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Associations of the *FTO* Gene and Risk of Acute Myeloid Leukemia

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by

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ABSTRACT

Background Acute Myeloid Leukemia (AML) is a blood cancer that affects ~70,000 people each year in the US. The Fat Mass and Obesity (*FTO*) gene on chromosome 16 has been reported to be associated with obesity, solid tumor cancers (lung, renal, breast, prostate, pancreatic, endometrial), and more recently with hematologic malignancies, like AML. In this thesis, we test the hypothesis that inherited variants within the *FTO* gene are associated with an increased risk of AML.

Methods Using the DISCOVeRY-BMT (Determining the Influence of Susceptibility COnveying Variants Related to one-Year mortality after Blood and Marrow Transplantation), a well-powered genome-wide association study consisting of 2 cohorts of BMT recipients with acute leukemias and their HLA-matched unrelated donors, reported to the Center for International Blood and Marrow Transplant Research, we leveraged 2959 AML cases and 3450 controls in a candidate gene study framework. We tested the association between SNPs located in the *FTO* gene using multivariable logistic regression assuming an additive model adjusting for age and sex. Further stratified was performed by genomic ancestry (European American, African American, and Hispanic), and body mass index (BMI). SNP and BMI interaction analysis was performed.

Results After performing standard candidate gene quality control measures, we found 130 SNPs in the *FTO* gene available for testing. We found 14 significantly associated *FTO* SNPs associated with increased risk of AML in obese European American cases compared to controls and a significant interaction between SNPs and BMI was detected.

While our power was limited, we did not find any significant associations between *FTO* SNPs and AML risk in other races/ethnicities.

Conclusion Our study provides evidence that *FTO* variants may contribute to AML, and not only solid tumors, while highlighting the importance of *FTO* variants in obesity and cancer. Identification of risk variants in AML by BMI is important for clinical risk stratification, as well as underlying functional molecular mechanisms of *FTO* in AML etiology.

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LIST OF ABBREVIATIONS

AML..... Acute Myeloid Leukemia

BM ..... Bone Marrow

BMT ..... Blood and Marrow Transplant

FTO ..... Fat Mass and Obesity Related Gene

HLA ..... Human Leukocyte Antigen

LD ..... Linkage Disequilibrium

MDS.....Myelodysplastic Syndrome

NGS..... Next Generation Sequencing

SNP ..... Single Nucleotide Polymorphism

CHAPTER 1

INTRODUCTION

Acute Myeloid Leukemia: Definitions and Diagnoses:

Acute Myeloid Leukemia (AML) is a type of hematopoietic cancer that affects people of varying ages.[1] In terms of diagnosis, AML is diagnosed by total bone marrow (BM) blast counts. Blasts are abnormal white blood cells that take over blood and marrow cells slowing the progression of normal blood cells and platelets making it difficult for the body to heal from infections.[36] AML is diagnosed when BM blast count is at least 20% with defective production of mature cells.

Acute leukemias affect blood cells, white blood cells, and platelets. In healthy adults, bone marrow creates blood stem cells that become mature blood cells over time and then become myeloid stem cells (red, white, or platelets) or a lymphoid stem cell (white blood cells).[5] In those who develop AML, myeloid stem cells can become a myeloblast which is an immature white blood cell that is abnormal and does not mature into a healthy white blood cell. These new abnormal cells are leukemic and can build up in the bone marrow and blood and creates less room for healthy cells. If left untreated, these cells can then migrate throughout the body causing more systemic damage.[5]

AML: Prevalence, Incidence, Survival:

AML remains incurable, there has been limited progress in detection, prognosis, or improving survival outcomes to date. The average age for AML diagnoses is 68 years old.[33] Only 16.6% of AML patients aged 60 or younger and only 2.4% of AML patients aged 60 or older are shown to be disease-free at 10 years post diagnosis.[6] Surveillance, Epidemiology, and End Results (SEER) has collected data on known cancer types for almost 50 years. Data shows that the age-adjusted rate of new cases is 4.3 per 100,000 men and women per year and lifetime risk of developing AML is about 0.5%. In 2018, an estimated 66,988 people were living with AML in the US.[7] In 2021, 20,040 new cases per year are expected in the US and the calculated 5-year survival rate for AML patients over the age of 20 is about 26% and those younger than 20 have a 5-year survival rate of 68%.[37,38] The expected number of deaths in the U.S in 2021 is 11,400. Also, by year 2040, deaths from AML are expected to rise to over 150,000 with 3.8 million life years lost globally.[9.5] AML accounts for just 1.1% of all cancer diagnoses, and according to the National Cancer Institute, AML is a cancer of the blood and bone marrow and is the most common type of acute leukemia in adults today.[5,33]

Risk Factors for AML

As previously mentioned, AML is a cancer that affects the blood and bone marrow of a patient. AML can affect people of all ages, but a common risk factor is older age. Sex is another risk factor; AML is shown to be more common in males compared to females, the pathology surrounding this is unknown. Out of the estimated 20,040 individuals in the U.S. to be diagnosed with AML in 2021, over half of them will be male (11,230).[37] Smoking is another risk factor for AML. It is the only well-established

lifestyle-related risk factor for AML. Obesity is shown to increase the likelihood of many cancers and diseases thus making it a possible risk factor for AML (see later section for further discussion of the role of obesity in AML).[10] Other risk factors include chemical exposures such as benzene (solvent found in rubber and oil), chemotherapy drugs such as alkylating agents and topoisomerase II inhibitors (etoposide, mitoxantrone, epirubicin, doxorubicin), radiation exposure from atomic bombs or nuclear reactor accidents, blood disorders such as chronic myeloproliferation disorders, and genetic syndromes such as Fanconi anemia, Bloom syndrome, and ataxia.[39]

Inherited germline risk of AML:

Identification of germline variants can lead to risk stratification, functional relevance to etiology, and development of preventative measures to reduce likelihood of developing cancer. technologies: genotyping or sequencing. While sequencing provides a more in-depth view of the genomic architecture, genotyping is an economical way to study germline variants in various diseases across large sample sets simultaneously.[41] There have been many studies looking at inherited germline risk of AML. Research has found genome-wide significant susceptibility loci for all AML and cytogenetically normal AML patients at 11q13.2 and 6p21.32. Significant heterogeneity for SNP rs3916765 was found with greater expression in cytogenetically normal AML females compared to males.[67] Research also found AML risk related to the *BIRCA* gene. SNP rs75797233 polymorphisms near the *BIRCA* gene were found to be associated with AML even though the expression of *BIRCA* is significantly lower in blood and lymphoblastic cell lines in individuals with rs75797233 risk allele.[69] The T allele at rs12203592 variant in intron 4 of *IRF4* was found to have increased risk of de novo abnormal cytogenetic

AML. SNP rs12203592 is shown to regulate *IRF4* transcription by physically interacting with an *IRF4* promoter.[52] These studies only account for a minimal amount of AML heritability, therefore it is likely other common, less common, and rare variants maybe contributing to missing heritability of AML.

