12-2019

The Associations Between Fat And Clinical Outcomes Among Lung Cancer Patients: Dietary Intake And Genetic Determinants

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THE ASSOCIATIONS BETWEEN FAT AND CLINICAL OUTCOMES AMONG LUNG CANCER PATIENTS: DIETARY INTAKE AND GENETIC DETERMINANTS

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Presented to the Faculty of The University of Texas

School of Public Health

in Partial Fulfillment

of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

THE UNIVERSITY OF TEXAS
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Houston, Texas
December, 2019

ACKNOWLEDGEMENTS

I would like to express my great appreciation to my dissertation committee. I could not complete my doctoral study and dissertation without the continuous support and guidance of Dr. Michelle Hildebrandt, Dr. Xifeng Wu, Dr. Kai Zhang, Dr. Lu-Yu Hwang and Dr. Yuanqing Ye. I would also thank Maosheng Huang, Dr. David Chang, Dr. Huakang Tu and Alem Belachew for their tremendous help to build my skills in nutritional and genetic analyses, and the intellectual and moral support of Su-Tsung Lin, Ming-Chu Hsiao, Hung-I Lin and Ching-Hsing Wang.

THE ASSOCIATIONS BETWEEN FAT AND CLINICAL OUTCOMES AMONG LUNG CANCER PATIENTS: DIETARY INTAKE AND GENETIC DETERMINANTS

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Higher dietary fat intake and alterations in fatty acid metabolism may affect cancer development and progression. This dissertation conducted a series of studies examining whether dietary fat intake and genetic variants in fatty acid metabolism genes have an impact on clinical outcomes among non-Hispanic whites newly diagnosed with non-small cell lung cancer (NSCLC). First, a cohort of 2,262 NSCLC patients was prospectively examined for intakes of total, saturated, monounsaturated, and polyunsaturated fat in relation to overall survival and recurrence. Dietary fat intake at diagnosis was assessed with a previously validated food frequency questionnaire and categorized by Dietary Reference Intakes. Multivariable Cox proportional hazard models were used to estimate adjusted hazard ratios (HRs) with 95% confidence intervals (CIs). Neither high intake of total, nor any subtype of fat, was associated with overall survival or recurrence for NSCLC. Analysis of stage-specific overall survival revealed that early-stage patients with high intake of saturated fat were at increased risk of poor survival compared to those who had intake less than the recommended amount (HR: 1.27; 95% CI: 1.02, 1.59). However, a protective effect was observed in

advanced-stage patients who received primary chemotherapy (HR: 0.84; 95% CI: 0.71, 0.99). This protective effect was also evident for high intake of monounsaturated fat in this same group of patients (HR: 0.64; 95% CI: 0.43, 0.96). In the second aim, the associations between single nucleotide polymorphisms (SNPs) in genes related to fatty acid metabolism and overall survival and recurrence among NSCLC patients were examined by using two-stage design, and further evaluated for differences by dietary fat intake. Among 1,593 NSCLC patients in the discovery set, candidate SNPs associated with overall survival or recurrence were identified, and were further validated in the replication set of 746 NSCLC patients. Four SNPs were associated with overall survival and one SNP was associated with recurrence in both datasets. Functional assessment identified three variants *ACSL1*:rs4862417, *CYP2C8*:rs1934953, and *FADS2*:rs174611 to be putatively functional. Early-stage patients with a G variant rs174611 were associated with 28% and 47% increased risk of death in the discovery (95% CI: 1.03, 1.59) and replication sets (95% CI: 1.03, 2.08), respectively. Monounsaturated fat intake was found to interact with rs174611 genotype in relation to overall survival (multiplicative $P_{\text{interaction}} = 0.03$). In summary, dietary fat intake and genetic variants in fatty acid metabolism genes play roles in survival among NSCLC patients. The association of dietary fat and overall survival is dependent on disease stage and treatment for NSCLC. Genetic variants and dietary fat intake may have multiplicative effect on overall survival in NSCLC. These findings provide evidence for potential genetically-targeted nutritional consultation on dietary fat intake for NSCLC patients.

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CHAPTER I: BACKGROUND

Epidemiology of Lung Cancer

Lung cancer has remained the leading cause of cancer deaths for several decades across the world (1). In 2018, a total of 1.76 million deaths from lung cancer were estimated, accounting for approximately one in five of cancer-related deaths worldwide (2). Lung cancer comprises a heterogeneous histology that can be divided into two major types: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC accounts for 15-20% of lung cancers, which behaves biologically aggressively and leads to poor survival (3, 4). About 80-85% of lung cancers are NSCLC that is the most common type of lung cancer (4). NSCLC can be further divided into three major subtypes: adenocarcinoma, squamous cell carcinoma and large cell carcinoma. Adenocarcinoma is the most prevalent type of NSCLC, accounting for 40% of lung cancers. Adenocarcinoma tends to grow slowly as opposed to other cell types (5). The incidence of adenocarcinoma has surpassed that of squamous cell carcinoma during the last several decades (6). Approximately 30% of lung cancers are squamous cell carcinoma. By contrast, large cell carcinoma occurs in only approximately 15% of people with lung cancer, which usually grows and spreads fast, similarly to SCLC (4). The prognosis of large cell carcinoma is usually poorer than the other NSCLCs (7).

It has been widely known that cigarette smoking is a major cause of lung cancer (8). Inhalation of cigarette smoke is the primary source of exposure to carcinogens among smokers. The potential carcinogens in cigarette smoke include polycyclic aromatic hydrocarbons (PAHs), N-nitroso compounds (NOCs), aromatic amines, and a large variety of organic and

inorganic chemicals (7). However, there is also a large fraction of lung cancer cases among never smokers (9). Evidence indicates that smokers and never smokers with lung cancer may have biological and genetics differences (10). Although smoking is a strong risk factor for all histological types of lung cancer, squamous cell of lung cancer is the most prevalent subtype among male smokers (11). Lung cancer in never smokers occurs more frequently among women, and the subtype of adenocarcinoma is seen more often in never smokers and females (10, 11). It is noteworthy to mention that lung cancer in smokers and never smokers display distinct patterns of oncogenic aberrations. Lung cancer in never smokers tends to carry mutations in the epidermal growth factor receptor (*EGFR*) as well as anaplastic lymphoma kinase (*ALK*) alterations compared to smokers, whereas kirsten rat sarcoma viral oncogene homologue (*KRAS*) mutations occur less frequently among never smokers (10). In addition to cigarette smoking, there are other important environmental factors related to lung cancer, including secondhand smoke (12), radon (13), ambient air pollution (14), and occupational exposures (15).

Genetic factors can also come into play in the development of lung cancer. Numerous studies observed familial aggregation of lung cancer, wherein multiple family members are diagnosed with lung cancer (16, 17). Family members share environmental exposures and genetic factors, both of which account for familial lung cancer. Nevertheless, individuals with a family history of lung cancer have significantly increased risk of lung cancer than those without such a history, and this relationship remains after adjusting for shared environmental exposures such as cigarette smoking (17, 18).

Single nucleotide polymorphisms (SNPs), the most common type of DNA variation among humans, are single base pair changes throughout the genome occurring at every 1,000 nucleotides on average (19). Single base pair alterations may alter the function or structure of the encoded proteins, and thus have a genetic predisposition to certain diseases, including cancer (19, 20). These SNPs usually occur within a gene or in regulatory region near a gene. However, most SNPs have a negligible effect on health, which generally occur in the non-coding region. Candidate gene studies have been the focus prior to the era of genome-wide association studies (GWAS). Candidate gene studies are relatively inexpensive and easy to perform, and most of the effort focused on identifying risk variants in genes related to the mechanism of a particular trait (21, 22). For lung cancer, this has included studies focusing on alterations in genes related to nicotine metabolism, DNA repair, inflammation, and cell-cycle pathways (23). Nevertheless, the results from many of these candidate gene studies have been inconsistent (24). In contrast, a large number of genetic variants implicated in the risk of lung cancer have been identified by GWAS. A typical GWAS uses a large sample of unrelated individuals and thousands of different loci in the human genome to distinguish common genetic variants for complex diseases, and focuses on the associations of SNPs with common human diseases (25). GWAS is a discovery-driven approach, without any prior knowledge of disease processes. The primary goal of GWAS is to discover novel genetic associations that could develop better strategies to detect, treat and prevent diseases. Over the past decade, GWAS has found that variants on nicotinic acetylcholine receptor subunits, telomerase, and DNA damage response were associated with lung cancer susceptibility (26-30). Only SNPs with p value $<5\times10^{-8}$ are considered statistically significant due to issues arising from multiple

testing and type I errors. Several SNPs that are truly but weakly associated with risk of lung cancer may fail to be detected. In addition, earlier GWAS often focused on the effect of genetic variants in a small number of genes on lung cancer susceptibility. The advancement of genotyping technology greatly increases the number of genetic variants that can be examined for association in GWAS, and began to be incorporated into pathway analysis (31). Pathway-based GWAS has become a popular approach to investigating the relationship between groups of genetic variants in the same biological pathway and disease. This approach substantially reduces the multiple testing burden and makes it possible to examine the true associations reporting lower effect size at individual genes. Pathway-based approach facilitates the understanding of genetic susceptibility to lung cancer and is conducive to unraveling the genomic complexity of lung cancer (32, 33).

The overall 5-year survival rate for lung cancer in the United States is only 18% (34), which is much lower than that of other common cancers (7). Research efforts have been made to identify prognostic factors that can be used in routine clinical practice to best take care of patients with lung cancer. Tumor stage is considered the predominant prognostic factor in lung cancer to guide treatment decision and to determine overall survival (35). Up to 56% of patients who are diagnosed with localized lung cancer have survived 5-year or more, compared to 30% and 5% for regional and distant cancers, respectively (34). Other established factors include tumor histology, age, gender, and performance status, which can be considered alongside with tumor stage to refine prognosis (36, 37). However, these factors remain insufficient to predict survival in lung cancer patients (38).

Poor survival in lung cancer can be partially explained by high prevalence of smoking (39) and high proportion of patients with advanced stage at diagnosis (40). Recent evidence demonstrates that smoking cancer patients limits the efficacy of cancer treatment and increases the risk of undesired side effects (41, 42), suggesting a potential causal relationship between smoking and adverse clinical outcomes in cancer patients (43). In addition, curative resection of lung cancer may no longer be a treatment option for patients with advanced-stage of lung cancer as a result of metastases. Accordingly, the implementation of smoking cessation and screening for early detection of lung cancer have been of great interest (44, 45). The more recent advent of low-dose computed tomography (CT) allows for more accurate detection of lung cancer at an early stage than does standard chest radiography (46). Evidence from the National Lung Screening Trial showed a 20% reduction in mortality from lung cancer in the low-dose CT group compared to the standard chest radiography (47). Although great progress has been made to decrease lung cancer mortality, long-term survival rate in lung cancer patients has remained low (48). Therefore, there is a pressing need to identify modifiable determinants of survival in patients with lung cancer.

Dietary Assessment Tools

Measurement of dietary exposure is an important process to understand the link between diet and cancer. However, it is challenging to accurately assess dietary intake because of the within and between subject variations in food consumption, and people may not accurately report the amount or the type of food that they have eaten. Although several dietary assessment tools are available to estimate a person's dietary intake, the choice of tool used to assess dietary

intake may affect varying types and degrees of measurement error (49). Dietary assessment can be either objective or subjective. Duplicate diet approach is a type of objective dietary assessment which collects duplicate portion of all the foods that subjects have eaten during the study period, and the amount of food consumed is weighted and recorded. This approach is considered as the most precise method for estimating dietary intake because it can capture the actual dietary intake information. One disadvantage is that the duplicate diet approach is costly in both time and money, and thus is not suitable for large-scale studies.

Subjective dietary assessment can be performed using the three main methods: 24-hour dietary recall, diet record, and food frequency questionnaire. The 24-hour dietary recall asks individuals to provide the actual intake information during the previous 24-hour period. The diet record method is similar to the 24-hour recall, but has a much longer observation period, which is only one difference between the diet record method and 24-hour dietary recall. The diet record method collects the actual intake information over a period, usually three to five consecutive days. Both methods use open-ended questions to assess food consumption, providing detailed intake information and can be easily used in different populations. The 24-hour dietary recall and diet record method provide typical meal and food consumption immediately prior to a study. Recall bias may not be a serious issue, but has a substantial burden on the respondents. While eating behavior may change from day to day, multiple records or recalls over several days are required to assess usual routine eating patterns. However, it is time-consuming and expensive to collect repeated measures. On the other hand, food frequency questionnaires (FFQ) ask the respondents to report how often and how much food were consumed over a relatively long period of time, typically one year. This method

enables the assessment of habitual dietary intake rather than that of current intake. FFQ enables researchers to perform comprehensive assessment of various nutrients and dietary components in a relatively simple, cost-effective, and time efficient manner. The burden on the respondents is lower and trained interviewers may not be required. Therefore, the FFQ has been widely used in epidemiological studies. The major limitation of the FFQ is that the questionnaire should be specifically developed for study population because diet may be influenced by ethnicity, culture, and economic status. Additionally, FFQ is prone to recall bias since individuals are asked to report their diet intake retrospectively for a prolonged period of time.

Dietary Intake and Lung Cancer Risk

Previous research has suggested that dietary factors may modulate lung cancer risk. The associations between dietary factors and lung cancer risk have been extensively examined for red meat (50), fruits and vegetables (51), but less studied for the other factors. High consumption of red meat has been associated with increased risk of lung cancer (50). Because of high levels of heme iron and saturated fat in red meat, the mutagenic byproducts such as heterocyclic amines, polycyclic aromatic hydrocarbons (PAHs) and N-nitroso compounds (NOCs) are produced when cooking at high temperatures, leading to carcinogenesis (52-54). On the other hand, a higher intake of fruits and vegetables has been associated with decreased risk of lung cancer (51), because fruits and vegetables contain carotenoids and other phytochemicals that may act as antioxidants in prevention of carcinogenesis.

Dietary fat intake is an emerging research interest and also of importance because it is a modifiable risk factor (55). Several observational studies have evaluated the role of dietary fat

in lung cancer risk, but the findings have remained inconclusive. The four case-control studies from Hawaii (56), Iowa (57), and Uruguay (58, 59) observed a positive association of dietary fat with lung cancer risk among ethnically diverse populations. Specifically, the study in Hawaii found the effect of dietary fat on lung cancer risk among men, but not among women (56). The odds ratio (OR) of lung cancer risk for the highest fat consumption quartile compared with the lowest quartile among male cases of lung cancers and male persons without lung cancer was 2.2-fold (95% confidence interval [CI], 1.3-3.7), after adjusting for age, ethnicity and pack-years of cigarette smoking. In the Iowa study that only targeted women, the excess risk of lung cancer was associated with fat intake (OR= 2.0; 95% confidence interval 1.3-3.1), adjusting for age and nutrient density calories (57). Two additional case-control studies of fat intake and lung cancer susceptibility were conducted in Uruguay. One study found that the highest fat intake was associated with a 128% increased risk of lung adenocarcinoma (95% CI, 1.5-3.5) than the lowest fat intake with adjustments for age, residence, urban or rural status, education attainment, body mass index (BMI), smoking status and total energy intake (58). The other study showed a 190% increased risk of lung cancer (95% CI, 1.8-3.9) associated with the highest fat intake after adjusting for age, residence, urban or rural status, pack-years of cigarette smoking, total energy intake, vegetables and fruits intake, and alpha-carotene intake (59). Nonetheless, the findings from another two case-control studies in China (60) and Missouri (61) showed null association between fat intake and lung cancer risk. A recent meta-analysis of the above-mentioned case-control studies demonstrated that high-fat intake was positively associated with lung cancer risk (OR= 1.64; 95% CI, 1.2-2.3) (62).

Given that dietary assessment in case-control studies is carried out after cancer diagnosis, recall bias may arise. To circumvent recall bias, several cohort studies with prediagnostic dietary assessment have been conducted to examine the role of fat intake in the development of lung cancer in the 1990s (63-68). A positive association between high consumption of fat and elevated lung cancer risk was observed in the three cohorts including Finland (65), Norway (67) and New York (63). However, only the result from the New York cohort was statistically significant (63). The New York cohort demonstrated a 44% increased risk of lung cancer (95% CI, 1.11-1.87) for the highest tertile versus the lowest tertile intake of fat only among males after adjusting for age, education attainment, pack-years of cigarette smoking and total energy intake. The New York cohort also observed that the effect of fat intake was confined to squamous cell carcinomas of the lung. The two cohort studies in Finland and Norway reported a 40-60% increased risk of lung cancer associated with the highest fat intake, but the risk was not statistically significant (65, 67). By contrast, another three cohort studies found no evidence for the effect of fat intake on lung cancer risk (64, 66, 68). It was anticipated that these earlier cohort studies may not have enough statistical power of assessment for dietary intake and lung cancer risk, since only a few cases of lung cancer would have developed during the follow-up periods given a limited number of study participants. In 2002, a pooled analysis was conducted of 8 prospective cohorts comprised of about half a million total participants (69). There were 3,188 incident cases of lung cancer that were identified during the follow-up periods of 6-16 years. The pooled analysis revealed that dietary fat intake was not associated with lung cancer risk in the pooled multivariate model after adjusting for age, education attainment, BMI, alcohol intake, total fruit and vegetable intake, energy intake and smoking status. Such a

conclusion had muted subsequent reports since then. In 2017, a larger pooled analysis including 10 prospective cohorts from the United States, Europe and Asia generated attention to address the association of dietary fat intake with lung cancer risk (70). This recent pooled analysis looked at approximately 1.4 million participants with a median follow-up of 9.4 years. In addition to adjusting for the empirical confounding variables, such as age, sex, smoking status, pack-years of cigarette smoking, education attainment, obesity status, alcohol intake, intakes of total energy and vegetables, this pooled analysis also controlled for family history of lung cancer, race, physical activity level and menopausal status in women. After adjusting for the above-mentioned potential confounders, it showed that the highest fat intake was associated with a 7% increased risk of lung cancer (95% CI, 1.00-1.15). Although this study showed a much lower risk estimate than previous studies, the large sample size of the international cohort consortium with the prospective study design pointed out the potential increased risk of high fat diet for the development of lung cancer.

Specific Types of Dietary Fat and Lung Cancer Risk

Fat is one of the main macronutrients, which is made up of glycerol and fatty acids. Fatty acids play an important role in energy storage, membrane synthesis, and signaling processes (71). Most fatty acids can be derived from either diet or synthesis by humans, except for essential fatty acids such as linoleic acid (also known as omega-6 acid) and alpha-linolenic acid (also known as omega-3 acid) that must be obtained from food intake. Fatty acids are long aliphatic chain, and they may be saturated or unsaturated. The difference between saturated and unsaturated fat lies in the number of double bonds within the fatty acid chain. Saturated

fat lacks double bond, while unsaturated fat has at least one double bond between the individual carbon atoms. Specifically, unsaturated fat can be divided into three major categories: monounsaturated, polyunsaturated and *trans* fat. Monounsaturated fat contains one double bond within the fatty acid chain, polyunsaturated fat contains more than one double bonds, and *trans* fat contains one or more double bonds in a trans geometric configuration.

