ethnic groups due to gene-disease association?

H3$_o$: There are no differences between ethnic groups due to gene-disease

H3$_a$: There is a difference between ethnic groups due to gene-disease association.

Methodology of Meta-Analysis

A meta-analysis research combines multiple studies to determine results from a group of articles with a common hypothesis. The unit of meta-analysis is the study; increasing the number of studies increases the power of the test. The power of the test in a meta-analysis is greater than an individual study. All included studies were a case-control study with a number of cases and controls in different geographical regions and ethnic groups. Although there may not be a matched case-control study, the statistical analysis was used to estimate the association between the risk of prostate cancer and one-carbon, metabolism gene polymorphisms (MTHFR, MTR, and MTRR).

Overview of Meta-Analysis

In this meta-analysis study, I examined the association between one-carbon, metabolism gene polymorphisms and the risk of prostate cancer. There were three genes involved in this study with their polymorphisms: MTHFR gene with two polymorphisms of C677T (rs1801133), A1298C (rs1801131), MTR gene with a polymorphism of A2756G (rs1805087), and MTRR with a polymorphism of A66G (rs1801394). The total number of samples for both groups of cases and controls in the 20 included studies were 8,675 cases and 9,207 controls. These cases and controls were the combined samples from each individual study for meta-analysis of the genetic-prostate cancer association. In

some studies, the different genes had different numbers. In this meta-analysis, I

conducted an analysis based on the results from each individual study: the result of effect

size (OR) association and CI were at the 95 % level of the test.

Data Collection

The data were collected from 20 individual studies from the years 2000 to 2014

with the number of cases and controls in variants and wild genotypes. The wild

genotypes were those genes without mutation, and the variant genotypes were those

genes with mutation. The analysis was on the raw collected data. Although each

individual study had an effect size and its CI results in their article, the size effect and its

boundaries were estimated based on an OR formula for a case-control study. The general

formula for OR estimation and its confidence intervals were presented in Chapter 3. Each

individual study was conducted in a different region using various ethnic groups. In this

study, I estimated the significant differences between the studies regarding these two

variables (regions and ethnic groups).

Eligibility: Inclusion/Exclusion Criteria

The basis of eligibility criteria was on studies testing relationships between one-

carbon, metabolism gene polymorphisms (MTHFR, MTR and MTR) and prostate cancer

risk. According to meta-analysis method, the results of this study should be able to be

generalized to the real world, due to the validity of the study. The inclusion criteria

involved defining the subject based on the study design. The individual study included

had to have an explanation of the method used to obtain the results, and the article also

had to include the needed information, such as number of samples in cases and controls,

ORs and CI boundaries, and the details of the data collection. The exclusion criteria were those studies with a defined subject and a lack of needed information. There were some studies in languages other than the English language, which were translated to English and were included in this study.

In the MTHFR gene, there was a total number of 20 individual studies involved in C667T polymorphism with a total of 8,675 cases and 9,207 control participants in each group of studies. In the MTHFR gene, the A1298C polymorphism, 11 studies were included in the analysis, of which there were 3,026 cases and 3,754 control participants included in the analysis. The MTR gene, A2756G polymorphism, had four included studies with a total number of 701 case participants and the total number of 739 control participants. The MTRR gene, A66G polymorphism, included four studies with the total of 698 case and 737 control participants.

Data Analysis

The data analysis of this meta-analysis study was based on a quantitative component of independent studies to yield an overall OR of the genotype-prostate cancer association. The analysis for this study included a step-by-step process. At first, the sample size in each individual study for both groups, cases and controls, was indicated by each genotype and allele frequencies. In the second step, I analyzed the homogeneity between studies by Q value and I^2 estimation and then a fixed and random effect models for the overall size effect estimation. A funnel plot was used to examine the publication bias to ensure the validity of the results of each individual study. In the fixed-effects model, the combined studies' results for the set of studies were estimated and only within

study variance was examined. In the random-effects model, the results were generalized to other studies where within and between studies variations were examined.

The meta-regression analysis helps to understand the source of variation that is indicated by the homogeneity test. In this meta-analysis, a meta-regression analysis was conducted after significant value for homogeneity was reached. The meta-regression analysis showed the related variation among the region of studies or the related variation among the different ethnic groups. The regions were in three groups of continents: America, Europe, and Asia. The ethnic groups were in four groups of Caucasian, African American, Asian, and Spanish. The forest plot in-text results show a general view of each individual study's effect size and its boundaries, in addition to the overall size effect.

Homogeneity

The homogeneity analysis tests the variation of effect size in different studies. In this meta-analysis, the variation of estimated OR within and between 20 selected individual studies was of concern. The homogeneity test was based on a Q statistical test with a chi-square distribution and degree of freedom, which valued the total number of study minus 1 (df =19). The description of variation percentage of homogeneity was calculated by I^2, which indicates the percentage of variation related to the homogeneity rather than by chance. A I^2 close to 0 means the variation in effect size is due to sampling error within studies, and a value of close to 100 means a high heterogeneity among effect sizes (Borenstein et al., 2009).

Publication Bias

The publication bias included the articles that were published rather than those not published among the total available studies. I showed the publication bias by a funnel plot. The funnel plot is a visual tool to show the biases in this meta-analysis, and it described the eligible selected studies involved in this meta-analysis study. The results of a funnel plot from small studies will typically be wide at the bottom of the graph and will be narrow at the bottom from large studies. The largest studies have the smallest value on the vertical axis (standard error of log odds ratios). The funnel plot will look like a symmetrical in absence of publication bias (Sterne & Harbord, 2004).

Weights

The weighting strategies in this meta-analysis were based on two methods of variance estimation, within- and between-studies variances. The inverse variance method is common in meta-analysis studies. In the fixed effect model, the weight is inversely proportional to the within-study variance, and in the random model the weight is the inverse of the sum of the within-plus-between studies variances (Pigott, 2012).

Validity

The validity of this meta-analysis was based on questions asked in each individual study. Each individual study contained the same inclusion and exclusion criteria as this meta-analysis study. The validity of each included individual study was based on the study design and the performance of analytic process of each study. The research question in each individual study was in line with the goal of this meta-analysis on gene-prostate cancer association.

Results

The results of this study were based on the results of statistical procedures on the association between prostate cancer risk and different alleles and genotype frequencies among selected populations in different regions, from 20 individual selected studies. The regions were subgroups in three continents: America, Europe, and Asia. The important issues in the analysis regarding different regions were related to different ancestry in the regions. The incidence rate of prostate cancer is different in different regions, such as 213.7 in North America, Western Europe 167.9, and 133.2 in Asia (Dorr et al., 2013). The different ancestry may affect the genotype because they had a different environmental effect on the gene-environment interaction. For this reason, I tested different ancestries' and regions' associations with prostate cancer risk.

The results are presented in subgroups of three different genes: MTHFR, MTR, and MTRR; the MTHFR gene sub grouped in two polymorphisms of C667T and A1298C. In the analysis, each polymorphism had its own results of test of homogeneity, fixed and random effects, meta-regression, forest plot, and funnel plot. The meta-regression analysis did not show in some of the analysis because of the value of the Q statistic, in cases where the value of Q test result was smaller than the degree of freedom. A multiple test adjustment, due to Type I error, was applied in each polymorphism analysis. The Holm-Bonferroni multiple testing corrections was applied for fixed and random models of each polymorphism, which had five related analyses. The study weight in the forest plots was based on an inverse proportional of the within-study variance.

MTHFR

The methylenetetrahydrofolate reductase (MTHFR) gene is involved in folate metabolism and has two polymorphisms: C677T (rs1801133) and A1298C (rs1801131).

The C677T polymorphism. In this study, the C677T polymorphism was in four subgroups of T Allele versus the C Allele, the genotype of TT versus CC, the genotype of CT versus CC, recessive model, and dominant model.

The T allele versus C allele. Table 2 shows the results of the homogeneity test analysis based on 18 eligible included studies. Table 6 shows a significant heterogeneity among effect sizes ($P<0.05$), which may be evidence of heterogeneity related to regions or ethnic groups where the study took place. The I^2 is another index of heterogeneity with 57.45 % of the variation being due to the effect sizes rather than sampling variance, a moderate degree of heterogeneity.

Table 2

Test for Homogeneity of Effects, Allele T vs. C

Q	df	ProbQ	I^2
39.95	17	0.0013	57.45

Note. The homogeneity results of MTHFR gene, C667T Polymorphism, Allele T vs. C

Table 3 shows the results of analysis in both fixed and random models. The fixed effect model indicates a significant association ($p<0.001$) between Allele T frequency and prostate cancer risk, but the random model indicates an association on the border of the significant ($p=0.051$). The OR indicates an inverse association of Allele T frequency and prostate cancer risk.

Table 3

Summary Effect Size, Allele T vs. C

Model	*OR*	Lower 95% limit	Upper 95% limit	*p*-value	*Adjusted p-value*
Fixed	0.92	0.88	0.97	0.0009	0.0027
Random	0.91	0.83	1.00	0.0513	0.1539

Note. The summary effect size of MTHFR gene, C667T polymorphism, Allele T vs. C

Figure 13 displays a publication bias by a funnel plot. The solid vertical line represents a summary of size effect, a log-OR derived using fixed-effect meta-analysis. This figure shows that there may be a slight evidence of publication bias, at the right bottom side of the graph, which may indicate some missing published studies.
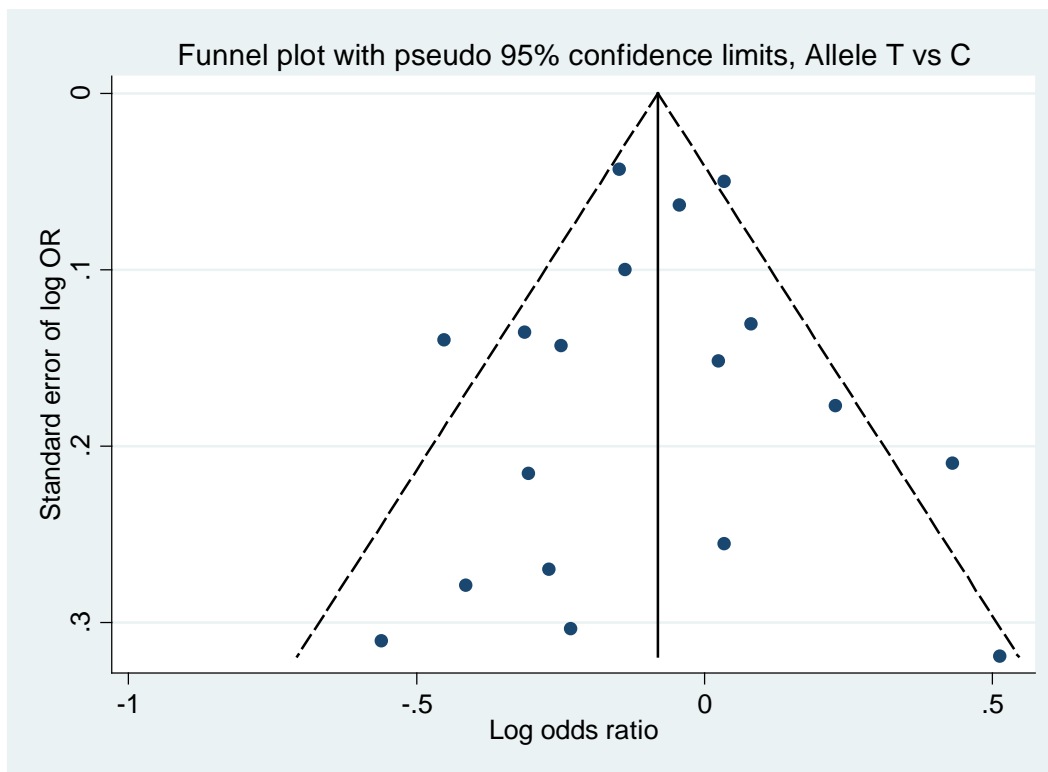
Figure 13. Funnel plot for risk of prostate cancer data, Allele T vs. C solid line is fixed effect model.

The overall effect size, based on the forest plot, shows a slight inverse association

between Allele T and prostate cancer risk (Figure 14).
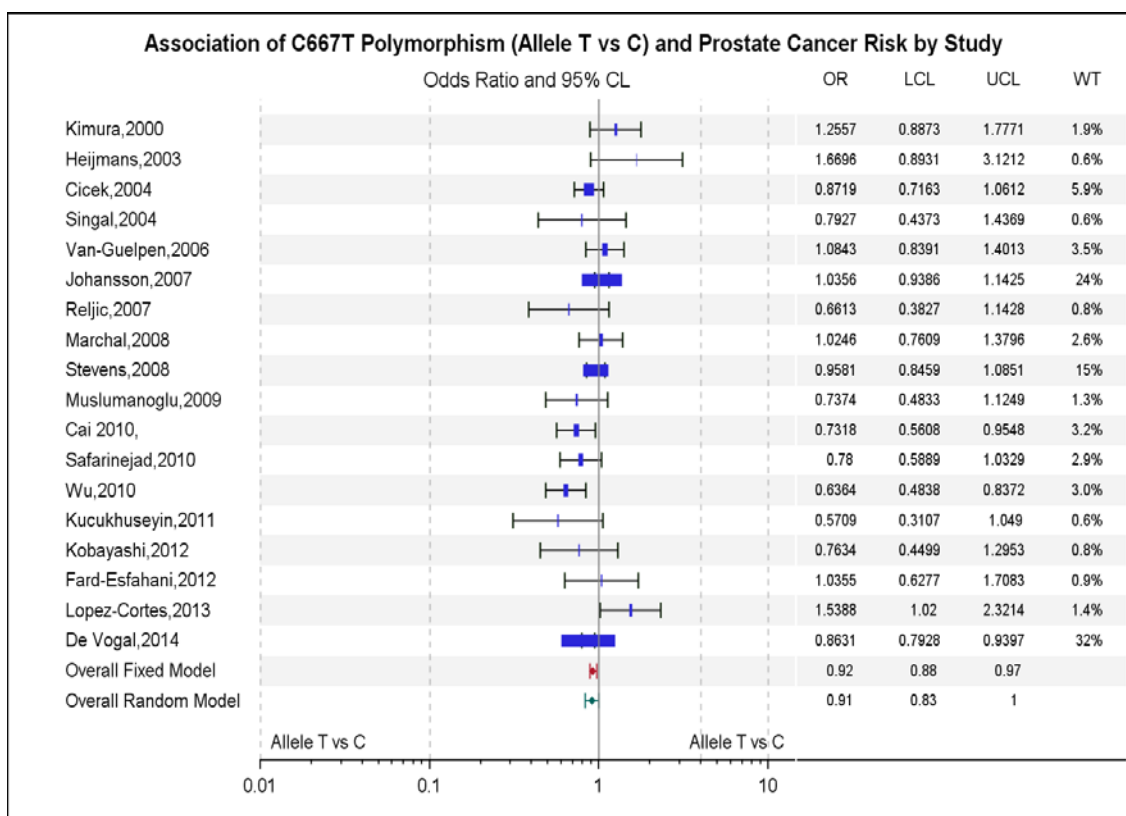
*Figure 14.*Forest plot of the association under allele T vs. C model

Table 4 shows a slight evidence of heterogeneity related to the regions.

Table 4

Result of Weighted Multiple Regression Analysis of Effect Size on Region of Study and

Ethnicity Groups, Allele T vs. C

Variable	*DF*	Parameter Estimate		*T* Value	*Pr>|t|*
		Parameter Estimate	Standard Error		
Intercept	1	0.18	0.16	1.09	0.29
Region	1	-0.13	0.07	-1.86	0.08
Ethnicity	1	-0.00	0.06	-0.02	0.98

Note. The summary of MTHFR gene, C667T polymorphism, Allele T vs. C

Figure 15 shows the log OR of the study decreased by the region from America to Asia (America=1, Europe=2, and Asia=3).
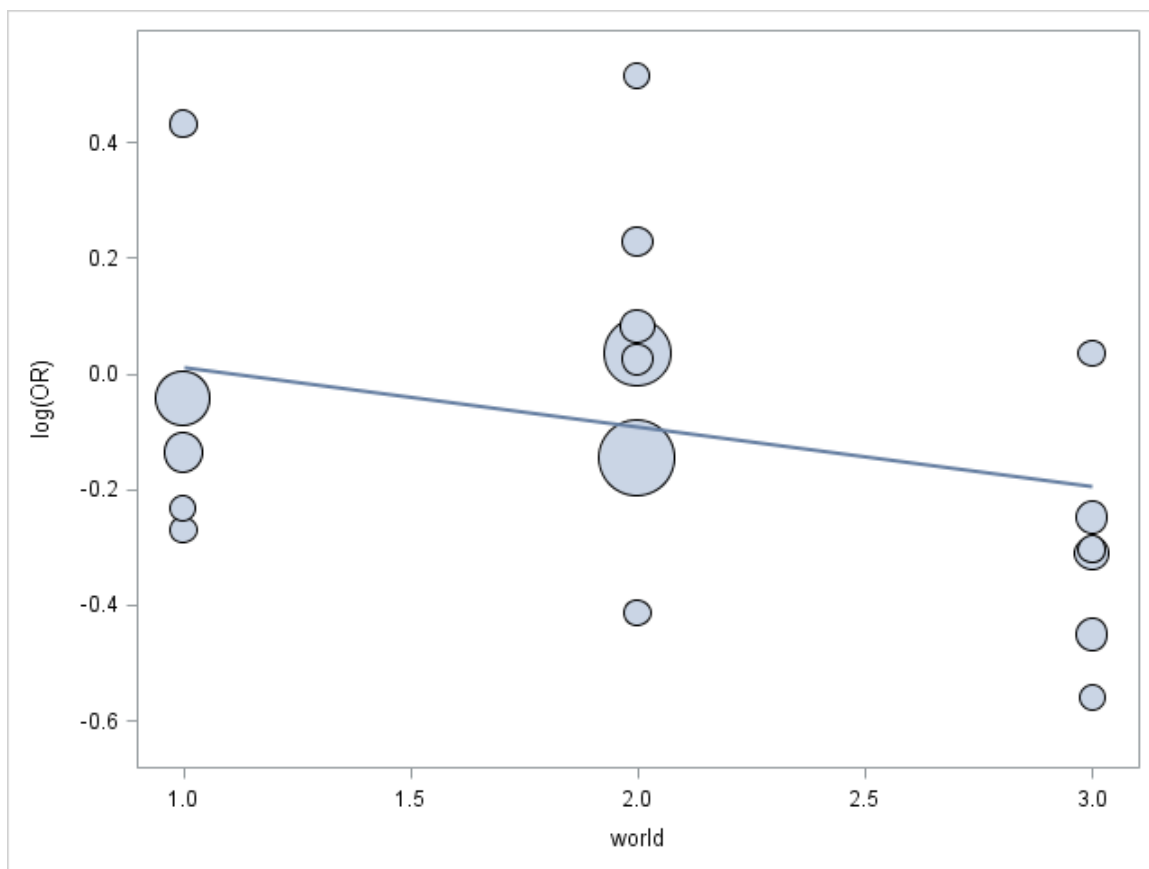
*Figure 15.*Plot of observed effect size for the risk of prostate cancer data against Allele T frequency.

The genotype TT versus CC. The heterogeneity of association of effect sizes

among studies, between genotype TT and prostate cancer risk, is small (28.54 %) and not

significant ($p>0.05$; Table 5).

Table 5

Test for Homogeneity of Effects, Genotype TT vs. CC

Q	df	ProbQ	I^2
23.79	17	0.13	28.55

Note. The homogeneity results of MTHFR gene, C667T Polymorphism, Genotype TT vs. CC

Although the results of heterogeneity were not significant (Table 5), it may be due to the result from the fixed effect model. The results from the random model show the same result as the fixed effect model due to significant association. The fixed effect model shows (Table 6) a significant association between TT genotype frequency and prostate cancer risk ($p<0.05$), which it is a significant association in the random model too ($P<0.05$).

Table 6

Summary Effect Size, Genotype TT vs. CC

Model	*OR*	Lower 95% limit	Upper 95% limit	*p*-value	*Adjusted p-value*
Fixed	0.81	0.73	0.91	0.0002	0.0010
Random	0.78	0.66	0.92	0.0035	0.0175

Note. The summary effect size of MTHFR gene, C667T polymorphism, Genotype TT vs. CC.

Figure 16 shows an asymmetric shape. The publication bias in Figure 16 is on missing studies in the top right side of the figure. Although the density of studies is more on the upper left hand side of the funnel plot, there are still missing studies on the right hand side of the funnel plot with the increase of OR and a decrease of the standard error.
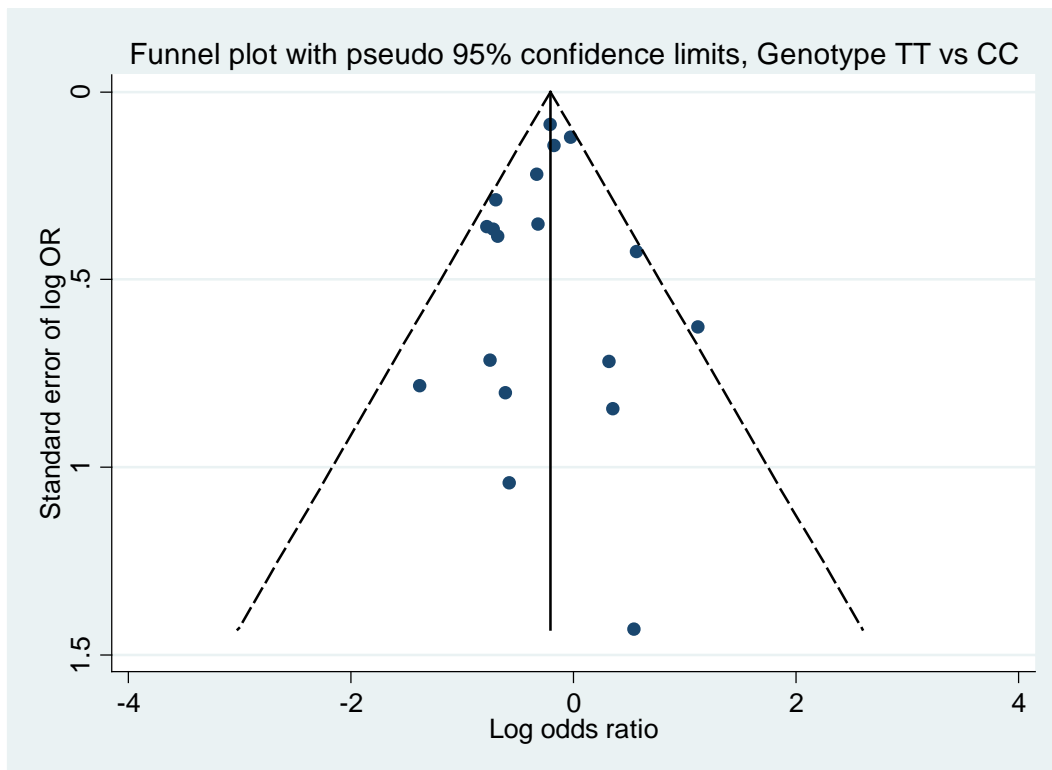
Figure 16. Funnel plot for risk of prostate cancer data and Genotype TT vs. CC.