Obesity and AML Risk:

Obesity causes physiological and hormonal changes that stimulate many diseases including cancer. Being overweight or obese has been shown to increase death rates for different types of cancers including kidney, gallbladder, endometrium, breast, esophagus, colon, rectum, pancreas, non-Hodgkin's lymphoma, and multiple myeloma for men and women.[9] Obesity is a disease that increases the likelihood of other morbidities and severe illnesses. In 2017-2018, 42.4% of Americans were classified as obese by the Center of Disease Control (CDC). In less than 20 years (1999-2018), the rate of obese Americans rose from 30.5% to 42.4% and prevalence of severe obesity rose from 4.7% to 9.2% .[10] Of all Americans diagnosed with cancer in 2014, 40% were overweight or obese according to BMI cutpoints.[11] There are mixed results in the literature about how obesity affects leukemia patients. In a French study on over 600 AML patients of varying BMIs, results showed that 91% of obese subjects achieved complete response (defined as meeting the following criteria: <5% blast counts, no blasts with Auer rods, no extramedullary disease; neutrophils ≥ 100,000/μL; platelets ≥100,000/μL; transfusion independent) and only 8.7% saw an early death compared to underweight or normal-weighted participants. Multivariate analysis showed that BMI did not influence complete response achievement or early death rate or post-mortality rate. Growing evidence shows

that obese patients with AML should be treated relative to their body size since dose under capping (a method using body size to measure the amount of chemotherapy to administer aiming to reduce a toxic overdose of treatment) of the chemotherapy could be more detrimental compared to those who are underweight or of normal weight.[44,45] Other studies show that obesity is indeed associated with an increased risk of developing adult AML but no association between obesity and survival outcomes of AML.[46]

FTO Gene:

The *FTO* gene is located on chromosome 16 spanning 53701692 BP to 54158512 BP. The *FTO* gene and SNPs have been identified by GWAS to be associated with many phenotypes[8] , including; rs9939609 (age-related diseases, mortality, and related endophenotypes); rs11642015 (BMI variance); rs62033406 (body fat % variance); rs1558902 (BMI); rs1421085 (childhood body fatness); rs1558902 (obesity, early onset, extreme); and rs56094641 (childhood obesity); and rs17254042-C, rs16953300-C, rs17175655-C, rs16953241-G, rs7191857-A, rs17254362-C, rs8052052-T, rs17175620-G, rs4423418-G are all risk alleles with a reported trait of core binding factors for AML. Additionally, there are hundreds of variants and risk alleles associated with expected obesity, % body fat, and even being a morning person.[47]

Herein our study aims are:

1. To examine associations between SNPs within the *FTO* gene and risk of Acute Myeloid Leukemia (AML).

2. To evaluate whether there are differences in inherited germline risk of AML based on race/ethnicity through SNPs within the *FTO* gene.

3. To test for interactions with BMI among SNPs within the *FTO* gene

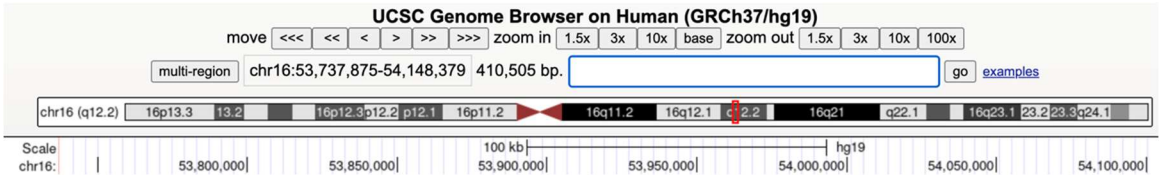

Figure 1.1 Human *FTO* Genome as displayed in UCSC Genome Browser (hg19/b37)

CHAPTER 2

LITERATURE REVIEW

AML Clinical manifestations and treatment

AML is sometimes preceded by a precursor disease called Myelodysplastic Syndrome (MDS), about 30% of MDS cases progress to AML.[1,35] The prognosis for these types of cancers is not based on tumor size or whether the cancer spreads throughout the body but is based off blood cell counts and blast cell counts. The WHO uses a Prognostic Scoring System (WPSS) to describe cancer progression and the optimal treatment for the patient.[12] The scoring system is based on three factors, the type of MDS based on WHO classification, chromosome abnormalities, and whether the patient needs regular blood transfusions. Each factor present is given a score and deciphers what MDS risk is appropriate. Lower scores equate to better prognosis for the patient. There are other factors that influence prognosis such as age, severity of low blood counts, serum ferritin levels, and certain gene or chromosomal changes not accounted for in the scoring systems.[12]

In a study done by Shi et al., 151 patients with MDS were followed over eight years and seven months. Of the 151 patients, 21 of them progressed to AML with a median survival of only 5 months. There were five factors positively associated with the leukemic transformation into AML including younger age (40 years or younger),

pancytopenia (lower than normal count of red and white blood cells and platelets) of three lineages, more than 15% blasts in the bone marrow, at least two abnormal karyotypes, and treatment combined with chemotherapy.[32] Only 31.25% completed remission and median survival time was only 6 months in patients treated with chemotherapy. MDS has a high risk of developing into secondary AML, either gradually or rapidly. Patients with MDS-to-AML progression have specific biological characteristics and a worse prognosis.[13] Statistics by the Aplastic Anemia and MDS International Foundation (AAMDS) show that patients with low-risk MDS have up to a 2 in 10 chance in progressing and developing AML, and those with a high-risk MDS status have more than a 4 in 10 chance of progressing to AML.[14]

Symptoms of AML are a result of blasts making copies of themselves at a faster rate which, in turn, slows the production of red blood cells and platelets. This causes many patients to be fatigued from anemia and puts them at a higher risk for severe bleeding from low platelet counts. Since white blood cell counts are also low, infection risk is also higher than normal. Early AML symptoms are similar the common flu and unspecific: fever, tiredness, loss of appetite, and shortness of breath. As AML progresses, symptoms include pale complexion, prolonged bleeding, bruising easily, frequent infections, swollen gums, weight loss, discomfort in bones or joints, and possibly enlarged spleen or liver. More serious symptoms include bleeding in the brain or lungs, serious infections, and myeloid sarcoma which is a tumor mass of AML cells that can form in the brain or spinal cord.[1]