Saturated fat is found in both animal and plant sources, but it is mainly found in animals. Rich sources of dietary saturated fat include cheese, butter, animal fat, processed meat, palm oil and coconut oil. It has been reported that saturated fat intake is associated with increased risk of cardiovascular disease by increasing serum low-density lipoprotein cholesterol (LDL-C) level (72). The World Health Organization (WHO) suggests that saturated fat intake should be less than 10% of total energy intake, and individuals whose intake of saturated fat is greater than 10% of total energy should reduce their saturated fat intake. Moreover, saturated fat has been identified as a possible risk factor for lung cancer. The majority of case-control studies have found a positive association between saturated fat intake and lung cancer risk (56-59). The risk of lung cancer in subjects with the highest intake of saturated fat was 1.9 to 3-fold higher than that of the lowest intake group. Only two case-control studies reported no association of saturated fat intake with lung cancer (60, 61). The positive association between saturated fat and lung cancer risk was also found in a few prospective cohort studies (63, 70), while most other cohort studies found no positive association (66-69).

Monounsaturated fats can be derived from plant-based foods and animal products. Specifically, monounsaturated fats from plant-based foods include olive, canola and peanut oils, avocados, cashews, almonds, as well as pumpkin and sesame seeds. Animal products from

red meat and high-fat dairy are not only rich in saturated fat but also monounsaturated fat. Monounsaturated fats, especially from plant-based foods, have been suggested to have a beneficial effect on heart diseases (73). In contrast, the evidence for the effect of dietary monounsaturated fat on lung cancer risk showed that high intake of monounsaturated fat is unlikely to provide benefit (58, 59, 63, 67). The findings from epidemiological studies revealed that high intake of monounsaturated fat was associated with a 1.4 to 2.1-fold increased risk of lung cancer. However, some studies did not confirm the relationship between monounsaturated fat intake and lung cancer risk (60, 69, 70). The inconsistent findings may be due to the fact that previous studies on monounsaturated fat intake in relation to lung cancer risk did not differentiate the sources of monounsaturated fats.

Polyunsaturated fats include omega-6 and omega-3 fatty acids that are needed for brain function and cell growth. Polyunsaturated fats have been shown to lower the risk of heart diseases, along with monounsaturated fats. Polyunsaturated fats can be found mostly in nuts, plant-based oils and fatty fish. Food sources of high concentration in polyunsaturated fats include walnuts, sunflower seeds, canola and sunflower oils, salmon and tuna. The results from observational studies have been inconclusive in terms of association between polyunsaturated fat and lung cancer risk. Furthermore, although the majority of cohort studies indicated no association between polyunsaturated fat intake and lung cancer risk (63, 69, 74-76), a few cohort studies demonstrated the potential benefit of polyunsaturated fat intake for lung cancer (70, 77). One cohort study conducted in Japan observed that high fish consumption was associated with an 81% reduced risk of lung cancer (95% CI, 0.08-0.46) (77). A recent pooled analysis of cohort studies also indicated that participants who consumed high amounts of

polyunsaturated fats had an 8% lower risk of lung cancer than those who consumed low amounts (95% CI, 0.87-0.98) (70). Moreover, several case-control studies reported a significantly lower risk of lung cancer that was linked with high consumption of polyunsaturated fats (78, 79). Nonetheless, some case-control studies displayed either no association (58, 60, 80) or a positive association (59) between polyunsaturated fat intake and lung cancer risk.

Dietary *trans* fat is mainly obtained from industrially produced food, especially in hardened vegetable fats used for frying, baked goods, processed snack foods, and margarine. A small amount of *trans* fat can also be found naturally in high-fat dairy products (such as butter and whole milk) and meat from ruminants animals (such as cattle and sheep). Studies have demonstrated that *trans* fat has an unfavorable effect on cardiovascular diseases because *trans* fat increases serum LDL-C level and decreases serum high-density lipoprotein cholesterol (HDL-C) level (81). Intake of *trans* fat may also be associated with cancer development through its effects on inflammation and oxidative stress (82). On the other hand, the relationship between *trans* fat intake and lung cancer risk has not been extensively investigated in epidemiological studies, although the available evidence suggests no association between *trans* fat intake and lung cancer risk (66, 83). Therefore, more studies are needed to clarify the relationship between *trans* fat intake and lung cancer risk.

Dietary Intake and Survival for Lung Cancer Patients

Lung cancer patients often experience malnutrition that can reduce their survival (84, 85). Some patients already have nutritional deficiencies when they are diagnosed with lung cancer,

particularly for those with advanced stage or metastatic disease (86). The low blood nutrient levels may result from unbalanced diets. Cancer can cause profound metabolic and physiological alterations, and thus patients may consume inadequate proportions of macronutrients (such as carbohydrates, fats and proteins) or micronutrients (such as vitamins and minerals) (87). In addition, lung cancer patients commonly experience adverse effects of treatment such as loss of appetite, nausea, vomiting, changes in taste or smell, pain and fatigue (88). These symptoms may have a negative impact on appetite and food intake, leading to alteration in nutritional status. On the other hand, adverse effects of smoking could also play a role in low blood nutrient levels that may begin prior to cancer diagnosis (89). Smokers tend to have a low concentration of micronutrients such as ascorbic acid (vitamin C) compared to non-smokers. Research has identified adverse effects of smoking on changing the intake, absorption, transport, utilization, and excretion of ascorbic acid (90).

Cancer patients are highly motivated to change their eating patterns to improve their survival. Few epidemiological studies have been done to understand the relationships between dietary intake and survival among lung cancer patients. In 1992, Goodman et al. conducted the first study to assess the dietary determinants of lung cancer survival among lung cancer patients (91). The retrospective cohort study comprised 675 patients who were newly diagnosed with lung cancer in Hawaii. The result of this study noted that increasing consumption of vegetables or fruits prior to cancer diagnosis was associated with a longer survival among women with lung cancer, but not among men. The finding was consistent with investigations of dietary intake and lung cancer risk that have shown the protective effects of vegetables and fruits against lung cancer. Besides, one prospective cohort study conducted in the Danish population,

which included 353 patients with lung cancer, found a tendency towards lower risk of death for patients with higher intake of fruits or vegetables before the diagnosis of lung cancer, but the finding did not reach statistical significance (92). In contrast, high intake of potatoes showed a tendency toward increased hazard of dying. A cohort study in Hong Kong comprising 1,208 patients with lung cancer found that men who consumed preserved and dried foods frequently prior to the diagnosis of lung cancer had an increased risk of death (93). Nevertheless, the associations between meat, fruits and vegetables consumption and lung cancer survival were not statistically significant. This study also found that men who drank alcohol 1-3 days per week before lung cancer diagnosis had a lower risk of death, but the favorable effect on prognosis of lung cancer disappeared in men who drank alcohol more than 3 days per week.

Although previous studies have attempted to investigate the relationship between dietary intake and lung cancer survival (91-93), only one of them has examined the effect of dietary fat intake on survival among lung cancer patients to date (93). However, the study did not differentiate what types of meat were consumed that could mask the real association for lung cancer survival. The types of meat differ significantly in the quality of fats. Higher intake of red meat from animal sources contain high amounts of saturated fat. Few studies have found that higher intakes of red meats were associated with poorer cancer survival (94, 95). Moreover, the relationships between dietary fat intake and survival in patients with breast (96), colorectal (97), prostate (98), gastric (99) and laryngeal cancers (100) have been documented. It is reasonable to look at how dietary fat intake influences survival and prognosis among lung cancer patients.

Potential Mechanisms Relating Dietary Fat to Cancer

Approximately seven decades ago, Knudson postulated that cancer occurs only when it contains two damaged alleles, also known as “two-hit” theory of cancer causation (101). The first “hit” occurs when individuals carry a cancer susceptibility gene or receive a somatic mutation from cancer-causing agents. Some individuals may experience additional somatic mutation in other normal genes, and hence cancer initiates. Cancer is the uncontrolled growth of abnormal cells that involve uncontrolled cellular proliferation, dysregulation of apoptosis, loss of differentiation, angiogenesis, invasion, and metastasis. Certain dietary behaviors containing food mutagens can cause DNA damage or affect cellular control of DNA regulation via methylation (102). The imbalance of forces regulating normal cellular functions can contribute to increased risk of cancer. Previous observational studies have suggested an important role of dietary fat intake in the etiology of lung cancer, and several reports indicated a dose-response relationship between total dietary fat intake and risk of lung cancer (56-59, 62, 63, 70). As mentioned earlier, a previous study in United States reported a significant positive association of red meats contributing to high amounts of saturated fat with lung cancer mortality (94). Besides, several lines of evidence have demonstrated that fat intake was associated with prognosis for breast, colorectal, prostate, gastric and laryngeal cancers (96-100). Dietary fat intake should come into play in terms of the prognosis of lung cancer (103). Although the mechanisms involving dietary fat intake with lung cancer initiation and progression are not fully understood, a growing body of evidence indicates that dietary fat

intake may promote cancer development and progression by altering inflammatory responses and oxidative stress.

It is well-known that inflammation plays a critical role in tumorigenesis by supplying important pro-growth signals to the tumor microenvironment. Increased dietary intake of saturated fat and *trans* fat could induce chronic inflammation and thereby promote tumor development. It has been consistently found that saturated fat and *trans* fat in diet are associated with up-regulation of inflammatory markers, such as interleukin-6 (IL-6), C-reactive protein (CRP) and adhesion molecules (104, 105). The mechanism of how saturated fat and *trans* fat mediated inflammation is complex and poorly understood. Substantial research has explored how saturated fat intake exacerbates inflammation. The inflammatory response appears to take place following saturated fat intake, and could last for 4-8 hours. Moreover, it could recur after repeated consumption of saturated fat (106). The positive association between saturated fat intake and concentration of inflammatory markers is observed particularly in overweight participants (107), possibly through activation of nuclear factor-kappa B (NF-kB) signaling (105). NF-kB is a transcription factor involving in several key cellular and organismal processes, such as immune and inflammation responses, cell survival and proliferation. NF-kB activation is constitutively seen in many solid tumors, including lung cancer (108), either by mutation or epigenetic mechanisms (109). The activation of NF-kB supplies tumor-promoting cytokines to the tumor microenvironment that favors cancer initiation, progression and metastasis (110).

Additionally, dietary intake of linoleic and alpha-linolenic acids may induce inflammation through modulation of eicosanoids that may in turn influence tumorigenesis. It is widely

considered that linoleic acids have a proinflammatory effect, whereas alpha-linolenic acids have an anti-inflammatory effect. However, not all of the available evidence supports this contention. Several studies revealed no association between linoleic and alpha-linolenic acids intake and inflammatory markers such as IL-6 or CRP (111-113). Only one study reported an inverse association between alpha-linolenic acids intake and IL-6 concentrations (114). Eicosanoids are bioactive signaling lipids, which have been implicated in the inflammation processes. Elevated levels of prostaglandin E2 (PGE2), a proinflammatory eicosanoid, are found in several human malignancies, including lung cancer (115). The up-regulation of PGE2 could promote proliferation, migration and invasion of tumor cells, and is associated with decreased survival among these cancer patients (116).

Notably, high-fat intake is linked to increased levels of oxidative stress (117, 118) that could lead to tumorigenesis. Oxidative stress is an imbalance between reactive oxygen species (ROS) and antioxidants. The accumulation of ROS not only leads to chronic inflammation, but also increases DNA damage, thus cancer initiation (119). The ROS-induced DNA damage involves DNA strand breaks and DNA cross-links, which induces changes in transcription and signaling factors, replication errors and genomic instability (120). For example, p53, a transcription factor considered guardian of the human genome which regulates several important intracellular pathways, is frequently mutated in solid tumors, including lung cancer (121). Mutations of *TP53* gene are induced by ROS, and would lead to loss of its tumor suppression functions (122). Therefore, mutant p53 is unable to trigger cell cycle arrest, DNA repair and apoptosis in response to oncogenic stimuli. In addition, mutant p53 could also gain oncogenic

functions that facilitate the energy supplies of tumor cells. As a consequence, p53 mutations are related to rapid tumor cell proliferation and increased cell migration and invasion (123).

Genetic Variants Modify Lung Cancer Prognosis and Fatty Acid Metabolism

As detailed above, evidence from candidate gene and GWAS approaches have indicated that several SNPs are associated with the risk of lung cancer. However, few studies have focused on lung cancer disease prognosis. Unlike identification of susceptibility loci or genes for the risk of lung cancer, identification of SNPs for cancer prognosis must account for additional confounding variables in the analysis, such as clinical characteristics, histology, treatment regimens and follow-up information. These requirements may hinder progress in the search for identifying genetic variants as prognostic factors for lung cancer using the GWAS approach. Even with these extra hurdles, a number of SNPs related to lung cancer survival have been identified by GWAS, although some findings may be false positive genetic associations. Several findings provide promising results that are reproducible in additional studies and in different ethnic groups. For example, SNP rs1878022 in the chemokine-like receptor 1 (*CMKLR1*) gene is associated with decreased overall survival in NSCLC patients who are treated with platinum-based chemotherapy. The association between the genetic variation and lung cancer survival was first identified and validated in non-Hispanic whites with NSCLC, and then replicated in African Americans (124, 125).

Moreover, multiple lines of evidence from GWAS suggested that fatty acid metabolism is modified by gene variation among healthy individuals. Specifically, SNPs in fatty acid synthase (*FASN*), fatty acid desaturase 1 (*FADS1*), fatty acid desaturase 2 (*FADS2*), fatty acid

elongase 2 (*ELOVL2*), fatty acid elongase 6 (*ELOVL6*), acyl-CoA dehydrogenase short chain (*ACADS*), acyl-CoA dehydrogenase medium chain (*ACADM*) and acyl-CoA dehydrogenase long chain (*ACADL*) have been found to be associated with alterations in fatty acid metabolism (126-129). These genetic variants across the different genes are enzymes in *de novo* fatty acid synthesis, thereby affecting both systemic and localized fatty acid metabolism.

Fatty Acid Metabolism and Cancer

The tumorigenesis process has a profound impact on the metabolic status of the cell (130, 131). Instead of anabolic and catabolic pathways that are regulated by nutrient availability in normal cells, tumor cells grow in an uncontrolled manner even under nutrient scarcity (132). Tumor cells alter metabolism of carbohydrates, fats and proteins to meet increased energy demands and accumulate the metabolic needs to support cell growth and proliferation (131, 133). Previously, less research efforts have been made to investigate alterations in fat metabolism in tumor cells, while more recently the importance of alterations in fat metabolism is increasingly being recognized (134, 135).

There are several enzymes involved in *de novo* fatty acid synthesis that have been related to tumorigenesis consisting of three stages: initiation, progression and metastasis. First, ATP-citrate lyase (ACLY) is one of the main enzymes involved in fatty acid synthesis. It has been shown that ACLY plays a role in tumorigenesis and is highly expressed in several cancers, including lung (136), bladder (137), colorectal (138) and glioblastoma cancers (139). Inhibition of ACLY could promote cell apoptosis and differentiation, which is a promising therapeutic target for cancer treatment (140). Second, acetyl-CoA carboxylases (ACACs) are

upregulated in several cancers, which have been postulated to be a therapeutic window for cancer. ACACA, one subtype of ACACs, is enriched in lipogenic tissues and mainly controls fatty acid synthesis. Knockdown of ACACA by small interfering RNA (siRNA) triggers apoptosis in prostate cancer and breast cancer cells (141, 142). With regard to lung cancer, inhibition of ACACs expression causes detrimental effect of tumor growth in preclinical models (143). Third, fatty acid synthase (FASN) is the most studied fatty acid enzyme with regard to cancer (144). Overexpression of FASN is observed in patients with sarcomas (145), endometrial (146) and colorectal (147) cancers. In addition, FASN is strongly correlated with tumor aggressiveness in breast cancer and lung cancer cells (148, 149). Lastly, stearoyl-CoA desaturase (SCD) is an enzyme involved in the synthesis of unsaturated fatty acids (150). SCD1 is the predominant isoform, and is ubiquitously expressed among tissues (150). SCD1 plays a key supporting role in many cancers such as lung (151), breast (152) and prostate (153). Studies have indicated that reduction of SCD1 in lung cancer cells contributes to a decrease in the proliferation rate, invasiveness and survival (154, 155).

Public Health Significance

Lung cancer is a disease of great public health concern and the number of deaths still continues to grow worldwide (1). It is widely recognized that environmental factors contribute to tumorigenesis (130). Diet can be one important domain of environmental exposures that can modulate a key hallmark capability of tumor cells (156). Growing evidence indicates a plausible role of dietary fat in cancer development and progression (157). While much scholarly attention has been paid to the association of dietary fat with breast cancer, few

investigations have been carried out in terms of lung cancer. Several studies have looked into the relationships between total and specific types of dietary fat and lung cancer risk, but only one study to date has examined the link between meat intake and survival among lung cancer patients (93). Lung cancer is a heterogeneous disease, and non-small cell lung cancer (NSCLC) is the most common type of lung cancer. Although more treatment options are available for NSCLC, the overall survival of patients with NSCLC remains low (158). As a result, there is a need to better understand the effect of dietary fat intake on overall survival and recurrence among NSCLC patients, which can provide specific dietary recommendations for NSCLC patients in order to improve survival and prognosis.

In addition to acquiring fatty acids from dietary fat intake, fatty acids can be derived from *de novo* synthesis that can be modulated by genetic variation. Previous studies have identified that several single nucleotide polymorphisms (SNPs) across fatty acid metabolism-related genes could affect fatty acid synthesis (126-129), and some of these genes could be potential lung cancer biomarkers (159). Nevertheless, there is limited evidence to support the associations between SNPs in fatty acid metabolism genes and survival and prognosis of patients with NSCLC (160), and the study simply focused on the genetic variants in a few genes related to fatty acid metabolism. Since fatty acids are essential for tumor cells to perform rapid cell membrane production and intracellular signaling transduction (71), it is required to conduct a systematic search for genetic variants in all genes involved in the fatty acid metabolism that could be a promising biomarker to improve survival and prognosis prediction of NSCLC.

Research Question and Hypotheses

The purpose of this dissertation is to examine the relationships between dietary fat and clinical outcomes among patients with NSCLC. It has been documented that fatty acids, one component of fat, are not only energy sources and membrane constituents, but also regulate a variety of biological activities that influence human health, including carcinogenesis and cancer progression. The different types of fat can be consumed through foods and are determined by the chemical structure of fatty acids. Fatty acids can also be synthesized in the human body, which could be affected by genetic variation in fatty acid metabolism-related genes. Therefore, this research aims to investigate the roles of dietary fat intake and single nucleotide polymorphisms (SNPs) in fatty acid metabolism-related genes in overall survival and recurrence among patients with NSCLC.

Hypothesis 1: Dietary fat intake is associated with clinical outcomes in NSCLC patients.

Dietary fat may promote carcinogenesis by altering oxidative stress and inflammatory responses, thereby providing the microenvironment that favors cancer initiation, recurrence and progression. Several epidemiologic studies have evaluated the role of dietary fat in lung cancer risk, demonstrating that high intake of total fat is associated with an increased risk of lung cancer. However, whether high intake of total fat and specific types of fat in diet has an impact on overall survival and recurrence in patients with NSCLC remains unclear.