Table 7 indicates no relationship between regions and Genotype TT frequency; there is not a significant association between prostate cancer and regions or ethnic groups, as heterogeneity was not significant (p=0.13).

Table 7

Result of Weighted Multiple Regression Analysis of Effect Size on Region of Study and Ethnicity Groups, Genotype TT vs. CC

Variable	*DF*	Parameter Estimate		*T* Value	*Pr>|t|*
		Parameter Estimate	Standard Error		
Intercept	1	0.18	0.27	0.68	0.50
Region	1	-0.13	0.15	-0.85	0.41
Ethnicity	1	-0.15	0.15	-0.97	0.35

Note. The summary of MTHFR gene, C667T polymorphism, and Genotype TT vs. CC

Figure 17 shows an inverse significant association between the overall OR of genotype TT and prostate cancer risk.
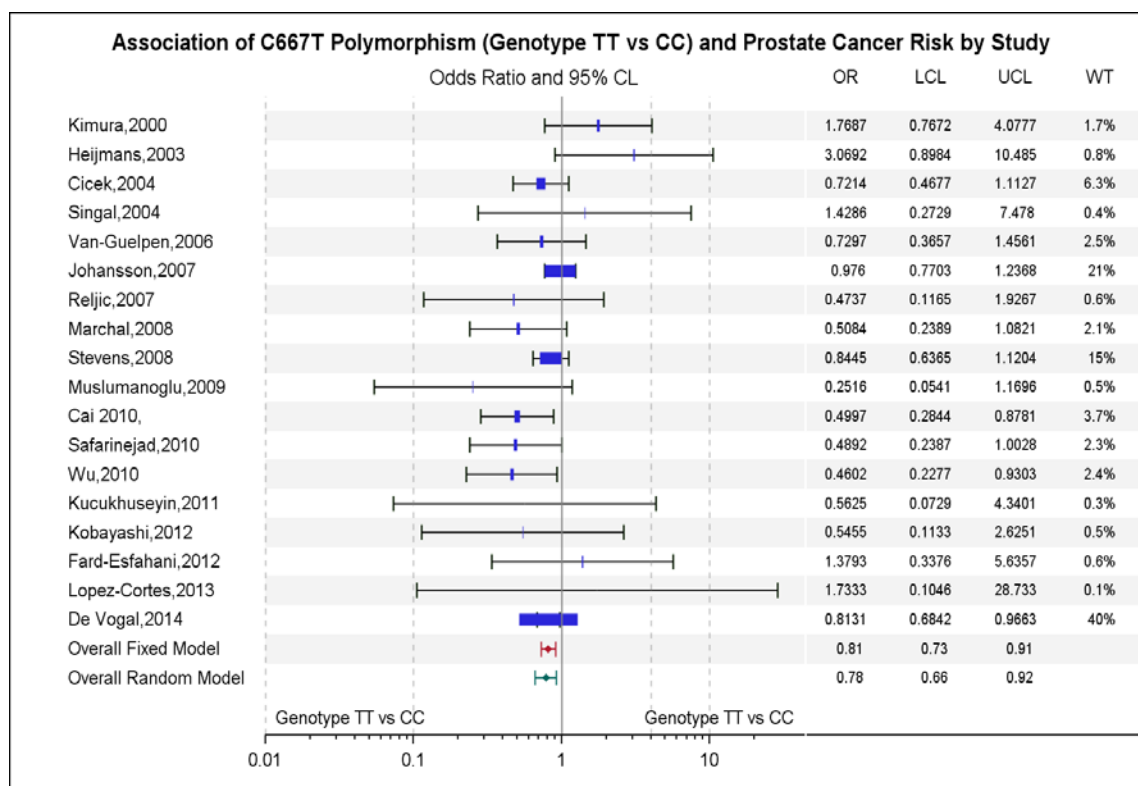
Figure 17. Forest plot of the association under Genotype TT vs. CC model.

The genotype CT versus CC. The test of homogeneity (Table 8) shows a highly significant heterogeneity among studies' effect sizes ($P<0.001$) with a moderated degree.

Table 8

Test for Homogeneity of Effects, Genotype CT vs. CC

Q	*df*	ProbQ	I^2
53.70	18	<.0001	66.48

Note. The homogeneity results of MTHFR gene, C667T Polymorphism, and Genotype CT vs. CC

There was not a significant association between genotype CT frequency and prostate cancer ($P>0.05$) in both fixed and random models (Table 9).

Table 9

Summary Effect Size, Genotype CT vs. CC

Model	OR	Lower 95% limit	Upper 95% limit	*p*-value	*Adjusted p-value*
Fixed	0.97	0.91	1.03	0.34	0.34
Random	0.96	0.83	1.12	0.63	0.70

Note. The summary effect size of MTHFR gene, C667T polymorphism, Genotype CT vs. CC

The funnel plot regarding the publication bias on genotype CT versus CC shows some

missing studies on the right side of the plot (Figure 18).
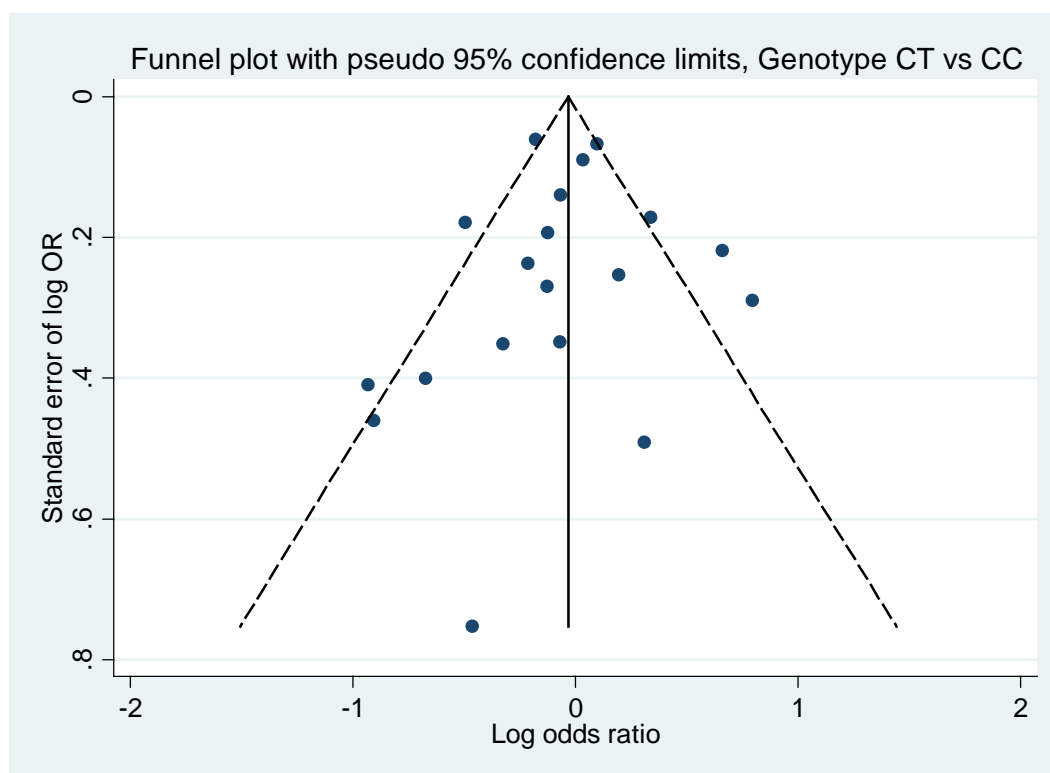
Figure 18. Funnel plot for risk of prostate cancer data, Genotype CT vs. CC.

Figure 19 shows no significant overall estimation of OR for both fixed and random models.
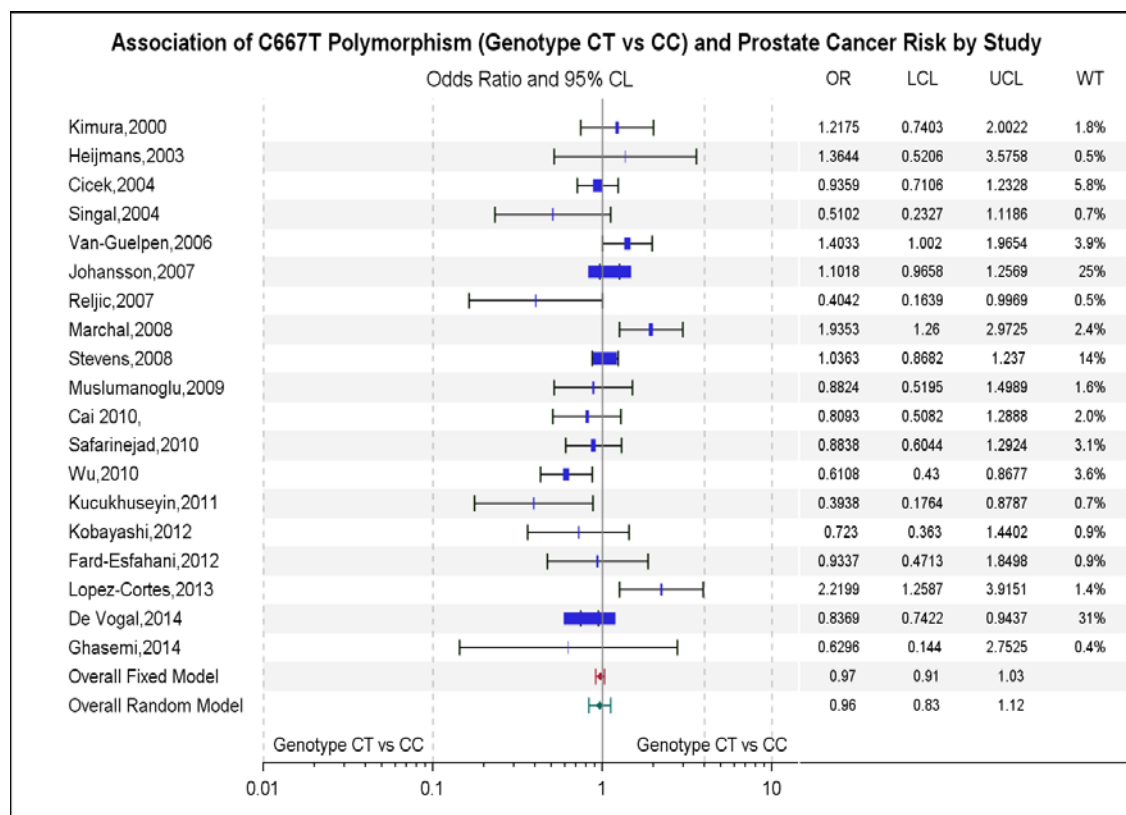
Figure 19. Forest plot of the association under Genotype CT vs. CC model.

Table 10 shows that the region and ethnic group did not relate to effect size.

Table 10

Result of Weighted Multiple Regression Analysis of Effect Size on Region of Study and

Ethnic Groups, Gynotype CT vs. CC

Variable	DF	Parameter Estimate		T Value	Pr>|t|
		Parameter Estimate	Standard Error		
Intercept	1	0.25	0.28	0.90	0.38
Region	1	-0.16	0.12	-1.36	0.19
Ethnicity	1	0.03	0.11	0.26	0.80

Note. The summary of MTHFR gene, C667T polymorphism, and Genotype CT vs. CC

The recessive model (TT vs. CT+CC). The homogeneity test does not show a

significant heterogeneity among studies (Table 11), a small degree of heterogeneity

among effect sizes (27.57%).

Table 11

Test for Homogeneity of Effects, Recessive Model (TT vs. CT+CC)

Q	df	ProbQ	I^2
23.47	17	0.1344	27.58

Note. The homogeneity results of MTHFR gene, C667T Polymorphism, and recessive
model (TT vs. CT+CC)

The results from the fixed and random effect models shows a highly significant

effect sizes for both models at 0.05 levels of test ($p<0.05$; Table 12). This significant

association is between the recessive model and the reduction of risk of prostate cancer.

Table 12

Summary Effect Size, Recessive Model (TT vs. CT+CC)

Model	OR	Lower 95% limit	Upper 95% limit	*P*-value	*Adjusted p-value*
Fixed	0.83	0.75	0.92	0.0003	0.0012
Random	0.79	0.68	0.93	0.0035	0.0175

Note. The summary effect size of MTHFR gene, C667T polymorphism, and recessive model (TT vs. CT+CC)

Figure 20 shows a publication bias at the right part of the plot. The population bias had missing studies with more than overall ORs.
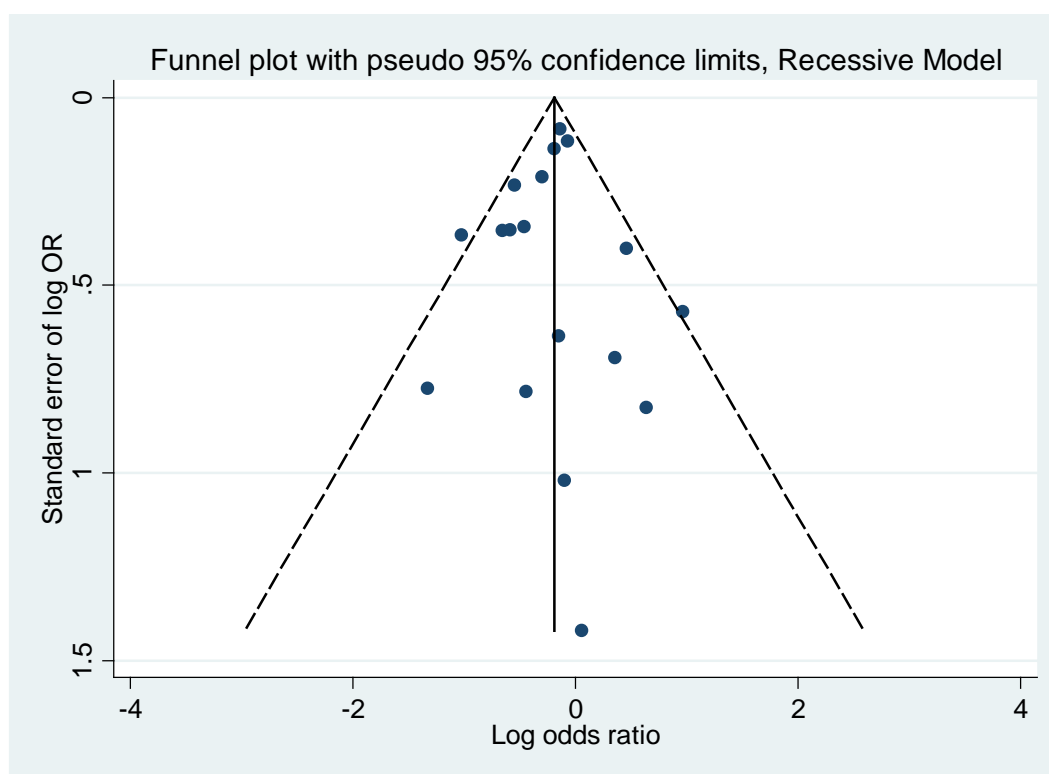
Figure 20. Funnel plot for risk of prostate cancer data and recessive model (TT vs. CT+CC).

Figure 21 indicates an overall significant association between the recessive model and a reduction of the risk of prostate cancer.

		OR	LCL	UCL	WT
	Association of C667T Polymorphism (Recessive Model) and Prostate Cancer Risk by Study				
	Odds Ratio and 95% CL				
Kimura,2000		1.5862	0.7212	3.4885	1.7%
Heijmans,2003		2.6353	0.8613	8.0635	0.9%
Cicek,2004		0.7441	0.492	1.1254	6.3%
Singal,2004		1.8919	0.3752	9.5394	0.4%
Van-Guelpen,2006		0.6303	0.3212	1.2371	2.4%
Johansson,2007		0.9348	0.7431	1.176	20%
Reljic,2007		0.8634	0.2488	2.9963	0.7%
Marchal,2008		0.359	0.1748	0.7372	2.1%
Stevens,2008		0.829	0.6345	1.0832	15%
Muslumanoglu,2009		0.2656	0.0581	1.2139	0.5%
Cai 2010,		0.5793	0.3657	0.9176	5.1%
Safarinejad,2010		0.5196	0.2597	1.0399	2.2%
Wu,2010		0.5566	0.2787	1.1116	2.2%
Kucukhuseyin,2011		0.9057	0.1228	6.6819	0.3%
Kobayashi,2012		0.6423	0.1383	2.9836	0.5%
Fard-Esfahani,2012		1.4315	0.3681	5.5672	0.6%
Lopez-Cortes,2013		1.0583	0.0653	17.141	0.1%
De Vogal,2014		0.8714	0.738	1.0289	39%
Overall Fixed Model		0.83	0.75	0.92	
Overall Random Model		0.79	0.68	0.93	

Figure 21. Forest plot of the association under genotype recessive model.

Table 13 shows that there was not any significant relationship between regions and ethnic groups to the variation on the recessive model.

Table 13

Result of Weighted Multiple Regression Analysis of Effect Size on Region of Study and Ethnicity Groups, Recessive Model (TT vs. CT+CC)

		Parameter Estimate			
Variable	*DF*	Parameter Estimate	Standard Error	*T* Value	*Pr>|t|*
Intercept	1	0.08	0.26	0.31	0.76
Region	1	-0.10	0.15	-0.64	0.53
Ethnicity	1	-0.10	0.15	-0.67	0.51

Note. The summary of MTHFR gene, C667T polymorphism, and recessive model (TT vs. CT+CC)

The dominant model (CT+TT) vs. CC. Table 14 indicates that there was a highly significant heterogeneity among effect sizes ($P<0.001$); the index value of heterogeneity was moderate (65.49%).

Table 14

Test for Homogeneity of Effects, Dominant Model (CT+TT) vs. CC

Q	df	ProbQ	I^2
52.15	18	<.0001	65.49

Note. The homogeneity results of MTHFR gene, C667T polymorphism, and dominant model (CT+TT) vs. CC

Table 15 shows no significant association between the dominant model and the risk of prostate cancer at the fixed and random effect models.

Table 15

Summary Effect Size, Dominant Model (CT+TT) vs. CC

Model	OR	Lower 95% limit	Upper 95% limit	*p*-value	*Adjusted p-value*
Fixed	0.94	0.88	1.00	0.05	0.10
Random	0.94	0.82	1.07	0.35	0.70

Note. The summary effect size of MTHFR gene, C667T polymorphism, and dominant model (CT+TT) vs. CC

Figure 22 was almost symmetric, just short of small number of studies at the right side of the graph.
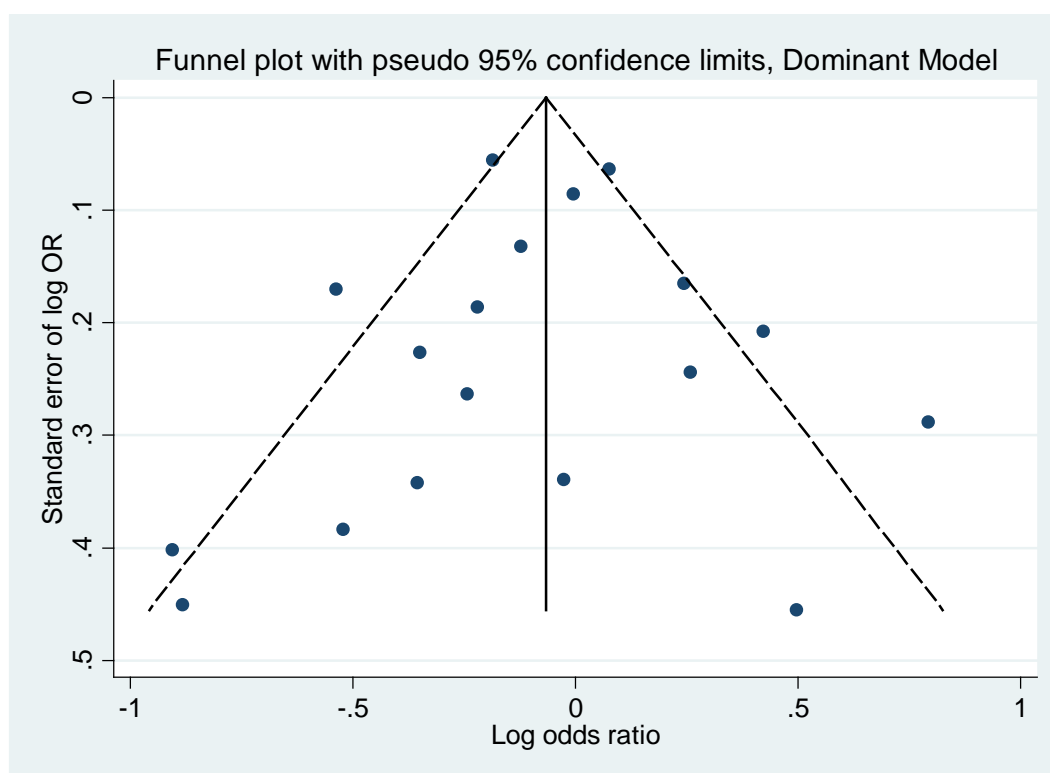
Figure 22. Funnel plot for risk of prostate cancer data, dominant model (CT+TT) vs. CC.

