SYMPTOMS, GENETICS, AND HEALTH-RELATED QUALITY OF LIFE IN PERSONS WITH NONALCOHOLIC FATTY LIVER DISEASE

By

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ABSTRACT

SYMPTOMS, GENETICS, AND HEALTH-RELATED QUALITY OF LIFE
IN PERSONS WITH NONALCOHOLIC FATTY LIVER DISEASE

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Background: Nonalcoholic fatty liver disease (NAFLD) is a highly prevalent condition strongly associated with obesity. NAFLD is characterized by the presence of fatty deposits in the liver and can result in premature death. Little is known about a symptoms experience in this progressive disease, preventing health-care providers from intervening in the early stages of the disease. **Hypothesis:** The purpose of this study is to explicate symptoms and health-related quality of life (HRQOL) in persons with NAFLD hypothesized to be at higher risk of disease progression based on *PNPLA3* (rs738409) genotype. **Methods:** A cross-sectional descriptive design, guided by the symptoms experience model, was used to recruit 42 persons 21 years of age or older with diagnosed NAFLD from gastroenterology and bariatric surgery offices in western Michigan. Genotyping for the presence of *PNPLA3* gene, (rs738409)-G allele was used to stratify the population. The Memorial Symptom Assessment Scale (MSAS), the Charlson Comorbidity Index, and the Centers of Disease Control and Prevention's Healthy Days Measure were used to measure symptoms, comorbid conditions, and HRQOL. Multiple linear regression techniques were used to analyze the data using PASW 17 software. **Results:** Frequency of the G allele was .369. Participants (97%) experienced one or more symptoms (M = 12.02, SD = 8.817). Significant predictors were obtained for mean frequency, severity, and distress of symptoms using the subscales of the Memorial Symptom Assessment Scales [TMSAS] (F = 2.609, df1 = 15,

*df*2 = 25, *p* = .016), Overall distress [MSAS-GDI] (*F* = 3.331, *df*1 = 15, *df*2 = 25, *p* = .004), Physical subscale [MSAS-PHYS] (*F* = 2.726, *df*1 = 15, *df*2 = 25; *p* = .013), and Psychological subscale [MSAS-PSYCH] (*F* = 2.944, *df*1 = 15, *df*2 = 25; *p* = .008). The frequency, intensity, and distress of symptoms did not differ according to *PNPLA3* (rs738409) genotype. However, persons with one or two copies of the *PNPLA3* gene, (rs738409)-G allele had poorer HRQOL than persons with no copies of the *PNPLA3* gene, (rs738409)-G allele (*F* = 5.068, *df*1 = 15, *df*2 = 25; *p* < .001). Distress of symptoms greatly influenced HRQOL when the *PNPLA3* gene, (rs738409)-G allele was removed (*F* = 11.057, *df*1 = 15, *df*2 = 39, *p* < .001). **Clinical Implications**: Persons with NAFLD experience symptoms and have nearly 3 times poorer HRQOL than the general U.S. population. The critical role of nursing in treating the human response derived from increased symptoms and poorer HRQOL is key to optimizing health in all stages of the NAFLD disease trajectory. The CDC Healthy Days tool along with the MSAS would be beneficial in screening NAFLD patients for changes in health before and after nursing interventions, and they may be beneficial in preliminary screening of obese patients for the presence of NAFLD. **Research Implications:** Studies with larger sample sizes are needed to explicate symptoms in persons with NAFLD. In addition, longitudinal studies of symptoms with a larger population are needed to explicate the symptoms experience in persons with NAFLD over time and through the disease trajectory. Further research is needed to determine the genetic contribution to symptom production in persons with NAFLD.

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ALT	Alanine transaminase
APOC3	Apolipoprotein C3
AST	Aspartate Aminotransferase
BMI	Body Mass Index
CC	Two copies of the *PNPLA3* gene, (rs738409) C-allele or CC genotype
CCI	Charlson Comorbidity Index
CG	One copy of the *PNPLA3* gene, (rs738409)-C allele and one copy of the *PNPLA3* gene, (rs738409)-G allele; or CG genotype
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
DNA	Deoxyribonucleic acid
ETOH	Alcohol
FNP-BC	Family Nurse Practitioner-Board Certified
GG	Two copies of the *PNPLA3* gene, (rs738409) G-allele or GG genotype
HCV	Hepatitis C
HDL	High Density Lipoprotein Cholesterol
HRQOL	Health-Related Quality of Life
ID	Identification Number
IL-6	Interleukin-6
LDL	Low-Density Lipoprotein Cholesterol
NAFLD	Nonalcoholic Fatty Liver Disease

NASH	Nonalcoholic Steatohepatitis
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institute of Health
NINR	National Institute of Nursing Research
NRSA	National Research Service Award
MSAS	Memorial Symptom Assessment Scale
MSAS-GDI	Memorial Symptom Assessment Scale-Global Distress Index
MSAS-PHYS	Memorial Symptom Assessment Scale-Physical
MSAS-PSYCH	Memorial Symptom Assessment Scale-Psychological
MSN	Master of Science in Nursing degree
MSU	Michigan State University
OR	Odds Ratio
P3NP	Procollagen-3 N-Terminal Peptide
PASW	Predictive Analytics SoftWare Statistics, formerly known as SPSS.
PCR	Polymerase Chain Reaction
PhDc	PhD Candidate or Doctor of Philosophy Candidate
PI	Primary Investigator
PNPLA3	Patatin-like Phospholipase Domain-Containing Protein 3 gene
RN	Registered Nurse
SD	Standard Deviation
SDS	Sequence Detection System
SE	Standard Error
SEM	Symptoms Experience Model

TIMP-1	Tissue Inhibitor of Metalloproteinase 1
TMSAS	Total Memorial Symptom Assessment Scale
TNF-α	Tumor Necrosis Factor-Alpha
TOUS	The Theory of Unpleasant Symptoms

Chapter 1: Overview

Nonalcoholic fatty liver disease (NAFLD) is a rapidly growing public health concern, closely associated with obesity, insulin resistance or Diabetes Mellitus Type 2, and the metabolic syndrome (Adler & Schaffner, 1979; Stranges, et al., 2004; Vernon, Baranova, & Younossi, 2011; Younossi, et al., 2011). NAFLD is often identified in the later stages of disease, resulting in premature death (Adams, Lymp, et al., 2005; Hui, et al., 2003). Consequently, the outcome of premature death because of NAFLD is devastating financial loss (Baumeister, et al., 2008; Kim, Brown, Terrault, & El-Serag, 2002). Research is needed to support early identification, early treatment, and improvement or maintenance of health status through multidisciplinary interventions in persons with NAFLD in order to prevent premature death.

Significance

Incidence and prevalence. The incidence of NAFLD is unknown. To date, no prospective studies have been conducted to determine the development of NAFLD over time (Angulo, 2007a). In addition, current diagnostic laboratory and imaging tests, other than liver biopsy, are not highly specific in the detection and staging of NAFLD. Nonetheless, NAFLD is considered a worldwide health concern with an estimated worldwide prevalence between 2.8% (diagnosed by aminotransferase elevation) and 46% (diagnosed by imaging or liver biopsy).

NAFLD is coincident with the worldwide obesity epidemic. According to the World Health Organization, more than 1 billion adults are either overweight or obese worldwide (World Health Organization, 2010). As a result, researchers have observed NAFLD in countries all over the globe including Australia (Adams, Waters, Knuiman, Elliott, & Olynyk, 2009), Belgium (Francque, et al., 2010), Brazil (Cotrim, et al., 2011),

Canada (Myers, et al., 2010), China (Hui, et al., 2005; Yu, et al., 2011), France (Aron-Wisnewsky, et al.; Mathurin, et al., 2006), India (Amarapurkar, et al., 2007), Italy (Targher, et al., 2007; Targher, et al., 2008), Japan (Kimura, et al., 2011), Mexico (Roldan-Valadez, et al., 2010), Saudi Arabia (AlQaraawi, et al., 2011), South Korea (Sung, et al., 2007), Spain (de Luis, Aller, Izaola, Gonzalez Sagrado, & Conde, 2010), Sweden (Kotronen, Yki-Jarvinen, et al., 2009; Söderberg, et al., 2010), Taiwan (Hsiao, et al., 2007), Turkey (Alper, et al., 2008), and the United States (Schwimmer, et al., 2006; Williams, et al., 2011; Younossi, et al., 2005). This worldwide prevalence underscores the need for research of this rapidly growing disease.

Approximately 30 to 70 million people are affected by NAFLD in the United States (Adams, Lymp, et al., 2005; American Liver Foundation, 2007; Angulo, 2007b; Chan, de Silva, Leung, Lim, & Farrell, 2007; Choudhury & Sanyal, 2004; Preiss & Sattar, 2007). U.S. obesity rates have risen a dramatic 50% since 1980, and today 66% of Americans are either overweight or obese (Ogden, et al., 2006). Persons with obesity are at greatest risk for NAFLD, as 71 – 97.8% of those with a body mass index (BMI) of 30 or greater have NAFLD (Beymer, et al., 2003; Colicchio, et al., 2005; Dixon, Bhathal, & O'Brien, 2001; Luyckx, et al., 1998; Silverman, et al., 1990; Spaulding, Trainer, & Janiec, 2003).

Race/ethnicity, sex, and age. NAFLD afflicts 1 in every 3 adults and 1 in every 10 children according to a comprehensive review (Angulo, 2007a). NAFLD affects all ages from adolescents to adults, with relatively equal prevalence in males and females, and slightly higher prevalence in male adolescents versus females, and in white adult

males (American Liver Foundation, 2007; Angulo, 2007b; Oh, Winn, & Poordad, 2008; Schwimmer, et al., 2006).

Hispanics are at higher risk, followed by Caucasians and African Americans (Browning, et al., 2004). Further study is needed to determine the risk of American Indians, and Alaskan Natives in the United States (Fischer, Bialek, Homan, Livingston, & McMahon, 2009).

Natural History

NAFLD can progress from simple fatty liver disease to an inflammatory stage known as nonalcoholic steatohepatitis (NASH) with or without fibrosis, to cirrhosis and liver cancer or liver failure resulting in premature death [See Figure 1 for NAFLD Illness Trajectory], (Adams, Angulo, & Lindor, 2005; Adams, Lymp, et al., 2005; Angulo, 2007b; Salt, 2004). Recent studies suggest that progression to cirrhosis or liver cancer may be related to the presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele (Falleti, et al., 2011; Romeo, et al., 2008; Romeo, et al., 2010; Sookoian, et al., 2009; Tian, Stokowski, Kershenobich, Ballinger, & Hinds, 2009; Valenti, et al., 2010). The *PNPLA3* gene "Patatin-like Phospholipase domain containing 3" participates in energy balance in the adipocytes (NCBI Entrez Gene, 2009). The *PNPLA3* gene, (rs738409)-G allele is strongly associated with liver steatosis and NAFLD severity (Romeo, et al., 2008; Sookoian, et al., 2009; Valenti, et al., 2010).

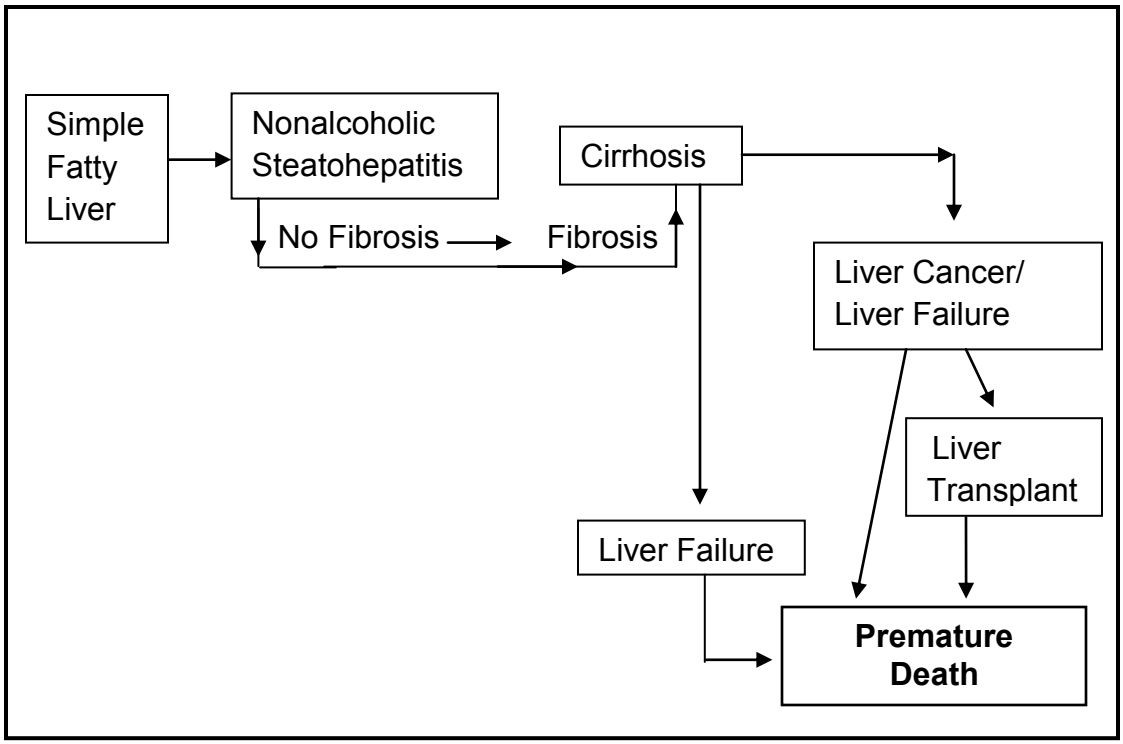

Figure 1. Nonalcoholic fatty liver disease (NAFLD) illness trajectory.

NAFLD, a progressive and potentially fatal disease, is defined as the presence of fatty deposits in the hepatocytes comprising 5% or more of the total liver weight (Angulo, 2007a). In most cases, if NAFLD is identified early and successful interventions such as weight loss are applied, NAFLD may reverse (Dixon, Bhathal, Hughes, & O'Brien, 2004).

Unfortunately, NAFLD is not only a rapidly emerging condition strongly associated with obesity, (Adams & Angulo, 2006; Angulo, 2007a; Oh, et al., 2008), but also associated with insulin resistance (Adams & Angulo, 2006; Oh, et al., 2008) and the metabolic syndrome (Adams & Angulo, 2007; Adams, Angulo, et al., 2005; Marchesini & Babini, 2006; Tsai, Li, & Lin, 2008), which increases risk for cardiovascular complications (Targher, 2007).

Persons from varying ethnic groups experience NAFLD (Adams, Waters, Knuiman, Elliott, & Olynyk, 2009; Alper, et al., 2008; Amarapurkar, et al., 2007; Angulo, 2007a; Araujo, De Oliveira, & Nunes, 1998; Berasain, et al., 2000; Browning, et al., 2004; el-Hassan, Ibrahim, al-Mulhim, Nabhan, & Chammas, 1992; Hamaguchi, et al., 2005; Hilden, Christoffersen, Juhl, & Dalgaard, 1977; Hui, et al., 2005; Lonardo, Bellini, Tartoni, & Tondelli, 1997; Luyckx, et al., 1998; Mathurin, et al., 2006; Sung, et al., 2007; Targher & Arcaro, 2007; Wanless & Lentz, 1990). Hispanics have a high prevalence of NAFLD, perhaps because of a higher frequency of the *PNPLA3* gene, (rs738409)-G allele compared to Caucasians and African Americans (Romeo, et al., 2008; Sookoian, et al., 2009; Tian, et al., 2009). Further examination of the link between NAFLD and genetic background, such as the presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele is needed in other racial and ethnic populations.

Public Health Implications

The potential impact of NAFLD progression is a significant threat to public health. As the disease progresses from simple fatty liver disease to NASH to liver cirrhosis, more medical treatment is needed. Persons that progress to the end stage of NAFLD, such as liver cirrhosis, may medically qualify for liver transplantation in an attempt to avoid premature death. However, liver transplantation is a significant cost to patients and third party payers. For example, the California Pacific Medical Center estimates the cost of transplantation to be $314,600 during the year of transplantation and approximately $22,000 for antirejection medications and follow-up appointments in subsequent years (California Pacific Medical Center, 2002). Indirect costs, including family members' time away from work to transport the patient to follow-up appointments

and provide other caregiving activities, loss of work for the patient and loss of future income as a result of premature death (Sandler, et al., 2002) must be considered in the financial costs of liver transplantation. Emotional costs to consider are the individual's or family members' worry about survival of the patient until a liver transplant is received (Miyazaki, et al., 2010); the ability of the patient's caregiver to balance household responsiblities, child care and other responsiblities (Miyazaki, et al., 2010); and worry about the additional cost incurred related to liver transplantation (Watanabe & Inoue, 2010). Therefore, with the increased prevalence of obesity, and the emerging prevalence of NAFLD in children, the number of persons needing transplantation to avoid premature death from NAFLD and the resultant costs of NAFLD to society will be astronomical (Angulo, 2006; California Pacific Medical Center, 2002; Flegal, Carroll, Ogden, & Curtin, 2010; Ogden, Carroll, Curtin, Lamb, & Flegal, 2010; Schwimmer, et al., 2006) .

In summary, NAFLD is a significant public health concern strongly associated with obesity, that can result in premature death (Angulo, 2007a) because of liver failure as a result of disease progression (Adams & Angulo, 2005; American Liver Foundation, 2007; Angulo, 2007b; Choudhury & Sanyal, 2004). Emotional and financial costs are significant as the disease progresses.

Significance to Nursing

Nurses are experts at preventing disease, promoting health, managing chronic disease, and treating the human response (American Nurses Association, 2003). While studies for potential treatment of NAFLD are ongoing, current practice is to treat NAFLD with weight loss through diet and exercise. Nurses mentor and monitor patients during

the weight loss program. If NAFLD can be identified early, nursing may provide important interventions to halt or reverse the disease process.

Given that interventions can halt or reverse the disease process in the early stages of the disease, (Mummadi, Kasturi, Chennareddygari, & Sood, 2008) and treatment of subtle symptoms in the late stages of the disease, such as cirrhosis, can prolong and enhance quality of life in the remaining years of life, identifying symptoms is critical in providing nurses with noninvasive tools to recognize those persons at risk of progression. Once identified and nursing interventions are implemented, symptoms and health-related quality of life (HRQOL) can be used to measure the effectiveness of nursing interventions aimed at influencing the frequency, intensity, and distress of symptoms in persons with NAFLD.

Contribution to Science

This study contributes to nursing and the National Institute of Nursing Research's initiatives of "advancing the quality of life through symptom management" by identifying symptoms in persons with NAFLD (National Institute of Nursing Research, 2011) [p.14]. This is the first study to identify a comprehensive list of symptoms in adults with NAFLD. The identification of symptoms will provide a means in which to monitor progression of NAFLD throughout the illness trajectory. Second, through the use of the *PNPLA3* gene, (rs738409)-G allele, an attempt is made to further knowledge of the relationship of a gene polymorphism associated with NAFLD and progressive stages of NAFLD, with symptoms, in accordance with NINR strategic plan (National Institute of Nursing Research, 2011). Third, through the use of the Centers of Disease Control and Prevention's Healthy Days tool, HRQOL will be quantified in this study. Quantification

of HRQOL will provide another measure in which to manage chronic illness as noted in the strategic plan of the National Institute of Nursing Research (National Institute of Nursing Research, 2011).

Problem

Despite the growing prevalence of NAFLD, little is known about symptoms for persons at risk of NAFLD progression as NAFLD is thought to be an asymptomatic disease (Salt, 2004). In addition, little is known about how symptoms influence HRQOL in adult persons with NAFLD.

Symptoms. Currently, there is a lack of knowledge regarding symptoms in persons with NAFLD (Angulo, 2007a; Salt, 2004). Some studies report fatigue, malaise and right upper quadrant pain or discomfort in persons with NAFLD (Angulo, 2007a), while other studies report that NAFLD is asymptomatic, calling for longitudinal studies to examine the presence of symptoms over time (Salt, 2004). Recently, in a study of obese children, persons with NAFLD reported difficulty sleeping, fatigue, and sadness, further suggesting that adults should be assessed for symptoms (Kistler, et al., 2010).

HRQOL. Research to examine the impact of NAFLD on HRQOL in persons with NAFLD is emerging in the literature. Persons with advanced stages of NAFLD have poorer HRQOL than persons with early stage disease (David, et al., 2009). In addition, persons with NAFLD have poorer HRQOL than persons with hepatitis B or C (Dan, et al., 2007). U.S. citizens report an average 5.3 unhealthy days per month (Centers for Disease Control and Prevention, 2000). However, more research is needed to compare HRQOL of the general population to those with NAFLD.

Genetics. As presented, evidence suggests that a genetic variant, the *PNPLA3* gene, (rs738409)-G allele contributes to the risk for developing NAFLD (Romeo, et al.,

2008) and the risk for disease progression (Sookoian, et al., 2009). The risk of severity of NAFLD increases with homozygosity of the polymorphism compared to the heterozygous genotype (Valenti, et al., 2010). In addition, the *PNPLA3* gene, (rs738409)-G allele variant is more prevalent in those of Hispanic descent (Tian, et al., 2009) which correlates with epidemiologic studies noting increased prevalence in Hispanics (45%) followed by Caucasians (33%) and African Americans (24%) (Browning, et al., 2004). Little is known, however, about symptoms, genetics and HRQOL in persons with NAFLD. This knowledge gap prevents adequate monitoring of subtle changes in health as a result of changes in symptoms or HRQOL in persons with NAFLD. Identification of symptoms and HRQOL in persons with NAFLD would provide a means of measuring changes in health as a result of nursing interventions.

Purpose

The purpose of this study is to describe symptoms and HRQOL in persons with NAFLD who are hypothesized to be genetically predisposed for progression of the disease to liver cirrhosis or liver cancer. A comparison will be made between symptoms in persons with NAFLD who have one or two copies of the *PNPLA3* gene, (rs738409)-G allele and symptoms in persons with NAFLD who do not have the *PNPLA3* gene, (rs738409)-G allele. If a difference in symptoms and HQROL is found in participants with the *PNPLA3* gene*,* (rs738409)-G allele, these results may be used as a foundation for screening and implementation of early interventions to prevent premature death from NAFLD.

The long-term goal of this research program is to develop assessment and intervention strategies to prevent the development of NAFLD, to prevent or reverse

(Mummadi, et al., 2008) disease progression in persons with existing NAFLD through assessment and intervention, and to prevent or manage symptoms from a multidisciplinary and patient-centered perspective. This study will provide a foundational understanding of symptoms and HRQOL in persons with NAFLD to support future intervention work. The rationale for this research is that, once known, symptoms in persons at risk of NAFLD disease progression can be identified noninvasively (i.e. without liver biopsy) by multiple disciplines; therefore, multidisciplinary interventions can be implemented to provide anticipatory guidance, support lifestyle modifications, and treat symptoms as measured by symptom levels and the nurse-sensitive outcome of HRQOL.

Specific Aims

The following four hypothesis-driven, specific aims will be pursued in this descriptive, correlational and cross-sectional study:

Aim 1. To identify the presence of symptoms in persons with NAFLD at higher risk of disease progression based on the presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele compared to those at lower risk of disease progression, that is, no copies of the *PNPLA3* gene, (rs738409)-G allele.

Hypothesis 1: Persons at higher risk of progression of NAFLD as determined by the presence of one or two copies of the PNPLA3 gene, (rs738409)-G allele will exhibit symptoms compared to persons at lower risk of progression of NAFLD, that is, with no copies of the PNPLA3 gene, (rs738409)-G allele

Aim 2. To compare the extent to which the frequency, intensity, and distress of symptoms in persons with NAFLD differ between those at higher risk of disease progression based on the presence of one or two copies of the *PNPLA3* gene,

(rs738409)-G allele compared to those at lower risk of disease progression, that is, no copies of the *PNPLA3* gene, (rs738409)-G allele.

Hypothesis 2: Persons at higher risk of disease progression will have more symptom intensity, frequency and/or distress than those at lower risk of disease progression.

Aim 3. To determine the difference in HRQOL in persons at higher risk of NAFLD progression based upon the presence of one or two copies of *PNPLA3* gene, (rs738409)-G allele versus those at lower risk of disease progression, that is, no copies of the *PNPLA3* gene, (rs738409)-G allele.

Hypothesis 3: Persons with NAFLD at higher risk of disease progression will have poorer HRQOL than those at lower risk of disease progression.

Aim 4. To describe the relationship between symptom distress and HRQOL in persons at higher risk of NAFLD progression based on the presence of one or two copies of *PNPLA3* gene, (rs738409)-G allele versus persons at lower risk of NAFLD progression, that is, no copies of the *PNPLA3* gene, (rs738409)-G allele.

Hypothesis 4: There will be a negative correlation between the distress of symptoms' total sum of scores and HRQOL score in those at higher risk of NAFLD progression.

Expected Outcomes and Overall Impact

Persons with NAFLD, who carry one or two copies of the *PNPLA3* gene, (rs738409)-G allele will have symptoms and poorer HRQOL than those without the *PNPLA3* gene, (rs738409)-G allele. The overall impact of this study will be the identification of symptoms and the quantification of HRQOL in persons with NAFLD who are hypothesized to be genetically predisposed for progression of NAFLD based on the

presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele. The findings of this study will provide the foundation for future interventional studies that may be targeted toward individual characteristics such as genotype.

Chapter 2: Conceptual Framework

In 2001, the Institute of Medicine published "Crossing the Quality Chasm" outlining six aims to improve health-care delivery in the United States. These aims -- Safe, Effective, Patient-Centered, Timely, Efficient, and Equitable care -- if implemented, would improve patient outcomes as patients would experience more responsive care in a timely fashion (Tarcin, et al., 2008).

Prior to this time, the National Center for Nursing Research, now known as the National Institute for Nursing Research (NINR), included the importance of measuring outcomes or consequences of symptom management, noting this as a high priority for nursing research in 1988 (Hegyvary, 1993). Currently, NINR defines the research focus of this agency to "encompass health promotion and disease prevention, quality of life, health disparities and end-of-life" (Charlson, Pompei, Alex, & MacKenzie, 1987).

Together, the initiatives from the IOM and NINR support the impetus for a foundational study of symptoms in persons with NAFLD. Symptoms, the "red flags of threats to health," can be used to trigger interventions for persons with NAFLD (Hegyvary, 1993). Identification of a symptoms experience in persons with NAFLD is important, as NAFLD can progress from simple fatty liver disease to cirrhosis and liver failure resulting in premature death (Angulo, 2007a). Unfortunately, little is known about symptoms of NAFLD beyond "fatigue, malaise, and right upper quadrant discomfort" often found in later disease stages (Salt, 2004). In addition, little is known about how symptoms influence HRQOL in adult persons with NAFLD. Given that early interventions can halt or reverse the disease process in the early stages of the disease (Mummadi, et al., 2008), and treatment of subtle symptoms in the late stages of the

disease, such as cirrhosis, can prolong and enhance quality of life in the remaining life years, identification of symptoms is critical in providing nurses with noninvasive tools to identify those persons at risk of progression. Once identified and nursing interventions are implemented, symptoms and HRQOL can be used to measure the effectiveness of nursing interventions aimed at influencing the frequency, intensity and distress of symptoms in persons with NAFLD.

Conceptual models provide the architecture or a means of organizing the design and methods of a study (Fawcett, 2005; Mock, et al., 2007). Armstrong's symptoms experience model, a middle-range theory, will be used as the conceptual framework for this research (Armstrong, 2003). The purpose of this chapter is to (a) describe the symptoms experience model, (b) critique the model using Fawcett's framework for analysis and evaluation of nursing models (Fawcett, 2005), (c) provide adaptations to the model that will be used in this study, and (d) present rationale for use of the symptoms experience model as a conceptual framework for the study of Symptoms, Genetics and HRQOL in Persons with NAFLD.

Description of Armstrong's Symptoms Experience Model: A Middle-Range Theory

Peterson identifies three levels of theories: grand theories, middle-range theories and nursing practice theories (Peterson, 2009). A grand theory is very broad, describing a world view of nursing (Fawcett, 2005; Peterson, 2009). A middle-range theory describes a specific phenomenon of a client's health, such as the symptoms experience, and may describe nursing interventions or predict an outcome or consequence such as a decrease in HRQOL as found in the symptoms experience model (Armstrong, 2003; Fawcett, 2005; Walker & Avant, 2005b). Finally, a nursing

practice theory is more specific and more narrow in scope than a middle range theory and often guides interventions for a specific nursing issue (Peterson, 2009; Walker, 1986).

Middle-range theories are optimal frameworks for research because of the narrow scope and limited number of variables in the model, such as the symptoms experience model (Peterson, 2009). The symptoms experience model is a middle-range theory developed for use by nurses for empirical research of a symptoms experience in caring for patients with cancer (Armstrong, 2003).

Armstrong, a neuro-oncology nurse practitioner and nurse researcher, developed the symptoms experience model to explicate the definition of symptoms experience. Armstrong purported that the meaning of symptoms should be considered when evaluating the patient's symptom occurrence and distress from the symptoms (Armstrong, 2003). Armstrong used Walker and Avant's concept analysis framework (Walker & Avant, 2005a) to define the construct of a symptoms experience.

While this research will determine a symptoms experience in persons with NAFLD rather than cancer, the symptoms experience model is a pragmatic framework that can provide guidance for the study of symptoms experience beyond the cancer population.

Historical background. The symptoms experience model was derived from the theory of unpleasant symptoms (Gift, 2009; Lenz, Pugh, Milligan, Gift, & Suppe, 1997; Lenz, Suppe, Gift, Pugh, & Milligan, 1995), the theory of self-regulation (Leventhal & Johnson, 1983), the symptom management model (Dodd, 2001; Larson, et al., 1994), the symptom work of Rhodes and Watson (Rhodes & Watson, 1987), and the meaning

of symptoms work of Richer and Ezer (Richer, 2000). Together, these models formed a common theme in conceptualization of a symptoms experience (Armstrong, 2003). The common premise among these models include “the subjective nature of symptoms, the occurrence seen as a departure from normal function, the multidimensional nature, and the inclusion of an emotional response to the symptom” (Armstrong, 2003) [p.602]. The term “symptoms experience” is written in the plural form to denote that symptoms occur in clusters. Thus, Armstrong defines the symptoms experience as the “perception of the frequency, intensity, distress, and meaning occurring as symptoms are produced and expressed” (Armstrong, 2003) [p.602].

The symptoms experience model reads from left to right (See Figure 1). The antecedents, derived from an extensive literature review, include the demographic characteristics, disease characteristics, and individual characteristics. Each of the antecedents influences one another and together influence the production or occurrence of the symptoms (Armstrong, 2003). Once symptoms are produced, symptoms are perceived by the individual as defined by the frequency, intensity, distress and meaning of the symptoms (Armstrong, 2003). The perception of the symptoms is also influenced by the situational meaning of the symptoms and the existential meaning of the symptoms (Armstrong, 2003). The response to the symptoms is known as the expression of symptoms in this model. As symptoms are expressed, changes occur in “physiological, psychological, sociocultural and the behavioral components” of the person, resulting in the consequences or outcomes of adjustment to illness, quality of life, mood, functional status, disease progression or survival (Armstrong, 2003; Dodd, 2001)

Characteristics of the antecedents. Each of the three antecedent categories contains specific characteristics of the patient. The demographic characteristics include age, gender, marital status, race, culture, role, education, and socioeconomic status. Characteristics related to the disease are the type and state such as cancer stage, or in this study, the stage of NAFLD. The type of treatment and comorbid medical and clinical factors also are disease characteristics within the antecedent category. The type of treatment may be chemotherapy in the cancer patient; but in the study of persons with NAFLD, treatment type includes weight loss interventions (Dixon, et al., 2004) or use of Vitamin E (Sanyal, et al., 2010). Comorbid medical and clinical factors of cancer include diabetes or anorexia. In NAFLD, comorbid medical and clinical factors include obesity (Beymer, et al., 2003; Colicchio, et al., 2005; Dixon, Bhathal, & O'Brien, 2001; Luyckx, et al., 1998; Silverman, et al., 1990; Spaulding, Trainer, & Janiec, 2003) and diabetes (Targher, et al., 2007; Targher, et al., 2010). Individual characteristics include health knowledge, values, past experiences, and sense of coherence. Health knowledge in this study is the prior knowledge about NAFLD.

Defining characteristics of symptoms. Armstrong notes that defining characteristics of symptoms seem to be consistent across many studies in the oncology population. These defining characteristics are found in the symptom perception of her model as distress, intensity, frequency and meaning as noted in her definition (Armstrong, 2003). These defining characteristics describe the extent to which the symptoms are perceived by the individual and will be used to define characteristics of potential NAFLD symptoms in this study.

Symptom production. Symptom production is not specifically defined by Armstrong; however, symptoms are defined as "concrete representation of disease experienced by individuals as a component of cognitive processing" as influenced by Leventhal & Johnson's theory of self-regulation (Armstrong, 2003; Leventhal & Johnson, 1983). Symptom production is defined by others as the physiological and neurobiological mechanisms that result as triggered by the disease process (Dalal, Del Fabbro, Bruera, Arnold, & Liao, 2006; Kim, Bruera, & Jenkins, 2004; Reyes-Gibby, et al., 2008). An example of symptom production includes stimulation of pain receptors (nociceptors) in the tissues such as distention of Gleason's capsule in the liver as hepatocarcinoma progresses. Kim notes that symptom production cannot be easily measured by clinicians (Kim, et al., 2004).

Symptom perception and symptom expression. The "perception of the symptoms experience requires the ability to understand what is obscure" (Armstrong, 2003). The demographic, disease and individual characteristics may influence the perception. The symptoms affect the person's situational meaning, that is, the perception of a new event and one's capacity to handle it or existential meaning, which is the global representation of one's place in the world (Armstrong, 2003; Richer, 2000). Symptoms may catalyze one other, producing the symptom expression or emotional response to the symptoms.

Consequences. The consequences of Armstrong's symptoms experience model are adjustment to illness, quality of life, mood, functional status, disease progression and survival.

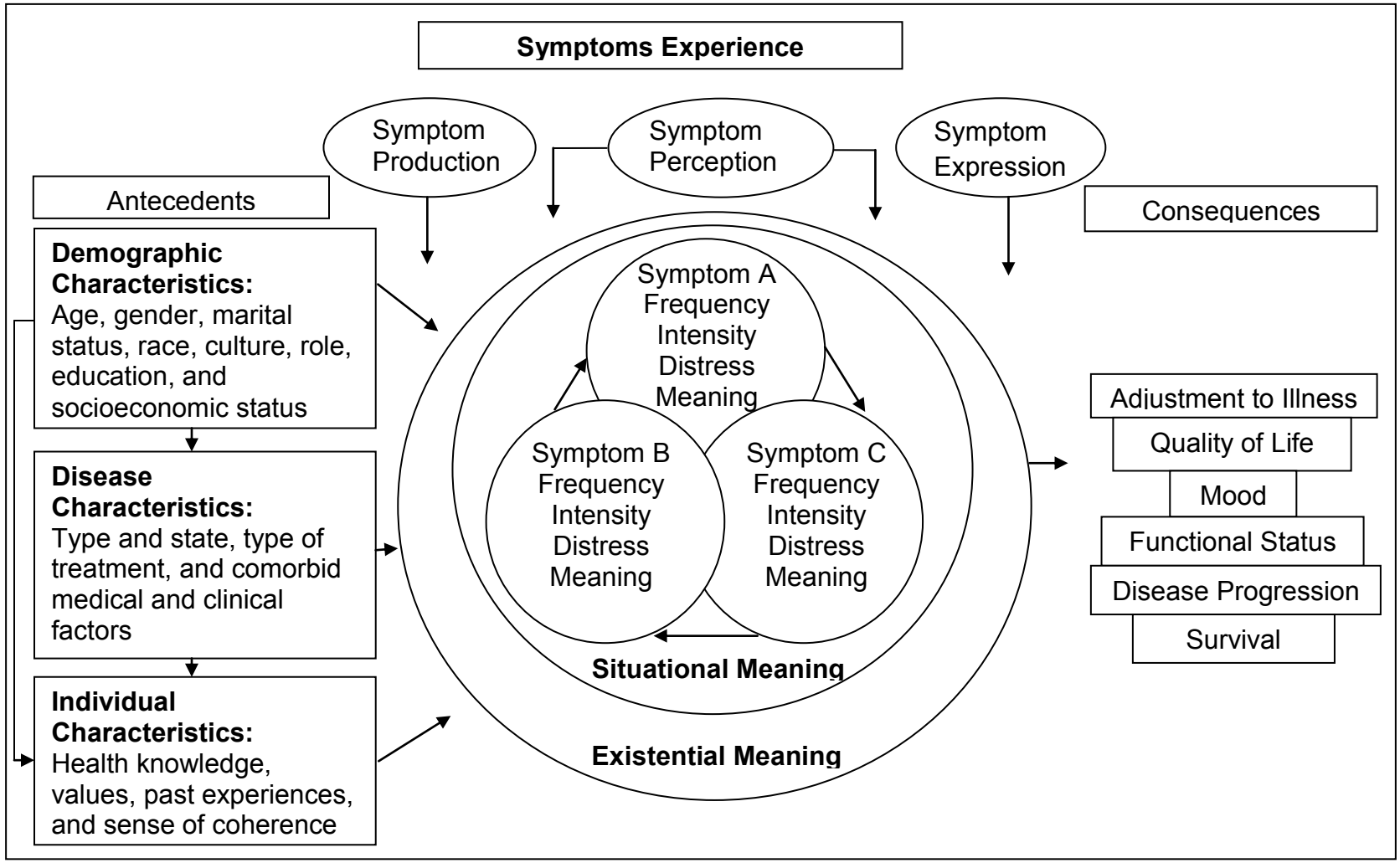

Figure 2. Symptoms experience model. From "Symptoms Experience: A Concept Analysis," by Terri S. Armstrong, MS, APRN, BC, 2003, *Oncology Nursing Forum, 30*(4), p. 603. Copyright 2003 by the Oncology Nursing Society. Reprinted with permission (see Appendix D).

Conceptual definition. The word, “symptom or symptoms” originated in Middle English, Medieval and Late Latin as “sinthoma” or “symptoma” to such as a symptom of a disease. The Greek form “sumptoma” or “sumptomat” refers to a happening, a symptom of a disease. These words originate from “sympiptein” or “sumpto,” which means “to coincide” or “to fall” such as to fall into illness (The American Heritage Dictionary of the English Language, 2007).

Experience, the second term found in the symptoms experience originated in Middle English from Latin somewhere between 1350 and 1400 from the words “experientia, experient, or experiéns meaning to try, or test.” Experience is defined as “what one lives through, the act of living through an event or events; personal involvement in or observation of events as they occur, the effect on a person of anything or everything that has happened to that person; individual reaction to events, feelings” (Webster's New World College Dictionary, 2005). Experience is also defined as “the totality of the cognitions given by perception; all that is perceived, understood, and remembered” (Dictionary.com Unabridged, 2011).

Several conceptual definitions of symptoms are found in the literature. For example, symptoms are the “red flags of threats to health” and “perceived indicators of change in normal functioning as experienced by patients” as compared to signs which are “objective measures of abnormal functioning” (Hegyvary, 1993) [p. 146]. Armstrong paraphrases a description of symptoms from Leventhal and Johnson’s theory of self-regulation work as “concrete representations of disease experienced by individuals as a component of cognitive processing” (Armstrong, 2003) [p.601].

Symptoms may occur alone or in clusters (Dodd, 2001). Symptom clusters are defined as "three or more concurrent symptoms that are related to each other; however the symptoms in the cluster are not required to have the same etiology" (Dodd, Miaskowski, & Paul, 2001) [p.465]. Dodd and others provide the example of pain, fatigue, and sleep insufficiency in cancer. The "pain is caused by the cancer, and the fatigue is caused by the cancer and the cancer treatment, and sleep insufficiency by the types of chemotherapy agents or anxiety" (Dodd, et al., 2001).

Given and others found that cancer patients with fatigue had an average of 4.4 other symptoms and that with pain and fatigue together, the patients had 6.3 other symptoms (Given, Given, Azzouz, Kozachik, & Stommel, 2001). In addition, those patients with three or more comorbid conditions were more likely to have pain, fatigue or both (Given, et al., 2001). According to Armstrong, "Symptoms may occur in clusters, may be multiplicative in nature, and act as catalysts for other symptoms" (Armstrong, 2003; Dodd, 2001; Lenz, et al., 1997). However, in this study, the presence of symptoms will be examined.

While there is a minimal amount of research in the study of symptoms in persons with NAFLD, the research that has been conducted notes that fatigue, malaise and right upper pain, fullness or discomfort may be present (Angulo, 2007a). Furthermore, consequences (or outcomes) such as quality of life are decreased in persons with NAFLD especially as the disease progresses and persons may experience more fatigue (David, et al., 2009). However, further work is needed to fully explicate the symptoms experience in persons with NAFLD. Before a study can be initiated, the theory or

conceptual framework must be evaluated. The following sections will provide an analysis and evaluation of the symptoms experience model.

Analysis and Evaluation of the Nursing Middle-Range Theory

Analysis and evaluation of nursing theories are essential to determine the strengths and weaknesses of a theory to be used to guide one's research (Fawcett, 2005). Theory analysis provides an objective means in which to describe a nursing theory (Fawcett, 2005). Criteria developed by Fawcett is used to provide a systematic and consistent means in which to evaluate the author's writings about the theory as well as other's critiques of the theory (Fawcett, 2005).

Fawcett's framework for analysis and evaluation of nursing theories has several steps. Three steps in the analysis of nursing theories are (1) theory scope, (2) theory context, and (3) theory content. The evaluation of nursing theories has six steps: (1) significance, (2) internal consistency, (3) parsimony, (4) testability, (5) empirical adequacy, and (6) pragmatic adequacy. An analysis of the symptoms experience model will be provided using Fawcett's framework for analysis and evaluation of nursing theories. The theory of unpleasant symptoms will be used as a comparison model (Lenz, et al., 1997; Lenz, et al., 1995).

Analysis

Historical background of the symptoms experience model. The Symptoms Experience Model was developed by a neuro-oncology nurse practitioner and researcher for patients with brain cancer (Armstrong, 2003). Armstrong used Walker and Avant's concept analysis to develop and define her model, building upon common themes of symptoms from the theory of unpleasant symptoms (Lenz, et al., 1997), the

theory of self-regulation (Leventhal & Johnson, 1983), the symptom management model (Dodd, 2001; Leventhal & Johnson, 1983), the symptom work of Rhodes and Watson (Rhodes & Watson, 1987), and the meaning of symptoms work of Richer and Ezer (Richer, 2000). In her analysis of existing symptom models, Armstrong noted that meaning of the symptoms, the situational meaning of the symptoms, and the existential meaning of symptoms were missing in these models, an important concept to include in a symptoms experience of those with brain cancer.

Armstrong defined symptoms experience as the "perception of the frequency, intensity, distress and meaning as symptoms are produced and expressed" (Armstrong, 2003). Antecedents in this model are the demographic, disease, and individual characteristics, which influence the symptom production and perception. The symptoms are defined by the symptom attributes of frequency, intensity, distress, and meaning as well as the situational and existential meaning. Symptoms are expressed and influence the consequences of adjustment to illness, quality of life, mood, functional status, disease progression, and survival.

Historical background of the theory of unpleasant symptoms. The theory of unpleasant symptoms originated from the work of two nursing scholars, Gift and Pugh, while working on a chapter of a book to describe symptoms of dyspnea and fatigue (Lenz et al., 1995). Gift and Pugh noted that the outlines developed to write about each of these different symptoms were quite similar, initiating the thought process of developing a theory to describe these potential similarities. Lenz was contacted to work with these scholars to assist in the creation of this theory because of her prior work with

theory development. The theory was developed using concept derivation, concept synthesis and concept analysis (Lenz et al., 1997).

The theory of unpleasant symptoms evolved from using one symptom to multiple symptoms with the defining attributes of intensity, quality, distress, and duration (Lenz et al., 1997). The antecedents were identified as the physiological, psychological, and situational factors that interact and influence the unpleasant symptoms. The intensity, quality, distress, and duration of the unpleasant symptoms influence the outcome or consequence of physical functioning, which in turn can influence the unpleasant symptoms or symptom cluster and influence the antecedents (See Figure 3).

Antecedents. Specific factors within the antecedent categories require further description. The physiologic factors include normal systems such as cardiovascular, pulmonary or gastrointestinal systems; pathological problems such as inflammation as is found in NAFLD; and the provision of adequate energy substrate as an example of nutrition (Lenz, et al., 1995). Psychological factors include mental state, such as depression, and reaction to illness state such as social support. Situational factors include life style and personal experiences.

Defining characteristics of the symptoms. Symptoms are described by the timing of the symptoms, the intensity or how severe the symptom is, the distress or bother it causes the persons, and the quality of the symptom often associated with "what the symptom feels like," such as sharp versus dull (Lenz, et al., 1997).

Performance (outcome). The outcome or consequence of the theory of unpleasant symptoms model is performance. Performance refers to the functional status, such as role performance of the individual; the cognitive functioning or problem-

solving capabilities; and the physical performance, such as the activity level of the individual. The outcome or consequence has a reciprocal influence on symptoms groups and the antecedents in a feedback system type of model rather than a linear model, such as the symptoms experience model (Brant, Beck, & Miaskowski, 2010).