Treatment for AML

Once an AML diagnosis has been given, prognosis and treatment are the next things to consider. Treatment options vary depending on type and severity of AML and patient's white blood cell count prior to treatment. Typical treatments include chemotherapy, stem cell transplants blood and marrow transplants, and radiation therapy.[40] There are three phases to AML treatment according to St. Jude's Children's Hospital. The first phase is called 'Induction', this phase is to kill leukemic cells in the blood and marrow and hopefully puts the disease into remission. After the induction phase, doctors search for cancer cell counts out of 1,000. Patients who have more than one cancer cell out of 1,000 after completing the induction phase are at the highest risk of relapse. This is when patients are offered other treatments such as stem cell transplants (gives the patient new blood cells from a donor's blood or marrow to replace lost healthy cells), immunotherapy (uses cell types called natural killer (NK) cells obtained from parents to illuminate leukemia cells), or radiation therapy (kills leukemic cells or stops them from growing). After leukemic cells leave the body, doctors proceed with the other two phases. Phase two is the central nervous system prophylaxis. This phase is necessary to kill leukemia cells that remain in the brain and spinal cord after standard chemotherapy. This treatment involves chemotherapy medications being injected into fluid-filled spaces around the brain and spinal cord. The third phase is the consolidation phase where the body disposes of any remaining cancer cells that could begin to grow and cause relapse. This phase only begins after remission has been confirmed and can continue for up to three years.[36]

The goal of AML treatment is full remission by having less than 5% blast cells, back to normal blood cell counts, and no signs or symptoms of the leukemia. Chemotherapy is aimed to destroy the DNA in cancer cells, but it also tends to destroy normal cell DNA in the process.[74] Using allogenic (unrelated) blood and marrow stem cell transplants as consolidation has higher success rates of remission but a higher risk of death due to complications. Consolidation is a part of the treatment process in administering chemotherapy. Using allogenic blood and marrow stem cell transplants as consolidation is a chance for doctors to administer a higher dose of chemotherapy compared to those who did not undergo a transplant.[40] The study population included in this thesis are AML cases who received unrelated BMT and their unrelated donors as controls.

Blood and Marrow Transplant Basics:

Blood and marrow transplants (BMTs) are possible treatment options for those in early stages of AML, in remission, who have responded well to treatment, and/or when the patient is in overall good health. BMTs replace unhealthy blood-forming cells (blasts) with healthy ones from a matched healthy donor. Blood stem cells are immature cells that grow into red and white blood cells and platelets and reside in the soft tissue in bones which is also called bone marrow. Once these cells mature, they leave the bone marrow and enter the blood stream. Before patients undergo transplantation, they receive chemotherapy and sometimes radiation to destroy the diseased cells and marrow. After less than 5% of blasts remain, BMT is available and healthy cells are given to the patient[53].

BMT is delivered via intravenous catheter or tube directly into the bloodstream of the AML patient. From there, the cells find their own way into the patient's BM. There are two main types of BMT. The first is autologous transplant which uses the patient's own healthy blood-forming cells as treatment. The second is allogenic transplant where healthy blood-forming cells come from a donor. Donors are most likely family members who have closely matched human leukocyte antigens (HLA). Unrelated adults 18 years and older can also be donors but must meet a rigorous health examination along with matching at least half of a patient's HLA[53].

Epidemiology

AML has been shown to affect people of all ages, but mainly impacts those who are aged 45 and older. According to the American Cancer Society (ACS) it is rare for AML to be diagnosed before the age of 45, but if detected in childhood, it usually presents within the first two years of life.[33,34] There are about 730 individuals under the age of 20 diagnosed in the U.S. with AML every year. Although survival rates vary depending on the subtype of AML, for children under the age of 15, the 5-year survival rate estimate is 68% and those aged 15-19 is about 66%. For those aged 20 and older, the survival rate is about 26%.[37] There are racial and ethnic differences in AML prognosis and survival rates. Okasuzyan et al. evaluated risk of ALL and AML of children aged 16 years based on their race/ethnicity and their parent's race/ethnicity using birth certificates and cancer registry data. Risk for AML was higher among Asian children with Asian fathers compared to White children with White fathers with an adjusted OR=1.34; 95%CI: [0.83-2.17]. Researchers found that African American children were also shown

to have a higher risk for AML compared to White children with an adjusted OR= 1.19; 95%CI: [0.75-1.90] and had a slightly higher risk if their parents were also of African descent OR=1.35; 95%CI: [0.77-2.37].[19] Using the Surveillance Epidemiology End Results (SEER) Program, AML diagnosis was studied among adults aged 18-60 between the years 1986-2015 among African Americans and European Americans where results presented different genes being affected based upon race.[20]

Early data on health risks of the *FTO* gene had only examined European populations for the risk of *FTO* alleles, but in recent years, it has expanded to include other races/ethnicities. According to Speliotes et al., *FTO* has the greatest impact on obesity expression, is the most common, and has the greatest variance among people of European ancestry compared to those of other races/ethnicities. Their study shows that approximately 43% of European descendants carry 1 risk allele of *FTO* and 20% carry two.[15] *FTO* SNPs have been identified in Asian populations as well. Specifically, East Asian populations show similar *FTO* allele frequencies to European American populations resulting in a 1.25-fold increased risk of obesity with each minor allele present in Asian populations. *FTO* was shown to increase BMI by .26kg/m^2 per minor allele present in Asian populations suggesting the effect of *FTO* is reduced compared to the effect *FTO* has on European populations. The main explanation for these differences can be explained by the different adiposity phenotypes, or the amount of innate fat accumulation, present in Asian populations compared to the phenotypes that are present within European populations.[16]

In a PAGE study performed on over 20,000 African Americans, results showed African Americans have a weaker association to *FTO* polymorphisms and obesity compared to European Americans due to a weakened linkage disequilibrium (LD) between *FTO* SNPs. Compared to European populations who saw a cluster of about 103 SNPs per *FTO* gene, African American populations saw only a cluster of 29 SNPs.[17] In a separate study, the sample population included 968 African Americans and 517 West Africans where researchers evaluated *FTO* variation among 262 SNPs across the *FTO* gene. Results showed a weaker LD pattern and significant differences in allele frequency compared to other European populations confirming the weaker association of *FTO* in African ancestry populations.[18] To date there are very few substantial studies on *FTO* SNPs in adult Hispanic populations and the studies listed above were of the few for Asian and African ancestry populations and *FTO* presence. A separate study found that among Mexican obese individuals, *FTO* risk allele rs9939609-A frequencies in overall Mexican populations are less than European Americans (20.4-21.2%) but was shown to be associated with increased total cholesterol in obese subjects. *FTO* polymorphisms showed a highly significant association with obesity suggesting *FTO* is a major risk factor for the Mexican population[48].