Hypothesis 2: SNPs in fatty acid metabolism-related genes are associated with clinical outcomes in NSCLC patients.

The importance of *de novo* fatty acid metabolism in tumorigenesis has been confirmed. Numerous studies have found that genetics and gene expression involving fatty acid

metabolism can contribute to tumor cell growth and survival. In particular, SNPs in fatty acid metabolism genes could modify fatty acid metabolism. Given the close link between fatty acids and cancer, dysregulation of fatty acid metabolism may lead to malignancy and increase migration and invasion of tumor cells. Nevertheless, the associations between SNPs in fatty acid metabolism-related genes and overall survival and recurrence in NSCLC patients have not been systematically investigated in the past.

CHAPTER II: METHODS

Study Population and Data Collection

Study participants were accrued from a large ongoing cohort study of lung cancer at University of Texas MD Anderson Cancer Center. All study participants had to be newly-diagnosed ($\leq$ 1 year before recruitment) with histologically confirmed lung cancer prior to treatment between 1995 and 2008. There were no recruitment restrictions on age, sex, ethnicity, histology and stage. At study enrollment, all study participants underwent a 45-minute in-person interview by trained staff. A structured questionnaire was used to collect information on demographics, smoking status, personal medical history, physical activity, and family history of cancer. A separate nutrition questionnaire (see below) was administered to collect dietary information. In addition, a 40 mL peripheral blood sample was drawn at the end of each interview. Clinical and follow-up information was abstracted from medical records by trained staff, including date of diagnosis, clinical stage, pathologic stage, tumor grade, treatment type, tumor recurrence, dates of recurrence, vital status, and date of last follow-up or death.

Because non-small cell lung cancer (NSCLC) accounts for the majority of lung cancers and the small number of cases were either Hispanic or other race/ethnic groups, the analysis of the study participants was restricted to non-Hispanic whites with NSCLC. In the first part of this study, participants with incomplete food frequency questionnaire, outlying or implausible energy intakes were excluded (Figure 1). An outlying energy intake was a value outside the interval delimited by the 25^{th} percentile minus 1.5 times the interquartile range

(IQR) and the 75th percentile plus 1.5 times the IQR. The values for energy intake below 500 kcal/d or above 5000 kcal/d were defined as implausible energy intakes. In the second part of this study, participants were restricted to patients with genotyping results from either genome-wide association study (GWAS) or OncoArray approach. This study employed a two-phase design. Participants were divided into the discovery set and the replication set. Patients who were included for genotyping in GWAS were in the discovery set, whereas those who were genotyped in OncoArray were in the replication set (Figure 2). In the discovery phase, genotype data from GWAS were used to identify genetic variants of fatty acid metabolism genes that were associated with overall survival and recurrence among NSCLC patients. Single nucleotide polymorphisms (SNPs) that reached statistical significance were candidates for further validation in the replication set.

Figure 1: Schematic of study design for the first aim

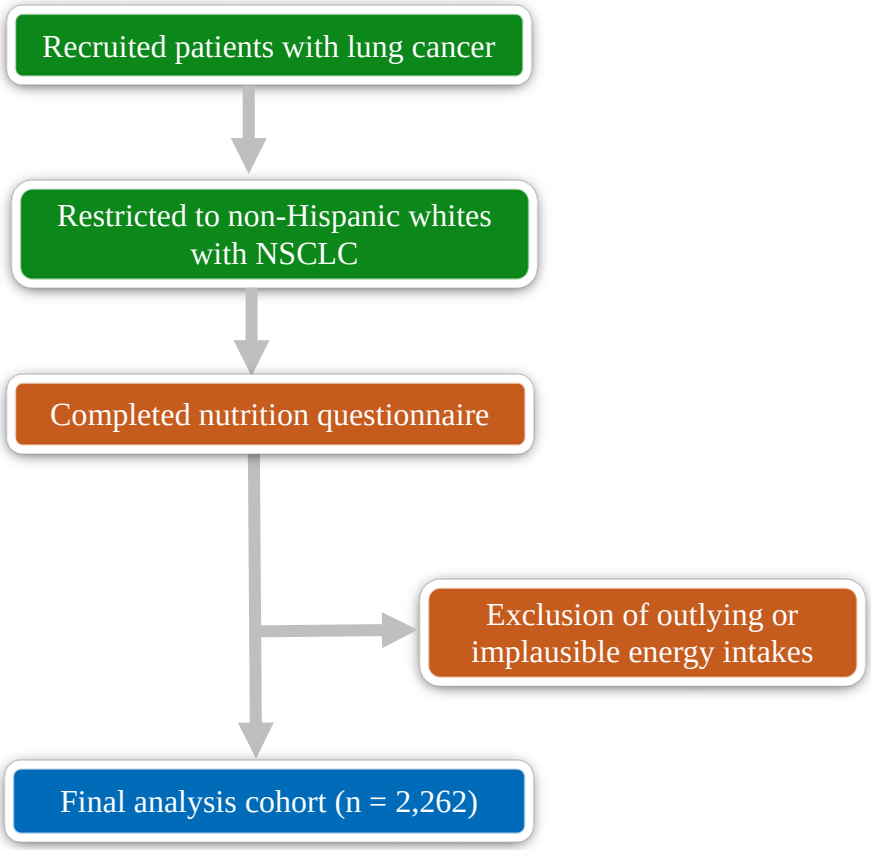
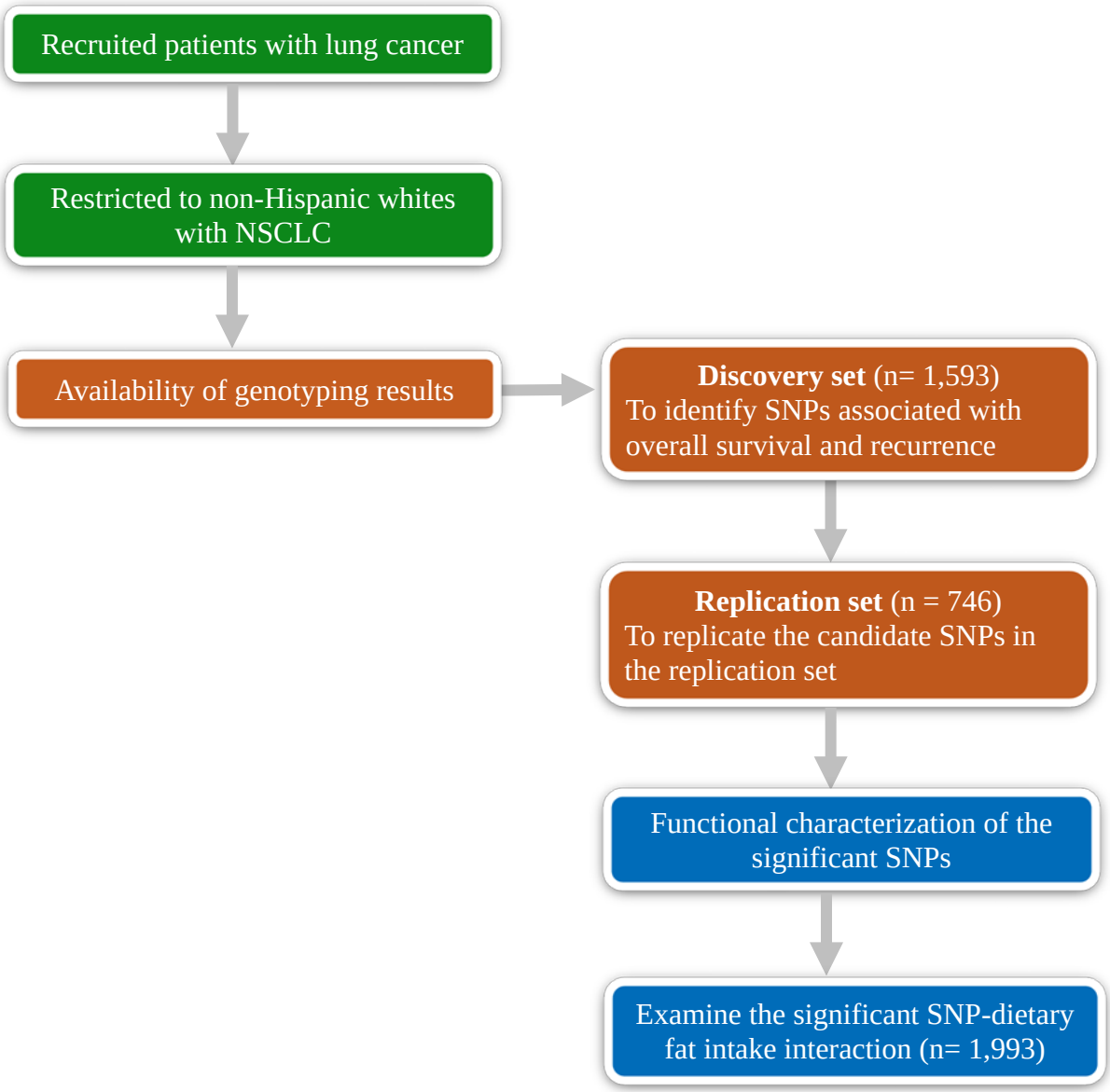


Figure 2: Schematic of study design for the second aim



Dietary Assessment

A modified version of the National Cancer Institute's Health Habits and History Questionnaire (HHHQ) was used to assess dietary intake during the year before cancer diagnosis. The HHHQ is a food frequency questionnaire designed to assess the role of dietary factors in disease morbidity and mortality (161), consisting of a wide array of nutrients and food groups. It has been shown that the HHHQ is a valid food questionnaire and has been widely used in numerous studies in the United States (162, 163). In our modified food frequency questionnaire, 165 food and beverage items and groups were listed, including the major nutrients in the American diet as well as ethnic foods commonly consumed in the Houston area. The food questionnaire asked information about the frequency (rare or no, the number of times a day, week, month or year) and portion sizes of food and beverage items frequently consumed (the number of pieces 1, 2, 3, or 4, and the number of cups ¼, ½, 1, or 2), and eating behaviors other than the frequency and portion size of intake of specific items, such as food preparation methods and dining at restaurants, either in categories or in an open-ended manner. Based on the United States Department of Agriculture (USDA) National Nutrient Database for Standard Reference (164) and USDA Food and Nutrient Database for Dietary Studies (165), total energy intake and energy amount consumed for each item were calculated.

Fatty acids are a group of lipids that are available from a variety of dietary sources. This study collected information about the consumption of foods and beverages that were associated with fat intake using our modified food frequency questionnaire. For example, how many times and portion sizes of meat, poultry and fish items (beef steaks, beef roasts, pork chops or roasts,

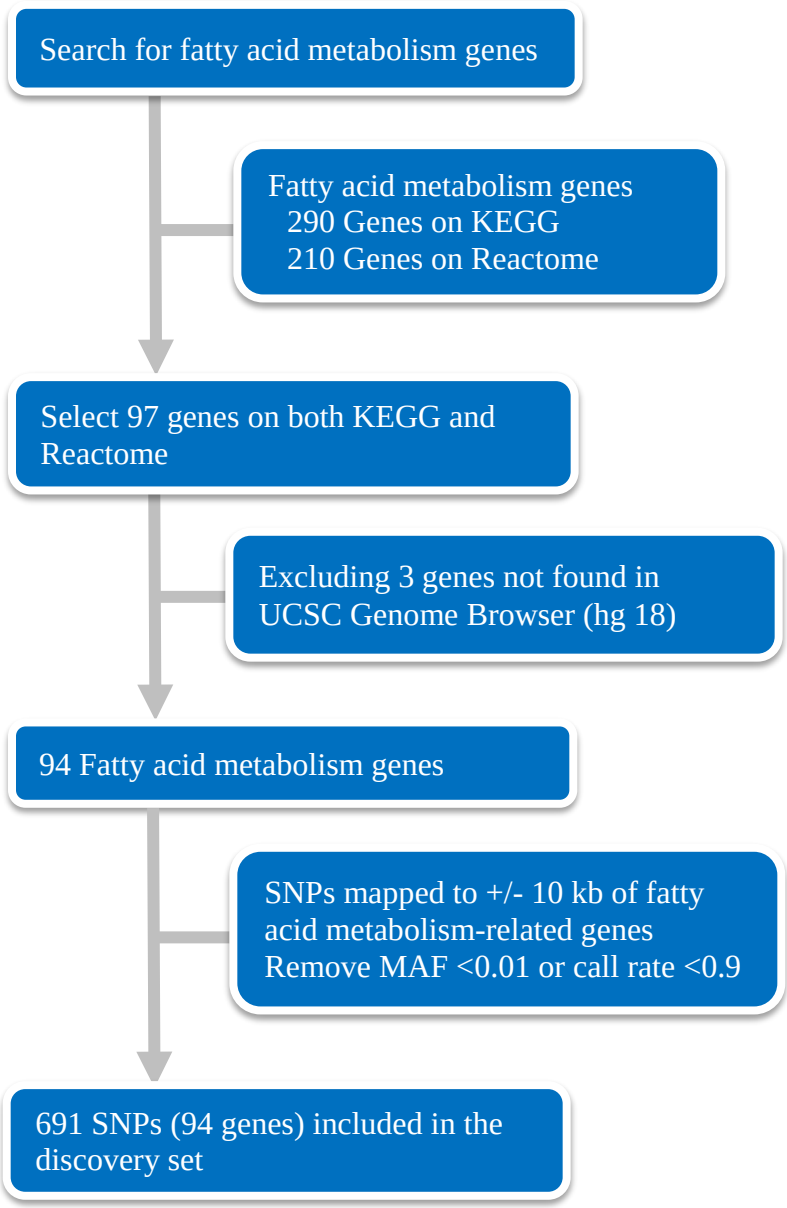
dinner ham, hot dogs, bacon, sausage or chorizo, chicken, tuna fish, shellfish, gravies, and hamburgers), desserts, sweets and snacks (nachos with cheese or potato skins with topping, potato chips, pretzels, corn chips, tortilla chips or popcorn, peanut butter, peanuts, sunflower seeds, soy nuts, power bars, and breakfast bars or granola bars), and dairy (glasses of milk, yogurt or frozen yogurt, cottage cheese, cream cheese, sour cream, margarine, eggs, biscuits or muffins, and pancakes, waffles or French toast) were consumed during the year before cancer diagnosis? Fat intake was expressed as the actual quantity of fatty acid in g per day.

Genes and SNPs selection

The schematic of fatty acid metabolism genes and SNPs selection is displayed in Figure 3. In order to analyze genetic variants of a whole genes involved in fatty acid metabolism, bioinformatics resources were applied. Both bioinformatics resources from Reactome (reactome.org/) and Kyoto Encyclopedia of Genes and Genomes [(KEGG) genome.jp/kegg/] were used to search for fatty acid metabolism genes (accessed December 19, 2018). The search included genes involved in fatty acid biosynthesis, elongation and degradation, arachidonic acid metabolism, alpha-linolenic and linoleic acid metabolism, glycerolipid metabolism, glycerophospholipid metabolism, and sphingolipid metabolism. A total of 97 genes were identified that were included in both bioinformatics resources. After excluding genes that were not found in The University of California Santa Cruz (UCSC) Genome Browser [genome.ucsc.edu, (n=3)], 94 genes were reported to be associated with fatty acid metabolism. A total of 691 SNPs mapped to 10 kb upstream or 10 kb downstream of these 94 fatty acid metabolism genes were included in the discovery set (see Appendix A). rAggr (raggr.usc.edu/)

was used to identify proxy linked SNPs as substitutes for those SNPs that were not directly genotyped in the replication set. The 1000 Genomes Phase III database was used to identify the linked SNPs that were in linkage disequilibrium with minor allele frequency at least 1% and r^2 of 80% or higher among European ancestry.

Figure 3: Schematic of genes and SNPs selection



Genotyping and Quality Control

DNA for each patient was isolated from peripheral whole blood using the QIAamp DNA extraction kit (QIAGEN, Valencia, CA). In the discovery set, genotyping was previously performed using the HumanHap 300k and HumanHap 660k BeadChips (Illumina, San Diego, CA) and analyzed using BeadStudio software (Illumina). In the replication set, Infinium Oncoarray-500K BeadChip on the iScan system (Illumina) was previously used for genotyping and the array data were analyzed using GenomeStudio software (Illumina). All genotypes were performed according to the manufacturers' instructions. All individuals had a call rate of 90% or higher and a minor allele frequency at least 1%.

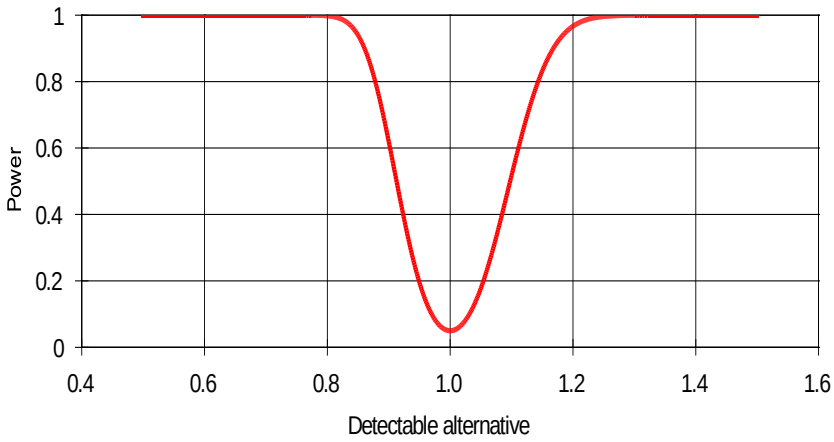
Power Calculations

Since this study used existing data, the sample size was fixed. The detectable alternative was the true hazard ratios of overall survival in the exposed group relative to that in the non-exposed group. Specifically, the detectable alternative was detected with a specified power given the type I error probability, the number of sample size for exposed group, the ratio of non-exposed group to exposed group, and median survival time on the non-exposed group. The detectable alternative was calculated individually for hypothesis 1 and hypothesis 2. In both hypotheses, an 80% probability of correctly rejecting the null hypothesis was set and a recruitment period of 1 year was chosen after which a subsequent 5-year period was allowed for follow-up.

In hypothesis 1, the study had 2,262 patients with NSCLC. To simplify the calculation of the detectable alternative, fat intake was considered in binary categories using median intake

of fat as a cutoff point. Accordingly, 1,131 patients had low fat intake with a median survival time of 2.5 years. Under this assumption, the detected true hazard ratios of failure for patients with high fat intake relative to those with low fat intake were 0.88 or 1.14 with a power of 0.8 and a two-tailed significance level of 0.05 (Figure 4).