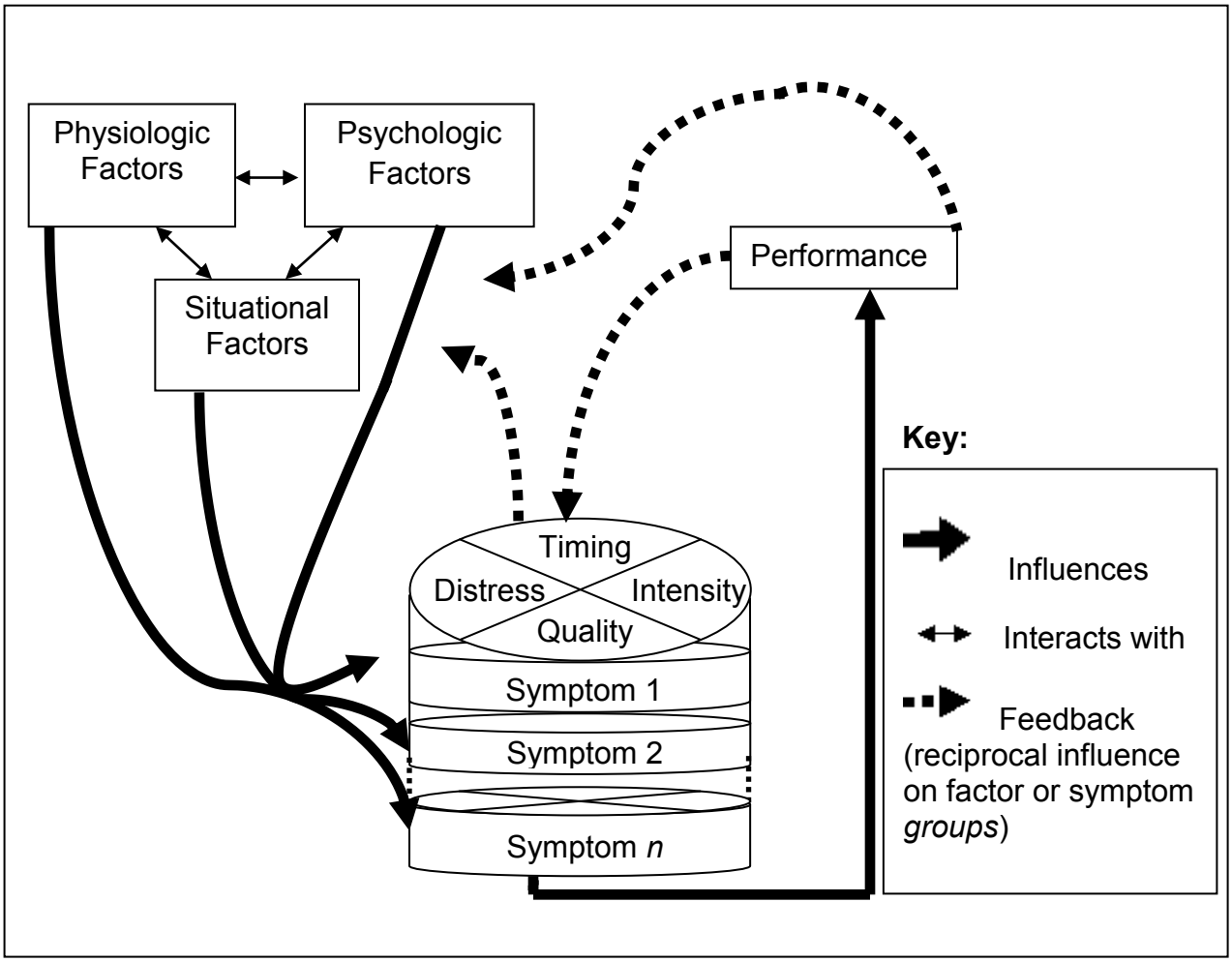

Figure 3. The theory of unpleasant symptoms. From Lenz, E.R., Pugh, L.C. Milligan, R.A., Gift, A. & Suppe, F. (1997). The middle-range theory of unpleasant symptoms: An update. *Advance in Nursing Science, 19*(3): 14-27. Reprinted with permission (see Appendix E).

Theory Scope, Context, and Content of the Symptoms Experience Model and the Theory of Unpleasant Symptoms

The following paragraphs will use the three steps of Fawcett's theory analysis to further describe and analyze the symptoms experience model in comparison to the theory of unpleasant symptoms.

Theory scope of the symptoms experience model. The scope refers to the breadth of the theory. As presented earlier, the symptoms experience model is a middle-range theory that is narrow in scope as it defines a patient's experience of symptoms. Armstrong does not address the scope in her concept analysis, as Walker and Avant's technique for concept analysis is different from that of Fawcett's theory analysis. However, it is implied that the symptoms experience model is a middle-range theory as it is specific to symptoms experience; the conceptual definitions are concrete, such as Armstrong's definition of the symptoms experience.

Middle-range theories are descriptive, explanatory, or predictive (Fawcett, 2005). Descriptive theories are those that provide grouping or classifications of the characteristics of the phenomenon (Peterson, 2009). Explanatory theories crisply define the relationships between characteristics of the phenomenon, explaining what is occurring between the antecedents, concepts, and outcomes (or consequences) (Peterson, 2009). Descriptive and explanatory theories are needed to develop predictive and prescriptive theories. Predictive theories describe situations, whereas prescriptive theories are situation-producing theories (Peterson, 2009).

The symptoms experience model is not defined by the author as descriptive, explanatory or predictive. However, it appears to be a predictive model. As one reads the model from left to right, the antecedents influence symptom production, which

influences the symptom perception and meaning, resulting in symptom expression and consequences. Hypothetically, in the person with advanced stage NAFLD (antecedent), the presence of daily fatigue (symptoms experience) that is moderate in intensity (defining characteristic) will decrease the quality of life (consequence).

Scope of the theory of unpleasant symptoms. In comparison, the theory of unpleasant symptoms is also a middle-range theory, a theory with a narrow scope (the description of unpleasant symptoms), and is specific. The theory of unpleasant symptoms is a predictive and, perhaps, a prescriptive model in that "preventative measure may be used to modify some of the factors that produce symptoms" (Lenz, et al., 1995). However, the scope is broad enough to include one or many symptoms known as clusters (Adams, et al.; Dickoff & James, 1968; Dodd, 2001; Lenz, et al., 1997).

Context of the symptoms experience model. The context of the theory is examined for: "(a) the identification of the concepts and propositions of the nursing metaparadigm addressed by the theory, (b) the philosophical claims on which the theory is based, (c) the conceptual model from which the theory was derived, and (d) the contribution of knowledge from nursing and other disciplines in the theory development effort" (Fawcett, 2005). Although not explicitly defined by Armstrong (2003), the metaparadigm concepts of nursing, health, environment and human beings are interwoven throughout the model. For example, the symptoms experience model is designed to model the symptoms experience of human beings. Armstrong discusses the importance of monitoring the patient's symptoms experience as a cornerstone for oncology nursing. Health is implied in the model through the disease characteristics

listing type and stage of disease and comorbid conditions. The environment is implied in the demographic and individual characteristics, such as the socioeconomic status and the sense of coherence within a community or family.

Fawcett notes that four relational propositions of the metaparadigm of nursing should be addressed in the theory. Relational propositions link the four concepts within the metaparadigm of nursing (human beings, environment, health, and nursing). These relational propositions are that "the discipline of nursing is concerned with the (a) human processes of living and dying, (b) patterning of human health experiences within the context of the environment, (c) nursing actions or processes that are beneficial to human beings, and (d) human processes of living and dying, recognizing that human beings are in continuous relationship with their environment" [p. 6] (Fawcett, 2005).

Armstrong addresses nursing actions or processes in the concept analysis of the symptoms experience model as she writes, "Symptoms are the guidepost for oncology nursing practice" [p. 601] (Armstrong, 2003).The processes of living and dying, noted as "adjustment to illness, quality of life, disease progression, and survival" [p.602] (Armstrong, 2003) are evident in the consequences of the symptoms experience model.

Although a worldview is not identified, the reaction worldview is also implied in which humans are bio-psycho-social-spiritual beings. The reaction worldview is comprised of a mechanistic and particulate-deterministic worldview (Fawcett, 2005), and it is depicted in Armstrong's model in the symptom production in which symptoms are produced as influenced by the antecedents. Interaction from the reciprocal worldview is noted in the interaction of the antecedents and the interaction of multiple symptoms. Symptoms often occur together (multiplicative effect or symptom clusters).

Symptoms may trigger the occurrence of other symptoms (catalyst effect). This model was originally influenced by other nursing theories as previously mentioned: the theory of unpleasant symptoms (Lenz et al., 1997), the self-regulation theory (Leventhal & Johnson, 1983) and Rhodes & Watson's work with symptom experience (1987). Although the symptoms experience model is designed for use in cancer patients, this study will utilize the symptoms experience model to examine symptoms, genetics and HRQOL in patients with NAFLD.

Context of the theory of unpleasant symptoms. The metaparadigm concepts of nursing, health, human beings, and the environment are addressed in the description of the theory of unpleasant symptoms (Lenz et al., 1997). Although there is not an intervention step in the model to depict nursing interventions in the treatment of the unpleasant symptoms, it is implied in the narrative (Adams, et al.). Components of health and the environment are noted in the antecedents of the situational, physiological, and psychological factors. The model is designed for human beings whose health is influenced by their environment. Nursing influences the human beings' health through identification and measurement of the intensity, distress, duration, and quality of the unpleasant symptoms. Although implicit and similar to the symptoms experience model (Fawcett, 2005), the theory of unpleasant symptoms reflects the reciprocal worldview in which there is an interaction between the metaparadigm concepts. However, the symptoms experience model also includes the reactive worldview.

Content of the symptoms experience model. Fawcett describes the theory content step of the analysis of nursing theories as "the subject matter of the theory,"

which is “described through the theory’s concepts and propositions” (Fawcett, 2005). The nonrelational proposition of the symptoms experience model is that “symptoms are multiplicative in nature and may act as catalysts for the occurrence of other symptoms.” There are several relational propositions in the symptoms experience model. An example of a relational proposition in the symptoms experience model is that “each individual symptom, as well as the interaction of multiple symptoms, has the ability to affect patients’ situational meaning or existential meaning” (Armstrong, 2003; Richer, 2000). The “meaning of the symptoms may influence the perception of the symptoms regardless of the frequency or distress of the symptom” is a relational proposition (Armstrong, 2003). Finally, another relational proposition in the symptoms experience model is the definition of symptoms experience: “the perception of the frequency, intensity, distress, and meaning occurring as symptoms are produced and expressed” (Armstrong, 2003).

Content of the theory of unpleasant symptoms. The theory of unpleasant symptoms contains (a) antecedents, (b) unpleasant symptoms which may occur alone or in clusters that are multiplicative and synergistic in nature, (c) the outcome (or consequence) of functioning and (d) the influence between the outcome (or consequence) and the symptoms, and back to the antecedents.

In this section, a theory analysis was described using the symptoms experience model and the theory of unpleasant symptoms (Armstrong, 2003; Fawcett, 2005; Lenz, et al., 1997) The following section will compare and contrast the symptoms experience model with the theory of unpleasant symptoms using theory evaluation (Fawcett, 2005).

Evaluation

Fawcett (2005) describes six criteria upon which to evaluate theories: (a) significance, (b) internal consistency, (c) parsimony, (d) testability, (e) empirical adequacy, and (f) pragmatic adequacy. This section will compare and contrast two middle-range theories the symptoms experience model and the theory of unpleasant symptoms using Fawcett's theory evaluation method (Fawcett, 2005).

Significance. Significance refers to the importance of the model to nursing with evidence of explicit metaparadigmatic, philosophical, conceptual, or paradigmatic origins (Fawcett, 2005). Both the theory of unpleasant symptoms and symptoms experience model are significant to nursing in that they incorporate the metaparadigm concepts of nursing, health, environment, and human beings (Fawcett, 2005). While these concepts of the metaparadigm of nursing are implicit in both theory models, the theory of unpleasant symptoms explicitly incorporates access to health care and lifestyle changes as concepts of nursing, health, environment, and human beings in the model. However, both theories explicitly address nursing in the narrative (Armstrong, 2003; Lenz, et al., 1997).

The theory of unpleasant symptoms and the symptoms experience model is used to study unpleasant symptoms or symptoms experiences of patients with lung, brain, colon, breast, and prostate cancers (Armstrong, 2006; Brant, 2008; Fox et al. 2007, Fox & Farce 2006; Hoffman, 2007, Keehne-Miron, 2007). In addition, the theory of unpleasant symptoms is used to study symptoms of patients undergoing bariatric surgery, a population in which 71 to 97.8% have NAFLD (Beymer, et al., 2003; Colicchio, et al., 2005; Dixon, Bhathal, & O'Brien, 2001; Luyckx, et al., 1998; Silverman,

et al., 1990; Spaulding, Trainer, & Janiec, 2003; Tyler & Pugh, 2009). The theory of unpleasant symptoms is also used in the study of other gastrointestinal diseases such as irritable bowel syndrome, (Farrell & Savage, 2010), in the study of heart failure (Jurgens, et al., 2009), and in the study of chronic obstructive pulmonary disease (Reishtein, 2005). However, neither has been used in the study of NAFLD or liver cirrhosis.

The symptoms experience model expands upon the theory of unpleasant symptoms (Gift, 2009) highlighting the symptoms experience of symptom production, symptom perception, and symptom expression. Recent studies report that genetic polymorphisms are involved in both disease risk and symptom production in the cancer population (Kurzrock, 2001; Maier & Watkins, 2003; Reyes-Gibby, et al., 2007; Reyes-Gibby, et al., 2008). The discovery of genetic polymorphisms influencing symptom production provides the rationale for the use of the symptoms experience model in the study of symptoms, genetics, and HRQOL in persons with NAFLD.

Internal consistency. Internal consistency, in the context of theory evaluation, is the semantic congruency and clarity of a model (Fawcett, 2005). Both models are internally consistent with the metaparadigm concepts of nursing, the philosophical claims, and the propositions. The philosophical claims made by Armstrong and Lenz note that both theories contribute to the science of nursing in the study of symptoms. Both claim that symptoms are multiplicative in nature and that symptoms are catalytic. The definitions of the concepts of symptoms experience and unpleasant symptoms are clearly defined in the respective models. Nursing interventions are missing from both the symptoms experience model and the theory of unpleasant symptoms (Brant, 2008).

The symptoms experience model was influenced by the theory of unpleasant symptoms (Armstrong, 2003). However, in personal communication, Armstrong noted that the inclusion of nursing interventions is one of the "next steps" in the revision of the model. Furthermore, some researchers have included nursing interventions as an adaptation of the model (Keehne-Miron, 2007).

Parsimony. Parsimony refers to the simplicity of the theory description, that is, how succinct the concepts and propositions are without becoming too simplistic (Fawcett, 2005). The descriptions of both models are parsimonious, although the symptoms experience model is more clearly defined. For example, the symptoms experience is defined as the "perception of the frequency, intensity, distress, and meaning as symptoms are produced and expressed" (Armstrong, 2003, p. 602). The definition of unpleasant symptoms is "the perceived indicators of change in normal functioning as experienced by patients"(Lenz, et al., 1997). Unpleasant symptoms are the "subjective indicators of threats to health" (Tolman, Fonseca, Dalpiaz, & Tan, 2007). Both models are parsimonious as the definitions are succinct and clearly defined. Brant noted, however, that "the model may be so parsimonious that some elements are missing" such as self-efficacy (Brant, 2008). However, middle range-theories are not designed to be "all-encompassing" (Peterson, 2009).

Testability. Testability is the ability of the theory to be used in research. Both models have been tested in nursing research. The symptoms experience model is currently used in the study of the oncology population (Brant, 2008; Keehne-Miron, 2007). The theory of unpleasant symptoms has been used in the oncology population, such as in lung cancer patients (Hoffman, 2007), postoperative gynecological cancer

populations (Liu, 2008), and persons with brain tumors (Linendoll, 2008), as well as many diverse populations, such as children following tonsillectomy surgery (Huth & Broome, 2007); persons with Alzheimer's disease (Hutchinson & Wilson, 1998); persons with chronic obstructive pulmonary disease (Reishtein, 2005) , multiple sclerosis (Motl & McAuley, 2009), heart failure (Jurgens, et al., 2009) and bariatric surgery (Tyler & Pugh, 2009); and postpartum, active-duty military women (Rychnovsky, 2007).

Both the theory of unpleasant symptoms and the symptoms experience model provide antecedents, defining characteristics of the symptoms, and the consequences of the symptoms experience (Armstrong, 2003; Lenz, et al., 1997). Several tools exist to measure the middle-range propositions of these models, such as the Memorial Symptom Assessment Scale (MSAS) (Portenoy, et al., 1994), the MD Anderson Symptom Inventory (Zhang & Wang, 2004), the Healthy Days Measure (Centers for Disease Control and Prevention, 2000), the Symptom Distress Scale (McCorkle & Young, 1978), and the McGill Pain Questionnaire (Melzack, 1975). Unfortunately, to date, no tools are available to measure the meaning of symptoms, situational meaning or existential meaning of symptoms in the symptoms experience model. Future research is needed using qualitative methods to explore and describe the situational and existential meaning of symptoms to determine the influence of meaning upon symptom perception, expression and HRQOL.

Other tools can be used to measure the consequences of the symptoms experience model. For example, consequences can be measured using a HRQOL tool to capture the mood, functioning, and quality-of-life outcomes or consequences, such as

the SF-36 (Ware, n.d.) and the Chronic Liver Disease Questionnaire (Younossi, Guyatt, Kiwi, Boparai, & King, 1999).

Empirical adequacy. Empirical adequacy is the congruency between the assertions of the theory and the findings of research studies to support the assertions (Fawcett, 2005). Theories should be evaluated for the "fit" and "nonfit" by consideration of other theories for interpretation of the results of the study (Fawcett, 2005).

To date, two dissertations have used the symptoms experience model as a conceptual framework: "The Associations of Frequency, Intensity and Distress Level of Fatigue, Pain and Insomnia and Effect of Fatigue Management on Pain and Insomnia for Chemotherapy Patients" (Keehne-Miron, 2007) and "Symptom Trajectories in Patients with Lung Cancer, Colorectal Cancer, and Lymphoma during Chemotherapy and Post treatment: A Latent Growth Curve Analysis" (Brant, 2008). Keehne-Miron (2007) used an adapted symptoms experience model to include time and an intervention to study frequency, intensity and distress of fatigue, pain and insomnia in a secondary analysis of cancer patients at baseline and at 10 weeks. The assertions of the theory that symptoms occur together were influenced by age, and the number of comorbid conditions, which were the antecedents measured in this study (Keehne-Miron, 2007). Brant used the symptoms experience model with adaptations to the individual and disease characteristics similar to the adapted model in this chapter; however, demographic characteristics were subsumed into the two remaining antecedents (Brant, 2008).

Several studies have used the theory of unpleasant symptoms (Hoffman, 2007; Hutchinson & Wilson, 1998; Huth & Broome, 2007; Jurgens, et al., 2009; Linendoll,

2008; Liu, 2008; Motl & McAuley, 2009; Reishtein, 2005; Rychnovsky, 2007). In all, the theory of unpleasant symptoms provided a strong framework for guidance of the studies.

Pragmatic adequacy. Pragmatic adequacy is the use of the theory in the practice setting. While no studies were found using the symptoms experience model in practice, it has been used to refine the MD Anderson symptom inventory for brain cancer patients (Armstrong, 2006). The theory of unpleasant symptoms has been used as a conceptual framework for the development of a tool to measure symptoms in a cardiac patient being cared for in the emergency room (Lenz et al., 1997).

The theory of unpleasant symptoms and the symptoms experience model have been used in both research and practice. This study will use the symptoms experience model to guide the study of symptoms, genetics, and HRQOL in persons with NAFLD.

Use of the Symptoms Experience Model in Research

The symptoms experience model is a good fit to guide the study of a potential symptoms experience in the NAFLD population. The antecedents are clearly defined in the symptoms experience model compared to the theory of unpleasant symptoms. In addition, the stage and type of disease and comorbid conditions are delineated. For example, NAFLD has four stages (simple fatty liver, NASH, cirrhosis, and liver failure or liver cancer/). The symptom production may be influenced by the inflammatory process; the symptoms are perceived and expressed, which influences the consequence of HRQOL.

While the theory of unpleasant symptoms was established earlier and has been used in research and practice, the newer symptoms experience model (as compared to

the theory of unpleasant symptoms) has been used in two recent doctoral dissertations (Brant, 2008; Keehne-Miron, 2007). Further, the symptoms experience model offers a unique concept of symptom production which is important in this researcher's study.

Symptom production in NAFLD is hypothesized to occur as waist circumference increases, and fat in the form of triglycerides is deposited into the hepatocytes. Fatty tissue acts like an endocrine organ, releasing cytokines such as IL-6 and TNF-α thought to produce "sickness behavior" (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008). This is in contrast to the production of IL-6 by muscle contraction, which is associated with health benefits (Pedersen & Fischer, 2007). In addition, persons at higher risk of disease progression are hypothesized to have one or two copies of the *PNPLA3* gene, (rs738409-G) allele, a disease characteristic within the symptoms experience model.

According to the assumptions of the symptoms experience model, the antecedents of demographic, disease and individual characteristics interact to influence symptom production (Armstrong, 2003). This sickness behavior or symptom may influence HRQOL. Therefore, the symptoms experience model provides a framework for the study of symptoms and potential contributors to the production of symptoms, such as genetic polymorphisms contributing to symptoms in the cancer population (Kurzrock, 2001; Maier & Watkins, 2003; Reyes-Gibby, et al., 2007; Reyes-Gibby, et al., 2008). In addition, the symptoms experience model provides a consequence or outcome of HRQOL to evaluate in persons with NAFLD.

In conclusion, the symptoms experience model and the theory of unpleasant symptoms were compared and contrasted using Fawcett's evaluation (2005). The symptoms experience model provides more clearly defined concepts than the theory of

unpleasant symptoms, making the symptoms experience model more appropriate for use by a novice researcher in the study of symptoms in an emerging disease.

Adaptation of the Model

For the purpose of this study, "Symptoms, Genetics and Health-Related Quality of Life in Persons with Nonalcoholic Fatty Liver Disease," Armstrong's symptoms experience definition is adapted to the "perception of the frequency, intensity, and distress of symptoms as they are produced and expressed" (Armstrong, 2003). Within the antecedent of disease characteristics, the NAFLD stages are noted as simple fatty liver disease and NASH without and with fibrosis, causing progression to cirrhosis. The stage of NAFLD is moderated by the inflammatory process and the genetic predisposition as represented by the gene variants of *PNPLA3* (rs738409) resulting in a proposed symptom production. The symptom production occurs, resulting in the symptom perception of the symptoms. The symptoms may occur alone or in clusters which interact with one another, influencing the frequency, intensity, and distress produced by the symptoms, which are expressed as the presence of the symptoms. The symptom expression is the impact of the symptoms which influences the HRQOL of the individual (Armstrong, 2003). The following paragraphs will describe each component of Armstrong's symptoms experience model in this proposed study of persons with NAFLD.

Antecedents. Antecedents of the model are the characteristics of patients (Armstrong, 2003) which include the demographic characteristics, the disease characteristics and the individual characteristics of persons with NAFLD.

Demographic characteristics. Demographic characteristics include the age, sex, race/ethnicity, education level, employment, and access to health care. The demographic characteristics, such as access to health care, will influence whether or not the individual seeks care to acknowledge that he or she has symptoms.

Disease characteristics. Disease characteristics are the stages of NAFLD, including simple fatty liver, NASH with or without fibrosis, and cirrhosis (if available in the medical record) and the risk of NAFLD progression as determined by the presence of the *PNPLA3* gene, (rs738409)-G allele. The *PNPLA3* gene, (rs738409)-G allele will be used to stratify the population into persons at higher risk (one or two copies present of [rs738409]-G allele) versus persons at lower risk (no alleles of [rs738409]-G present) of NAFLD disease progression. Known comorbid conditions of diabetes, metabolic syndrome, and obesity, as well as additional potential comorbid conditions, such as cardiac and lung diseases, cancers, metabolic disorders and autoimmune categories as listed in the Charlson Comorbidity Index (CCI) are included as disease characteristics. Also included as disease characteristics are alcohol intake and medication use. In addition, waist and hip circumference, BMI, Aspartate aminotransferase (AST) elevation, and Alanine aminotransferase (ALT) elevation, are included as disease characteristics.

Individual characteristics included in this study are the knowledge about NAFLD prior to diagnosis and cognitive state. Cognitive state will be used as part of the inclusion criteria as determined by the ability to converse with the intake coordinator. The changes to the antecedents in this model were made to reflect specific conditions unique to the individual with NAFLD.

Symptoms experience. The symptoms experience in this model encompasses symptom production, symptom perception, and symptom response. Symptoms experience is a plural term used to describe the multiplicative nature of symptoms (Armstrong, 2003). That is, symptoms rarely occur alone (Armstrong, 2003; Dodd, 2001).

Symptom production. Symptoms are the "objective, concrete representations of disease experienced by individuals as a component of cognitive processing" (Armstrong, 2003; Leventhal & Johnson, 1983). As presented earlier, symptom production is the physiological and neurobiological mechanisms that result as triggered by the disease process (Dalal, et al., 2006; Kim, et al., 2004; Reyes-Gibby, et al., 2008). Symptom production may occur as a result of changes in the pro-inflammatory cytokines and influence of genetic predisposition (Dantzer, et al., 2008; Kurzrock, 2001; Maier & Watkins, 2003; Reyes-Gibby, et al., 2008). Genotyping will be completed to measure a sequence variant within the *PNPLA3 gene*.

Symptom perception. Symptom perception is the recognition of the occurrence of the symptoms and the defining characteristics of the symptoms, which are the frequency, intensity, and distress that the person experiences (Armstrong, 2003). Frequency is defined as how often the symptom occurs over the week prior to being surveyed (Portenoy, et al., 1994). Intensity is defined as the severity or strength of the symptom, determined by the severity of the symptom, usually over the past week (Rhodes & Watson, 1987). Distress of the symptom is the feeling of a need to make adjustments in one's life in response to the symptom occurrence (Rhodes & Watson, 1987) -- for example, the extent to which the symptom caused distress or bothered the

person over the past week (Portenoy, et al., 1994). Symptom meaning, situation meaning and existential meaning will not be measured in this study. The symptoms experience proposes symptoms occurring in clusters with defining attributes of frequency, intensity, and distress.

Symptom expression. Symptom expression is defined using a modified symptom expression definition of Keehne-Miron which is the physical, psychological, and emotional response to a symptom (Keehne-Miron, 2007). Symptom expression influences or impacts the consequence in this study, the HRQOL, but measurement of the symptom expression is beyond the scope of this study.

Consequences. Armstrong lists six consequences (outcomes) or dependent variables in the symptoms experience model: adjustment to illness, quality of life, mood, functional status, disease progression, and survival. For this study, the term *health-related* quality of life rather than quality of life will be used as this study will only examine those items that are health-related, not quality of life related to components such as income or living environment. HRQOL will provide an understanding of how symptoms will affect the person's perceived physical and mental health.

HRQOL. The consequence or outcome in this adapted model is HRQOL in persons with NAFLD. HRQOL will be defined using an adaptation of the Center for Disease Control and Prevention's definition, "an individual's perceived physical and mental health" (Centers for Disease Control and Prevention, 2000) as measured by the number of healthy physical and mental health days in a 30 day period in those individuals with NAFLD (p. 8). Figure 4 depicts an adapted symptoms experience model. Table 1 depicts the differences between the symptoms experience model and

the adaptations to symptoms experience model for the study of persons with NAFLD. At the end of this chapter, Table 2 provides a summary of definitions that will be used in this study.

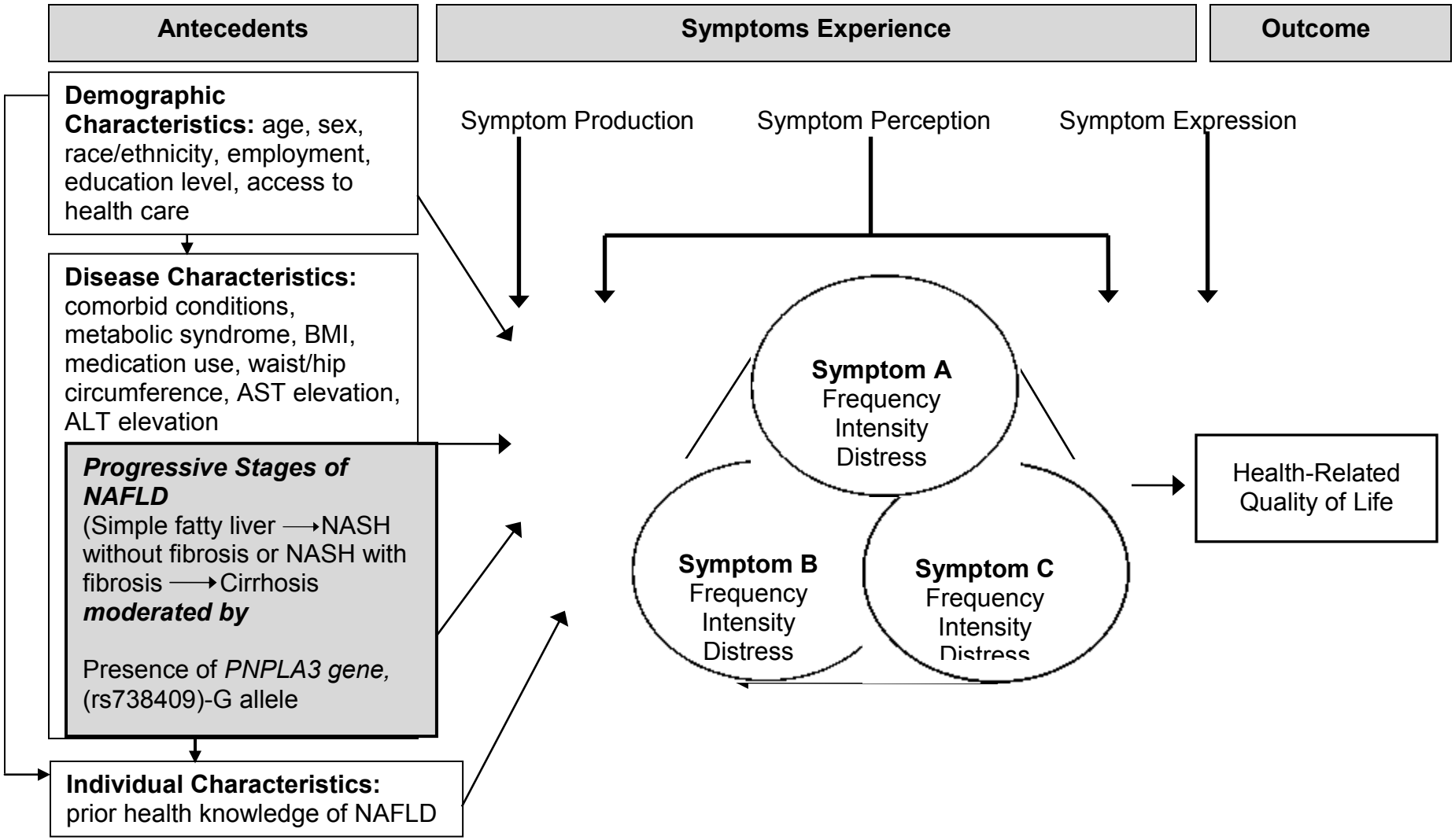

Figure 4. Adapted symptoms experience model. BMI = body mass index; AST = aspartate aminotransferase; ALT = alanine aminotransferase; NAFLD = nonalcoholic fatty liver disease; NASH= nonalcoholic steatohepatitis. From "Symptoms Experience: A Concept Analysis," by Terri S. Armstrong, MS, APRN, BC, 2003, *Oncology Nursing Forum, 30*(4), p. 603. Copyright 2003 by the Oncology Nursing Society. Adapted with permission (see appendix D).

Table 1

Adaptations to the Symptoms Experience Model

Armstrong's symptoms experience model	**Proposed study with adaptations to symptoms experience model**
Demographic characteristics: • Age • Gender • Marital status • Race • Culture • Role • Education • Socioeconomic status	**Demographic characteristics:** • Age • Sex • Race/ethnicity • Employment • Education level • Access to health care
Disease Characteristics: • Type and state • Type of treatment • Comorbid medical and clinical factors	**Disease Characteristics:** • *PNPLA3 gene,* (rs738409)-G allele • Stage of NAFLD (if available) • Comorbid conditions • (DM/insulin resistance, CVD, obesity, metabolic syndrome, thyroid disease) • Waist/hip circumference (as a measure of fat distribution) • BMI • AST elevation • ALT elevation • ETOH Intake • Medication Use
Individual Characteristics: • Health knowledge • Values • Past experiences • Sense of coherence	**Individual Characteristics:** • Prior health knowledge of NAFLD

Table 1 (cont'd)

Adaptations to the Symptoms Experience Model

Armstrong's symptoms experience model	**Proposed study with adaptations to symptoms experience model**
(Symptoms Experience: Symptom Production, Symptom Perception, Symptom Expression)	**Symptom Perception:** • (Presence) • Frequency • Intensity • Distress
Symptom Production **Symptom Perception** • Frequency • Intensity • Distress • Meaning • Situation meaning • Existential meaning **Symptom Expression**	
Consequences: • Adjustment to illness • Quality of life • Mood • Functional status • Disease progression • Survival	**Consequences:** • Health-related quality of life

Note. NAFLD = nonalcoholic fatty liver disease; DM = diabetes mellitus BMI = body mass index; AST = aspartate aminotransferase; ALT = alanine aminotransferase; CVD=cardiovascular disease; ETOH=alcohol use.

Summary

The symptoms experience model is determined to be an appropriate middle-range theory to guide this study, "Symptoms, Genetics, and Health-Related Quality of Life in Persons with Nonalcoholic Fatty Liver Disease." Middle-range theories are narrow in scope and ideal for guiding research questions because of the specificity of the model. According to the analysis described above, the concepts and propositions of the symptoms experience model are sufficiently concrete. The content either implicitly

or explicitly incorporates the metaparadigm of nursing, such as the four concepts of nursing, environment, health, and human beings and the propositions.

The worldview of mechanistic and particulate-deterministic (or reductionism) supports the use of genetics in this study as it deduces the influences of symptom production to a smaller level (i.e., gene allele variants) as a contributor to symptom production. The understanding of the cellular level influences is important to the comprehension of the larger picture, in this case, the symptoms experience of persons with NAFLD.

The symptoms experience model also incorporates the reciprocal worldview in the interaction of the antecedents and the interaction of multiple symptoms. In clinical practice, the researcher has seen multiple symptoms of pain, fatigue, and nausea, along with unpleasant symptoms of leg and torso edema (multiplicative effects of symptoms) in persons with liver cancer or liver failure, the advanced stages of NAFLD.

Finally, the additive or multiplicative effect of the symptoms experience influencing the consequences or outcomes of HRQL has also been noted in the clinical area. Persons experiencing the advanced stages of NAFLD such as liver cirrhosis, liver failure or liver cancer have decreased HRQOL, as they are no longer able to assume their roles in their households because of extreme fatigue and unpleasant symptoms of leg and torso edema.

In all, the analysis and evaluation of the middle-range theory, along with the similar worldview of the theory to this researcher's and the reciprocity noted in the antecedents and symptoms experience ultimately influencing the consequences of HRQOL, are the rationale for the use of the symptoms experience model in this study,

"Symptoms, Genetics and Health-Related Quality of Life in Persons with Nonalcoholic Fatty Liver Disease."

Table 2

Conceptual Definitions

Symptoms experience is the perception of the frequency, intensity, distress, and meaning occurring as symptoms are produced and expressed.
Demographic characteristics • Age is the number of years lived. • Sex is the sex of the person; male or female. • Race/ethnicity category in which one identifies based on ancestry or cultural group. • Employment is that the person has a paid job. • Education level is the attainment of elementary, high school, or college education. • Access to health care is the proximity to organized health care as determined by zip code.
Disease characteristics: • *PNPLA3* gene, (rs738409)-G allele is the presence or absence of one or two copies of the *PNPLA3* gene, *(rs738409)-G allele* • Stage and grade of NAFLD (if available) is simple fatty liver, NASH without fibrosis, NASH with fibrosis, cirrhosis, or liver cancer. Grade is the presence of steatosis (fat in the liver) plus lobular inflammation, ballooning degeneration, and/or Mallory bodies. Comorbid conditions are those diseases or conditions that may coexist with NAFLD such as diabetes mellitus/insulin resistance, cardiovascular disease, arthritis, obesity, metabolic syndrome, thyroid disease • ETOH intake is the amount and type of alcohol that one ingests. • Medication use is any medications such as prescriptive medications, over-the-counter, vitamins, herbal medications, or street drugs that are currently being taken by the person. • Waist circumference is the distance around the natural waist of the person. • Hip circumference is the distance around the fullest part of the hips of the person. • Waist/hip circumference ratio is the quotient of waist circumference and hip circumference. • Body mass index (BMI) is a measure of one's weight in relation to height. • Aspartate aminotransferase (AST) is an enzyme released into the blood when cells are damaged such as in the liver.

Table 2 (cont'd)

Conceptual Definitions

• Alanine aminotransferase (ALT) is an enzyme released into the blood when the liver cells are damaged.
Individual characteristics: • Prior health knowledge of NAFLD is one's awareness of NAFLD prior to this study.
Symptom perception: • Presence is the occurrence of the symptom. • Frequency is how often the symptom occurs. • Intensity is the severity of the symptom. • Distress is the feeling of a need to make adjustments in one's life in response to the symptom occurrence.
Consequences: • Health-related quality of life is an individual's perceived physical and mental health.

Note. NASH= nonalcoholic steatohepatitis.

Chapter 3: Literature Review

NAFLD is a rapidly emerging disease strongly associated with obesity (Adler & Schaffner, 1979). Physicians originally discovered NAFLD in the 1950s when they noted fatty changes in liver biopsy samples of patients with obesity, similar to fatty changes in livers of patients with alcoholic hepatitis (Westwater & Fainer, 1958). Few articles have been published regarding fatty liver changes similar to alcoholic hepatitis and cirrhosis (i.e., NAFLD) since 1958 (Adler & Schaffner, 1979; Massarrat, et al.,1974; Miller, 1979). It was not until 1980 that Dr. Ludwig, a physician, at Mayo Clinic presented NAFLD as a new disease termed *nonalcoholic steatohepatitis* (Ludwig, Viggiano, McGill, & Oh, 1980) or NASH.

The term, NASH, now known as the inflammatory stage of NAFLD, described the lobular inflammation, focal necrosis and Mallory bodies found in liver biopsy samples, but in patients who did not use alcohol (Ludwig, et al., 1980). Since Ludwig's acknowledgement of NAFLD, numerous studies have ensued, especially in the last 10 years, a timeframe that corresponds with the increased incidence of obesity over the last two decades (Oh, et al., 2008).

Therefore, the purpose of this chapter is to present a literature review for this study, providing the epidemiology, pathophysiology, risk for disease, and staging of NAFLD. A literature review of the antecedents such as genetics and the outcome of HRQOL, along with nursing's potential influence on HRQOL will be presented. Symptoms production, symptoms perception, and symptoms expression as depicted in the adapted symptoms experience model have been addressed in chapter 2.

Epidemiology and Overview of NAFLD

Pathophysiology. NAFLD is a disease in which 5% or greater of the total liver weight is fat, in the absence of or with minimal alcohol use (Angulo, 2007a; Kistler, et al., 2010). NAFLD is thought to develop from excess circulating fatty acids that are deposited in the liver in the form of triglycerides, likely caused by obesity as a result of overnutrition and decreased physical activity (Cave, et al., 2007; Day, 2006; Ouyang, et al., 2008). Excess protein and carbohydrates are converted to triglycerides and stored in the liver for future use (McCance & Huether, 2002).

NAFLD can progress from simple fatty liver to NASH without fibrosis/with fibrosis, to cirrhosis and liver cancer or liver failure as noted in chapter 1. Edmison and McCullough (2007) described the histopathological stages of the disease differently and more specifically categorized as Types 1 through 4, correlating these stages with the clinical diagnoses noted in Figure 1 (see Chapter 1). Type 1 is known as simple steatosis, the deposits of fat in the liver without disease progression (Edmison & McCullough, 2007). Type 2 is defined as steatosis plus lobular inflammation, that is inflammation of liver lobes (Edmison & McCullough, 2007). Type 2 is thought to be benign and most likely not NASH. Types 3 and 4 are considered NASH (Edmison & McCullough, 2007). Type 3 is steatosis, lobular inflammation, and ballooning degeneration, which is NASH without fibrosis and may progress to cirrhosis and liver failure (Edmison & McCullough, 2007). Steatosis, ballooning degeneration, and fibrosis or Mallory bodies are present in Type 4 (Edmison & McCullough, 2007). Thus, Type 4 is NASH with fibrosis, which may also progress to cirrhosis and liver failure.

While researchers have hypothesized several theories to describe the pathology of NAFLD progression, the most commonly accepted is the *two-hit* theory (Day & James, 1998; Gentile & Pagliassotti, 2008; Lewis & Mohanty, 2010). Normally, the liver has the capability to regenerate after injury; however, in NAFLD, it is bombarded with secondary "hits" or insults inducing injury. Genetic predisposition for progression of disease precedes this process (Sookoian, et al., 2009; Tian, et al., 2009; Valenti, et al., 2010). The first hypothesized hit, as discussed, is the dysregulated free fatty acid uptake, resulting in fat accumulation in the hepatocytes strongly associated with visceral obesity. The second hit is theorized to be a sum of multiple hits: (a) an inflammatory response of increased tumor necrosis factor alpha (TNF-α), increased transforming growth factor beta (TGF-β), and decreased adiponectin levels, along with increased oxidative damage, decreased glutathione, and deregulated apoptosis resulting in NASH and (b) the inflammatory stage of NASH, which results in increased death of hepatocytes, increased stellate cell activity and TGF-β, and decreased hepatic regeneration and abnormal hepatocyte repair, resulting in fibrosis. Clinically, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), enzymes that degrade proteins and are made by the liver, are released into the bloodstream as the hepatocytes necrose, stimulating the inflammatory process. If the inflammatory process leading to fibrosis is allowed to continue, cirrhosis -- the result of successive rounds of damage, hepatocyte necrosis, and repair and regeneration -- may result (Edmison & McCullough, 2007; Lewis & Mohanty, 2010).

Diagnosis and staging. Early diagnosis of NAFLD is difficult, as NAFLD is not often suspected unless liver enzymes, such as ALT and AST, are elevated. Often,

obese patients are diagnosed with NAFLD if liver enzymes such as ALT and AST are elevated and other causes for ALT and AST elevation are excluded. Unfortunately, many persons with the early stages of NAFLD may not have elevated liver enzymes. In a study of diabetes in Italy, more than 70% of patients had NAFLD, but did not have elevated liver enzymes (Targher, et al., 2007). Consequently, liver biopsy remains the gold standard for staging and grading of the stages of NAFLD (Yan, Durazo, Tong, & Hong, 2007). However, only a very small percentage of persons with NAFLD receive liver biopsy to stage the disease because of the risk of bleeding (Sanyal, 2002) as a result of the procedure.

Brunt (2007) developed grading criteria for NAFLD for use in quantifying liver biopsies known as the NAFLD Activity Score. The NAS is a composite score of the percentage of steatosis, ballooning of the hepatocytes, and the amount of lobular inflammation from the liver biopsy sample (Brunt, 2007). The staging criteria adapted by Kleiner, Brunt, and others (2005) describes the degree of fibrosis present in the liver biopsy sample. The staging criteria ranges from 0 (*no fibrosis*) to 4 (*cirrhosis*) (Kleiner, et al., 2005).

Ultrasound, MRI, and CT scans can confirm the presence of fat in the liver, but these imaging tests are not able to determine fibrosis (Mehta, Thomas, Bell, Johnston, & Taylor-Robinson, 2008), the scarring that occurs with disease progression. Furthermore, ultrasound, MRI, and CT scans are inadequate for use in longitudinal studies, as water content in the tissues and obesity around the abdomen --including the internal organs -- decrease the sensitivity and specificity of the tests. Two technologies could be used in diagnosing and staging: magnetic resonance spectroscopy, which

discerns fat in the liver and magnetic resonance elastography, which has potential for measurement of liver stiffness to determine fibrosis (Huwart, et al., 2008; Szczepaniak, et al., 2005). Currently, neither method is readily available.

Researchers are developing other methodologies to stage and grade NAFLD, such as serum-based biomarkers. Guha and others have proposed a combined biomarker panel of the European Liver Fibrosis Panel along with a simple biomarker panel as an alternative to liver biopsy for stratification of fibrosis severity (Guha, et al., 2008). These combined biomarker panels contain the following components: BMI, presence of diabetes mellitus or insulin resistance, and age, along with serum levels of AST and ALT, platelets, albumin, fasting glucose, hyaluronic acid, procollagen-3 N-terminal peptide (P3NP), and tissue inhibitor of metalloproteinase 1 (TIMP1) (Guha, et al., 2008). While these algorithms are very promising in the stratification of NAFLD, P3NP and TIMP-1 panels are currently only available in Europe.

Prevalence and incidence. The incidence of NAFLD is unknown, as to date, no prospective studies have been conducted to determine the development of NAFLD over time (Angulo, 2007a). However, with the obesity epidemic emerging worldwide, the prevalence of the obesity-related disease of NAFLD has been noted in Westernized countries all over the globe, as presented in chapter 1. It is estimated that there are 30 to 70 million people with NAFLD in the United States alone (Angulo, 2007a; Browning, et al., 2004). Further, 71 to 97.8% of those with a BMI of 30 or greater have NAFLD (Beymer, et al., 2003; Colicchio, et al., 2005; Dixon, et al., 2001; Luyckx, et al., 1998; Silverman, et al., 1990; Spaulding, et al., 2003). While the worldwide prevalence is not known, researchers predict that as affluent countries continue to incorporate Western

diet in their cultures, obesity will continue to rise, and subsequently, the prevalence of NAFLD will increase worldwide.