Functional Genetic Mechanisms of *FTO*:

FTO has been shown to be substantially expressed in adipose tissues, skeletal tissues, mesenteric fat, pancreatic cells, liver cells, and at its highest expression, in the hypothalamus, specifically in the area that controls energy balance and appetite regulation as well as body size and body fat accumulation.[21,22] *FTO* is an RNA

demethylase (it modifies RNA and removes certain methyl groups) which links amino acid availability to regulate cell growth, mRNA translation, and autophagy which is a necessary catabolic system for removal of dysfunctional cellular pieces.[23] *FTO* has been shown to decrease m^6A activity.[25] M^6A is an mRNA and DNA modifier which can control oncogene expression by RNA processing, splicing, translocation and degradation which can affect biological regulation.[24] M^6A has been shown to be the most favorable nucleobase substrate of *FTO* and studies show that targeting *FTO* inhibits the deletion of m^6A levels which can show anti-leukemic properties in AML cell lines.[25,26,27]

In a study done performed by Villalobos-Comparán et al., researchers found that the *FTO* risk allele rs9939609-A is shown to be upregulated in subcutaneous adipose tissue in very obese individuals.[48] A separate study done on Scandinavian women also looking at the risk allele rs9939609 showed that the adipose tissue level of *FTO* mRNA was increased in obesity and was similar in subcutaneous and omental adipose tissue both being higher in fat cells than in fat tissue. Obesity was also induced at an earlier stage in the differentiation process and suggests a role of the *FTO* gene in fat cell lipolysis possibly explaining the gene's implication in body weight regulation. This study also took tissue biopsies to study fat cell metabolism and found that in homozygous carriers of the T-allele, the in vitro basal adipocyte glycerol release was increased by 22% and in vivo plasma glycerol level increase by ~30% compared to A-allele carriers.[49]

<u>*FTO* and AML Risk:</u>

Even though it has been over 100 years since the discovery of AML, we still have limited literature on AML risk and its association with inherited genetic risk variants. Lin

et al. conducted a meta-analysis of three independent genome-wide studies (GWAS) with a fourth GWAS used as validation with AML cases and controls of United Kingdome and German ancestry. For each GWAS, association tests were performed for all AML cases and cytogenetically normal AML subjects assuming an additive genetic model. Researchers found genome-wide significant susceptibility loci in all AML and cytogenetically normal AML patients at 11q13.2 and 6p21.32. Although once stratified by age and sex separately, there was no significant heterogeneity of AML risk from the four risk variants tested. Results showed SNP rs4930561 having a significant association with risk of AML irrespective of subtype. Strong statistical evidence was also found for an association with cytogenetically normal AML for SNP rs3916765 which maps to the HLA locus. None of the four AML susceptibility variants were significantly associated with either relapse-free or overall survival in univariate analyses including all AML or cytogenetically normal AML subjects.[67] A study by Walker et al used two independent American case control cohorts testing for associations in five independent loci with risk of AML along with testing if the rs75797233 genotype correlates with the BIRCA gene expression. After performing a fixed-effects meta-analysis, researchers identified rs75797233 as the first common risk allele for AML. Polymorphisms of rs75797233 near the BIRCA gene were found to be associated with risk of AML.[69] A study performed by Wang et al also used the DISCOVeRY-BMT cohort and performed a genome-wide association study to identify loci that shows associations with AML based on subtype. A novel SNP was found to have an association with AML at the T allele of rs12203592 variant in intron 4 of IRF4 which validated an increased risk of de novo abnormal cytogenetic AML, de novo normal cytogenetic AML, MDS, and t-MDS (treatment

related MDS). Analyses also identified rs12203592 in the regulatory region of IRF4. The A allele on rs12203592 shown to have an association with AML and MDS risk.[52]

Functional role of *FTO* in hematologic malignancies

FTO is illustrated to play a critical role in hematopoietic cell transformation and leukemogenesis. A study by Li et al shows that *FTO* expression can be upregulated by certain oncogenic genes (*ASB2* and *RARA*) at both protein and RNA levels which has been shown to increase in certain types of AML.[29] *FTO* was studied in vitro and in vivo and results on *FTO* in vitro showed that forced expression of the *FTO* gene significantly enhances viability and growth of human AML cells while inhibiting apoptosis cells. Among normal karyotype AML subjects, *FTO* is expressed at a significantly higher level in AML with *FLT3-ITD* and/or *NPM1* mutations compared to those without.[29] This same study used MLL-rearranged AML as a model to further investigate whether *FTO* is directly upregulated by oncogenic proteins. In cases, *FTO* was significantly higher in MLL- rearranged AML subjects compared to normal controls. Among non-MLL-rearranged AML subjects, *FTO* is expressed at a significantly higher level in t(11q23) and t(15;17) AML patients. *FTO* was shown to be expressed at a significantly higher level in CD34 bone marrow cells isolated from primary MLL-rearranged AML patients and was relatively normal CD34+ BM cells detached from healthy donors. *FTO* was further enhanced after primary MLL-AF9 leukemic BM cells were transplanted into secondary recipients.[29]

In a separate study looking at the relationship between *FTO* and AML, researchers stated that ‘epitranscriptic RNA methylation holds potential to treat AML and

that *FTO* can be a druggable target'.[28,30] *FTO* has been found to be the first RNA demethylase that can remove m^6A from RNA. Research suggests that m^6A is a reversible and dynamic RNA modification that can impact biological regulation and could possibly be a cause to leukemogenesis.[29,30] *FTO* targets a specific gene, *R-2HG*, which is a metabolite that is produced by *IDH1/S* mutants. By suppressing *FTO*, *R-2HG* shows anti-leukemic effects.[31] *FTO* inhibitors alone or with standard chemotherapy radiation are possible effective therapies for treatment of AML.[30]

Conclusions:

AML is a life-threatening cancer that affects those of all ages but mainly those aged 60 and older.[33] MDS can be a precursor disease to AML in about a third of those diagnosed with MDS.[1] Populations affected by increased *FTO* variants alleles include mainly those of European descent, with fewer SNPs identified in those of Asian descent, African descent, or Hispanic populations.[48] Those affected by AML were shown to be of older ages, average age being 68, and those of Asian and African descent had higher odds of developing AML compared to European Americans.[16,17,18,19,20,33] These increased odds were dependent on different inherited germline variants that varied by races/ethnicities.[19,20]

Through my review of the literature, I found limited sources that specifically studied the effects of the *FTO* gene and risk of AML. Many of these studies solely looked at those of European descent. Generalizability is low and more research is needed on different races/ethnicities relating to *FTO* susceptibility and AML risk. Another gap in the literature was most of the studies I found were not primary research. Reviews of all

kinds were saturated in the data pool and many failed to cite original sources. Most AML studies do not focus on inherited risk but instead AML somatic mutations, *FTO* expression including m^6A activity, and possible *FTO* inhibitors that could be used in combination with other AML treatments. Further research is needed on the inherited impact of *FTO* gene variants and risk of AML and other related cancers and diseases. Additional research is needed to help bring awareness and increase survival rates of AML. In conclusion, AML is a life-threatening cancer that kills thousands of individuals in the U.S. each year. Studying germline inherited risk for *FTO* and AML can help risk stratification/personalized medicine and provide a better understanding of AML etiology which could lead to future prevention targets.