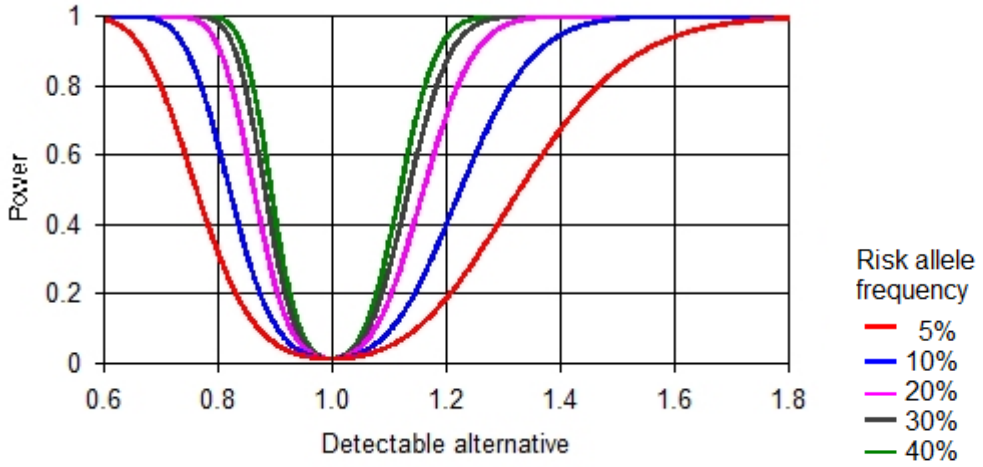
Figure 4: A plot of detectable alternative hazard ratio for the first aim



In hypothesis 2, the study had 2,339 patients with NSCLC whose peripheral blood samples were genotyped using either GWAS or the OncoArray approach. Assuming that a SNP relative to allelic frequency for the risk allele is 10%, 234 patients carried the risk allele and 2,105 patients did not have the risk allele. The median survival time for those who carried genotype without the risk allele was 2.5 years. Under this context, the detected true hazard ratios of failure for patients with the risk allele relative to those with the risk allele were 0.77 or 1.32 with a power of 0.8 and a two-tailed significance level of 0.01. A plot of detectable alternative

hazard ratios of overall survival under the risk allele frequency of 5%, 10%, 20%, 30% and 40% is shown in Figure 5.

Figure 5: A plot of detectable alternative hazard ratio for the second aim



Statistical Analysis

All analyses were performed with Stata (College Station, TX), and 2-sided $p<0.05$ was considered statistically significant. In the first part of this study, the dietary intake was energy-adjusted by using the nutrient density method prior to further analysis (166). Intakes of total fat, saturated fat, monounsaturated fat and polyunsaturated fat in diet were categorized according to the Dietary Reference Intakes (DRIs). To determine whether demographic and behavioral characteristics lead to different amounts of fat intake in patients with NSCLC, the ANOVA test and chi-square test were used to compare the differences. The Cox proportional hazards model was performed to calculate the hazard ratios (HRs) and 95% confidence intervals for the associations between total fat and specific types of fat in diet and clinical

outcomes among NSCLC patients. Person-time was calculated individually for overall survival and disease recurrence. Overall survival was defined as the period from the date of diagnosis to the date of death from any cause, or the last follow-up, whichever came first. Disease recurrence was defined as the date of diagnosis to first recurrence, or the date of the last follow-up, depending on which date came first. Recurrence was defined as tumor growth in adjacent to the planning target volume or surgical resection in the ipsilateral hilum or mediastinum, or new sites of involvement in lymph nodes or distant organ after curative resection. The multivariable Cox model was adjusted for potential confounders based on a priori knowledge, such as age at cancer diagnosis, sex, education attainment, BMI, smoking status, clinical stage, pathology, and total energy intake.

In the second part of this study, a two-phase design was employed. First, the associations between SNPs in fatty acid metabolism genes and overall survival and recurrence among NSCLC patients was examined in the discovery set. SNPs with significant associations from the discovery set was examined whether they had consistent associations and reach statistical significance in the replication set. The comparison of selected characteristics in the discovery and replication sets was performed using the chi-square test for categorical variables and Student *t* test for continuous variables. Person time was calculated individually for overall survival and recurrence from the date of study enrollment to the date of death/recurrence, or the last follow-up, depending on which date came first. The Cox proportional hazards model with the estimates of HRs and 95% confidence intervals were employed to assess the associations between SNPs in fatty acid metabolism genes and clinical outcomes for each phase among patients with NSCLC. The multivariable Cox proportional hazards model with

adjustment for age at diagnosis, sex, smoking status, clinical stage, performance status, and treatment regimen were estimated in both sets. All genetic models of inheritance including the dominant, recessive and additive models were taken into consideration in the assessment. The genetic model with the smallest p value is considered as the best-fitting model. Q value was used to control the false discovery rate. The significant SNPs were selected if the SNP had p value < 0.05 and Q value < 0.20 in the discovery set, and p value remained less than 0.05 in the replication set as well as consistent associations with overall survival or recurrence in both discovery and replication sets. We conducted functional characterization of the significant SNPs that were associated with clinical outcomes among patients with NSCLC. Finally, we examined the associations of the significant SNPs in fatty acid metabolism genes and dietary fat intake with overall survival and lung cancer recurrence.

Human Subjects Considerations

The data set was comprised of data collected by at the University of Texas MD Anderson Cancer Center. All study participants provided written informed consent prior to participating in the study. The study has been approved by the University of Texas MD Anderson Cancer Center Institutional Review Board. All identifying variables were removed and only de-identified patient data were provided to the researcher. No risk of identification to study participants nor any further contact with study participants occurred during the conduct of this study. The dataset was stored on the University of Texas MD Anderson Cancer Center server with secure firewalls and accessed using secure passwords. This study has been granted exempt

status by the University of Texas Health Science Center Committee for Protection of Human Subjects (study number: HSC-SPH-19-0085, reference number: 183036) (see Appendix B).

CHAPTER III: JOURNAL ARTICLE 1

Title of Journal Article: Is Dietary Fat Intake Associated with Overall Survival and Recurrence in Non-small Cell Lung Cancer? An Evidence-based Cohort Study

Name of Journal Proposed for Article Submission: ***The American Journal of Clinical Nutrition***

Abstract

Higher dietary fat intake is associated with increased risk of lung cancer. It remains unclear whether total and subtypes of dietary fat intake have an impact on prognosis among patients with non-small cell lung cancer (NSCLC). We prospectively examined intakes of total, saturated, monounsaturated, and polyunsaturated fat in relation to overall survival and recurrence in NSCLC. In a cohort of 2,262 patients newly diagnosed with NSCLC, dietary fat intake at diagnosis was assessed with a previously validated food frequency questionnaire and categorized by Dietary Reference Intakes. During median follow-up of 23 months, we observed 1,594 deaths and 276 recurrences. Multivariable Cox proportional hazard models were used to estimate adjusted hazard ratios (HRs) with 95% confidence intervals (CIs). Neither high intake of total, nor any subtype of fat, was associated with overall survival or recurrence for NSCLC. Analysis of stage-specific overall survival revealed that early-stage patients with high intake of saturated fat were at increased risk of death compared to those who had intake less than the recommended amount (HR: 1.27; 95% CI: 1.02, 1.59; P = 0.03). However, a protective effect was observed in advanced-stage patients who received primary

chemotherapy (HR: 0.84; 95% CI: 0.71, 0.99; P = 0.04). This protective effect was also showed for high intake of monounsaturated fat in this same group of patients (HR: 0.64; 95% CI: 0.43, 0.96; P = 0.03). The association of dietary fat and overall survival is dependent on disease stage and treatment for NSCLC, which suggest that high intake of saturated fat may be harmful for early-stage patients, and sufficient amounts of saturated and monounsaturated fat may have survival benefits for advanced-stage patients who receive primary chemotherapy. These findings provide evidence for nutritional consultation on fat intake for NSCLC patients.

Keywords: dietary factor, fat, total fat, saturated fat, monounsaturated fat, polyunsaturated fat, lung cancer, non-small cell lung cancer, recurrence, survival, clinical outcome

Introduction

Lung cancer is the leading cause of cancer death in the world (1). Up to 56% of patients who are diagnosed with early-stage lung cancer have survived for 5 years or more, compared to 30% and 5% for those with locally advanced and advanced cancers, respectively (2). Of these patients, non-small cell lung cancer (NSCLC) is the most common type of lung cancer (3). Nearly 70% of patients with advanced-stage NSCLC at the time of diagnosis undergo chemotherapy, often combined with radiation therapy (3). Surgical resection remains the optimal treatment for patients with early-stage NSCLC, but 30% to 70% of patients with NSCLC develop recurrent lesions after surgical resection (4). Survival after lung cancer diagnosis greatly depends on tumor stage, and other factors, including tumor histology, age at diagnosis, gender, and performance status (5) which are non-modifiable. Due to a high recurrence rate and low survival rate for lung cancer, there is increasing interest in identifying modifiable risk factors, particularly dietary factors, which may affect lung cancer development and survival.

It has been suggested that dietary fat plays an important role in cancer development and progression (6), and several studies have investigated the link between dietary fat and risk of lung cancer with inconsistent findings (7-13). Case-control studies have shown that high-fat intake was associated with a moderate increased risk of lung cancer (7-10). Yet the findings from small-scale cohort studies have been null (11, 12). A recent pooled analysis using prospective cohorts from the United States, Europe, and Asia with 1.4 million participants suggested a potential increased risk of high fat diet for the development of lung cancer (13). However, there has been very little epidemiologic research on the association between fat

intake and survival for lung cancer patients. To the best of our knowledge, only one study examined this association but showed no evidence for overall survival from lung cancer patients with higher intakes of meats (14). Meat is one kind of dietary source containing various types of fatty acids. Different types of fatty acids are available from a wide variety of foods. This is the first study to prospectively investigate how intakes of specific types of fat as nutrients affect overall survival and risk of recurrence among a large cohort of NSCLC patients.

Methods

Patients and data collection

Study participants were accrued from a large ongoing cohort study of lung cancer at University of Texas MD Anderson Cancer Center. All study participants were newly-diagnosed ($\leq$ 1 year before recruitment) with histologically confirmed lung cancer treated between 1995 and 2008. There were no recruitment restrictions on age, sex, ethnicity, histology, and stage. Because NSCLC accounts for the majority of lung cancers diagnosed and the limited number of cases from Hispanic or other ethnic groups, study participants for the present study were restricted to non-Hispanic whites with NSCLC.

At study enrollment, all study participants underwent a 45-minute in-person interview by trained staff. A structured questionnaire was used to collect information on demographic characteristics, smoking status, personal medical history, physical activity, and family history of cancer. Clinical and follow-up information was abstracted from medical records by trained staff, including date of diagnosis, clinical stage, pathologic stage, tumor grade, treatment type,

tumor recurrence, dates of recurrence, vital status, and date of last follow-up or death. Recurrence was defined as tumor recurring locally, regionally, and at distant metastasis sites after curative resection. Patients with clinical stages I and II were identified as early-stage NSCLC, and those with stages III and IV were classified as advanced-stage NSCLC. Each participant enrolled in this study provided written informed consents, and the study was approved by Institutional Review Board of The University of Texas MD Anderson Cancer Center.

Dietary assessment

During the in person interview, dietary intake during the year prior to NSCLC diagnosis was measured with a modified version of the National Cancer Institute's Health Habits and History Questionnaire that was designed to capture habitual dietary intake. This food frequency questionnaire (FFQ) was previously validated to provide reliable assessments of nutrients and dietary components across various populations (15, 16). The details about our dietary assessment have been previously described (17, 18). Briefly, our modified FFQ consisted of 165 food and beverage items and groups, and participants were asked information about the frequency and portion sizes of food and beverage items frequently consumed. From the dietary information obtained in the FFQ, the United States Department of Agriculture (USDA) National Nutrient Database for Standard Reference (19) and USDA Food and Nutrient Database for Dietary Studies (20) were used to determine the energy content and grams consumed per day for each food item. The caloric values of all food items consumed by the individuals were summed to calculate daily total energy intake. For those participants with

incomplete answers to the food frequency questionnaire, both outlying and implausible energy intakes were excluded. An outlying energy intake was a value that lied outside the interval delimited by the 25th percentile minus 1.5 times the interquartile range (IQR) and the 75th percentile plus 1.5 times the IQR. The values for energy intake below 500 kcal/d or above 5,000 kcal/d were defined as implausible energy intakes.

Since fatty acids are a group of lipids that are available from a variety of dietary sources, fat from red meat, processed meats, dairy products, fish and shellfish, desserts, and snacks were included in this study. Daily fat intakes were calculated by multiplying the fat content of each food item of the specific portion size by the frequency of consumption, and then were summed and expressed as the quantity of specific fatty acid in grams per day. Energy-adjusted dietary intakes were obtained by nutrient density method on intakes of total, saturated, monounsaturated and polyunsaturated fat. They were calculated by the percentage of calorie intakes from total and specific types of fat over total energy intake. Based on the Dietary Reference Intakes (DRIs), the recommended daily intake is 20-35% for fat, 6-10% for saturated fat, 10-15% for monounsaturated fat, and 5-10% for polyunsaturated fat (21). High intakes of total and specific types of fat were defined as the percentage of fat intakes being greater than the recommended intakes, whereas low intakes were the percentage of fat intakes below the recommended amounts.

Statistical analysis

Dietary intakes of total, saturated, monounsaturated, and polyunsaturated fat were energy-adjusted prior to further analysis (22), and then were classified into three categories based on

DRIs for fat. Chi-square tests were used to compare the proportion fat intake by different groups for categorical variables, and the ANOVA test was used to compare the differences between the means of different groups for continuous variables. Analyses of the associations between dietary fat and covariates with clinical outcomes among NSCLC patients were conducted by using Cox proportional hazards models. Person-time was calculated individually for overall survival and disease recurrence. Overall survival was defined as the period from the date of diagnosis to the date of death from any cause, or the last follow-up, whichever came first. Time to recurrence was defined as the date of diagnosis to first recurrence, or the date of the last follow-up among early-stage patients, depending on which date came first. Because of few patients with low fat intake prior to cancer diagnosis, we combined low and recommended categories into one category in multivariable analysis. The multivariable Cox model estimated hazard ratios (HRs) and 95% confidence intervals (CIs) adjusting for potential confounders based on a priori knowledge, including age at cancer diagnosis, sex, body mass index (BMI) at diagnosis, smoking status, clinical stage, tumor grade, pathology, and cancer treatment. In addition, analyses were conducted stratified by patients diagnosed with early-stage and advanced-stage NSCLC as well as by smoking status, BMI, and cancer treatment type. All analyses were performed with Stata (College Station, TX), and 2-sided p value <0.05 was considered statistically significant.

Results

Host characteristics of NSCLC patients

The distribution of fat intake of the 2,262 NSCLC patients is presented in Figure 1. More than two-thirds of patients met the recommended intakes of total, monounsaturated, and polyunsaturated fat, whereas 62% of patients exceeded the recommendations for saturated fat. Overall, study participants had a higher proportion of current or former smokers, lower BMI, advanced clinical stage, poorly differentiated tumors, and adenocarcinomas (Table 1). Patients with high intakes of total, saturated, or monounsaturated fat were more likely to be younger at cancer diagnosis, male, current smokers and obese (Table 1 and Supplemental Tables 1-3). In contrast, patients with high intake of polyunsaturated fat were more likely to be female. There were no significant differences between the three groups of dietary fat intake (low, recommended, and high) and other demographic, behavioral, and clinical characteristics, or cancer treatment, except that patients with chemoradiation therapy had a higher proportion of low total fat intake.

Dietary fat intake and risk of overall survival and recurrence

After a median follow-up of 23 months, 1,594 patients died and 276 tumor recurrences were recorded. Table 2 shows the multivariable-adjusted associations of intakes of total, saturated, monounsaturated, and polyunsaturated fat with risk of overall survival and recurrence. The associations between fat intake and overall survival and recurrence were not significant among NSCLC patients. The risk of overall survival by fat intake was further stratified by cancer stage. Compared to early-stage patients with recommended or lower intake of saturated fat,

those with high intake of saturated fat had a 27% (HR: 1.27; CI: 1.02, 1.59) increased risk of death (Table 2). This unfavorable effect on survival was slightly increased among ever smokers (HR: 1.31; CI: 1.03, 1.67), and the association was stronger among obese patients who had more than double the risk of death (HR: 2.06; CI: 1.16, 3.63; Table 3). Moreover, high intake of saturated fat was suggestive of an increased risk of death among early-stage patients who received surgery only (HR: 1.25; CI: 0.91, 1.72) and combined surgery and chemotherapy (HR: 1.32; CI: 0.95, 1.84) (Table 4). In contrast, although a direct relationship between fat intake and survival was not observed for advanced-stage patients, weight- and treatment-specific effects were observed with high fat intake on overall survival that was in the opposite direction than that shown for early-stage patients. A favorable effect on overall survival was observed in overweight patients with advanced-stage NSCLC who had high intake of monounsaturated fat, with a 47% (HR: 0.53; CI: 0.29, 0.97) lower risk of death (Table 3). In advanced-stage patients receiving primary chemotherapy, high intake of saturated or monounsaturated fat was associated with a 16% (HR: 0.84; CI: 0.71, 0.99) and 36% (HR: 0.64; CI: 0.43, 0.96) decreased risk of death, respectively (Table 4). Findings for all other stratified analyses by stage, smoking status, BMI, and treatment type were null.

Discussion

This prospective cohort study showed no evidence for an association between intakes of total, saturated, monounsaturated, and polyunsaturated fat and overall survival and recurrence in NSCLC patients when patients who were diagnosed with early-stage and advanced-stage were pooled together. Nonetheless, we observed that high intake of saturated fat was associated

with poor overall survival in patients with early-stage NSCLC, whereas patients who had advanced cancer and received primary chemotherapy had improved overall survival. This favorable effect on survival was also identified for monounsaturated fat intake in this same subgroup.

The concerns over dietary fat intake and lung cancer have focused on the risk rather than survival. There has been limited research investigating the effect of dietary fat intake on lung cancer survival. Even though saturated fat has been suggested to play a role in lung cancer development, epidemiologic evidence for the association is inconsistent. Individual studies have shown positive and null association of saturated fat and risk of lung cancer (7-11). The findings from a recent pooled analysis of previous studies showed that the highest consumption of saturated fats was associated with a 14% increased risk of lung cancer (13). Additionally, previous nationally representative studies in United States reported a significant positive association of red meats contributing to high amounts of saturated fat with lung cancer mortality (94, 95). Nevertheless, a cohort study in China showed no significant association between high meat intake and overall survival in lung cancer patients (14). Different types of meat contain varying concentration of fatty acids. Because the study did not specify the types of meat consumed, it could actually hide a real association between meat intake and survival in lung cancer patients. The relationship between high intake of saturated fat and worse survival in patients with breast (23, 24) and prostate cancers (25) has been documented in the United States. Similar to previous studies in other cancer sites, we found a poor overall survival in early-stage NSCLC patients with high intake of saturated fat that exceeded 10% of total energy intake. In addition, the effect of high saturated fat intake on overall survival was more

evident among former and current smokers. It has been known that smoking at the time of diagnosis is an important predictor of poor outcome in patients with early-stage NSCLC (26, 27). Cigarette smoking contains chemical carcinogens which may cause lung carcinogenesis and recurrence (28). Although the mechanisms of how saturated fat intake leads to lung cancer initiation and progression are not fully understood, a growing body of evidence indicates that increased saturated fat may promote cancer development and progression by altering inflammatory responses and oxidative stress (29). It is likely that the association between poor survival and high intake of saturated fat may be exaggerated by smoking.