Risk factors for disease. NAFLD can occur from other secondary causes. Nutritional causes of NAFLD, as noted in a literature review by Angulo, are protein-caloric malnutrition, rapid weight loss, gastric bypass surgery, and total parental nutrition use (Angulo, 2007a). Drugs that are potentially harmful to the liver, such as glucocorticoids, estrogens, tamoxifen amiodarone, methotrexate, diltiazem, zidovudine, valproate, aspirin, tetracycline, and cocaine, can also contribute to NAFLD (Angulo, 2007a). Metabolic conditions such as lipodystrophy, hypopituitarism, dysbetalipoproteinemia, and Weber-Christian disease have been associated with NAFLD (Angulo, 2007a). Toxins such as Amanita phalloides mushroom, phosphorus poisoning, petrochemicals, and bacillus cereus may cause NAFLD. Infections such as HIV, hepatitis C, and small bowel diverticulosis with bacterial overgrowth are also known causes of NAFLD (Angulo, 2007a). In fact, some hypothesize that some of these factors may contribute to the two-hit theory presented earlier (Wigg, et al., 2001). Wigg and others (2001) found that more than 50% of persons with NAFLD had bacterial overgrowth in the small intestine compared to 22% of the control group (p = .048). Others found that NAFLD may coexist with other liver disease because of the increase in obesity within the world population. For example, NAFLD is comorbid with hepatitis C (22%), and is found in less than 1% of persons with other nonhepatitis liver diseases, such chronic hepatitis B, drug-induced hepatitis, hemochromatosis, primary biliary cirrhosis, and alpha-1 antitrypsin deficiency (Brunt, et al., 2003; Powell, Jonsson, &

Clouston, 2005). NAFLD, comorbid with other liver diseases, can accelerate fibrosis (Powell, et al., 2005). However, NAFLD due to obesity will be the focus of this study.

Heritability and genetic risk factors for presence of disease. Genetic background may also predispose persons to NAFLD and disease progression. In heritability estimates, Struben and others noted that patients with varying stages of NAFLD also had a positive family history for NAFLD, including parent-children, sibling or cousin relationships (Struben, Hespenheide, & Caldwell, 2000). Schwimmer and others found high heritability (h^2 = .850; standard error, 0.325, p<.001) in a study of obese adolescent patients with NAFLD (*n* = 33) confirmed by liver biopsy compared to obese adolescent patients without NAFLD (*n* = 11). In the Schwimmer study, of the adolescents with NAFLD, 59% of siblings and 78% of parents also had NAFLD; of the obese adolescents (*n* = 11) without NAFLD, 17% of siblings and 37% of parents had NAFLD (Schwimmer, et al., 2009).

NAFLD and the PNPLA3 gene. Although several genes are related to the comorbid condition of obesity (Thorleifsson, et al., 2009; Willer, et al., 2009), only one gene*, PNPLA3,* has been strongly associated with NAFLD, that is, liver fat content (Romeo, et al., 2008; Romeo, et al., 2009; Sookoian, et al., 2009). *PNPLA3* encodes the protein adiponutrin, a "triacylglycerol lipase that mediates triacylglycerol hydrolysis in adipocytes. The encoded protein, which appears to be membrane bound, may be involved in the balance of energy usage/storage in adipocytes" (NCBI Entrez Gene, 2010). The *PNPLA3* polymorphism inhibits normal breakdown of triglycerides (hydrolysis) resulting in increased storage of triglyceride in the hepatocytes (He, et al., 2010).

A nonsynonymous polymorphism (rs738409), that is, a substitution in nucleotide sequencing of cytosine to guanine, results in an amino acid change in the *PNPLA3* gene from isoleucine to methionine. This *PNPLA3* polymorphism was strongly associated with hepatic fat content in a genome-wide study (N = 2,111) to determine the association with hepatic fat and genetic contributions of NAFLD (Romeo, et al., 2008). In this genome-wide association study, 9,222 nonsynonymous variants were examined in 2,111 people. NAFLD was diagnosed using imaging of the proton magnetic resonance spectroscopy. The Dallas Heart Study found that the presence of the gene *PNPLA3 gene* (rs738409)-G allele was strongly associated with fat content in the liver ($p = 5.9 \times 10^{-10}$]) and more significant after adjusting for BMI, diabetes, alcohol use, and ancestry, $p = 7.0 \times 10^{-14}$ (Romeo, et al., 2008). Furthermore, the study found a strong association between elevated ALT levels and the *PNPLA3* gene, (rs738409-G) allele in Hispanics, $p = 3.7 \times 10^{-4}$ (Romeo, et al., 2008). This finding corroborates with Browning and others that Hispanics have a higher prevalence of NAFLD than do Caucasians and African Americans (Browning, et al., 2004).

In the Dallas Heart Study, a protective allele within the *PNPLA3* gene (rs6006460-T) was identified in African Americans, a finding that supports the decreased prevalence of NAFLD in the African American population in epidemiology studies (Browning, et al., 2004; Romeo, et al., 2008). The gene variant *PNPLA3* (rs6006460) substitutes an isoleucine for serine in codon 453. The protective T-allele was common in African Americans, but rare in Caucasians and Hispanics (Romeo, et al., 2008). Furthermore, the liver fat content was 18% lower in African Americans with

the *PNPLA3* gene (rs6006460-T) allele compared to those with the wild type allele, 3.3% versus 2.7%, $p = 6.0 \times 10^{-4}$ (Romeo, et al., 2008). Finally, *PNPLA3* gene variant (rs738409-G) allele was also found to influence plasma levels of ALT and AST, liver function tests used to denote liver inflammation (Weiskirchen & Wasmuth, 2009; Yuan, et al., 2008).

The association of the polymorphism (rs738409-G) allele in the *PNPLA3* gene has subsequently been replicated in a European population (Kotronen, Johansson, et al., 2009). In the Kotronen study, 291 Finnish persons were genotyped after confirmation of NAFLD using proton magnetic resonance spectroscopy (Kotronen, Johansson, et al., 2009). The *PNPLA3* gene, (rs738409)-G allele was associated with increased hepatic fat ($p = .011$) and increased liver enzymes in serum, $p = .002$. (Kotronen, Johansson, et al., 2009).

Tian and others studied 1,221 Mestizo (European and Native American ancestry) individuals to evaluate the association of the *PNPLA3* gene, (rs738409)-G allele to liver damage. They compared a control group of 305 Mestizo individuals with a history of alcohol abuse and normal liver enzymes, 434 Mestizo individuals with intermediate alcoholic liver disease, and 482 with cirrhosis due to alcoholic liver disease. Ancestry was also determined using genetic analysis. The Tian study found that the *PNPLA3* gene, (rs738409)-G allele is strongly associated with alcoholic liver disease and alcoholic cirrhosis, unadjusted $OR = 2.25$, $p = 1.7 \times 10^{-10}$, ancestry-adjusted $OR = 1.79$, $p = 1.9 \times 10^{-5}$ (Romeo, et al., 2008). These findings suggest that the gene *PNPLA3* gene, (rs738409-G) allele contributes to liver injury. It is noteworthy that Hispanics have

a higher prevalence of NAFLD when compared to Caucasians and African Americans (Browning, et al., 2004). Tian and others (2010) found that the (rs738409-G) allele, normally the minor allele, was common in this Hispanic population with alcoholic liver disease.

Genetics and risk of NAFLD progression. Results from the Dallas Heart Study genome-wide study have shown a strong association between genetic variants within the *PNPLA3* gene and risk of NAFLD disease progression (Romeo, et al., 2008). Those persons with two copies of the *PNPLA3* gene, (rs738409)-G alleles were found to have a higher incidence of liver cirrhosis (i.e., disease progression) than those with one allele (Romeo, et al., 2009).

Sookoian and others (2009) found that the gene variant *PNPLA3* gene, (rs738409)-G allele was strongly associated with disease severity. In this study, the researchers studied 266 patients, including 172 cases and 94 controls, to examine the potential influence of disease severity and the *PNPLA3* gene, (rs738409)-G allele (Sookoian, et al., 2009). They found that the *PNPLA3* gene, (rs738409)-G allele was strongly associated with NAFLD, $p < .001$, *OR* 2.8, 95% CI [1.5, 5.2]. Liver cirrhosis, an end stage of NAFLD, was strongly associated with the presence of one or more copies of *PNPLA3* gene, (rs738409-G) allele. No copies of the G-allele resulted in a lower steatosis score as measured by Brunt's criteria (14.9% $\pm$ 3) compared to those with CG genotype (26.3% $\pm$ 3.5) and GG genotype (33.3% $\pm$ 4.0), $p = 0.002$ (Sookoian, et al., 2009).

Severity of NAFLD and the presence of *PNPLA3* gene, (rs738409-G) allele was particularly prominent in a related study with Mestizo Hispanics with alcoholic liver

disease (Tian, et al., 2009). Mestizo Hispanics are of European and Native American ancestry and are more apt to develop liver damage due to alcohol use (Tian, et al., 2009). The Tian study found that the G-allele of the *PNPLA3* gene polymorphism (rs738409), usually the minor allele in the general population, was more prominent than the C-allele in the cirrhosis group (Tian, et al., 2009). In addition, in an urban population study, 45% of Hispanics were found to have NAFLD compared to 33% Caucasians and 24% of African Americans (Browning, et al., 2004).

Valenti and others (2010) noted that persons with two copies of the *PNPLA3 gene,* (rs738409)-G allele had an increased odds for severity of NAFLD, adjusted *OR* = 3.29, 95% CI [1.8, 6.9] (Valenti, et al., 2010). In this study, 253 patients with NAFLD from Italy and the United Kingdom were compared with 179 healthy, Italian control subjects (Valenti, et al., 2010). The Italian patients were matched by age, sex, and geographic location. *PNPLA3* genotype (CC, CG, and GG) influenced the severity of steatosis as determined by liver biopsy. There was no significant difference between the frequency distribution of *PNPLA3* genotypes CG and GG between the Italian and the United Kingdom patients (Valenti, et al., 2010). However, the homozygous genotype (GG) of *PNPLA3* gene, (rs738409) was more common in patients with diagnosed NAFLD (14%) than in persons without NAFLD (3%).

Valenti and others (2010) also found that as the presence of one or more copies of *PNPLA3* gene, (rs738409)-G increased, so did the severity of NAFLD. As discussed earlier, NAFLD progresses from steatosis (fatty liver) to NASH to NASH with fibrosis and cirrhosis. In this study, Valenti and others found that the GG genotype increased the risk of steatosis grade >1 (*OR* = 1.35, 95% CI [1.04, 1.76]), NASH (*OR* = 1.5, 95%

CI [1.12, 2.04]), and fibrosis stage >1 (*OR* = 1.5, 95% CI [1.09, 2.12]) (Valenti, et al., 2010). The *PNPLA3* genotype was found to have a dose effect on steatosis and fibrosis of NAFLD with the presence of one or more copies of the *PNPLA3* gene, (rs738409)-G) allele ($p = 0.0007$) in the Italian group, as evidenced by elevated ALT levels (Valenti, et al., 2010). Similarly, in the U.K. group, those with the GG genotype had higher ALT levels compared to those with the CC or CG genotype [76.0 $\pm$ 54 IU/L CC; 74.5 $\pm$ 56 CG; 123.0 $\pm$ 103 GG; $p < 0.0001$ at ANOVA, $p < 0.05$ for GG vs. either CC or CG genotypes] (Valenti, et al., 2010). However, in the U.K. patients, there was no association with abnormal HDL and LDL as there was in the Italian patients.

Other potential susceptibility genes of NAFLD. The *PNPLA3* polymorphisms and the association of the presence of NAFLD, progression and severity of NAFLD are the most important genetic discoveries in the study of NAFLD. Recently, the APOC3 gene, found on chromosome 11q23, -455-C (rs2854116) and -482C-T (rs2854117), has been associated with NAFLD. Persons with the variants had 60% higher plasma triglyceride levels when compared to the homozygous wild type. Thirty-eight percent of the persons with the variants had NAFLD compared to the NAFLD-free persons with the homozygous wild type (Petersen, et al., 2010).

In sum, the presence of one or more copies of the *PNPLA3* gene, (rs738409)-G allele plays an important role in the susceptibility to and rate of progression to advanced stages of NAFLD. The presence of one or more copies of the *PNPLA3* gene, (rs738409)-G allele will provide a means to stratify the population of persons with NAFLD to determine those with a higher risk of progression in the study of symptoms and HRQOL.

Symptoms in Persons with NAFLD

Researchers have noted a need for the study of symptoms in persons with NAFLD. Salt (2004) wrote, "Only limited data on symptomatology are available from longitudinal studies, and both the likelihood of developing symptoms over time as well as the predictors of the future development of symptoms are not known [in persons with NAFLD]" (Salt, 2004) (p. 33). The limited research conducted to date suggests that persons with NAFLD are often asymptomatic, while others note that persons with NAFLD have vague symptoms of fatigue, malaise, right upper quadrant pain, fullness, or discomfort (Angulo, 2007b). Persons with NAFLD often do not present with symptoms until NAFLD has progressed to the later stages, such as cirrhosis (Angulo, 2007a).

A recent study of children with NAFLD disputes the theory that persons with NAFLD are asymptomatic (Kistler, et al., 2010). Fifty percent of 239 children ages 5 to 17 years with biopsy-diagnosed NAFLD were found to have five or more symptoms as measured by the NIDDK Symptoms of Liver Disease questionnaire and the PedsQL quality of life measurement. Symptoms with the highest frequency were "irritability (73%), fatigue (68%), headache (60%), trouble concentrating (55%), and muscle aches or cramps (53%)" (Kistler, et al., 2010). The most severe symptoms experienced by the children included irritability, trouble concentrating, and fatigue (Kistler, et al., 2010). In comparison, David and others (2009) found that adults with NASH experienced role limitations due to physical health (p = .036), less vitality (p = .043), more bodily pain (p = .043), and poorer general health (p = .023) than in persons with NAFLD, but without NASH (David, et al., 2009).

The findings from the Kistler (2010) and the David (2009) studies support the symptom work conducted with cancer patients in that symptoms often coexist with other symptoms. For example, Given and others found that cancer patients with fatigue had an average of 4.4 other symptoms, and those patients with pain and fatigue together experienced 6.3 other symptoms (Given, et al., 2001).

Other research also suggests symptoms may be present earlier in the disease course. Banks and others noted arthritic symptoms in a small, cross-sectional study of 23 liver biopsy-diagnosed NAFLD patients when compared to 54 patients with hepatitis C, a liver disease population known to have symptoms of arthritis and arthralgias (Banks, Riley III, & Naides, 2007). Both groups experienced equal prevalence of joint pain (HCV = 66.6%, NAFLD = 65.2%, with p = .90, no significant difference). The joints involved included hands, wrists, knees, elbows, shoulders, hips, ankles, and back with similar distribution across the two groups. Thirty-six percent of persons with hepatitis C and 40% of persons with NAFLD reported joint inflammatory complaints such as morning stiffness lasting longer than one hour and daily or weekly symmetrical, painful joint swelling that is better with exercise (Banks, Riley III, et al., 2007).

Inflammatory processes and symptoms. Despite the equivocal results of symptom research done to date, there are physiological reasons to hypothesize that symptoms do occur throughout the different stages of NAFLD. For example, several biomarkers may contribute to the symptoms experience in NAFLD. Adipocytokines, such as adiponectin which decrease with morbid obesity, interleukin-6 which increases in morbid obesity, and TNF-α, are cytokines produced by the adipocytes or fat cells and are associated with the chronic inflammatory disease of obesity (Rogge, 2002), the

precursor to NAFLD development (Day, 2006). Adiponectin is released from the fat cells and is regulated by TNF-α (Fantuzzi, 2005; Rogge, 2002). Adiponectin has anti-inflammatory effects; however, in persons with NAFLD, hypoadiponectinemia, or lower levels of adiponectin, occurs (Hui, et al., 2004).

In addition to the dysregulation of adiponectin levels in persons with obesity and NAFLD, TNF-α has pro-inflammatory effects, and, in conjunction with interleukin-6, has been found to contribute to sickness behavior in which these peripheral inflammatory markers cross the blood-brain barrier to perpetuate behaviors of sickness, such as fatigue, depression, irritability, and nausea (Dantzer, et al., 2008). Furthermore, serum TNF-α was found to be more elevated in obese patients with NASH, the inflammatory stage of NAFLD, compared to obese patients without NASH (Baranova, et al., 2007). Elevated TNF-α and Interleukin-6 stimulate serotonin uptake resulting in depression (Zhu, Blakely, & Hewlett, 2006) and fatigue (D'Mello & Swain, 2011). The elevated TNF-α in persons with NASH provides rationale to hypothesize a potential symptoms experience in persons at risk of NAFLD disease progression.

In prior research, sickness behavior has been described in the context of acute illnesses. Sickness behaviors may also occur in chronic illness, such as NAFLD. In fact, other chronic liver diseases such as hepatitis B, hepatitis C, and primary biliary cirrhosis, have inflammatory symptoms (Banks, Riley, & Naides, 2007).

In summary, patients with the inflammatory stage of NAFLD known as NASH were found to have joint pain, depression, and fatigue (Banks, Riley, et al., 2007; Habib & Saliba, 2001). Elevated TNF-α has been noted in synovial fluid of NASH patients with joint pain (Crespo, et al., 2001). In the NAFLD population, elevated TNF-α may suggest

a biological reason for symptoms, and TNF- α may mediate the inflammatory response in both NASH and arthritis (Habib & Saliba, 2001). In addition, elevated pro-inflammatory biomarkers such as TNF- α along with low adiponectin levels found in NAFLD may provide a biological explanation for the current symptoms experience of fatigue, malaise, and right upper quadrant pain or fullness as the sickness behavior of patients with NAFLD. However, current literature noted that patients often do not notice pain or fullness until the later stages or inflammatory stages of the disease (Angulo, 2007b). Therefore, further study is needed to fully explicate the symptoms experience in persons with NAFLD.

Symptoms and comorbid conditions of NAFLD. NAFLD, formerly known as the *hepatic manifestation of the metabolic syndrome* is strongly associated with obesity, insulin resistance, and metabolic syndrome (Angulo, 2002). Most recently, hypothyroidism was linked to a higher incidence of disease progression in NAFLD. Each of these comorbid diseases may yield symptoms themselves. Persons with obesity have reported symptoms of joint pain (Heo, Pietrobelli, Wang, Heymsfield, & Faith, 2009) and mental health problems such as stress, depression, and problems with emotions for one week or more during a 30 day period (Heo, et al., 2009). Patients described joint pain as pain, aching, stiffness, or swelling around a joint.

Persons with diabetes mellitus type 2 may not experience symptoms or may have symptoms of polyphagia, polyuria, and/or polydipsia; blurred vision; weight loss; fatigue; or irritability (American Diabetes Association, 2010). Although, this list is neither predictive nor conclusive (Clark, Fox, & Grandy, 2007). The American Heart Association lists no symptoms for metabolic syndrome (American Heart Association, 2009);

however, depressive symptoms have been associated with metabolic syndrome (Akbaraly, et al., 2009).

Finally, symptoms common with hypothyroidism include fatigue; weakness; weight gain or increased difficulty losing weight. Persons with hypothyroidism may also experience coarse, dry hair; dry, rough pale skin; hair loss; cold intolerance; muscle cramps and frequent muscle aches; constipation; depression; irritability; memory loss; abnormal menstrual cycles; and decreased libido (Norman, 2009). There are numerous symptoms associated with comorbid conditions of NAFLD which include obesity, hypothyroidism, diabetes mellitus Type 2 and metabolic syndrome which may actually be attributed to undiagnosed NAFLD.

Studies are needed to develop a foundational understanding of symptoms, genetics, and HRQOL in persons with NAFLD. Once known, persons at risk of NAFLD and NAFLD progression can be identified noninvasively (i.e., without liver biopsy). These findings will support further scientific research in the study of NAFLD to prevent disease progression in all stages of this disease process -- especially the early stages -- initially through the identification of symptoms in this population, to promote optimal quality of life for these patients in all stages of this disease, and to promote public awareness of NAFLD.

The long-term goals of this emerging research are to develop assessment and intervention strategies to prevent the development of NAFLD, to prevent or reverse disease progression in persons with NAFLD through assessment and intervention, and to prevent or manage symptoms from a patient-centered perspective. From these data, an NAFLD registry in the western Michigan area will be created to enhance knowledge

and future shared-research opportunities beyond western Michigan in the study of this very concerning public health threat.

No studies have examined symptoms of those at risk of NAFLD progression compared to those not at risk of NAFLD progression using the gene variant of *PNPLA3* and the Healthy Days Measure, nor do studies control for comorbid conditions. Therefore, research is needed to fully describe the symptoms experience in persons with NAFLD.

Factors that influence symptoms (antecedents). Antecedents are factors that are known or hypothesized to influence symptoms in persons with NAFLD, such as demographic characteristics, disease characteristics, and individual characteristics. As discussed in chapter 2, symptoms become known to the provider by the individual acknowledging the symptoms (Rhodes & Watson, 1987). Perception or awareness of symptoms "requires the ability to grasp and comprehend what is obscure" (Rhodes & Watson, 1987). Antecedents such as demographic, disease, and individual characteristics influence the awareness or perception of symptoms (Armstrong, 2003).

Demographic characteristics. As defined in chapter 2, demographic characteristics of the individual are the age, sex, race/ethnicity, occupation, education level, access to health care, environment, and socioeconomic status of the individual with NAFLD.

Age, sex, race/ethnicity, and symptoms. Age and sex may influence symptoms. Middle-aged women may have menopausal symptoms. A study of 325 Ecuadorian (Hispanic) postmenopausal women, ages 40 to 70 years (median 54 years), and screened for metabolic syndrome, reported symptoms of hot flushes (53.3%), sweating

(49.2%), poor memory (80.6%), depression (67.4%), muscle and joint aching (84%), skin dryness (85.5%), intimacy avoidance (76.2%), and changes in sexual desire (76.5%). Those with central obesity had more severe symptoms of menopause, such as hot flushes, than in persons without central obesity (Chedraui, et al., 2007). Furthermore, those with high triglyceride levels had higher rates of sweating (54% vs. 42.9%) and depression (73.5% vs. 59.3%) than those with normal triglyceride levels ($p < 0.05$). Women with increased waist circumference, that is a waist circumference greater than 88 cm, were twice as likely to have muscle and joint aches compared to those without higher waist circumference, $OR = 2.1$, CI [1.1, 4.1], $p < 0.05$ (Chedraui, et al., 2007).

Stages of disease, genotype, and inflammation (disease characteristics). Disease characteristics for this study are the alcohol intake, medication use, and body fat distribution as measured by waist-to-hip circumference and BMI. Most importantly, disease characteristics are the stages of the disease, genotype and inflammation. NAFLD can progress from simple fatty liver disease to NASH without fibrosis, or NASH with fibrosis, to cirrhosis and then to liver failure or liver cancer resulting in premature death. It is estimated that 67.2 million people out of 280 million people in the United States have the simple fatty liver stage of NAFLD. Out of the 67.2 million, it is estimated that 5.6 to 8.4 million will progress onto NASH, and from the 5.6 to 8.4 million people that have NASH, it is estimated that 890,000 to 2.52 million will develop cirrhosis (Aouizerat, 2004).

Progressive fibrosis to cirrhosis is influenced by a combination of the presence of fat in the liver “(steatosis), oxidative stress, and genetic predisposition” (Clouston &

Powell, 2002). This inflammatory process triggers reuptake of serotonin in the brain, producing symptoms (D'Mello & Swain, 2011). As discussed earlier, persons at higher risk of disease progression have one or two copies of the gene *PNPLA3* gene, (rs738409)-G alleles (Sookoian, et al., 2009; Valenti, et al., 2010). I hypothesize that those with one or two gene alleles or those with advanced disease will have more symptoms.

In the recent and ongoing prospective study, the Framingham Heart Study, 2,589 individuals were evaluated for fatty liver disease using multidetector-computed tomography of the abdomen. Most participants in the study were middle-aged women (51%) with an average BMI of 27.6 kg/m^2. Seventeen percent of participants were found to have fatty liver disease strongly associated with visceral obesity. Once visceral obesity was controlled, fatty liver disease was most strongly associated with dyslipidemia ($p < 0.0001$) and dysglycemia, $p < 0.0001$ (Speliotes, et al., 2010). Individuals with fatty liver disease were nearly 3 times more likely to have diabetes than those without fatty liver disease, adjusted *OR* 2.98, 95% CI [2.12, 4.21]. Persons with fatty liver also had a 6 times higher prevalence of insulin resistance, *OR* 6.16, 95% CI [4.90, 7.76]; more than 5 times higher prevalence of metabolic syndrome, *OR* 5.22, 95% CI [4.15, 6.57]; and approximately 3 times higher incidence of hypertension, *OR* 2.73, 95% CI [2.16, 3.44] and impaired fasting glucose levels, *OR* 2.95, 95% CI [2.32, 3.75], than persons without fatty liver disease, $p < .001$ (Speliotes, et al., 2010).

Physical characteristics, such as increased BMI and increased waist-to-hip circumference, are observed in persons with NAFLD. Persons with a BMI greater than 30 (obese category) are more likely to have NAFLD than those with a normal BMI

(Browning, et al., 2004). Visceral obesity and increased waist-to-hip circumference are strongly related to NAFLD (Clark, 2006). Visceral obesity often suggests insulin resistance, a contributor to NAFLD (Day, 2006), although recent genetic studies noting that the presence of a single nucleotide variation (rs738409) in the *PNPLA3* gene has been found in higher prevalence in persons with NAFLD (Romeo, et al., 2008) did not find a correlation of insulin resistance (Romeo, et al., 2009).

Alcohol (Etoh) intake. Alcohol intake will be considered for exclusion criteria for NAFLD. NAFLD is defined as 5% or greater of the total liver weight consisting of fat with minimal or no alcohol intake (Angulo, 2007a). Alcohol intake should be relatively low, such as less than 40 grams of alcohol per week in men and less than 20 grams of alcohol per week in women. Note that 10 grams of alcohol is equal to one 330 ml can of beer, one 100 ml glass or wine or 1 ounce of liquor (Alcohol Advisory Council of New Zeeland, 2008).

Medication use. Medications may reverse or halt the progression of NAFLD. For example, metformin is being used in conjunction with dietary changes to decrease hepatic fat (Garinis, et al., 2010). Combination therapy of fenofibrate and atorvasatin showed a decrease in liver enzymes and hepatic fat (Athyros, et al., 2006). The use of pioglitazone was also found to improve liver function studies in a small group of patients with NAFLD (Shadid & Jensen, 2003).

Recently, vitamin E was shown to reduce AST and ALT (p = .005) and lobular inflammation (p = .02) in comparison to placebo, in which p values less than .025 were considered significant (Sanyal, et al., 2010). In the Sanyal study, 247 adults with NASH and without diabetes were randomly assigned to either 30 mg of pioglitazone per day (*n*

= 80), 800 IU of vitamin E per day (n = 84), or placebo (n = 83) for 96 weeks. Persons taking vitamin E had a higher rate of improvement (43%) than did persons taking the placebo (17%), $p < .001$. AST and ALT were significantly decreased in persons taking vitamin E ($p = .001$) or pioglitazone ($p < .001$) when compared to placebo. Both vitamin E ($p = .005$) and pioglitazone ($p < .001$) were found to reduce fat deposits in the liver (hepatic steatosis). However, neither vitamin E ($p = .024$) nor pioglitazone ($p = .12$) showed an improvement in fibrosis scores (Sanyal, et al., 2010).

Comorbid conditions. NAFLD is associated not only with obesity but also with other obesity-related diseases. For example, those with NAFLD are more likely to develop other comorbid conditions, such as diabetes mellitus, 300% more likely, and metabolic syndrome, 50% more likely (Adams, et al., 2009). In addition, those with NAFLD will also eventually develop cardiovascular disease (Akabame, et al., 2008).

Body fat distribution. In addition to diabetes and metabolic syndrome, visceral obesity, a component of metabolic syndrome as defined by the World Health Organization, has also been associated with NAFLD (Marchesini, et al., 1999) suggesting waist-to-hip circumference may be an important measurement. Some consider NAFLD to be “the hepatic manifestation of the metabolic syndrome” (Angulo & Lindor, 2002). Recent studies suggest that intrahepatic fat measured as intrahepatic triglycerides rather than visceral fat may be associated with the metabolic syndrome (Fabbrini, et al., 2009). Body fat distribution of fat deposits in the liver (NAFLD) may be the missing link noted by Sowers and others in determining cardiometabolic abnormality in 24% of normal-weight adults compared to 51% of overweight adults and 32% of obese adults (Wildman, et al., 2008).

Individual characteristics. Individual characteristics of persons with NAFLD include prior health knowledge of NAFLD. Prior health knowledge of NAFLD will be measured in this study as I hypothesize that if an individual has an awareness of the disease process, one may be more apt to perceive symptoms. Rhodes and Watson write, "symptom awareness is being cognizant, having knowledge of something" (Rhodes & Watson, 1987). One may be more apt to be aware of symptoms associated with NAFLD if one has had experience or health knowledge about NAFLD.

HRQOL

HRQOL studies are needed to assess the burden of illness in persons with NAFLD (Angulo, 2007a; Kistler, et al., 2010). However, HRQOL must be defined. Several definitions of HRQOL exist in the literature. HRQOL is frequently referred to in the literature as "self-reported health status, quality of life, and functional health" (Crosby, Kolotkin, & Willams, 2003) or "physical functioning, symptoms, psychosocial adjustment, well-being, life satisfaction, and happiness" (Ferrans, Zerwic, Wilbur, & Larson, 2005). Others define quality of life as a holistic, self-determined evaluation of satisfaction with issues important to the person [which are] influenced by finances, housing, employment, spirituality, social support networks, and health" (Curtis, Martin, & Martin, 1997).

Patrick and Erickson define HRQOL as the "value assigned to duration of life as modified by the impairments, functional states, perceptions, and social opportunities that are influenced by diseases, injury, treatment, or policy" (Patrick & Erickson, 1993). For example, in persons with NAFLD, those with advanced disease such as liver cirrhosis may have decreased functional states that are due to extreme fatigue as

influenced by the severity of NAFLD in the later stages. Persons with cirrhosis and extreme fatigue are unable to conduct normal activities such as shopping, house cleaning, and attending social events without pacing activities with periods of rest. In addition, those with NAFLD may perceive their general state of health as poor or fair due to the decreased functional state.

According to Ferrans and colleagues (2005) and Armstrong (2003), biological function (Ferrans, et al., 2005) or disease characteristics (Armstrong, 2003) can influence HRQOL (Armstrong, 2003; Ferrans, et al., 2005). Ferrans and colleagues (2005) define biological function as the "molecular, cellular and whole organ level processes… from ideal functioning to life-threatening pathological function." Biological function of the individual can be measured through indicators of how the body is working, such as through the evaluation of biomarkers, genetic predisposition, vital signs, laboratory values, imaging tests, and physical assessment. Disease characteristics such as the stage of NAFLD and genetic predisposition such as the presence of one or more copies of the *PNPLA3* gene, (rs738409)-G allele noted in the adaptation of the symptoms experience model (Armstrong, 2003) can denote severity of disease and ultimately influence HRQOL.

Very few studies have addressed HRQOL in adults with NAFLD (Dan, et al., 2007; David, et al., 2009) and no studies have measured HRQOL as a nurse-sensitive outcome or consequence in this population. HRQOL in persons with NAFLD decreases as the disease progresses (David, et al., 2009). A recent study using the SF-36 (Ware, n.d.) as a measure of HRQOL found that patients with cirrhosis have poorer HRQOL when compared to the less severe state of NASH, and that those with NASH have

poorer HRQOL than those with simple fatty liver disease (David, et al., 2009). In addition, patients with NAFLD have poorer HRQOL compared with hepatitis B and hepatitis C patients when using the Chronic Liver Disease Questionnaire as a measurement of HRQOL (Dan, et al., 2007). This study will use the Healthy Days Measure, which has been used in study of persons with arthritis (Mielenz, Jackson, Currey, DeVellis, & Callahan, 2006) and obesity (Heo, Allison, Faith, Zhu, & Fontaine, 2003), to assess HRQOL. For the purposes of this study, HRQOL will be defined as an "individual's perceived physical and mental health" (Centers for Disease Control and Prevention, 2000). To avoid multicollinearity between healthy days and symptoms, physical and mental health will not be used in the linear regression.

Nursing and HRQOL

In 1996, President Clinton formed the Advisory Commission on Consumer Protection and Quality in the Health Care Industry to keep abreast of changes in the health-care system and make recommendations for improvement (Garinis, et al., 2010). The Health Care Quality Commission defined the purpose of the health care system "is to continuously reduce the impact and burden of illness, injury and disability and to improve the health and functioning of the people of the United States" (Garinis, et al., 2010). A great deal of work needs to be done to reduce the impact and burden of illness for persons with NAFLD and their families.

Subsequently, in 2008, the Robert Wood Johnson Foundation, a long-time supporter of the nursing profession, formed a partnership with the Institute of Medicine and began work on the 2-year *Initiative on the Future of Nursing.* The charge of this partnership was to develop recommendations of policy change at institutional, local,

state, and federal levels to support the role of the professional nurse in the fullest extent (Institute of Medicine, 2011).

Nurses comprise more than 3 million people of the health-care workforce and by the nature of their work, provide more time-intensive care to the public. Nurses have the most opportunities, being the most-trusted health-care professional by patients, to make significant changes in the quality, safety, affordability, and accessibility of health care, but are stifled by state laws and by third-party reimbursement policies, in their abilities to practice to the fullest extent of their educations (Institute of Medicine, 2011). These changes are timely with the current worldwide epidemic of NAFLD.

The *Initiative on the Future of Nursing* produced four key messages and more than 600 hundred pages of recommendations to reform factors that impact nursing (Institute of Medicine, 2011). These four key messages are as follows (Institute of Medicine, 2011):

1) Nurses should practice to the full extent of their education and training.
2) Nurses should achieve higher levels of education and training through an improved education system that promotes seamless academic progression.
3) Nurses should be full partners, with physicians and other health care professionals, in redesigning health care in the United States.
4) Effective workforce planning and policy making require better data collection and an improved information infrastructure. (p. 4)

Nursing is vital in redefining health care in the United States and establishing care for the epidemic of persons with NAFLD. The American Nurses Association defines the role of the professional nurse to “protect, promote, and optimize health and abilities,

prevent illness and injury, alleviate suffering through the diagnosis and treatment of human response and advocacy in the care of individuals, families, communities, and populations." (American Nurses Association, 2003). Through these actions, nurses are crucial in reducing the impact and burden of illness, injury, and disability of NAFLD which is important to payers, consumers, providers, and policy makers (Tarcin, et al., 2008).

Reducing the impact and burden of illness may reduce cost and improve quality of care for persons with NAFLD. Measurement of HRQOL is one means for nurses and other health-care professionals to monitor the burden of illness, injury, and disability in the aggregate and individual populations of persons with NAFLD. Once the impact of the burden of illness, injury, and disability is identified through a change in HRQOL, interventions can be implemented to improve care in persons with NAFLD.

Intervention Work in NAFLD

Little intervention work has been done in the treatment of NAFLD. Weight loss has been shown to improve liver histology in NASH. In a randomized control trial, overweight and obese participants were randomized into a lifestyle change group versus a control group (Promrat, et al., 2010). Individuals in the lifestyle change group who lost greater than 7% of their body weights had an improvement in their NASH upon liver biopsy. The percentage of weight loss correlated with an improved NAS, $r = 0.497$, $p = .007$ (Promrat, et al., 2010). Vitamin E has been found to reduce fat deposits in the liver and lower ALT and AST, but not to decrease fibrosis as discussed earlier in the medication section (Sanyal, et al., 2010). A systematic review and meta-analysis of the effect of bariatric surgery in persons with NAFLD found that steatosis, 91.6%, 95% CI

[82.4, 97.6]; NASH, 81.3%, 95% CI [61.9, 94.9]; and fibrosis, 65.5%, 95% CI [38.2, 88.1], seem to either improve or resolve after bariatric surgery (Mummadi, et al., 2008).

All of these studies (Mummadi, et al., 2008; Promrat, et al., 2010; Sanyal, et al., 2010) show improvement in NAFLD. The Institute of Medicine charges researchers and clinicians to have a patient-centered focus (Tarcin, et al., 2008). Thus, the inclusion of the patient's perception of symptoms and HRQOL may provide researchers with earlier opportunities for intervention through the assessment of symptoms and HRQOL in the early stages of NAFLD.

Problem Statement

There is a dearth of knowledge regarding the symptoms experience and level of HRQOL in persons at risk of disease progression as defined by the presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele. Identification of symptoms along with the combined influence of the frequency, intensity, and distress of these symptoms need to be studied to lay the foundation for nursing interventions to be provided to persons with NAFLD in order to treat the human response of these symptoms (Rhodes & Watson, 1987). Establishing the burden of illness through measurement of HRQOL in persons with NAFLD will provide a foundation to measure the impact of nursing interventions in the future studies. Further, the recent association of the variants within the *PNPLA3* gene and disease progression provides an innovative strategy for examining the symptoms experience according to risk for progression of NAFLD.

Significance and Impact of Addressing Problem

Significance. NAFLD, if allowed to progress, can result in premature death. As presented earlier, an estimated prevalence of NAFLD determined from the 2000 U.S.

Census of 280 million people projects that 24% will have simple fatty liver disease (*n* = 67 million), and of these persons, 2 to 3% will progress to the second stage of NAFLD, known as NASH (*n* = 5.6-8.4 million) and 0.3 to 0.9% will develop fibrosis leading to cirrhosis, *n*= 0.89-2.52 million (Aouizerat, 2004). NAFLD can also lead to liver failure and liver cancer (Angulo, 2007b).

Nursing and symptoms. Symptoms are often the "red flags" that something is wrong with one's health (Hegyvary, 1993) or a "perceived indicator of change in normal functioning"(Lenz, et al., 1997). Researchers have noted a need for the study of symptoms in NAFLD. Salt (2004) wrote, "Only limited data on symptomatology are available from longitudinal studies, and both the likelihood of developing symptoms over time as well as the predictors of the future development of symptoms are not known [in persons with NAFLD]"(Salt, 2004) (p. 33).

Thus, through the study of symptoms using genetic markers for stratification of the population, I hope to build a symptom profile for this significant health problem linking genetic markers and HRQOL with symptoms. The information gained from the successful completion of this study is expected to provide the foundation for the development of symptom and HRQOL assessment tools that can be used to measure the impact of interventions for those patients with NAFLD at risk of disease progression.

Most important, symptom identification can be used for early detection of persons at risk for disease progression and to manage the care of persons with later stages of disease using the identified symptom burden experience as early red flags of disease progression by nurses, nurse practitioners, and other health care professionals. Nurses can play a critical role in the early identification and intervention of symptoms of NAFLD.

In addition to symptoms, changes in health status as measured by HRQOL can be used to evaluate interventions in persons in the later stages of NAFLD. Early detection of NAFLD will trigger multidisciplinary interventions for NAFLD, preventing costly liver transplants for those who progress to cirrhosis, and will possibly prevent premature death.

Impact of the Study

Through the study of symptoms, using genetic markers for stratification of the population, we expect to identify a list of symptoms for this significant health problem, linking genetic markers and HRQOL with symptoms. Changes in health status as measured by HRQOL can be used to measure interventions in persons in the later stages of NAFLD. Early detection of NAFLD will trigger multidisciplinary interventions for NAFLD, preventing costly liver transplants for those who progress to cirrhosis, and will possibly prevent premature death.

Summary. There are currently few studies describing symptoms in NAFLD (Kistler, et al., 2010), no studies linking genetic or biological markers of NAFLD with symptoms or HRQOL (Banks, Riley III, et al., 2007), and adult studies linking a symptoms experience with HRQOL are also lacking. This study will provide critical foundational work for nurses to use symptom and HRQOL instruments to measure the effectiveness of nursing interventions in the treatment of NAFLD.

Chapter 4: Design and Methods

Chapter 4 will present the design and methods for this study. A description of the sample, setting, predictors, covariates and dependent variables, instruments, data collection procedures, data analysis plan, and a human subject protection plan including a description of the plan for inclusion of minorities and children will be presented.

The purpose of this research was to determine a symptoms experience in persons with NAFLD hypothesized to be at higher risk of disease progression as evidenced by the presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele.

Design

The research strategy proposed for this dissertation research was a cross-sectional, descriptive design to explore a symptoms experience and HRQOL in persons with NAFLD hypothesized to be at higher risk of disease progression versus persons at lower risk of progression as determined by genotype at the *PNPLA3* gene, (rs738409) locus.

Aims. The aims of this study were as follows:

Aim 1. To identify the presence of symptoms in persons with NAFLD at higher risk of disease progression based on the presence of one or two copies of *PNPLA3* gene, (rs738409)-G allele variant compared to those at lower risk of disease progression, that is, no copies of the *PNPLA3* gene, (rs738409)-G allele.

Aim 2. To compare the extent to which the frequency, intensity, and distress of symptoms in persons with NAFLD differ between those at higher risk of disease progression based on the presence of one or two copies of *PNPLA3* gene, (rs738409)-

G allele variant compared to those at lower risk of disease progression, that is no copies of the *PNPLA3* gene, (rs738409)-G allele.

Aim 3. To determine the difference in HRQOL in persons at higher risk of NAFLD progression based upon the presence of one or two copies of *PNPLA3* gene, (rs738409)-G allele variant versus those at lower risk of disease progression, that is, no copies of the *PNPLA3* gene, (rs738409)-G allele.

Aim 4. To describe the relationship between symptom distress and HRQOL in persons at higher risk of NAFLD progression based on the presence of one or two copies of *PNPLA3* gene, (rs738409)-G allele versus persons at lower risk of NAFLD progression, that is, no copies of the *PNPLA3* gene, (rs738409)-G allele.

Setting. The participants for this study were recruited from two clinical sites in Grand Rapids: Michigan. These private practices were located in the same hospital-affiliated, health-park medical office building in Wyoming, MI, serving patients from at least three different counties.

Site one was a general surgery office that specializes in gastric-banding of the bariatric population and in general surgery. This site provided care for approximately 15 persons with NAFLD per month. Site one had a health professional staff of three surgeons, a nurse practitioner, a registered dietician, a behavioral counselor specializing in addictive behavior, several medical assistants, a receptionist, sonography technician, and an office manager. The nurse practitioner was a national consultant for adjustable banding company. Site one had been recognized as a center of excellence for its multidisciplinary approach to supporting its patients in pre and postgastric banding surgery. In addition, this office provided lifelong support for its

surgery patients, most of whom have comorbid NAFLD. Providers at site one conducted imaging of the liver on most of the gastric banding candidates and on many abdominal surgery candidates. At site one, the surgeon also obtained liver biopsies for some patient populations, such as cholecystectomy patients.

Site two is a gastroenterology practice that also serviced several counties and provided care to persons with disease processes such as NAFLD, hepatitis, cirrhosis, and Crohn's disease in Grand Rapids, MI. Staff at site two estimated that the office cared for approximately 75 persons with NAFLD per month and performed five liver biopsies per week on patients with a variety of liver diseases. This office supported one gastroenterologist and two internal medicine physicians. Site two employed several medical assistants, registered nurses, receptionists, billing clerks, and an office manager.

Combined, it was estimated that these recruitment sites would yield a minimum of 90 patients per month for potential recruitment. Recruitment of participants occurred from September 2010 through June 2011. Approximately 64 patients were approached about the study, and 9.3% declined to participant. Of the 9.3% who declined, five stated that they did not have time to complete the questionnaire and to provide a saliva sample, and one was not comfortable providing a saliva sample. Nearly 5% (n = 3) identified that they weren't eligible because of presence of hepatitis or a history of alcohol abuse. In addition, two were identified by the provider, but did not show up for their scheduled appointment at the provider's office. One patient was recruited by word-of-mouth and is designated as recruited from home. Table 3 compares estimated recruitment versus participants recruited from each site.

Table 3

Estimated vs. Recruited Number of Participants with Diagnosed NAFLD Per Recruitment Site

Recruitment site	Number of participants with NAFLD per month	
	Estimated	Recruited
Site one	15	0-8
Site two	75	0-3
	0	1

Note. NAFLD = nonalcoholic fatty liver disease.

Sample. A sample size of 54 participants was recruited for this study using convenience sampling. The medical assistant approached patients with diagnosed NAFLD, supplied a marketing flyer, and asked if they would be interested in participating in the study. If the patient was interested, the participant signed a *patient authorization for disclosure of health information for research*, which allowed the researcher to discuss the study with the participant. Once recruited, the patient provided consent to the researcher, who determined eligibility based on the inclusion and exclusion criteria listed in the Inclusion/Exclusion Criteria section. Data collection ensued using a questionnaire, anthropometric measurements and a saliva collection device as a source of DNA for the genetic analysis. If potential participants had NAFLD, but were being seen for surgical pre or postoperative care, the participants were enrolled, but were contacted again at least 6 weeks postoperatively to answer the symptoms and HRQOL portion of the questionnaire, so as not to skew the symptom results of the study. Saliva samples for genetic analysis were obtained at the initial visit,

as many of the surgical patients would not be returning to this office once their surgical post-operative visits were completed. (Kotronen, Johansson, et al., 2009; Kotronen, Peltonen, et al., 2009; Romeo, et al., 2008; Romeo, et al., 2010) and data from the supplier of the gene Single Nucleotide Polymorphism (SNP) kits (Life Technologies Corporation, 2010). Of the 42 participants enrolled, 55.69% (*n* = 25) were expected to have one or two copies of the *PNPLA3* gene, (rs738409)-G allele and 42.26% (*n* = 17) will have no copies of the *PNPLA3* gene, (rs738409)-G allele. Figure 5 describes the Hardy-Weinberg equilibrium equation used to calculate allele frequencies.

$$P^2 + 2pq + q^2,$$

where p is the major allele (C-allele) of the *PNPLA3* gene, (rs738409) polymorphism; and q is the minor allele (G-allele) of the *PNPLA3* gene, (rs738409) polymorphism.