Figure 23 indicated no significant association between the dominant model and the risk of prostate cancer, but there was a border significant association (*p*=0.05) for the fixed effect model.
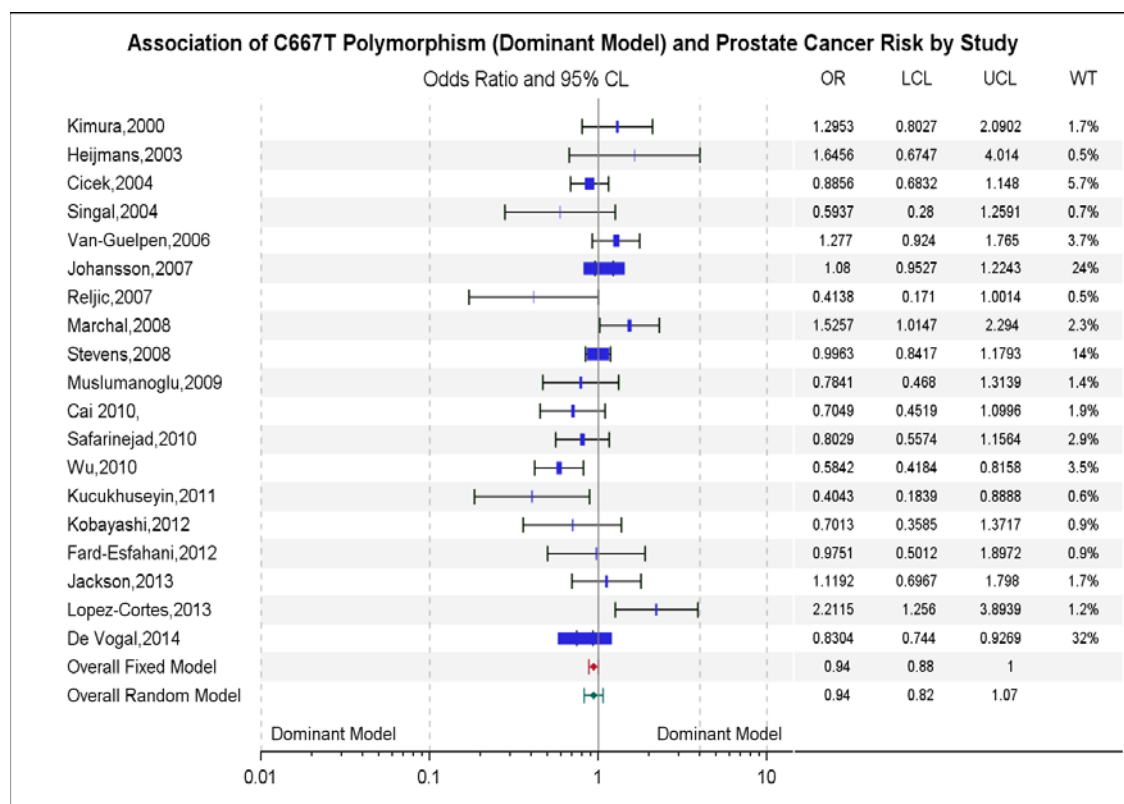
Figure 23. Forest plot of the association under dominant model.

Table 16 shows that there was not an explanation of variation of effect size related to the regions or ethnic groups.

Table 16

Result of Weighted Multiple Regression Analysis of Effect Size on Region of Study and Ethnicity Groups, Dominant Model (CT+TT) vs. CC

Variable	*DF*	Parameter Estimate		*T* Value	*Pr>|t|*
		Parameter Estimate	Standard Error		
Intercept	1	0.26	0.26	1.00	0.33
Region	1	-0.18	0.11	-1.66	0.12
Ethnicity	1	0.01	0.08	0.18	0.86

Note. The summary effect size of MTHFR gene, C667T polymorphism, and dominant model (CT+TT) vs. CC

The A1298C polymorphism. In this study, the polymorphisms were analyzed in five subgroups of Allele C versus Allele A, Genotype CC versus AA, Genotype AC versus AA, recessive model, and dominant model.

The C Allele versus A allele. There was a significant heterogeneity of effect sizes at 0.05 levels of test ($P<0.05$; Table 17), which indicated a moderate degree of heterogeneity. There was a 52.63 % of heterogeneity in this study due to heterogeneity among effect sizes rather than sampling variance.

Table 17

Test for Homogeneity of Effects, Allele C vs. A

Q	df	ProbQ	I^2
19	9	0.03	52.63

Note. The homogeneity results of MTHFR gene, A1298C polymorphism, Allele C vs. A

Table 18 indicates that there was not a significant association between the Allele

C and risk of prostate cancer (*P*>0.05).

Table 18

Summary Effect Size, Allele C vs. A

Model	*OR*	Lower 95% limit	Upper 95% limit	*p*-value	*Adjusted p-value*
Fixed	1.04	0.96	1.13	0.36	0.72
Random	1.06	0.93	1.22	0.37	1.00

Note. The summary effect size of MTHFR Gene, A1298C Polymorphism, and

Allele C vs. A

Figure 24 shows a slight publication bias on the bottom left side of the plot, with a

decrease of log OR values.
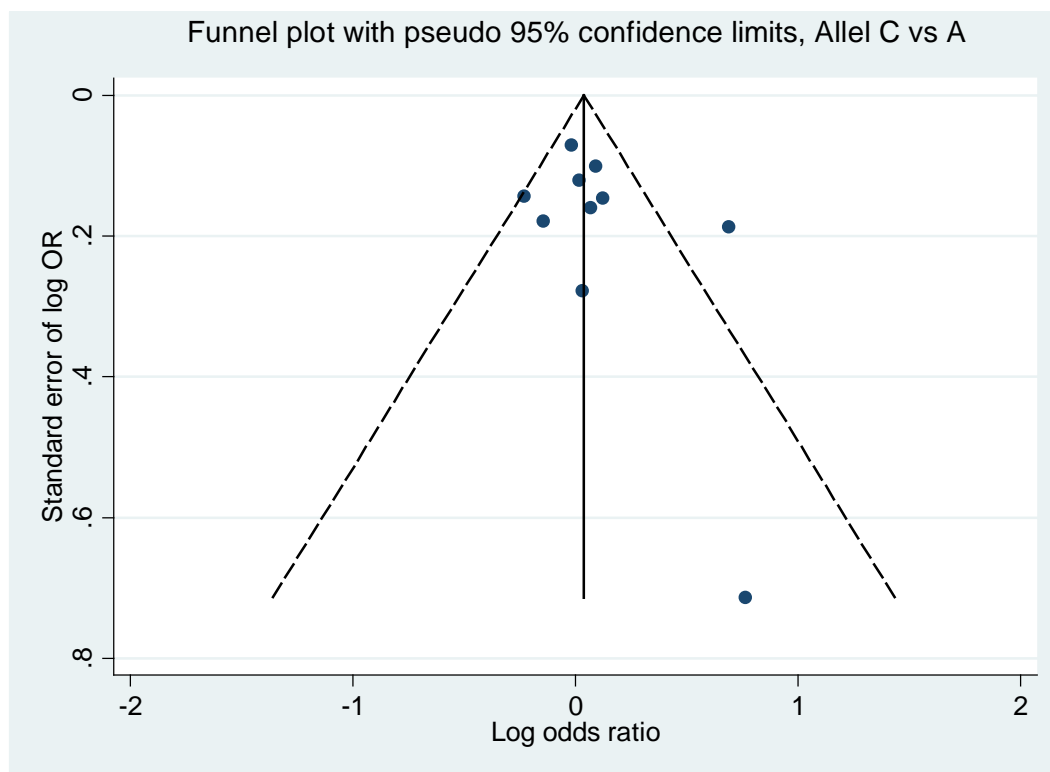
Figure 24. Funnel plot for risk of prostate cancer data, Allele C vs. A.

Figure 25 shows no significant association between the overall OR of Allele C and prostate cancer risk.
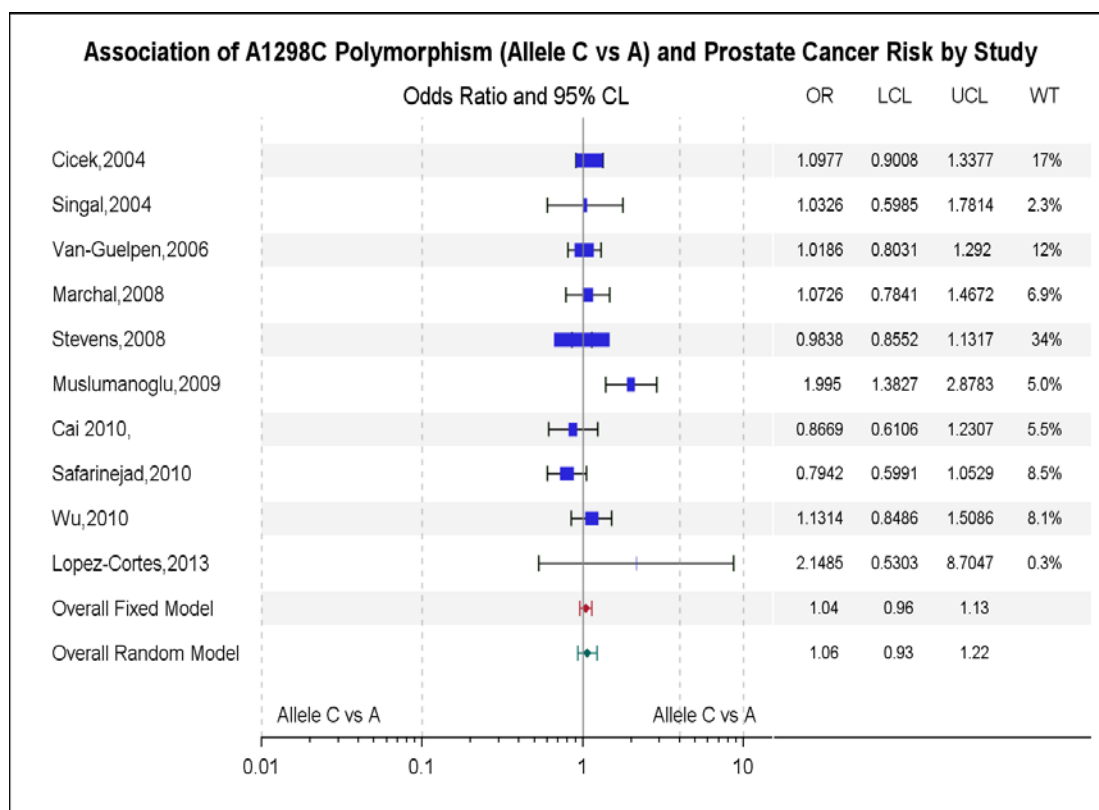
Figure 25. Forest plot of the association under Allele C vs. A model.

Table 19 shows that there was not a variation by regions or ethnic groups.

Table 19

Result of Weighted Multiple Regression Analysis of Effect Size on Region of Study and

Ethnicity Groups, Allele C vs. A

Variable	*DF*	Parameter Estimate		*T* Value	*Pr>|t|*
		Parameter Estimate	Standard Error		
Intercept	1	0.04	0.23	0.17	0.87
Region	1	0.02	0.11	0.22	0.83
Ethnicity	1	-0.02	0.11	-0.16	0.88

Note. The summary of MTHFR gene, A1298C polymorphism, and Allele C vs. A

The Gynotype CC vs. AA. Table 20 shows a moderate degree of heterogeneity of effect sizes (I^2=31.40%).

The estimation of the association between Genotype CC frequency and risk of prostate cancer shows a slight association with the fixed effect model with unadjusted value (p=0.08), but there was no significant association at the random effect model (p>0.05; Table 21).

Table 20

Test for Homogeneity of Effects, Gynotype CC vs. AA

Q	*df*	ProbQ	I^2
13.12	9	0.16	31.42

Note. The homogeneity results of MTHFR gene, A1298C polymorphism, and Gynotype CC vs. AA

Table 21

Summary Effect Size, Genotype CC vs. AA

Model	OR	Lower 95% limit	Upper 95% limit	*p*-value	*Adjusted p-value*
Fixed	1.18	0.98	1.41	0.08	0.24
Random	1.14	0.89	1.46	0.30	1.00

Note. The summary effect size of MTHFR Gene, and A1298C Polymorphism, Genotype CC vs. AA

Figure 26 showed a publication bias with missing studies on the right side of the plot, with an increase in the ORs.

Figure 26 showed a publication bias with missing studies on the right side of the plot, with an increase in the ORs.
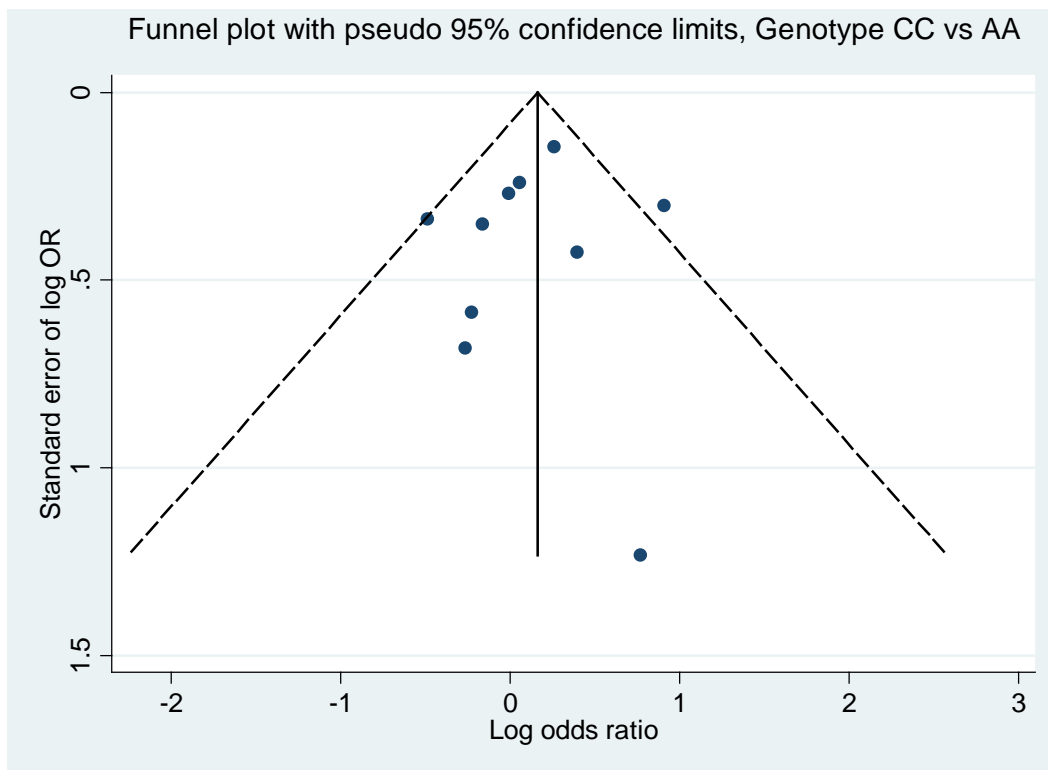
Figure 26. Funnel plot for risk of prostate cancer data and Gynotype CC vs. AA.

Figure 27 describes the positive association between Genotype CC frequency and prostate cancer, although it was not statistically significant.
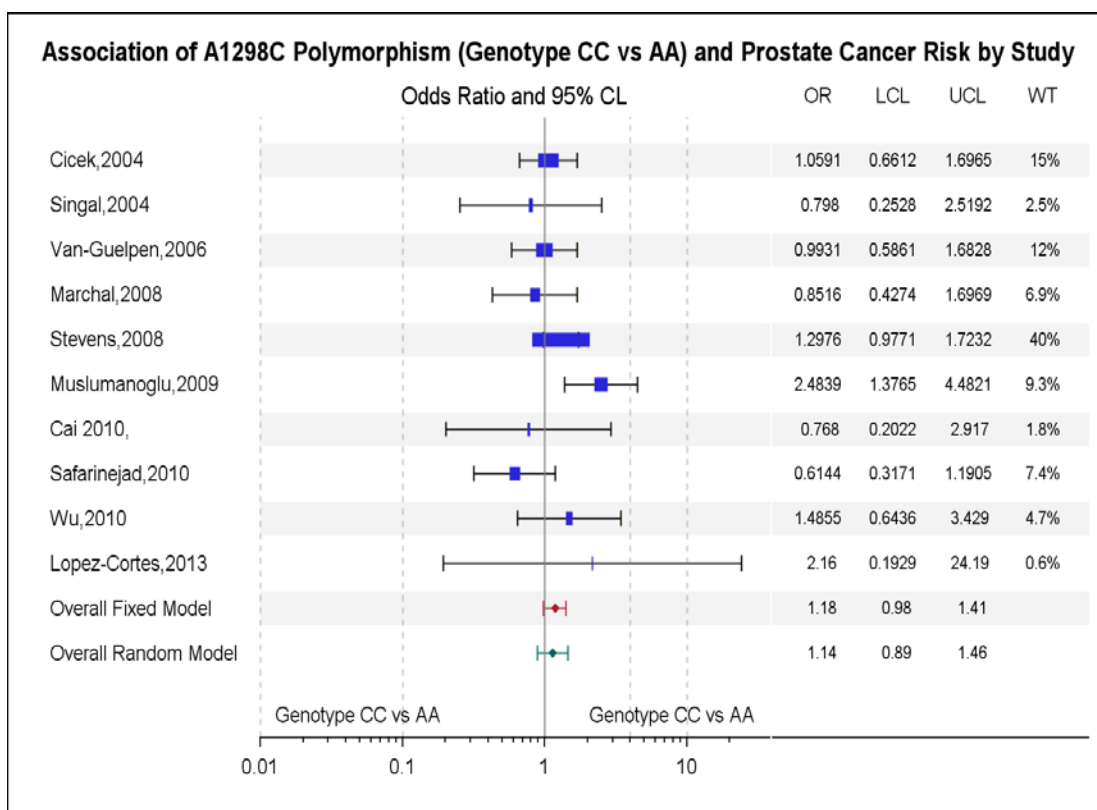
Figure 27. Forest plot of the association under Genotype CC vs. AA model.

Table 22 does not show any significant association that the variation between studies was because of the regions or ethnic groups.

Table 22

Result of Weighted Multiple Regression Analysis of Effect Size on Region of Study and

Ethnicity Groups, Gynotype CC vs. AA

Variable	*DF*	Parameter Estimate		*T* Value	*Pr>|t|*
		Parameter Estimate	Standard Error		
Intercept	1	0.05	0.39	0.13	0.90
Region	1	0.03	0.17	0.15	0.88
Ethnicity	1	0.03	0.22	0.12	0.90

Note. The summary of MTHFR gene, A1298C polymorphism, and Genotype CC vs. AA

    The Genotype AC vs. AA. Table 23 shows a significant value of the homogeneity

test ($p<0.05$), with a moderate degree of heterogeneity. There was a significant

heterogeneity in the effect size of the different studies ($I^2=60.66$).

Table 23

Test for Homogeneity of Effects, Genotype AC vs. AA

Q	*df*	ProbQ	I^2
22.88	9	0.007	60.66

Note. The homogeneity results of MTHFR gene, A1298C polymorphism, and Genotype
AC vs. AA

    Table 24 shows a highly significant effect size ($p<0.05$), but the random effect

model shows no significant effect sizes.

Table 24

Summary Effect Size, Genotype AC vs. AA

Model	OR	Lower 95% limit	Upper 95% limit	*p*-value	*Adjusted p-value*
Fixed	1.24	1.11	1.38	0.0001	0.0005
Random	1.10	0.90	1.34	0.36	1.00

Note. The summary effect size of MTHFR Gene, A1298C Polymorphism, and Genotype AC vs. AA

    Figure 28 shows asymmetry and that there were some missing studies at the top right side of the graph, studies with more log OR than the overall size effect, and low standard error of log OR.
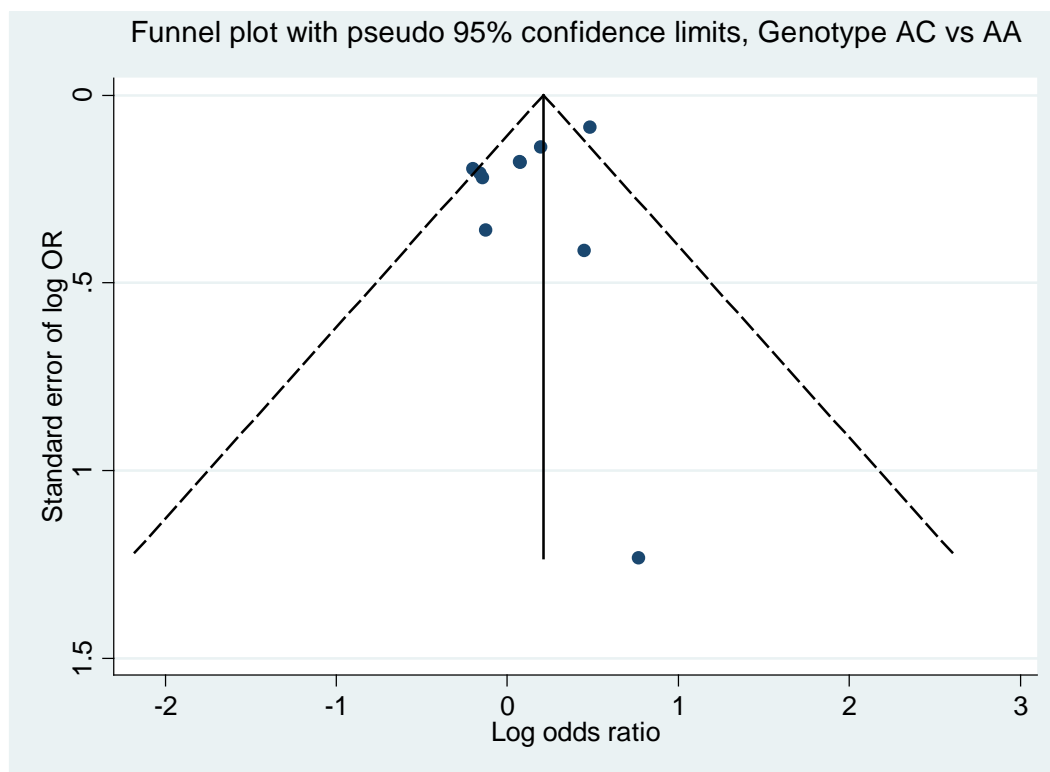
Figure 28. Funnel plot for risk of prostate cancer data and Genotype AC vs. AA.