CHAPTER 3

METHODS

Study Design and Population:

This is a nested case control study from the parent study "Determining the Influence of Susceptibility Conveying Variants Related to 1-year Mortality" (DISCOVeRY-BMT) after unrelated donor Blood and Marrow Transplant (URD-BMT)(Figure 1).[50] Briefly, this parent study was intended to find germline genetic variations associated with survival after URD-BMT which is used as a curative therapy for Acute Myeloid Leukemia (AML), Acute Lymphoblastic Leukemia (ALL), and Myeloid Dysplastic Syndrome (MDS) patients. DISCOVeRY-BMT is made up of two cohorts of patients from 151 clinics within the U.S. through the Center for International Blood and Marrow Transplant Research (CIBMTR). Patients within these cohorts have been diagnosed with AML, ALL, or MDS along with their human leukocyte antigen (HLA)-matched unrelated healthy donors. We will solely be looking at those who have been diagnosed with AML in this study. Cohort 1 collected data between 2000-2008 and cohort 2 collected from 2009-2011. In the first cohort, we have 1277 cases who are AML patients and 2219 controls and cohort 2 has 487 cases of AML with 809 matched controls from DISCOVeRY-BMT. Controls are matched based on sex, race/ethnicity, and genetic composition (HLA) of the case and are unrelated healthy donors aged 18-66 years who passed a comprehensive medical screening and were disease-free at the time of donation.

All patients and donors provided written informed consent for their clinical data to be used for research purposes and subjects were not compensated for their participation[51,52].

Out of all cases in cohort 1, 5.0% were pediatric AML patients and 8.9% were older adult AML patients above the age of 65. In cohort 2, out of all cases 4.7% were pediatric AML patients and 9.6% were older adult AML patients. Young adults accounted for 6.6% of all cases and 4.3% of all controls in cohort 1. In cohort 2, young adults made up 6.5% of all cases and 8.5% of all controls in cohort 2. Adults aged from 21-64 years were 79.5% of all cases and 95.7% of all controls in cohort 1. In cohort 2, adults made up 78.5% of all cases and 91.5% of all controls. In relation to race/ethnicity, African Americans in cohort 1 accounted for 5.9% of all cases and 7.8% of all controls. In cohort 2, African Americans made up 1.4% of all cases and 1.98% of all controls. European Americans in cohort 1 made up 91.6% of all cases and 87.7% of all controls. In cohort 2, European Americans comprised 90.6% of all cases and 89.1% of all controls. In Hispanic AML patients in cohort 1, there was 3.5% of all cases and 3.1% of all controls. In cohort 2, 3.1% of all cases and 2.2% of all controls were Hispanic. We also stratified by BMI status where those who are underweight in cohort 1 were 5.9% of all cases and 7.8% of all controls. In cohort 2, underweight individuals were 6.1% of all cases and 7.3% of all controls. Normal weight individuals in cohort 1 were 31.7% of all cases and 33.6% of all controls. In cohort 2, normal-weight subjects were 29.2% of all cases and 29.2% of all controls. Of overweight individuals in cohort 1, there were 31.1% of all cases and 30.6% of all controls. In cohort 2, overweight individuals were 33.2% of all cases and 32.8% of all controls. In obese individuals in cohort 1, they were 30.6% of all

cases and 27.7% of all controls. In cohort 2, obese subjects were 30.5% of all cases and 30.7% of all controls.

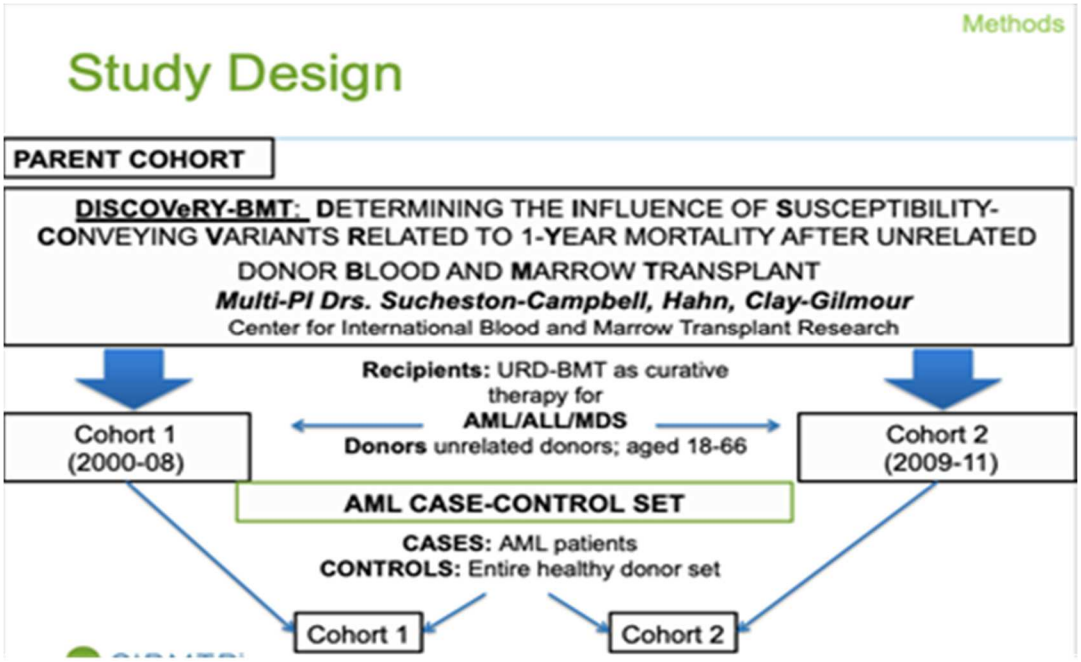

Figure 3.1 DISCOVeRY-BMT Information and Cohort Separation between AML, ALL, and MDS Patients