Figure 5. Hardy-Weinberg equilibrium equation (Tolman & Dalpiaz, 2007):

Methods

Power. Using G-power to estimate a sample size for this study, I used *F* test, linear multiple regression, fixed model, R^2 increase. An estimated sample size of 59, participants will yield 0.80 power to detect a medium effect size of 0.15 with α error probability of 0.05 using the Healthy Days tool as the measure of effect, along with 37 potential predictors: 32 symptoms, waist-hip circumference ratio, CCI, presence of one or two copies of the *PNPLA3* gene, (rs738409)-G alleles, presence of obesity, and race/ethnicity. Participant's data were stratified into two groups based on genotype at the *PNPLA3* gene, (rs738409) locus: (a) persons at higher risk of NAFLD disease progression that is the presence of one or two copies of the *PNPLA3* gene, (rs738409)-

G allele and (b) persons at lower risk of disease progression, that is no copies of the *PNPLA3* gene, (rs738409)-G allele.

Inclusion/exclusion criteria. The inclusion criteria were as follows: (a) All patients 21 years of age and older with a diagnosis of NAFLD, (b) English as primary reading and speaking language, and (c) confirmed NAFLD through imaging or liver biopsy as noted in the medical record.

Participants were excluded from the study for the following reasons: (a) history of alcohol or drug abuse; (b) presence of liver disease or other liver disease such as hepatitis or alcoholic fatty liver disease; (c) physical or cognitive limitations that prevent participation requirements, such as being weighed or completing the questionnaire, which was assessed by the participant's responses to eligibility questions on the brochure describing the study; (d) diagnosis of psychosis; (e) surgery within the last 6 weeks; and (f) acute condition requiring surgery such as acute cholecystitis.

Instruments.

Demographic characteristics. Demographics were obtained using a self-report questionnaire. Patient demographics gathered from the patient data questionnaires from each site and the medical record audits provided the individual's employment status and education level to measure socioeconomic status, and zip code to measure access to health care (see Michigan State University study questionnaire in Appendix A). Demographic characteristics such as age, sex, and race/ethnicity were obtained from the questionnaire.

Disease characteristics. Disease characteristics of this population included the genotype at the *PNPLA3* locus according to the presence of one or two copies of the

PNPLA3 gene, (rs738409)-G allele or the absence of the *PNPLA3* gene, (rs738409)-G allele. The presence of NAFLD as noted by imaging and stages of NAFLD (simple fatty liver, NASH without or with fibrosis, cirrhosis, liver failure, and liver cancer) as determined by liver biopsy, if conducted, was also considered a disease characteristic and collected through chart review. The presence of comorbid conditions such as diabetes mellitus or insulin resistance, cardiovascular disease, arthritis, obesity, metabolic syndrome, thyroid disease, alcohol intake, and medication use were extracted through chart review.

Genotype measurement. Saliva samples were collected to extract genomic DNA for genotyping of the *PNPLA3* gene, (rs738409) variant. Specific genotyping methods are described in the Data Collection Procedures section.

Stages of NAFLD. Stages of NAFLD, if available, were obtained from the medical record by the primary investigator to be used as supplemental information about disease stage. Staging of NAFLD as found from the chart audit was recorded as the diagnosis from pathology report, such as NASH without fibrosis, NASH with stage 0-1, NASH with stage 3-4 fibrosis, or cirrhosis.

Individual characteristics. Individual characteristics included one variable: prior health knowledge of NAFLD. Prior health knowledge was the awareness of NAFLD in that the participant had heard about NAFLD through a lecture, had read about NAFLD, had a family member or friend that had the disease or had been told by a provider that he or she had NAFLD prior to the study.

Prior health knowledge about NAFLD. Prior health knowledge about NAFLD was measured by the addition of a categorical question to the combined Michigan State

University study questionnaire: “Have you heard of nonalcoholic fatty liver disease before today? (Yes/No).” This question measured any prior health knowledge of NAFLD in that the participant may have prior knowledge about NAFLD, but may not have been aware that he or she had a diagnosis of NAFLD prior to recruitment into the study.

Height and weight measurements were obtained as part of the normal intake process for the appointment and used to calculate BMI. Hip and waist circumferences were obtained to calculate waist-to-hip ratios.

Comorbid conditions. The Charlson Comorbidity Index, a widely studied comorbidity index, was used to control for confounding comorbid conditions associated with NAFLD (Charlson, et al., 1987). A list of 10 categories of diseases, (e.g., myocardial, vascular, pulmonary, neurologic, endocrine, renal, liver, gastrointestinal, cancer/immune, and miscellaneous) comprised of 19 conditions is found in the CCI. The conditions are summed and weighted to create an index score. These scores yield four comorbidity grades: 0, 1-2, 3-4, and $\geq$5 (Birim, et al., 2003; Charlson, et al., 1987). A comorbidity grade (i.e., the CCI) was calculated for each participant and used in the regression equation as a predictor variable. To determine the CCI, the participant was asked to complete the patient questionnaire, which embedded the comorbid conditions of the CCI. In addition, comorbid conditions were confirmed by the Primary Investigator through a medical record audit as some comorbid conditions, such as obesity, are not included in the CCI.

Psychometrics of the CCI. The CCI has high reliability and validity for use in controlling for comorbid conditions and for predicting mortality (Birim, et al., 2003;

Charlson, et al., 1987; de Groot, Beckerman, Lankhorst, & Bouter, 2003). In the original study, comparisons were conducted between two cohorts of 559 medical patients over 1 year and 685 patients over 10 years (Charlson, et al., 1987). Cox regression method was used to determine proportional hazards analysis in each cohort using the CCI as one measure of the 10 categories of comorbid conditions in a regression model. As the comorbidity index increased, the mortality rates increased in stepwise fashion (log rank chi-square = 165, $p < .0001$) depicting high validity. The population in this study included patients with cancer, immune diseases, and liver diseases.

The CCI has high reliability as well, and has been used in several studies to control for comorbid conditions, to predict mortality or as criterion validity for indices development (Jepsen, Vilstrup, Andersen, Lash, & Sørensen, 2008). These studies include the use of the CCI in persons with liver cirrhosis (Jepsen, et al., 2008), in persons with cardiac rehabilitation postacute coronary syndrome (Dunn, Stommel, Corser, & Holmes-Rovner, 2009), and in comparison of pioglitazone with other antidiabetic medications for incidence of liver failure (Rajagopalan, Iyer, & Perez, 2005).

The CCI has been used to control for comorbid conditions for subjects with a diagnosis of liver cirrhosis (Jepsen, et al., 2008). In this population of persons with liver cirrhosis, persons with NAFLD were not identified. However, those with a CCI of 1 had a 1.17 higher mortality rate (95% CI [1.11, 1.23]) than those persons with a Charlson Comorbidity of 0 (Jepsen, et al., 2008). Likewise, those with a CCI of 2 had a 1.51 higher mortality rate ([95% CI [1.42, 1.62]) when compared to persons with a CCI of 0. A CCI of 3 resulted in a mortality rate of 2, 95% CI [1.85, 2.15] (Jepsen, et al., 2008).

Symptoms experience. The MSAS (Portenoy, et al., 1994) was used to determine the symptoms experience in persons with NAFLD. The MSAS measures 32 symptoms using Likert scales to evaluate the frequency, intensity, and distress that the patient perceives from the symptom production within the domains of high-prevalence physical symptoms, low-prevalence physical symptoms, and psychological symptoms. Scores are calculated by summing and averaging each subscale.

Measurement of the symptoms experience; frequency, intensity, and distress. Four subscales of the MSAS measured the defining attributes of the symptoms experience; the Total Memorial Symptom Assessment Scale (TMSAS), the Memorial Symptom Assessment Scale-Global Distress Index (MSAS-GDI), the Memorial Symptom Assessment Scale-Physical (MSAS-PHYS) and the Memorial Symptom Assessment Scale-Psychological (MSAS-PSYCH). TMSAS measured the frequency, intensity, and distress of 24 symptoms and the intensity and distress of eight symptoms for a total of 32 symptoms. MSAS-GDI measured the frequency of four psychological symptoms -- feeling sad, worrying, feeling irritable, and feeling nervous -- along with the distress of six physical symptoms: lack of appetite, lack of energy, pain, feeling drowsy, constipation, and dry mouth. MSAS-PHYS measured the frequency, intensity, and distress of 12 physical symptoms: lack of appetite, lack of energy, pain, feeling drowsy, constipation, dry mouth, nausea, vomiting, change in taste, weight loss, feeling bloated, and dizziness. MSAS-PSYCH measured the frequency, intensity, and distress of feeling sad, worrying, feeling irritable, feeling nervous, difficulty sleeping, and difficulty concentrating.

In addition, as part of the MSAS, participants added symptoms experienced that are not listed in the scale. The MSAS has high internal consistency in the psychological symptom subscale and most frequently occurring physical symptoms with Cronbach alphas of 0.83 to 0.88. However, less frequently occurring physical symptoms had moderate internal consistency with a Cronbach alpha of 0.58 (Portenoy, et al., 1994) in the cancer population. Table 4 lists the 32 symptoms included in the MSAS.

The MSAS-GDI is a combination of the frequency of four symptoms and distress of six symptoms within the MSAS. The MSAS-GDI is the average of the frequency scores for the four psychological symptoms of feeling sad, worrying, feeling irritable, and feeling nervous; and the six physical symptom distress scores for lack of appetite, lack of energy, pain, feeling drowsy, constipation, and dry mouth (Portenoy, et al., 1994). The MSAS-GDI was found to have moderate to high criterion validity with two subscales of the Revised Rand Mental Health Inventory (RAND); showing a moderately strong inverse relationship with the RAND well-being subscale score (-0.66), and a strong positive correlation with the RAND distress subscale score (0.79). The MSAS-GDI also had a high negative correlation with a quality of life scale, the Functional Living Index-Cancer Scale (-.078), and showed a moderate negative correlation with the Karnofsky Performance Status Scale (-0.60), a scale measuring physical performance as measured by health professional ratings. The MSAS has not been used in the study of NAFLD.

Table 4

Symptoms Listed in the Memorial Assessment Symptom Scale

Difficulty concentrating	Constipation	Sweats
Pain	Difficulty sleeping	Worrying
Lack of energy	Feeling bloated	Changes in skin
Cough	Problems with urination	Itching
Feeling nervous	Vomiting	Lack of appetite
Dry mouth	Shortness of breath	Dizziness
Nausea	Diarrhea	Difficulty swallowing
Feeling drowsy	Feeling sad	Feeling irritable
Mouth sores	Hair loss	"I don't look like myself"
Changes in the way food tastes	Numbness/tingling in hands/feet	Problems with sexual interest or activity
Weight loss	Swelling of arms or legs	

The MSAS has high criterion validity when correlated with the quality of life instruments such as the Functional Living Index-Cancer (-0.78), and moderate correlation with functional status such as the Karnofsky Performance Status Scale (-0.58). The MSAS had moderate correlation with the RAND well-being subscale score (0.60), and the RAND distress subscale score [0.65] (Portenoy, et al., 1994).

HRQOL instruments. The outcome, HRQOL, was measured by The Center for Disease Control and Prevention Healthy Days (four items) tool. The Healthy Days tool measures the patient's perception of his or her general health, physical and mental health and how these perceptions affect usual activities of daily living (Centers for Disease Control and Prevention, 2000). The Healthy Days Measure consists of four questions: mental unhealthy days over 30 days, physical unhealthy days over 30 days, general health using a Likert scale, and activity limitation over 30 days. The HRQOL Healthy Days score is calculated using the sum of the mental health and physical health *unhealthy* days and subtracted from 30 days to create a healthy day score.

Healthy days psychometrics. The test-retest reliability of the Healthy Days four-item tool is moderate to excellent (Andresen, Catlin, Wyrwich, & Jackson-Thompson, 2003). The Healthy Days tool demonstrated moderate correlations with the physical (r = .78, p < .0001) and mental health domains (r = .71, p < .0001) of the Short Form Health Survey [SF-36] (Mielenz, et al., 2006). The Healthy Days tool demonstrated strong convergent validity between the SF-36 and the physical (Cronbach alpha of 0.84) and mental (Cronbach alpha of 0.91) components of the two tools (Mielenz, et al., 2006). The four-item Healthy Days tool is able to predict mortality and hospitalization at 1 month and 12 months (Dominick, Ahern, Gold, & Heller, 2002). Table 5 provides a summary of the key study variables and corresponding instruments. (See Appendix A for a copy of the combined Michigan State University study questionnaire containing the data collection instruments: demographic data, the CCI, the MSAS, and the four-item Healthy Days measure).

Table 5

Summary of Variables and Instrumentation Used to Measure Variables

Variable	Instrument used to measure
Demographic characteristic: • Age • Sex • Race/ethnicity • Employment • Education level • Access to health care	• MSU study questionnaire and • Medical record audit. • Access to health care will be assessed by capturing the participant's zip code.
Disease characteristic: • Gene variant *PNPLA3* (rs738409) • Stage of NAFLD (if available) • ETOH intake • Medication use • AST elevation now or in past • ALT elevation now or in past • Waist/hip circumference	• Saliva sample as DNA source • Taqman® quantitative PCR (Applied Biosystems)– for downstream genotyping • Medical record audit • Waist/hip circumference – manually measured by PI or

Table 5 (cont'd)

Summary of Variables and Instrumentation Used to Measure Variables

Variable	Instrument used to measure
• BMI • Comorbid conditions (DM/insulin resistance, CVD, obesity, metabolic syndrome, thyroid disease)	• Medical assistant in office using tape measure • Height and weight measured in office by medical assistant during office visit. Will be used to calculate BMI. • Charlson Comorbidity Index • Patient questionnaire and medical record audit
Individual characteristic: • Health knowledge of NAFLD	• Patient questionnaire and medical record audit
Symptoms: • Presence, frequency, intensity, and distress of 32 symptoms	Memorial Symptom Assessment Scale
Health-related quality of life outcome: • General health • Mental health • Physical health • Activity limitation	Healthy Days Measure [Healthy Days = 30 days – (mentally +physically healthy days)].

Note. MSU = Michigan State University; NAFLD = nonalcoholic fatty liver disease; AST = aspartate aminotransferase; ALT = alanine aminotransferase; BMI = body mass index; DM = diabetes mellitus; CVD = cardiovascular disease; PI=primary investigator; PCR = polymerase chain reaction

***Moderators and Mediators of Symptoms and HRQOL*.**

Moderators. Moderators influence the effect of other variables to alter an outcome (Baron & Kenny, 1986; Kenny, 2008). Moderators have an interaction affect and can influence the "direction and/or strength of the relationship between two variables to influence the outcome (Baron & Kenny, 1986). For example, there is a dose effect of one or two copies of the *PNPLA3* gene, (rs738409)-G allele in persons with NAFLD (Valenti, et al., 2010). Persons with NAFLD who have one copy of the

PNPLA3 gene, (rs738409)-G allele are more likely to progress to cirrhosis than those with no copies of the *PNPLA3* gene, (rs738409)-G allele. In turn, persons with NAFLD who have two copies of the *PNPLA3* (rs738409)-G allele are more likely to progress to cirrhosis than persons with one copy of the *PNPLA3* gene, (rs738409)-G allele (Valenti, et al., 2010). It is hypothesized that the presence of one or more copies of the *PNPLA3* gene, (rs738409)-G allele (variable) that influences disease progression of NAFLD (variable) results in decreased HRQOL (outcome). Therefore, one or two copies of the *PNPLA3* gene, (rs738409)-G allele were considered to be a moderator in this study.

Mediators. Mediators are variables that intervene to influence the outcome (Kenny, 2008). The hypothesized mediator in this study was the prior knowledge of NAFLD. If a person is aware of NAFLD, he or she may be more cognizant of the potential symptoms. Patients perceive symptoms through cognitive processing (Leventhal & Johnson, 1983). Thus, if one is aware of the potential disease process, one may be more perceptive of vague symptoms.

Procedures

Recruitment procedures. Potential participants were recruited during their clinic appointments at the offices of Grand River Surgery and Gastroenterology Associates by the intake medical assistant who reviews the client list for those previously diagnosed with NAFLD by the providers, as evidenced by the imaging results of the liver. Once a potential participant was identified, the intake medical assistant provided the patient with a brochure describing the study. If the patient was interested in participating, the medical assistant notified the primary investigator who was in the

office. The primary investigator explained the study. The participant was asked to read and sign a consent form.

Data collection procedures. Once the patient had consented to participate in the study, the participant was given a saliva-sample collection kit and instructions; a packet of questionnaires by the primary investigator, which included a demographic data sheet; the CCI; the MSAS; and the four-item Healthy Days tool. This group of questionnaires took approximately 20-25 min to complete. Often, the primary investigator read the questionnaire to the participant while the participant was providing the saliva sample to optimize the participant's time. Once completed, the questionnaire was reviewed for completeness by the primary investigator. If the questionnaire was complete, the primary investigator asked the participant if he had missed those questions and would like to complete them. Originally, the primary investigator planned to ask the participants to complete the forms while waiting for their appointments. However, the procedure changed to accommodate the flow of patients through the clinic, as the participants might not have been waiting long enough to complete the forms or they might have been identified as they were being seen by the provider. Instead, the participants completed the forms after their appointments. Once completed, the questionnaire was stored in a sealed envelope coded with the participant's preprinted identification sticker. The primary investigator collected the envelope and stored it in a briefcase locked in a secured room in her home.

Genetic saliva sample collection. Participants were asked if they had anything to eat or drink 30 min prior to providing the saliva sample. If the participant had water to drink or was chewing gum, the sample was still obtained, but noted on the chart audit

form. The participants were asked to provide approximately 2 ml of saliva for the genetic analysis by spewing saliva into a premarked Oragene®-DNA kit (DNA Genotek, Ottawa Canada) while in the presence of the primary investigator. The primary investigator assessed the amount of saliva to ensure 2 ml of saliva (rather than saliva bubbles) reached the fill line in the Oragene-DNA self-collection kit. Once the saliva was obtained and mixed according to manufacturer's directions, the sample was placed in a biohazard bag for transportation to Dr. Debra Schutte's laboratory at a later date. Dr. Debra Schutte is the primary investigator's dissertation chairperson at Michigan State University. She provided oversight during the dissertation process, was the sponsor of the National Research Service Award grant funding from the National Institute of Nursing Research, National Institute of Health for this study, and is the co-primary investigator of this study.

The primary investigator ensured that the coded participant number on the saliva collection tube matched the coded participant number on the questionnaires. The collection of the saliva usually took 2-5 min, although some took up to 15 min.

Saliva samples were stored at room temperature in a plastic container and transported to Michigan State University by the primary investigator weekly or biweekly. The primary investigator delivered the samples to Dr. Schutte's laboratory, where they were batched at room temperature for processing and analysis.

Anthropometric measurement data collection. The primary investigator obtained anthropometric measurements after the consent was signed. Height, weight, blood pressure, and heart rate were obtained by the medical assistant during the intake process for the participant's provider appointment. For standardization of

measurement, the primary investigator compared weights between weight scales using a 20-pound, circular barbell weight. Both scales at each site measured 20 pounds. Blood pressure techniques were not observable, as the participants' blood pressures were obtained prior to signing both the patient authorization for disclosure of health information for research and consent to participate in the study.

Waist and hip circumference. The primary investigator obtained waist and hip circumference measurements were obtained. Waist circumference was obtained by locating the top of the iliac crest, placing a cloth measuring tape on top of each iliac crest, and measuring comfortably around the waist. Note that the tape measure was placed around the waist, rather than under a pendulous abdomen.

Hip circumference was obtained around the fullest part of the hips, usually at the level of the greater trochanter, using a cloth measuring tape. The primary investigator obtained all but two measurements, which were obtained by a qualified registered nurse who was trained by the primary investigator).

All participants were fully clothed, but lifted bulky sweaters or removed coats for waist and hip circumference measurements. The participants removed wallets or cell phones from their pockets to facilitate accurate hip circumference measurements.

Height and weight for calculation of BMI. As part of the regular intake procedure, the intake medical assistant obtained height measurements with the participant's bare or stocking feet against the wall, body erect, and head level and looking forward (Jarvis, 2004). A measurement bar was adjusted on the top of the head to obtain the height (Jarvis, 2004). Weight was obtained using the respective recruitment site's standing digital or balance scale, and by measuring to the nearest tenth of a pound. As

mentioned previously, scales were assessed for consistent readings between sites using a weight standard for comparison.

Molecular Genetic Assays

Sample processing methods. Saliva samples from the participants were transported from Grand Rapids to Michigan State University in a cooler or plastic, handled bin, batched at room temperature, and processed using the Oragene-DNA kit reagents and protocols (DNA Genotek, Ontario, Canada). Once in Dr. Schutte's lab, the Oragene-DNA protocol for purifying the DNA was followed with minor adaptations from Dr. Schutte (Levy, Clore, & Stevens, 2004).

The Oragene-DNA/saliva sample was inverted several times to ensure that viscous saliva samples were thoroughly mixed with the Oragene-DNA solution. After mixing, the sample was incubated at 50°C in a water incubator for a minimum of 8 hr. Five hundred microliters (500 µL) of the Oragene-DNA/saliva sample were transferred into a 1.5 ml microcentrifuge tube and 20 µL, that is 1/25th of the volume of the Oragene-DNA purifier (OG_L2P), was added to the microcentrifuge tube. The microcentrifuge tube was mixed by vortexing for 3-5 s, incubated on ice for 10 min, and centrifuged at room temperature for 15 min at 13,000 rpm. The clear supernatant was transferred by pipette into a new microcentrifuge tube and the pellet containing impurities was discarded.

Finally, 5 µL of glycogen was added to each tube. The glycogen made the forming purified DNA pellet more visible. To the 500 µL of supernatant, 500 µL of 95-100% ethanol was added to remove additional impurities, mixed by inversion 10 times, and centrifuged at room temperature for 3 min at 13,000 rpm.

After centrifuging, the supernatant was removed from the tube by pipette and discarded. The remaining DNA pellet was washed by adding 70% ethanol to the DNA pellet in the microcentrifuge tube which then stood at room temperature for 1 min. The ethanol solution was carefully poured off or pipetted out of the microcentrifuge tube, so as not to disturb the purified DNA pellet, and then discarded. If the DNA detached from the wall of the tube, the sample was centrifuged again for 5 min at 13,000 rpm. One sample required additional centrifuging as a result of DNA detachment.

Next, 70 µL of DNA buffer (DNA hydration solution) was added to dissolve the DNA pellet. This solution was mixed via vortex for 5 s or more. Finally, the DNA microcentrifuge tube was incubated for 1 hr in a 50°C water bath and removed briefly for vortexing every 15 min.

Additional incubation at room temperature for 1-2 days was also conducted to ensure adequate hydration of the DNA. The fully hydrated DNA was stored in TE buffer (10 mM Tris-HCl, 1mM EDTA, with a pH of 8.0) in 1 µL aliquots at -20°C in Dr. Schutte's frost-free freezer in her laboratory.

Quantification of DNA. DNA was quantified using the NanoDrop ND 1000 spectophotometer (Thermo Fisher Scientific Inc. Wilmington, Delaware). Absorbency rates between 50 and 300 nm for a 1-2 µL DNA sample were expected. The Oragene quantification of DNA procedure for spectrophotometry was used. This method involved diluting a 10 µL aliquot of purified DNA with 90 µL of TE (1:10 dilution), mixing the DNA and the DNA hydration solution (TE) by pipetting up and down and waiting for bubbles to clear. TE was also added to a blank cell to be used as a control. For the

spectrophotometry reading, the NanoDrop ND1000 spectrophotometer was calibrated using 2 µL of double-distilled water.

Genotyping methods. The Taqman® quantitative Polymerase Chain Reaction [PCR] (Applied Biosystems) platform was used for allele discrimination in the *PNPLA3* gene, (rs738409) variant. The Taqman assays used allele-specific PCR amplification to detect SNPs in candidate genes. The Taqman system used two short, invariant primers to amplify the target DNA, which was then interrogated with two allele-specific probes. These allele-specific probes consist of a fluorescent tag at one end, the specific nucleotide sequence difference midprobe, and a quenching dye at the other end. When the allele-specific probe matched the polymorphic sequence, the probe bound tightly to the DNA, the quencher was cleaved by the 5' exonuclease activity of Taq polymerase, and the reporter subsequently fluoresced. One reporter was released in homozygous samples; both reporters were released in heterozygous samples. The resulting gradient of fluorescence was read by an automatic sequence-detection system (Applied Biosystems) in the Michigan State University genomics core facility.

Several strategies were used to establish the reliability of the genotype data. Six duplicate samples were analyzed in the PCR stage of the analysis. Samples were chosen from the GG and GC genotypes. In addition, the Hardy-Weinberg equilibrium equation was used to calculate genotype frequencies from the participants' saliva samples, to assess whether the observed genotypes in this study were consistent with those expected by Hardy-Weinberg Equilibrium. Table 5 provides a summary of the study's instrumentation.

Data-Collection Training and Ongoing Quality Monitoring

Prior to the initiation of the study, clinic employees involved in the study participated in an inservice. The inservice provided protocols for obtaining accurate waist and hip circumference measurements and height and weight for BMI calculation. It also instructed on correct blood pressure techniques and the completion of questionnaires. However, after discussion with these employees, it was determined that the primary investigator would obtain hip and waist measurements after consent was obtained. In addition, brief obesity-sensitivity training was provided by the primary investigator as all patients with NAFLD are (or have been) obese or overweight. Clinic employees involved in the study and the registered nurse who assisted the primary investigator during the her absence completed the online human subject protection tutorial provided by Michigan State University prior to the initiation of the study.

Quality monitoring. Data quality was monitored throughout the project. To protect the patients' confidentiality, preprinted stickers with coded identification numbers were applied to each patient questionnaire, the saliva sample tubes within the Oragene DNA saliva kits, and the consent forms. A master list of all participants' names and the corresponding coded identification numbers were kept in a locked cabinet in Dr. Schutte's Laboratory. In addition, raw data, including signed consent forms, were secured in a locked file cabinet drawer in Dr. Schutte's laboratory and separated from the coded data after participants who had surgery were recontacted for postsurgery symptom information. Only the primary investigator and Dr. Debra Schutte had access to this file.

Data management. All data were coded and entered into a Microsoft Excel spreadsheet. The database was stored on the primary investigator's laptop computer and password protected. Raw data were secured in a locked filing cabinet in Dr. Schutte's office once the data were entered.

Data analysis plan for specific aims

General data analysis strategies. Data analysis was conducted by the primary investigator using PASW 17 in consultation with and under the supervision of Dr. Alex von Eye and Dr. Debra Schutte. Descriptive statistics were used to check assumptions for statistical tests and to provide a sample description.

Stratification of participants into NAFLD risk group by PNPLA3 genotype. Participants' data were stratified by risk of NAFLD progression using the gene variant *PNPLA3* (rs738409). The group at higher risk for progression was defined as the presence of one or two copies of the gene variant *PNPLA3* gene, (rs738409)-G allele. The group at lower risk of progression was defined as the absence of the *PNPLA3* gene, (rs738409)-G allele. Once stratification of data from persons with confirmed NAFLD was achieved, the analysis of the data for the specific aims was conducted.

Aim 1. To identify the presence of symptoms in persons with NAFLD at higher risk of disease progression based on the presence of one or two copies of *PNPLA3* gene, (rs738409)-G allele variant compared to those at lower risk of disease progression, that is no copies of the *PNPLA3* gene, (rs738409)-G allele.

Each of the 32 symptoms (the dependent variable) listed in the MSAS was grouped into dichotomous variables (present or absent). The *PNPLA3 gene,* (rs438409) variant was grouped into dichotomous variables as well (present or absent).

Odds ratios and Fisher's exact tests were used to determine the likelihood of the presence of each symptom in those with the presence of one or two copies of the t*PNPLA3* gene, (rs738409)-G allele (independent variable) compared to the absence of the *PNPLA3* gene, (rs738409)-G allele.

Aim 2. To compare the extent to which the frequency, intensity, and distress of symptoms in persons with NAFLD differ between those at higher risk of disease progression based on the presence of one or two copies of *PNPLA3* gene, (rs738409)-G allele versus those at lower risk of NAFLD progression, that is no copies of the *PNPLA3* gene, (rs738409)-G allele.

Scores of the Likert scales of frequency, intensity, and distress of each symptom present were summed and averaged according to the subscales of the TMSAS score, the MSAS-GDI, the MSAS-PHYS, and the MSAS-PSYCH. These scores were compared within groups of those at risk of disease progression by the presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele (independent variable) compared to the absence of the gene variant *PNPLA3* gene, (rs738409)-G allele using linear regression. Comorbid conditions were controlled for by using the CCI score as a variable in the linear regression equation. Predictors in the linear regression model also included demographic characteristics of age, sex, race/ethnicity binned as "white/nonwhite," education level (high school or less vs. some college education or more), unemployed/retired/disabled versus employed, and access to health care as determined by zip code. Disease characteristics in the linear regression model included no copies versus one or two copies of the *PNPLA3* gene, (rs738409)-G allele, BMI, waist/hip circumference, metabolic syndrome (CCI without the diagnosis of NAFLD as

previously noted), AST elevation now or in the past, ALT elevation now or in the past, and number of medications (15 maximum medications) taken. Individual characteristics of prior knowledge of NAFLD (yes/no) were also included in the linear regression model.

Aim 3. To determine the difference in HRQOL in persons at higher risk of NAFLD progression based upon the presence of one or two copies of *PNPLA3* gene, (rs738409)-G allele versus those at lower risk of disease progression, that is no copies of the *PNPLA3* gene, (rs738409)-G allele.

The Healthy Days score and domain scores of the Healthy Days tool of general health, activity limitation, mental health, and physical health were used to compare differences in HRQOL in those at risk of disease progression by the presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele (independent variable) compared to the absence of the *PNPLA3* gene, (rs738409)-G allele. Linear regression modeling was used to evaluate the Healthy Days score with the potential predictors presented in Aim 2 equation. Obesity measured by BMI was included as an additional predictor as it is not included in the CCI. These potential predictor variables were added to the model to evaluate the variance influencing HRQOL as the dependent variable. The modeling equation in Figure 6 depicts the outcome variable (y) of HRQOL with predictors of age, sex, race, comorbid conditions, and obesity.

HRQOL (outcome or “y”)i = b0 + b1(age)i + b2(sex)i + b3(race/ethnicity)i + b4(Charlson Comorbidity index)i + b5(obesity) + εi

Figure 6. Example modeling equation.

Aim 4. To describe the relationship between symptom distress and HRQOL in persons at higher risk of NAFLD progression based on the presence of one or two copies of *PNPLA3* gene, (rs738409)-G allele versus persons at lower risk of NAFLD progression, that is no copies of the *PNPLA3* gene, (rs738409)-G allele.

Symptom distress was summed and averaged for each symptom and was compared against the HRQOL (i.e., Healthy Days) score binned into groupings of (a) fewer than 23 Healthy Days per month and (b) 24 and greater Healthy Days per month in those at risk of disease progression by the presence of one or two copies of the gene variant *PNPLA3* gene, (rs738409)-G allele (independent variable) compared to the absence of the gene variant *PNPLA3* gene, (rs738409)-G allele.

Protection of human subjects

Human subjects' involvement and characteristics. Participants in this study were men and women with diagnosed NAFLD. Application to the Biomedical Institutional Review Board was sought and obtained prior to the initiation of this study.

Sources of material. Anthropometric measures were obtained from the participants using equipment normally found in gastroenterology offices, such as scales and blood pressure machines. Cloth tape measures were purchased by the primary investigator. DNA for genotyping was obtained from saliva samples collected from each participant. Demographic, symptom assessment, and HRQOL questionnaires was completed by each participant. The primary investigator obtained the subjects' consent, screened their cognitive function (through general conversation), and enrolled subjects in the study. Medical record reviews were conducted for accurate description of the stage of NAFLD, if available. Information regarding medication use was also obtained

from the medical record. Data were collected for research purposes only. Questionnaires and saliva samples were number-coded to protect the identities of the participants. A master list of names and coded identifiers was created and stored in the Dr. Debra Schutte's office in a locked file once the participant had been contacted for postsurgical symptoms. Only the primary investigator and the dissertation chair have access to the file.

Potential risks. Potential risks for participants involved in this study were minimal. This study was a cross-sectional study of persons with NAFLD. Potential psychological risks in this study were as follows:

1. Participants had anthropometric measurements taken of their heights, weights, waist circumferences, and hip circumferences. NAFLD is strongly associated with obesity, and there is a societal stigma with obesity. Thus persons with NAFLD may feel psychologically uncomfortable having these measurements taken. Every measure was taken to protect the participant's privacy and dignity. For instance, anthropometric measures took place in a private exam room and those involved in the study avoided commenting negatively about weight or anthropometric measurements. No participants verbalized psychological concerns of having measurements obtained.
2. In addition, participation in this study may increase awareness of NAFLD and potential long-term ramifications of the disease, triggering the participants to conduct additional reading on NAFLD outside of the study. As a result, psychological stress may occur. Education and basic counseling about NAFLD

were available from the primary investigator, although no participants expressed concern.

3. Finally, participants may be concerned about participating in genetic research in relationship to insurability or in terms of concerns regarding the heritability of NAFLD. No genotype information was entered into the medical record or shared with the participant, the health-care provider, or the insurance company. The participants were informed that the genetic analyses conducted in this study are not predictive or diagnostic.

The physical risks of the study were associated with the collection of saliva samples. Saliva samples were obtained from participants by asking each participant to spew into a small specimen-collection container. No physical harm, such as infection, was anticipated. Participants may feel awkward spewing into a specimen collection container. Every measure was taken to protect the participant's privacy and dignity, such as obtaining saliva samples in the privacy of an exam room. One patient declined participating in the study after consenting as he believed that he would become nauseated by providing a saliva sample.

Adequacy of protection against risks.

Recruitment and informed consent. Potential eligible participants with NAFLD or at risk of NAFLD were identified by the medical assistant or the provider at each recruitment site. The medical assistant provided information about the project. If the potential participant was interested in more information, the medical assistant asked the potential participant to sign the *patient authorization for disclosure of health information for research* form and advised the primary investigator that the potential participant was

interested. The primary investigator would then approach the potential participant for consent at the recruitment site. The consent listed a brief description of NAFLD, the rationale of determining a symptoms experience and the level of HRQOL in persons with NAFLD who have one or two copies of the *PNPLA3* gene, (rs738409)-G allele versus those who have none. Risks and benefits to society were also presented in the consent.

If the participant consented to enroll into the study, the primary investigator asked the participant to complete the questionnaires. The completion of the questionnaires took place after the participant had seen the provider. Anthropometric measurements and saliva samples were obtained in the exam room after seeing the provider. Missing data regarding history, diagnosis, and medications were retrieved by the primary investigator from the medical record, if needed.

Protection against risk. Physical risks to the participant related to saliva sample collection were not anticipated. However, strategies for the protection of participants were planned. Counseling would be provided by the primary investigator and her dissertation chair, if necessary, for psychological distress as a result of concerns about the heritability of NAFLD. However, this counseling was not needed. All data were coded to protect the participants' identities. Only the primary investigator and her major professor had access to the master list after the data were coded. The paper copy of the master list of names and assigned codes was stored in a locked drawer in the primary investigator's office. The computer on which the data are stored is password protected.

The data collection process was also designed to protect against risk. Identification numbers were placed on questionnaires, saliva collection containers, and anthropometric measurement forms, all of which were stored in a 6 x 8 in. (16.24 x 20.32 cm) biohazard bag. In collecting data from each participant, the primary investigator removed the questionnaires and saliva collection container from the bag. She then obtained the saliva sample and anthropometric measures and placed the completed anthropometric measurement form and saliva sample into the biohazard bag for storage. The biohazard bag with saliva sample was stored in a designated container. DNA vials in the lab were also labeled with the participant's identification number.

The participants were informed that they could withdraw from participating at any time. After the completion of the study, the DNA samples were stored for future study if the participant so desired. All participants provided consent to store DNA samples for future study Otherwise, DNA samples would have been destroyed according to the policies of Michigan State University and blood-borne pathogen policies of the Centers for Disease Control and Prevention and OISHA.

Potential benefits to human subjects. There was no direct benefit of this research to the participants; however, there is potential benefit for society at large. Results from this study will provide a description of symptoms and HRQOL in those with NAFLD who may be at risk of disease progression. Better understanding of symptoms and HRQOL may lead to early intervention of all stages of NAFLD in the future, resulting in a decrease in disease burden for society.

Inclusion of women, minorities, and children

Recruitment of women. Both genders and all ethnicity groups that met the inclusion/exclusion criteria were eligible for recruitment into this study. According to 2008 census data from Kent county in the state of Michigan where the recruitment sites were located, 50.5% of the population was of female gender (Ip, Farrell, Hall, Robertson, & Leclercq, 2004). The primary investigator monitored the recruitment of women as the study proceeded. If similar numbers of women and men were not found, the primary investigator planned to meet with the staff at the recruitment sites to develop another approach for recruitment of women. As noted in the literature, the incidence of NAFLD in women and men is similar. Thus, I anticipated having a fairly similar distribution of men and women in this study. However, I recruited many more women than men.

***Recruitment of minorities*.** All patients presenting to the recruitment sites and meeting the inclusion/exclusion criteria were approached for inclusion into the study, regardless of race or ethnicity. Flyers written in English were available at the site. Flyers were not available in other languages for this small study, as there was no funding for an interpreter. The primary investigator will also be rotated to each site to assist with recruitment, especially of minorities and women. The primary investigator planned that if the recruitment of minorities was lower than projected from census data, then the primary investigator would meet with the site and possibly former Hispanic clinic contacts in the community for additional recruitment advice. Recruitment of minorities was not lower than the census data projections.

Census data from Kent county in the state of Michigan noted that approximately 22% of the population is of a race or ethnicity other than Caucasian or white (Ip, et al., 2004). Based on this data, I anticipated that the ethnic/racial percentages of our sample population would reflect those of Kent county, MI. Furthermore, this study was a small study. In the event that our sample population did not reflect that of Kent county, strategies would be developed to recruit minorities, especially those at higher risk of NAFLD, such as the Hispanic population, in subsequent studies. If this study of symptoms and HRQOL using the *PNPLA3* gene, (rs738409)-G allele to stratify the population was successful, future plans would be to heavily recruit those of Hispanic ethnicity from Grand Rapids-area Hispanic clinics and to approach the neighboring American Indian Nation or American Indian community groups for potential collaboration in the study of NAFLD using *PNPLA3* gene, (rs738409)-G allele for stratification purposes. Table 6 provides a projection of anticipated enrollment of participants by racial and ethnic categories.

Inclusion of children. Although NAFLD afflicts adolescents and adults, this small study focused on adult men and women. Currently, there are not enough children with diagnosed NAFLD to recruit for this study. In addition, including children in this study introduces complexities in anthropometric measurements such as BMI calculations, adolescent self-body-image challenges, and parental consent. Therefore, children were not included in this study.

Table 6

Targeted/Planned Enrollment by Ethnic/Racial Categories and Sex.

Targeted/Planned Enrollment (*N* = 59)			
Ethnic category	Sex		
	Female	Male	Total
Hispanic or Latino	3	3	6
Non-Hispanic or Latino	27	26	53
Ethnic category: total of all subjects	30	29	59
Racial categories			
American Indian or Alaskan Native	1	1	2
Asian	1	1	2
Native Hawaiian or Other Pacific Islander	0	0	0
African American	3	3	6
White	25	24	49
Racial categories: total of all subjects	30	29	59

Facilities and resources

Facilities. All DNA purification and PCR analyses were conducted in Dr. Schutte's genetic laboratory in the College of Nursing at Michigan State University. Supplies for the genetic analyses were provided by Dr. Schutte.

Resources. The endpoint genotype reads were completed at the genomics technology support facility at Michigan State University. The Sequence Detection System (SDS software files were returned to the lab for genotype data management and analysis. The College of Nursing research center at Michigan State University provided computers, printer access, and telephones for all doctoral students. All computers had PASW 17 and Endnote software installed for the students to use for data analysis. The primary investigator used PASW 17 and Endnote on her laptop

computer. A desk and lab space was also available for the primary investigator's use in Dr. Schutte's lab.

Summary

The design and methods of this study were presented in Chapter 4. The purpose of this research was to determine a symptoms experience and the level of HRQOL in persons with NAFLD at higher risk of disease progression as evidenced by the presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele.

CHAPTER 5: RESULTS

The purpose of this cross-sectional study was to determine the symptoms experience and level of HRQOL in persons with NAFLD. The sample was stratified by the risk for disease progression as determined by the absence versus presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele. The goals of the study were to determine whether persons with NAFLD have symptoms; how the frequency, intensity, and distress of the symptoms and HRQOL differ between the two groups; and finally, how symptom distress influences HRQOL.

In this chapter, descriptive statistics -- organized by the antecedents of demographic, disease, and individual characteristics from the symptoms experience model -- will be used to define the sample. Descriptive statistics of the sample will include participants remaining in the study and data from participants who were ineligible. Descriptive statistics of each of the variables used in this study will also be presented. In addition, the relationships between the variables and demographic variables, the disease characteristic, such as the presence of the *PNPLA3* gene, (rs738409)-G allele; along with the relationship between important comorbid conditions, such as obesity, will be presented. A thorough description of symptoms reported by participants will be examined through comparison with demographic, disease, and individual characteristics, as well as the defining attributes of the symptoms' frequency, intensity, and distress. Descriptive statistics of the outcome of HRQOL will be provided. Finally, the results for each aim and hypothesis will be addressed.

Descriptive Statistics of Sample

A total of 54 participants were recruited for this study. Thirty-seven (68.5%) participants were from Grand River Surgery and 15 (27.8%) participants were from Gastroenterology Associates of Western Michigan, both of which are located in Wyoming, MI. Two (3.7%) participants were recruited by word-of-mouth from neither office.

Sixty-seven percent (*n* = 36) of the participants were being seen by the provider for routine appointments, such as follow-up for care of NAFLD or standard Lap-Band® follow-up appointments. However, 33% (*n* = 17) of participants were being seen for presurgical or postsurgical visits. Nineteen percent (*n* = 10) of the participants were being seen for pre or postoperative cholecystectomies. Other surgeries included mass excisions, Lap-Band insertion, gynecological operations, and herniorrhaphy. The surgical participants in this study completed a second symptom survey and HRQOL questionnaire 6 weeks to 5 months postsurgery in order to avoid skewing data with gallbladder inflammation symptoms or other pre or postsurgical symptoms.

Ineligible participants. Twelve participants (22%) were determined to be ineligible for the study upon medical record review and were not included in the analysis (See Table 7). Of the twelve participants, eight (66.7%) were removed from this analysis for ineligibility due to a diagnosis of hepatitis B or hepatitis C after the chart audit, a history of alcohol abuse, or incomplete data (e.g., missing an imaging confirmation of NAFLD or postsurgical symptom survey). When contacted by the primary investigator for postsurgical symptoms, one participant requested to be removed from the study because of a death in the family. Three participants decided

not to have a cholecystectomy, but each had an acute process (biliary dyskinesia), and they were also removed from the study.

Table 7

Ineligibility Criteria- Frequencies of 12 Participants Removed from Analysis

Ineligibility Criteria	*n*	%
Hepatitis B	3	25.0
Hepatitis C	2	16.7
Alcohol abuse	2	16.7
Biliary dyskinesia (acute process)	3	25.0
Incomplete data/requested	2	16.7
Total	12	100

Demographics of data from 12 participants removed from the analysis. Sex was evenly distributed between males (*n* = 6) and females (*n* = 6) of the 12 removed from the analysis. The majority of the 12 participants were recruited from Grand River Surgery. Ninety-two percent (*n* = 11) of these 12 participants were white and 8.3% (*n* = 1) were Black. Ages in this subset spanned 45 years ranging from 39 years to 84 years. The mean age of this group was 55.92 years (*SD* 13.97) with a median of 53.50 years. Forty-two percent were unemployed or retired. Seven (58.3%) of the participants had a high school education or less and five (41.7%) had some college level classes or a bachelor's or a master's degree.

Genotype. Of the 12 participants removed from the study, 11 had genotyping conducted prior to removal. Of the 11, 54.5% had no copies of the *PNPLA3* gene, (rs738409)-G allele (genotype CC).

Symptoms. Thirty-two symptoms from the MSAS were measured, in addition to symptoms of fatigue and ache or discomfort in the right lower rib or below the rib area. Ten of the 12 participants whose data were removed from this analysis had complete symptom data. These 10 participants experienced a minimum of five symptoms and a maximum of 34 symptoms with a mean of 18.50 symptoms. Table 8 provides comparisons between the 12 participants removed from analysis versus the 42 participants remaining in the analysis. Age and the prevalence of metabolic syndrome were the only significant differences between the two groups as noted in Table 9.