Figure 29 shows a significant positive association by fixed effect model, but no significant association for the random model.
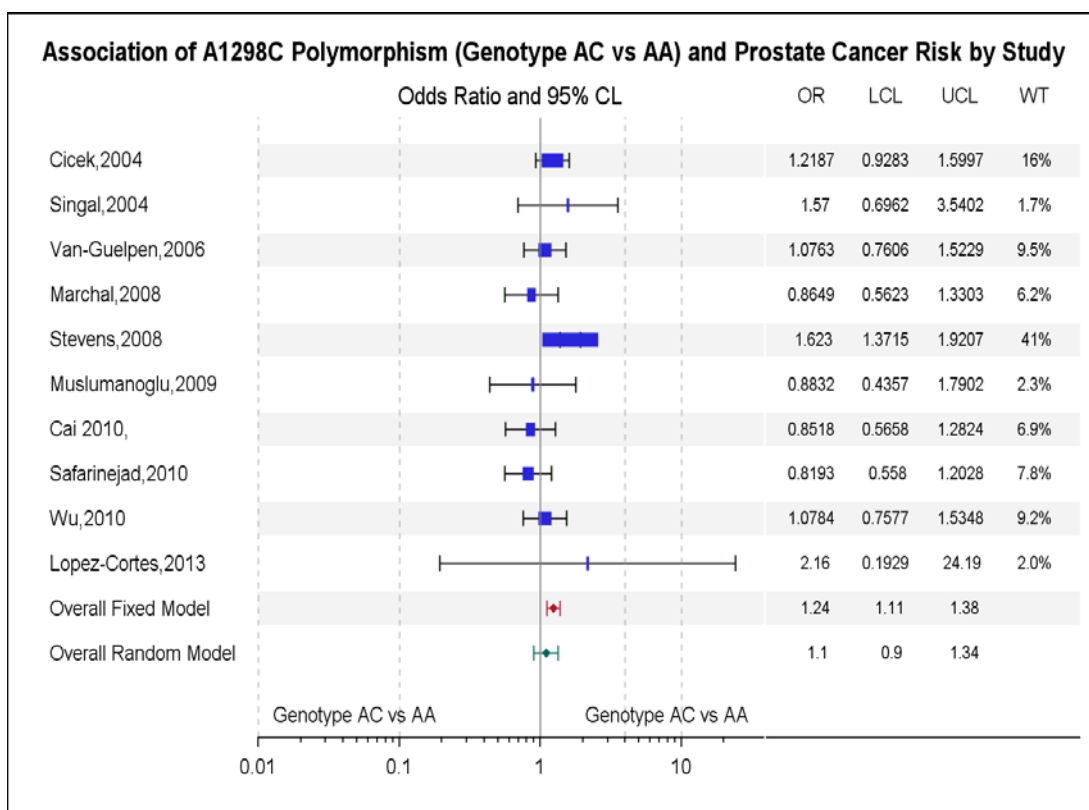
Figure 29. Forest plot of the association under Genotype AC vs. AA model.

Table 25 indicates a significant variance in effect size that related to the regions of studies placed.

Table 25

Result of Weighted Multiple Regression Analysis of Effect Size on Region of Study and Ethnicity Groups, Genotype AC vs. AA

		Parameter Estimate			
Variable	*DF*	Parameter Estimate	Standard Error	*t* Value	Pr>|t|
Intercept	1	0.51	0.12	4.26	0.004
Region	1	-0.28	0.06	-4.70	0.002
Ethnicity	1	0.10	0.06	1.69	0.136

Note. The summary of MTHFR gene, A1298C polymorphism, and Genotype AC vs. AA

Figure 30 shows a scatter plot of effect size against regions, which was evidenced in the relationship between regions and effect size variance. The effect size decreased from the American region to the Asian region; the samples in Asia had an inverse association with an increased risk of prostate cancer related to AC genotype frequency.
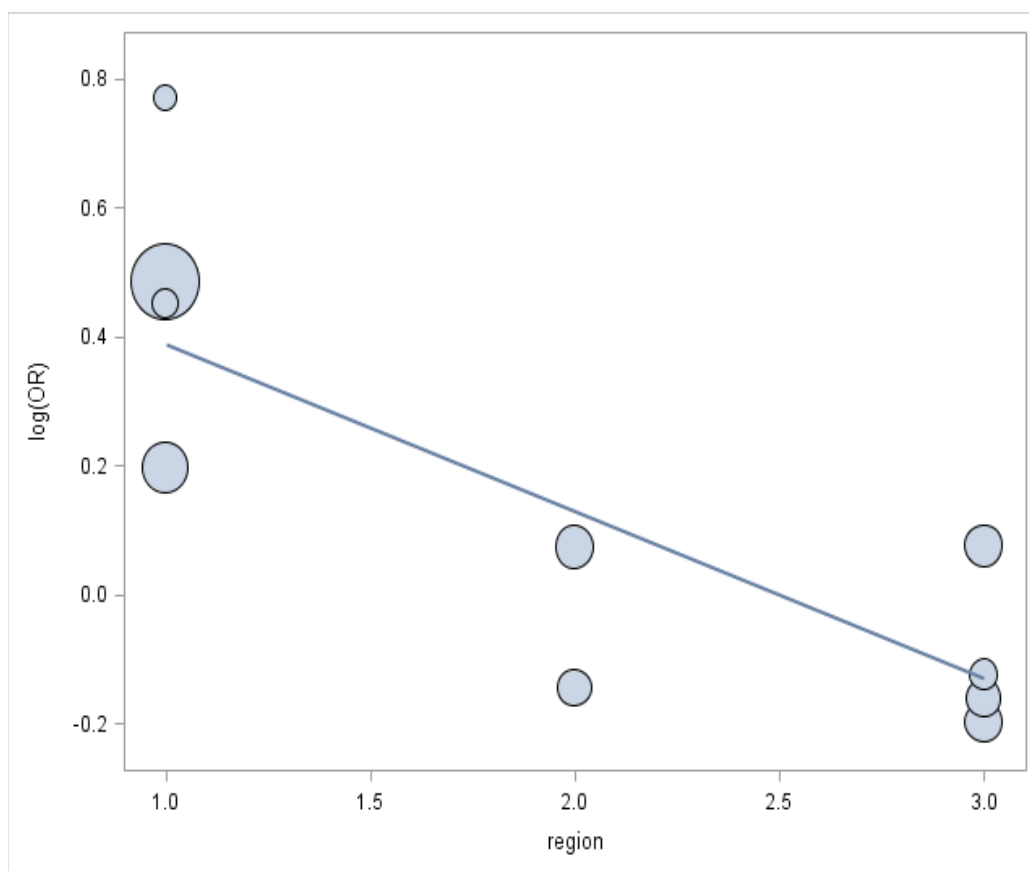
Figure 30. Plot of observed effect size for the risk of prostate cancer data against Genotype AC frequency.

The recessive model (CC vs. AC+AA). Table 26 shows no signs of heterogeneity of effect size.

Table 26

Test for Homogeneity of Effects, Recessive Model (CC vs. AC+AA)

Q	df	ProbQ	I^2
15.19	9	0.09	40.76

Note. The homogeneity results of MTHFR Gene, A1298C Polymorphism, and recessive model (CC vs. AC+AA)

The test of association between recessive model and prostate cancer risk for both fixed and random models shows no significant association (Table 27).

Table 27

Summary Effect Size, Recessive Model (CC vs. AC+AA)

Model	OR	Lower 95% limit	Upper 95% limit	*p*-value	*Adjusted p-value*
Fixed	1.07	0.90	1.27	0.42	0.72
Random	1.07	0.83	1.39	0.59	1.00

Note. The summary effect size of MTHFR Gene, A1298C Polymorphism, and recessive model (CC vs. AC+AA)

Figure 31 describes the missing studies on the right hand side of the funnel.
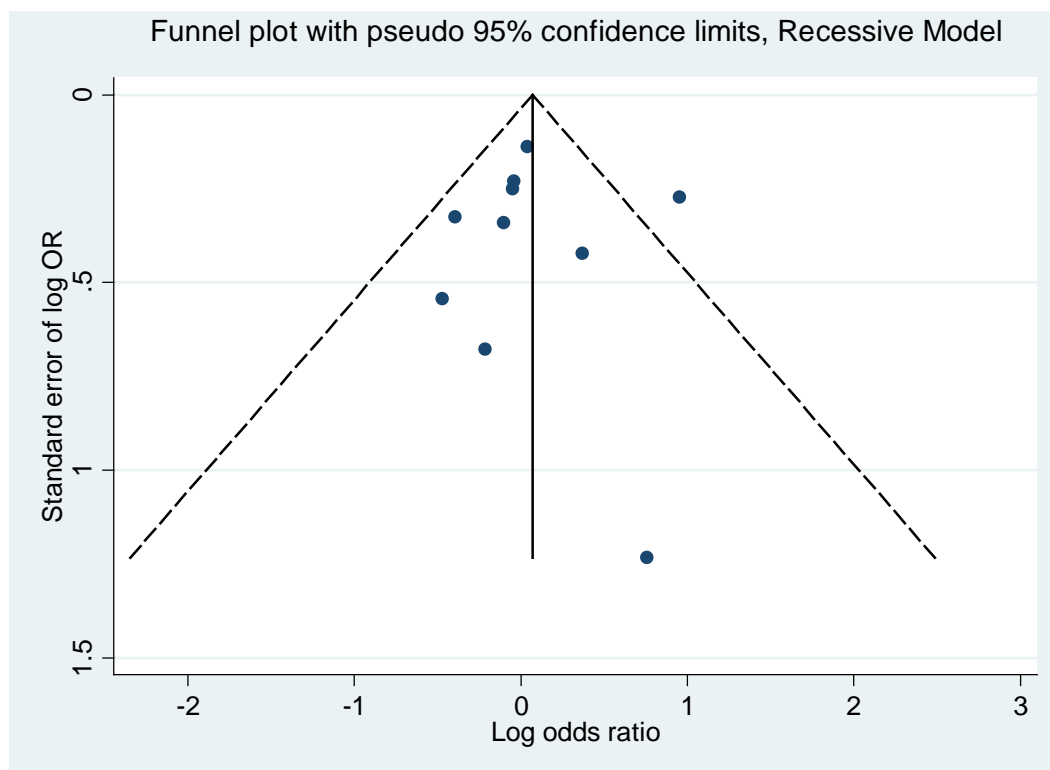
Figure 31. Funnel plot for risk of prostate cancer data, recessive model (CC vs. AC+AA).

Figure 32 shows an overview of odds of studies and the overall effect size for both fixed and random effects with no significant association between the recessive model and prostate cancer risk.
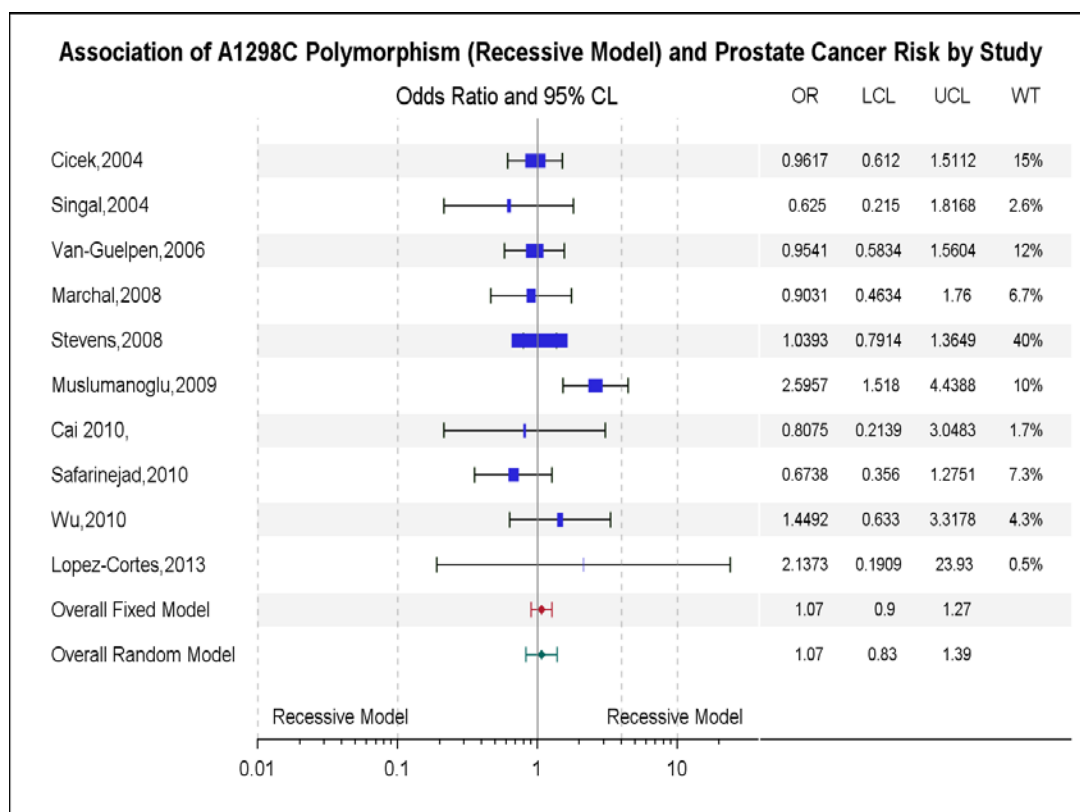
Figure 32. Forest plot of the association under a recessive model.

Table 28 shows no relationship between regions and ethnic groups to the variation in effect size.

Table 28

Result of Weighted Multiple Regression Analysis of Effect Size on Region of Study and

Ethnicity Groups, Recessive Model (CC vs. AC+AA)

		Parameter Estimate			
Variable	*DF*	Parameter Estimate	Standard Error	*T* Value	Pr>|t|
Intercept	1	-0.22	0.39	-0.57	0.59
Region	1	0.16	0.17	0.95	0.37
Ethnicity	1	-0.01	0.21	-0.04	0.97

Note. The summary of MTHFR gene, A1298C polymorphism, recessive model (CC vs. AC+AA)

The dominant model (AC+CC) vs. AA. Table 29 shows a highly significant

heterogeneity between studies (58.21%), a large degree of heterogeneity of effect size.

Table 29

Test for Homogeneity of Effects, Dominant Model (AC+CC) vs. AA

Q	*df*	ProbQ	I^2
23.93	10	0.008	58.21

Note. The homogeneity results of MTHFR Gene, A1298C Polymorphism, dominant model (AC+CC) vs. AA

Table 30 shows a highly significant association between dominant model and

prostate cancer risk at the fixed effect model.

Table 30

Summary Effect Size, Dominant Model (AC+CC) vs. AA

Model	OR	Lower 95% limit	Upper 95% limit	*p*-value	*Adjusted p-value*
Fixed	1.24	1.12	1.36	<.0001	0.0005
Random	1.15	0.96	1.36	0.13	0.65

Note. The summary effect size of MTHFR Gene, A1298C Polymorphism, dominant model (AC+CC) vs. AA

Figure 33 shows missing studies at the bottom left hand side of the graph.
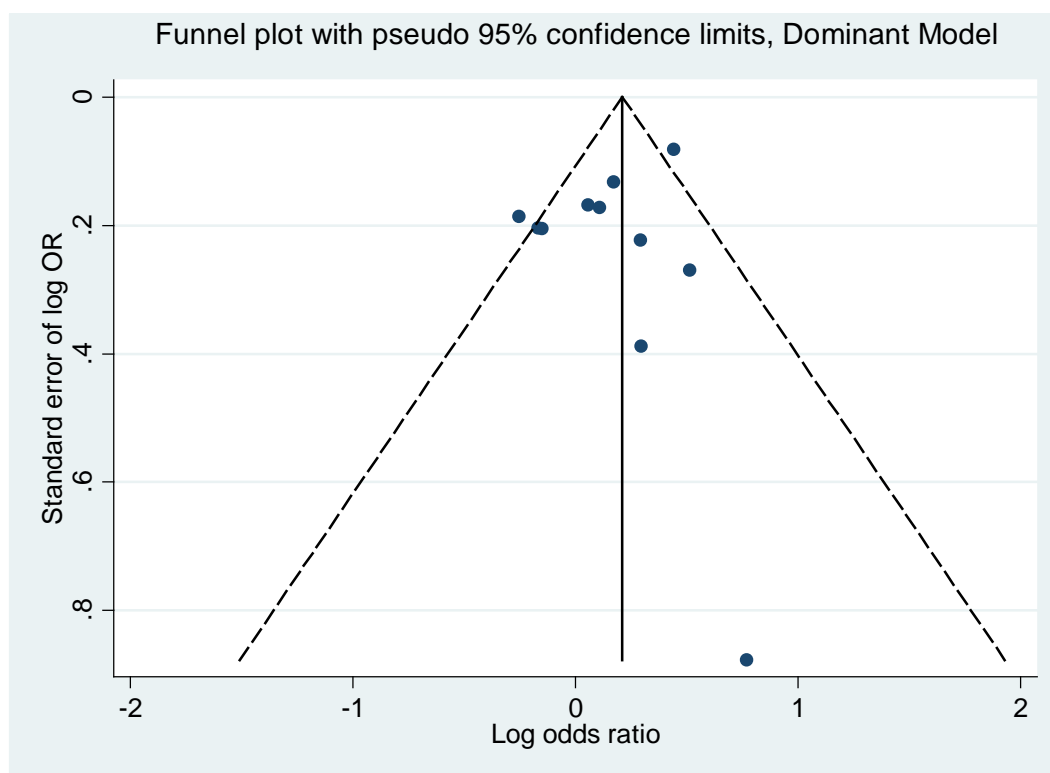
Figure 33. Funnel plot for risk of prostate cancer data, dominant model (AC+CC) vs. AA.

Figure 34 shows no significant association between the dominant model and

prostate cancer risk.
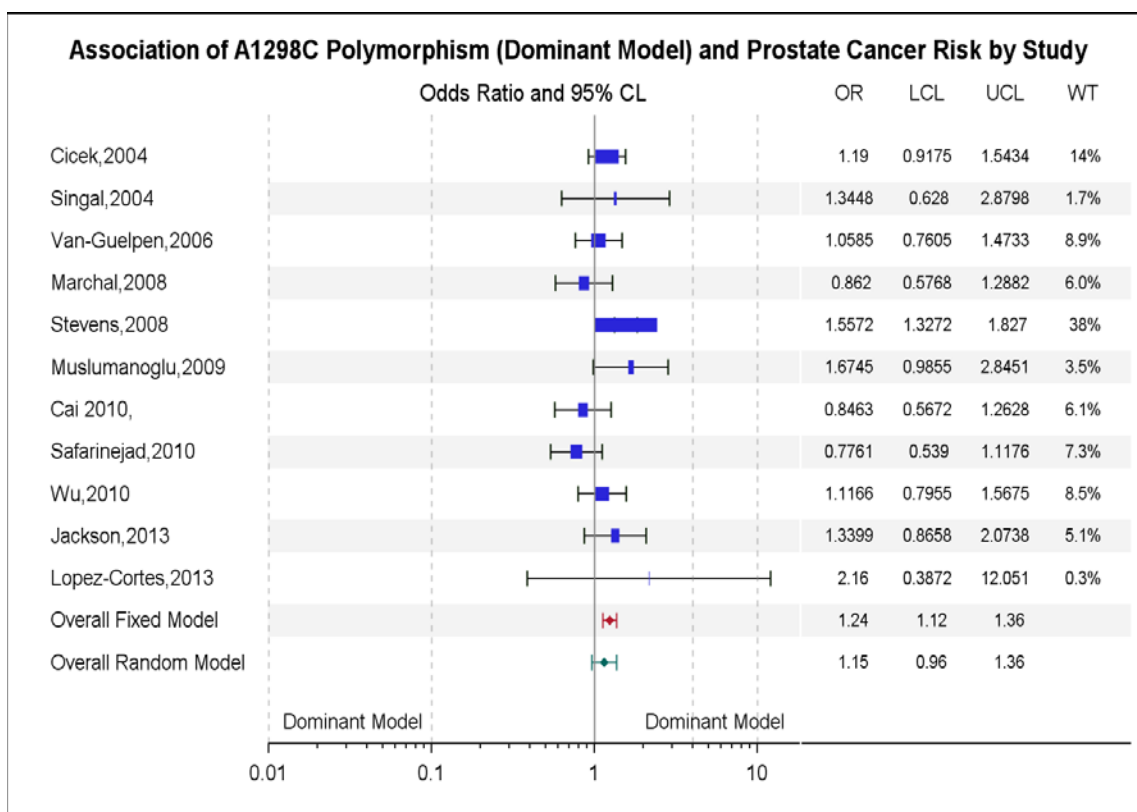
Figure 34. Forest plot of the association under dominant model.

Table 31 shows that there was not any relationship between the variance of the

effect size and by the regions and ethnic groups.

Table 31

Result of Weighted Multiple Regression Analysis of Effect Size on Region of Study and

Ethnicity Groups, Dominant Model (AC+CC) vs. AA

Variable	DF	Parameter Estimate		t Value	Pr>|t|
		Parameter Estimate	Standard Error		
Intercept	1	0.42	0.21	2.03	0.08
Region	1	-0.16	0.08	-1.88	0.09
Ethnicity	1	0.01	0.06	0.23	0.82

Note. The summary effect size of MTHFR Gene, A1298C Polymorphism, dominant model (AC+CC) vs. AA

MTR (A2756G)

In this study, the association of the MTR gene, including A2756G (rs1805087) polymorphism with prostate cancer risk, was analyzed. The number of studies was not large enough to make a more accurate result, but the results provide information for future research.

The G Allele versus A Allele. Table 32 shows no significant results of homogeneity on the effect size of studies.

Table 32

Test for Homogeneity of Effects, Allele G vs. A

Q	df	ProbQ	I^2
2.49	2	0.29	19.68

Note. The homogeneity results of MTR Gene, A2756G polymorphism, Allele G vs. A

The results of fixed and random model analysis (Table 33) show no significant effect sizes.

Table 33

Summary Effect Size, Allele G vs. A

Model	OR	Lower 95% limit	Upper 95% limit	*p*-value	*Adjusted p-value*
Fixed	1.17	0.89	1.54	0.27	1.00
Random	1.19	0.86	1.65	0.29	1.00

Note. The summary effect size of MTR Gene, A2756G Polymorphism, Allele G vs. A

Figure 35 shows missing studies at the right side of the graph.
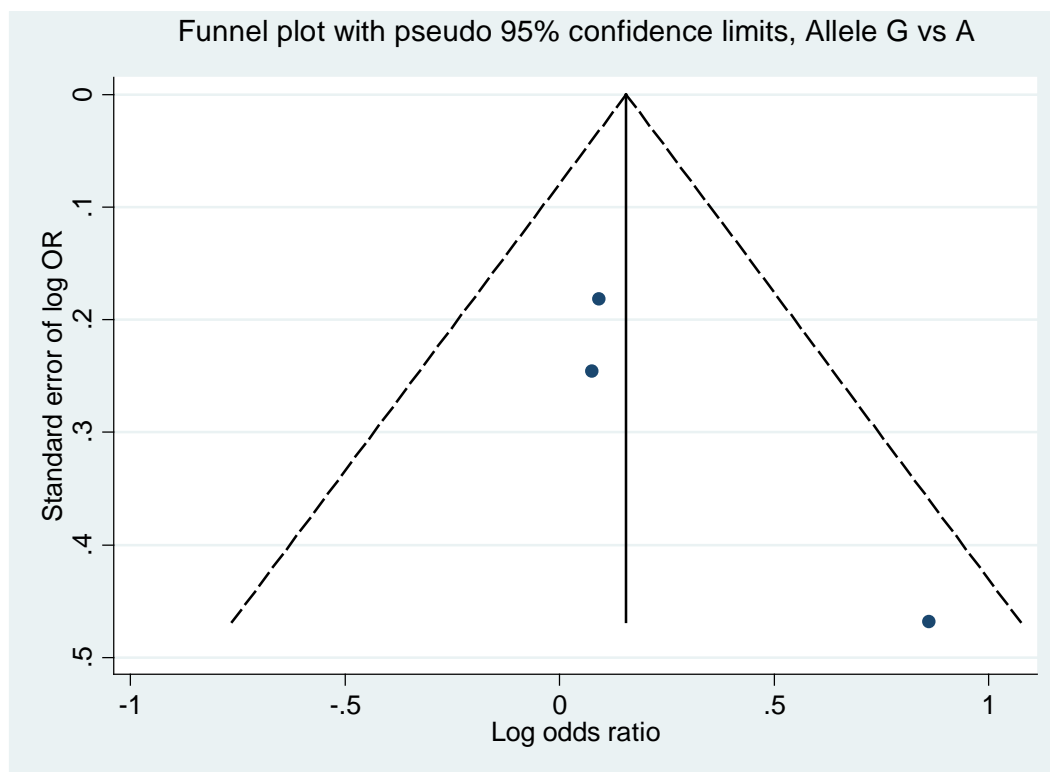
Figure 35. Funnel plot for risk of prostate cancer data, Allele G vs. A.