Nevertheless, we observed a non-significant impact of improvement in overall survival among advanced-stage NSCLC patients with high intakes of saturated fat. The role of dietary fat in cancer prognosis is likely to be a complex association, depending on the stage of cancer. In a cohort of Swedish men with prostate cancer, greater intake of saturated fat was associated with increased risk of prostate cancer death, particularly among patients with localized disease, but no association was observed among those with advanced disease (30). Besides, our findings seemed to suggest that the role of saturated fat intake depends on disease stage and treatment for NSCLC. We found a significant overall survival improvement for high intakes of saturated fat among advanced-stage patients who received primary chemotherapy, but high intake of saturated fat among early-stage patients was associated with worse overall survival. The excess fat in diet have different effects on survival varying by stage of cancer. Among patients with early-stage cancer, excess fat intake may accumulate energy stored in adipose tissue resulting in chronic inflammation (31). As a consequence, proliferation of blood vessels may facilitate tumor cell growth and progression (32). By contrast, among patients with advanced-stage

cancer, excess adipose tissue leads to a better longevity compared to normal or underfed patients because excess adipose tissue may provide nutritional reserve to counterattack the damage made by treatment for advanced cancer (33).

Diet high in monounsaturated fat has been suggested to replace saturated fat due to beneficial effects on heart diseases (34), whereas previous studies have reported no association and a positive association of monounsaturated fat intake with risk of lung cancer (9-11, 13, 35, 36). The effect of monounsaturated fat on risk of lung cancer does not yield a similar association for survival in lung cancer patients. Although the association between monounsaturated fat and survival has not been examined in lung cancer patients, there are many studies looking at the role of monounsaturated fat in other cancer sites, including breast (24), prostate (25) and stomach (37). These studies showed that higher monounsaturated fat intake was neither associated with all-cause nor cancer-specific mortality, although the protective association was observed for prostate and gastric cancers, and the detrimental association was reported for breast cancer. Our study found that advanced-stage patients who had high intake of monounsaturated fat and received primary chemotherapy had a lower risk of death. Previous research indicated that monounsaturated fat intake has anti-inflammatory properties and thus may suppress tumorigenesis (38), which might explain the improvement in survival for patients with high intake of monounsaturated fat. Further research is needed to confirm the protective effect of high intake of monounsaturated fat for patients with advanced-stage NSCLC.

In addition, a greater magnitude of improved survival for high intake of monounsaturated fat that was more than 15% of total energy intake was found in overweight patients who were

diagnosed with advanced-stage, but was not observed in obese patients. Excess weight has been linked to greater mortality of various cancers, including gastrointestinal, prostate, and breast cancers (39). However, it has been suggested that increased BMI in patients with advanced-stage lung cancer conferred favorable survival (40, 41), and early-stage patients with a higher BMI demonstrated a significant improvement of overall survival (42), both of which were in line with our findings. Our study observed a poor overall survival for the influence of high intake of saturated fat among early-stage patients with obesity. It is possible that excess weight has different effects on overall survival varying by stage of cancer. Similar to excess fat intake in early-stage patients, excess weight increases the storage of energy in adipose tissue and alters metabolic and inflammatory characteristics that facilitate the microenvironment favoring tumor initiation and progression. Patients with advanced-stage cancer may benefit from the metabolic reserves of body fat to withstand treatments (33).

There are several strengths of this study. On the one hand, we prospectively explored the relations between total fat and subtypes of fat intake at diagnosis and clinical outcomes of NSCLC patients, and moreover examined the effects of fat intake under different disease stages, treatments, BMI and smoking status. On the other hand, this study used a previously validated FFQ to collect food intake, and was able to adjust for potential confounding factors to minimize the possibility of confounded associations. There are also several limitations of this study. First, the dietary information was based on self-reported data, and study participants were asked to recall what they had eaten 1 year prior to cancer diagnosis. It is likely that a person may be unable to precisely recall and answer the habitual food that he/she actually consumed over the past year, resulting in measurement errors in dietary data. Nevertheless, the

measurement errors tend to be non-differential of clinical outcomes. Second, study participants were limited to non-Hispanic whites, and thus the study results may not be directly generalizable to the other ethnic groups. Third, although the sample size of this study was large, the number of patients with low fat intake was relatively small, providing limited statistical power. Therefore, we combined low fat intake with recommended fat intake to evaluate the effects of dietary fat intake on clinical outcomes by comparing patients with high fat intake with those who consumed the least amounts. Besides, only a small proportion of patients consumed high polyunsaturated fat and developed recurrent disease, which indicated a lower precision and statistical power. Lastly, despite this study took potential epidemiologic and clinical factors into consideration for adjustment in the analysis, the possibility of uncontrolled confounding factors could not be completely excluded. Although this study has the above-mentioned limitations, the findings of this study may fill a gap of knowledge about dietary fat and clinical outcomes in patients with NSCLC.

To sum up, our results indicate that the relationship between dietary fat intake and overall survival among NSCLC seems to depend on the subtypes of fat and clinical stage. We observed a poor overall survival for early-stage NSCLC patients with high intake of saturated fat, but did not find similar patterns for total fat and other subtypes of fat. Besides, high intakes of saturated and monounsaturated fat were associated with improved overall survival among advanced-stage NSCLC patients who received primary chemotherapy. However, there were no consistent associations between fat intake and disease recurrence. Our study suggests that early-stage NSCLC patients may need to reduce consumption of saturated fat,

and those who have advanced-stage and receive primary chemotherapy may need to get enough fat intake.

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Table 1: Characteristics of NSCLC patients by total fat intake

Characteristics		All (n = 2,262)	Total fat intake[1], n (%)[2]			*p* value[3]
			Low (n = 53)	Recommended (n = 1,402)	High (n = 807)	
Age at diagnosis, year		63.0 ± 10.8[4]	64.2 ± 11.6	63.3 ± 11.0	62.4 ± 10.4	0.03
Sex	Men	1161 (51.3)	29 (54.7)	689 (49.1)	443 (54.9)	
	Women	1101 (48.7)	24 (45.3)	713 (50.9)	364 (45.1)	0.03
Education	High school or less	850 (37.6)	21 (39.6)	488 (34.9)	341 (42.4)	
	Some college	741 (32.8)	18 (34.0)	470 (33.6)	253 (31.4)	
	Complete college or greater	667 (29.5)	14 (26.4)	442 (31.6)	211 (26.2)	0.009
Smoking status	Never	399 (17.6)	3 (5.7)	286 (20.4)	110 (13.6)	
	Former	1033 (45.7)	32 (60.4)	653 (46.6)	348 (43.1)	
	Current	830 (36.7)	18 (34.0)	463 (33.0)	349 (43.2)	< 0.0001
BMI, kg/m^2	< 25	940 (41.6)	21 (39.6)	621 (44.3)	298 (36.9)	
	25-30	776 (34.3)	17 (32.1)	479 (34.2)	280 (34.7)	
	≥ 30	546 (24.1)	15 (28.3)	302 (21.5)	229 (28.4)	0.002
Total energy, kcal/d		2005.2 ± 719.5	1935.4 ± 847.8	1975.7 ± 735.4	2061.2 ± 678.9	0.0002
Clinical stage	I and II	780 (34.5)	17 (32.1)	484 (35.9)	279 (36.3)	
	III and IV	1392 (61.5)	36 (67.9)	866 (64.1)	490 (63.7)	0.82
Grade	Well differentiated	154 (6.8)	5 (9.4)	101 (7.2)	48 (5.9)	
	Moderately differentiated	509 (22.5)	8 (15.1)	313 (22.3)	188 (23.3)	
	Poorly differentiated	934 (41.3)	24 (45.3)	574 (40.9)	336 (41.6)	
	Undifferentiated	23 (1.0)	0	9 (0.6)	14 (1.7)	
	Unknown	642 (28.4)	16 (30.2)	405 (28.9)	221 (27.4)	0.23
Pathology	Adenocarcinoma	1276 (56.4)	34 (64.2)	827 (59.0)	415 (51.4)	
	Squamous cell	538 (23.8)	10 (18.9)	309 (22.0)	219 (27.1)	
	Large cell	313 (13.8)	6 (11.3)	180 (12.8)	127 (15.7)	
	Others	135 (6.0)	3 (5.7)	86 (6.1)	46 (5.7)	0.02
Surgery	No	1382 (61.1)	32 (60.4)	848 (60.5)	502 (62.2)	
	Yes	880 (38.9)	21 (39.6)	554 (39.5)	305 (37.8)	0.72
Radiation	No	1750 (77.4)	44 (83.0)	1083 (77.2)	623 (77.2)	
	Yes	512 (22.6)	9 (17.0)	319 (22.8)	184 (22.8)	0.61
Chemotherapy	No	1174 (51.9)	21 (39.6)	716 (51.1)	437 (54.2)	
	Yes	1088 (48.1)	32 (60.4)	686 (48.9)	370 (45.8)	0.07
Chemoradiation	No	1892 (83.6)	21 (39.6)	716 (51.1)	437 (54.2)	
	Yes	370 (16.4)	32 (60.4)	686 (48.9)	370 (45.8)	0.02

[1]Fat intake was energy-adjusted by using the nutrient density method. Low (< 20 % kcal), recommended (20-35 % kcal) and high (> 35 % kcal).
[2]Numbers may not add up to the total because of missing data.
[3]Derived by using chi-square test for categorical variables and ANOVA for continuous variables.
[4]Mean ± SD (all such values).

Table 2: Risk of overall survival and recurrence by total and specific types of dietary fat intake among NSCLC patients

			Overall survival			Recurrence		
		Dietary fat intake[1]	**Events/ total**	**HR (95% CI)**[2]	***p* value**	**Events/ total**	**HR (95% CI)**[2]	***p* value**
All participants	Total fat[3]	Recommended or lower	1041/1455	Reference		176/501	Reference	
		High	553/807	0.95 (0.86, 1.06)	0.35	100/279	1.12 (0.86, 1.45)	0.41
	Saturated fat[4]	Recommended or lower	597/870	Reference		105/300	Reference	
		High	997/1395	1.03 (0.93, 1.14)	0.58	170/477	1.12 (0.86, 1.45)	0.40
	Monounsaturated fat[5]	Recommended or lower	1015/1570	Reference		202/569	Reference	
		High	64/114	0.86 (0.66, 1.11)	0.23	12/40	0.94 (0.52, 1.71)	0.84
	Polyunsaturated fat[6]	Recommended or lower	1061/1646	Reference		205/589	Reference	
		High	20/37	0.78 (0.50, 1.23)	0.29	7/15	1.33 (0.57, 3.09)	0.50
Early-stage	Total fat[3]	Recommended or lower	254/501	Reference				
		High	123/279	0.92 (0.73, 1.15)	0.47			
	Saturated fat[4]	Recommended or lower	133/300	Reference				
		High	242/477	**1.27 (1.02, 1.59)**	**0.035**			
	Monounsaturated fat[5]	Recommended or lower	242/569	Reference				
		High	14/40	0.93 (0.54, 1.62)	0.80			
	Polyunsaturated fat[6]	Recommended or lower	253/589	Reference				
		High	4/15	0.76 (0.27, 2.10)	0.59			
Advanced-stage	Total fat[3]	Recommended or lower	749/902	Reference				
		High	398/490	0.96 (0.85, 1.09)	0.54			
	Saturated fat[4]	Recommended or lower	446/542	Reference				
		High	703/854	0.95 (0.84, 1.07)	0.40			
	Monounsaturated fat[5]	Recommended or lower	744/953	Reference				
		High	45/69	0.78 (0.58, 1.07)	0.12			
	Polyunsaturated fat[6]	Recommended or lower	776/1006	Reference				
		High	14/20	0.78 (0.45, 1.34)	0.37			

[1]Fat intake was energy-adjusted by using the nutrient density method.
[2]Multivariable model adjusted for age, gender, BMI, smoking status, stage, grade, pathology, and treatment.
[3]Recommended or lower (≤ 35 % kcal) and high (> 35 % kcal).
[4]Recommended or lower (≤ 10 % kcal) and high (> 10 % kcal).
[5]Recommended or lower (≤ 15 % kcal) and high (> 15 % kcal).
[6]Recommended or lower (≤ 10 % kcal) and high (> 10 % kcal).

Table 3: Overall survival among dietary fat intake categories in early-stage and advanced-stage NSCLC patients by smoking status and body mass index

	Dietary fat intake[1]		Early-stage			Advanced-stage		
			Events/total	HR (95% CI)[2]	*p* value	Events/total	HR (95% CI)[2]	*p* value
Never smokers	Saturated fat[3]	Recommended or lower	24/55	Reference		126/155	Reference	
		High	23/49	1.62 (0.81, 3.25)	0.18	96/123	1.07 (0.80, 1.44)	0.64
	Monounsaturated fat[4]	Recommended or lower	38/86	--	--	176/227	Reference	
		High	0/3			10/14	1.22 (0.63, 2.37)	0.55
Ever smokers	Saturated fat[3]	Recommended or lower	109/245	Reference		320/387	Reference	
		High	219/428	**1.31 (1.03, 1.67)**	**0.027**	607/731	0.91 (0.79, 1.05)	0.19
	Monounsaturated fat[4]	Recommended or lower	204/483	Reference		568/726	Reference	
		High	14/37	1.03 (0.59, 1.82)	0.91	35/55	0.72 (0.50, 1.02)	0.064
Normal weight	Saturated fat[3]	Recommended or lower	61/131	Reference		210/248	Reference	
		High	97/176	1.23 (0.87, 1.74)	0.24	302/351	0.98 (0.81, 1.18)	0.82
	Monounsaturated fat[4]	Recommended or lower	95/216	Reference		313/390	Reference	
		High	6/11	2.30 (0.94, 5.65)	0.069	14/23	0.80 (0.47, 1.39)	0.43
Overweight	Saturated fat[3]	Recommended or lower	51/103	Reference		138/171	Reference	
		High	89/178	1.02 (0.70, 1.48)	0.91	235/295	0.92 (0.74, 1.15)	0.48
	Monounsaturated fat[4]	Recommended or lower	95/207	Reference		240/313	Reference	
		High	4/13	0.45 (0.16, 1.29)	0.14	12/22	**0.53 (0.29, 0.97)**	**0.039**
Obese	Saturated fat[3]	Recommended or lower	21/66	Reference		98/123	Reference	
		High	56/123	**2.06 (1.16, 3.63)**	**0.012**	166/208	0.92 (0.70, 1.20)	0.53
	Monounsaturated fat[4]	Recommended or lower	52/146	Reference		191/250	Reference	
		High	4/16	1.10 (0.36, 3.37)	0.87	19/24	0.97 (0.58, 1.62)	0.91

[1]Fat intake was energy-adjusted by using the nutrient density method.
[2]Multivariable model adjusted for age, gender, BMI, smoking status, stage, grade, pathology, and treatment where appropriate.
[3]Recommended or lower ($\leq$ 10 % kcal) and high (> 10 % kcal).
[4]Recommended or lower ($\leq$ 15 % kcal) and high (> 15 % kcal).

Table 4: Risk of overall survival by total and specific types of dietary fat intake stratified by stage and treatment type

	Early-Stage Surgery only			Early-Stage Surgery and chemotherapy			Advanced-Stage Primary chemotherapy		
Dietary fat intake[1]	**Dead**	**HR (95% CI)**[2]	***p* value**	**Dead**	**HR (95% CI)**[2]	***p* value**	**Dead**	**HR (95% CI)**[2]	***p* value**
Total fat[3]									
Recommended or lower	131	Reference		123	Reference		434	Reference	
High	65	0.81 (0.59, 1.11)	0.20	58	1.11 (0.78, 1.56)	0.57	221	0.90 (0.76, 1.07)	0.24
Saturated fat[4]									
Recommended or lower	66	Reference		67	Reference		261	Reference	
High	129	1.25 (0.91, 1.72)	0.17	113	1.32 (0.95, 1.84)	0.10	397	**0.84 (0.71, 0.99)**	**0.036**
Monounsaturated fat[5]									
Recommended or lower	119	Reference		123	Reference		454	Reference	
High	11	1.23 (0.65, 2.34)	0.52	3	0.51 (0.16, 1.68)	0.27	27	**0.64 (0.43, 0.96)**	**0.030**
Polyunsaturated fat[6]									
Recommended or lower	128	Reference		125	Reference		477	Reference	
High	2	0.86 (0.20, 3.66)	0.83	2	0.55 (0.12, 2.40)	0.42	8	0.63 (0.31, 1.28)	0.20

[1]Fat intake was energy-adjusted by using the nutrient density method.
[2]Multivariable model adjusted for age, gender, BMI, smoking status, stage, grade, pathology and treatment where appropriate.
[3]Recommended or lower (≤ 35 % kcal) and high (> 35 % kcal).
[4]Recommended or lower (≤ 10 % kcal) and high (> 10 % kcal).
[5]Recommended or lower (≤ 15 % kcal) and high (> 15 % kcal).
[6]Recommended or lower (≤ 10 % kcal) and high (> 10 % kcal).