Table 8

Differences Between Participants Remaining in Analysis vs. Removed

Variable	Participants		
	42 remaining *n* (%)	12 removed *n* (%)	*p* value
Age in years Mean (*SD*), Minimum-Maximum	45.67 (10.42) (24-69)	55.92 (SD 13.97) (39-84)	*.008**
Sex Male Female	12 (28.6) 30 (71.4)	6 (50.0) 6 (50.0)	*.184*

Table 8 (cont'd)

Differences Between Participants Remaining in Analysis vs. Removed

Variable	Participants		
	42 remaining *n* (%)	12 removed *n* (%)	*p* value
Race/ethnicity			
White	36 (85.7)	11 (91.7)	*1.000*
Hispanic	2 (4.8)	0 (0.0)	*1.000*
African American/Black	1 (2.4)	1 (8.3)	*.398*
Multiracial	3 (7.1)	0 (0.0)	*1.000*
Education level			
High school or less	23 (54.8)	7 (58.3)	
Some college or more	19 (45.2)	5 (41.7)	*1.000*
Employment			
Unemployed/retired/disabled	18 (42.9)	5 (41.7)	*.513*
Employed	24 (57.1)	7 (58.3)	
Recruitment site			
Gastroenterology Associates/home	12 (28.6)	4 (33.3)	*1.000*
Grand River Surgery	29 (69.0)	8 (66.7)	
Access to healthcare			
City or suburb with ≥2 hospitals	18 (42.9)	5 (41.6)	*1.000*
Outlying areas	24 (57.1)	7 (58.4)	
PNPLA3 gene, (rs738409)-G allele			
CC	18 (42.8)	6 (50.0)	*.518*
CG	17 (40.5)	2 (16.7)	*.290*
GG	7 (16.7)	3 (25.0)	*.416*
BMI			
Mean (*SD*)	39.68 (10.41)	37.36 (13.85)	*.531*
Waist/hip circumference ratio			
Mean (*SD*)	.932 (.075)	.945 (.087)	.596
Metabolic syndrome	14 (33.3)	10 (83.3)	.003*

Table 8 (cont'd)

Differences Between Participants Remaining in Analysis vs. Removed

Variable	Participants		
	42 remaining *n* (%)	12 removed *n* (%)	*p* value
Comorbid conditions			
Mean (*SD*)	2.09 (1.41)	3.25 (2.09)	.030*
AST elevation now or past	15 (36.6)	2 (20.0)	.463
ALT elevation now or past	24 (58.5)	3 (30.0)	.160
Medications			
Mean (*SD*)	6.40 (3.72)	8.83 (4.59)	.064
Prior health knowledge of NAFLD?			
Yes	28 (66.7)	6 (50.0)	.326
No	14 (33.3)	6 (50.0)	
Symptoms	12.02 (8.82)	18.5 (9.58)	.217
Mean (*SD*)			
Unhealthy Days	15.67 (11.53)	15.81 (11.03)	.969
Mean (*SD*)			

Note. SD = standard deviation; BMI = body mass index; AST = aspartate aminotransferase; ALT = alanine aminotransferase; NAFLD = nonalcoholic fatty liver disease; CC = no copies of the *PNPLA3* gene, (rs738409)-G allele; CG = one copy of the *PNPLA3* gene, (rs738409)-G allele; GG = two copies of the *PNPLA3* gene, (rs738409)-G allele.
*$p < .05$

Demographic Characteristics of Sample

Age and sex. Forty-two participants, meeting inclusion criteria and providing complete data, were included in these analyses. Of these 42 participants, the majority (*n* = 30, 71.4%) were female. Participants ranged in age from 24 to 69 years, with a mean age of 45.67 years and standard deviation of 10.424.

Race/ethnicity. Nearly 86% (*n* = 36) of the participants were white non-Hispanic. More than 2% (*n* = 1) of the participants were African American and nearly 5% (*n* = 2) were Hispanic. However, 7% (*n* = 3) of the participants stated that they also had some multiracial background, such as Indian-African American-Hispanic.

Educational level and employment. The majority of participants had a high school education or more. Seven percent of participants had less than a high-school education. Forty-five percent of participants had some level of college education (see Table 8). Most of the participants were unemployed, retired, or disabled.

. **Access to health care.** Zip codes were used to estimate miles traveled to access health care, that is, accessibility to a large city with two or more hospitals. The Zip Codes were compared against 49503 and 49509 (zip codes for Grand Rapids area hospitals, as well as the office locations that patients were recruited), in addition to a Lansing zip code of 48912. Zip codes were binned by 15 miles (24.14 km) or less for city or suburb with more than one hospital (Grand Rapids or Lansing) and 16 - 40 miles (25.7 - 64.3 km) for outlying areas. A calculator on www.zipcodes.com was used to calculate mileage from the center of the zip code using to Lansing hospitals as well as Grand Rapids. Both were calculated because some participants sought health care in Lansing or lived closer to Lansing but traveled to Grand Rapids for health care (Datasheer, 2003-2011). Fifty-seven percent (*n* = 24) of participants lived in rural communities or villages. Nearly 43% (*n* = 18) of participants lived within 15 miles of a major city that had two or more hospitals, such as the Grand Rapids/Wyoming area.

Disease Characteristics

Imaging/liver biopsy. All participants had a diagnosis of NAFLD determined via ultrasound (*n*=32, 76.2%), CT scan (*n*=19, 45.2%), MRI (*n*=1, 2.4%) or a combination of imaging techniques. Thirty-three percent of participants (*n* = 14) also had a liver biopsy. In addition, one participant had a diagnosis of cryptogenic cirrhosis due to NASH, apparently diagnosed from a liver biopsy several years ago; however, the liver biopsy results were not available for this study. Table 9 provides liver biopsy results.

Table 9

Liver Biopsy Results and Frequencies of 14 Participants

Biopsy results	Frequency *n* (%)
NASH without fibrosis	4 (28.0)
NASH with stage 0-1 fibrosis	5 (35.7)
NASH with stage 3-4 fibrosis	5 (35.7)
Totals	14 (99.4)

BMI. The majority of participants were overweight or obese as classified by the World Health Organization. The BMI range was 24-80 kg/m^2. Only 2.3% (*n* = 1) of participants had a normal BMI, 14.3% (*n* = 6) were classified as overweight and 83.3% (*n* = 35) were obese. Of the participants who were categorized as obese, 21.4% (*n* = 9) had a BMI of 30-34.9 kg/m^2, 16.7% (*n* = 7) had a BMI of 35-39.9 kg/m^2 and 45.2 % (*n* = 19) had a BMI equal to or greater than 40 kg/m^2.

Waist/hip circumference ratio. Waist/hip circumference ratios ranged from .75 to 1.09 with a mean of .9318 (*SD* .07539). Waist/hip circumference ratios of 1.01 and greater for men and 0.86 and greater for women suggest high risk for obesity-related diseases, such as diabetes, heart disease, and hypertension (BMI Calculator, nd; Centers for Disease Control and Prevention, 2010; de Koning, Merchant, Pogue, & Anand, 2007; Yusuf, et al., 2005).

Metabolic Syndrome. A diagnosis of metabolic syndrome was derived from data collected through medical record review, using the World Health Organization's definition (World Health Organization, 1999). According to the World Health Organization's definition of metabolic syndrome, the person should have three of following conditions: (a) impaired glucose regulation or diabetes, (b) insulin resistance, (c) hypertension, (d) elevated triglycerides or low HDL, (e) central obesity, or (f) microalbuminuria. Thirty-three percent (*n* = 14) of participants met these criteria for a diagnosis of metabolic syndrome. Metabolic syndrome is included in Table 10 for comparison with other comorbid conditions.

Comorbid Conditions. The CCI was used to measure comorbid conditions (Charlson, Szatrowski, Peterson, & Gold, 1994). Thirty-six percent (*n* = 15) of participants identified 0-1 comorbid conditions, 50% (*n* = 21) of participants noted that they had 2-4 comorbid conditions, and only 14% (*n* = 6) had 5-6 comorbid conditions. Comorbid condition frequencies are noted in Table 10.

Table 10

Identified Comorbid Conditions

Comorbid conditions	*n*	%
Hypertension	23	54.8
High total cholesterol	20	47.6
Diabetes mellitus	14	33.3
Metabolic syndrome[a]	14	33.3
Asthma	10	23.8
High triglycerides	7	16.7
Hypothyroidism	5	11.9
Ulcer disease	3	7.1
Any tumor	2	4.8
Lymphoma	1	2.4

Note. Participants identified up to six comorbid conditions.
[a]Determined from chart audit based upon three characteristics of the World Health Organization's definition of metabolic syndrome.

Alcohol intake. Most participants in this study (*n*= 22, 52.4%) reported that they never drink alcoholic beverages or have alcoholic beverages twice a year. Participants were asked to quantify the amount that they drank during the times noted earlier. Thirty-three percent (*n*=14) of participants reported that they don't drink alcohol at all as noted in Table 11. Seven percent (*n*=3) reported 4 or more drinks during "week-end" drinking.

Table 11

Self-Reported Alcohol Intake of Participants

	none	1 drink	2 drinks	3 drinks	4 drinks or more
Never or twice a year	13	4	3	1	1
3-6 times per year	1[a]	4	3	0	0
Once per month	0	0	4	0	1
Once per week	0	2	2	1	0
2-4 times per week	0	0	1	0	1[b]
Daily	0	0	0	0	0

[a]Participant stated used to drink 3-6 times per year prior to diagnosis of nonalcoholic fatty liver disease, but after diagnosis, none. [b]Participant stated this was "Friday and Saturday night" drinking.

Medications. The number of medications, including over-the-counter medications and herbal medications, taken by the participant were summed. The mean number of medications taken was 6.62 (*SD* 3.754) with a range of 0 to15.

AST elevation now or in the past. The most recent AST level was obtained from chart audits. Time since the AST level was obtained varied greatly between participants, from a few weeks to 3-4 years prior to enrollment into the study. To capture this fluctuation of elevation, a dichotomous variable was created, AST elevation now or in the past versus no elevation. One participant did not have AST levels available in the medical record. However, of the remaining 41 participants, 36.6% had elevated AST levels recently or in the past.

ALT elevation now or in the past. One ALT level was obtained from the chart for each participant. As with AST levels, the time since the ALT level was obtained varied greatly between participants, from a few weeks to 3-4 years prior to enrollment into the study. Likewise, a dichotomous variable was created, ALT elevation now or in the past versus no elevation. One participant had no ALT levels available. Of the remaining 41 participants, 58.5% of participants had ALT elevation recently or in the past.

Genotype. All participants provided saliva samples as a DNA source for genotyping of the *PNPLA3* gene, (rs738409) variant. The genotype CC is considered the wild type in the Caucasian population. In this study, 18 (42.9%) of the participants had no copies of the *PNPLA3* gene, (rs738409)-G allele (genotype CC), 17 (40.5%) had one copy of the *PNPLA3* gene, (rs738409)-G allele (genotype CG), and 7 (16.7%) had two copies of the *PNPLA3* gene, (rs738409)-G allele (genotype GG). Therefore, 42.9% (*n* = 18) had no copies of the *PNPLA3* gene, (rs738409)-G allele, and 57.2% (*n* = 24) had one or two copies of the *PNPLA3* gene, (rs738409)-G allele (see Table 12).

Observed genotype frequencies were compared with those expected by Hardy-Weinberg equilibrium, using chi-square analysis. Genotype frequencies were consistent with those expected. Allele frequencies were calculated using the Hardy-Weinberg equilibrium equation in which frequencies of the alleles, p + q = 1. The Hardy-Weinberg equilibrium equation states that allele and genotype frequencies remain constant over time when assumptions, such as random mating, are met (Hartl & Jones, 2009). The frequencies of the *PNPLA3* gene, (rs738409)-C alleles are .631, and the frequencies of

the *PNPLA3* gene, (rs738409)-G alleles are .369. Calculations and frequencies are provided in Table 12.

Table12

Observed Genotype and Allele Frequencies of PNPLA3 *Gene, (rs738409)-G*

Genotype	No copies vs. one or two copies of G allele	*n* (%) per genotype	C alleles	G alleles
CC	18 (42.9)	18 (42.9)	36	0
CG	24 (57.1)	17 (40.5)	17	17
GG		7 (16.7)	0	14
Total alleles			53	31
Frequencies		42 (100)	.631[a]	.369[b]

Note. CC = no copies of the *PNPLA3* gene, (rs738409)-G allele; CG = one copy of the *PNPLA3* gene, (rs738409)-G allele; GG = two copies of the *PNPLA3* gene; (rs738409)-G allele.
[a]Frequency of C allele = [(2 x 18) + 17] / 84 total alleles = .631.[b]Frequency of *PNPLA3* gene, (rs738409)-G allele = [(2 x 7) = 17] / 84 total alleles = .369.p = .631; q = .369; p + q = 1, therefore, 0.631 + 0.369 = 1.000.

Individual Characteristics.

Prior health knowledge of NAFLD. Approximately 67% (*n* = 28) of participants were told by their physician, nurse practitioner, or physician's assistant that they had NAFLD prior to the day that they were enrolled in the study. However, 33.3% (*n* = 14) of participants learned that they had NAFLD the day of their enrollment into the study.

Descriptive Statistics and Relationships Between Key Variables

Genotype versus sex and ethnicity. Participant genotypes are compared with demographic characteristics in Table 13. The demographic characteristic, age, was

binned by decade for easier comparison with copies of the *PNPLA3* gene, (rs738409)-G allele.

Table 13

Comparison of Participant Characteristics by Presence of the PNPLA3 *Gene, (rs738409)-G Allele*

Participant information	*n* (%)	No copies *n* (%)	One or two copies *n* (%)
Age in years			
Mean (*SD*)	45.67(10.424)	18(42.9)	24 (57.1)
Median in years	46.50		
Range in years	24-69		
24-30 years	4 (9.5)	2 (4.8)	2 (4.8)
31-40 years	9 (21.4)	6 (14.3)	3 (7.1)
41-50 years	16 (38.1)	6 (14.3)	10 (23.8)
51-60 years	11 (26.2)	3 (7.1)	8 (19.0)
61-69 years	2 (2.8)	1 (2.4)	1 (2.4)
Sex			
Male	12 (28.6)	4 (9.5)	8 (19.0)
Female	30 (71.4)	14 (33.3)	16 (38.1)
Ethnicity			
American Indian/Alaskan Native	0 (0.0)	0 (0)	0 (0)
Asian	0 (0.0)	0 (0)	0 (0)
African American/Black (non-Hispanic)	1 (2.4)	1 (2.4)	0 (0)
Hispanic	2 (4.8)	0 (0)	2 (4.8)
Native Hawaiian or other Pacific Islander	0 (0.0)	0 (0)	0 (0)
White (non-Hispanic)	36 (85.7)	14 (33.3)	22 (52.4)
Other race (including multiracial)	3 (7.1)	3 (7.1)	0 (0)
Recruitment location			
Gastroenterology Associates/Home[a]	12 (28.6)	5 (11.9)	8 (19.1)
Grand River Surgery	29 (69.0)	13 (31.0)	16 (38.1)

Table 13 (cont'd)

Comparison of Participant Characteristics by Presence of the PNPLA3 *Gene, (rs738409)-G Allele*

Participant information	*n* (%)	No copies *n* (%)	One or two copies *n* (%)
Education level			
Less than Grade 9	1 (2.4)	0 (0.0)	1 (2.4)
Grades 9-11[b]	2 (4.8)	0 (0)	2 (4.8)
High school graduate/GED or equivalent	20 (47.6)	11 (26.2)	9 (21.4)
Some college or associate's degree	11 (26.2)	4 (9.5)	7 (16.7)
College graduate (bachelor's degree)	5 (11.9)	2 (4.8)	3 (7.1)
Master's degree	3 (7.1)	1 (2.4)	2 (4.8)
Doctoral or post-doctoral degree	0 (0.0)	0 (0)	0 (0)
Employment			
Employed	24 (57.1)	9 (21.4)	15 (35.7)
Unemployed or retired	18 (42.9)	9 (21.4)	9 (21.4)
Access to health care[c]			
Outlying areas	24 (57.1)	11 (26.2)	13 (31.0)
City or suburb with ≥2 hospitals	18 (42.9)	7 (16.7)	11 (26.2)

Note. SD = standard deviation.
[a]One participant was recruited by word of mouth, designated here as "home." [b]Includes Grade 12, no diploma. [c]Determined by zip code.

Fisher's Exact comparisons between sex and the *PNPLA3* gene, (rs738409)-G allele portray nearly equal distribution of females between the no copies of the *PNPLA3* gene, (rs738409)-G allele group (*n* = 14, 33.3%) and the one or two copies of the *PNPLA3* gene, (rs738409)-G allele group (*n* = 16, 38.1%). However, males have twice as many participants with one or two copies of the *PNPLA3* gene, (rs738409)-G allele (*n* = 8, 19.0%) than participants with no copies of the *PNPLA3* gene, (rs738409)-G

allele (*n* = 4, 9.5%). There was no significant relationship between sex and the *PNPLA3* gene, (rs738409)-G allele (*p* = .348, Fisher's Exact Test).

Hispanics have a higher frequency of the *PNPLA3* gene, (rs738409)-G allele and a higher frequency of the risk of NAFLD (Browning, et al., 2004; Tian, Stokowski, Kershenobich, Ballinger, & Hinds, 2010). Race/ethnicity comparisons with the *PNPLA3* gene, (rs738409)-G allele groups depicted that the two (4.8%) participants of Hispanic dissent in this study had one or two copies of the *PNPLA3* gene, (rs738409)-G allele. Although there was only one Black or African American (non-Hispanic) and three multiracial participants, all had no copies of the *PNPLA3* gene, (rs738409)-G alleles. There were more white, non-Hispanic participants in the one or two copies of the *PNPLA3* gene, (rs738409)-G alleles (*n* = 22, 52.4%) group, compared to the no copies of the *PNPLA3* gene, (rs738409)-G allele group (*n* = 14, 33.3%). Dichotomous variables were created for each of the race/ethnicity categories. There was no significant relationship between the *PNPLA3* gene, (rs738409)-G allele and the following characteristics: white/nonwhite (*p* = .375, Fisher's Exact Test); multiracial/not multiracial (*p* = .071, Fisher's Exact Test); Hispanic/non-Hispanic (*p* = .498, Fisher's Exact Test); and Black/non-Black (*p* = .429, Fisher's Exact Test).

Access to health care and *PNPLA3* gene, (rs738409)-G allele. Thirty-one percent (*n* = 13) of participants living in outlying areas had one or two copies of the *PNPLA3* gene, (rs738409)-G allele compared to 26.2% (*n* = 11) of participants living in a city or suburb with two or more hospitals. Likewise, 26.2% (*n* = 11) of participants living in outlying areas had no copies of the *PNPLA3* gene, (rs738409)-G allele compared to 16.7% (*n* = 7) of participants with no copies of the *PNPLA3* gene,

(rs738409)-G allele living in the city or suburb with two or more hospitals. However, the relationship between the *PNPLA3 gene,* (rs738409)-G allele and access to healthcare as determined by zip codes was not significant (p = .757, Fisher's Exact Test).

Body Mass Index (BMI) and genotype. The presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele was associated with increased BMI (F = 4.808, $df1$ = 1, $df2$ = 40, p = .034). The percentage of participants with one or two copies of the *PNPLA3* gene, (rs738409)-G allele was higher in the Obese Class 1 (n = 7, 16.7%) than the no copies of the *PNPLA3* gene, (rs738409)-G allele (n = 2, 4.8%) group. There was also a higher percentage of participants with one or two copies of the *PNPLA*3 gene, (rs738409)-G alleles in the Obese Class 2 (n = 6, 14.3%) versus no copies of the *PNPLA3* gene, (rs738409)-G allele (n = 1, 2.4%). However, in the Obese Class 3, 28.6% (n = 12) of the participants had no copies of the *PNPLA3* gene, (rs738409)-G allele compared to 16.7% (n = 7) who had one or two copies of the *PNPLA3* gene, (rs738409)-G allele (see Table 14). BMI Obesity Class 3 frequencies are delineated in Table 15.

Waist/hip circumference ratio and genotype. Waist/hip circumference ratios were calculated for each participant. Waist/hip circumference ratios were also converted into dichotomous variables based on sex and high risk of obesity-related diseases (males with waist/hip circumference ratios greater than 1.00 and females with waist/hip circumference ratios greater than .85). Waist circumference, waist/hip circumference ratios, waist circumference, and dichotomous waist/hip circumference ratios describing high risk of obesity-related diseases based on sex were compared by *PNPLA3* gene, (rs738409) genotype. There was no significant relationship between one

or two copies of the *PNPLA3* gene, (rs738409)-G alleles and waist circumference (F = 1.852, $df1 = 1$, $df2 = 40$, $p = .181$), or high risk of obesity-related diseases based on waist/hip circumference ratios ($F = .002$, $df1 = 1$, $df2 = 40$, $p = .924$).

Table 14

Obesity Classification and Body Mass Index (BMI) Frequencies by PNPLA3 *Gene, (rs738409)-G Allele*

Obesity classification	BMI (kg/m^2)	Frequency *n* (%)	No copies *n* (%)	One or two copies *n* (%)
Underweight	<18.5	0 (0)	0 (0)	0 (0)
Normal weight	18.5-24.99	1 (2.4)	1 (2.4)	0 (0)
Overweight	25-29.99	6 (14.3)	2 (4.8)	4 (9.5)
Obese Class 1	30-34.99	9 (21.4)	2 (4.8)	7 (16.7)
Obese Class 2	35-39.99	7 (16.7)	1 (2.4)	6 (14.3)
Obese Class 3	≥ 40	19 (45.2)	12 (28.6)	7 (16.7)

Table 15

Obesity Class 3 Body Mass Index (BMI) Frequencies by Copies of the PNPLA3 *Gene, (rs738409)-G Alleles*

BMI (kg/m^2)	Frequency within all BMIs *n* (%)	Frequency of BMI subcategory *n* (%)	No copies *n* (%)	One or two copies *n* (%)
40-44.99	10 (23.8)	10 (100)	6 (60)	4 (40)
45-49.99	5 (11.9)	5 (100)	2 (40)	3 (60)
50-79.99	4 (9.5)	4 (100)	4 (100)	0 (0)

Recruitment sites. Data from participants recruited at Gastroenterology Associates and from the one participant recruited by word of mouth (home) were combined into one category, Gastroenterology Associates, to maintain anonymity, and for dichotomous comparison with participants recruited from Grand River Surgery. Fisher's exact test was used for categorical comparisons. For continuous variable comparisons with recruitment sites, independent *t*-tests were used for the analysis. Results are summarized in Table 16. Significant findings are presented in this section.

***Recruitment site and employment*.** The majority of participants (*n* = 9, 69.2%) from Gastroenterology Associates or home were more often unemployed, retired, or disabled, compared to Grand River Surgery (*n* = 9, 31%). The relationship between recruitment site and unemployment was significant (*p* = .041, Fisher's Exact Test). Those from Gastroenterology Associates or home were twice as likely to be unemployed, retired, or disabled (*OR* 2.231, 95% CI [1.162, 4.284]).

Recruitment site versus AST elevation now or in the past. Nearly 62% (*n* = 8) of participants recruited from site two had elevated AST levels either during the recruitment period or in the past, as noted in the medical record, compared to 25% (*n* = 7) of participants recruited from site one. The relationship between recruitment site and AST elevation was significant (*p* = .038, Fisher's Exact Test). Participants from site 2or home were 2.5 times more likely to have elevated AST levels (*OR* 2.462, 95% CI [1.137, 5.328]).

Recruitment site versus ALT elevation now or in the past. Nearly 85% (*n* = 11) of participants recruited from site two or home had ALT elevation either during the recruitment period or in the past, as noted in the medical record, compared to 46.4% (*n*

= 13) of participants recruited from site one. There is a significant relationship between recruitment site and ALT elevation now or in the past (p = .039, Fisher's Exact Test). Participants recruited from site two or Home were 1.8 times as likely to have ALT elevation than those recruited from site one (*OR* 1.822, 95% CI [1.150, 2.888]).

Table 16

Comparison of Participant Characteristics by Recruitment Site

Variables	Site two or home *n* = 13	Site one *n*= 29	*p* value (Fisher's Exact or t Test)
Age in years			
Mean (*SD*)	49.54 (10.16)	43.93 (10.24)	.108
Sex			
Male	3 (23.1)	9 (31.0)	.722
Female	10 (76.9)	20 (69.0)	
Race/ethnicity			
White	10 (76.9)	26 (89.7)	.353
African American/Black	0 (0.0)	1 (3.4)	1.000
Hispanic	2 (15.4)	0 (0)	.091
Multiracial	1 (7.7)	2 (6.9)	1.000
Employment			
Unemployed/retired/disabled	9 (69.2)	9 (31.0)	.041*
Employed	4(39.8)	20 (69.0)	
Education			
High school or less	10 (76.9)	13 (44.8)	.093
Some college or more	3 (23.1)	16 (55.2)	
Access to health care[a]			
Outlying area	6 (46.2)	18 (62.1)	.501
City or suburb with ≥2 hospitals	7 (53.8)	11 (37.9)	

Table 16 (cont'd)

Comparison of Participant Characteristics by Recruitment Site

Variables	Site two or home *n* = 13	Site one *n*= 29	*p* value (Fisher's Exact or t Test)
PNPLA3 gene, (rs738409)-G allele			
No copies	5 (38.5)	13 (44.8)	.748
One or two copies	8 (61.5)	16 (55.2)	
BMI			
Mean (*SD*)	40.0 (9.94)	39.54 (10.78)	.897
Waist/hip circumference ratio			
Mean (SD)	.916 (.072)	.939 (.077)	.358
High risk *n* (%)	9 (69.2)	21 (72.4)	1.000
Metabolic syndrome	5 (38.5)	9 (31.0)	.729
Comorbid conditions	2.5 (1.61)	1.8 (1.28)	.134
AST elevation now or past	8 (61.5)	7 (25.0)	.038*
ALT elevation now or past	11 (84.6)	13 (46.4)	.039*
Medications	7.69 (3.68)	6.14 (3.75)	.219
Prior health knowledge of NAFLD?	10 (76.9)	18 (62.1)	.485

Note. SD = standard deviation; BMI = body mass index; AST = aspartate aminotransferase; ALT = alanine aminotransferase; NAFLD = nonalcoholic fatty liver disease.
[a]Determined by zip code.
*$p < .05$

Descriptive Statistics of Symptoms

The MSAS was used to assess the participants for the presence of 32 symptoms, the frequency, intensity, and distress that the symptom caused the participant. As noted in chapter 2, for this study, intensity is defined as the severity of

the symptoms. The symptoms of fatigue and ache or discomfort in right lower rib or below rib area were also assessed.

Participants were asked if they experienced symptoms over the last week. If they experienced symptoms, further descriptors were asked, such as how often they experienced the symptom, how severe (intense) it usually was, and how much the symptom distressed or bothered them. Ninety-seven percent of participants reported one or more symptoms (mean 12.02, *SD* 8.817). Symptom frequencies are discussed in detail under Aim 1.

Four (9.5%) participants added additional symptoms of stomach pains or abdominal discomfort, back/renal pain, left upper shoulder/scapular pain, lower back pain radiating to right front, and leg and feet numbness. One participant mentioned having “stomach grumbling” that lead to frequent trips to the restroom during the night. This symptom occurred over many years prior to a diagnosis of NASH cirrhosis. These symptoms were not compared to genotype as one person wrote each symptom in on the questionnaire, and other participants were not given an opportunity to respond to these symptoms.

Symptoms and sex. On average, females reported more symptoms than males. Females reported a mean number of 12.50 (*SE* = 1.477) symptoms. The mean number of symptoms for males was 10.83 (*SE* = 3.097). An independent *t*-test comparing means of symptoms between males and females revealed that there was no significant difference between the mean number of symptoms between the sexes (t = -.549, df = 40, p = .586).

Table 17

Frequencies and Mean Number of Symptoms Comparison by Age Groups

Age groups	Frequencies of age *n* (%)	Mean number of symptoms	SEM	*t* test, *df* =, *p* value
30 years or less	4 (9.5)	14.75	4.66	.646, 40, .522
vs. 31 years and older	38 (90.4)	11.74	1.43	
40 years or less	13 (30.9)	12.54	2.21	.250. 40, .804
Vs. 41 years and older	29 (69.0)	11.79	1.73	
50 years or less	29 (69.0)	12.93	1.81	.996, 40, .325
vs. 51 years and older	13 (30.9)	10.00	1.71	
60 years or less	40 (95.2)	12.18	1.41	.492, 40, .625
vs. 61 years and older	2 (4.8)	9.00	6.000	

Note. SEM = standard error of the mean; df = degrees of freedom.

Symptoms and age. On average, younger participants experienced more symptoms. However, there was no statistically significant difference between the means in the age groups (see Table 17).

Symptoms and race/ethnicity. On average, persons that identified race or ethnicity as Black, Hispanic or multiracial (*n* = 6) experienced more symptoms than persons that identified their race as white (*t* = 3.716, *df* = 40, *p* = .001). Persons that identified Black, Hispanic or multiracial race reported a mean number of 22.83 (*SE* = 2.97) symptoms. In contrast, persons that identified their race as white (*n* = 36) had a mean number of 10.222 (*SE* = 1.29) symptoms.

Symptoms and recruitment site. Participants recruited from Gastroenterology Associates and home reported more symptoms (mean 14.85, *SE* = 2.53) than participants recruited from Grand River Surgery (mean 10.76, *SE* = 1.58). However, there was no statistically significant difference between the mean number of symptoms

in participants recruited from Gastroenterology Associates or home compared to participants recruited from Grand River Surgery (t = 1.405, df = 40, p = .168).

Table 18

Frequencies and Mean Number of Symptoms Comparisons by Body Mass Index (BMI) Obesity Groups

BMI groups	Frequencies of BMI	Mean number of symptoms	SEM	*t* test, *df*=, *p* value
BMI less than 30	7	11.86	3.62	-.054, df=40, .957
vs. BMI of 30 or greater	35	12.06	1.49	
BMI less than 40	23	11.04	1.89	-.789. df=40, .435
vs. BMI of 40 or greater	19	13.21	1.97	
BMI less than 50	38	11.87	1.45	-.348, df=40, .729
vs. BMI of 50 or greater	4	13.50	4.33	

Note. SEM = standard error of the mean; df= degrees of freedom.

Symptoms and BMI. There was no significant difference between BMI and the number of symptoms experienced by participants in this study (F = 1.122, df1 = 1, df2 = 40, p = .296). In addition, there was no significant difference in the mean number of symptoms comparing normal and overweight participants (BMI less than 30) to obese participants (BMI of 30 and greater), and between participants in obese classes, as noted in Table 18.

Symptoms and waist/hip circumference ratio. Males with waist/hip circumference ratios greater than 1.0 and females with waist/hip circumference ratios greater than .85 are at higher risk for obesity-related diseases, such as diabetes or heart disease. While 80% of females (n = 24) and 50% of males (n = 6) in this study were considered high risk (p = .069, Fisher's Exact Test), fewer symptoms were reported by the high risk participants (n = 30 males and females, mean 11.13, SE =

1.70) than participants who were not at high risk based on waist/hip circumference ratio by sex (*n* = 12 males and females, mean 14.25, *SE* =2.10). However, there was no significant difference between the mean number of symptoms in participants who were at high risk of obesity-related diseases compared to participants not at high risk of obesity-related diseases as determined by waist/hip circumference ratio (*t* = 1.036, *df* = 40, *p* = .306).

Symptoms, sex and comorbid conditions. On average, participants in this study had 2.5 comorbid conditions. Males had a mean of 2.08 (*SE* = .583) comorbid conditions, and females had a mean of 2.67 (*SE* = .312) comorbid conditions. There was no statistically significant difference between sex and mean comorbid conditions (*t* = -.949, *df* = 40, *p* = .348).

The mean number of symptoms increased with the number of comorbid conditions; however, there was a significant difference between mean symptoms and three or less comorbid conditions. Participants with three or more comorbid conditions reported fewer symptoms than participants with four or five comorbid conditions (*p* = .012). However, the mean number of symptoms between persons with four (*p* = .057) or five (*p* = .078) comorbid conditions approached statistical significance.

Symptoms and metabolic syndrome. Thirty-three percent (*n* = 14) of the participants had a diagnosis of metabolic syndrome. Of these, 21.4% (*n* = 3) were males and 78.6% (*n* = 11) were females (p = .719, Fisher's Exact Test). On average, participants with metabolic syndrome experienced more symptoms (mean 14.36, *SE* = 2.59) compared with participants without metabolic syndrome (mean 10.86, *SE*= 1.57).

However, there was not a statistically significant difference between the mean symptoms of the two groups (t = -1.220, df = 40, p = .230).

Symptoms and AST elevation now or in the past. Fifteen participants that had elevated AST levels now or in the past experienced a mean of 12.07 (SE = 2.23) symptoms compared to mean symptoms of 26 participants without elevated AST levels now or in the past (mean of 11.42, SE = 1.71). There was no statistically significant difference in mean symptoms between these two groups (t = -.228, df = 39, p = .821).

Symptoms and ALT elevation now or in the past. Twenty-four (57%) participants that had ALT elevation now or in the past experienced a mean of 11.63 (SE = 1.68) symptoms compared to 17 (40%) participants without ALT elevation now or in the past, with a mean of 11.71 (SE = 2.27) symptoms. There was no statistically significant difference in the mean symptoms between these two groups (t = .029, df = 39, p = .977).

Table 19

Frequencies and Mean Number of Symptoms by PNPLA3 *Gene, (rs738409)-G Genotype*

PNPLA3 (rs738409)-G allele (genotype)	Frequency *n* (%)	Mean number of symptoms	SEM	*t* test, *df*, *p* value
No copies of G alleles (CC)	18	11.667	2.24	225, *df* = 40, .823
vs. one or two copies of G alleles (not CC)	24	12.292	1.72	
1 copy of G allele (CG)	17	11.582	1.88	.261, *df* = 40, .795
vs. no copies and 2 copies of G alleles (not CG)	25	12.320	1.93	
2 copies of G allele (GG)	7	14.000	3.93	-.645, *df* = 40, .523
vs. 1 copy and no copies of G alleles (not GG)	35	11.629	1.45	

Note. SEM = standard error of the mean; *df* = degrees of freedom.

Symptoms and *PNPLA3 gene,* (rs738409)-G alleles. Participants with two copies of the *PNPLA3* gene, (rs738409)-G alleles reported more symptoms than those with one copy or no copies of the *PNPLA3* gene, (rs738409)-G alleles, but none of the mean comparisons between genotypes were statistically significant (see Table 19). Note that Figure 7 is displayed by genotype to assess dose effect of G-alleles.

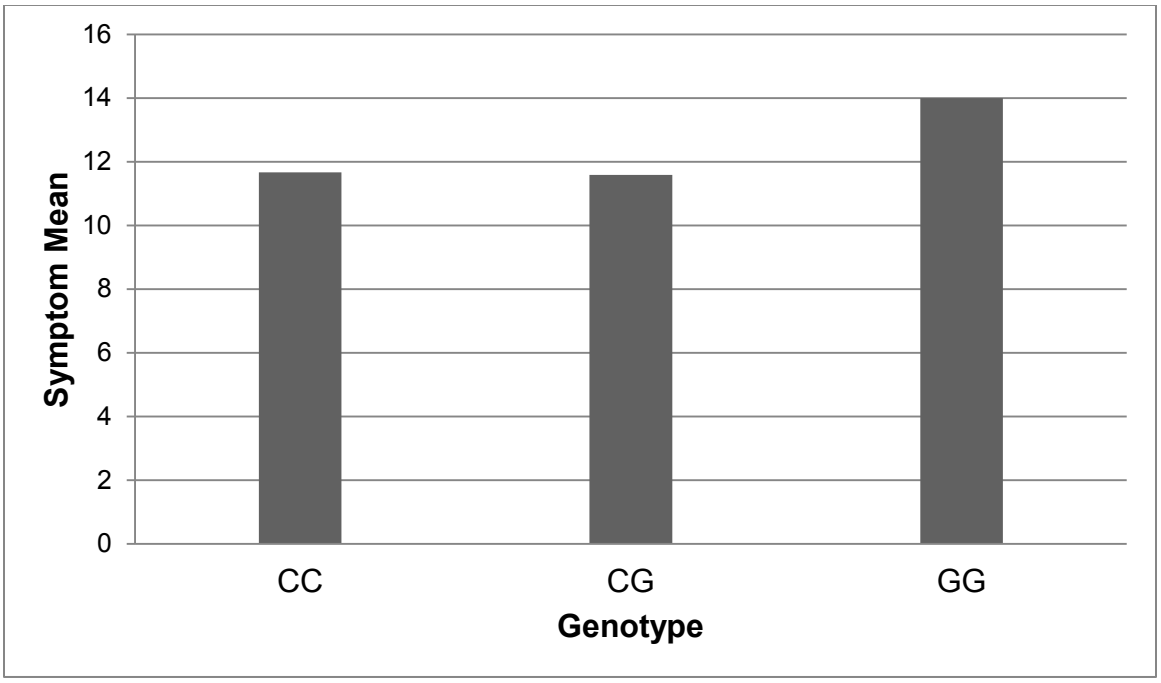

Figure 7. Comparison of mean number of symptoms by *PNPLA3* gene, (rs738409) genotype.

Symptoms and prior health knowledge of NAFLD. Participants that had health knowledge of NAFLD prior to recruitment into the study reported more symptoms (mean = 13.75, *SE* = 1.56) than participants who had learned about NAFLD from their providers on the day they were recruited into the study (mean = 8.57, *SE* = 2.45). There was no statistically significant difference between the mean of these two groups (t = -1.846, df = 40, p = .072).

In summary, nearly all participants experienced symptoms in this study. The mean number of symptoms was 12. Race/ethnicity and having three or less comorbid conditions were significantly associated with symptoms. The relationship between prior knowledge of NAFLD and number of symptoms approached statistical significance.

Outcome of HRQOL.

This section will present descriptive statistics of HRQOL. General health, physical health, mental health, activity limitation, and healthy days, as defined by the Healthy Days tool, will be presented.

General Health. Participants were asked to rate their general health. Almost 5% (*n* = 2) of participants rated their general health as excellent and 14.3% (*n* = 6) rated their general health as very good as noted in Figure 8. Forty-five percent (*n* = 19) of participants rated their general health as good. Nearly 24% (*n* = 10) rated their health as fair and almost 12% (*n* = 5) rated their health as poor. The mean general health was 3.24 (*SD* 1.008), with a range 1 (excellent) to 5 (poor).

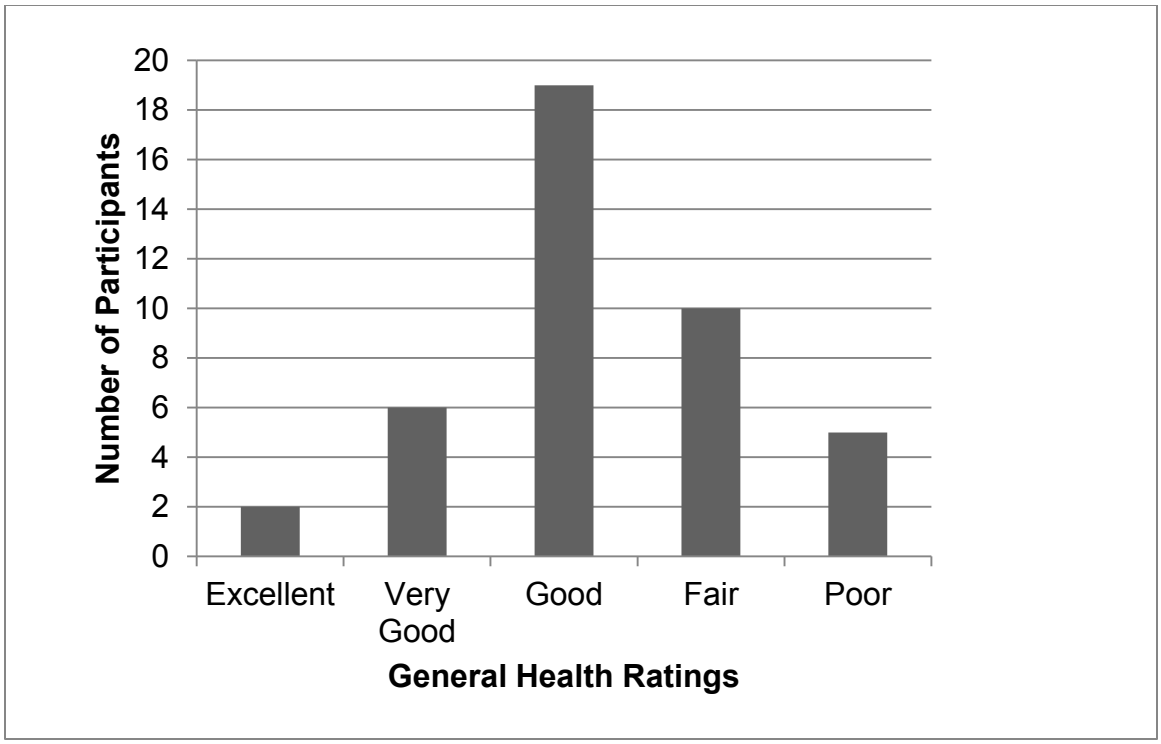

Figure 8. Frequency distribution of general health ratings.

Physical health. Physical health was assessed by asking the participants to determine how many days over the last 30 days that their physical health was not good. Physical health included physical illness and injury. Although the Healthy Days tool provides days 1-30 as choices, some participants wrote in "0" or "none" or said, "well, none, but I will answer 1 as 0 is not a choice." The mean number of physically unhealthy days reported by participants was 10.36 (*SD*10.903; Range: 0-30). Americans report an average of 24.7 healthy days or 5.3 unhealthy days per month, thus physically unhealthy days were binned similarly for comparison: 0-6 unhealthy days versus 7-30 unhealthy days (Centers for Disease Control and Prevention, 2000). Figure 9 depicts the distribution of physically unhealthy days.

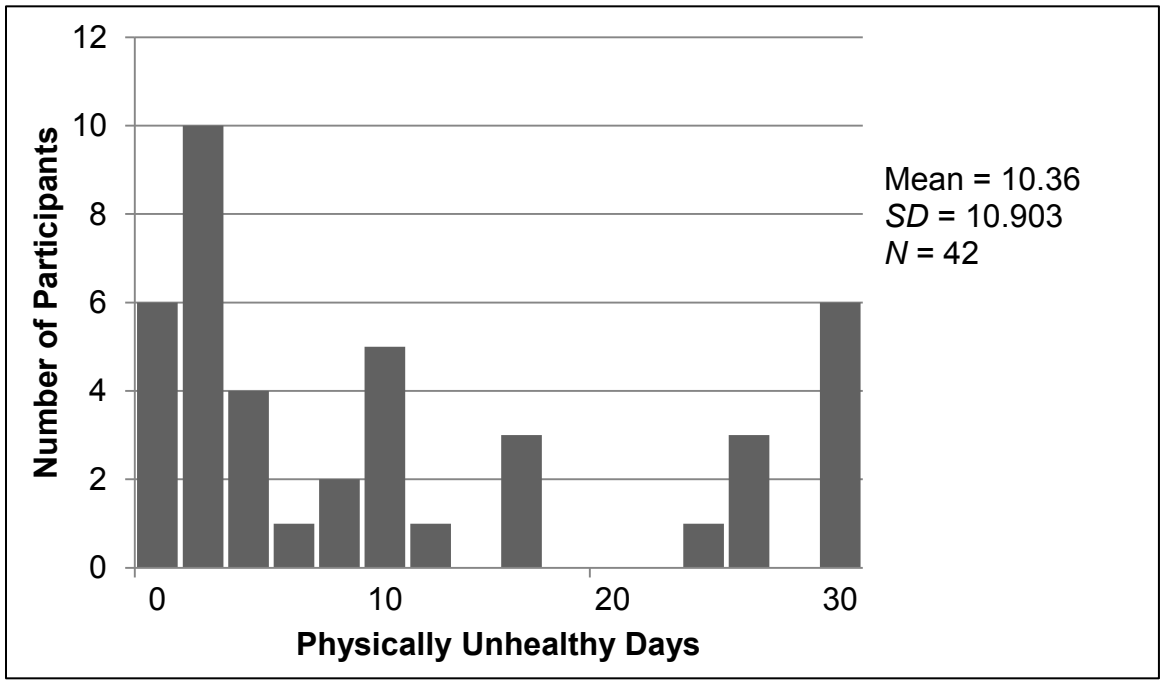

Figure 9. Physically unhealthy days.

Mental Health. Mental health was assessed by asking, "Now thinking about your mental health, which includes stress, depression, and problems with emotions, for how many days during the past 30 days was your mental health not good?" Participants responded "0" or "none" as noted earlier. Mentally unhealthy days were binned in the same manner as the physically unhealthy days: 0-6 mentally unhealthy days versus 7-30 mentally unhealthy days. The mean number of mentally unhealthy days reported by participants was 10.48 days (*SD* 10.712; Range 0-30), similar to the mean number of physically unhealthy days. The distribution of mentally unhealthy days is shown in Figure 10.

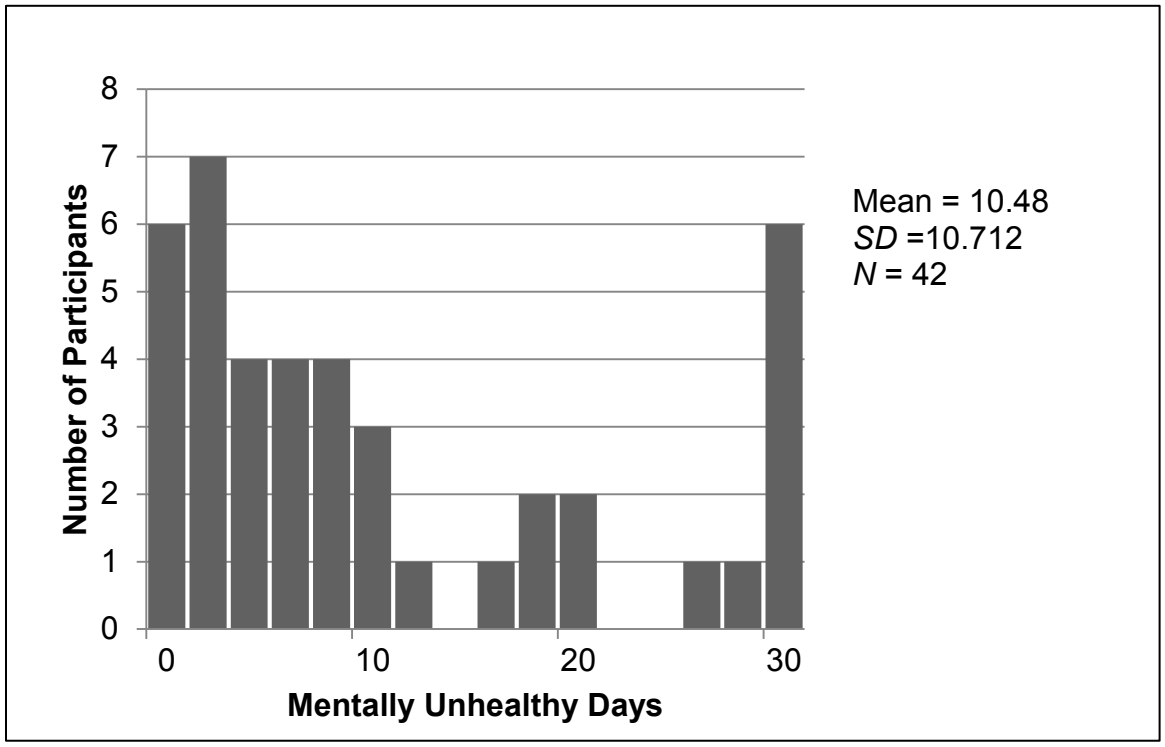

Figure 10. Mentally unhealthy days.