Genotyping and Quality Control

Genotyping was handled at the University of Southern California Genomics Facility using the Illumina Omni-Express BeadChip® containing ~733,000 single-nucleotide polymorphisms (SNPs). Samples were assigned to plates to ensure an even distribution of patient characteristics. SNPs were removed if missing values were >2.0%, Minor Allele Frequency (MAF) was <1%, or for any violation of the Hardy Weinberg equilibrium proportions ($P<1.0x10^{-4}$). Problematic samples were removed based on missing rates, duplicates, reported-genotyping mismatch, abnormal heterozygosity, cryptic relatedness, and population outliers. All quality-control (QC) measures were implemented in Plink statistical software. Genotype data were imputed on build hg19/37

using Impute2 v2.0 with a reference panel of haplotypes from 1000 Genomes phase 1v.3 QCTOOL was used to remove imputed genotypes with a MAF <1.0 and imputation quality scores <0.9.[51,52]

Statistical Methods:

First, descriptive statistics and counts were generated using R. PLINK statistical analysis software was used to test the association of SNPs located within the *FTO* gene in AML cases and controls. Controls consisted of all available donors of unrelated blood and marrow transplantation. Genotyping data consisted of the *FTO* gene ranging on the 16th chromosome from 53737875 to 54148379 base-pairs (build hg19/37) and consisted of a total of 130 SNPs. Multivariable logistic regression assuming an additive genetic model (0,1,2) was used to test the association between SNP and case/control status, adjusting for age, sex, and principal components (PCs). Primary SNP association tests were performed in three genetic ancestry groups: 1) European Americans (non-Hispanic White), 2) Black/African American (AA), and 3) Hispanic. Principal component analysis (PCA) was performed in PLINK to account for possible population stratification within each of the three race/ethnicity groups. Analyses was performed in cohort 1 (discovery) and separately in cohort 2 (validation). Meta-analyses between cohort 1 and cohort 2 was performed assuming fixed effect model and considered heterogeneity using Q and I^2 statistics. To correct for multiple testing in single SNP analysis, we used Bonferroni Correction (130 SNPs/0.05=Bonferroni p-value). Further, we performed stratified case-control analysis, using logistic regression framework for SNP association between BMI categories (underweight, normal, overweight, obese). Lastly, we explored the possible

interaction between SNPs and BMI by adding the interaction term (BMI*SNP) in the primary logistic regression models.

CHAPTER 4

RESULTS

After quality control, we found 130 SNPs to test for an association with risk of AML and identified some borderline significant and significantly associated results. Testing for significance, we used our Bonferroni value of 0.000387 as our significance value to account for multiple testing. We also used the standard significance level of 0.05 to define borderline significance. Study demographics are presented in Table 4.1.

Within European Americans, we found a total of 22 SNPs that were borderline significantly associated with risk of AML (Table 4.2). The Hispanic population had five SNPs that were borderline significantly associated with risk of AML (Table 4.3), while among African Americans, we only found two SNPs to be borderline significantly associated with risk of AML (Table 4.4). When stratified by BMI, we saw an increased risk of AML from *FTO* SNPs with the most increased risk being mainly in obese individuals. In under-weight European Americans, we found one SNP to be borderline significantly associated with risk of AML (Table 4.5). In normal-weight European Americans, we found 13 SNPs to be borderline significantly associated with risk of AML in cases vs. controls (Table 4.6) and in overweight European Americans, we found eight SNPs to be borderline associated with risk of AML (Table 4.7). Within obese European Americans, we found 15 significant and 12 borderline significant *FTO* SNPs associated with risk of AML (Table 4.8). We also included an interaction term between independent

SNPs and BMI in the model to test for interaction. We identified 18 borderline significant and 12 significant *FTO* SNPs associated with risk of AML (Table 4.9).

Table 4.1 Study demographics of Acute Myeloid Leukemia (AML) cases and healthy unrelated controls

Variables	Cohort 1 (N=3970)			Cohort 2 (N=1462)		
N (%)	Cases N=1428 (36)	Controls N=2542 (64)	P*	Cases N=554 (38)	Controls N=908 (62)	P*
Age[1]						
Pediatric (0-14)	90 (6)	0	<0.05	36 (6)	0	<0.05
Adolescent/Young Adult (15-39)	367 (26)	1858 (73)		156 (28)	685 (75)	
Adult (>40)	971 (68)	684 (27)		362 (65)	223 (25)	
Sex						
Female	673 (47.1)	840 (33.0)	<0.05	258 (46.6)	263 (29.0)	<0.05
Male	753 (52.7)	1702 (66.95)		294 (53.1)	645 (71.0)	
BMI[2]						
Underweight (<18.5)	88 (5.9)	199 (7.8)	0.07	34 (6.1)	66 (7.3)	0.92
Normal (18.5-<25)	452 (31.7)	854 (33.6)		162 (29.2)	265 (29.2)	
Overweight (25-<30)	444 (31.1)	777 (30.6)		184 (33.2)	298 (32.8)	
Obese (30+)	437 (30.6)	705 (27.73)		169 (30.5)	279 (30.7)	
Race/Ethnicity[3]						
African American	25 (1.8)	51 (2.0)	<0.05	8 (1.4)	18 (2.0)	<0.05
European American	1308 (91.6)	2229 (87.7)		502 (90.6)	809 (89.1)	
Hispanic	50 (3.5)	79 (3.1)		17 (3.1)	20 (2.2)	
AML Subtype[4]						
DeNovo	1163 (81.4)	1135 (44.7)	<0.05	1134 (49)	446 (49.1)	< 0.05
Treatment Related	112 (7.8)	112 (4.4)		32 (22)	31 (3.4)	
Diagnosis Status[5]						
Early	647 (45.3)	1170 (46.0)	0.90	304 (54.9)	441 (48.6)	<0.05
Intermediate	369 (25.8)	643 (25.3)		121 (21.8)	162 (17.8)	
Advanced	412 (28.9)	729 (28.7)		129 (23.3)	305 (33.6)	

1.The Academy of Pediatrics 2. WHO categorization 3. Stratified to see population stratification of *FTO* gene. 4. De novo are patents with no clinical history of AML, MDS, or other leukemic agents; TxRel is treatment related AML. 5. Stage of AML

*significance <0.05 between cases and controls

Table 4.2 Borderline significant associations of *FTO* SNPs and risk AML in European Americans