Figure 36 shows the individual studies, ORs, and CIs, plus the overall effect sizes and their boundaries.
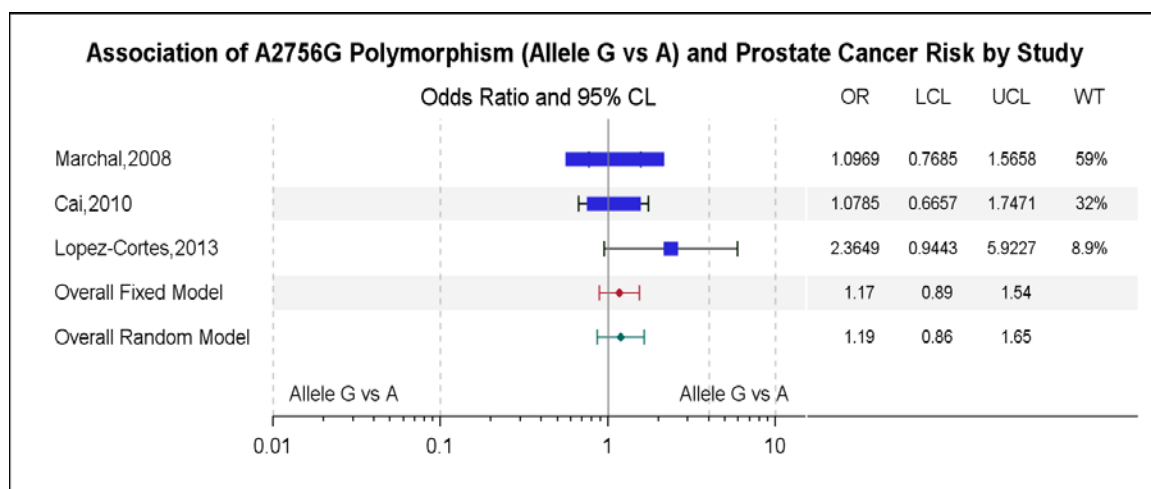
Figure 36. Forest plot of the association under Allele G vs. A model.

The Genotype GG vs. AA. The homogeneity test does not come up with a result

because the degree of freedom was more than the *Q* test result (Table 34).

Table 34

Test for Homogeneity of Effects

Q	*df*	ProbQ	I^2
1.23	2	0.54	Q<df

Note. The homogeneity results of MTR Gene, A2756G Polymorphism, Genotype GG vs. AA

Table 35 shows no significant association between Genotype GG and prostate

cancer risk.

Table 35

Summary Effect Size, Genotype GG vs. AA

Model	OR	Lower 95% limit	Upper 95% limit	*p*-value	*Adjusted p-value*
Fixed	1.26	0.61	2.62	0.53	1.00
Random	1.26	0.61	2.62	0.53	1.00

Note. The summary effect size of MTR Gene, A2756G Polymorphism, Genotype GG vs. AA

Figure 37 shows missing studies at the left side of the graph.
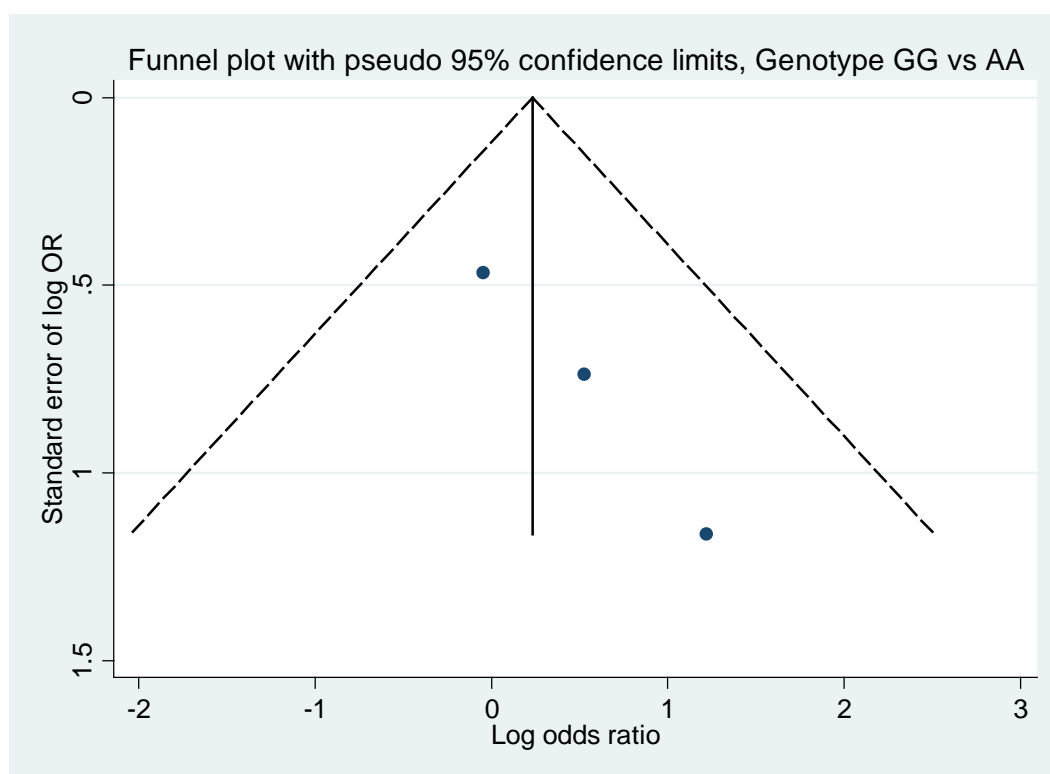
Figure 37. Funnel plot for risk of prostate cancer data, Genotype GG vs. AA.

Figure 38 shows the effect sizes and their boundaries with the results of no significant association between Genotype GG and prostate cancer risk.
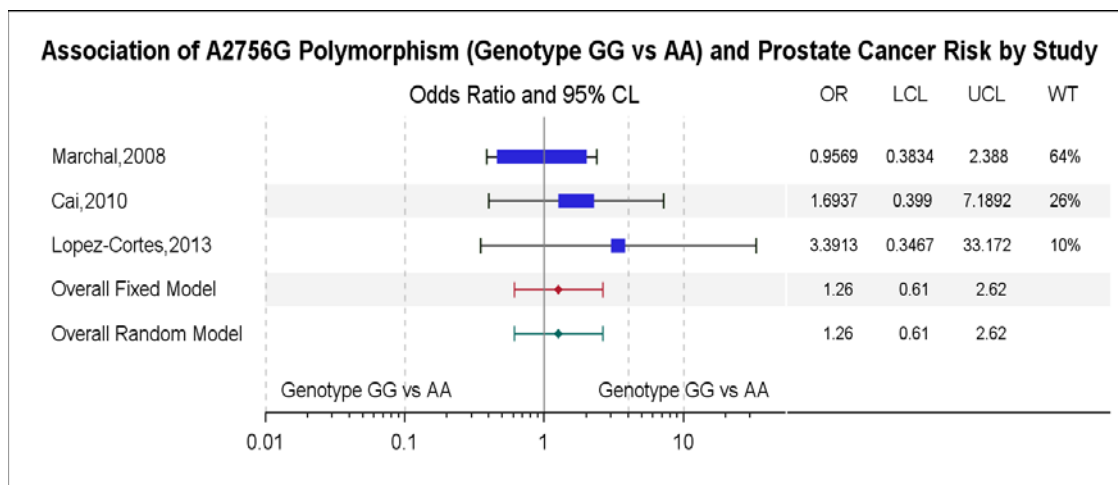
Figure 38. Forest plot of the association under Genotype GG vs. AA.

The Genotype AG vs. AA. Table 36 shows no results because of a lesser value of *Q*-test than *df*.

Table 36

Test for Homogeneity of Effects, Genotype AG vs. AA

Q	df	ProbQ	I^2
1.43	2	0.49	Q<df

Note. The homogeneity results of MTR gene, A2756G polymorphism, Genotype AG vs. AA

There was no significant association of effect sizes and prostate cancer risk in both fixed and random models (Table 37).

Table 37

Summary Effect Size, Genotype AG vs. AA

Model	OR	Lower 95% limit	Upper 95% limit	*p*-value	*Adjusted p-value*
Fixed	1.13	0.81	1.58	0.48	1.00
Random	1.13	0.81	1.58	0.48	1.00

Note. The summary effect size of MTR Gene, A2756G Polymorphism, Gynotype AG vs. AA

Figure 39 shows missing studies at the left side of the graph.
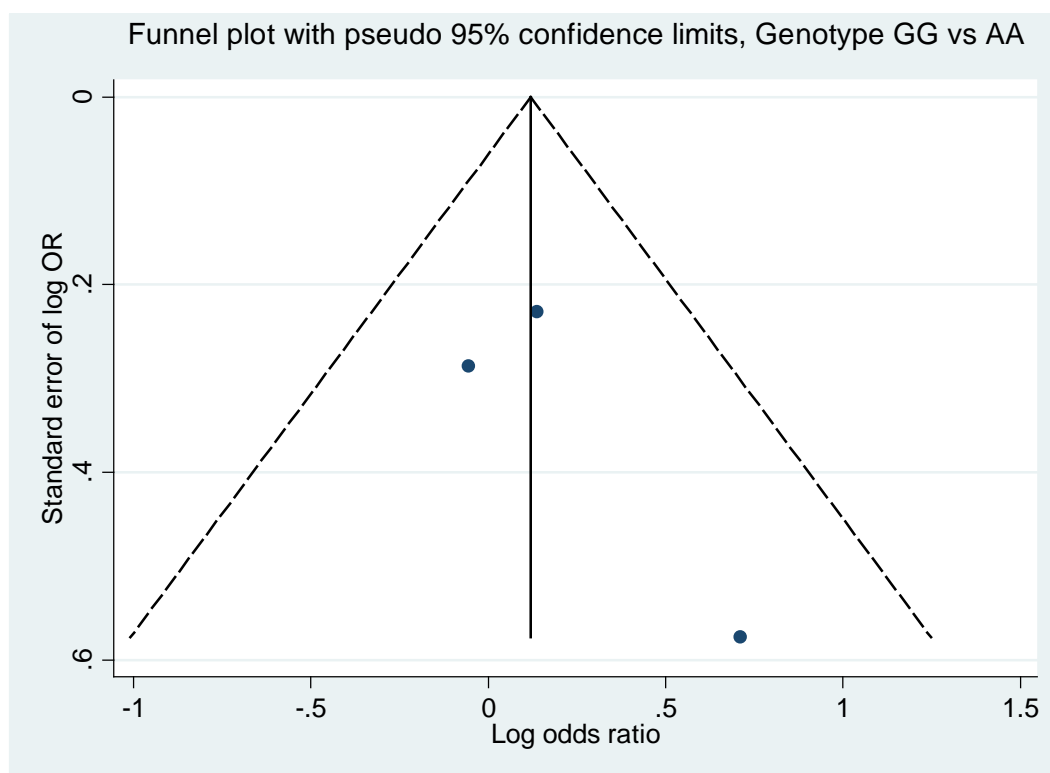
Figure 39. Funnel plot for risk of prostate cancer data, Genotype AG vs. AA.

Figure 40 shows the ORs and their boundaries, including both fixed and random effect sizes.
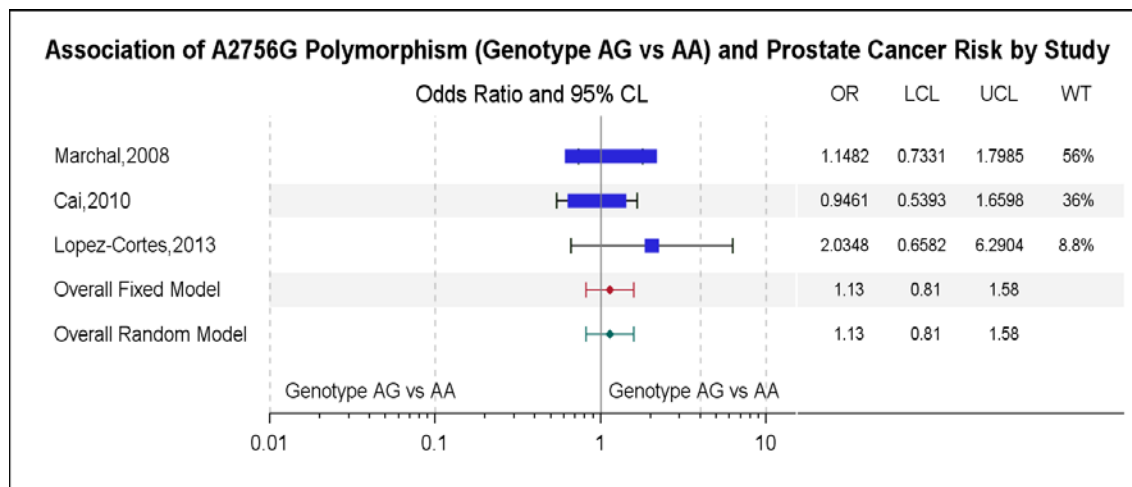
Figure 40. Forest plot of the association under Genotype GG vs. AA.

The recessive model (GG vs. AA+AG). Table 38 does not show the homogeneity result of effect size because the value of the *Q*-test was less than *df*.

Table 38

Test for Homogeneity of Effects, Recessive Model (GG vs. AA+AG)

Q	df	ProbQ	I^2
1.29	2	0.52	*Q<df*

Note. The homogeneity results of MTR Gene, A2756G Polymorphism, recessive model (GG vs. AA+AG)

Table 39 shows the same value that there was no significant association.

Table 39

Summary Effect Size, Recessive Model (GG vs. AA+AG)

Model	OR	Lower 95% limit	Upper 95% limit	*p*-value	*Adjusted p-value*
Fixed	1.22	0.59	2.52	0.59	1.00
Random	1.22	0.59	2.52	0.59	1.00

Note. The summary effect size of MTR Gene, A2756G Polymorphism, recessive model (GG vs. AA+AG)

Figure 41 shows missing studies at the left side of the graph.
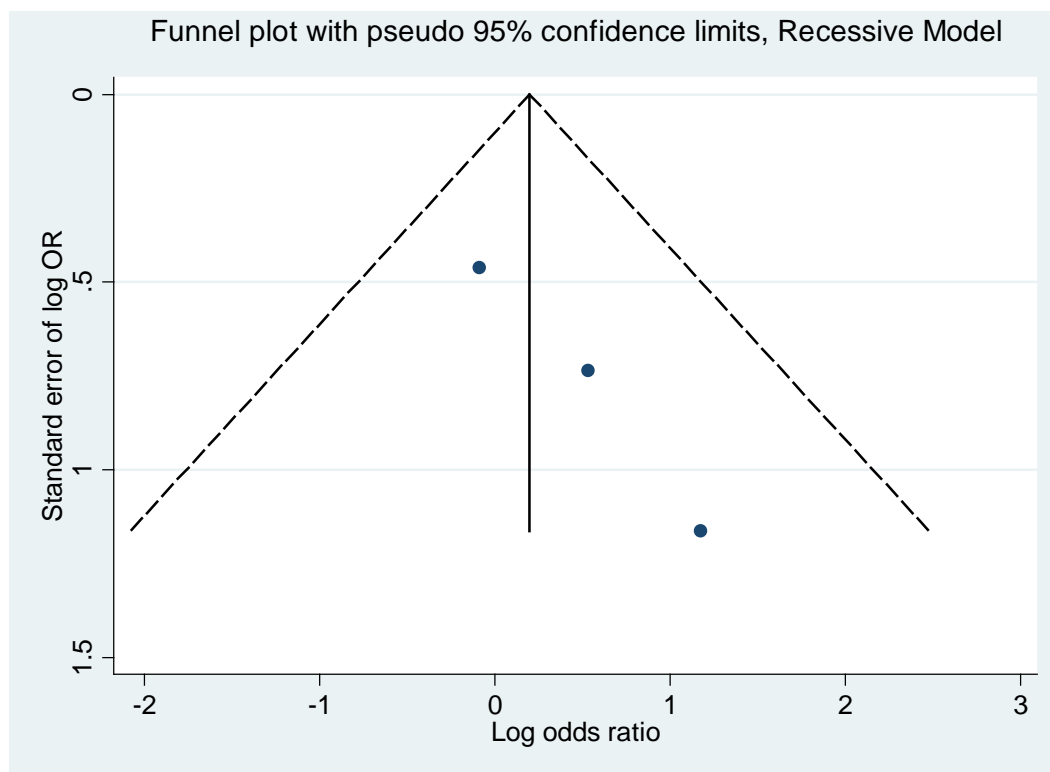
Figure 41. Funnel plot for risk of prostate cancer data, recessive model (GG vs. AA+AG).

The forest plot describes the OR and their boundaries (Figure 42).

Figure 42. Forest plot of the association under recessive model (GG vs. AA+AG).

The dominant model (AG +GG vs. AA). Table 40 shows no significant result

because the value of the *Q*-test was less than *df*. Table 41 shows no significant

association between dominant model and prostate cancer risk.

Table 40

Test for Homogeneity of Effects, Dominant Model (AG +GG vs. AA)

Q	*d*f	ProbQ	I^2
2.32	3	0.51	*Q<df*

Note. The homogeneity results of MTR Gene, A2756G Polymorphism, dominant model
(AG +GG vs. AA)

Table 41

Summary Effect Size, Dominant Model (AG +GG vs. AA)

Model	OR	Lower 95% limit	Upper 95% limit	*p*-value	*Adjusted p-value*
Fixed	1.08	0.85	1.38	0.52	1.00
Random	1.08	0.85	1.38	0.52	1.00

Note. The summary effect size of MTR Gene, A2756G Polymorphism, dominant model (AG +GG vs. AA)

Figure 43 shows missing studies on the left side of the graph with a low log of OR on the top right of the graph.
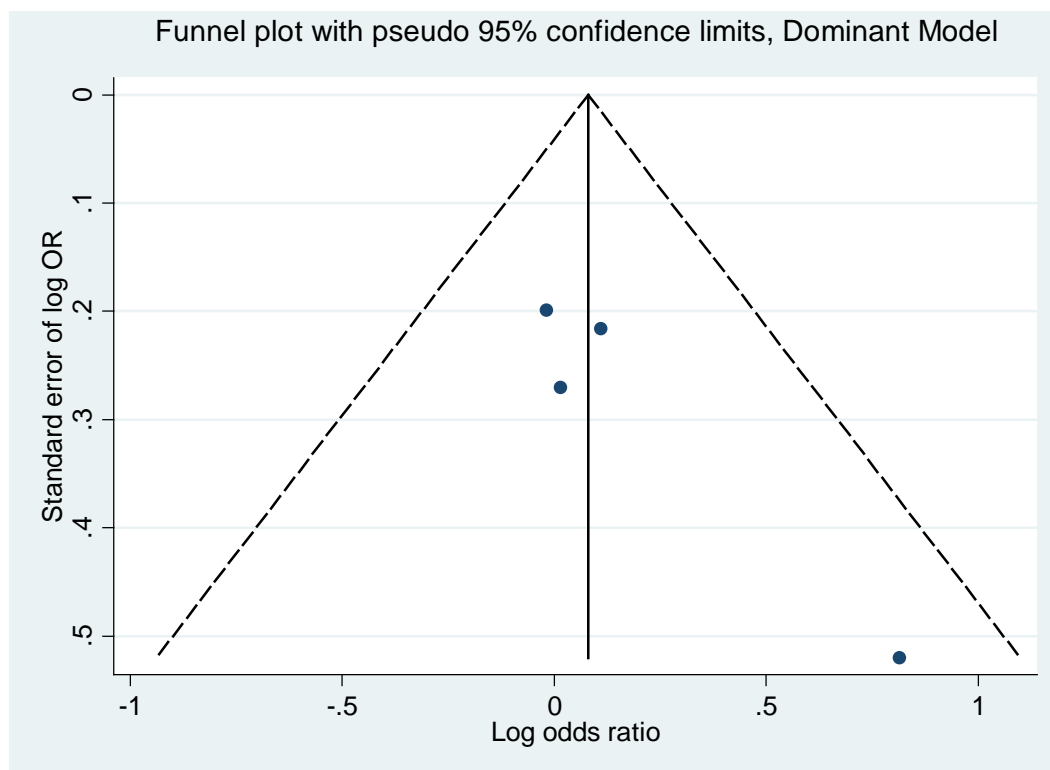
Figure 43. Funnel plot for risk of prostate cancer data, dominant model (AG +GG vs. AA).

The forest plot shows the odds and their boundaries, including both effect sizes (Figure 44).
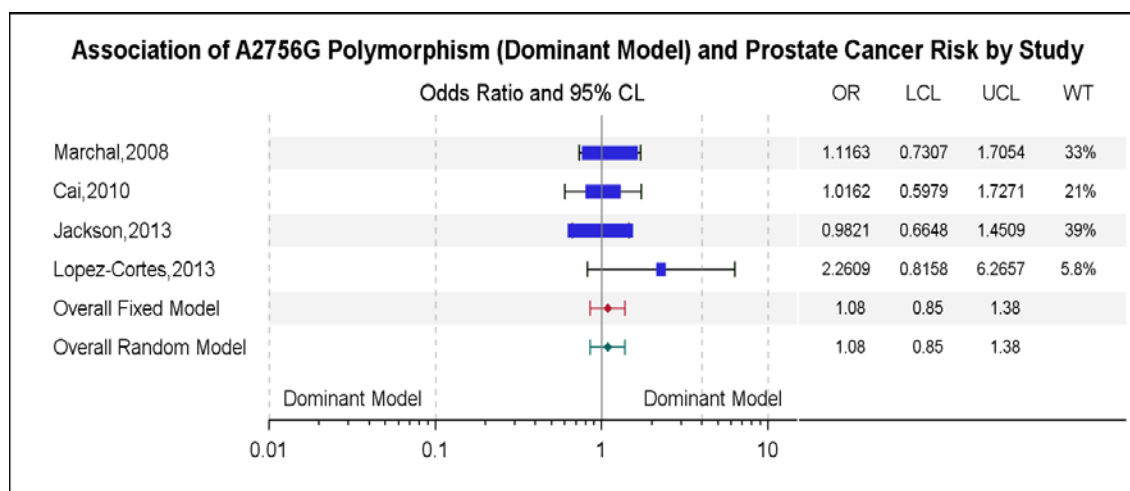
Figure 44. Forest plot of the association under dominant model (AG +GG vs. AA).