Activity Limitation. Activity Limitation was measured by asking, "During the past 30 days, for about how many days did poor physical or mental health keep you from doing your usual activities, such as self-care, work, or recreation?" Again, participants responded with a "0" or "none" that they wrote in because the scale begins at 1. The average number of activity-limited days was 7.81 with a range of 0 to 30. Figure 11 depicts the days that activity was limited because of physically and mentally unhealthy days.

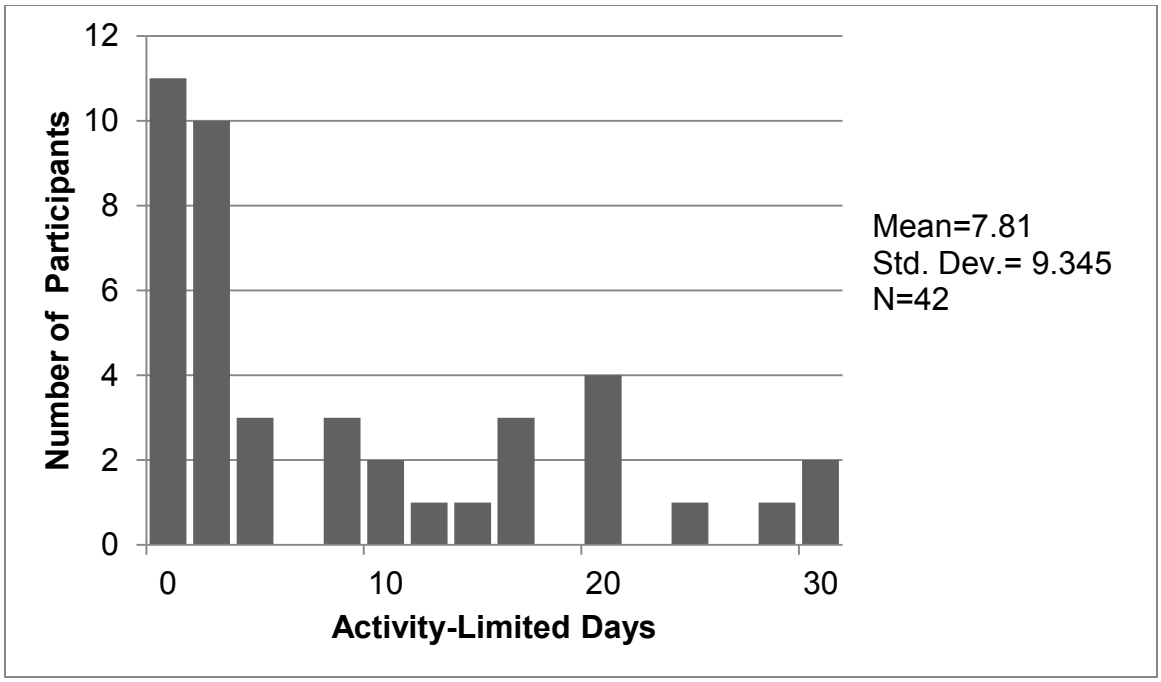

Figure 11. Activity-limited days due to physically and mentally unhealthy days.

Participants in this study reported that they experience physical or mental unhealthy days more than one-third of the month. Activity is limited one week of the month from these physically or mentally unhealthy days. Over one half of the month, participants experience unhealthy days. Table 20 provides a summary of the means, standard deviations, and ranges for general health, physical health, mental health, and activity limitation.

Table 20

Mean, Standard Deviation, and Range of Unhealthy Days: Physical, Mental, Activity-Limited, and Total Unhealthy Days

Unhealthy days	Mean	*SD*	Range
Physically unhealthy days (0-30 days/month)	10.36	(10.903)	0-30
Mentally unhealthy days (0-30 days/month)	10.48	(10.712)	0-30
Activity-limited days due to physically and mentally unhealthy days (0-30 days/month)	7.81	(9.345)	0-30
Total unhealthy days physically + mentally unhealthy days up to a maximum of 30 days/month).	15.67	(11.531)	0-30

Note. SD = standard deviation. Physical Health, Mental Health and Activity Limitation had multiple modes. The top three modes are shown.

Healthy Days. The Healthy Days tool calculated healthy days by summing the physically unhealthy days and mentally unhealthy days. If the sum exceeds 30 days, a maximum unhealthy days score is assigned as 30 total unhealthy days. Then, to determine healthy days, the unhealthy days are subtracted from 30 healthy days. For example, if a participant has 15 physically unhealthy days and 20 mentally unhealthy days, the maximum number of unhealthy days is 30 rather than 35. Thirty unhealthy days are subtracted from thirty healthy days to equal 0 healthy days per month. Likewise, if a participant has 8 physically unhealthy days and 3 mentally unhealthy days, 11 total unhealthy days would be subtracted from 30 healthy days to equal 19 healthy days.

In this study, 31% (*n* = 13) of participants reported 0 healthy days; that is, all 30 days of the month were either physically or mentally unhealthy days. Nineteen percent

(*n* = 8) of participants reported that only 6 to15 days were healthy. Nearly 17% (*n* = 7) reported healthy days between 16 and 24. Twenty-six percent (*n* = 11) of participants reported healthy days of 26 to 30. Figure 12 provides the distribution of healthy days for this study.

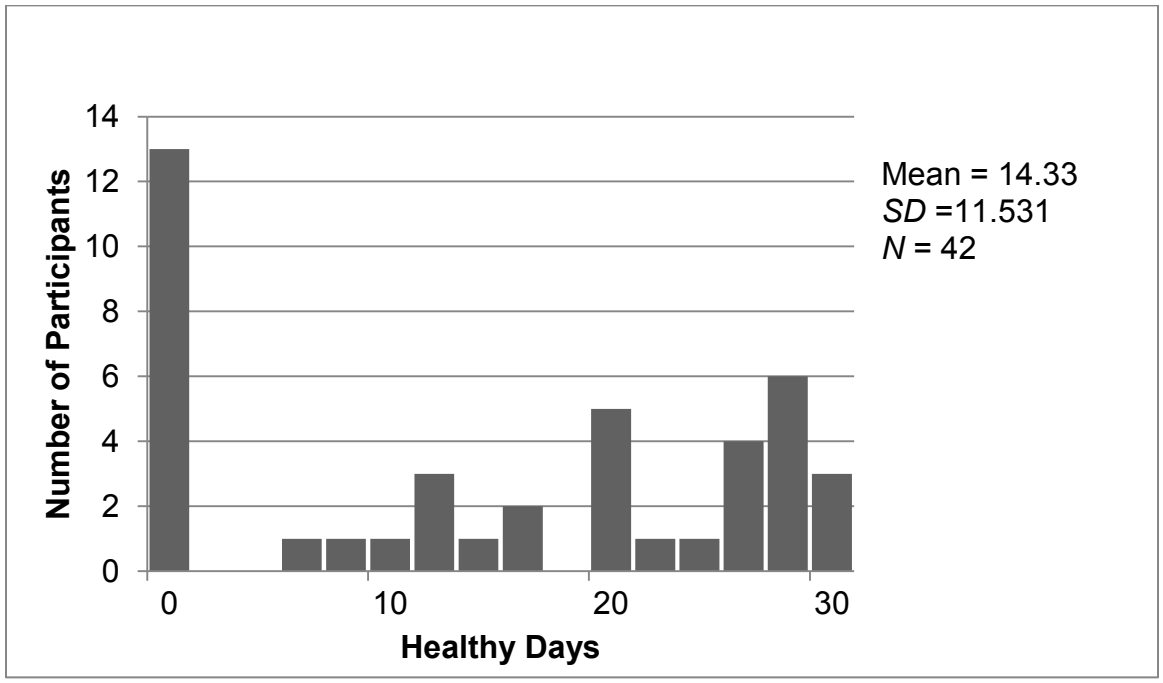

Figure 12. Healthy days.

Table 21 provides race/ethnicity by sex and healthy days. The majority of females reported 0 to23 healthy days. All participants identifying as Black, Hispanic, and multiracial reported 0 to 23 healthy days.

Table 21

Healthy Days Binned Frequencies vs. Race/Ethnicity vs. Sex

Race/ethnicity[a]	Frequency *n* (%)	Healthy days	
		0-23 healthy days *n* (%)	24-30 healthy days *n* (%)
African American/Black (non-Hispanic)	1 (2.4)	1 (2.4)	0 (0)
Male	0 (0)[b]	0 (0)[b]	0 (0)[b]
Female	1 (3.3)[b]	1 (3.3)[b]	0 (0)[b]
Hispanic	2 (4.8)	2 (4.8)	0 (0)
Male	1 (8.3)[b]	1 (8.3)[b]	0 (0)[b]
Female	1 (3.3)[b]	1 (3.3)[b]	0 (0)[b]
White (non-Hispanic)	36 (85.7)	22 (52.4)	14 (33.3)
Male	11 (91.7)[b]	5 (41.7)[b]	6 (50.0)[b]
Female	25 (83.3)[b]	17 (56.7)[b]	8 (26.7)[b]
Other race – including multiracial	3 (7.1)	3 (7.1)	0 (0)
Male	0 (0)[b]	0 (0)[b]	0 (0)[b]
Female	3 (10.0)[b]	3 (10.0)[b]	0 (0)[b]
Total	42 (100)	28 (66.7)	14 (33.3)
Male	12 (100)[b]	6 (50.0)[b]	6 (50.0)[b]
Female	30 (100)[b]	22 (73.3)[b]	8 (26.7)[b]

[a]There were no participants of American Indian/Alaskan Native, Asian, Native Hawaiian or Other Pacific Islander race/ethnicity in this study. [b]Percentages for male are within male and percentages for female are within female.

Females generally report fewer healthy days than males (Centers for Disease Control and Prevention, 2000). In this sample, 73% (*n* = 22) of females compared to 50% (*n* = 6) of males reported 0 to 23 healthy days (see Table 22).

Table 22

*Healthy Days Binned Frequencies vs. Sex (*n = *42)*

Healthy days	Frequency *n* (%)	Male *n* (%)	Female *n* (%)
0-23 healthy days	28 (66.7)	6 (14.3)	22 (52.4)
24-30 healthy days	14 (33.3)	6 (14.3)	8 (19.0)
Total	42 (100)	12 (28.6)	30 (71.4)

In summary, descriptive statistics and frequencies were presented for the variables in this study. The next section will present results organized by the Specific Aims.

Specific Aims

Aim 1. The purpose of Aim 1 was to identify the presence of symptoms in persons with NAFLD at higher risk of disease progression based on the presence of one or two copies of the *PNPLA3 gene,* (rs738409)-G allele compared to those at lower risk of disease progression, that is, no copies of the *PNPLA3* gene, (rs738409)-G allele.

Hypothesis 1. Persons at higher risk of progression of NAFLD as determined by the presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele will exhibit symptoms compared to persons at lower risk of progression of NAFLD, that is, with no copies of the PNPLA3 gene, (rs738409)-G allele.

Symptoms were elicited from participants using the MSAS (Portenoy, et al., 1994). Participants were asked if they had experienced any of the 32 symptoms in the

scale plus two additional symptoms found in the NAFLD literature: fatigue and ache or discomfort in the right lower rib or below the rib area.

Results for Hypothesis 1. Ninety-seven percent of participants *(n* = 41) experienced one or more symptoms (see Table 23). The majority of symptom frequencies were more prevalent in participants with one or two copies of the *PNPLA3* gene, (rs738409)-G allele. Symptoms of feeling sad, nausea, lack of appetite, hair loss, vomiting, and ache or discomfort in the right lower rib or below the rib area were more prevalent in participants with no copies of the *PNPLA3* gene, (rs738409)-G allele.

Many of the symptoms identified by participants with NAFLD in this study are also found in chronic liver disease patients (Younossi, et al., 1999). Fatigue and lack of energy have been noted in adults and children with NAFLD (Dan, et al., 2007; Kistler, et al., 2010). Further discussion of similarities of chronic liver disease patients and symptoms found in this study will be presented in Chapter 6.

While symptoms were found to be present in persons with NAFLD, and higher percentages of participants with one or two copies of the *PNPLA3* gene, (rs738409)-G alleles experienced certain symptoms, there was no significant relationship between the symptoms and genotype (see Table 23). Sample size in this study was small, with 42 participants, thus Fisher's Exact test was used rather than chi-square.

Although not statistically significant, trends in odds ratios suggest that participants may be more likely to experience certain symptoms if they have one or two copies of the *PNPLA3* gene,(rs738409)-G allele, as noted in Table 23. For example, participants with one or two copies of the *PNPLA3* gene, (rs738409)-G allele may be twice as likely to experience fatigue as those with no copies of the *PNPLA3* gene,

(rs738409)-G allele (*OR* 2.000, 95% CI [.570, 7.013]). Another example is that participants with one or two copies of the *PNPLA3* gene, (rs738409)-G allele may also be nearly twice as likely to experience pain compared to participants with no copies of the *PNPLA3* gene,(rs738409)-G allele (*OR* 1.943, 95% CI [.540, 6.990]). It should be noted that these confidence intervals have a wide range, thus, further study is needed with a larger sample to determine the accuracy of these odds ratios.

Table 23

Symptom Frequencies vs. PNPLA3 *Gene, (rs738409)-G Genotype Odds Ratios*

Symptom	Frequency *n* (%)	One or two copies *n* (% within symptom)	*OR*	95% CI	Fisher's Exact two-sided
Lack of energy	30 (71.4)	16 (53.3)	.571	[.141, 2.313]	.506
Pain	27 (64.3)	17 (63.0)	1.943	[.540, 6.990]	.347
Fatigue	25 (59.5)	16 (64.0)	2.000	[.570, 7.013]	.348
Feeling irritable	24 (57.2)	13 (54.2)	.752	[.217, 2.604]	.757
Worrying	22 (52.4)	13 (59.1)	1.182	[.347, 4.019]	1.000
Feeling drowsy	21 (50.0)	13 (61.9)	1.477	[.432, 5.046]	.756
Diarrhea	21 (50.0)	13 (61.9)	1.477	[.432, 5.046]	.756
Difficulty sleeping	20 (47.6)	12 (60.0)	1.250	[.367, 4.262]	.764
Feeling bloated	19 (45.2)	11 (57.9)	1.058	[.310, 3.613]	1.000
Numbness/tingling in hands/feet	18 (42.8)	10 (55.6)	.893	[.260, 3.067]	1.000
Feeling sad	17 (40.4)	8 (47.1)	.500	[.143, 1.753]	.348

Table 23 (cont'd)

Symptom Frequencies vs. PNPLA3 *Gene, (rs738409)-G Genotype Odds Ratios*

Symptom	Frequency *n* (%)	One or two copies *n* (% within symptom)	*OR*	95% CI	Fisher's Exact two-sided
Feeling nervous	16 (38.1)	9 (56.3)	.943	[.268, 3.315]	1.000
Swelling of arms or legs	16 (38.1)	10 (62.5)	1.429	[.400, 5.099]	.750
Dry mouth	15 (35.7)	8 (53.3)	.786	[.220, 2.804]	.754
Shortness of breath	15 (35.7)	10 (66.7)	1.857	[.500, 6.899]	.517
Sweats	15 (35.7)	8 (53.3)	.786	[.220, 2.804]	.754
Ache or discomfort in right lower rib (or below the rib area)	15 (35.7)	7 (46.7)	.515	[.143, 1.852]	.347
Difficulty concentrating	14 (33.3)	8 (57.1)	1.000	[.274, 3.656]	1.000
Cough	14 (33.3)	8 (57.1)	1.000	[.274, 3.656]	1.000
Constipation	13 (30.9)	8 (61.5)	1.300	[.342, 4.943]	.748
Problems w/sexual activity/interest	12 (28.6)	7 (58.3)	1.071	[.276, 4.154]	1.000
Itching	12 (28.6)	8 (66.7)	1.750	[.432, 7.084]	.506
Dizziness	12 (28.6)	7 (58.3)	1.071	[.276, 4.154]	1.000
Weight loss	12 (28.5)	8 (61.5)	1.300	[.342, 4.943]	.748
Nausea	11 (26.2)	5 (45.5)	.526	[.131, 2.112]	.483

Table 23 (cont'd)

Symptoms Frequencies vs. PNPLA3 *Gene, (rs738409)-G Genotype Odds Ratios*

Symptom	Frequency *n* (%)	One or two copies *n* (% within symptom)	*OR*	95% CI	Fisher's Exact two-sided
Problems with urination	10 (23.8)	7 (70.0)	2.059	[.450, 9.416]	.473
Change in the way food tastes	10 (23.8)	7 (70.0)	2.059	[.450, 9.416]	.473
Changes in skin	10 (23.8)	7 (70.0)	2.059	[.450, 9.416]	.473
Lack of appetite	9 (21.4)	3 (33.3)	.286	[.060, 1.355]	.139
"I don't look like myself"	8 (19.0)	6 (75.0)	2.667	[.470, 15.136]	.431
Hair loss	7 (16.6)	3 (42.9)	.500	[.097, 2.584]	.438
Vomiting	5 (11.9)	2 (40.0)	.455	[.068, 3.057]	.636
Difficulty swallowing	5 (11.9)	4 (80.0)	3.400	[.346, 33.397]	.371
Mouth sores	4 (9.5)	3 (75.0)	2.429	[.231, 25.510]	.623

Note. OR = odds ratio; CI = confidence level.

Summary of results for Aim 1, Hypothesis1. We hypothesized that persons at higher risk of progression of NAFLD as determined by the presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele would exhibit symptoms compared to persons at lower risk of progression of NAFLD, that is, with no copies of the *PNPLA3* gene, (rs738409)-G allele. While 97% (*n* = 41) of participants with NAFLD experienced one or more symptoms, none of the symptoms correlated with the presence of the

PNPLA3 gene, (rs738409)-G allele, despite a mean of 12 symptoms in persons with NAFLD. Further analysis needs to be conducted to identify a symptoms experience in the NAFLD population using a larger sample size. Aim 2 will examine the defining attributes of the symptoms.

Aim 2. In Aim 2, the goal was to compare the extent to which the frequency, intensity, and, distress of symptoms in persons with NAFLD differ between those at higher risk of disease progression based on the presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele compare to those at lower risk of disease progression, that is no copies of the *PNPLA3* gene, (rs738409)-G allele.

Hypothesis 2. It was hypothesized that persons at higher risk of disease progression would have more symptom intensity, frequency, and/or distress than those at lower risk of disease progression.

Preparation for analysis of Aim 2. *To* analyze this aim, scores of the Likert scales of frequency, intensity, and distress of each symptom in the MSAS were summed and averaged according to the subscales of the MSAS-GDI, the MSAS-PHYS, the MSAS-PSYCH and the TMSAS score. These scores were compared between participants in the group with no *PNPLA3* gene, (rs738409)-G allele and those with one or two copies of the *PNPLA3* gene, (rs738409)-G allele using linear regression.

The CCI was used to control for comorbid conditions. The presence or absence of the comorbid condition was multiplied by a factor found in the scoring directions for the CCI to create an index for each comorbid conditions (Charlson, et al., 1994). The indexes of the comorbid conditions were summed and averaged to create the CCI. Liver disease and severe liver disease were not included in the equation to avoid

multicollinearity issues, as all participants in the study had a diagnosis of NAFLD and those with hepatitis or other liver diseases were excluded from the study.

Creating categorical variables. The values of categorical variables were changed to reflect coding of 0 and 1 to avoid skewing results of the linear regression model. Variables with more than two categories were binned into dichotomous variables. Age and BMI were included in the analyses as continuous variables. Waist/hip circumference was left as a ratio for the linear regression modeling. The maximum number of medications taken by the participants was 15. The number of medications that each participant was taking was summed and averaged. Race/ethnicity was binned into white (*n* = 36, 85.7%) versus nonwhite (*n* = 6, 14.3%). The nonwhite group consisted of one African American participant, two Hispanic participants and three multiracial (Hispanic, Indian, African American) participants to create a dichotomous variable. Although Hispanics have a higher incidence of NAFLD and are more apt to carry the polymorphic *PNPLA3* gene, (rs738409)-G allele, followed by whites and Blacks/African Americans, the white population of this sample is considerably larger (Browning, et al., 2004; Tian, et al., 2010). In addition, the multiracial group included more Hispanic ethnicity, thus the groups were dichotomized accordingly for the linear regression.

MSAS. The four subscales of the MSAS were used to evaluate the defining attributes of frequency, intensity, and distress of symptoms. As noted in Chapter 2, intensity is defined as the severity of the symptoms for this study. These four subscales are TMSAS, MSAS-GDI, (MSAS-PHYS, and MSAS-PSYCH.

TMAS. The TMSAS was calculated by summing the dimensions of frequency, severity (intensity), and distress, -- or severity (intensity) and distress, depending on the symptom -- and averaging the sum of each of the 32 symptoms of the MSAS. These scores were averaged using the total number of symptoms (*n* = 32) to create the TMSAS (Chang, Hwang, Feuerman, Kasimis, & Thaler, 2000; Ingham & Portenoy, 1996; Portenoy, nd; Portenoy, et al., 1994). Table 24 displays the frequencies of the means of the symptom-defining attributes of frequency, severity, and distress.

MSAS-GDI. The MSAS-GDI measures overall distress (Portenoy, et al., 1994). It was calculated by averaging the frequency of four psychological symptoms and the distress of see physical symptoms (see Table 24).

MSAS-PHYS. The MSAS-PHYS is the average of the frequency, severity (intensity), and distress of 12 physical symptoms (Portenoy, nd; Portenoy, et al., 1994). These prominent physical symptoms are: lack of appetite, lack of energy, pain, feeling drowsy, constipation, dry mouth, nausea, vomiting, change in taste, weight loss, feeling bloated, and dizziness.

MSAS-PSYCH. The Psychological Symptom Subscale is the average of the frequency, severity (intensity), and distress of six psychological symptoms. These symptoms include: worrying, feeling sad, feeling nervous, difficulty sleeping, feeling irritable and difficulty concentrating (Portenoy, et al., 1994) .

Multiple linear regression. Multiple linear regression measures the influence of several independent variables on the dependent variable (George & Mallery, 2010). Ten subjects are recommended for each variable; however, in this study, the sample

size was relatively small (*n* = 42, 100%). Results will be presented using 15 variables as noted in the model.

Table 24

Comparison of Prevalence, Means, and Standard Deviations of Symptom Frequency, Intensity, and Distress

Symptom	Overall prevalence *n* (%)	Frequency mean (*SD*) range (1-4)	Intensity mean (*SD*) range (1-4)	Distress mean (*SD*) range (0-4)
Lack of energy	30 (71.4)	1.98 (1.423)	1.45 (1.152)	1.55 (1.452)
Pain	27 (64.3)	1.88 (1.611)	1.43 (1.213)	1.52 (1.469)
Fatigue	25 (59.5)	1.52 (1.469)	1.33 (1.300)	1.31 (1.456)
Feeling irritable	24 (57.2)	1.26 (1.326)	1.14 (1.221)	.98 (1.316)
Worrying	22 (52.4)	1.36 (1.495)	1.17 (1.324)	1.00 (1.288)
Feeling drowsy	21 (50.0)	1.17 (1.305)	.93 (1.068)	.74 (1.061)
Diarrhea	21 (50.0)	1.17 (1.342)	1.02 (1.259)	.81 (1.110)
Difficulty sleeping	20 (47.6)	1.31 (1.522)	1.05 (1.248)	1.10 (1.445)
Feeling bloated	19 (45.2)	1.10 (1.445)	.83 (1.102)	.79 (1.260)
Numbness/tingling in hands/feet	18 (42.8)	1.05 (1.431)	.74 (1.037)	.81 (1.194)
Feeling sad	17 (40.4)	.90 (1.284)	.74 (1.061)	.71 (1.215)
Feeling nervous	16 (38.1)	.83 (1.208)	.71 (1.066)	.74 (1.191)
Swelling of arms or legs	16 (38.1)	-	.81 (1.234)	.76 (1.284)
Dry mouth	15 (35.7)	.88 (1.365)	.69 (1.115)	.62 (1.188)
Shortness of breath	15 (35.7)	.79 (1.116)	.71 (1.154)	.71 (1.175)

Table 24 (cont'd)

Comparison of Prevalence, Means, and Standard Deviations of Symptom Frequency, Intensity, and Distress

Symptom	Overall prevalence *n* (%)	Frequency mean (*SD*) range (1-4)	Intensity mean (*SD*) range (1-4)	Distress mean (*SD*) range (0-4)
Sweats	15 (35.7)	.79 (1.317)	.71 (1.195)	.64 (1.226)
Ache or discomfort in right lower rib (or below the rib area)	15 (35.7)	.98 (1.423)	.86 (1.299)	.83 (1.286)
Difficulty concentrating	14 (33.3)	.76 (1.206)	.57 (.941)	.50 (.994)
Cough	14 (33.3)	.81 (1.234)	.50 (.804)	.43 (.859)
Constipation	13 (30.9)	-	.52 (.862)	.38 (.764)
Problems w/sexual activity/interest	12 (28.6)	.79 (1.389)	.62 (1.168)	.190 (1.231)
Itching	12 (28.6)	.67 (1.183)	.55 (.993)	.43 (.831)
Dizziness	12 (28.6)	.57 (.991)	.45 (.861)	.40 (.828)
Weight loss	12 (28.5)	-	.39 (.666)	.17 (.543)
Nausea	11 (26.2)	.57 (1.063)	.48 (.862)	.38 (.825)
Problems with urination	10 (23.8)	.40 (.828)	.31 (.749)	.24 (.656)
Change in the way food tastes	10 (23.8)	-	.45 (.889)	.45 (.916)
Changes in skin	10 (23.8)	-	.43 (.914)	.38 (.909)
Lack of appetite	9 (21.4)	.45 (.993)	.33 (.687)	.21 (.645)
"I don't look like myself"	8 (19.0)	-	.43 (1.016)	.38 (.987)
Hair loss	7 (16.6)	-	.43 (1.063)	.43 (1.107)

Table 24 (cont'd)

Comparison of Prevalence, Means, and Standard Deviations of Symptom Frequency, Intensity, and Distress

Symptom	Overall prevalence *n* (%)	Frequency mean (*SD*) range (1-4)	Intensity mean (*SD*) range (1-4)	Distress mean (*SD*) range (0-4)
Vomiting	5 (11.9)	.19 (.594)	.17 (.581)	.19 (.707)
Difficulty swallowing	5 (11.9)	.29 (.835)	.29 (.835)	.26 (.857)
Mouth sores	4 (9.5)	-	.19 (.707)	.14 (.683)

Note. The Memorial Symptom Assessment Scale has eight symptoms where frequency is not assessed. *SD* = standard deviation.

Correlations. Pearson correlations measure the strength of the relationship between two variables in the analysis (George & Mallery, 2010). Pearson correlations were conducted between the variables. Variables included in the correlation analysis were age in years, sex, white vs. nonwhite, education level, unemployed or retired, access to health care by zip code, presence or absence of G alleles, BMI, waist/hip circumference, metabolic syndrome, CCI without NAFLD, medications (15 maximum) patients took, AST elevation now or in the past, ALT elevation now or in the past, and prior knowledge of NAFLD. The correlation matrix is too large to reproduce for this section, but Table 25 highlights the significant relationships found between the variables.

Table 25

Summary of Pearson Correlations and Significance Between Predictor Variables

Variables	Pearson Correlation	Significance *p* value
BMI		
PNPLA3 gene, (rs738409)-G	-.328	.034
Metabolic syndrome	.548	.001
Medications		
Metabolic syndrome	.481	.001
Knowledge of NAFLD	.458	.002
Charlson Comorbidity Index	.458	.002
TMSAS	.400	.009
MSAS-GDI	.393	.010
MSAS-PHYS	.332	.032
Race/ethnicity (white/nonwhite)[a]		
TMSAS	.517	.001
MSAS-GDI	.517	.001
MSAS-PHYS	.612	.001
MSAS-PSYCH	.482	.001
Educational level		
MSAS-PSYCH	-.341	.027
Waist/hip circumference ratio		
Sex	-.531	.001
Charlson Comorbidity Index		
Access to health care	.347	.024

Note. BMI = body mass index; NAFLD = nonalcoholic fatty liver disease; TMSAS = Total Memorial Symptom Assessment Score; MSAS-GDI = Memorial Symptom Assessment Scale - Global Distress index; MSAS-PHYS = Memorial Symptoms Assessment Physical subscale; MSAS-PSYCH = Psychological subscale of the Memorial Symptom Assessment Scale.

[a]The term *white* refers to all Caucasian participants. The term *nonwhite* refers to participants of all other races, including African American, Hispanic, and multiracial participants.

A negative relationship existed between BMI and the presence/absence of *PNPLA3* gene, (rs738409)-G alleles (r = -.328, p = .034). In addition, a positive relationship was found between BMI and metabolic syndrome (r = .548, p = 0.001).

Medications had a positive relationship with metabolic syndrome (r = .481, p = .001). Medications also correlated with knowledge about NAFLD (r = .458, p = .002). Medications correlated with the CCI (r = .458, p = .002). Finally, medications also correlated with three of the symptom subscales; TMSAS (r = .400, p = .009), MSAS-GDI (r = .393; p =.010), and MSAS-PHYS (r = .332, p = .032).

Race/ethnicity was categorized into white (n = 36) versus nonwhite (n = 6). The nonwhite group consisted of Hispanic, African American, and multiracial (Hispanic, African American, and Indian) participants. Race/ethnicity had a positive relationship with TMSAS (r =.517, p <.001), MSAS-GDI (r = .517, p <.001), MSAS-PHYS (r = .612, p <.001), and MSAS-PSYCH (r = .482, p = .001). Educational level correlated with the MSAS-PSYCH score (r = -.341, p = .027). Waist/hip circumference ratio had a negative relationship with sex (r = -.531, p <.001). In this study, males were coded as 0 and females were coded as 1. CCI had a positive relationship with access to health care by zip code (r = .347, p = .024).

Linear regression was used to analyze Aim 2 to compare the extent to which frequency, intensity, and distress of the symptoms in persons with NAFLD differed between those at higher risk of disease progression based on the presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele variant compared to those at lower risk of disease progression, that is no copies of the *PNPLA3* gene, (rs738409)-G allele. Symptom subscales of TMSAS, MSAS-GDI, MSAS-PHYS, and MSAS-PSYCH correlated with one another because of multicollinearity, as several variables are represented in each of the subscales. Therefore, these subscales were not used simultaneously in the linear regression models.

Aim 2. Again, the goal was to compare the extent to which the frequency, intensity, and distress of symptoms in persons with NAFLD differ between those at higher risk of disease progression based on the presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele compared to those at lower risk of disease progression, that is no copies of the *PNPLA3* gene, (rs738409)-G allele.

Hypothesis 2. *It was hypothesized that persons at higher risk of disease progression would have more symptom intensity, frequency, and/or distress than those at lower risk of disease progression*.

Results for Aim 2, Hypothesis 2. *TMSAS.* The TMSAS was utilized as the dependent variable in the first linear regression analysis. Predictors used in the linear regression model were attempted according to the symptoms experience model. Predictors included the demographic characteristics of age, race/ethnicity, sex, education level, employment, and access to health care by zip code; the disease characteristics of presence or absence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele, medication average, BMI, metabolic syndrome, waist/hip Circumference ratio, CCI, AST elevation now or in the past, and ALT elevation now or in the past; and the individual characteristic, prior health knowledge of NAFLD.

Table 26

Predictor of Total Memorial Symptom Assessment Score (*TMSAS)*

Predictor variable (coding)	Beta (unstandardized)	*p* value
White (1) vs. nonwhite (0)[a]	-.812	.003

[a]The term *white* refers to all Caucasian participants. The term *nonwhite* refers to participants of all other races, including African American, Hispanic, and multiracial participants.

Using the enter method, white versus nonwhite was a significant predictor of the TMSAS model (F = 2.609, df1 = 15, df2 = 25, p = .016). Significant predictors of this model are presented in Table 26.

MSAS-GDI. In the next step of the analysis of Aim 2, the dependent variable of the MSAS-GDI was compared to the predictors according to the conceptual model, the symptoms experience model. Predictors included the demographic characteristics of age, race/ethnicity, sex, education level, employment, and access to health care by zip code; the disease characteristics of presence or absence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele, medication average, BMI, metabolic syndrome, waist/hip circumference ratio, CCI, AST elevation now or in the past, and ALT elevation now or in the past; and the individual characteristic, prior health knowledge of NAFLD.

Using the enter method, white versus nonwhite and unemployed were the only significant predictors of the MSAS-GDI model as noted in Table 27. The MSAS-GDI explained 66.7% of the variability of the model (F = 3.331, df1 = 15, df2 = 25, p = .004) as noted in Table 27.

Table 27

Predictors of Memorial Symptom Assessment Scale - Global Distress index (MSAS-GDI)

Predictor variable (coding)	Beta (unstandardized)	*p* value
White (1) vs. nonwhite (0)[a]	-.954	.002
Unemployed, retired, disabled (1) vs. employed (0)	.529	.036

[a]The term *white* refers to all Caucasian participants. The term *nonwhite* refers to participants of all other races, including African American, Hispanic, and multiracial participants.

MSAS-PHYS. The Memorial Symptoms Assessment Physical Subscale was analyzed as the dependent variable in the multiple linear regression analysis with predictors. Predictors included the demographic characteristics of age, race/ethnicity, sex, education level, employment, and access to health care by zip code; the disease characteristics of presence or absence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele, medication average, BMI, metabolic syndrome, waist/hip circumference ratio, CCI, AST elevation now or in the past, and ALT elevation now or in the past; and the individual characteristic, prior health knowledge of NAFLD, as noted in the symptoms experience model.

Using the enter method, significant predictors of MSAS-PHYS model were race/ethnicity dichotomous classification of white versus nonwhite and health knowledge of NAFLD prior to recruitment into the study (F = 2.726, df1 = 15, df2 = 25, p = .013), as noted in Table 28. Sixty-two percent of the variability was explained by the model.

Table 28

Predictors of Memorial Symptoms Assessment Physical Subscale (MSAS-PHYS)

Predictor variables	Beta	*p* value
White (1) vs. nonwhite (0)[a]	-.983	<.001
Health knowledge of NAFLD prior to recruitment into study (1=Yes)	.448	.036

Note. NAFLD = nonalcoholic fatty liver disease.

[a]The term *white* refers to all Caucasian participants. The term *nonwhite* refers to participants of all other races, including African American, Hispanic, and multiracial participants.

MSAS-PSYCH. Finally, the MSAS-PSYCH was analyzed against predictors using multiple linear regression. Demographic characteristics of age, race/ethnicity, sex, education level, employment, and access to health care by zip code; the disease characteristics of presence or absence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele, medication average, BMI, metabolic syndrome, waist/hip circumference ratio, CCI, AST elevation now or in the past, and ALT elevation now or in the past; and the individual characteristic, prior health knowledge of NAFLD as noted in the symptoms experience model were used in the analysis versus the MSAS-PSYCH.

Using the enter method in the linear regression model, significant predictors were the race/ethnicity category of white versus nonwhite and unemployment (F=2.944, df1=15, df2=25, *p*=.008) as noted in Table 29.

Table 29

Predictors of Psychological Subscale of the Memorial Symptom Assessment Scale (MSAS-PSYCH)

Predictor variables (coding)	Beta	*p* value
White (1) vs. nonwhite (0)[a]	-.983	.003
Unemployed, retired, disabled (1) vs. employed (0)	.945	.006

[a]The term *white* refers to all Caucasian participants. The term *nonwhite* refers to participants of all other races, including African American, Hispanic, and multiracial participants.

Summary of results of Aim 2 and Hypothesis 2. In summary, multiple linear regression models of TMSAS, MSAS-GDI, MSAS-PHYS, and MSAS-PSYCH, were analyzed with 15 predictors of the demographic characteristics of age, race/ethnicity, sex, educational level, employment, and access to healthcare by zip code; the disease

characteristics of presence or absence of one or two copies of the *PNPLA3 gene,* (rs738409)-G allele, medication average, BMI, metabolic syndrome, waist/hip circumference ratio, CCI, AST elevation now or in the past, and ALT elevation now or in the past; and the individual characteristic, prior health knowledge of NAFLD, as noted in the symptoms experience model.

The sum and average of the frequency, intensity, and distress of all 32 symptoms as measured by the TMSAS were influenced by race/ethnicity. There was no difference between participants with one or two copies of the *PNPLA3* gene*,* (rs738409)-G allele versus no copies of the *PNPLA3* gene, (rs738409)-G allele

The frequency of feeling sad, worrying, feeling irritable and feeling nervous along with the distress of lack of appetite, lack of energy, pain, feeling drowsy, constipation, and dry mouth, as measured by the MSAS-GGDI, provided a measure of overall distress. Race/ethnicity and unemployed, retired, or disability status influenced the distress of symptoms. There was no difference between participants with one or two copies of the *PNPLA3* gene, (rs738409)-G allele versus no copies of the *PNPLA3* gene, (rs738409)-G allele.

The sum and average of the frequency, intensity, and distress of 12 physical symptoms (lack of appetite, lack of energy, pain, feeling drowsy, constipation, dry mouth, nausea, vomiting, change in taste, weight loss, feeling bloated, and dizziness) was measured by the MSAS-PHYS. Race/ethnicity and prior health knowledge of NAFLD were significant predictors. There was no difference between participants with one or two copies of the *PNPLA3* gene, (rs738409)-G allele versus no copies of the *PNPLA3* gene, (rs738409)-G allele.

Finally, the MSAS-PSYCH measured the sum and average of six psychological symptoms (worrying, feeling sad, feeling nervous, difficulty sleeping, feeling irritable, and difficulty concentrating). Race/ethnicity and unemployment, retired, or disability status influenced the frequency, intensity, and distress of psychological symptoms. There was no difference between participants with one or two copies of the *PNPLA3* gene, (rs738409)-G allele versus no copies of the *PNPLA3* gene, (rs738409)-G allele. Table 30 summarizes the linear models of TMSAS, MSAS-GDI, MSAS-PHYS, and MSAS-PSYCH.

Table 30

Summary of TMSAS, MSAS-GDI, MSAS-PHYS, and MSAS-PSYCH Linear Models

MSAS subscales	Significance of model
TMSAS	(F = 2.609, df1 = 15, df2 = 25, p = .016).
MSAS- GDI	(F = 3.331, df1 = 15, df2 = 25, p = .004).
MSAS-PHYS	(F = 2.726, df1 = 15, df2 = 25, p = .013).
MSAS-PSYCH	(F = 2.944, df1 = 15, df2 = 25, p = .008).

Note. MSAS = Memorial Symptom Assessment Scale; TMSAS = Total Memorial Symptom Assessment Score; MSAS-GDI = Memorial Symptom Assessment Scale - Global Distress index; MSAS-PHYS = Memorial Symptoms Assessment Physical subscale; MSAS-PSYCH = Psychological subscale of the Memorial Symptom Assessment Scale; F = F distribution, .df = degrees of freedom;

Aim 3. The intent of the analysis in Aim 3 was to determine a difference in HRQOL in persons at higher risk of NAFLD progression based upon the presence of one or two copies of *PNPLA3* gene, (rs738409)-G allele versus those at lower risk of disease progression, that is, no copies of the *PNPLA3* gene, (rs738409)-G allele.

Hypothesis 3. It was hypothesized that persons with NAFLD at higher risk of disease progression would have poorer HRQOL than those at lower risk of disease progression.

Preparation for analysis of Aim 3. To prepare for this analysis, domain scores of the Healthy Days tools of general health, physical health, mental health and activity limitation as well as the total Healthy Days score were used to compare differences in HRQOL in those at risk of disease progression by the presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele (independent variable) compared to the absence of the *PNPLA3* gene, (rs738409)-G allele.

Results of Aim 3, Hypothesis 3.

General health and PNPLA3 *gene, (rs738409)-G allele.* Participants who described their general health as good or fair had a higher percentage of one or two copies of the *PNPLA3* gene, (rs738409)-G allele (*n* = 12, 63.2% vs. *n* = 6, 60%, respectively), compared to no copies of the *PNPLA3* gene, (rs738409)-G allele (38.6% and 40%, respectively) as noted in Table 31.

Table 31
Frequencies and Fisher's Exact Comparisons Between General Health and Copies of the PNPLA3 *Gene, (rs738409)-G Allele*

General health	Frequencies *n* (%)	Frequency within subcategory *n* (%)	No copies *n* (%)[a]	One or two copies *n* (%)[a]
Excellent	2 (4.8)	2 (100)	1 (50.0)	1 (50.0)
Very good	6 (14.3)	6 (100)	3 (50.0)	3 (50.0)
Good	19 (45.2)	19 (100)	7 (38.6)	12 (63.2)
Fair	10 (23.8)	10 (100)	4 (40.0)	6 (60.0)
Poor	5 (11.9)	5 (100)	3 (60.0)	2 (40.0)

[a]Frequency of copies of *PNPLA3* gene, (rs738409)-G alleles within general health subcategory.

General health was binned into two groups: excellent, good, and good, (1) versus fair and poor (0) health. Multiple linear regression techniques were used to evaluate the general health ratings with the comorbid conditions of obesity, diabetes mellitus/insulin resistance, metabolic syndrome, age, sex and race. Comorbidities were controlled for by using the CCI score as a predictor in the multiple linear regression equation. BMI was included as an additional predictor for obesity, as it is not included in the CCI. These potential predictor variables were added to the model to evaluate the variance influencing general health as the dependent variable. Variables in this model included demographic characteristics of age, race/ethnicity, sex, education level, employment, and access to healthcare by zip code; the disease characteristics of presence or absence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele, medication average, BMI, metabolic syndrome, waist/hip circumference ratio, CCI, AST elevation

now or in the past and ALT elevation now or in the past; and the individual characteristic, prior health knowledge of NAFLD

Using the enter method in a linear regression, the race/ethnicity category of white versus nonwhite was a significant predictor (Beta .527, p = .012) as was education level (Beta .296, p = .035). Sixty-three percent of the variability was explained by the model (F =3.012, $df1$ = 15, $df2$ = 25, p = .007).

Physical Health. Fifty percent (n = 21) of participants reported 7 to 30 physically unhealthy days over a 30 day period in the study. More than 21% (n = 9) of participants with no copies of the *PNPLA3* gene, (rs738409)-G alleles responded that they had 0 to 6 physically unhealthy days per month, and 21.4% (n = 9) of participants responded that they experienced 7 to30 physically unhealthy days per month. Likewise, 28.6% (n = 12) of participants with one or two copies of the *PNPLA3* gene, (rs738409)-G allele responded that they had 0 to 6 physically unhealthy days per month and 28.6% (n = 12) of participants with one or two copies of the *PNPLA3* gene, (rs738409)-G allele responded that they experienced 7 to 30 physically unhealthy days per month (see Table 32). The relationship between physical health and one or two copies of the *PNPLA3* gene, (rs738409)-G alleles was not significant (p = 1.000, Fisher's Exact Test).

Table 32

Comparison of Physical Unhealthy Days Frequencies for Total Sample by Copies of the PNPLA3 *Gene, (rs738409)-G Genotype*

Physical health	Frequency *n* (%)	No copies *n* (%)	One or two copies *n* (%)
0-6 unhealthy days	21 (50)	9 (21.4)	12 (28.6)
7-30 unhealthy days	21 (50)	9 (21.4)	12 (28.6)
Total	42 (100)	18 (42.8)	24 (57.2)

Physically unhealthy days was used as a dependent continuous variable. Physical health, in terms of physically unhealthy days, was compared with the demographic characteristics of age, race/ethnicity, sex, educational level, employment, and access to healthcare by zip code; the disease characteristics of presence or absence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele, medication average, BMI, metabolic syndrome, waist/hip circumference ratio, CCI, AST elevation now or in the past, and ALT elevation now or in the past; and the individual characteristic, prior health knowledge of NAFLD.

Using the enter method in the multiple linear regression analysis, there were significant predictors for the model (F = 2.235, $df1$= 15, $df2$= 25, p = .036). Unemployment (i.e., unemployed, retired, or disabled coded as 1) was the only significant predictor (Beta 8.771, p = .046). Therefore, participants who were unemployed, retired, or disabled had more physically unhealthy days than participants who were employed.

Mental Health. The frequencies of mentally unhealthy days were identical to the physically unhealthy days in that 21.4% (*n* = 9) of participants with no copies of the *PNPLA3* gene, (rs738409)-G allele responded that they experienced 0 to 6 mentally unhealthy days, as did 21.4% (*n* = 9) of participants with one or two copies of the *PNPLA3 gene,* (rs738409)-G allele responded that they experience 0 to 6 mentally unhealthy days. Likewise, 28.6% (*n* = 12) of participants with no copies of the *PNPLA3* gene, (rs738409)-G allele responded that they experienced 0 to 6 mentally unhealthy days and 28.6% (*n* = 12) of participants with one or two copies of the *PNPLA3* gene, (rs738409)-G allele responded that they experienced 7 to 30 mentally unhealthy days (see Table 33). The relationship between mentally unhealthy days and one or two copies of the *PNPLA3* gene, (rs738409)-G allele was not significant (*r* =.000, *p* = 1.000).