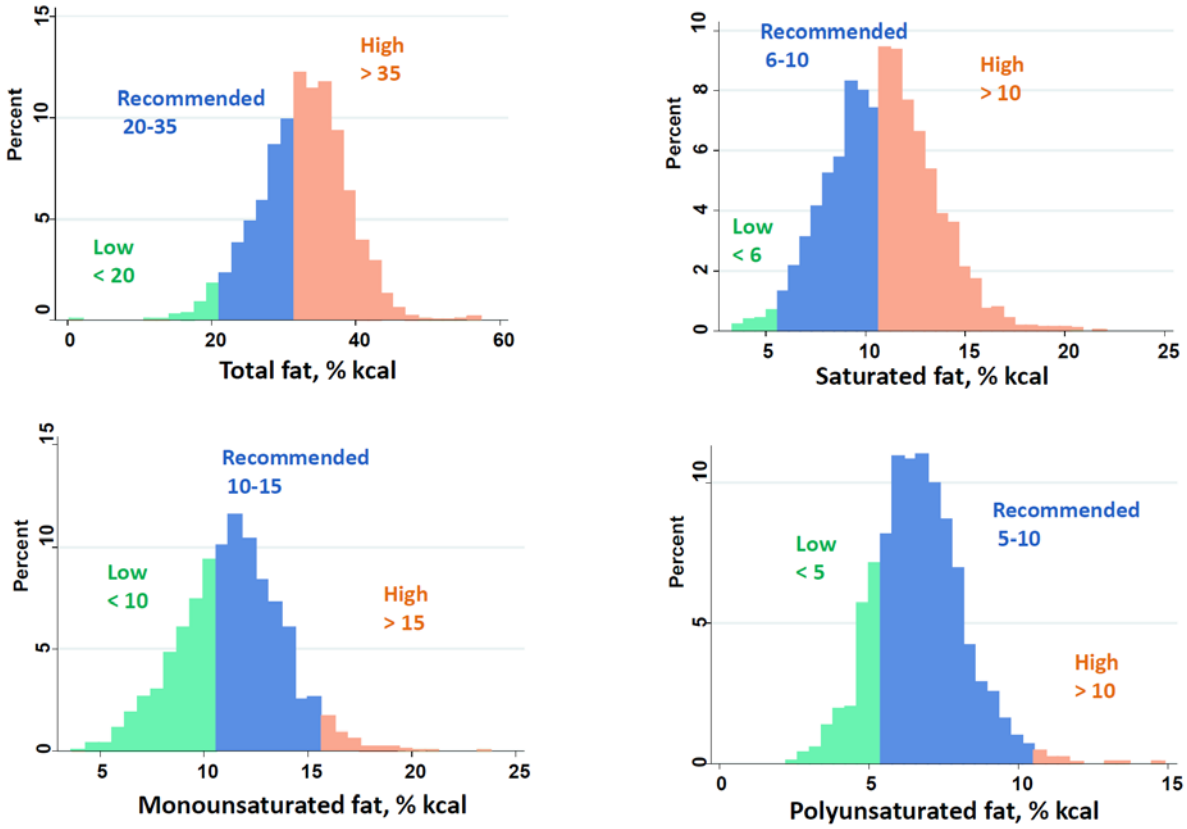

Figure: Distribution of fat intake in the study population

Supplemental

Supplemental Table 1: Characteristics of NSCLC patients by saturated fat intake

Characteristics		Saturated fat intake[1], n (%)[2] Low (n = 60)	Recommended (n = 810)	High (n = 1,395)	*p* value[3]
Age at diagnosis, year		64.7 ± 9.7[4]	64.3 ± 10.7	62.3 ± 10.9	< 0.0001
Sex	Men	24 (40.0)	400 (49.4)	738 (52.9)	
	Women	36 (60.0)	410 (50.6)	657 (47.1)	0.06
Education	High school or less	19 (31.7)	281 (34.7)	548 (39.4)	
	Some college	19 (31.7)	267 (33.0)	455 (32.7)	
	Complete college or greater	22 (36.7)	261 (32.3)	389 (27.9)	0.10
Smoking status	Never	10 (16.7)	211 (26.0)	180 (12.9)	
	Former	35 (58.3)	382 (47.2)	622 (44.6)	
	Current	15 (25.0)	217 (26.8)	593 (42.5)	< 0.0001
BMI, kg/m^2	< 25	29 (48.3)	364 (44.9)	547 (39.2)	
	25-30	19 (31.7)	265 (32.7)	498 (35.7)	
	≥ 30	12 (20.0)	181 (22.3)	350 (25.1)	0.08
Total energy, kcal/d		1772.9 ± 704.3	1973.6 ± 770.4	2046.8 ± 713.4	< 0.0001
Clinical stage	I and II	22 (37.3)	278 (35.5)	477 (35.8)	
	III and IV	37 (62.7)	505 (64.5)	854 (64.2)	0.96
Grade	Well differentiated	4 (6.7)	69 (8.5)	81 (5.8)	
	Moderately differentiated	11 (18.3)	184 (22.7)	310 (22.2)	
	Poorly differentiated	26 (43.3)	328 (40.5)	585 (41.9)	
	Undifferentiated	0	4 (0.5)	19 (1.4)	
	Unknown	19 (31.7)	225 (27.8)	400 (28.7)	0.19
Pathology	Adenocarcinoma	36 (60.0)	489 (60.4)	757 (54.3)	
	Squamous cell	15 (25.0)	176 (21.7)	344 (24.7)	
	Large cell	6 (10.0)	101 (12.5)	206 (14.8)	
	Others	3 (5.0)	44 (5.4)	88 (6.3)	0.19
Surgery	No	35 (58.3)	474 (58.5)	876 (62.8)	
	Yes	25 (41.7)	336 (41.5)	519 (37.2)	0.13
Radiation	No	47 (78.3)	636 (78.5)	1069 (76.6)	
	Yes	13 (21.7)	174 (21.5)	326 (23.4)	0.58
Chemotherapy	No	26 (43.3)	400 (49.4)	744 (53.3)	
	Yes	34 (56.7)	410 (50.6)	651 (46.7)	0.08
Chemoradiation	No	54 (90.0)	691 (85.3)	1155 (82.8)	
	Yes	6 (10.0)	119 (14.7)	240 (17.2)	0.13

[1]Fat intake was energy-adjusted by using the nutrient density method. Low (< 6 % kcal), recommended (6-10 % kcal) and high (> 10 % kcal).
[2]Numbers may not add up to the total because of missing data.
[3]Derived by using chi-square test for categorical variables and ANOVA for continuous variables.
[4]Mean ± SD (all such values).

Supplemental Table 2: Characteristics of NSCLC patients by monounsaturated fat intake

Characteristics		Monounsaturated fat intake[1], n (%)[2] Low (n = 475)	Recommended (n = 1,095)	High (n = 114)	*p* value[3]
Age at diagnosis, year		64.2 ± 11.1[4]	62.9 ± 11.4	62.0 ± 10.4	0.02
Sex	Men	205 (43.2)	583 (53.2)	62 (54.4)	
	Women	270 (56.8)	512 (46.8)	52 (45.6)	0.0008
Education	High school or less	162 (34.1)	392 (35.9)	44 (38.9)	
	Some college	158 (33.3)	383 (35.1)	37 (32.7)	
	Complete college or greater	155 (32.6)	317 (29.0)	32 (28.3)	0.62
Smoking status	Never	119 (25.1)	206 (18.8)	18 (15.8)	
	Former	229 (48.2)	467 (42.6)	53 (46.5)	
	Current	127 (26.7)	422 (38.5)	43 (37.7)	0.0001
BMI, kg/m^2	< 25	214 (45.1)	410 (37.4)	35 (30.7)	
	25-30	155 (32.6)	380 (34.7)	36 (31.6)	
	≥ 30	106 (22.3)	305 (27.9)	43 (37.7)	0.002
Total energy, kcal/d		1932.1 ± 777.4	2080.9 ± 765.3	1938.3 ± 549.6	0.0001
Clinical stage	I and II	162 (35.2)	407 (38.3)	40 (36.7)	
	III and IV	298 (64.8)	655 (61.7)	69 (63.3)	0.51
Grade	Well differentiated	38 (8.0)	68 (6.2)	6 (5.3)	
	Moderately differentiated	101 (21.3)	276 (25.2)	26 (22.8)	
	Poorly differentiated	184 (38.7)	409 (37.4)	52 (45.6)	
	Undifferentiated	1 (0.2)	11 (1.0)	1 (0.9)	
	Unknown	151 (31.8)	331 (30.2)	29 (25.4)	0.27
Pathology	Adenocarcinoma	309 (65.1)	633 (57.8)	49 (43.0)	
	Squamous cell	90 (18.9)	266 (24.3)	41 (36.0)	
	Large cell	54 (11.4)	150 (13.7)	21 (18.4)	
	Others	22 (4.6)	46 (4.2)	3 (2.6)	0.0004
Surgery	No	290 (61.1)	646 (59.0)	64 (56.1)	
	Yes	185 (38.9)	449 (41.0)	50 (43.9)	0.57
Radiation	No	376 (79.2)	856 (78.2)	89 (78.1)	
	Yes	99 (20.8)	239 (21.8)	25 (21.9)	0.90
Chemotherapy	No	228 (48.0)	511 (46.7)	54 (47.4)	
	Yes	247 (52.0)	584 (53.3)	60 (52.6)	0.89
Chemoradiation	No	401 (84.4)	899 (82.1)	98 (86.0)	
	Yes	74 (15.6)	196 (17.9)	16 (14.0)	0.36

[1]Fat intake was energy-adjusted by using the nutrient density method. Low (< 10 % kcal), recommended (10-15 % kcal) and high (> 15 % kcal).
[2]Numbers may not add up to the total because of missing data.
[3]Derived by using chi-square test for categorical variables and ANOVA for continuous variables.
[4]Mean ± SD (all such values).

Supplemental Table 3: Characteristics of NSCLC patients by polyunsaturated fat intake

Characteristics		Polyunsaturated fat intake[1], n (%)[2] Low (n = 205)	Recommended (n = 1,441)	High (n = 37)	*p* value[3]
Age at diagnosis, year		63.1 ± 12.1[4]	63.2 ± 11.2	62.5 ± 12.1	0.88
Sex	Men	111 (54.1)	725 (50.3)	12 (32.4)	
	Women	94 (45.9)	716 (49.7)	25 (67.6)	0.05
Education	High school or less	68 (33.2)	515 (35.8)	14 (37.8)	
	Some college	69 (33.7)	495 (34.4)	13 (35.1)	
	Complete college or greater	68 (33.2)	427 (29.7)	10 (27.0)	0.86
Smoking status	Never	33 (16.1)	302 (21.0)	6 (16.2)	
	Former	95 (46.3)	633 (43.9)	20 (54.1)	
	Current	77 (37.6)	506 (35.1)	11 (29.7)	0.40
BMI, kg/m^2	< 25	94 (45.9)	549 (38.1)	13 (35.1)	
	25-30	62 (30.2)	501 (34.8)	10 (27.0)	
	≥ 30	49 (23.9)	391 (27.1)	14 (37.8)	0.14
Total energy, kcal/d		2007.0 ± 796.1	2041.2 ± 759.1	1708.8 ± 492.8	0.77
Clinical stage	I and II	73 (36.0)	516 (37.1)	15 (42.9)	
	III and IV	130 (64.0)	876 (62.9)	20 (57.1)	0.74
Grade	Well differentiated	13 (6.3)	98 (6.8)	2 (5.4)	
	Moderately differentiated	40 (19.5)	355 (24.6)	8 (21.6)	
	Poorly differentiated	95 (46.3)	538 (37.3)	13 (35.1)	
	Undifferentiated	0	12 (0.8)	1 (2.7)	
	Unknown	57 (27.8)	438 (30.4)	13 (35.1)	0.25
Pathology	Adenocarcinoma	137 (66.8)	836 (58.0)	18 (48.6)	
	Squamous cell	40 (19.5)	343 (23.8)	12 (32.4)	
	Large cell	21 (10.2)	198 (13.7)	5 (13.5)	
	Others	7 (3.4)	64 (4.4)	2 (5.4)	0.24
Surgery	No	118 (57.6)	863 (59.9)	23 (62.2)	
	Yes	87 (42.4)	578 (40.1)	14 (37.8)	0.78
Radiation	No	170 (82.9)	1123 (77.9)	30 (81.1)	
	Yes	35 (17.1)	318 (22.1)	7 (18.9)	0.25
Chemotherapy	No	94 (45.9)	681 (47.3)	14 (37.8)	
	Yes	111 (54.1)	760 (52.7)	23 (62.2)	0.50
Chemoradiation	No	167 (81.5)	1198 (83.1)	32 (86.5)	
	Yes	38 (18.5)	243 (16.9)	5 (13.5)	0.71

[1]Fat intake was energy-adjusted by using the nutrient density method. Low (< 5 % kcal), recommended (5-10 % kcal) and high (> 10 % kcal).
[2]Numbers may not add up to the total because of missing data.
[3]Derived by using chi-square test for categorical variables and ANOVA for continuous variables.
[4]Mean ± SD (all such values).

CHAPTER IV: JOURNAL ARTICLE 2

Title of Journal Article: Genetic polymorphisms in fatty acid metabolism genes, dietary fat intake, and clinical outcomes in non-small cell lung cancer

Name of Journal Proposed for Article Submission: *American Journal of Epidemiology*

Abstract

Alterations in fatty acid metabolism may affect cancer development and progression. In this two-stage study, we assessed associations between 691 single nucleotide polymorphisms (SNPs) in 94 genes involved in fatty acid metabolism and overall survival and recurrence among non-Hispanic whites with non-small cell lung cancer (NSCLC), and further evaluated whether the associations varied by dietary fat intake. Among 1,593 NSCLC patients in the discovery set, we identified candidate SNPs associated with overall survival or recurrence. Those SNPs were further validated in the replication set of 746 NSCLC patients. We identified four SNPs associated with overall survival and one SNP associated with recurrence that were consistently significant in both datasets. Functional assessment identified three variants *ACSL1*:rs4862417, *CYP2C8*:rs1934953, and *FADS2*:rs174611 to be putatively functional. Early-stage patients with a G variant of rs174611 were associated with 28% and 47% increased risk of death in the discovery (95% confidence interval: 1.03, 1.59) and replication sets (95% confidence interval: 1.03, 2.08), respectively. Monounsaturated fat intake was found to interact with the rs174611 genotype in relation to overall survival (multiplicative $P_{\text{interaction}} = 0.03$).

Genetic variants and dietary fat intake may have multiplicative effect on overall survival in NSCLC.

Keywords: fatty acids, single nucleotide polymorphism, genetics, non-small cell lung cancer, survival, recurrence, monounsaturated fat

Introduction

Lung cancer continues to be the most frequently diagnosed cancer and the leading cause of cancer death in the United States (1). Although progress has been made in the treatment for lung cancer, the overall 5-year survival rate is only 18% (2). Non-small cell lung cancer (NSCLC) is the major histological subtype of lung cancer, accounting for 80-85% of all lung cancer cases (1). Prognostic factors for survival of NSCLC include age at diagnosis, gender, performance status, and tumor stage (3). In addition, a variety of genes and signaling pathways have been shown to be associated with survival and tumor progression in NSCLC patients (4, 5).

The importance of fatty acid metabolism in cancer has been increasingly recognized (6, 7). Fatty acids are one of the main macronutrients, which can be externally derived through daily diet intake and internally derived through *de novo* fatty acids synthesis. Tumor cells often have fatty acid metabolic abnormalities (7), and rely mostly on *de novo* fatty acid synthesis instead of uptake of exogenous fatty acid (8). Alterations in fatty acid metabolism in tumor cells favor excessive fatty acids for synthesis of membranes and signaling molecules to meet the demands of cancer cell proliferation (6). The rate of fatty acids synthesis is controlled by a series of enzymatic regulation, including ATP citrate lyase (*ACLY*), acetyl-CoA carboxylase-α (*ACACA*), and fatty acid synthase (*FASN*) (9). These enzymes are highly up-regulated in lung cancer cells (10, 11), as well as prostate, breast, colorectal, stomach, and endometrial cancer cells (12-15). Moreover, overexpression of *FASN* shows an aggressive clinical behavior in patients with lung cancer (16).

Previous research has documented that single nucleotide polymorphisms (SNPs) in fatty acid synthesis genes are associated with prognosis among NSCLC patients who received surgery (17), but the scope was limited to a small subset of variants in a few genes related to fatty acid synthesis. The broader impact of genetic variants in genes involved in the fatty acid metabolism in clinical outcomes among NSCLC patients remain unclear. Therefore, we aimed to systematically investigate the associations between SNPs in fatty acid metabolism genes and overall survival and recurrence in NSCLC by using two-stage design, and further examine whether the associations were modified by dietary fat intake.

Methods

Study population and recruitment

Study participants were accrued from an ongoing epidemiology study of lung cancer at The University of Texas MD Anderson Cancer Center (MDACC) between 1995 and 2008. The study was approved by Institutional Review Board of MDACC and all study participants provided written informed consent. There were no recruitment restrictions on age, sex, ethnicity, histology, and stage. All participants were newly-diagnosed within 1 year of recruitment, histologically confirmed, and had not previously received cancer treatment prior to study recruitment. Each study participant underwent a 45-minute in-person interview by trained staff, and at the end of interview a 40 mL peripheral blood sample was drawn for molecular analysis. To be eligible for this current analysis, study participants must have had existing genotyping data available from previous genome-wide association studies (GWAS) and the OncoArray project (18-20). The previous studies only included non-Hispanic white

NSCLC patients for a total of 1,593 in the discovery (GWAS) and 746 in the replication (OncoArray).

Data collection

A structured questionnaire was used to collect epidemiological data from all study participants, including demographic characteristics, smoking status, and weight at diagnosis. Trained staff abstracted clinical and follow-up information from medical records. Early-stage NSCLC was categorized as clinical stages I and II, and those with stages III and IV were classified as advanced-stage NSCLC.

Nutrient data were collected in a subset of study participants using a modified version of the National Cancer Institute's Health Habits and History Questionnaire that was previously validated (21). The details about our dietary assessment have been previously described (22, 23). Our food frequency questionnaire (FFQ) asked study participants about the frequency and portion sizes of 165 food and beverage items consumed during the year prior to NSCLC diagnosis. Nutrient calculations were done using the US Department of Agriculture National Nutrient Database for Standard Reference and Food and Nutrient Database for Dietary Studies (24, 25). Energy-adjusted dietary intakes were obtained by nutrient density method on intakes of total, saturated, monounsaturated, and polyunsaturated fat.

SNP selection

We selected fatty acid metabolism genes that were included in both Reactome (reactome.org/) and Kyoto Encyclopedia of Genes and Genomes [(KEGG) genome.jp/kegg/] databases. These fatty acid metabolism genes included those involved in fatty acid

biosynthesis, elongation, and degradation; arachidonic acid metabolism; alpha-linolenic and linoleic acid metabolism; glycerolipid metabolism; glycerophospholipid metabolism; and sphingolipid metabolism. There were 97 genes including in both bioinformatics resources. After excluding genes that were not found in The University of California Santa Cruz (UCSC) Genome Browser [genome.ucsc.edu, (n=3)], 94 genes were reported to be associated with fatty acid metabolism. A total of 691 SNPs mapped 10 kb upstream or 10 kb downstream of 94 genes were included in the discovery set. rAggr (raggr.usc.edu/) was used to identify proxy SNPs (with $r^2 \geq 80\%$) as substitutes for those SNPs that were not directly genotyped in the replication set.

Statistical analysis

Person-time was calculated individually for overall survival and recurrence. Overall survival was defined as the period from the date of diagnosis to the date of death from any cause, or the last follow-up, whichever came first. Time to recurrence was defined as the date of diagnosis to first recurrence, or the date of the last follow-up among early-stage patients, depending on which date came first. Recurrence was defined as tumor recurring locally, regionally, and at distant metastasis sites after curative resection. Analyses of the associations between SNPs in fatty acid metabolism genes and clinical outcomes among NSCLC patients were conducted by using multivariable Cox proportional hazards regression models with adjustment for age, sex, smoking status, clinical stage, performance status, and treatment regimen in the discovery and replication sets. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated from the Cox regression model. All genetic models of inheritance including the dominant, recessive, and additive models were taken into

consideration in the assessment. The genetic model with the smallest *P* value was considered as the best-fitting model. We used Q value to control the false discovery rate since many SNPs were tested for the associations with clinical outcomes. All the significant SNPs had Q value < 0.20 in the discovery set.

Expression quantitative trait loci (eQTL) analysis was utilized to conduct functional characterization of the significant SNPs that had consistent associations with overall survival or recurrence in both discovery and replication sets using genomic data from the Genotype-Tissue Expression (GTEx) project (26). We also considered functional annotations of the proxy variants in high linkage disequilibrium (LD) to the significant SNPs using HaploReg v4.1 (27).