MTRR (A66G)

This study included analysis of the MTRR Gene, A66G Polymorphism (rs1801394). Although the number of studies was not enough for an accurate result, the results of this study can be used for the future studies.

The G Allele versus A Allele. Table 42 does not show a significant effect; the degree of homogeneity of effect size was small (I^2 <50%).

Table 42

Test for Homogeneity of Effects

Q	df	ProbQ	I^2
3.04	2	0.22	34.21

Note. The homogeneity results of MTRR Gene, A66G Polymorphism, Allele G vs. A

Table 43 shows no significant result of effect sizes at 0.05 levels of test.

Table 43

Summary Effect Size, Allele G vs. A

Model	OR	Lower 95% limit	Upper 95% limit	*p*-value	*Adjusted p-value*
Fixed	0.95	0.80	1.14	0.60	1.00
Random	0.94	0.75	1.18	0.61	1.00

Note. The summary effect size of MTRR Gene, A66G Polymorphism, Allele

G vs. A

Figure 45 shows missing studies at the left side of the graph.
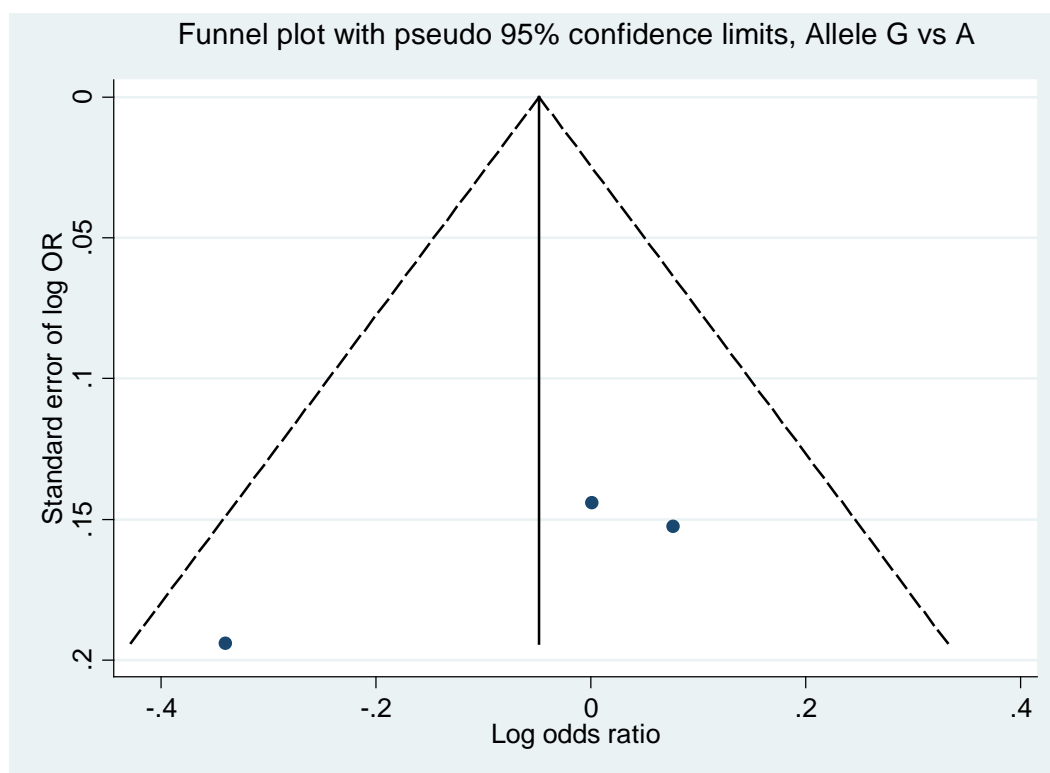
Figure 45. Funnel plot for risk of prostate cancer data, Allele G vs. A.

Figure 46 shows the ORs and the boundaries of the weight of studies including the overall size effect as a square shape.
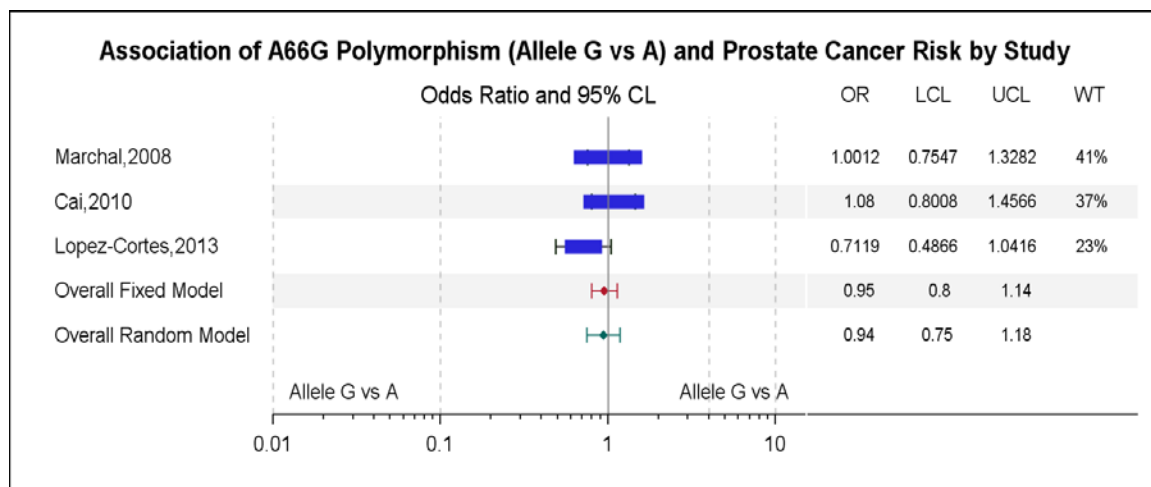
Figure 46. Forest plot of the association under Allele G vs. A model.

The Genotype GG vs. AA. Table 44 shows a significant result with the moderate degree of homogeneity of effect size.

Table 44

Test for Homogeneity of Effects, Genotype GG vs. AA

Q	df	ProbQ	I^2
6.10	2	0.05	67.21

Note. The homogeneity results of MTRR Gene, A66G Polymorphism, Genotype GG vs. AA

Table 45 does not show any significant association.

Table 45

Summary Effect Size, Genotype GG vs. AA

Model	OR	Lower 95% limit	Upper 95% limit	*p*-value	*Adjusted p-value*
Fixed	0.87	0.55	1.38	0.56	1.00
Random	0.70	0.29	1.72	0.44	1.00

Note. The summary effect size of MTRR Gene, A66G Polymorphism, Genotype GG vs. AA

Figure 47 shows missing studies at the left side of the graph.
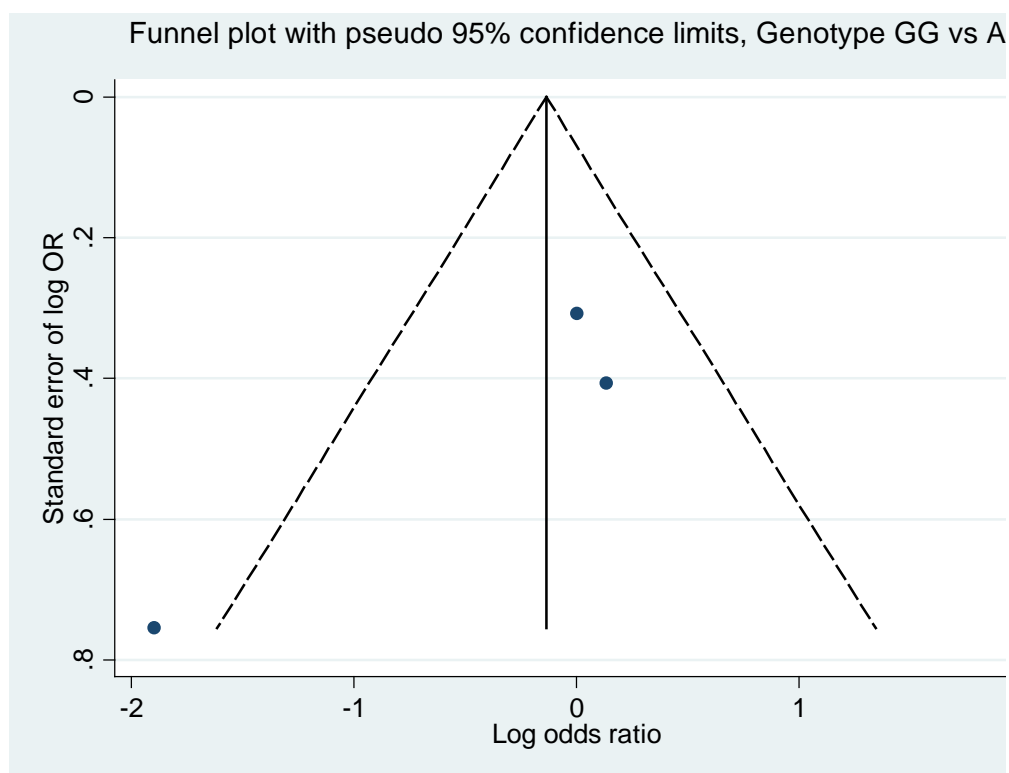
Figure 47. Funnel plot for risk of prostate cancer data, Genotype GG vs. AA.

Figure 48 shows the individual OR and their boundaries, including fixed and random effect sizes.
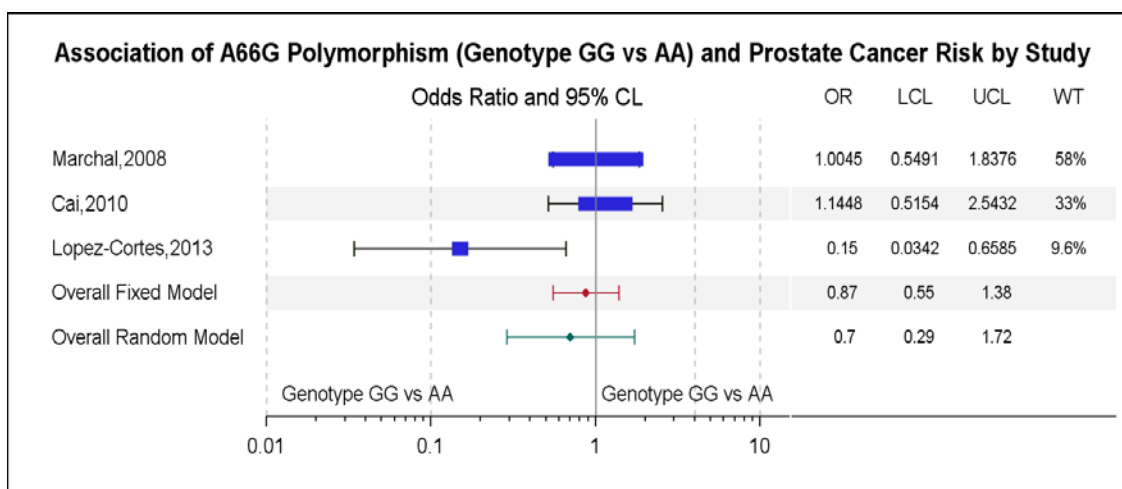
Figure 48. Forest plot of the association under Genotype GG vs. AA.

The Genotype AG vs. AA. Table 46 shows a significant heterogeneity of effect

size with a large degree of heterogeneity (*p*<0.05).

Table 46

Test for Homogeneity of Effects, Genotype AG vs. AA

Q	df	ProbQ	I^2
13.63	2	0.0011	85.33

Note. The homogeneity results of MTRR Gene, A66G Polymorphism, Genotype AG vs.
AA

Table 47 does not show a significant association of effect sizes.

Table 47

Summary Effect Size, Genotype AG vs. AA

Model	OR	Lower 95% limit	Upper 95% limit	*p*-value	*Adjusted p-value*
Fixed	0.97	0.72	1.31	0.84	1.00
Random	0.63	0.25	1.58	0.33	1.00

Note. The summary effect size of MTRR Gene, A66G Polymorphism, Genotype AG vs. AA

Figure 49 shows missing studies at the left side of the graph.
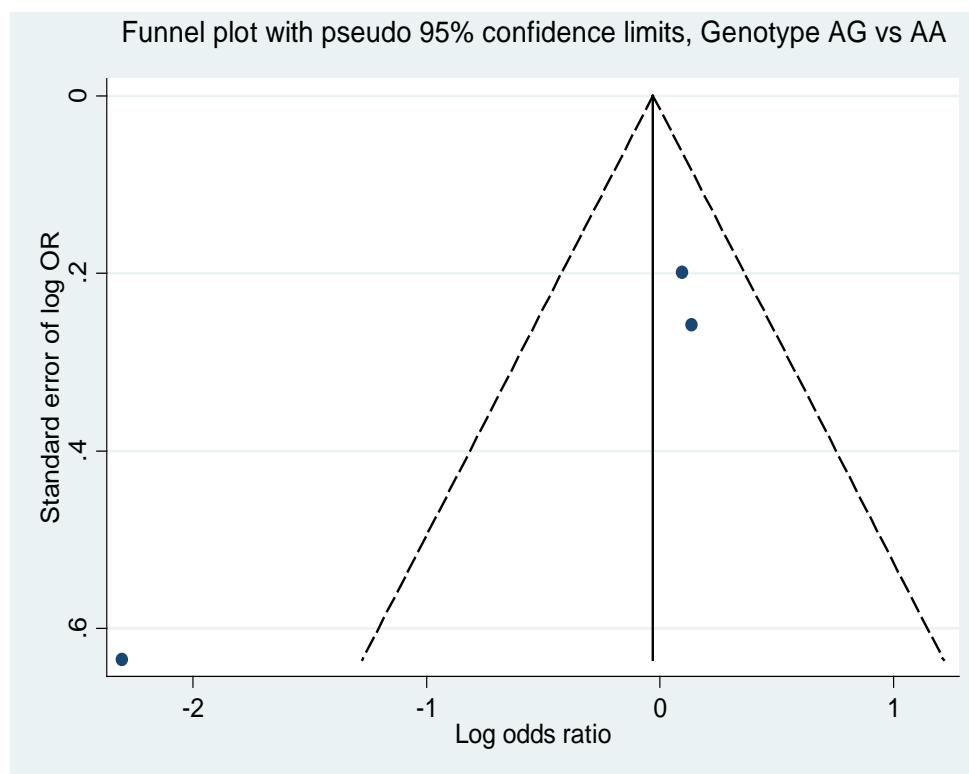
Figure 49. Funnel plot for risk of prostate cancer data, Genotype AG vs. AA.

Figure 50 shows the OR and their boundaries of effect sizes, including the overall

effect sizes for both fixed and random models.
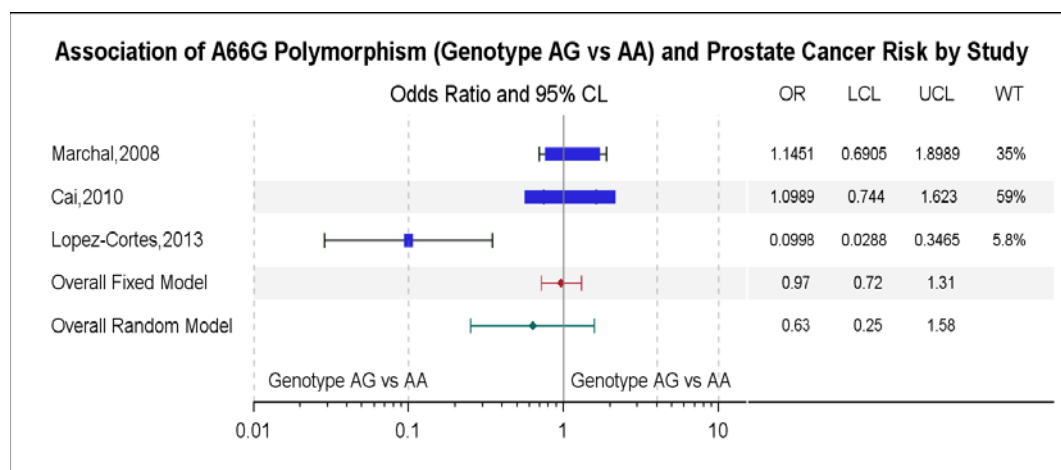
Figure 50. Forest plot of the association under Genotype AG vs. AA.

The recessive model (GG vs. AG+AA). Table 48 does not show a significant

result because the *Q*-test value was smaller than *df*.

Table 48

Test for Homogeneity of Effects, Recessive Model (GG vs. AG+AA)

Q	df	ProbQ	I^2
0.33	2	0.85	Q<df

Note. The homogeneity results of MTRR Gene, A66G Polymorphism, recessive model
(GG vs. AG+AA)

Table 49 shows no sign of significant of either effect size.

Table 49

Summary Effect Size, Recessive Model (GG vs. AG+AA)

Model	OR	Lower 95% limit	Upper 95% limit	*p*-value	*Adjusted p-value*
Fixed	1.00	0.68	1.44	0.98	1.00
Random	1.00	0.68	1.44	0.98	1.00

Note. The summary effect size of MTRR Gene, A66G Polymorphism, recessive model (GG vs. AG+AA)

Figure 51 shows missing studies at the left side of the graph.
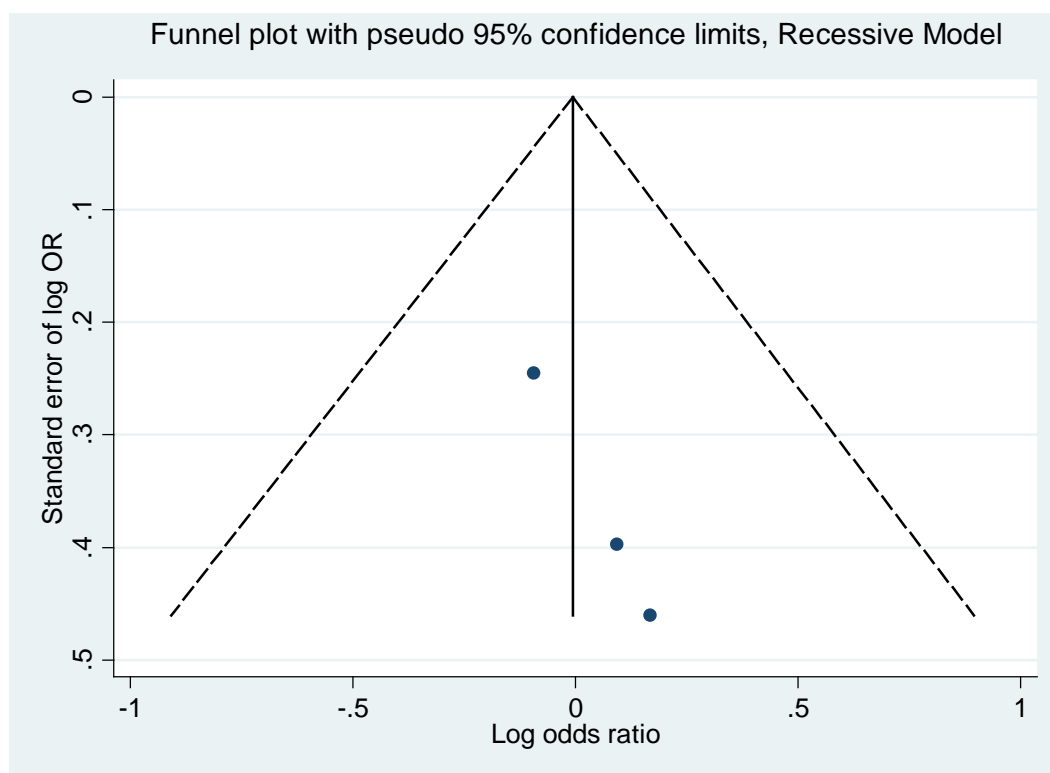
Figure 51. Funnel plot for risk of prostate cancer data, recessive model (GG vs. AG+AA).

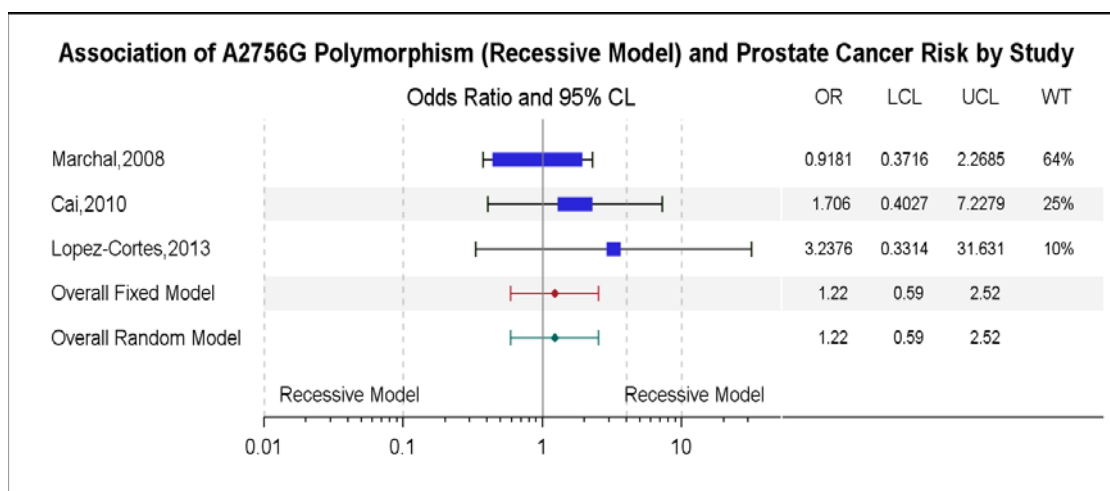Figure 52 shows no significant association on recessive model and prostate cancer risk.
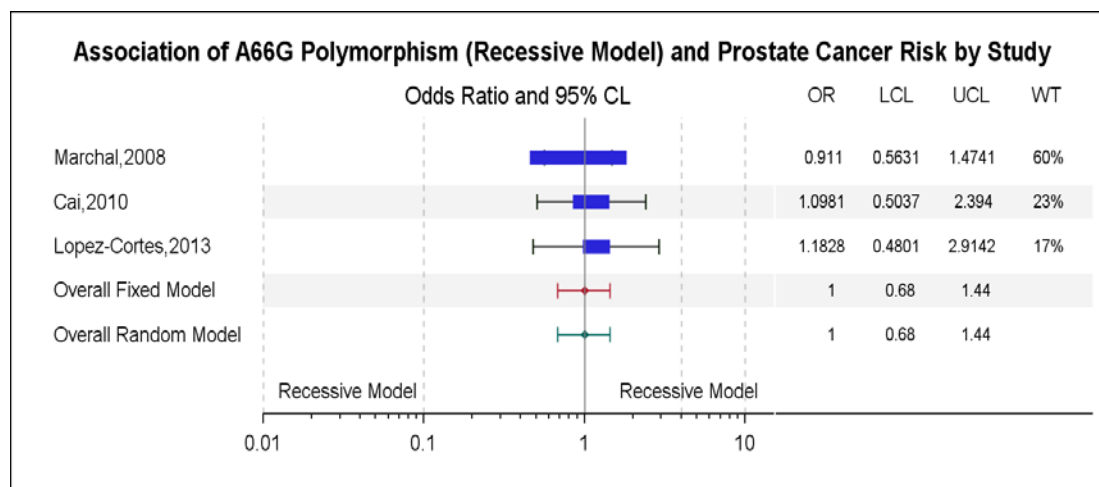
Figure 52. Forest plot of the association under recessive model (GG vs. AG+AA).