Table 33

Comparison of Mentally Unhealthy Days Frequencies and Copies of the PNPLA3 *Gene, (rs738409)-G Allele*

Mental health	Frequency *n* (%)	No copies of *n* (%)	One or two copies *n* (%)
0-6 unhealthy days	21 (50)	9 (21.4)	12 (28.6)
7-30 unhealthy days	21 (50)	9 (21.4)	12 (28.6)
Total	42 (100)	18 (42.8)	24 (57.2)

Mentally unhealthy days was used as a continuous dependent variable in a multiple linear regression model using the demographic characteristics of age, race/ethnicity, sex, educational level, employment, and access to health care by zip code; the disease characteristics of presence or absence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele, medication average, BMI, metabolic syndrome,

waist/hip circumference ratio, CCI, AST elevation now or in the past, and ALT elevation now or in the past; and the individual characteristic, prior health knowledge of NAFLD. Significant predictors in the model were BMI (Beta .697, $p < .001$) and white vs. nonwhite (Beta -18.204, $p < .001$). AST elevation now or in the past was nearly significant (Beta 5.546, $p = .054$). The predictors explained 79% of the variability in the model ($F = 6.425$, $df = 15$, $df = 25$, $p < .001$).

Activity limitation due to physically and mentally unhealthy days. As noted in Table 34, 57% ($n = 24$) of participants responded that physically and mentally unhealthy days limited their activity 0 to 6 days per month. Of these participants, nearly 24% ($n = 10$) had no copies of the *PNPLA3* gene, (rs738409)-G allele and 33.3% ($n = 14$) had one or two copies of the *PNPLA3* gene, (rs738409)-G allele. Almost 43% of participants responded that they had 7 to 30 activity-limited days. Of these participants, 19% ($n = 8$) had no copies of the *PNPLA3* gene, (rs738409)-G allele, and 23.8% ($n = 10$) had one or two copies of the *PNPLA3* gene, (rs738409)-G allele. The relationship between activity limitation and copies of the *PNPLA3* gene, (rs738409)-G allele was not significant ($\chi^2 = .032$, $p = .857$).

Table 34

Comparison of Activity Limitation due to Unhealthy Days and Copies of the PNPLA3 *Gene, (rs738409)-G Alleles*

Activity limitation	Frequency *n* (%)	No copies *n* (%)	One or two copies *n* (%)
0-6 limited days	24 (57.1)	10 (23.8)	14 (33.3)
7-30 limited days	18 (42.9)	8 (19.0)	10 (23.8)
Total	42 (100)	18 (42.9)	24 (57.1)

Activity limitation, the dependent variable was analyzed in a multiple linear regression model as a continuous variable. Predictors used in the model included demographic characteristics of age, race/ethnicity, sex, educational level, employment, and access to health care by zip code; the disease characteristics of presence or absence of one or two copies of the *PNPLA3* gene*,* (rs738409)-G allele, medication average, BMI, metabolic syndrome, waist/hip circumference ratio, CCI, AST elevation now or in the past, and ALT elevation now or in the past; and the individual characteristic, prior health knowledge of NAFLD.

Using the enter method in the multiple linear regression analysis, significant predictors were found for the activity limitation model (F = 3.338, df1 = 15, df2 = 25, p = .004). Significant predictors of activity limitation due to physically and mentally unhealthy days were the race/ethnicity category of white versus nonwhite (Beta -9.852, p = .012) and employment (Beta 11.883, p = .001).

Healthy days versus copies of the PNPLA3 *gene, (rs738409)-G allele.* For a chi-square comparison of healthy days versus one or two copies of the *PNPLA3* gene, (rs738409)-G allele, healthy days were binned into dichotomous variables similar to the average of 24.7 healthy days for the general U.S. population: 0 to 23 healthy days and 24 to 30 healthy days (Centers for Disease Control and Prevention, 2000). Sixty-six percent (n = 28) of this sample had 0 to 23 healthy days, and 33.3% (n = 14) had 24 to 30 healthy days. Of the 66.7% (n = 28) of participants that reported 0 to 23 healthy days, 40.5% (n = 17) of these participants had one or two copies of the *PNPLA3* gene, (rs738409)-G allele (see Table 35). However, the relationship between healthy days

and copies of the *PNPLA3* gene, (rs738409)-G alleles was not significant (r = .438, p = .508).

Table 35

Healthy Days Binned Frequencies Comparison by PNPLA3 *Gene, (rs738409)-G Allele*

Healthy days	Frequency *n* (%)	No copies *n* (%)	One or two copies *n* (%)
0-23 Healthy days	28 (66.7)	11 (26.2)	17 (40.5)
24-30 Healthy days	14 (33.3)	7 (16.7)	7 (16.7)
Total	42 (100)	18 (42.9)	24 (57.1)

Table 36

Healthy Days Binned Frequencies vs. Sex vs. Copies of PNPLA3 *Gene, (rs738409)-G Allele*

		Healthy days	
Sex	Frequency *n* (%)	0-23 healthy days *n* (%)	24-30 healthy days *N* (%)
Male	12 (100)	6 (50.0)	6 (50.0)
No copies	4 (33.3)	2 (16.7)	2 (16.7)
One to two copies	8 (66.7)	4 (33.3)	4 (33.3)
Female	30 (100)	22 (73.3)	8 (26.7)
No copies	14 (46.7)	9 (30.0)	5 (16.7)
One or two copies	16 (53.3)	13 (43.3)	3 (10.0)

To evaluate whether the 0 to 23 healthy days were comprised primarily of females, a three-way comparison was conducted using healthy days, sex, and copies of the *PNPLA3* gene, (rs738409)-G allele groups. Although 73.3% (n = 22) of females compared to 50% (n = 6) of males reported 0-23 healthy days, 43.3% (n = 13) of the 73.3% (n = 22) of females and 33.3% (n = 4) of the 50% (n = 6) of males had one or two

copies of the *PNPLA3* gene, (rs738409)-G allele (see Table 36). Furthermore, when genotype was compared with healthy days and sex, 100% of the male and female participants with the GG genotypes reported 0 to 23 healthy days, that is, HRQOL (see Table 37).

Table 37

Healthy Days Comparisons of Males and Females by PNPLA3 *(rs738409) Genotype*

		Healthy days	
Sex	Frequency *n* (%)	0-23 healthy days *n* (%)	24-30 healthy days *n* (%)
Male	12 (100)	6 (50.0)	6 (50.0)
CC	4 (33.3)	2 (16.7)	2 (16.7)
CG	5 (41.7)	1 (8.3)	4 (33.3)
GG	3 (25.0)	3 (25.0)	0 (0)
Female	30 (100)	22 (73.3)	8 (26.7)
CC	14 (46.7)	9 (30.0)	5 (16.7)
CG	12 (40.0)	9 (30.0)	3 (10.0)
GG	4 (13.3)	4 (13.3)	0 (0)

Note. CC = no copies of the *PNPLA3* gene, (rs738409)-G allele; CG = one copy of the *PNPLA3* gene, (rs738409)-G allele; GG = two copies of the *PNPLA3* gene, (rs738409)-G allele.

Healthy days were evaluated as a continuous dependent variable using multiple linear regression. Predictors used in the model included demographic characteristics of age, race/ethnicity, sex, educational level, employment, and access to healthcare by zip code; the disease characteristics of presence or absence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele, medication average, BMI, metabolic syndrome, waist/hip circumference ratio, CCI, AST elevation now or in the past, and ALT elevation now or in the past; and the individual characteristic, prior health knowledge of NAFLD. The healthy days model was significant ($F = 5.068$, $df1 = 15$, $df2 = 25$, $p<.001$). There

were several significant predictors in this model, including BMI, *PNPLA3* gene, (rs738409)-G allele, white/nonwhite, employment, and prior health knowledge of NAFLD. Table 38 summarizes the significance values of the predictors of healthy days. Lower BMI was a predictor of more healthy days per month. Participants with no copies of the *PNPLA3 gene,* (rs738409)-G allele had more healthy days. Participants who identified white as their race had more healthy days than others (African American, Hispanic and multiracial races/ethnicities). Unemployed, retired, and disabled participants had fewer healthy days than those participants who were employed. Participants with no knowledge of NAFLD prior to the day they were recruited into the study had healthier days than those who were aware that they had a diagnosis prior to the study.

Table 38

Significant Predictors of Healthy Days

Predictors	Beta	Significance
BMI	-.358	$p = .024$
0 copies (0) vs. one or two copies (1) of the *PNPLA3* gene, (rs738409)-G allele	-5.812	$p = .040$
White (1) /nonwhite (0)[a]	16.731	$p < .001$
Unemployed, retired, disabled (1) vs. employed (0)	-8.844	$p = .015$
Prior health knowledge of NAFLD (1=yes)	-8.061	$p = .018$

Note. BMI = body mass index; NAFLD = nonalcoholic fatty liver disease; (0) and (1) = dichotomous coding designations.

[a]The term *white* refers to all Caucasian participants. The term *nonwhite* refers to participants of all other races, including African American, Hispanic, and multiracial participants.

Summary of results for Aim 3 and Hypothesis 3. Significant predictors were found for healthy days, general health, physical health, mental health, and activity limitation as noted in Table 39. Only the healthy days model depicted a statistically significant relationship with the *PNPLA3* gene, (rs738409)-G genotype.

Table 39

Healthy Days, General Health, Physical Health, Mental Health, and Activity Limitation Model Summary

Model	Regression	Predictors
Healthy days	(F = 5.068, $df1$ = 15, $df2$ = 25, p < .001)	BMI, *PNPLA3 gene,* (rs738409)-G allele, White[a] race/ethnicity, Unemployed, Health knowledge of NAFLD
General health	(F = 3.012, $df1$ = 15, $df2$ = 25, p = .007)	White race/ethnicity[a], Education level
Physical health	(F = 2.235, $df1$ = 15, $df2$ = 25, p = .036)	Unemployed
Mental health	(F = 6.425, $df1$ = 15, $df2$ = 25, p < .001)	BMI, White race/ethnicity[a]
Activity limitation	(F = 3.338, $df1$ = 15, $df2$ = 25, p = .004)	White race/ethnicity[a], Unemployed

Note. F = F distribution, df = degrees of freedom; BMI = body mass index; NAFLD = nonalcoholic fatty liver disease.
[a]The term *white* refers to all Caucasian participants.

Aim 4. In aim 4, the purpose of the analysis was to describe the relationship between symptom distress and HRQOL in persons at higher risk of NAFLD progression based on the presence of one or two copies of *PNPLA3* gene, (rs738409)-G allele

versus persons at lower risk of NAFLD progression, that is no copies of the *PNPLA3* gene, (rs738409)-G allele.

Hypothesis 4. It was hypothesized that there would be a negative correlation between the distress of symptoms total sum of scores and HRQOL score in those at higher risk of NAFLD progression.

Preparation for analysis of Aim 4. Multiple linear regression was used for the analysis. Healthy days was the dependent variable in this multiple linear regression analysis and was left as a continuous variable.

Distress of 32 symptoms within the MSAS were summed and averaged to be utilized as a predictor, along with the presence or absence of *PNPLA3* gene, (rs738409)-G alleles. Using the enter method in the multiple linear regression analysis, significant predictors were found for the model and explained 36.2% of the variability (F = 11.057, $df1$ = 15, $df2$ = 39, p <.001). The significant predictor in this model was the distress average of all 32 symptoms (Beta -.385, p = .001). The correlation table shows a negative relationship between healthy days and the distress of all 32 symptoms (r^2 = - .385, p = .001).

Summary of results of Aim 4 and Hypothesis 4. A significant relationship was found between symptom distress average and healthy days. However, the *PNPLA3* gene, (rs738409)-G allele was not a predictor of symptom distress or healthy days when compared with the distress average of symptoms in the model, although it was nearly significant (p = .057)

Summary of Aims 1-4 Results. Overall, persons with NAFLD experienced symptoms. Persons with NAFLD had more than 10 physically or mentally unhealthy

days per month and more than 15 total unhealthy days per month. Persons with NAFLD rated their health as good to fair. The number of healthy days per month was influenced by BMI, the *PNPLA3* gene, (rs738409)-G allele, white race, unemployment (including retired and disabled), and prior health knowledge of NAFLD. The more symptom distress a person with NAFLD experienced, the fewer healthy days he or she experienced. This study had a small sample size of 42 participants. Further research is needed to evaluate these findings with a larger sample.

Chapter 6: Discussion

This chapter will discuss the findings of this study. Findings will be presented for each aim along with a discussion of literature relative to each aim. Study limitations will be presented, followed by implications for nursing practice and for research.

Main Findings

The overall purpose of the study was to determine the presence of symptoms and the level of HRQOL in persons with NAFLD and to compare the symptoms experience and HRQOL by *PNPLA3* (rs738409) genotype. It was hypothesized that persons with genetic predisposition of NAFLD, that is, one or two copies of the *PNPLA3* gene, (rs738409)-G allele, would have symptoms and poorer HRQOL than those without the *PNPLA3* gene, (rs738409)-G allele. The findings of this study will provide foundational work for future interventional studies.

Aim 1. The purpose of Aim 1 was to identify the presence of symptoms in persons with NAFLD at higher risk of disease progression based on the presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele compared to those at lower risk of disease progression, that is no copies of the *PNPLA3* (rs738409)-G allele. It was hypothesized that persons at higher risk of progression of NAFLD will exhibit symptoms.

Discussion. Ninety-seven percent of participants with NAFLD in this study experienced at least one symptom. Furthermore, participants experienced 12 symptoms on average. However, no relationship was found between the presence of individual symptoms and one or two copies of the *PNPLA3* gene, (rs738409)-G allele using Fisher's Exact Test.

Although, there were no statistically significant relationships between the *PNPLA3* gene, (rs738409)-G allele and individual symptoms, trends in odds ratios

suggest that participants may be more likely to have certain symptoms if one or two copies of the *PNPLA3* gene, (rs738409)-G allele is present. For example, participants were twice as likely to experience fatigue, a known symptom in NAFLD, (OR= 2.000; 95% CI [570-7.013; p=.348]) if they had one or two copies of the *PNPLA3* (rs738409)-G allele, (Angulo, 2007a). Further study with a larger sample size is needed to evaluate a potential relationship between symptoms and copies of the *PNPLA3* gene, (rs738409)-G allele.

Many of these symptoms identified by participants in this study have also been found in persons with other liver diseases (Younossi, et al., 1999). In the development of the Chronic Liver Disease Questionnaire (CLDQ) 29 symptoms were identified and categorized into domains of fatigue, abdominal pain, emotional function, systemic symptoms, activity, and worry (Younossi, et al., 1999). The CLDQ was developed through survey of 60 patients with liver diseases, such as hepatitis B and C and cirrhosis, then reduced through survey of 75 patients, and retested in 133 patients (Younossi, et al., 1999). Symptoms in the CLDQ were deemed important by 50% (*n* = 35) of 70 participants who participated in the item reduction phase of the CLDQ reflected in Table 40. Likewise, in a recent study of children with liver biopsied-confirmed NAFLD, symptoms were also found (Kistler, et al., 2010). Table 40 outlines the similarities between symptoms found in (a) the CLDQ development, (b) in Kistler's study of symptoms and HRQOL in children alongside (c) this study (Kistler, et al., 2010; Younossi, et al., 1999).

Table 40

Comparison of Symptoms of Chronic Liver Disease with Symptoms Found in Children and Adults with NAFLD

Symptoms found in development of Chronic Liver Disease Questionnaire (%)	Symptoms found in children with NAFLD (%)	Symptoms, Genetics, and HRQOL in Persons with NAFLD (%)
	Symptom mean= 5	Symptom mean=12
Fatigue domain		
Tiredness or fatigue (80)	Fatigue (68)	Fatigue (59.5)
Sleepiness during the day (80)		Feeling drowsy (50.0)
Decreased strength (69)		
Decreased level of energy (81)		Lack of energy (71.4)
Felt drowsy (63)		
Abdominal pain domain		
[Feeling bloated][a] (66)	Swelling abdomen (20)	Feeling bloated (45.2)
Abdominal bloating (58)	Liver pain (41)	Ache/discomfort in right lower rib/below rib area (35.7)
Abdominal pain		Diarrhea (50)
Abdominal discomfort (55)	Diarrhea (35)	
		Others: Constipation (30.9)
Emotional function		
Anxious (69)		Feeling irritable (57.2)
Unhappy		Feeling nervous (38.1)
Irritable (55)	Irritability (73)	
Difficulty sleeping (61)		Difficulty sleeping (47.6)
Mood swings (52)		
Inability to fall asleep at night (58)		
Felt depressed (56)		Feeling sad (40.4)
Problems concentrating	Trouble concentrating (55)	Difficulty concentrating (33.3)

Table 40 (cont'd)

Comparison of Symptoms of Chronic Liver Disease with Symptoms Found in Children and Adults with NAFLD

Symptoms found in development of Chronic Liver Disease Questionnaire (%)	Symptoms found in children with NAFLD (%)	Symptoms, Genetics, and HRQOL in Persons with NAFLD (%)
	Symptom mean= 5	Symptom mean=12
Systemic symptoms		
Bodily pain	Headache (60)	Pain (64.3)
Shortness of breath (53)		Shortness of breath(35.7)
Muscle cramps (56)	Muscle aches or	
Dry mouth (61)	cramps (53)	Dry mouth (35.7)
Itching (53)		Itching (28.6)
		<u>Others:</u>
		Numbness/tingling in hands/feet (42.8)
	Swelling ankles (15)	Swelling of arms/legs (38.1)
		Sweats (35.7)
		Cough (33.3)
		Dizziness (28.6)
		Problems with sexual activity/interest (28.6)
		Weight loss (28.5)
		Problems with urination (23.8)
	Nausea (50)	Nausea (26.2)
		Vomiting (11.9)
		Changes in skin (23.8)
		"I don't look like myself" (19.0)
		Hair loss (16.6)
		Mouth sores (9.5)

Table 40 (cont'd)

Comparison of Symptoms of Chronic Liver Disease with Symptoms Found in Children and Adults with NAFLD

Symptoms found in development of Chronic Liver Disease Questionnaire (%)	Symptoms found in children with NAFLD (%)	Symptoms, Genetics, and HRQOL in Persons with NAFLD (%)
	Symptom mean= 5	Symptom mean=12
Activity		
Unable to eat as much as preferred (56)		Lack of appetite (21.4)
Trouble lifting or carrying heavy Objects (53), (55)		Difficulty swallowing (11.9)
Limitation of diet		Change in the way food tastes (23.8)
Worry		Worrying (52.4)
Concern about liver disease impact on family (64)		
Worried symptoms will develop into major problems (73)		
Worried conditions will get worse (83)		
Worried never going to feel better (52)		
Concerned about transplant availability (55)		

Note. NAFLD = nonalcoholic, fatty liver disease. Adapted from Younossi, Z. M., Guyatt, G., Kiwi, M., Boparai, N., & King, D. (1999). Development of a disease specific questionnaire to measure health related quality of life in patients with chronic liver disease. *Gut, 45*(2), 295-300. Adapted from Kistler, K. D., Molleston, J., Unalp, A., Abrams, S. H., Behling, C., & Schwimmer, J. B. (2010). Symptoms and quality of life in obese children and adolescents with non-alcoholic fatty liver disease. *Alimentary Pharmacology & Therapeutics, 31*(3), 396-406.

[a]Symptom combined with others in final Chronic Liver Disease Questionnaire.

These findings purport that further study is warranted in the study of symptoms in NAFLD. The identification of symptoms in persons with NAFLD is an extremely important contribution to science. Prior studies and literature reviews report few symptoms or that NAFLD is asymptomatic (Adams & Angulo, 2005; Angulo, 2007a; Sanyal, 2002). However, more recently, evidence has emerged to support the hypothesis that persons with NAFLD do indeed have symptoms, and perhaps, have symptoms in the earlier stages of disease, concurring with studies of symptoms with children (Kistler, et al., 2010). For example, symptoms of fatigue, trouble sleeping, irritability, sadness, trouble concentrating, diarrhea, nausea, swelling abdomen, swelling ankles, headache, liver pain, and muscle aches or cramps were recently reported in children with liver biopsied-confirmed NAFLD as noted in Table 40 (Kistler, et al., 2010). This study is important in that most children do not have comorbid conditions that would suggest that symptoms are not occurring from causes other than NAFLD. Secondly, as children grow up, they may exhibit similar symptoms as found in this study as their NAFLD progresses.

In adults, evidence suggests that the symptoms of NAFLD may be present in the early stages. Autonomic dysfunction related to falls, postural dizziness, syncope and cognitive symptoms have been found in persons with NAFLD (Newton, 2010). These symptoms were not related to the severity of the disease, suggesting that symptoms are present in earlier stages of NAFLD (Newton, 2010). Likewise, in this study only 26% (*n* = 11) of participants had known fibrosis or cirrhosis from previous liver biopsies. Together, the findings from Newton's study and the results of this study suggest that

participants who did not have liver biopsies either had unknown fibrosis or cirrhosis or that NAFLD symptoms are present in early stages.

Symptoms identified in this study have also been found in symptoms experiences of patients with liver cirrhosis from other liver disease, such alcoholic liver disease or hepatitis (Kim, Oh, & Lee, 2006; Younossi, et al., 1999). Therefore, it is possible that persons with NAFLD experience similar symptoms to persons with liver cirrhosis and cirrhosis from hepatitis B and C.

The potential causes of symptoms in persons with NAFLD and other liver diseases are not fully understood. One theory is that symptoms of chronic diseases, such as obesity-related NAFLD, are caused from inflammatory processes in response to the deposition of fat in the liver. In turn, proinflammatory cytokines are released and travel to the brain resulting in a sickness behavior that includes fatigue, one of the symptoms found in NAFLD in this study (D'Mello & Swain, 2011; Dantzer, et al., 2008). While newer physiological studies of the liver-brain inflammatory axis provide an explanation for symptom production, further study is needed to determine the biologic mechanisms underlying symptoms in persons with NAFLD. In addition, studies with a larger sample size are needed to explicate symptoms in persons with NAFLD.

Summary for Aim 1. Symptoms were identified in persons with NAFLD in this study and these symptoms were similar to symptoms in a recent study of symptoms and HRQOL in children (Kistler, et al., 2010). In addition, the symptoms experience identified in this study was similar to persons with other chronic liver diseases as found in a survey used to develop the CLDQ. However, there was no relationship between the *PNPLA3* gene, (rs738409)-G allele and symptoms. While the sample size in this

study was small, trends suggested that persons with certain symptoms were more likely to have one or two copies of the *PNPLA3* gene, (rs738409)-G allele. Further study is needed with a larger sample size to explicate symptoms and a possible relationship of symptoms with the *PNPLA3* gene, (rs738409)-G allele.

Additional findings: Relationship Between Demographic Characteristics and Symptoms

PNPLA3 genotype was associated with BMI in this sample. Specifically, persons with higher BMI had no copies of the *PNPLA3* gene, (rs738409)-G allele. Participants with no copies of the *PNPLA3* gene, (rs738409)-G allele (CC genotype) had a mean BMI of 43.57 compared to participants with one or two copies of the *PNPLA3* gene, (rs738409)-G allele (M = 36.76, p = .034). Incidentally, the mean BMI was 35.62, for participants with two copies of the *PNPLA3* gene, (rs738409)-G alleles (GG genotype) and 40.49 for the combined CC and CG genotypes, suggesting that the C allele may contribute to obesity. However, this relationship was not statistically significant (p = .264). This result of no copies of the *PNPLA3* gene, (rs738409)-G alleles and higher mean BMI is the opposite of what was expected. In a meta-analysis of 16 studies reflecting data from 4,141 subjects, there was no relationship between BMI and the *PNPLA3* gene, (rs738409)-G alleles (Romeo, et al., 2008).

The symptoms experience in persons with NAFLD differed by race/ethnicity. Persons who identified their race/ethnicity as Hispanic or multiracial experienced more symptoms (M = 22.83) compared to persons who identified their race/ethnicity as Caucasian (M = 10.22, p = .001). Race/ethnicity did not affect symptom domain scores in a quality of life study of 237 persons with chronic liver disease, 106 with NAFLD (Dan,

et al., 2007). This observation may be explained by the higher prevalence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele in Hispanics, which predisposes them to higher risk of liver injury (Browning, et al., 2004; Tian, et al., 2010).

Persons recruited from Gastroenterology Associates or from home were 2.5 times more likely to have elevated AST levels and 1.8 times more likely to have elevated ALT levels. Elevated AST and elevated ALT levels are often triggers for referral to a gastroenterology office (Sanyal, 2002). This finding suggests that these participants had more severe liver disease or that persons with more severe liver disease were being seen at the gastroenterologist office. However, limited liver biopsy results are available to examine this assumption.

Aim 2. In Aim 2, a comparison was made to determine the extent to which the frequency, intensity, and distress of symptoms in persons with NAFLD differ between those at higher risk of disease progression based on the presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele compared to those at lower risk of disease progression, that is no copies of the *PNPLA3* gene, (rs738409)-G allele. The hypothesis was that persons at higher risk of disease progression would have more symptom intensity, frequency and/or distress than those at lower risk of disease progression. This aim was examined using subscales of the MSAS: the Global Distress Index, the Physical subscale, the Psychological subscale and the TMSAS.

Discussion of Aim 2. The frequency, intensity and distress of 32 symptoms found in the TMSAS contribute to the symptoms experience in persons with NAFLD. Race/ethnicity of non-White (p = .003) was the only significant predictor of TMSAS in the linear model, a finding that provides further evidence that persons with NAFLD have

symptoms. Participants who identified their race/ethnicity as African American, Hispanic or multiracial, (categorized as nonwhite) had higher TMSAS averages of combined frequency, intensity, and distress of 32 symptoms than participants who identified their race as white.

There are minimal symptom studies of persons with NAFLD. Davis and others found education level to influence the degree of psychological distress in persons with chronic liver disease diagnosed as NAFLD or hepatitis (Davis, De-Nour, Shouval, & Melmed, 1998). In NAFLD-related diseases such as liver cancer or cardiovascular disease, the MSAS was used to determine frequency, severity, and distress of symptoms in 135 cancer versus non-cancer patients, that is, those with chronic obstructive pulmonary disease, congestive heart failure and cirrhosis (Tranmer, et al., 2003). The non-cancer group, which included cirrhosis, experienced weight loss more frequently, more distress with dizziness, more distress with coughing, and less severe diarrhea than the cancer group. While there was no difference in demographics between groups, no statistical analysis between demographics and distress was presented in this paper (Tranmer, et al., 2003).

The MSAS-GDI is the average of the frequency of four psychological symptoms and the distress of six physical symptoms: feeling sad, worrying, feeling irritable, feeling nervous, lack of appetite, lack of energy, pain, feeling drowsy, constipation, and dry mouth. Nonwhite race/ethnicity and being unemployed, retired or disabled were significant predictors of symptom distress. Nonwhites and the unemployed, retired, or disabled participants experienced higher levels of global distress from identified symptoms.

Persons who identified their race/ethnicity as African American, Hispanic or multiracial had more frequent, intense and distress-producing physical symptoms than did persons identifying their race as white. The frequency, intensity, and distress of 12 physical symptoms using the MSAS-PHYS subscale included lack of appetite, lack of energy, pain, feeling drowsy, constipation, dry mouth, nausea, vomiting, change in taste, weight loss, feeling bloated, and dizziness. Health knowledge of NAFLD prior to recruitment into the study also played a significant role in the identification of more frequent, intense, and distress-producing physical symptoms. Perhaps, prior health knowledge of NAFLD suggests that participants may have had NAFLD longer. In addition, persons with prior health knowledge of NAFLD may also have had more advanced disease, or they were more aware of potential symptoms as they had time to be more aware of their body's signals, that is, the "red flags of threat to health" (Hegyvary, 1993).

Participants that identified their race/ethnicity as African American, Hispanic, or multiracial were more apt to have more frequent, intense, and distress-producing psychological symptoms than participants that identified their race as white, as measured by the MSAS-PSYCH subscale. The MSAS-PSYCH subscale measured the average of the defining attributes of six psychological symptoms: worrying, feeling sad, feeling nervous, difficulty sleeping, feeling irritable, and difficulty concentrating. In addition, unemployed, retired, or disabled participants experienced more frequent, intense, and distress-producing psychological symptoms than participants who were employed, as measured by the MSAS-PSYCH subscale.

The frequency, intensity, and distress of symptoms did not differ according to *PNPLA3* genotype. One or two copies of the *PNPLA3* gene, (rs738409)-G allele has been associated with advanced disease in adults (Valenti, et al., 2010) and with earlier presentation in children (Rotman, Koh, Zmuda, Kleiner, & Liang, 2010). However, the *PNPLA3* gene, (rs738409)-G allele may not correlate with symptoms in this study for several reasons. First, the *PNPLA3* gene, (rs738409) genotype may not be associated with symptom production despite the relationship between the *PNPLA3* gene, (rs738409)-G allele and disease severity noted in earlier studies (Rotman, et al., 2010; Valenti, et al., 2010). Second, other variants in the *PNPLA3* gene may be better predictors of symptoms. Finally, only 14 (33.3%) of the 42 participants in this study had liver biopsies. Thus the severity of disease in each participant is unknown. It is possible that only 10 (23.8 %) of the 42 participants that have liver biopsy-confirmed fibrosis or cirrhosis are the only participants with advanced disease. Genetics and environmental factors jointly influence traits of genes to be expressed; therefore, even though participants had one or two copies of the *PNPLA3* gene, (rs738409)-G allele, perhaps they did not have advanced disease yet, because of the influence of environmental factors over time (Hartl & Jones, 2009).

Summary of Aim 2. In all, the defining attributes of frequency, intensity, and distress of all symptoms (TMSAS); the frequency and distress of prevalent physical symptoms (MSAS-GDI); the average of the defining attributes of 12 prevalent physical symptoms (MSAS-PHYS); and the average of the defining attributes of six prevalent psychological symptoms (MSAS-PSYCH) were evaluated noting several significant

predictors. These results provide crucial insight into the symptoms experience of persons with NAFLD.

There was no difference in the frequency, intensity and distress of symptoms in persons with NAFLD between those at higher risk of disease progression based on the presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele compared to those at lower risk of disease progression (no copies of the *PNPLA3* gene, [rs738409]-G allele).

Aim 3. Aim 3 was to determine the difference in HRQOL in persons at higher risk of NAFLD progression based upon the presence of one or two copies of *PNPLA3* gene, (rs738409)-G allele versus those at lower risk of disease progression, that is no copies of the *PNPLA3* gene, [(rs738409)-G allele.

Hypothesis 3: It was hypothesized that persons with NAFLD at higher risk of disease progression would have poorer HRQOL than those at lower risk of disease progression.

Discussion of Aim 3. Persons with NAFLD in this study had an average of 15.67 unhealthy days per month. Persons with one or two copies of the *PNPLA3* gene (rs738409)-G allele experienced poorer HRQOL. Likewise, persons with a higher BMI had more unhealthy days than those with lower BMI. Persons who reported their race and ethnicity as Hispanic, African American, or multiracial, which include Hispanic, African American, and American Indian, had more unhealthy days than those who reported their race/ethnicity as White. Persons who were unemployed, retired or disabled had poorer HRQOL than those who were employed. Finally, persons with prior

health knowledge of NAFLD had poorer HRQOL than those who hadn't heard about NAFLD prior to recruitment into the study.

The relationship between the PNPLA3 gene (rs738409)-G allele and HRQOL was an important finding. This is the first study to examine this gene so strongly correlated with advanced stages of NAFLD and the quantification of HRQOL measured in number of unhealthy days per month.

Poor HRQOL as measured by healthy days was a critical finding. The average of 15.67 unhealthy days in this sample is *triple* that of the 5.3 average unhealthy days reported for the general U.S. population (Centers for Disease Control and Prevention, 2000).

Results from the 1996 Behavioral Risk Factor Surveillance System (BRFSS), a national study of 109,076 participants using the Healthy Days tool, revealed that unhealthy days increased as BMI increased (Ford, Moriarty, Zack, Mokdad, & Chapman, 2001). After adjusting for age, gender, race/ethnicity, education, employment, smoking status, and physical activity, researchers found that persons with normal weight range (BMI 18.5 to 24.99) reported 4.9 unhealthy days and that persons with a BMI of 40 or greater reported 8.3 unhealthy days (Ford, et al., 2001). Unadjusted unhealthy days ranged from 4.8 for those with a BMI of 18.5 to 24.99 up to 9.9 for those with a BMI of 40 or greater (Ford, et al., 2001). However, 26.6% (*n* = 24,651) of persons with a BMI of at least 35 but less than 40 were 1.8 times more likely to report unhealthy days of 14 or greater. Likewise, 33.2% (*n* = 36,213) of persons with a BMI of 40 or greater were nearly 2.3 times more likely to report at least 14 or more unhealthy days.

The BRFSS results polled persons in 1996 (Ford, et al., 2001). However, NAFLD has dramatically increased from the 1990s because of the obesity epidemic in the United States (Younossi, et al., 2011). National averages of unhealthy days of persons with NAFLD may have been substantially underreported in 1996. Recently, Younossi and others used elevated AST and ALT markers and exclusion of other chronic liver diseases to identify persons with NAFLD within the National Health and Nutrition Examination Surveys, or NHANES (Younossi, et al., 2011). Younossi and others found that the prevalence of NAFLD has doubled from 1994 (5.51%) to 2008 (11.1%) along with the increases in obesity and obesity-related diseases (Younossi, et al., 2011). Using AST and ALT levels underestimates the population with NAFLD, as up to 70% of persons with NAFLD do not have elevated AST and ALT levels, but these variables are available predictors of NAFLD within the NHANES database (Kotronen, Peltonen, et al., 2009; Targher, et al., 2007).

This finding of 15.67 unhealthy days in persons with NAFLD has very important implications for policy. The Healthy Days measure predicts mortality in the general population (Centers for Disease Control and Prevention, 2000). For example, persons with 15.67 unhealthy days are more apt to succumb to premature death than persons of 5.3 unhealthy days (Centers for Disease Control and Prevention, 2000). Increased funding for research needs to be allocated for those studying modalities for the prevention of NAFLD and for halting the progression of NAFLD.

Activity limitation. Activity limitation is a comprehensive measure of the perception of one's disability or lack of productivity due to physically and mentally unhealthy days (Centers for Disease Control and Prevention, 2000). Of the 15.67

unhealthy days reported by participants, activity was limited 7.81 days of the month compared to the U.S. mean of activity-limited days of 1.7 days (Centers for Disease Control and Prevention, 2000). Participants of Hispanic, Black or Multiracial race/ethnicity have more activity limited days due to physically and mentally unhealthy days than do participants of white race. Unemployed, retired or disabled participants have more activity limited days than do participants that were employed.

Activity limitation increases with obesity (Ford, et al., 2001). Nearly 4.5% (*n* = 1,747) of persons with a BMI of 25 to 29.99 (overweight) reported activity-limited days of 14 days or greater, compared to 6.7% (*n* = 818) of persons with a BMI of 30-34.99 (obese) reporting 14 or more activity-limited days (Ford, et al., 2001). Likewise, 11.3% (*n* = 382) of persons with a BMI of 35 to 39.99 reported 14 days or more of activity limitation, as did 11.9% (*n*= 181) of persons with a BMI of 40 or greater (Ford, et al., 2001). Activity limitation resulting in a loss of more than one week of work per month for those with NAFLD would have severe negative consequences, from lost productivity to increased health care costs, on an already struggling U.S. economy (Centers for Disease Control and Prevention, 2000).

General health. Twenty-nine (69%) persons with NAFLD rated their general health as good to fair (mean 3.25) with 11.9% (*n*=5) of participants reporting their general health as poor; that is, 34 (81%) of persons with NAFLD in this study reported their health as good, fair, or poor. Persons in this study who identified their race as White had higher ratings of general health such as excellent, very good , and good, compared to persons who identified their race or ethnicity as Hispanic, African American or multiracial (Hispanic, African American and Indian).

Physically unhealthy days. Participants in this study reported a mean of 10.36 physically unhealthy days per month. This finding is more than 3 times the national mean of 3.1 physically unhealthy days for adults per month (Centers for Disease Control and Prevention, 2000). Persons in this study who were unemployed, retired, or disabled experience more physically unhealthy days than persons who were employed.

Mentally unhealthy days. Participants reported a mean of 10.48 mentally unhealthy days per month. Nationally, the mean number of mentally unhealthy days for adults was 2.8 days per month (Centers for Disease Control and Prevention, 2000). Persons with a higher BMI had more mentally unhealthy days. Persons who reported their race/ethnicity as Hispanic, African American, or multiracial (Hispanic, African American and Indian) had more mentally unhealthy days per month that persons who reported their race as white.

Additional analysis of Healthy days, the *PNPLA3 gene,* (rs738409)-G alelles and other variables. Nonwhites in this study had a mean of *27.0* unhealthy days compared to whites (*M* = 13.8). Participants who were unemployed, retired, or disabled had 20.89 unhealthy days compared to the 11.75 mean unhealthy days of participants who were employed. Nationally, persons who reported their race as white had much lower mean unhealthy days (5.2) compared to African Americans (5.8), Asian/Pacific Islanders (4.2), American Indian/Alaskan Native (7.3), and Hispanic (5.9) versus non-Hispanic (5.2) respondents (Centers for Disease Control and Prevention, 2000). Interestingly, NAFLD and the presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele are more prevalent in Hispanics, which may explain the higher mean unhealthy days of Hispanics nationally and nonwhites (Hispanic, African

American, and multiracial participants) in this study (Browning, et al., 2004). In addition, American Indians and Alaskan Natives have higher incidences of liver injury due to alcohol use, but few studies have examined the presence of NAFLD in these two populations (Bialek, et al., 2008; Livingston, et al., 2010; Livingston, et al., 2006). However, further research is needed to determine the influence of the *PNPLA3* gene, (rs738409)-G allele upon the presence of NAFLD and ultimately the influence of HRQOL in the American Indian and Alaskan Native populations.

Other HRQOL studies note that patients having NAFLD at more advance stages, such as with cirrhosis, had poorer HRQOL than those with fibrosis or no fibrosis (David, et al., 2009). In addition, persons with NAFLD reported poorer quality of life than those with other liver diseases of hepatitis B and C (Dan, et al., 2007).

Studies of HRQOL in other populations, such as those with metabolic syndrome and obesity, reported poor HRQOL (Ford & Li, 2008; Ford, et al., 2001; Hassan, Joshi, Madhavan, & Amonkar, 2003). These populations have very high prevalence of NAFLD, as discussed earlier. For example, persons with metabolic syndrome, or obesity were more likely to have 14 or more unhealthy days using the Healthy Days tool as a measurement of HRQOL (Ford & Li, 2008; Hassan, et al., 2003). However, in this study, metabolic syndrome and obesity were not significant predictors, which suggest that poor HRQOL is related to NAFLD.

Summary of Aim 3. In summary, participants in this study experienced good to fair general health. Participants in this study reported 3 times as many physically unhealthy days and nearly 5 times as many mentally unhealthy days as the national mean. Participants who identified their race/ethnicity as nonwhite had many more

unhealthy days than whites. Finally, physically or mentally unhealthy days contributed to nearly 5 times more activity-limited days than the national average. Persons with NAFLD have critically low levels of HRQOL compared to the national average. Further research is needed to enhance health and resultant HRQOL in this population.

Aim 4. The fourth aim was to describe the relationship between symptom distress and HRQOL in persons at higher risk of NAFLD progression based on the presence of one or two copies of *PNPLA3* gene, (rs738409)-G allele versus persons at lower risk of NAFLD progression, that is no copies of the *PNPLA3* gene, (rs738409)-G allele.

Hypothesis 4. It was hypothesized that there would be a negative correlation between the distress of symptoms total sum of scores and HRQOL score in those at higher risk of NAFLD progression.

Discussion of Aim 4. Participants with higher levels of symptom distress had poorer HRQOL as measured by the Healthy Days tool. Persons with no copies of the *PNPLA3* gene, (rs738409)-G alleles had more healthy days per month than participants with one or two copies of the *PNPLA3* gene, (rs738409)-G allele.

Summary of Aim 4. Persons with NAFLD have more symptom distress and poorer HRQOL. The *PNPLA3* gene, (rs738409)-G allele was not a significant contributor to HRQOL when symptom distress was included in the model. While all participants with two copies of the *PNPLA3* gene, (rs738409)-G allele had poorer HRQOL than those with no copies or one copy of the G allele, the G allele was not a statistically significant independent predictor when distress was included in the model. This may reflect a Type II error (i.e., false negative result) because of a small sample

size. Further research using a larger sample size is needed to determine the contribution of the *PNPLA3* gene, (rs738409)-G allele to HRQOL when evaluating symptom distress.

NAFLD Symptoms and the Symptoms Experience Model.

This study represents the first examination of symptoms within a population with diagnosed NAFLD using the symptoms experience model (Brant, 2008; Keehne-Miron, 2007). In addition, this model provides a framework to guide bio-behavioral studies such as those incorporating genetics with symptoms and HRQOL.

Demographic, disease and individual characteristics influenced symptoms. For example, using HRQOL as the outcome, significant antecedents in this model were demographic characteristics of white/nonwhite race/ethnicity, and Unemployed/retired/disabled. Disease Characteristics of BMI and one or two copies of the *PNPLA3* gene, (rs738409)-G allele were also significant antecedents of poorer HRQOL as well as the individual characteristics of health knowledge of NAFLD. The defining characteristic of symptom distress strongly influenced HRQOL. We did not evaluate the meaning of symptoms, situational meaning or existential meaning of the symptoms. Nor did we evaluate other consequences of the symptoms experience such as adjustment to illness, mood, functional status, disease progression, or survival. Further research is needed to evaluate the situational and existential meaning of symptoms in persons with NAFLD.

Although the *PNPLA3 gene,* (rs738409)-G genotype was hypothesized to influence symptom production as a mediator of risk of disease progression in NAFLD, symptom production, per se, was not studied in this model. Future studies are needed

to determine potential genes or other SNPS within the *PNPLA3* gene that may be responsible for symptom production in persons with NAFLD.

Study Limitations

There were several limitations in this study. First, the sample size was small. The statistical program, G-Power (Faul, Erdfelder, Buchner, & Lang, 2009) was used to calculate a post-hoc power analysis for a sample size of 42 using an *F*-test for linear regression with an increase in R^2. The power analysis was 81.2% for a large effect size of 0.35 and an alpha probability of 0.05 with 15 predictors. Eighty-six participants would be needed to maintain a power of 80% for a medium effect size of 0.15 with 15 predictors.

Second, this study was a cross-sectional, descriptive design. Therefore, causality could not be determined.

Third, only one polymorphism within the *PNPLA3* gene, (rs738409)-G allele was examined. Other DNA variants within the PNPLA3 gene may be better predictors of symptoms. While *PNPLA3* is a strong candidate gene for the presence of fibrosis and severity of NAFLD, other genes likely play a role in the symptoms experience. For example, other genes may encode proteins that influence symptom production, and no known symptom-producing genes, such as genes coding for cytokines, were examined in this study.

Fourth, participants were diagnosed for NAFLD via imaging techniques of ultrasound, CT scan, or MRI. While imaging can determine the presence of NAFLD, it cannot determine staging of NAFLD (Mehta, et al., 2008). In addition, the American Association for the Study of Liver Diseases has recently recommended that liver biopsy should be used as the determinant for diagnosis of NAFLD, as a means of

standardization across studies (Sanyal, et al., 2011). Participants in this sample had varying stages of NAFLD.

Fifth, exclusion of participation by hepatitis B and C was conducted from self-report and medical chart audit. There were no consistent laboratory tests available in this sample population for exclusion of participants with hepatitis B and C, as not all participants had been previously screened. Therefore, it is possible, that a few participants may have had undiagnosed hepatitis C.

Sixth, assessment of comorbid conditions was conducted from self-report and from the medical chart audit. Participants reported several comorbid conditions. While the CCI was used to control for comorbid conditions, it is possible that some comorbid conditions were not accounted for such as sickle cell anemia or rheumatoid arthritis.

Seventh, by design, all participants in this sample had a diagnosis of NAFLD. There was no comparison against those without NAFLD. In addition, nearly all participants were obese; therefore, minimal comparisons could be made between those with and without obesity to discern if symptoms and HRQOL are attributed to NAFLD or obesity.

Eighth, participants reported their race/ethnicity. Genomic, ancestry-specific sequence variations analyses were not conducted in this study to assess and control for potential population stratification.

Ninth, nearly all participants were recruited from one location. The symptoms experience and levels of HRQOL that were found in this study may be unique to persons living in this area of western Michigan or who seek health care in western

Michigan. Therefore, findings cannot be generalized to other populations or the public at large.

Tenth, comorbid conditions and medications could also be responsible for the symptoms experience found in this NAFLD population. The CCI and BMI were used to control for comorbid conditions and obesity in this study. It is possible that symptoms are because of side effects of medications or conditions other than NAFLD.

Other Considerations. There may be other reasons for the results of this study. First, there was no variability in the predictors. Nearly all of the HRQOL models had white/nonwhite race as either the only or one of two significant predictors. This result may be a result of sample size and this may not be true using a larger sample.

Second, participants with liver biopsy confirmed NAFLD, rather than imaging diagnosed NAFLD, may have different findings. For example, persons with NAFLD as diagnosed by liver biopsy may not have a symptoms experience or changes in HRQOL.

Third, the waist/hip circumference ratio as a measure of central obesity was not a significant predictor of HRQOL in persons with NAFLD. Increased waist/hip circumference ratios place persons with NAFLD at higher risk of cardiovascular diseases. One would expect a difference in symptoms; however none was found. Sample homogeneity may account for this finding. That is, all participants in this study are at risk of cardiovascular disease.

Fourth, the *PNPLA3* gene, (rs738409)-G allele did not correlate with symptoms. Only 2 groups were used to study this population (a) participants with one or two copies of the *PNPLA3* gene (rs738409)-G allele and (b) participants with no copies of the *PNPLA3* gene (rs738409)-G allele. It is possible that with a larger sample, relationships

may be discovered using three groups of participants based on the *PNPLA3* gene (rs738409) genotype of CC (no copies of the G allele), CG (one copy of the G allele) and GG (two copies of the G allele).