The putatively functional SNPs were further examined the potential impact of dietary fat intake on the associations between the significant SNPs and overall survival and recurrence in a subset of study participants. A total of 1,993 participants were included after excluding four participants with outlying energy intake with values that laid outside the intervals delimited by the 25th percentile minus 1.5 times the interquartile range (IQR) and the 75th percentile plus 1.5 times the IQR, and implausible energy intake with values for energy intake below 500 kcal/d or above 5,000 kcal/d. Total and subtypes of dietary fat intake were categorized by their medians. Dietary intakes of total and specific types of fat were energy-adjusted prior to further analysis (28). The associations between dietary fat intake and overall survival and recurrence were examined via the multivariable Cox regression model adjusting for age, sex, body mass index (BMI) at diagnosis, smoking status, clinical stage, performance status, and treatment regimen. We tested for additive and multiplicative interaction between the significant SNPs and dietary fat intake in the multivariable Cox regression model. All analyses were performed

with Stata (College Station, TX), and two-sided P value < 0.05 was considered statistically significant.

Results

The characteristics of the 1,593 NSCLC patients in the discovery set and 746 NSCLC patients in the replication set are shown in Table 1. The mean age at diagnosis in the discovery set was 62 years and 65 years in the replication set. The majority of study participants were males, former or current smokers, and reported performance status score of one indicating limitations in physically strenuous activity.

For overall survival, there were 70 SNPs that were significantly associated with overall survival in the discovery set, of which one SNP was validated in the replication set. This variant *CYP2C19*:rs7916649 was associated with a 15% increased risk of death in the discovery set (95% CI: 1.01, 1.32) and 46% increase in the replication set (95% CI: 1.02, 2.09) under the dominant model (Table 2). A total of 68 SNPs were associated with recurrence in the discovery set, and only one variant in *PLA2G4A* was consistently associated with increased recurrence of NSCLC. Patients with G allele (AG or GG genotypes) of rs10911933 had 109% and 67% increased risk of death in the discovery (95% CI: 1.48, 2.95) and replication sets (95% CI: 1.00, 2.79), respectively.

When we assessed the effect on survival by stage grouping, there were 57 SNPs and 87 SNPs significant in patients with early-stage and advanced-stage NSCLC in the discovery set, respectively. Among these SNPs, three SNPs remained consistent and significant in association with overall survival among early-stage patients and were predicted to be putatively functional,

but no SNP was replicated among advanced-stage patients. Early-stage NSCLC patients with variant *ACSL1*:rs4862417 conferred a 28% improvement in overall survival in the discovery set (95% CI: 0.54, 0.96), and 39% improvement in the replication set (95% CI: 0.37, 0.99) under the dominant model. The proxy SNP, rs2292899 in high LD with *ACSL1*:rs4862417 was predicted to be a weak transcriptional regulator based on its location in 3' untranslated region (3'UTR) and enhancer-like region. Those with two recessive alleles of rs1934953 in *CYP2C8* had 42% and 80% decreased risk of survival in the discovery (95% CI: 0.36, 0.95) and replication sets (95% CI: 0.06, 0.66), respectively. The predicted location of genotyped variant, *CYP2C8*:rs1934953, was in a transcription factor binding site for *IRF2* that modulates cellular responses and involves in tumorigenesis. This variant was also identified as a direct eQTL regulating expression of *CYP2C8* in thyroid, testis, stomach and muscle tissues. In the same subgroup, the variant *FADS2*:rs174611 was associated with a 28% increased risk of death in the discovery set (95% CI: 1.03, 1.59), and 47% increase in the replication set (95% CI: 1.03, 2.08) under the additive model. The variant *FADS2*:rs174611 was predicted to be located in the enhancer-like region as well as the transcription factor binding site for *ELF1*, and a direct eQTL regulating expression of *FADS2* was found in lung tissues.

In a subset of study participants with nutrient data collected (N = 1,993), the multivariable-adjusted results for the associations between total and specific types of fat intake and clinical outcomes in NSCLC are shown in Table 3. High intake of saturated fat was associated with a 13% increased risk of death (95% CI: 1.00, 1.26), but associations between other types of dietary fat and overall survival were not significant among NSCLC patients. There was a marginally significant survival reduction of high intake of monounsaturated fat among early-

stage patients. Moreover, those with high intake of monounsaturated fat were associated with a 63% increased risk of recurrence (95% CI: 1.22, 2.19), and an unfavorable effect on recurrence was borderline significant with high intake of total fat.

To elucidate potential interactions between the putatively functional SNPs and dietary fat intake effects on overall survival among early-stage NSCLC patients, we further stratified the analysis (Table 4). Stratification by low and high intake of monounsaturated fat showed consistent associations for overall survival by *ACSL1* and *CYP2C8* SNPs, although neither of were significant. In contrast, low intake of monounsaturated fat was associated with poor survival for early-stage patients with the variant (AG or GG) rs174611 genotype (HR: 2.29, 95% CI: 1.32, 3.96), whereas null association was observed in the high intake of monounsaturated fat subgroup. The variant rs174611 showed evidence of no additive interaction, but a multiplicative interaction with monounsaturated fat intake (*P* for interaction = 0.035).

Discussion

In this present study, we found that genetic variants *CYP2C19*:rs7916649, *ACSL1*:rs4862417, *CYP2C8*:rs1934953 and *FADS2*:rs174611 were associated with overall survival among NSCLC patients, whereas the associations with SNPs in *ACSL1*, *CYP2C8,* and *FADS2* were only observed in early-stage patients. The variant *PLA2G4A*:rs10911933 was also associated with recurrence. These associations were consistent and significant in both discovery and replication sets. Moreover, the association between variant in *FADS2* and risk of death was modified by monounsaturated fat intake, and was seemingly confined to early-

stage NSCLC patients with G allele (AG and GG genotypes) of rs174611 who had low intake of monounsaturated fat.

Previous studies have investigated the role of genetic variation in genes encoding fatty acid metabolism enzymes and cancer outcomes. A study examined eight SNPs in *ACLY*, *ACACA*, and *FASN* as predictors of overall survival and recurrence among NSCLC patients treated with surgery in a Chinese population (17). Although they found no association between variants in *ACACA* and survival or recurrence, *ACLY*:rs9912300 was shown to be associated with overall survival, and two SNPs in *FASN*, rs4246444 and rs4485435, were associated with recurrence. Another group used data from another Chinese population, and identified that two SNPs in *ACACA* were associated with survival among hepatocellular carcinoma patients who underwent surgery, while also confirming the possible role of *FASN*:rs4485435 in survival (29). Finally, a study from European American men treated with radical prostatectomy showed a weak association between *FASN*:rs4246444 and disease recurrence (30). Unlike previous studies, our study took a more systematic approach by including 691 SNPs in 94 genes involved in fatty acid metabolism, but found that genetic variation in *ACLY*, *ACACA*, and *FASN* was not associated with overall survival or recurrence for NSCLC patients. Moreover, no such association was observed when the analysis was stratified by patients with early-stage and advanced-stage NSCLC. However, we identified five novel SNPs that were associated with overall survival and recurrence in NSCLC patients, and three of them in *ACSL1*, *CYP2C8*, and *FADS2* were predicted to be functional.

FADS2 located on 11q12.2 encodes a desaturase enzyme that controls the biosynthesis of unsaturated fatty acids from polyunsaturated fatty acids. *FADS2* has been found to be highly

expressed in lung tumor tissue (31). *FADS2*:rs174611 was predicted to be located in a transcription factor binding site for *ELF1*, a member of *ETS* transcription factor family that has been shown to regulate cell proliferation in epithelial cells (32). *ETS* is highly expressed in lung cancer patients with advanced clinical stage and is associated with poor survival (33). The expression of *ETS* in lung cancer tissue is positively associated with *VEGF* (34). Previous studies have suggested that *ELF1* serves as an intermediate in the oncogenesis process through *VEGF* (35, 36). *VEGF* is a key mediator of angiogenesis by binding to specific *VEGF* receptors, leading to subsequent signal transduction in facilitating proliferation and migration in common cancers, including NSCLC (37). The *VEGF* signaling activates *ETS1* that regulates the expression of *TEK* (38), and *TEK* has also been reported to be regulated by *ELF1* (39). Moreover, eQTL analysis for *FADS2*:rs174611 in lung tissue suggested that the risk allele of rs174611 was associated with a significantly increased *FADS2* expression.

Previous studies have indicated that genetic variations in *FADS2* influenced fatty acid composition in circulation, cells, and tissues (40). In addition, a significant decrease in triglyceride and low density lipoprotein cholesterol levels following monounsaturated fatty acids-rich diets has been observed (41). Dietary recommendations for fatty acid intake have been suggested to replace saturated fat with monounsaturated fat to prevent heart diseases (42). It has been recognized that monounsaturated fat intake has anti-inflammatory action that may contribute to suppress tumor cell proliferation and progression (43). The association between monounsaturated fat intake and prognosis of common cancers has been examined, not including lung cancer, but studies yield inconsistent results (44-46). Our study showed no evidence for associations between high intake of monounsaturated fat and overall survival and

recurrence in NSCLC patients. We observed that high intake of monounsaturated fat was associated with worse survival among early-stage NSCLC although the association was borderline significant. Nevertheless, the unfavorable effect on survival disappeared among early-stage patients with high intake of monounsaturated fat who carry at least one copy of G allele of rs174611 compared to those with no risk allele. Given that the variant *FADS2*:rs174611 affects tumorigenesis through activation of *VEGF*, patients with high intake of monounsaturated fat exhibit alleviated inflammation in response to proinflammatory stimuli of *VEGF* among those who carry the variant. In contrast, those with low intake of monounsaturated fat who carry the variant may not have the ability to impede the inflammation via *VEGF* resulting in a poorer survival. Our finding showed that the association between rs174611 genotype and overall survival in early-stage NSCLC was modified by monounsaturated fat intake. Further research is needed to explore the biological mechanism of how monounsaturated fat and the variant rs174611 interact to cause differences in survival among NSCLC patients.

ACSL1 is located on chromosome 4q35.1 encoding for a long-chain fatty acid enzyme that converts long-chain fatty acid into fatty acyl-CoA ester and plays a vital role in fatty acid synthesis and degradation. *ACSL1* has been shown to be downregulated in lung cancer tissue, and lower expression of *ACSL1* is associated with better survival (47). A variant in high LD with *ACSL1*:rs4862417, rs2292899, was predicted to have functional effects on transcriptional regulation through methylation, although the predicted effect was weak.

CYP2C8 located on chromosome 10q24 is a multifunctional enzyme involved in drug metabolism and lipid synthesis, particularly the conversion of long-chain polyunsaturated fatty

acids to epoxyeicosatrienoic acids or monounsaturated fatty acids. *CYP2C8*:rs1934953 was predicted to be located in a transcription factor binding site for *IRF2* that plays an anti-oncogenic role. *IRF2* has been shown to promote cell apoptosis, and inhibit cell proliferation and migration in lung cancer (48). A direct eQTL regulating expression of *CYP2C8* was observed in thyroid, testis, stomach and muscle tissues. Additional eQTL data from lung tissue is needed to unravel the underlying relationship.

The present study had some strengths and limitations. To the best of our knowledge, this is the first study to systematically investigate the associations between genetic variation in genes involved in fatty acid metabolism and overall survival and recurrence in NSCLC. It is also the first study to evaluate the potential impact of dietary fat intake on theses associations. Additionally, the significant SNPs associated with overall survival and recurrence were identified and validated by using two datasets to minimize false positive associations. However, a potential weakness is recall bias because information about dietary fat intake over the past year prior to diagnosis was based on self-report. However, it is unlikely that the measurement error was the differential of clinical outcomes. In addition, this study includes only non-Hispanic whites, and thus our study results may not be directly generalizable to other ethnic populations. Lastly, we should be cautious about the generalization from the findings of our study, since external validation is lacking.

In summary, our study provides support for the hypothesis that germline genetic variants in fatty acid metabolism genes play a critical role in overall survival among patients with early-stage NSCLC. Specifically, early-stage patients with variants *ACSL1*:rs4862417 and *CYP2C8*:rs1934953 may improve their survival. By contrast, those with variant

FADS2:rs174611 may have poor survival, and this risk may be further modified by monounsaturated fat intake. This study suggests that the effect of genetic variants in fatty acid metabolism genes on overall survival in NSCLC is likely to be complex, and further investigation should be conducted to provide insights into the mechanisms underlying the association.

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Table 1: Characteristics of NSCLC patients in discovery and replication sets

		Discovery (*n* = 1593)		**Replication** (*n* = 746)	
Characteristics		**No.**	**%**	**No.**	**%**
Age at diagnosis[1]		62.03 (11.06)		65.20 (9.78)	
Sex	Men	820	51.5	381	51.1
	Women	773	48.5	365	48.9
Smoking status	Never	278	17.5	38	9.3
	Former	699	43.9	226	55.5
	Current	616	38.7	143	35.1
Clinical stage	I and II	492	31.8	405	54.3
	III and IV	1057	68.2	341	45.7
Performance status	0	334	29.8	157	28.6
	1	638	56.9	328	59.9
	2-4	150	13.4	63	11.5
Surgery	Yes	593	37.2	335	44.9
	No	1000	62.8	411	55.1
Radiation	Yes	379	23.8	189	25.3
	No	1214	76.2	557	74.7
Chemotherapy	Yes	803	50.4	380	50.9
	No	790	49.6	366	49.1
Chemoradiation	Yes	292	18.3	120	16.1
	No	1301	81.7	626	83.9

Abbreviations: NSCLC, non-small cell lung cancer.
[1]Values are expressed as mean (standard deviation).

Table 2: Overall survival and recurrence by fatty acid metabolism gene variants in NSCLC patients

				Discovery				Replication			
SNP	**Gene**	**MOD**	**Genotype**	**Events**	**Nonevents**	**HR[1] 95% CI**	***P* Value**	**Events**	**Nonevents**	**HR[1] 95% CI**	***P* Value**
Overall survival											
rs7916649	*CYP2C19*	Dom	GG\GA\AA	323\577\223	171\222\75	1.15 1.01, 1.32	0.036	95\166\64	149\189\83	1.46 1.02, 2.09	0.039
Overall survival among early-stage NSCLC											
rs4862417	*ACSL1*	Dom	AA\AG\GG	119\74\12	147\123\16	0.72 0.54, 0.96	0.027	78\43\4	154\108\18	0.61 0.37, 0.99	0.048
rs1934953	*CYP2C8*	Rec	AA\AG\GG	83\103\20	111\135\40	0.58 0.36, 0.95	0.029	58\60\7	120\111\47	0.20 0.06, 0.66	0.008
rs174611[2]	*FADS2*	Add	AA\AG\GG	87\99\20	145\117\24	1.28 1.03, 1.59	0.026	51\57\17	144\113\23	1.47 1.03, 2.08	0.031
Recurrence											
rs10911933[2]	*PLA2G4A*	Dom	AA\AG\GG	100\67\11	244\69\1	2.09 1.48, 2.95	$2.5x10^{-5}$	85\47\5	184\79\4	1.67 1.00, 2.79	0.049

Abbreviations: SNP, single nucleotide polymorphisms; MOD, model; MAF, minor allele frequency; Dom, dominant; Add, additive; HR, hazard ratio; CI, confidence interval; NSCLC, non-small cell lung cancer.

[1]Multivariable models were adjusted for age, gender, smoking status, clinical stage, performance status, and treatment regimen.

[2]Proxy SNP with high linkage disequilibrium ($R^2 > 0.8$) was used in replication set.

Table 3: Overall survival and recurrence by total and specific types of dietary fat intake among NSCLC patients

Dietary fat intake[1]		Events/ total	Multivariable-adjusted[6]		
			HR	**95% CI**	***P* Value**
Overall survival					
Total fat[2]	Low	615/980	1.00	Referent	
	High	645/979	1.03	0.92, 1.16	0.58
Saturated fat[3]	Low	605/980	1.00	Referent	
	High	656/979	**1.13**	**1.00, 1.26**	**0.042**
Monounsaturated fat[4]	Low	424/722	1.00	Referent	
	High	435/722	1.05	0.91, 1.20	0.51
Polyunsaturated fat[5]	Low	434/723	1.00	Referent	
	High	429/723	1.04	0.91, 1.19	0.59
Early-stage					
Total fat[2]	Low	134/374	1.00	Referent	
	High	139/348	1.22	0.95, 1.56	0.11
Saturated fat[3]	Low	126/364	1.00	Referent	
	High	146/356	1.19	0.93, 1.53	0.18
Monounsaturated fat[4]	Low	95/294	1.00	Referent	
	High	104/278	**1.32**	**0.99, 1.76**	**0.060**
Polyunsaturated fat[5]	Low	95/289	1.00	Referent	
	High	104/278	1.16	0.87, 1.55	0.31
Recurrence					
Total fat[2]	Low	129/374	1.00	Referent	
	High	132/348	**1.28**	**0.97, 1.66**	**0.064**
Saturated fat[3]	Low	122/364	1.00	Referent	
	High	138/356	1.24	0.96, 1.61	0.099
Monounsaturated fat[4]	Low	91/294	1.00	Referent	
	High	115/278	**1.63**	**1.22, 2.19**	**0.001**
Polyunsaturated fat[5]	Low	100/289	1.00	Referent	
	High	104/278	1.16	0.87, 1.55	0.32

Abbreviations: HR, hazard ratio; CI, confidence interval; NSCLC, non-small cell lung cancer.

[1]Fat intake was energy-adjusted by using the nutrient density method.

[2]Low ($\leq$ 32.9 % kcal) and high (> 32.9 % kcal).

[3]Low ($\leq$ 10.8 % kcal) and high (> 10.8 % kcal).

[4]Low ($\leq$ 11.3 % kcal) and high (> 11.3 % kcal).

[5]Low ($\leq$ 6.6 % kcal) and high (> 6.6 % kcal).

[6]Multivariable model adjusted for age, gender, BMI, smoking status, clinical stage, performance status, and treatment regimen.

Tables 4: Associations between genotype of the risk-associated SNPs and overall survival among early-stage NSCLC patients stratified by monounsaturated fat intake

		Monounsaturated fat intake						
		Low (n = 294)			**High** (n = 277)			**P for interaction**[2]
SNP	**Genotype**	**Events /Total**	**HR**[1]	**95% CI**	**Events /Total**	**HR**[1]	**95% CI**	
rs4862417	AA	60/161	1.00	Referent	57/152	1.00	Referent	0.60
(*ACSL1*)	AG or GG	34/132	0.66	0.38, 1.15	46/125	0.70	0.39, 1.25	
rs1934953	AA or AG	87/252	1.00	Referent	96/244	1.00	Referent	0.65
(*CYP2C8*)	GG	8/41	0.52	0.21, 1.26	7/33	0.75	0.28, 2.02	
rs174611	AA	33/142	1.00	Referent	54/142	1.00	Referent	**0.035**
(*FADS2*)	AG or GG	62/152	**2.29**	**1.32, 3.96**	49/135	1.00	0.57, 1.75	

Abbreviations: SNP, single nucleotide polymorphisms; HR, hazard ratio; CI, confidence interval; NSCLC, non-small cell lung cancer.

[1]Multivariable model adjusted for age, gender, BMI, smoking status, clinical stage, performance status, and treatment regimen.

[2]Multiplicative interaction from cross-product term in Cox regression model between monounsaturated fat (high/low) and each SNP.

CHAPTER V: CONCLUSION

Summary of Results

This dissertation provides a comprehensive investigation into the role of dietary fatty acids in clinical outcomes among patients with non-small cell lung cancer (NSCLC). Specifically, this dissertation starts with examining the associations of dietary fat intake with overall survival and recurrence, and then identifies genetic variants in fatty acid metabolism genes that are associated with NSCLC survival in non-Hispanic whites, and how this association is influenced by fat intake.