The dominant model (AG+GG) vs. AA. Table 50 shows a highly significant heterogeneity with a large degree of heterogeneity of effect size (I^2=77.53%).

Table 50

Test for Homogeneity of Effects, Dominant Model (AG+GG) vs. AA

Q	df	ProbQ	I^2
13.36	3	0.0039	77.54

Note. The homogeneity results of MTRR Gene, A66G Polymorphism, dominant model (AG+GG) vs. AA

Table 51 does not show a significant association of effect sizes on both fixed and random effect models.

Table 51

Summary Effect Size, Dominant Model (AG+GG) vs. AA

Model	OR	Lower 95% limit	Upper 95% limit	*p*-value	*Adjusted p-value*
Fixed	1.01	0.80	1.28	0.91	1.00
Random	0.82	0.48	1.41	0.48	1.00

Note. The summary effect size of MTRR Gene, A66G Polymorphism, dominant model (AG+GG) vs. AA

Figure 53 shows missing studies at the left side of the graph.
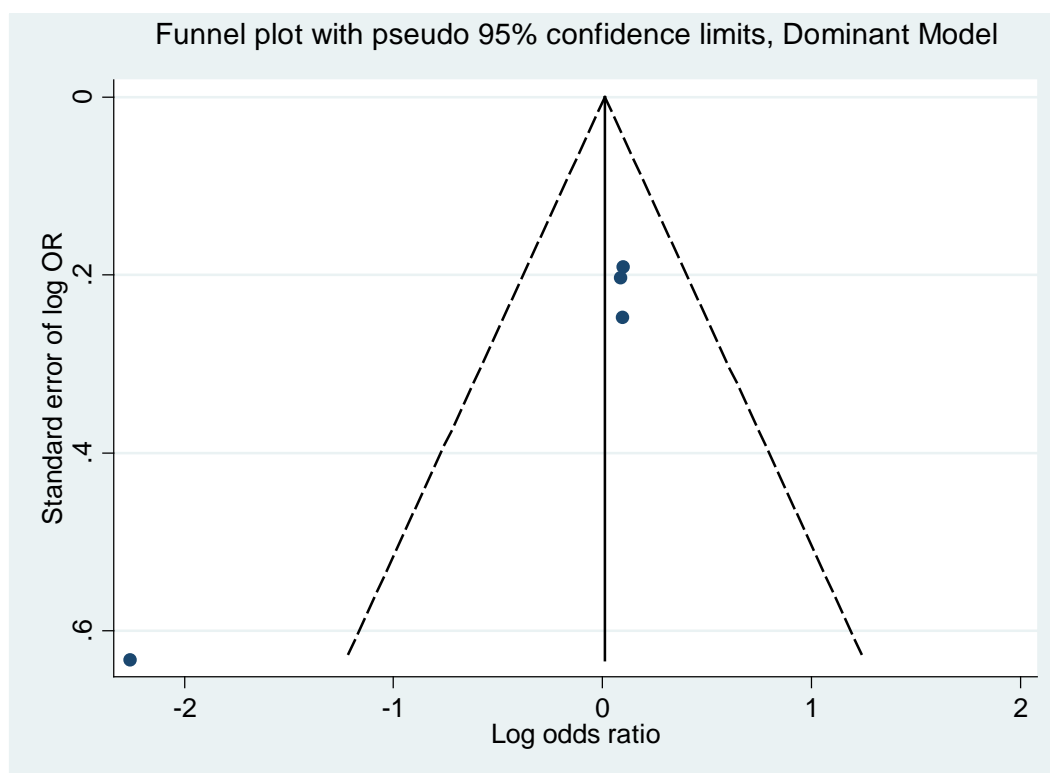
Figure 53. Funnel plot for risk of prostate cancer data, dominant model (AG+GG) vs. AA.

Figure 54 shows no significant association between dominant model and prostate cancer risk.
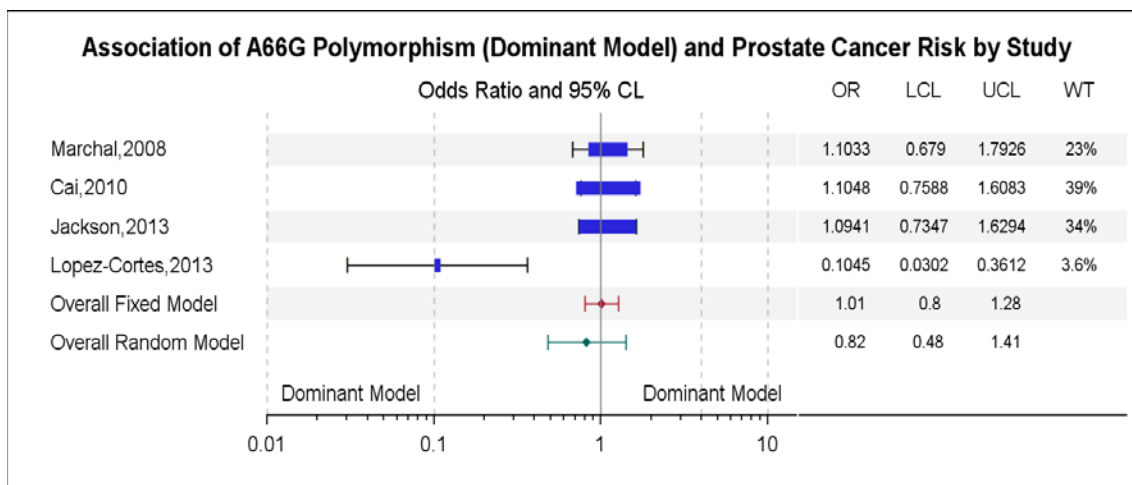
Figure 54. Forest plot of the association under dominant model (AG+GG) vs. AA.

Table 52 shows that there was not an association of variation of effect size related to the regions and ethnic groups.

Table 52

Result of Weighted Multiple Regression Analysis of Effect Size on Region of Study and

Ethnicity Groups, Dominant Model (AG+GG) vs. AA

Variable	*DF*	Parameter Estimate		*t* Value	*Pr>|t|*
		Parameter Estimate	Standard Error		
Intercept	1	-0.33	3.04	-0.11	0.93
Region	1	0.28	0.91	0.31	0.81
Ethnicity	1	-0.14	0.63	-0.22	0.86

Note. The summary effect size of MTRR Gene, A66G Polymorphism, dominant model (AG+GG) vs. AA

Summary

In this chapter, I presented the results of the statistical analysis based on the

research questions and the association of prostate cancer risk with the four genes of

MTHFR with two polymorphisms (C667T and A1298C), MTR (A2756G

polymorphism), and MTRR (A66G polymorphism). In the process of testing, I first

estimated the variation of effect size by a homogeneity test and then estimated the overall

effect size of both fixed and random models. In the case of heterogeneity of effect size, a

researcher usually looks at the overall randomness of the model. In this study, I placed

both fixed and random model results into a table.

I found that there was a highly significant association between variant allele

(Allele T) in the MTHFR Gene, C667T Polymorphism, and decreased prostate cancer

risk ($P<0.05$) in both fixed and random models. The homogeneity test for these associations was significant ($p<0.05$) with a moderate degree of heterogeneity. There was a highly significant association of Genotype TT and recessive model (TT vs. [CT+CC)]) with a decrease in prostate cancer risk in both fixed and random models. The sensitivity analysis revealed significance for Allele T, Genotype TT, and recessive model (TT vs. [CT+CC]) with a decrease in prostate cancer risk.

In A1298C Polymorphism, there was a significant association between Genotype AC and an increased risk of prostate cancer ($p<0.05$), and the same significant increase of risk in the dominant model in the fixed effect model only. The sensitivity analysis revealed a significant association between Genotype AC and an increased risk of prostate cancer in the fixed effect model only. The results showed a significant association between regions and risk of prostate cancer on Genotype AC versus AA in A1298C polymorphism of the MTHFR gene. The region and prostate cancer association showed a decrease of log ORs among Asian verses American (Figure 30) subjects. The number of samples used to test MTR and MTRR was small, and the related results were not significant, but a future researcher may examine this association. In Chapter 5, I present the discussion, conclusions, and recommendations for future study.

Chapter 5: Discussion, Conclusions, and Recommendations

Introduction

In Chapter 5, I discuss the study overview and results with conclusions and recommendations. This study involved 20 eligible articles of 2,800 reviewed studies from the years 2000 to 2014, based on inclusion and exclusion criteria. A total of 8,675 cases and 9,207 control participants were in the 20 involved studies with a common hypothesis on the association between one-carbon, metabolism gene polymorphisms (MTHFR, MTR, MTRR Gens) and prostate cancer risk.

The Objectives

The objective of this study was to examine the association between one-carbon metabolism gene polymorphisms and prostate cancer risk among men in the different regions of the world and from different ethnic groups. The results of this study could help meet the following long-term goals related to the prevention of prostate cancer:

- To develop clinical methods in preventing prostate cancer risk at an early stage of the disease

- To improve health care availability and accessibility for low-income families and those who need these services

- To develop the health literacy and knowledge of the public on the benefits of prostate cancer prevention and prostate cancer screening

- To recommend establishing policies on surveillance systems, practices, and quality assurance on genetic screening to detect prostate cancer

- To develop a positive change of people's attitude and behavior regarding the early detection of prostate cancer disease, prevention, and course treatment strategies

The prevention of prostate cancer at an early stage helps improve the quality of life in communities and increases life expectancy by decreasing the cost of treatment. Prostate cancer is one of the most serious diseases among males in the world, which affects life expectancy and life quality. In 2009, prostate cancer was the second leading cause of death among males in the United States ("CDC - Prostate Cancer," n.d.). The most important aspect of treating prostate cancer is diagnosis at an early stage ("CDC - Prostate Cancer," n.d.). The gene-disease association studies were developed by clinicians to test the technologies and the methods of analysis to improve diagnosis. The technologies helped scientists to make accurate results of gene mapping due to the gene-disease association (Gudmundsson et al., 2009).

The association between one-carbon metabolism gene polymorphisms and cancers was studied previously (Suzuki et al., 2007, 2008; Theodoratou et al., 2012), but the individual studies left a gap in the literature that could be filled with a meta-analysis of relevant research. In this study, the association between one-carbon, metabolism gene polymorphisms and prostate cancer risk was examined to find the best course of treatment methods and to develop policies to prevent the disease among males who are at risk of the disease. The key findings are the decrease risk of prostate cancer with TT genotype, and recessive model in MTHFR gene (C667T polymorphism). A significant

association was found in an increased risk of prostate cancer with AC genotype and dominant model in A1298C polymorphism.

Interpretation of the Findings

The findings of this study are related to the association of three selected genes (MTHFR, MTR, and MTRR) and prostate cancer risk. In addition, the findings of this study show an association between prostate cancer risk and different regions in the world.

Gene-Prostate Cancer Association

A human genome study could provide knowledge on reducing the incidence rate of diseases and promote health care and health services that benefit people. The gene and prostate cancer association was examined in previous studies (López-Cortés et al., 2013). The chromosomes or gene abnormalities that cause genetic diseases may or may not be transferred from parents to the offspring as an inherited disease (Frank, 2007). The gene-gene and gene-environment association is a detection method that can be studied in future studies. The folate intake, dietary, and genetic linkage disequilibrium should also be examined for a gene-prostate cancer association (Shirai et al., 2002; Yeager et al., 2009).

In this study, I examined three genes with their polymorphisms to determine if there was an association with prostate cancer risk. The genes studied in this study are involved in folate pathway, which are in DNA synthesis and methylation modification (Donkena et al., 2010). I examined the three following genes: MTHFR gene located at short arm of Chromosome 1, p36.3, Exon 4 with two polymorphisms C667T and A1285C; MTR gene located on long (q) arm of Chromosome 1 at Position of 43, Exon 33 A2756G polymorphism; and MTRR gene located at short arm (p) of Chromosome 5,

at Position 15.31 with A66G polymorphism. The results show that the MTHFR gene has an association with prostate cancer risk.

Regions and Ethnic Groups

The regions and ethnic groups are important in this study analysis because there was a different incidence rate of prostate cancer among regions (Figure 4) and ethnic groups (Crawford, 2003). People have different ancestry, which affects their genotype; different genotypes and environments effect (gene-environment interaction) make a different phenotype for people. These combinations of genotype and environment effect may affect a person's lifestyle, as Grönberg (2003) mentioned in the incidence rate of prostate cancer among those men who moved to The United States from Japan. Grönberg indicated that each different group has different incidence rates of prostate cancer. Grönberg suggested a difference between ethnic groups who live in different geographical areas and prostate cancer incidence. When a Japanese group emigrated from Japan to the United States, their previous low incidence rate of prostate cancer changed to a higher incidence rate of prostate cancer in the new home (Grönberg, 2003). The results of the meta-analysis study result were in alignment with region-prostate cancer risk association.

In this meta-analysis study, I examined the association between prostate cancer risk at different regions and ethnic groups on selected polymorphisms. I found a significant association between regions and prostate cancer risk on Genotype AC versus AA in A1298C Polymorphism of the MTHFR Gene. The AC Genotype frequency is different for people who live in Asia and America. The people who live in Asia are at less

risk of prostate cancer than those people who live in America. The results of this study are in alginment with studies by Li et al. (2013) and Ntais et al. (2003), who indicated less risk of prostate cancer for those who live in Asia than America. The gene frequency differences among different racial/ethical groups who live in different regions may be related to their diet and environmental factors. Future researchers should study gene-gene and gene-environmental interaction rch.

There are ethical issues related to prostate cancer risk and prevention. I believe that males who are at an increased risk of prostate cancer may not wish to share their genetic prevalence with third parties, such as insurance companies or even their family due to future problems in their lives. Males at risk may not wish to inform their insurance companies because they may fear an increase in their insurance rate, and they may not wish to inform family because of the potential economic burden of death or inability to provide for the family. Finally, males at risk of prostate cancer may not wish to inform their employers because the employers may not wish to employ a person who has an increased risk for disease.

There are also problems with the testing of prostate cancer among patients. Patients may experience stress when thinking about the physical exam (digital rectal exam), which is required to test prostate cancer diagnosis ("Information, Testing, Treatment, Research, Support Services - Prostate Cancer Canada," n.d.). If a male has a gene linked to prostate cancer risk, he may avoid the stress associated with a physical exam because a physical exam is more painful for the patients ("Information, Testing, Treatment, Research, Support Services - Prostate Cancer Canada," n.d.). The diagnosis of

prostate cancer by a genetic screening could help patients lessen their pain and have more accurate results when combined with other tests.

Limitations of the Study

This study was limited to the information included in each eligible study in this meta-analysis. There was some publication bias in each eligible study, as some eligible studies were not published. This study was also limited to the data collected from the individual studies based on crude ORs because only some of them had adjusted data for covariates, such as folate intake or alcohol consumption (Johansson et al., 2007). The robustness of the statistical technique that they used was included in each individual study. Some of the researchers included a small sample size in their study, which affected the power of the test (López-Cortés, 2013). This study had the limitation of small sample size, but the results will help for future research.

The unity of cases and control groups (matched case-control) in numbers and the quality of data collection were also another limitation to this study. The unity included related risk factors for prostate cancer. In some studies, the researcher did examine the effect of environmental factors, such as the folate intake, dietary intake, drinking, and smoking and the risk of prostate cancer, but others did not. Although I adjusted those environmental covariates by statistical methods, it was still a limitation to the study. The included studies were conducted in different geographical areas (different regions), which may have affected this analysis. Although I did examine the regional effect in this study as a covariate, there were differences between the groups of people in genetic concepts.

The limitation of the multiplicity test came through this study. The multiple comparisons among hypothesis testing of these three genes (MTHFR, MTR, and MTRR) impacted the validity of each hypothesis testing due to Type I error inflation. The *p*-value adjustment method by the Holm (Step-down Bonferroni) method for the multiplicity test of individual polymorphism was estimated (Aickin & Gensler, 1996). The dependency and correlation among these three genes has not been reported yet, so the multiplicity test adjustment was not estimated for the three combined genes in this study. The multiplicity adjustment *p*-value will apply among these three genes if there is an approved result of linkage disequilibrium among them in future studies. The simulation method would help for an appropriate adjustment method of the gene-disease association.

The strengths of this meta-analysis were in my ability to analyze and summarize a large amount of information from previous studies with a common hypothesis. This meta- analysis allowed for subgroup (regions) analysis. I clarified the heterogeneity between individual study results and did a subgroup analysis based on different regions of individual study. I considered the literature reviews in the studies and placed more attention on gene-gene and gene-environment interaction for future study. In this meta-analysis study on the association between MTHFR, MTR, and MTRR genes and prostate cancer, I included more studies than others did. In this study, I increased the sample size to increase the power of the test, and I reduced false negative results.

Recommendations

The limitations of this study provide grounds for future studies. I used only studies that had a common hypothesis of the association between one-carbon, metabolism

gene polymorphism and the risk of prostate cancer, but they had different covariates involved in the analysis. Some of the scholars examined the folate intake association with the hypothesis plus other variables, but some did not include those variables. Future researchers should take into account the effect of the environment (variables) as well as group, age, nutrient intake, and family history in the target population.

Future scholars should evaluate the association between folate intake levels and the risk of prostate cancer. I suggest that future studies include the ancestry of the involved people in the record. Although some of the included studies collected data from the U.S. or Canadian population, it would be important to know where future populations come from in Asia, Europe, or the Middle East. In addition, future scholars should record the chronic diseases in individual participants, such as diabetes, heart diseases, or other cancers. I recommend having a larger sample size in future studies to provide results that are more accurate.

Epidemiology Recommendation

One of the epidemiology recommendations for future study is to establish an epidemiological process to examine the prostate cancer risk in the male population by a common and more accurate test. There should be methods of surveillance of the disease at an early stage to improve an early detection of prostate cancer. Health professionals should inform males about the benefits of prostate cancer genetic screening.

Statistical Methods Recommendations

A meta-analysis is a complicated technique. Published papers and books supported the hypothesis and statistical techniques in this study. I found three previous

meta-analysis studies of this hypothesis; each study had a different point regarding this type of design. Bai et al. (2009) included seven studies on only the MTHFR gene and its two polymorphisms C677T and A1298C. Li et al. (2011) included nine studies and examined only the MTHFR gene and its A1298C Polymorphism. Zhang et al. (2012) included 15 individual studies in his study, but the assumption of independence between the studies for a meta-analysis was not reached in his study. Zhang et al. included the results of a meta-analysis study by Collin et al. (2009) as an individual study.

The unit of meta-analysis is the included individual studies. This study included 20 eligible studies to increase the sample size and provide a more accurate statistical analysis. The assumption of independence between studies was provided in this study. In future meta-analysis studies, researchers should increase the sample size to provide results that are more accurate. The weighting strategy, on studies with a different number of participants, is another statistical matter that should be addressed with future meta-analyses.

Recommendation for Policy Makers

The job of a policy maker is to create a policy to protect people from health problems. The policy makers in public health infrastructure must establish policies to help people in developing a healthy lifestyle. The policy makers must promote better health care services, emergency services, and increase the life expectancy. The policy should be on maximizing the benefits and minimizing the harms and costs.

Improve the Tests

One of the policies that should be implemented is to create improved tests for prostate cancer risk. There must be accurate tests that are affordable to all. Physicians and health professionals should inform the participant about the benefits and harms of the test based on the procedure by the policy. Policy makers should also improve prostate cancer diagnosis through genetic screening for prostate cancer.

Insurance Coverage

Insurance should cover the cost of testing for the disease without affecting the insurance premium cost. If the insurance company pays to diagnose prostate cancer at an early stage, then they will pay less compared to paying for developed prostate cancer treatment. The insurance company should cover the cost of course treatment for prostate cancer disease. Patients should not fear taking the test or informing their insurance company of the results.

Psychological Policy

Prostate cancer patients fear the progression of the disease, the stage of the disease, and the treatment course. Patients with prostate cancer may experience psychological stress, depression, pain, and anxiety ("Enhancing Your Quality of Life When You Have Advanced Prostate Cancer - Prostate Cancer Canada," n.d.). Patients with prostate cancer may feel hopeless, helpless, a fear of death, and a lack of pleasure. An effective treatment and psychotherapy may assist patients in reducing their symptoms and better adjust to their treatment. An accurate test and insurance coverage may decrease a patient's stress. The consultation center is recommended for those who are involved in

this disease, including sections for the family. A team of patients, psychologists, and urologists can help the patient to manage the disease.

Implications

A successful public health program is one that improves the health of a population and increases the health literacy among them. Social change is based on the role of societal factors related to the etiology of prostate cancer. This study could lead to positive social change by providing information on how to improve individual knowledge regarding the disease and by promoting the awareness of prostate cancer. I also provided information on how to prevent, detect, and treat prostate cancer in the early stage of the disease by genetic screening. This early stage diagnosis helps to decrease the cost of treatment and increase the chance of surviving (Krahn et al., 2010). The increased health literacy and knowledge of prostate cancer could lead to a better understanding of the disease to protect males from the progression of prostate cancer.

Genetic screening could be used to increase life expectancy. Health professionals must understand the process of prostate cancer genetic screening so that they can improve the surveillance system and increase the accuracy of the screening (Petersen, 2000). If more patients are informed about the benefits and harms of the genetic screening, public health workers may be able to better diagnose prostate cancer and provide better prevention and treatment strategies.