Fifth, a large effect size was used in the power calculation. It is possible that the large effect size of .35 may be so weak that a significant relationship between the *PNPLA3* gene, (rs738409)-G allele and symptoms is not be evident.

Numerous explanations for the results of this study exist. These comments only further substantiate the rationale for repeating this study with a larger sample size.

Implications for Nursing Practice

Symptoms. Symptoms are central to caregiving in nursing, as they are the "red flags to health" in the treatment of the human response (American Nurses Association, 2003; Hegyvary, 1993). It is imperative for nurses and other health care professionals to recognize symptoms of NAFLD and to mutually set goals with the patient for treatment of NAFLD to help alleviate symptoms found in this study, such as fatigue.

Health Knowledge of NAFLD. Prior health knowledge of persons with NAFLD was assessed in this study and was found to be a significant predictor of physical symptoms using the MSAS-PHYS subscale. Prior health knowledge of NAFLD and the identification of a symptoms experience and unhealthy days support the idea that awareness of the disease is imperative to adequately assess symptoms and HRQOL in persons with NAFLD. In addition, health-care providers' awareness for the presence of NAFLD in patients, especially those at high risk, should be of utmost importance in addressing this serious, worldwide health concern (de Silva & Dassanayake, 2009).

Most persons with NAFLD are seen by primary care providers, but primary care providers are less likely to screen for NAFLD in persons who, for example, are morbidly obese, diabetic, or have hyperlipidemia (Kallman, et al., 2009). While no studies note the nurse practitioner's awareness of NAFLD screening in overweight patients, several studies note that children are not evaluated for overweight and the resulting risk for comorbid conditions (such as NAFLD) by either nurse practitioners or primary care providers (Dilley, Martin, Sullivan, Seshadri, & Binns, 2007; Dorsey, Wells, Krumholz, & Concato, 2005; Larsen, Mandleco, Williams, & Tiedeman, 2006; Sharp, Santos, & Cruz, 2009).

Nurse practitioners and other health providers should screen all overweight and obese patients for NAFLD, but especially middle-aged, overweight or obese women as found in this study, regardless of AST or ALT elevation (Bellentani, et al., 2000; Dixon, et al., 2001; Targher, et al., 2007). Furthermore, previous literature reviews suggest that persons with diabetes types 1 or 2 are at high risk of developing NAFLD and may not have elevated liver enzymes (Targher, et al., 2007; Targher, et al., 2010). Therefore, diabetics should be monitored for the presence of NAFLD (de Silva & Dassanayake, 2009). Nurses can screen for the presence of NAFLD through assessment and monitoring of the symptoms experience and poorer HRQOL found in NAFLD. In turn, persons with NAFLD should also be evaluated for comorbid conditions of cardiovascular disease and insulin resistance or diabetes (Baumeister, et al., 2008; Targher, et al., 2007; Targher, Marra, & Marchesini, 2008). Assessing NAFLD and teaching patients and their families about NAFLD and related comorbid conditions are key roles of the nurse.

Race/ethnicity is an important assessment component in the care of persons with NAFLD. As presented earlier, persons of Hispanic ethnicity are at high risk for disease progression based on the presence of one or two copies of the *PNPLA3* gene, (rs738409)-G allele (Romeo, et al., 2008; Tian, et al., 2010). In this study, 5% of participants were of Hispanic ethnicity and all had one or two copies of the *PNPLA3* gene, (rs738409)-G allele. Hispanic ethnicity should be seen as a trigger for intervention in the prevention, identification, and treatment of NAFLD. It is critical for those diagnosed with NAFLD to receive and to implement interventions early in the disease trajectory to reverse or to improve the disease (Dixon, Bhathal, Hughes, & O'Brien, 2004).

Comprehensive, family-health histories that include three generation pedigrees should be obtained from all persons, especially from those at risk or that have NAFLD or comorbid conditions of obesity, diabetes, or cardiovascular disease. Pedigrees should be obtained upon entrance into the health-care system and updated annually. Examining family history related to NAFLD or the NAFLD illness trajectory may provide insight into potential health problems that the patient and family members may encounter (Calzone, 2010). For example, if there is a family history of liver cancer, one of the end-stage diagnoses of NAFLD, the health-care provider should monitor the patient for the presence or development of NAFLD. In addition, prevention interventions such as monitoring healthy diets and including physical activity should be applied for the children in this family. In turn, pedigrees will also provide data for researchers to study heritability of NAFLD.

Healthy days. Subtle changes in HRQOL as determined by the Healthy Days measure may provide insight into the slowly declining health of patients with NAFLD, which nursing interventions can positively impact through diet and exercise, advocacy for further testing for NAFLD, monitoring of HRQOL, and symptom management to prevent disease progression (McCorkle, et al., 2009). The Healthy Days measure should be used as a screening tool for patients at risk of NAFLD, such as those who are obese or have insulin resistance. If a patient has poor HRQOL, further evaluation for the presence of NAFLD should be conducted.

The Healthy Days tool should also be used by health-care providers at annual physicals to monitor the slowly declining health of the persons with NAFLD, especially those at greater risk of progression or premature death, such as persons with fibrosis or cirrhosis as charged by the NINR (National Institute of Nursing Research, 2011). The Healthy Days tool or a symptom survey can be used to identify symptoms or declining HRQOL that can be used by nurses and other health-care professionals in patient assessment, follow-up, or self-management, as utilized in cancer patients with fatigue or pain (Borneman, et al., 2010; Given, et al., 2002; McCorkle, et al., 2009).

Lastly, the Healthy Days tool has been shown to predict mortality and would be helpful in evaluating the effectiveness of interventions to promote optimal health in this population (Centers for Disease Control and Prevention, 2000). Use of the MSAS and the Healthy Days tool could be used by health care professionals to monitor symptoms and HRQOL in persons with cirrhosis or liver cancer. A decrease in HRQOL or an increase in the frequency, intensity, or distress of symptoms would be used to trigger interventions. Assessment of the symptoms experience such as measurement of

increasing fatigue or decreasing HRQOL by the gastroenterologist or nurse practitioner should trigger an intervention to rule out liver cirrhosis complications. Examples are complications of liver cirrhosis such as spontaneous bacterial peritonitis, a condition with high morbidity, or thrombocytopenia, a condition in which serum platelet levels drop placing the patient at higher risk of hemorrhaging, which increase mortality. The Healthy Days tool would capture these changes in health status.

Nursing is well-positioned to provide community interventions that will significantly impact the health of persons with NAFLD. In addition to increasing awareness of the NAFLD, health promotion programs initiated in the schools to increase physical activity through the use of motivational interviewing will increase weight loss and ultimately, prevent or improve obesity-related diseases such as NAFLD in children (Robbins, Gretebeck, Kazanis, & Pender, 2006; Robbins, Talley, Wu, & Wilbur, 2010). Role modeling obesity-sensitivity in schools, hospitals, clinics, and the community is another key intervention that nurses can provide. Nurses can also help patients and their families effectively navigate the health-care system to obtain timely care throughout the disease trajectory. Specifically, nurses can assist patients and families in the management of NAFLD and comorbid conditions to lengthen times between discharge and hospital readmission for treatment of advanced stages of chronic disease (Jack, et al., 2009; Naylor, et al., 1999). Altogether, nurses can assume many roles in the health promotion, disease prevention and management of NAFLD and comorbid conditions in persons and their families.

Implications for Research

Symptoms. Further studies of symptoms with a larger population are needed to explicate the symptoms experience in persons with NAFLD over time. Although patients with NAFLD may not report symptoms at office visits, symptoms are present as noted in this study. Symptoms identified in this study -- such as lack of energy, fatigue, diarrhea, dry mouth, cough, problems with urination, swelling of arms or legs, numbness or tingling in hands or feet, feeling nervous, and lack of appetite -- may be the foundation for a symptom screening tool for NAFLD. As charged by the NINR, further research is needed to "improve assessment and management of symptoms over disease trajectories, including the transition from acute to chronic illness" (p. 15).

Distress of symptoms. Additional research is needed to further examine the defining attributes of symptoms in persons with NAFLD. In this study, the defining attribute of distress from symptoms that the person experienced was a predictor of poorer HRQOL. In other populations, such as those with cancer, distress has been found to be a predictor of premature death when controlling for age and disease severity (Degner & Sloan, 1995). Distress of symptoms should trigger better management of symptoms. For example, distress of symptoms may warrant a psychological consult (McCorkle, et al., 2009). However, it is unknown if symptom distress is a predictor of premature death in persons with NAFLD. As a result, longitudinal studies are needed to determine if distress of symptoms predicts premature death in persons with NAFLD.

Genetics. Further research is needed in the genetic contribution to symptom production and the liver/gut-brain axis to discern if symptoms in addition to fatigue may

be linked to this pathway (D'Mello & Swain, 2011). There is a wealth of opportunities to study the genetics of symptoms -- such as lack of energy, fatigue, pain, diarrhea, dry mouth, cough, problems with urination, swelling of arms or legs, numbness or tingling in hands or feet, feeling nervous, and lack of appetite -- in the NAFLD population.

In line with the NINR initiatives of advancing quality of life through symptom management, further research is needed to understand the physiological mechanisms of symptoms and symptom clusters, such as those associated with genetics (Landmark-Hoyvik, et al., 2010; Lyon, McCain, Pickler, Munro, & Elswick, 2011; National Institute of Nursing Research, 2011). Some genetic studies are ongoing. Aouizerat, Dodd and others found the genetic association of TNF-α (TNFA-308 gene, [rs1800629]G>A) with severity of sleep and resultant morning fatigue in 253 patients with cancer (Aouizerat, et al., 2009). Likewise, Miaskowski and others have found associations with interleukin-6 (IL-6 c.-6101A>T [rs4719714]) and fatigue and sleep disturbances in the same cohort (Miaskowski, et al., 2010).

Next steps in research. This study raises further research questions such as If a larger sample size was used to study the NAFLD population, would the *PNPLA3* gene, (rs738409)-G allele influence symptom production as suggested by the trends in Aim 1? What is the symptoms experience of persons with simple fatty liver, NASH, and liver cirrhosis? What SNPs in the *PNPLA3* gene influence HRQOL and potentially, a symptoms experience?

This study needs to be repeated with a larger sample size recruiting persons who have had liver biopsies for the diagnosis of NAFLD. Study of a symptoms experience along with HRQOL needs further investigation to determine the association between

poorer HRQOL, increased symptoms and risk for NAFLD progression. A longitudinal study to measure symptoms over time and throughout the disease trajectory powered well enough to accurately measure the influence of the *PNPLA3* gene, (rs738409)-G allele on symptom production is included in future goals of this researcher. Systemic symptoms such as cognitive factors and measurement of falls as noted by Newton would be added (Newton, 2010). Future studies may also include measurement of other SNPs in the *PNPLA3* gene, (rs738409)-G allele to evaluate the contribution to symptoms.

Implications for Policy

Economics. Care and treatment of persons with NAFLD and their comorbid conditions are costly. While no U.S. health-care costs of NAFLD are available, health-care costs associated with NAFLD in Germany, which included related comorbid conditions of NAFLD, were found to be 32.9% higher than health-care costs for participants without NAFLD (Baumeister, et al., 2008). Higher health-care utilization of patients with NAFLD was due to increased likelihood of hospitalizations and more outpatient visits (Baumeister, et al., 2008). Untreated symptomatology may contribute to these costs as well.

The symptom of fatigue and poor HRQOL, found in this study and others, has far-reaching implications, not only as a symptom of NAFLD, but for detrimental ramifications of economic loss (Dan, et al., 2007; Ricci, Chee, Lorandeau, & Berger, 2007). In employees with fatigue, a common symptom of NAFLD, U.S. employers lose $101 billion in productive employee work time, compared to a $36.4 billion loss in productive work time for employees without fatigue annually (Ricci, Chee, Lorandeau, & Berger, 2007). In addition, employees with obesity, a cause of NAFLD, cost employers

$42.29 billion in the loss of productive work time compared to employees of normal weight, who cost employers $11.70 billion in loss of productive work time (Ricci & Chee, 2005). The implication for policy is that if symptoms and HRQOL can be treated early to (a) prevent NAFLD, (b) reverse NAFLD, or (c) halt NAFLD progression, over time, health care utilization will also decrease, resulting in decreased health care expenditures.

The treatment of symptoms in NAFLD, such as fatigue, demands the attention of health-care providers not only to treat the human response of the patient and family, but also to manage economic loss as well. Policy makers should focus health-care dollar expenditures on promotion of healthy behaviors, such as obesity prevention and treatment, to effectively prevent the development of NAFLD. In addition, symptomatology should be addressed and covered by health-care insurance so that intervention occurs early on to avoid lost productivity.

Access to care. Populations at high risk of disease progression, such as Hispanics, are not being seen at gastroenterology and bariatric surgical offices for treatment of NAFLD. Only 5% (*n* = 2) of this sample was Hispanic and 7% (*n* = 3) was multiracial (Hispanic, African American, and American Indian). This may be reflective of the lack of access to health care or the lack of health knowledge of NAFLD in the Hispanic population at large (Committee on Quality Health Care in America, 2001).

Other populations such as American Indian and Alaskan Native were rarely seen or not seen at all at gastroenterology clinics or bariatric surgical offices. American Indians and Alaskan Natives are at risk of liver injury, such as liver cirrhosis from alcoholism, and may be at higher risk of NAFLD based on the presence of one or two

copies of the *PNPLA3* gene,(rs738409)-G allele (Tian, Stokowski, Kershenobich, Ballinger, & Hinds, 2010). In addition, few studies report findings of NAFLD in the Native Alaskan population (Fischer, Bialek, Homan, Livingston, & McMahon, 2009; Livingston, et al., 2010; Livingston, et al., 2006). While 7% (*n* = 3) of participants identified their race/ethnicity as multiracial that included American Indian, further study of NAFLD is warranted in the American Indian and Native Alaskan populations to determine risk of NAFLD (Bialek, et al., 2008; Livingston, et al., 2006). As risk is determined and health care is desired, increased awareness of NAFLD in this population may result in increased access to care in a patient-centered manner which will also positively influence quality of care (Committee on Quality Health Care in America, 2001).

Efficient care. Development of registries for liver diseases that would assist in the data collection process would be beneficial in understanding more about NAFLD in the population, allowing for the tracking of symptoms and outcomes, disease severity, and HRQOL. It would be important to note how NAFLD was diagnosed in the registry, preferably by liver biopsy as recommended by the American Association for the Study of Liver Diseases, but it is not economical or feasible to perform liver biopsies on all persons with suspected NAFLD. However, once diagnosed, coding for NAFLD for easy data extraction from clinical sites would enhance data collection in existing electronic medical records. Expansion of large aggregate databases, such as the NHANES, is needed to provide a readily available source of data in which to correlate biological and behavioral components of NAFLD.

Timely and equitable care. As presented previously, early identification and intervention to prevent the development or progression of NAFLD is key in promoting the health of persons at risk of NAFLD. Quick referrals to gastroenterologists, hematologists, and bariatric surgeons with strong multidisciplinary teams who specialize in caring for persons with NAFLD are important to optimize the health of NAFLD patients. Multidisciplinary teams of advance-practice nurses, nutritionists, behavioral counselors, and physical activity experts can address the complex health-care issues of NAFLD patients with many comorbid conditions, enhance timely interventions, and encourage equitable care for these patients (Committee on Quality Health Care in America, 2001).

In summary, policy implications for populations with NAFLD reflect increased health-care expenditures related to symptoms such as fatigue and poorer HRQOL, which result in lost productivity in the workplace. Addressing the aims of the Institute of Medicine while planning care for this population should enhance health-care options and decrease cost. Furthermore, health-care appropriations should be directed toward behavioral interventions to prevent NAFLD and to optimize health in those at risk of progression.

Conclusion

This study was the first to identify a comprehensive symptoms experience in NAFLD. In addition, this was the first study to evaluate a relationship between HRQOL and the *PNPLA3* gene, (rs738409)-G alleles, a gene closely related to risk of progression in NAFLD.

NAFLD has a substantial impact on HRQOL. Integration of biological markers, such as genotype, will help researchers and clinicians develop a greater understanding of the physiological underpinnings of symptoms and HRQOL. Through understanding of the physiological underpinnings, effective therapies and interventions can be developed to enhance the health of persons with NAFLD.

The critical role of nursing in treating the human response derived from increased symptoms and poorer HRQOL is key to optimize health in all stages of the NAFLD disease trajectory. Nursing is pivotal in promoting healthy behavioral interventions to prevent NAFLD development and to prevent disease progression through early assessment and intervention.

APPENDICES

APPENDIX A

Questionnaire

MSU Study Questionnaire

Demographics

Instructions: *Please complete the following information.*

1. **Date of birth:** _____/_____/_____
   (month/date/year)
2. **Current Age:** _____ (in years)
3. **Sex:** *(Please check your sex below)*

   ____Female

   ____Male

4. **Ethnicity/Race:** *(Please check one of the following race/ethnic groups that best describes you)*

   ____American Indian/Alaska Native

   ____Asian

   ____Black or African American (non-Hispanic)

   ____Hispanic

   ____Native Hawaiian or Other Pacific Islander

   ____White (non-Hispanic)

   ____Other Race – Including Multi-racial

5. **Educational level:** *(Please check one of the following educational levels)*

   _____Less than 9^{th} grade

   _____9-11^{th} grade (includes 12^{th} grade with no diploma)

   _____High School Graduate/GED or Equivalent

_____Some College or Associates Degree

_____College graduate (bachelor's degree)

_____Master's degree

_____Doctoral or post-doctoral degree

6. **Are you unemployed or retired?**

   ____yes

   ____no

7. **Occupation: Please write in your current or most recent job** *(prior to unemployment or retirement).*

   _____

8. **Have you possibly been exposed to chemicals or pesticides in prior jobs or living conditions?**

   ____Yes or maybe

   ____Not that I'm aware of

9. **If you answered "yes or maybe" to question number 8, please describe your exposure to chemicals or pesticides:**

_____

(examples: working with flowers as a florist, spraying crops as a farmer, grew up next to a tannery)

10. In your work or daily life, are (were) you regularly exposed to any of the following?

If "yes", indicate the number of years exposed.

Table A1

MSU Questionnaire Environmental Exposure Checklist

Exposure to:	**Check One**		**Number of Years**	**Exposure to:**	**Check One**		**Number of Years**
	Yes	No			Yes	No	
Asbestos	☐	☐		Herbicides	☐	☐	
Chemicals/Acids/Solvents	☐	☐		Loud or Excessive Noise	☐	☐	
Coal or Stone Dusts	☐	☐		Pesticides	☐	☐	
Coal Tar/Pitch/Asphalt	☐	☐		Textile Fibers/Dust	☐	☐	
Diesel Engine Exhaust	☐	☐		Wood Dust	☐	☐	
Dyes	☐	☐		X-rays or radioactive materials	☐	☐	
Fertilizers	☐	☐			☐	☐	
Formaldehyde	☐	☐			☐	☐	
Gasoline Exhaust	☐	☐			☐	☐	

11. Where do you currently live most of the year? (please check only one)

☐ On a working farm or ranch
☐ In a rural/country home or hobby farm, not a working farm or ranch
☐ In a village
☐ In a suburb or city

12. How long have you lived at your current address? _____

13. Have you ever lived on a working farm?

☐ Yes ☐ No

14. What is the primary source of drinking water at your home?

- ☐ Private Well (complete questions 14a and 14b)
- ☐ Community Supply
- ☐ Bottled Water
- ☐ Other (Specify)_____ (Go to question 15)

<u>Private Well</u>

14a. What is the depth of your private well?

- ☐ Less than 50 Feet
- ☐ 50-100 Feet
- ☐ 101-150 Feet
- ☐ 151-250 Feet
- ☐ 251-500 Feet
- ☐ 501 Feet or More
- ☐ Don't Know

14b. Was the private well cased? [Casing is usually metal or plastic pipe that extends into the bedrock to prevent shallow ground water from entering the well.]

- ☐ Cased
- ☐ Not Cased
- ☐ Don't Know

15. Are any of the following water treatment devices used in your home (check all that apply)?

- ☐ Brita or other water filter
- ☐ Ceramic or charcoal filter
- ☐ Water softener
- ☐ Aerator
- ☐ Reverse Osmosis
- ☐ Don't Know

16. To determine how accessible health care is for you, please provide your zip code:

_____

(zip code)

Health knowledge of NAFLD and Past Experiences:

17. Have you heard of nonalcoholic fatty liver disease before today?

____Yes

____No

18. If you answered "yes" to question number 17, how did you learn about it?
(Please check all that apply)

____family member has or had nonalcoholic fatty liver disease

____friend has or had nonalcoholic fatty liver disease

____read about nonalcoholic fatty liver disease

____heard someone talk about nonalcoholic fatty liver disease

____attended lecture/conference/class about nonalcoholic fatty liver disease

____other_____

19. Comorbid conditions: *(Please check if you have or have had any of the following)*

____Myocardial Infarct (heart attack)
____Congestive Heart Failure
____Peripheral vascular disease
____Cerebrovascular disease (stroke)
____Dementia
____Chronic pulmonary Disease (lung disease like emphysema)
____Asthma
____Ulcer Disease
____Mild Liver Disease
____Diabetes
____Hemiplegia (paralyzed on one side or half of your body)
____Moderate or severe kidney disease
____Diabetes (with kidney disease, loss of sensation in your feet or vision loss)
____Any tumor
____Leukemia
____Lymphoma
____Moderate or severe liver disease
____Metastatic Solid Tumor

____AIDS
____Hypothyroidism
____Addison's Disease
____Goiter
____Hepatitis
____High Blood pressure or history of high blood pressure
____High Cholesterol or history of high cholesterol
____High Triglycerides or history of high triglycerides
____Rheumatoid Arthritis

20. Etoh intake (How often do you drink alcoholic beverages, if at all?)

____Never or twice a year

____3-6 times per year

____Once per month

____Once per week

____ 2-4 times per week

____Daily

21. If you drink alcohol, on average, how much do you drink during the times noted above?

____I don't drink alcohol at all

____1 oz. of liqueur, OR 1 small glass of wine OR 1 average can/bottle of beer

____2 oz. of liqueur, OR 2 small glasses of wine OR 2 average cans/bottles of beer

____3 oz. of liqueur, OR 3 small glasses of wine OR 3 average cans/bottles of beer

____4 oz. or more of liqueur, OR 4 or more small glasses of wine OR 4 or more average cans/bottles of beer

22. Medication use: *Please list all medications including vitamins, and herbal medications that you are taking:*

_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

23. Date of your last surgery _____/_____/_____
(Month/Date/Year)

24. Today's date _____/_____/_____
(Month/Date/Year)

Continue to the next page ….

Symptom and Health-Related Quality of Life Questions

Instructions: Below are 24 symptoms. Read each one carefully. If you have had the symptom during the **past week**, let us know how OFTEN you had it, how SEVERE it was usually and how much it DISTRESSED or BOTHERED you by **circling the appropriate number.** If you **DID NOT HAVE** the symptom, make an **"X"** in the box marked "**DID NOT HAVE**".

Table A2

Memorial Symptom Assessment Scale Questionnaire: Frequency, Severity (intensity) and Distress

DURING THE PAST week, **Did you have any of the following symptoms?**	**Did not have**	If **Yes**, How **OFTEN** did you have it?				If **Yes**, How **SEVERE** was it usually?				If **Yes**, How much did it **DISTRESS** OR **BOTHER** you?				
		Rarely	Occasionally	Frequently	Almost Constantly	Slight	Moderate	Severe	Very Severe	Not at all	A little bit	Somewhat	Quite a bit	Very Much
Difficulty concentrating		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Pain		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Lack of Energy		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Cough		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Feeling nervous		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Dry Mouth		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Nausea		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Feeling Drowsy		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Numbness/tingling in hands/feet		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Difficulty sleeping		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Feeling Bloated		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Problems with urination		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**

Table A2 (cont'd)

Memorial Symptom Assessment Scale Questionnaire: Frequency, Severity (Intensity) and Distress

DURING THE PAST week, **Did you have any of the following symptoms?**	**Did not have**	If **Yes**, How **OFTEN** did you have it?				If **Yes**, How **SEVERE** was it usually?				If **Yes**, How much did it **DISTRESS** OR **BOTHER** you?				
		Rarely	Occasionally	Frequently	Almost Constantly	Slight	Moderate	Severe	Very Severe	Not at all	A little bit	Somewhat	Quite a bit	Very Much
Vomiting		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Shortness of Breath		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Diarrhea		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Feeling sad		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Sweats		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Worrying		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Problems with sexual interest or activity		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Itching		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Lack of Appetite		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Dizziness		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Difficulty Swallowing		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Feeling Irritable		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Fatigue		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Ache or discomfort in right lower rib (or below rib) area		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**

Note. Adapted from "The Memorial Symptom Assessment Scale: An instrument for the evaluation of symptom prevalence, characteristics and distress," by Portenoy, R.K, et al., 1994, *European Journal of Cancer, 30A*(9), pp. 1326-1336.

Section 2

Instructions:
We have listed 8 symptoms below. Read each one carefully. If you have had the symptom during this **past week**, let us know how SEVERE it was usually and how much it DISTRESSED or BOTHERED you by **circling the appropriate number**. If you **DID NOT HAVE** the symptom, make a **"X"** in the box marked **"DID NOT HAVE."**

Table A3

Memorial Symptom Assessment Scale, Severity (Intensity) and Distress of 8 Symptoms

DURING THE PAST Week, **Did you have any of the following symptoms?**		If **Yes**, How **SEVERE** was it usually?				If **Yes**, How much did it **DISTRESS** OR **BOTHER** you?				
	Did not have	Slight	Moderate	Severe	Very Severe	Not at all	A little bit	Somewhat	Quite a bit	Very Much
Mouth sores		**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Change in the way food tastes		**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Weight loss		**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Hair loss		**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Constipation		**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Swelling of arms or legs		**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
"I don't look like myself"		**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Changes in skin		**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
If you had any other symptoms during the past week, Please list below and indicate how much the symptom has distressed or bothered you.										
Other:		**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Other:		**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Other:		**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Other:		**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**

Now, we would like to ask you some questions about your quality of life as is related to your health status.

Would you say that in general your health is *(Please circle one of the following answers):*

a. Excellent
b. Very good
c. Good
d. Fair
e. Poor

2 Now thinking about your physical health, which includes physical illness and injury, for how many days during the <u>past 30 days</u> was your physical health not good? *(Please circle one of the following number of days)*

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

3 Now thinking about your mental health, which includes stress, depression, and problems with emotions, for how many days during the <u>past 30 days</u> was your mental health not good? *(Please circle one of the following number of days)*

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

4 During the <u>past 30 days</u>, for about how many days did poor physical or mental health keep you from doing your usual activities, such as self-care, work, or recreation? *(Please circle one of the following number of days)*

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Today's date if you are completing this questionnaire on two different days:

_____/_____/_____
(Month/Date/Year)

This concludes the questionnaire for the study.

Thank you very much for participating.

APPENDIX B

Chart Audit Tool

Pt ID_____

Chart Audit Tool

____No Food or drink in the last 30 minutes?

____Saliva sample obtained?

____Sample Sufficient?

Most recent surgery date: _____/_____/_____

Waist circumference _____ Inches

Hip Circumference _____ Inches

Height _____ Inches

Weight _____ Pounds

Table B1

Chart Audit Tool: Three Most Recent Vital Sign Measurements

Measure	Today's	2nd most recent reading	3rd most recent reading
Blood Pressure			
Heart Rate			
Respirations			

_____Check medications

_____Checked history

Surgeries:

Table B2

Chart Audit Tool: Ultrasound Results

Ultrasound:	**Results**
Date: _____/_____/_____ Ordering Diagnosis:	

Table B3

Chart Audit Tool: CT Scan Results

CT Scan:	**Results**
Date: _____/_____/_____ ***Date of Comparison study, (if available)*** • ***Date: _____/_____/_____*** *Ordering Diagnosis:* _____ _____ ____with contrast? • IV Contrast_____ • Oral Contrast_____ ____Axial? Slice thickness: • _____2 mm & 5 mm • Other _____	_____fatty infiltration of liver focal hepatic abnormality? _____ _____ Gall Bladder _____ Pancreas _____ Common Bile Duct _____ _____ Spleen _____ Adrenal glands _____ Kidneys _____ Kidneys concentrate & excrete contrast bilaterally?_____

Table B3 (cont'd)

Chart Audit Tool: CT Scan Results

CT Scan:	**Results**
MPR coronal reconstructions with _____mm slice thickness Read by: _____ At: _____	_____ Abdominal aorta & periaorta regions _____ Masses? _____ Ascites? _____ Spine?_____ **Conclusion:**

Table B4

Chart Audit Tool: Liver Biopsy Results

Liver Biopsy	**Results**
Date_____/_____/_____ ____from needle core? ____ultrasound guided? ____Other *Ordering Diagnosis:* _____ _____ _____ _____ Read by: _____ At:_____	Portal triads _____ Inflammation_____ ____macro & ___microvesicular steatosis Central veins_____ Trichrome stain_____ Iron stain_____ Other: _____ _____ _____

Table B5

Chart Audit tool: MRI Results

MRI:	**Results**
Date_____/_____/_____	_____fatty infiltration of liver focal hepatic abnormality? _____ _____ Gall Bladder _____ Pancreas _____ Common Bile Duct _____ _____ Spleen _____ Adrenal glands_____ Kidneys _____ Kidneys concentrate & excrete contrast bilaterally?_____ Abdominal aorta & periaorta regions _____

Table B5 (cont'd)

Chart Audit tool: MRI Results

MRI:	**Results**
Read by: _____ At: _____	Masses? _____ Ascites? _____ Spine?_____ ___ **Conclusion:**

Table B6

Chart Audit Tool: Laboratory Results with Date of Testing and Normal Ranges

Labs	**Dates**	**Normal Ranges**	**Results**
Glucose		70-100 mg/dL	
BUN		7-18 mg/dL	
Creatinine		.6-1.0 md/dL	
BUN/Creat. Ratio		10-20 ratio	

Table B6 (cont'd)

Chart Audit Tool: Laboratory Results with Date of Testing and Normal Ranges

Labs	**Dates**	**Normal Ranges**	**Results**
Na		135-145 mmol/L	
K		3.5-5.1 mmol/L	
Cl		95-108 mmol/L	
TCO2		21-32 mmol/L	
Anion Gap		2-11 Gap	
Ca		8.5-10.1 mg/dL	
Protein		6.4-8.2 gm/dL	
Albumin		3.5-5.0 g/dL	
Globulin		2.0-3.8 gm/dL	
Albumin/Globulin Ratio		1.2-2.2 ratio	
Alkaline Phos.		50-136 U/L	
AST		15-37 U/L	
ALT		30-65 U/L	
GFR non-African		$\geq$ 60.1 mL/min/1.73 m^2	

Table B6 (cont'd)

Chart Audit Tool: Laboratory Results with Date of Testing and Normal Ranges

Labs	Dates	Normal Ranges	Results
GFR African American		≥ 60.1 mL/min/1.73 m²	
Bulirubin, Total		0.30-1.20 mg/dL	
WBC		4.0-10.8 K/uL	
RBC		3.8-5.2 million/uL	
Hgb		12-16 g/dL	
Hct.		35-47 %	
MCV		80-100 fL	
MCH		26-34 pg	
MCHC		32-36 g/dL	
RDW		11-16 %	
Plt. Ct.		130- 400 K/uL	
MPV		≤ 11 fL	
Neutrophils %		50-75 %	
Lymphocytes %		20-45 %	

Table B6 (cont'd)

Chart Audit Tool: Laboratory Results with Date of Testing and Normal Ranges

Labs	**Dates**	**Normal Ranges**	**Results**
Monocytes %		≤ 10 %	
Eosinophil %		≤ 5 %	
Basophil %		≤ 4 %	
Neutrophil Absolute Count		2.1 – 8.1 K/uL	
Lymphocyte Absolute Count		0.8-4.8 K/uL	
Monocyte Absolute Count		≤ 1.0 K/uL	
Eosinophil Absolute Count		≤ 0.5 K/uL	
Basophil Absolute Count		≤ 0.3 K/uL	
ESR		≤ 20 mm/hr	
PT		9.5-12 sec	
INR		0.9 – 1.2 sec	
PTT		22-32 sec.	

Any complaints of symptoms in the chart? Date _____/_____/_____

Describe:

APPENDIX C

Study Consent

Research Participant Information and Consent Form

You are being asked to participate in a research study. Researchers are required to provide a consent form to inform you about the study, to convey that participation is voluntary, to explain risks and benefits of participation, and to empower you to make an informed decision. You should feel free to ask the researchers any questions you may have.

Study Title: **Symptoms, Genetics, and Health-Related Quality of Life in Persons with Nonalcoholic Fatty Liver Disease.**

Researchers and Title: Debra L. Schutte, PhD RN
Michigan State University College of Nursing
Room 515-A West Fee Hall
East Lansing, MI 48824
(517) 432-4310

Lori D. Houghton-Rahrig, PhDc RN FNP-BC
Doctoral Candidate
Michigan State University College of Nursing
West Fee Hall
East Lansing, MI 48824
(616) 813-4008

1. **What is the purpose of this study?**

   The purpose of this research study is to learn if there are symptoms with nonalcoholic fatty liver disease, how these symptoms affect quality of life and how symptoms and health-related quality of life are influenced by genes associated with nonalcoholic fatty liver disease. This research study includes doing tests on DNA, a person's genetic material, collecting information about medical history, symptoms and health-related quality of life.

   Over the next 3-5 months, approximately 120 people will participate in this study. We estimate that participation in this study will take 30-40 minutes of your time; the majority of that time will be while you are waiting for your appointment or to see the provider for today's visit. No further information or involvement beyond today is needed unless you have had surgery within the last 6 weeks. If you have recently had surgery, we would like to contact you 6 weeks after your surgery to ask you about your symptoms. Allowing us to contact you 6 weeks after your surgery will help us learn if symptoms are possibly related to nonalcoholic fatty liver disease rather than from your recent surgery.

This consent form was approved by the Biomedical and Health Institutional Review Board (BIRB) at Michigan State University. Valid from 08/26/2010 – valid through 08/08/2011. This version supersedes all previous versions. IRB #10-714.

2. **What will happen during this study?**
   Data Collection Procedures
   The following procedures will be completed during this study.

   - If you decide to participate, the medical assistant or the nurse practitioner will provide you with a questionnaire to complete. Blood pressure, waist and hip measurements, height and weight will be taken. You will be asked to submit a saliva sample by "spewing" into a plastic tube. The medical record will be reviewed by the researcher to obtain lab values, medical history, prior symptoms, and liver scanning or liver biopsy reports, if available.

DNA Storage for this study about Symptoms, Genetics and Health-Related Quality of Life.

With your permission, the DNA obtained from your saliva sample will be stored indefinitely. We hope to expand this study in the future. Additional tests may be developed in the future that may help understand nonalcoholic fatty liver disease that would be helpful to have many results from many people, therefore we are asking for your permission to store your DNA so that we can study it in the future to better understand nonalcoholic fatty liver disease. It is unlikely that what we learn from these studies will have a direct benefit for you. The results of the studies that we do in the laboratory with your DNA will not be reported back to you in the current study or in future studies as we are conducting these tests in a research laboratory. Generally, research laboratories are not certified to provide genetic results for clinical use such as in the diagnosis or treatment of disease.

However, you may decide to participate in the current study, but do not wish to have your DNA stored for future studies. Permission to store or discard your DNA specimen after this study is found at the end of this consent.

3. **What are the benefits of this study?**
   You will not benefit personally from being in this study. However, we hope that, in the future, other people might benefit from this study by having a better understanding of the relationship between symptoms, health-related quality of life and genes related to nonalcoholic fatty liver disease in order to improve recognition and treatment of the disease.

4. **What are the risks of this study?**
   There are some risks to be included in this study. These possible risks include:
   - Psychological discomfort of having waist/hip measurements. We will take every step to ensure your privacy.

This consent form was approved by the Biomedical and Health Institutional Review Board (BIRB) at Michigan State University. Valid from 08/26/2010 – valid through 08/08/2011. This version supersedes all previous versions. IRB #10-714.

- Embarrassment of "Spewing" into a plastic tube to provide a saliva sample. There is little or no physical discomfort associated with the procedure of saliva sampling and has little or no possibility of infection.

- We cannot fully anticipate how information gained through research will affect the insurability or employability of persons with nonalcoholic fatty liver disease. However, insurance companies do not have routine access to our research data. The genetic analyses performed during this study are not a form of treatment, diagnosis, or prediction of nonalcoholic fatty liver disease for persons with nonalcoholic fatty liver disease or their family members. We will not place study information we generate (genetic information) or collect (clinical information) in your medical record. We will not release information about you unless you authorize us to do so or unless we are required to do so by law. However, if you tell your family doctor, nurse practitioner or physician's assistant or other health care professional, that you have participated in this study, he or she could put such information into their medical record. Since insurance companies commonly have access to medical records, an insurance company who saw that you participated in this study might assume you are at higher risk for insurance purposes. That could then hurt your access to health or medical insurance. However, your current diagnosis of nonalcoholic fatty liver disease is most likely in your medical record as is your other health history.

- Participation in this study may increase one's awareness of the role of genetic factors in diseases such as nonalcoholic fatty liver disease, which may cause anxiety. Basic education, counseling, and emotional support to relive anxiety are available through the researchers. Referrals for genetic counseling or psychological support may be made to a specialty genetics clinic; payment for these services is the responsibility of the study participants.

- As with any research study, there may be additional risks to the participant that are currently unforeseeable.

5. How will privacy and confidentiality be protected?

To help protect your privacy, measurements and saliva samples will be conducted in an exam room. Medical records will only be viewed by the researchers.

We will keep your participation in this research study confidential to the extent permitted by law through the use of code numbers, locked files, and restricted access to data. Members of our research team will have access to the coded study information, but not your name. However, federal government regulatory agencies and Michigan State University Institutional Review Board (a committee that review and approves research studies) also may inspect and copy records pertaining to this research. Some of these records could contain information that personally identifies you. In the event of any report or publication from this study, your identity will not be disclosed. If we write a report or article about this study, we will describe the study results in a summarized manner so that you cannot be identified.

To help protect your confidentiality, we will use identification code numbers only on data forms, on DNA samples and genetic results. The file that links the ID numbers to personal identifiers will be kept in a secure location separate from the data files. The information we collect for the study will be stored in locked filing cabinets and storage areas and in password—protected computer files.

6. **What are my rights to participate, say no, or withdraw?**
Taking part in this research study is completely voluntary. You may choose not to participate at all. If you decide to consent to participate in this study, you may request to stop participate at any time. Choosing not to participate or withdrawing from this study will not make any difference in the quality of care or treatment that you receive or in any benefits for which you may otherwise qualify. If you choose to stop participating while completing the questionnaire or while measurements are being taken or while saliva samples are being collected, your information will not be used. If you choose to stop any further DNA testing on stored samples in the future, analysis completed up to that point will continue to be used in the study, but no further analysis will be pursued, and the DNA sample will be destroyed.

If you decline participation today, but think that you may be interested after leaving the office, please contact doctoral candidate Lori Houghton-Rahrig at (616) 813-4008. Please take home the flyer describing this study as it lists the contact information as well.

7. **What are the costs and compensation for being in the study?**
You will not have any costs for being in the research study. You will not receive money or any other form of compensation for participating in this research study.

8. **What are my rights to get help if injured?**
If you are injured as a result of participation in this research study, Michigan State University will assist you in obtaining emergency care, if necessary, for their research related injuries. If you have insurance for medical care, your insurance carrier will be billed in the ordinary manner. As with any medical insurance, any costs that are not covered or are in excess of what are paid by your insurance, including deductibles, will be your responsibility. The University's policy is not to provide financial compensation for lost wages, disability, pain or discomfort, unless required by law to do so. This does not mean that you are giving up any legal right you may have. You may contact Dr. Debra Schutte (517-432-4310) or Doctoral Candidate Lori Houghton-Rahrig (616 -813-4008) with any questions or to report an injury.

This consent form was approved by the Biomedical and Health Institutional Review Board (BIRB) at Michigan State University.
Valid from 08/26/2010 – valid through 08/08/2011. This version supersedes all previous versions. IRB #10-714.

9. Conflict of Interest

The researchers do not have any conflict of interest to disclose related to this research study.

10. Contact Information for Questions and Concerns.

If you have questions or concerns about the role and rights of you as a research participant, would like to obtain information or offer input, or would like to register a complaint about this study, you may contact, anonymously if you wish, the Michigan State University's Human Research Protection Program at 517-355-2180, Fax 517-432-4503 or e-mail irb@msu.edu or regular mail at 207 Olds Hall, MSU, East Lansing, MI 48824

11. Documentation of Informed Consent.

Your signature below means that you voluntarily agree to participate in this research study.

Subject's Name (printed):

_____

_____

_____

(Signature of Subject) (Date)

You will be given a copy of this form to keep. ***Please continue to the next page.***

This consent form was approved by the Biomedical and Health Institutional Review Board (BIRB) at Michigan State University.
Valid from 08/26/2010 – valid through 08/08/2011. This version supersedes all previous versions. IRB #10-714.

DNA Storage for other Future Studies
It is possible that researchers at Michigan State University, National Institute of Health, and other scientific institutions may request the use of your DNA sample for some unforeseen research project.

We ask that you identify the conditions under which your DNA sample may be used in another research program. Please check one of the following statements to indicate whether your DNA sample can or cannot be used with your permission in some other research project:

____I agree to allow my DNA samples to be stored and used for future research. I may be contacted
for future studies. Initials_____ Date_____

____ I do not want my DNA samples to be stored and used for other future research.
Initials_____ Date _____

If you have recently had surgery, we would like to contact you either by phone or mail to obtain information about symptoms 6 weeks after your surgery date. This information will help us learn if symptoms are possibly related to nonalcoholic fatty liver disease rather than symptoms as a result of your recent surgery.

____I agree to be contacted. Initials_____ Date_____

____I do not want to be contacted. Initials_____ Date_____

This consent form was approved by the Biomedical and Health Institutional Review Board (BIRB) at Michigan State University.
Valid from 08/26/2010 – valid through 08/08/2011. This version supersedes all previous versions. IRB #10-714.

APPENDIX D

Permission to use Symptoms Experience Model

Oncology Nursing Society
125 Enterprise Drive ● Pittsburgh, PA 15275-1214
Toll Free: 866-257-4ONS ● Phone: 412-859-6100 ● Fax: 412-859-6163
pubpermissions@ons.org ● www.ons.org

April 12, 2010
Lori Houghton-Rahrig
500 West Fee Hall
East Lansing MI 48824
USA
616 813-4008 (home phone)
616-677-2615 (home fax)
hought15@msu.edu
Date of request: 3-26-10

IDENTIFICATION OF INFORMATION YOU WISH TO USE:
Title of publication (book or journal), including volume and issue numbers: ***Oncology Nursing Forum, 30*(4), p. 603, 2003**.
Title of book chapter or article: **Symptoms Experience: A Concept Analysis**
Author(s)/editor(s): **Terri S. Armstrong**
Figure or table number: **Symptoms Experience Model**
USE/REPUBLICATION INFORMATION:
Material will be [check one]
X Other [please explain**] I will describe the Symptoms Experience Model in my dissertation and use an adaptation of model as a conceptual framework in my grant and dissertation.**
Type of publication in which material will be reproduced: Dissertation and NRSA grant
Author: Lori Houghton- Rahrig
Title: Symptoms, Genetics and Health-Related Quality of Life In Persons with Nonalcoholic Fatty Liver Disease
Publisher: UMI
Expected publication date: May 2011, but NRSA grant will be submitted for April 8, 2010 deadline.
Permission to use material as stated herein is granted.

Permission is subject to the following conditions:
1. **A permission fee of $25 U.S. dollars** must be received by the Oncology Nursing Society and must be accompanied by a signed copy of this agreement before permission is considered valid. Keep proof of payment with this signed document. A check should be made payable to the **Oncology Nursing Society** and sent to:
   Natalie Aitken Tooch
   Oncology Nursing Society
   125 Enterprise Drive
   Pittsburgh, PA 15275

Payment also may be made by credit card. To pay by credit card, please complete the following and fax
the signed agreement to Natalie Aitken Tooch at 412-859-6163.
Type of card: Visa MasterCard American Express Discover
Credit Card No. Expiration Date Name as shown on card

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APPENDIX E

PERMISSION TO USE THE THEORY OF UNPLEASANT SYMPTOMS

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APPENDIX F

PERMISSION FOR USE OF MEMORIAL SYMPTOM ASSESSMENT SCALE

Promoting Excellence : Memorial Symptom Assessment Scale

Instrument Name:
Memorial Symptom Assessment Scale (MSAS)
Memorial Symptom Assessment Scale - Short Form (MSAS-SF)

Category:
Clinical Care Tools - Symptom Management

Authors:
Russell Portenoy, MD and colleagues

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Keywords:
symptom assessment, symptom level,mutliple symptoms, function, health status

References:
Portenoy RK, Thaler HT, Kornblith AB, Lepore JM, Friedlander-Klar H, Kiyasu E, Sobel K, Coyle N, Kemeny N, Norton L, et al. The Memorial Symptom Assessment Scale: an instrument for the evaluation of symptom prevalence, characteristics and distress. Eur J Cancer. 1994;30A(9):1326-36.
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Chang VT, Hwang SS, Feuerman M, Kasimis BS, Thaler HT. The memorial symptom assessment scale short form (MSAS-SF). Cancer. 2000 Sep 1;89(5):1162-71.

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type:symptom_management

Promoting Excellence in End-of-Life Care was a national program of the Robert Wood Johnson Foundation dedicated to long-term changes in health care institutions to substantially improve care for dying people and their families. Visit PromotingExcellence.org for more resources.

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