SNP	A1	OR (C1)	95%CI (C1)	P (C1)	OR (C2)	95%CI (C2)	P (C2)	P (Meta)
rs10521307	G	0.89	0.79-0.99	0.04	0.88	0.72-1.07	0.19	0.016
rs1121980	A	1.14	1.03-1.3	0.02	1.05	0.87-1.26	0.64	0.02
rs12149832	A	1.15	1.03-1.28	0.01	1.04	0.86-1.24	0.72	0.019
rs1421085	C	1.18	1.06-1.31	0.003	1.05	0.87-1.26	0.62	0.005
rs17218700	A	0.89	0.75-1.04	0.13	1.22	0.93-1.59	0.15	0.04
rs17817449	G	1.15	1.04-1.3	0.01	1.05	0.87-1.26	0.64	0.014
rs17819033	T	1.12	0.98-1.28	0.09	1.18	0.96-1.45	0.12	0.02
rs2111115	G	1.09	0.98-1.21	0.12	1.17	0.98-1.4	0.09	0.02
rs3751812	T	1.16	1.04-1.29	0.009	1.04	0.86-1.25	0.71	0.01
rs6499640	G	1.06	0.95-1.18	0.3	0.83	0.69-0.99	0.04	0.05
rs6499643	C	0.86	0.75-0.99	0.03	1.21	0.95-.15	0.11	0.008
rs6499652	T	1.09	0.98-1.22	0.11	1.17	0.98-1.40	0.09	0.02
rs7193144	C	1.15	1.03-1.28	1.28	1.04	0.86-1.25	0.67	0.01
rs7203572	C	1.09	0.97-1.25	0.15	1.17	0.95-1.44	0.13	0.04
rs7205009	T	1.08	0.97-1.21	0.15	1.17	0.98-1.40	0.09	0.03
rs7205986	A	1.06	0.95-1.18	0.32	1.29	1.08-1.54	0.006	0.02
rs8050136	A	1.16	1.04-1.29	0.009	1.05	0.87-1.26	0.60	0.01
rs8061518	G	0.90	0.81-1.01	0.08	0.92	0.77-1.11	0.38	0.05
rs9922619	T	1.12	1.00-1.25	0.04	1.05	0.87-1.26	0.65	0.05
rs9930333	G	1.14	1.02-1.27	0.02	1.05	0.87-1.26	0.64	0.02
rs9936385	C	1.16	1.04-1.29	0.008	1.04	0.87-1.25	0.66	0.01

SNP=single nucleotide polymorphism, A1=tested risk allele, C1=cohort 1, OR=odds ratio, 95% CI=95% confidence interval, C2=cohort 2, P-value= significance (0.000387), borderline significance (0.05), Meta=meta-analysis of cohort 1 and cohort 2.

Table 4.3 Borderline significant associations of *FTO* SNPs and risk of AML in Hispanics

SNP	A1	OR (C1)	95%CI (C1)	P (C1)	OR (C2)	95%CI (C2)	P (C2)	P (Meta)
rs10852525	A	0.26	0.09-0.78	0.01	0.67	0.12-3.71	0.65	0.02
rs1421091	C	0.52	0.29-0.90	0.02	2.13	0.83-5.44	0.11	0.005
rs17218700	A	2.87	1.09-7.52	0.03	2.27	0.69-7.47	0.18	0.01
rs17823199	C	1.69	0.98-2.89	0.06	0.53	0.19-1.48	0.23	0.02
rs7205986	G	1.89	1.10-3.25	0.02	1.13	0.41-3.10	0.82	0.03
rs9929152	G	0.54	0.29-0.99	0.047	0.63	0.19-1.98	0.42	0.03

SNP=single nucleotide polymorphism, A1=tested risk allele, C1=cohort 1, OR=odds ratio, 95% CI=95% confidence interval, C2=cohort 2, P-value= significance (0.000387), borderline significance (0.05), Meta=meta-analysis of cohort 1 and cohort 2.

Table 4.4 Borderline significant associations of *FTO* SNPs and risk of AML in African Americans

SNP	A1	OR (C1)	95%CI (C1)	P (C1)	OR (C2)	95%CI (C2)	P (C2)	P (Meta)
rs4784351	A	1.9	0.97-3.87	0.05	0.65	0.16-2.59	0.54	0.05
rs9936385	C	1.5	0.65-3.37	0.35	9.63	1.48-62.83	0.02	0.04

SNP=single nucleotide polymorphism, A1=tested risk allele, C1=cohort 1, OR=odds ratio, 95% CI=95% confidence interval, C2=cohort 2, P-value= significance (0.000387), borderline significance (0.05), Meta=meta-analysis of cohort 1 and cohort 2.

Table 4.5 Borderline significant associations of *FTO* SNPs and risk of AML in under-weight European Americans

SNP	A1	OR (C1)	95%CI (C1)	P(C1)	OR (C2)	95%CI (C2)	P (C2)	P (Meta)
rs8049933	T	2.22	0.87-5.61	0.093	1.79	0.54-5.94	0.33	0.05

SNP=single nucleotide polymorphism, A1=tested risk allele, C1=cohort 1, OR=odds ratio, 95% CI=95% confidence interval, C2=cohort 2, P-value= significance (0.000387), borderline significance (0.05), Meta=meta-analysis of cohort 1 and cohort 2.

Table 4.6 Borderline significant associations of *FTO* SNPs and risk of AML in normal weight European American

SNP	A1	OR (C1)	95%CI (C1)	P (C1)	OR (C2)	95%CI (C2)	P (C2)	P (Meta)
rs12931859	T	0.69	0.51-0.96	0.025	1.18	0.71-1.95	0.52	0.02
rs1345390	T	0.76	0.62-0.93	0.008	1.12	0.80-1.57	0.51	0.008
rs16952649	T	1.36	1.00-1.84	0.04	1.13	0.68-1.86	0.64	0.05
rs2003583	T	0.76	0.59-0.96	0.02	1.10	0.73-1.65	0.64	0.03
rs2111118	C	0.75	0.61-0.92	0.005	1.13	0.80-1.58	0.49	0.006
rs2665275	T	0.83	0.62-1.11	0.21	1.72	1.00-2.95	0.05	0.04
rs4396532	A	1.52	1.00-2.30	0.05	0.39	0.16-0.97	0.044	0.007
rs4783826	G	0.78	0.64-0.94	0.01	1.31	0.94-1.85	0.11	0.003
rs7186220	A	0.71	0.56-0.91	0.006	1.10	0.73-1.65	0.66	0.009
rs7194243	T	0.75	0.60-0.93	0.009	1.11	0.76-1.61	0.59	0.01
rs7200972	G	1.33	1.09-1.63	0.005	1.14	0.81-1.62	0.45	0.004
rs8046658	C	0.73	0.59-0.89	0.003	1.08	0.77-1.52	0.64	0.004
rs8053966	C	1.28	0.99-1.65	0.05	0.87	0.56-1.34	0.52	0.04

SNP=single nucleotide polymorphism, A1=tested risk allele, C1=cohort 1, OR=odds ratio, 95% CI=95% confidence interval, C2=cohort 2, P-value= significance (0.000387), borderline significance (0.05), Meta=meta-analysis of cohort 1 and cohort 2.