Conclusion

Genes play a role in diseases, especially in prostate cancer. In this study, I examined the association between one-carbon, metabolism gene polymorphisms and

prostate cancer risk. Researchers have published different results on the association between MTHFR, MTR, and MTRR genes and prostate cancer; some scholars supported the association (Küçükhüseyin et al., 2011; ópez-Cortés et al., 2013) and some researchers did not find support for the association (Johansson et al., 2007; Stevens et al., 2008). In this meta-analysis study, I combined the results of 20 eligible studies with a common hypothesis and an increased power of test to determine an association between MTHFR, MTR, and MTRR genes and prostate cancer risk.

I concluded that gene and prostate cancer risk have a significant association, and the region of the target population may influence this association. I found a highly significant association between variant Allele T of polymorphism C667T in the MTHFR Gene and a decreased risk of prostate cancer. The TT genotype, and the recessive model (TT vs. [CT+CC)]), in polymorphism C667T of MTHFR Gene had a significant association with a decrease in the risk of prostate cancer. The Genotype AC in A1298C polymorphism of MTHFR Gene had a significant association with an increased risk of prostate cancer, but only in the fixed effect model. There was a significant association between regions due to the association with prostate cancer on Genotype AC versus AA in A1298C polymorphism of the MTHFR Gene.

In this study, I collected information from America, Europe, and Asia. Each region had a specific gene pool, which led to gene variation among each population. The carcinogens that cause prostate cancer slightly alter the genetic code in a male's gene sequence. The different regions also had different habits, such as diet, smoking, or drinking that may affect carcinogens leading to cancer.

I concluded that there is a need for general and comprehensive health care policies on genetic screening of prostate cancer. The genetic screening could lead to a longer and healthier life for males. The genotype screening could help to prevent the progress of prostate cancer at an early stage and reduce the cost of course of treatment. The prevention of prostate cancer could also improve the quality of life for males and their families. The individual patient may go through a treatment program at an early stage and increase his chance of living. Economically, males who live longer can provide financial support for their families, for a longer period in their life. The results of this study could be used to improve the health of individuals and communities.

I recommend that a genetic screening test should be created to diagnose prostate cancer risk among males at an early stage of the disease. I recommend that more research should be conducted in a variety of geographical regions with a variety of carcinogen factors to find the gene-gene and gene-environment interaction related to prostate cancer risk. A meta-analysis is an appropriate method to increase the power of the test and to get better results than an individual study. If policy makers promote genetic screening and it becomes an accurate and active test in the diagnosis of prostate cancer at an early stage of the disease, my study results could promote positive social change due to increased life expectancy among males. This increased life expectancy could benefit individuals, their families, their communities, and society at large level.

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Appendix A: The Data Collected Summary of Prostate Cancer Risk Associated with selected genes, MTHFR, MTR, and MTRR

Table A1

Summary of Prostate Cancer Associated with MTHFR Gene and C677T Polymorphism, Allele, and Genotype Frequencies

First Author	Year	Region	Ethnicity	Cases C	T	CC	CT	TT	Total	Controls C	T	CC	CT	TT	Total
Kimura	2000	Europe	Caucasian	165	99	49	67	16	132	203	97	65	73	12	150
Heijmans	2003	Europe	Caucasian	25	17	8	9	4	21	1127	459	399	329	65	793
Cicek	2004	America	Caucasian	610	268	214	182	43	439	637	321	219	199	61	479
Singal	2004	America	Caucasian, African-American	123	39	28 / 21	21 / 4	7 / ---	56 / 25	60	24	10 / 10	15 / 5	2 / ---	27 / 15
Van Guelpen	2006	Europe	Caucasian	322	124	111	100	12	223	642	228	243	156	36	435
Johansson	2007	Europe	Caucasian	3808	1546	1340	1128	209	2677	2214	868	801	612	128	1541
Reljic	2007	Europe	Caucasian	124	66	38	48	9	95	41	33	8	25	4	37
Marchal	2008	Europe	Caucasian	238	126	67	104	11	182	269	139	96	77	31	204
Stevens	2008	America	Caucasian	1461	739	472	517	111	1100	1449	765	474	501	132	1107
Muslumanoglu	2009	Asia	Caucasian	144	42	53	38	2	93	225	89	80	65	12	157
Cai	2010	Asia	Asian	237	197	58	121	38	217	206	234	45	116	59	220
Safarinejad	2010	Asia	Caucasian	249	99	86	77	11	174	461	235	153	155	40	348
Wu	2010	Asia	Asian	346	90	139	68	11	218	619	253	221	177	38	436
Kucukhuseyin	2011	Asia	Caucasian	85	25	32	21	2	55	66	34	18	30	2	50
Kobayashi	2012	America	Caucasian	63	23	22	19	2	43	230	110	72	86	12	170
Fard-Esfahani	2012	Asia	Caucasian	91	43	29	33	5	67	103	47	32	39	4	75
Jackson	2013	America	African-American			157	(+)45		202			164	(+)42		206
Lopez-Cortes	2013	America	Caucasian Spanish	133	75	30	73	1	104	161	59	52	57	1	110
De Vogal	2014	Europe	Caucasian	3634	1410	1407	820	295	2522	3597	1617	1334	929	344	2607
Ebrahimi	2014	Asia	Caucasian	57		27	3		30	74		34	6		40

Table A2

Summary of Prostate Cancer Associated with MTHFR Gene and A1298C Polymorphism,

Allele, and Genotype Frequencies

First Author	Year	Region	Ethnicity	Cases						Controls					
				A	C	AA	AC	CC	Total	A	C	AA	AC	CC	Total
Cicek	2004	America	Caucasian	595	283	195	205	39	439	667	289	233	201	44	478
Singal	2004	America	Caucasian, African-American	101	61	18	32	6	56	53	31	10	11	6	27
						11	11	3	25			8	6	1	15
Van Guelpen	2006	Europe	Caucasian	282	162	87	108	27	222	555	313	176	203	55	434
Marchal	2008	Europe	Caucasian	258	106	98	62	17	177	295	113	108	79	22	209
Stevens	2008	America	Caucasian	1480	728	481	518	105	1104	982	743	491	493	125	1109
Muslumanoglu	2009	Asia	Caucasian	78	104	31	16	44	91	199	133	77	45	44	166
Cai	2010	Asia	Asian	363	71	150	63	4	217	359	81	144	71	5	220
Safarinejad	2010	Asia	Caucasian	250	98	90	70	14	174	466	230	158	150	40	348
Wu	2010	Asia	Asian	346	90	138	70	10	218	709	163	287	135	14	436
Jackson	2013	America	African-American			137	(+)62		199			151	(+)51		202
Lopez-Cortes	2013	America	Caucasian Spanish	202	6	100	2	2	104	217	3	108	1	1	110

Table A3

Summary of Prostate Cancer Associated with MTR Gene and A2756G Polymorphism,

Allele, and Genotype Frequencies

First Author	Year	Region	Ethnicity	Cases						Controls					
				A	G	AA	AG	GG	Total	A	G	AA	AG	GG	Total
Marchal	2008	Europe	Caucasian	290	74	118	54	9	181	331	77	138	55	11	204
Cai	2010	Asia	Asian	397	37	185	27	5	217	405	35	188	29	3	220
Jackson	2013	America	African-American			97	(+)102		199			99	(+)106		205
Lopez-Cortes	2013	America	Caucasian Spanish	193	15	92	9	3	104	213	7	104	5	1	110

Table A4

Summary of Prostate Cancer Associated with MTRR Gene and C677T Polymorphism,

Allele, and Genotype Frequencies

First Author	Year	Region	Ethnicity	Cases						Controls					
				A	G	AA	AG	GG	Total	A	G	AA	AG	GG	Total
Marchal	2008	Europe	Caucasian	181	183	38	105	39	182	203	205	46	111	47	204
Cai	2010	Asia	Asian	314	120	111	92	14	217	325	115	118	89	13	220
Jackson	2013	America	African-American			111	(+)84		195			120	(+)83		203
Lopez-Cortes	2013	America	Caucasian Spanish	115	93	22	71	11	104	103	117	3	97	10	110

Appendix B: The Summary of Statistical Analysis Association Between One-Carbon

Metabolism Gene Polymorphisms and Risk of Prostate Cancer

Gene (Polymorphism)	Genetic Model	Fixed effect model		Random effect model		Homogeneity test		
		OR [95% CI]	adjuste p-value	OR [95% CI]	adjuste p-value	Q-value	ProbQ	I^2 (%)
MTHFR	Allele T vs.C	.92[.88,.97]	.002	.91[.83,1]	.15	39.95	0.001	57.45
	TT vs. CC	.81[.73,.91]	.001	.78[.66,.92]	.01	23.79	.13	28.54
(C677T)	CT vs. CC	.97[.91,1.03]	.34	.96[.83,1.12]	.70	53.70	<.001**	66.48
	Recessive	.83[.75,.92]	0.001	.79[.68,.93]	.01	23.47	.134	27.57
	Dominant	.94[.88,1]	.10	.94[.82,1.07]	.70	52.15	<.001**	65.48
MTHFR	Allele C vs. A	1.04[.96,1.13]	.72	1.06[.93,1.22]	1.00	19	.03*	52.63
	CC vs. AA	1.18[.98,1.41]	.24	1.14[.89,1.46]	1.00	13.12	.16	31.40
(A1298C)	AC vs. AA	1.24[1.11,1.38]	<.001**	1.10[.90,1.34]	1.00	22.88	.007*	60.66
	Recessive	1.07[.90,1.27]	.72	1.07[.83,1.39]	1.00	15.19	.09	40.75
	Dominant	1.24[1.12,1.36]	<.001**	1.15[.96,1.36]	.65	23.93	.008*	58.21
MTR	Allele G vs. A	1.17[.89,1.54]	1.00	1.19[.86,1.65]	1.00	2.49	.29	19.68
	GG vs. AA	1.26[.61,2.62]	1.00	1.26[.61,2.62]	1.00	1.23	.54	Q<df
(A2756G)	AG vs. AA	1.13[.81,1.58]	1.00	1.13[.81,1.58]	1.00	1.43	.49	Q<df
	Recessive	1.22[.59,2.52]	1.00	1.22[.59,2.52]	1.00	1.29	.52	Q<df
	Dominant	1.08[.85,1.38]	1.00	1.08[.85,1.38]	1.00	2.32	.51	Q<df
MTRR	Allele G vs. A	.95[.80,1.14]	1.00	.94[.75,1.18]	1.00	3.04	.22	34.32
	GG vs. AA	.87[.55,1.38]	1.00	.70[.29,1.72]	1.00	6.10	.05	67.19
(A66G)	AG vs. AA	.97[.72,1.31]	1.00	.63[.25,1.58]	1.00	13.63	.001**	85.33
	Recessive	1[.68,1.44]	1.00	1[.68,1.44]	1.00	.33	.85	Q<df
	Dominant	1.01[.80,1.28]	1.00	.82[.48,1.41]	1.00	13.36	.003*	77.53

Appendix C: A Summary of Statistical Results from Individual Included Study on

Prostate Cancer Associated with One-Carbon Metabolism Gene Polymorphism, Based on

Genotype Frequency

First Author	Year	Region	Race/Ethnicity	Study base	Study design	Cases	Control	SNP	Odds Ratios (95%CI)
Kimura	2000	Europe	Caucasian	Hospital-base	Case-control study	132	150	MTHFR	C677T Crude CC Ref. CT 1.22 (0.74-2) TT 1.77 (0.77-4.08) Recessive 1.59 (0.72-3.49) Dominant 1.29 (0.80-2.09) T vs. C 1.25 (0.89-1.78)
Heijmans	2003	Europe	Caucasian	Population-base	Cohort study (Related Risk)	21	793	MTHFR	C677T Crude CC Ref. CT 1.36 (0.52-3.57) TT 3.07 (0.90-10.48) Recessive 1.59 (0.72-3.49) Dominant 2.63 (0.86-8.06) T vs. C 1.67 (0.89-3.12)
Cicek	2004	America	Caucasian	Population-base	Case-control study	439	479	MTHFR	C677T Crude CC Ref. CT 0.93 (0.71-1.23) TT 0.72 (0.47-1.11) Recessive 0.74 (0.49-1.12) Dominant 0.88 (0.68-1.15) T vs. C 0.87 (0.72-1.06) Crude A1298C AA(Ref) AC 1.22 (0.93-1.6) CC 1.06(0.66-1.70) Recessive 0.96 (0.61-1.51) Dominant 1.19 (0.92-1.54) C vs. A 1.1 (0.90-1.34)
Singal	2004	America	Caucasian, African-American	Hospital-base	Case-control study	81	42	MTHFR	C677T Crude CC Ref. CT 0.51 (0.23-1.12) TT 1.43 (0.27-7.48) Recessive 1.89 (0.37-9.54) Dominant 0.59 (0.28-1.26) T vs. C 0.79 (0.44-1.44) Crude A1298C AA(Ref) AC 1.56 (0.70-3.54) CC 0.80 (0.25-2.52) Recessive 0.62 (0.21-0.82) Dominant 1.34 (0.63-2.88) C vs. A 1.03 (0.60-1.78)
Van	2006	Europe	Caucasian	Population	Nested	22	435	MTHFR	C677T

Author	Year	Region	Ethnicity	Source	Study type	Cases	Controls	Gene	Results
Guelpen				ion-base	Case-control	3			Crude CC Ref. CT 1.40 (1.00-1.96) TT 0.73 (0.36-1.46) Recessive 0.63 (0.32-1.24) Dominant 1.28 (0.92-1.76) T vs. C 1.08 (0.84-1.40) Crude A1298C AA (Ref) AC 1.08 (0.76-1.52) CC 0.99 (0.59-1.68) Recessive 0.93 (0.74-1.17) Dominant 1.08 (0.95-1.22) C vs. A 1.02 (0.80-1.29)
Johansson	2007	Europe	Caucasian	Population-base	Case-control study	2677	1541	MTHFR	C677T Crude CC Ref. CT 1.10 (0.96-1.26) TT 0.98 (0.77-1.24) Recessive 0.93 (0.74-1.17) Dominant 1.08 (0.95-1.22) T vs. C 1.03 (0.94-1.14)
Reljic	2007	Europe	Caucasian	Population-base	Case-control study	95	37	MTHFR	C677T Crude CC Ref. CT 0.40 (0.16-1.00) TT 0.47 (0.12-1.93) Recessive 0.86 (0.25-3.00) Dominant 0.41 (0.17-1.00) T vs. C 0.66 (0.38-1.14)
Marchal	2008	Europe	Caucasian	Hospital-base	Case-control study	182	204	MTHFR	C677T Crude CC Ref. CT 1.93 (1.26-2.97) TT 0.51 (0.24-1.08) Recessive 0.36 (0.17-0.74) Dominant 1.53 (1.01-2.30) T vs. C 1.02 (0.76-1.38) Crude A1298C AA Ref. AC 0.86 (0.56-1.33) CC 0.85 (0.43-1.70) Recessive 0.90 (0.46-1.76) Dominant 0.86 (0.58-1.29) C vs. A 1.07 (0.78-1.47)
Marchal	2008	Europe	Caucasian	Hospital-base	Case-control study	181	204	MTR	Crude A2756G AA Ref. AG 1.14 (0.73-1.80) GG 0.96 (0.38-2.39) Recessive 0.91 (0.37-2.27) Dominant 1.12 (0.73-1.70) G vs. A 1.10 (0.77-1.56)
Marchal	2008	Europe	Caucasian	Hospital-base	Case-control study	182	204	MTRR	Crude A66G AA Ref. AG 1.14 (0.69-1.90) GG 1.00 (0.55-1.84) Recessive 1.10 (0.68-1.78) Dominant 1.03 (0.68-1.79)

Stevens	2008	America	Caucasian	Population-base	Nested Case-control study	1100	1107	MTHFR	G vs. A 1.00 (0.75-1.33) C677T Crude CC Ref. CT 1.04 (0.87-1.24) TT 0.84 (0.64-1.12) Recessive 0.83 (0.63-1.08) Dominant 1.00 (0.84-1.18) T vs. C 0.96 (0.84-1.08)
Muslumanoglu	2009	Asia	Caucasian	Hospital-base	Case-control study	93	157	MTHFR	C677T Crude CC Ref. CT 0.88 (0.52-1.50) TT 0.25 (0.05-1.17) Recessive 0.26 (0.06-1.21) Dominant 0.78 (0.47-1.31) T vs. C 0.74 (0.48-1.12) Crude A1298C AA(Ref) AC 0.88 (0.43-1.79) CC 2.48 (1.38-4.48) Recessive 2.59 (1.52-4.44) Dominant 1.67 (0.98-2.84) A vs C 0.50 (0.35-0.72)
Cai	2010	Asia	Asian	Hospital-base	Case-control study	217	220	MTHFR	C677T Crude CC Ref. CT 0.81 (0.51-1.29) TT 0.50 (0.28-0.88) Recessive 0.58 (0.36-0.92) Dominant 0.70 (0.45-1.10) T vs. C 0.73 (0.56-0.95) Crude A1298C AA(Ref) AC 0.85 (0.56-1.28) CC 0.77 (0.20-2.92) Recessive 0.81 (0.21-3.05) Dominant 0.85 (0.57-1.26) C vs A 0.87 (0.61-1.23)
Cai	2010	Asia	Asian	Hospital-base	Case-control study	217	220	MTR	Crude A2756G AA(ref) AG 0.95(0.54-1.66) GG 1.69(0.40-7.19) Recessive 1.7 (40-7.23) Dominant 1.02 (0.60-1.73) G vs A 1.08(0.67-1.75)
Cai	2010	Asia	Asian	Hospital-base	Case-control study	217	220	MTRR	Crude AA(Ref) A66G AG 1.10(0.74-1.62) GG 1.15(0.52-2.54) Recessive 0.91 (0.42-1.98) Dominance 1.10 (0.76-1.61) G vs A 1.08 (0.80-1.46)
Safarinejad	2010	Asia	Caucasian	Population-base	Case-control study	174	348	MTHFR	C677T Crude CC Ref.

CT 0.88 (0.60-1.29)
TT 0.49 (0.24-1.00)
Recessive 0.52 (0.26-1.04)
Dominant 0.80 (0.56-1.16)
 T vs. C 0.78 (0.59-1.03)

Crude
A1298C
AA (Ref)
AC 0.82 (0.56-1.20)
CC 0.61 (0.32-1.19)
Recessive 0.67 (0.36-1.27)
Dominant 0.78 (0.54-1.12)
C vs A 0.79 (0.60-1.05)

Author	Year	Continent	Ethnicity	Source	Design	Case	Control	Gene	Results
Wu	2010	Asia	Asian	Hospital-base	Case-control study	218	436	MTHFR	Crude C677T CC Ref. CT 0.61 (0.43-0.87) TT 0.46 (0.23- 0.93) Recessive 0.56 (0.28-1.11) Dominant 0.58 (0.42-0.82) T vs.C 0.63 (0.48-0.84) Crude A1298C AA Ref. AC 1.08(0.76-1.53) CC 1.48(0.64-3.43) Recessive 1.45(0.63-3.32) Dominant 1.12(0.79-1.57) C vs. A 1.13(0.85-1.51)
Kucukhuseyin	2011	Asia	Caucasian	Population-base	Case-control study	55	50	MTHFR	C677T Crude CC Ref. CT 0.39 (0.18-0.88) TT 0.56 (0.07-4.34) Recessive 0.91 (0.12-6.68) Dominant 0.40 (0.18-0.89) T vs. C 0.57 (0.31-1.05)
Kobayashi	2012	America	Caucasian	Hospital based	Case-control study	43	170	MTHFR	C677T Crude CC Ref. CT 0.72 (0.36-1.44) TT 0.54 (0.11-2.62) Recessive 0.64 (0.14-2.98) Dominant 0.70 (0.36-1.37) T vs. C 0.76 (0.45-1.29)
Fard-Esfahani	2012	Asia	Caucasian	Hospital-base	Case-control study	67	75	MTHFR	C677T Crude CC Ref. CT 0.93 (0.47-1.85) TT 1.38 (0.34-5.63) Recessive 1.43 (0.37-5.57) Dominant 0.95 (0.49-1.85) T vs. C 1.03 (0.63-1.71)
Jackson	2013	America	African-American	Hospital-base	Case-control study	202	206	MTHFR	Crude Dominant C677T 1.12 (0.70-1.80) A1298C 1.34 (0.86-2.07)
Jackson	2013	America	African-American	Hospital-base	Case-control	199	205	MTR	Dominant 0.98 (0.66-1.45)

Jackson	2013	America	African-American	Hospital-base	study Case-control study	195	206	MTRR	Dominant 1.09 (0.73-1.63)
Lopez-Cortes	2013	America	Caucasian Spanish	Population-base	Case-control study	104	110	MTHFR	Crude C677T CC Ref. CT 2.22 (1.26-3.91) TT 1.73 (.10- 28.73) Recessive 1.06 (0.06-17.14) Dominant 2.21 (1.25-3.89) T vs.C 1.54 (1.02-2.32)
									Crude A1298C AA Ref. AC 2.16 (0.19-24.19) CC 2.16 (0.19-24.19) Recessive 2.14 (0.19-23.93) Dominant 2.16 (0.39-12.05) C vs. A 2.15 (0.53-8.7)
Lopez-Cortes	2013	America	Caucasian Spanish	Population-base	Case-control study	104	110	MTR	Crude A2756G AA Ref. AG 2.03 (0.66-6.29) GG 3.4 (0.35-33.17) Recessive 3.24 (0.33-31.63) Dominant 2.26 (0.81-6.26) G vs. A 0.71 (0.48-1.04)
Lopez-Cortes	2013	America	Caucasian Spanish	Population-base	Case-control study	104	110	MTRR	Crude A66G AA Ref. AG 0.1 (0.03-0.35) GG 0.15 (0.03-0.66) Recessive 1.18 (0.48-2.91) Dominant 0.10 (0.03-0.36) G vs. A 2.36 (0.94-5.92)
Kobayashi	2012	America	Caucasian	Hospital-base	Case-control study	80	334	MTHFR	C677T Crude CC Ref. CT 0.72 (0.36-1.44) TT 0.54 (. 11- 2.62) Recessive 0.64 (0.14-2.98) Dominant 0.70 (. 36-1.37) T vs.C 0.76 (0.45-1.29)
De Vogel	2014	Europe	Caucasian	Population-base	Nested Case-control study	2522	2607	MTHFR	C677T Crude CC Ref. CT 0.84 (0.74-0.94) TT 0.81 (0.68- 0.97) Recessive 0.87 (0.74-1.03) Dominant 0.83 (0.74-0.93) T vs.C 0.86 (0.79-0.94)
Ebrahimi	2014	Asia	Caucasian	Hospital-base	Case-control study	30	40	MTHFR	C677T Crude CC Ref. CT 0.63 (0.14-2.75)