In Chapter III, a total of 2,262 NSCLC patients were asked to recall the frequency and portion sizes of fatty acids that they had eaten one year before lung cancer diagnosis using a modified food frequency questionnaire at study enrollment. They were followed from the time of diagnosis to death from any cause and recurrence, or the last follow-up during a median follow-up of 23 months. This study showed that neither high intake of total fat, nor any subtype of fat including saturated, monounsaturated and polyunsaturated fat, was associated with overall survival or recurrence for NSCLC. The association between dietary fat intake and overall survival was examined individually for early-stage patients and advanced-stage patients, and the results were different. In particular, high intake of saturated fat was associated with increased risk of death among early-stage NSCLC patients, whereas a protective effect was observed in advanced-stage patients who received primary chemotherapy. A protective effect of high intake of monounsaturated fat on overall survival was also found in these advanced-stage patients.

Previous studies demonstrated that red meat consumption was positively associated with increased risk of lung cancer mortality (94, 95). Red meat contains high amount of saturated fat. The previous findings lent support to the view that high intake of saturated fat may be linked to poor lung cancer survival. Previous studies observed an unfavorable effect of diets with high saturated fat on the prognosis of different types of cancer, including breast and prostate cancers (167-170). Increased saturated fat intake may promote tumorigenesis by altering inflammatory responses and oxidative stress (171). A cohort study of Swedish men showed that high intake of saturated fat increased the risk of prostate cancer death among patients with localized prostate cancer, whereas the association was not present among patients with advanced disease (170). The identified association was consistent with the findings of this dissertation, suggesting that stage of cancer could influence the association between saturated fat intake and cancer prognosis. Interestingly, this study also showed a poor outcome for early-stage patients with obesity. Excess weight and saturated fat intake may accumulate energy stored in adipose tissue, which promotes the release of inflammatory mediators resulting in the increase of oxidative stress and chronic inflammation (172). Oxidative stress and chronic inflammation are closely related to cancer development and progression (119). However, an improved survival for high intake of saturated fat was observed in advanced-stage patients who received primary chemotherapy. The excess fat intake is stored in adipose tissue that may provide nutritional reserve to counterattack the damage made by treatment for advanced cancer (173).

Most studies have focused attention to the relation of unsaturated fat intake with lung cancer risk. To the best of my knowledge, this is the first cohort study to evaluate the association

between unsaturated fat intake and lung cancer prognosis. The role of unsaturated fat in cancer prognosis has been examined in breast, prostate and stomach cancers, but the findings were inconsistent (99, 168, 169). This study found that high intake of monounsaturated fat was associated with better overall survival among advanced-stage NSCLC patients who received primary chemotherapy and were overweight. Monounsaturated fat intake is considered to have an anti-inflammatory property, which may suppress tumorigenesis (174). As mentioned earlier, patients with advanced-stage cancer may benefit from the metabolic reserves of body fat to withstand treatment (173). Further research is needed to confirm the protective effect of high intake of monounsaturated fat on this group of patients with advanced-stage NSCLC.

In Chapter IV, 2,339 NSCLC patients were divided into the discovery and the replication sets to identify significant single nucleotide polymorphisms (SNPs) in fatty acid metabolism genes that were associated with overall survival or recurrence. Those SNPs reaching statistical significance in the discovery set were further examined in the replication set to select the significant SNPs which have consistent associations in both datasets. This study identified four SNPs that were associated with overall survival and one SNP was associated with recurrence in NSCLC. These were novel SNPs and have not been found to be associated with cancer prognosis previously. Among these SNPs, three SNPs in *ACSL1*, *CYP2C8* and *FADS2* were predicted to be putatively functional, suggesting that they are candidates for further study.

ACSL1 plays a vital role in fatty acid synthesis and degradation. This study showed that early-stage patients with a G variant rs4862417 were associated with 28% and 39% decreased risk of death in the discovery and the replication sets, respectively. Variant rs2292899 in high linkage disequilibrium (LD) to rs4862417 was predicted to have functional effects on

transcriptional regulation through methylation. Erroneous methylation has been highly linked to the development and progression of several cancers, including lung cancer (175). *ACSL1* has been found to be lower expressed in lung cancer tissue that is associated with better survival (176). Nevertheless, there has been no expression quantitative trait loci (eQTL) analysis directly for rs4862417 nor its high LD SNPs, and thus further investigation is needed.

Both *CYP2C8* and *FADS2* regulate biosynthesis of unsaturated fatty acids from polyunsaturated fatty acids. This study showed that early-stage patients with two G variants *CYP2C8*:rs1934953 were associated with 42% decreased risk of death in the discovery set and 80% decreased risk in the replication set. rs1934953 was predicted to be located in the transcription factor binding site for *IRF2*. *IRF2* has been shown to inhibit cell proliferation and migration in lung cancer (177), but how eQTL regulates expression of rs1934953 in lung tissue is unknown and requires further research. In addition, this study found that early-stage patients who carry a G variant *FADS2*:rs174611 were increased the risk of death by 28% in the discovery set and 47% in the replication set. rs174611 was predicted to be located in the transcription factor binding site for *ELF1* that is an intermediate player involved in promoting proliferation and migration in NSCLC through *VEGF* (178, 179). The expression of *ELF1* has been shown to be positively associated with *VEGF* (180). A direct eQTL regulating expression of rs174611 showed that the number of risk alleles was correlated with increased *FADS2* expression in lung tissue. These findings strongly support the unfavorable effect on survival among early-stage NSCLC patients who carry the risk allele of rs174611. Moreover, the association between rs174611 genotype and overall survival in early-stage NSCLC was

modified by monounsaturated fat intake. A stronger unfavorable effect was observed in those who had low intake of monounsaturated fat and carried the risk allele.

Strengths and Limitations

This is the first study that systematically investigates how dietary fat intake and SNPs in all genes involved in fatty acid metabolism were correlated with overall survival and recurrence in NSCLC patients. The role of fatty acids in clinical outcomes among patients with NSCLC was prospectively examined. It is also the first study to examine the potential impact of dietary fat intake on the association between genetic variants and overall survival among NSCLC patients. This study used a previously validated food frequency questionnaire to collect dietary fat intake and was able to adjust for potential confounding factors to minimize the possibility of confounded associations. In addition, the sample size of this study was large, which gave more reliable results with greater precision and power. However, there are also several limitations to this study. First, the dietary information was based on self-reported data, and study participants were asked to recall what they had eaten during the past year. Participants may be unable to precisely recall the actual food they consumed, resulting in measurement errors in dietary data. It is unlikely that the measurement error was the differential of clinical outcomes. Second, the study participants only included non-Hispanic whites and external validation was not available, and thus we should be cautious about extrapolating our findings. Third, only a small proportion of patients had high intake of monounsaturated or polyunsaturated fat, which indicated a lower precision and statistical power. Lastly, although

this study adjusted for potential epidemiologic and clinical factors in the analysis, the possibility of uncontrolled confounding factors may not be completely excluded.

Future Directions

This dissertation was motivated by a lack of evidence on the influence of fatty acids on lung cancer prognosis. This dissertation utilized dietary, genetic and clinical data to explore how dietary fat intake and genetic variants in fatty acid metabolism genes were linked with overall survival and recurrence among patients with NSCLC. The results of this dissertation advance knowledge to improve prognosis among patients with NSCLC.

The results of this dissertation indicated that dietary fat intake was associated with overall survival among NSCLC patients, but the association seems to depend on the subtypes of fat, clinical stage, and types of treatment. There was a decreased overall survival for early-stage NSCLC patients with high intake of saturated fat. No similar patterns for total fat and other subtypes of fat were found. By contrast, there was an increased overall survival for advanced-stage NSCLC patients with primary chemotherapy who had high intake of saturated or monounsaturated fat. Nevertheless, there was no evidence for the association between fat intake and disease recurrence. This dissertation suggested that early-stage NSCLC patients may need to reduce consumption of saturated fat, and those who have advanced-stage and receive primary chemotherapy may need to get enough fat intake. Further studies are needed to confirm these findings and elucidate the mechanisms underlying the effect of saturated and monounsaturated fat intakes on lung cancer prognosis.

In addition, the results of this dissertation supported the involvement of genetic variants in fatty acid metabolism genes in overall survival, particularly for those with early-stage NSCLC. Variants *ACSL1*:rs4862417, *CYP2C8*:rs1934953 and *FADS2*:rs174611were important SNPs affecting survival among patients with early-stage NSCLC. These SNPs have not been identified with NSCLC prognosis in the previous study, and none of them has taken dietary fat intake into account. This study indicated a multiplicative interaction between an individual's genotype and habitual intake of monounsaturated fat that modulated the risk of survival in early-stage NSCLC. This dissertation suggested that early-stage NSCLC patients with G allele of rs174611 may need to increase the consumption of monounsaturated fat. More studies are needed to clarify how dietary factors interact with genetic variants to modify the prognosis of NSCLC.

Taken together, consuming fat in food and synthesizing fatty acids in human body both influence overall survival and recurrence among NSCLC patients. A novel gene-diet interaction with monounsaturated fat intake for NSCLC survival highlighted that diet may modify the effect of genetic variants on disease prognosis, which would be a key to achieving better patient care. However, in the absence of evidence supporting our findings, more studies across populations and solid experimental approaches will provide insights into the mechanisms underlying the association. This knowledge will contribute to better genetically-targeted nutrition advice to the public regarding NSCLC prognosis in the future.

APPENDICES

Appendix A: List of 94 fatty acid metabolism genes (691 SNPs) for this study

Gene	Gene full name	Chr	Start Position	End position	No. of SNPs
ACADM	acyl-CoA dehydrogenase medium chain	1	75962870	76001771	4
ACOT7	acyl-CoA thioesterase 7	1	6246919	6376413	10
CPT2	carnitine palmitoyltransferase 2	1	53434689	53452455	5
CYP2J2	cytochrome P450 family 2 subfamily J member 2	1	60131568	60165011	5
CYP4A11	cytochrome P450 family 4 subfamily A member 11	1	47167433	47180004	1
CYP4A22	cytochrome P450 family 4 subfamily A member 22	1	47375694	47387113	2
ELOVL1	ELOVL fatty acid elongase 1	1	43601659	43606286	2
MECR	mitochondrial trans-2-enoyl-CoA reductase	1	29391972	29430041	4
PLA2G4A	phospholipase A2 group IVA	1	1.85E+08	1.85E+08	23
PPT1	palmitoyl-protein thioesterase 1	1	40310969	40335555	6
PTGS2	prostaglandin-endoperoxide synthase 2	1	1.85E+08	1.85E+08	2
SCP2	sterol carrier protein 2	1	53165536	53289870	7
THEM4	thioesterase superfamily member 4	1	1.5E+08	1.5E+08	2
THEM5	thioesterase superfamily member 5	1	1.5E+08	1.5E+08	4
ACADL	acyl-CoA dehydrogenase long chain	2	2.11E+08	2.11E+08	2
ACSL3	acyl-CoA synthetase long chain family member 3	2	2.23E+08	2.24E+08	11
HADHA	hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha	2	26267008	26321098	6
HADHB	hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit beta	2	26321120	26366837	3
ACAA1	acetyl-CoA acyltransferase 1	3	38139211	38153619	5
EHHADH	enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase	3	1.86E+08	1.86E+08	7
GPX1	glutathione peroxidase 1	3	49369613	49370795	1
ACOX3	acyl-CoA oxidase 3	4	8418909	8493352	13
ACSL1	acyl-CoA synthetase long chain family member 1	4	1.86E+08	1.86E+08	12
CYP2U1	cytochrome P450 family 2 subfamily U member 1	4	1.09E+08	1.09E+08	4
ELOVL6	ELOVL fatty acid elongase 6	4	1.11E+08	1.11E+08	23
HADH	hydroxyacyl-CoA dehydrogenase	4	1.09E+08	1.09E+08	3
HPGDS	hematopoietic prostaglandin D synthase	4	95438730	95483050	6
SCD5	stearoyl-CoA desaturase 5	4	83769714	83939034	37
ACSL6	acyl-CoA synthetase long chain family member 6	5	1.31E+08	1.31E+08	9
ELOVL7	ELOVL fatty acid elongase 7	5	60083373	60175858	13
HSD17B4	hydroxysteroid 17-beta dehydrogenase 4	5	1.19E+08	1.19E+08	9
LTC4S	leukotriene C4 synthase	5	1.79E+08	1.79E+08	1

ACAT2	acetyl-CoA acetyltransferase 2	6	1.6E+08	1.6E+08	3
ECI2	enoyl-CoA delta isomerase 2	6	4060926	4080830	6
ELOVL2	ELOVL fatty acid elongase 2	6	11088978	11152610	6
ELOVL4	ELOVL fatty acid elongase 4	6	80681248	80713941	4
ELOVL5	ELOVL fatty acid elongase 5	6	53240155	53321901	9
PPT2	palmitoyl-protein thioesterase 2	6	32229279	32239430	4
TBXAS1	thromboxane A synthase 1	7	1.39E+08	1.39E+08	30
EPHX2	epoxide hydrolase 2	8	27404562	27458403	10
PTGDS	prostaglandin D2 synthase	9	1.39E+08	1.39E+08	2
PTGES	prostaglandin E synthase	9	1.32E+08	1.32E+08	5
PTGES2	prostaglandin E synthase 2	9	1.3E+08	1.3E+08	3
PTGS1	prostaglandin-endoperoxide synthase 1	9	1.24E+08	1.24E+08	10
ACSL5	acyl-CoA synthetase long chain family member 5	10	1.14E+08	1.14E+08	9
AKR1C3	aldo-keto reductase family 1 member C3	10	5126568	5139878	4
ALOX5	arachidonate 5-lipoxygenase	10	45189635	45261571	10
CYP2C19	cytochrome P450 family 2 subfamily C member 19	10	96512453	96602661	7
CYP2C8	cytochrome P450 family 2 subfamily C member 8	10	96786519	96819244	7
CYP2C9	cytochrome P450 family 2 subfamily C member 9	10	96688430	96739137	13
ECHS1	enoyl-CoA hydratase, short chain 1	10	1.35E+08	1.35E+08	1
ELOVL3	ELOVL fatty acid elongase 3	10	1.04E+08	1.04E+08	2
GPAM	glycerol-3-phosphate acyltransferase, mitochondrial	10	1.14E+08	1.14E+08	5
OLAH	oleoyl-ACP hydrolase	10	15125951	15155857	7
SCD	stearoyl-CoA desaturase	10	1.02E+08	1.02E+08	7
CPT1A	carnitine palmitoyltransferase 1A	11	68278664	68365881	12
FADS1	fatty acid desaturase 1	11	61323679	61340886	5
FADS2	fatty acid desaturase 2	11	61352289	61391401	11
HSD17B12	hydroxysteroid 17-beta dehydrogenase 12	11	43658719	43834745	12
ACACB	acetyl-CoA carboxylase beta	12	1.08E+08	1.08E+08	28
ACADS	acyl-CoA dehydrogenase short chain	12	1.2E+08	1.2E+08	2
LTA4H	leukotriene A4 hydrolase	12	94918742	94953496	14
PTGES3	prostaglandin E synthase 3	12	55343649	55368156	2
ACOT4	acyl-CoA thioesterase 4	14	73128163	73132223	2
GPX2	glutathione peroxidase 2	14	64475625	64479284	6
ACSBG1	acyl-CoA synthetase bubblegum family member 1	15	76250242	76313954	15
CYP1A2	cytochrome P450 family 1 subfamily A member 2	15	72828237	72835994	2
ECI1	enoyl-CoA delta isomerase 1	16	2229897	2241604	3
ACACA	acetyl-CoA carboxylase alpha	17	32516040	32841015	17
ACADVL	acyl-CoA dehydrogenase very long chain	17	7063877	7069309	2
ACOX1	acyl-CoA oxidase 1	17	71449183	71487039	7
ALDH3A2	aldehyde dehydrogenase 3 family member A2	17	19492656	19521500	4
ALOX12	arachidonate 12-lipoxygenase, 12S type	17	6840108	6854779	5
ALOX12B	arachidonate 12-lipoxygenase, 12R type	17	7916679	7931746	9
ALOX15	arachidonate 15-lipoxygenase	17	4480963	4491709	3

ALOX15B	arachidonate 15-lipoxygenase type B	17	7883083	7893177	7
FASN	fatty acid synthase	17	77629503	77649395	2
ACAA2	acetyl-CoA acyltransferase 2	18	45563873	45593900	4
ACSBG2	acyl-CoA synthetase bubblegum family member 2	19	6086710	6144112	6
CYP2B6	cytochrome P450 family 2 subfamily B member 6	19	46189044	46216141	6
CYP4F2	cytochrome P450 family 4 subfamily F member 2	19	15849834	15869884	5
CYP4F3	cytochrome P450 family 4 subfamily F member 3	19	15612707	15632570	5
CYP4F8	cytochrome P450 family 4 subfamily F member 8	19	15587029	15601448	7
TECR	trans-2,3-enoyl-CoA reductase	19	14501382	14537792	7
PTGIS	prostaglandin I2 synthase	20	47553818	47618114	6
CBR1	carbonyl reductase 1	21	36364155	36367332	3
CPT1B	carnitine palmitoyltransferase 1B	22	49354156	49363862	10
GGT1	gamma-glutamyltransferase 1	22	23309718	23354972	2
GGT5	gamma-glutamyltransferase 5	22	22945622	22971110	5
MCAT	malonyl-CoA-acyl carrier protein transacylase	22	41858156	41869347	5
ACSL4	acyl-CoA synthetase long chain family member 4	23	1.09E+08	1.09E+08	3

Appendix B: University of Texas Health Science Center Committee for Protection of Human Subjects Outcome Notice

UTHealth
The University of Texas
Health Science Center at Houston

Committee for the Protection of Human Subjects

6410 Fannin Street, Suite 1100
Houston, Texas 77030

Fang-Yu Lin
School of Public Health

February 05, 2019

HSC-SPH-19-0085 - *THE ASSOCIATIONS BETWEEN FAT AND CLINICAL OUTCOMES AMONG LUNG CANCER PATIENTS: DIETARY INTAKE AND GENETIC DETERMINANTS*

The above named project is determined to qualify for exempt status according to 45 CFR 46.101(b)

CATEGORY #4 *: Research, involving the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens, if these sources are publicly available or if the information is recorded by the investigator in such a manner that subjects cannot be identified directly or through identifiers linked to the subjects.*

CHANGES: Should you choose to make any changes to the protocol that would involve the inclusion of human subjects or identified data from humans, please submit the change via iRIS to the Committee for the Protection of Human Subjects for review.

INFORMED CONSENT DETERMINATION:
Waiver of Consent Granted

HEALTH INSURANCE PORTABILITY and ACCOUNTABILITY ACT (HIPAA):
Exempt from HIPAA

STUDY CLOSURES: Upon completion of your project, submission of a study closure report is required. The study closure report should be submitted once all data has been collected and analyzed.

Should you have any questions, please contact the Office of Research Support Committees at 713-500-7943.

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