Table 4.7 Borderline significant associations of *FTO* SNPs and risk of AML in overweight European Americans

SNP	A1	OR (C1)	95%CI (C1)	P (C1)	OR (C2)	95%CI (C2)	P (C2)	P (Meta)
rs1345390	T	1.07	0.87-1.32	0.49	1.67	1.18-2.35	0.003	0.03
rs2003583	T	1.09	0.86-1.37	0.48	1.81	1.22-2.67	0.003	0.02
rs2111118	C	1.08	0.88-1.33	0.44	1.70	1.20-2.41	0.002	0.02
rs4784351	G	1.08	0.86-1.34	0.51	1.74	1.20-2.52	0.003	0.03
rs7193938	T	1.18	0.92-1.53	0.19	1.53	0.99-2.36	0.05	0.03
rs7200972	G	0.96	0.78-1.19	0.74	0.55	0.38-0.78	0.0008	0.03
rs8046658	C	1.07	0.87-1.32	0.49	1.57	1.11-2.22	0.01	0.04
rs8056199	A	0.99	0.80-1.22	0.91	1.97	1.39-2.79	0.0001	0.03

SNP=single nucleotide polymorphism, A1=tested risk allele, C1=cohort 1, OR=odds ratio, 95% CI=95% confidence interval, C2=cohort 2, P-value= significance (0.000387), borderline significance (0.05), Meta=meta-analysis of cohort 1 and cohort 2.

Table 4.8 Borderline significant and significant associations of *FTO* SNPs and risk of AML in obese European Americans

SNP	A1	OR (C1)	95%CI (C1)	P (C1)	OR(C2)	95%CI (C2)	P (C2)	P(Meta)
rs10521307	G	0.57	0.44-0.73	6.95E-06	0.74	0.51-1.07	0.11	**3.30E-06**
rs10852521	T	0.69	0.56-0.86	0.0009	0.84	0.58-1.20	0.33	0.0008
rs10852523	C	1.34	1.1-1.68	0.008	1.24	0.87-1.78	0.23	0.004
rs11075994	A	0.78	0.62-0.98	0.03	0.87	0.60-1.26	0.46	0.02
rs11075996	T	1.36	1.09-1.70	0.005	1.25	0.87-1.79	0.22	0.002
rs11075997	T	1.34	1.07-1.67	0.009	1.24	0.87-1.76	0.24	0.004
rs1121980	A	1.55	1.25-1.93	6.15E-05	1.44	0.99-2.08	0.05	**1.03E-05**
rs11642841	A	1.55	1.24-1.93	0.0001	1.49	1.04-2.15	0.03	**8.97E-06**
rs12149832	A	1.64	1.32-2.04	1.01E-05	1.4	0.98-2.00	0.06	**2.40E-06**
rs1421085	C	1.66	1.34-2.07	5.05E-06	1.49	1.03-2.16	0.03	**5.77E-07**
rs17218700	A	0.65	0.48-0.88	0.006	1.16	0.67-2.0	0.60	0.009
rs17219084	G	1.33	1.07-1.67	0.01	1.32	0.92-1.91	0.13	0.003
rs17817449	G	1.66	1.33-2.07	5.84E-06	1.43	0.99-2.06	0.05	**1.22E-06**
rs3751812	T	1.64	1.32-2.05	9.32E-06	1.44	0.99-2.07	0.05	**1.73E-06**
rs3826169	G	1.26	0.99-1.60	0.05	0.89	0.61-1.32	0.57	0.05
rs6499652	T	1.24	1.001-1.55	0.04	1.24	0.86-1.78	0.25	0.02
rs6499653	T	1.28	1.00-1.63	0.04	1.14	0.78-1.67	0.48	0.03
rs7193144	C	1.64	1.32-2.05	8.27E-06	1.43	0.98-2.06	0.05	**1.75E-06**
rs7205009	T	1.25	1.01-1.56	0.04	1.28	0.88-1.83	0.18	0.01
rs8044769	T	0.69	0.56-0.86	0.001	0.87	0.61-1.24	0.45	0.001
rs8050136	A	1.66	1.33-2.07	7.32E-06	1.43	0.99-2.07	0.05	**1.48E-06**
rs8061518	G	0.57	0.45-0.72	4.46E-06	0.88	0.62-1.25	0.50	**2.24E-05**
rs9922619	T	1.55	1.24-1.93	9.96E-05	1.42	0.98-2.04	0.06	**1.85E-05**
rs9922708	T	1.57	1.26-1.95	5.46E-05	1.39	0.97-1.99	0.07	**1.26E-05**
rs9930333	G	1.57	1.26-1.95	4.47E-05	1.44	0.99-2.09	0.05	**7.25E-06**
rs9936385	C	1.64	1.32-2.04	9.65E-06	1.43	0.99-2.07	0.05	**1.90E-06**
rs9939973	A	1.57	1.26-1.95	4.59E-05	1.44	0.99-2.09	0.05	**7.39E-06**

SNP=single nucleotide polymorphism, A1=tested risk allele, C1=cohort 1, OR=odds ratio, 95% CI=95% confidence interval, C2=cohort 2, P-value= significance (0.000387), borderline significance (0.05), Meta=meta-analysis of cohort 1 and cohort 2. **Bold=Significant**

Table 4.9 Borderline significant and significant *FTO* SNPs associated with risk of AML using an interaction term in European Americans

SNP	BP	A1	P (Meta)
rs9922708	53831146	T	7.13E-05
rs9939973	53800568	A	0.0001
rs9930333	53799977	G	0.0001
rs1121980	53809247	A	0.0001
rs1421085	53800954	C	0.0002
rs12149832	53842908	A	0.0002
rs9922619	53831771	T	0.0002
rs17817449	53813367	G	0.0002
rs8050136	53816275	A	0.0003
rs3751812	53818460	T	0.0003
rs8061518	53861024	G	0.0003
rs7193144	53810686	C	0.0004
rs11642841	53845487	A	0.0007
rs9936385	53819169	C	0.0004
rs11642841	53845487	A	0.0007
rs10521307	53865701	G	0.0008
rs6499653	53877592	T	0.002
rs3826169	53860481	G	0.007
rs8044769	53839135	T	0.01
rs10852521	53804965	T	0.02
rs17820875	53926790	G	0.02
rs7199716	54033248	T	0.02
rs2665271	54127879	C	0.02
rs7191718	53911023	C	0.02
rs4783826	53961354	G	0.03
rs17236708	54128426	G	0.03
rs7200972	54036352	G	0.03
rs7194243	54056159	T	0.03
rs17219084	53855600	G	0.04
rs6499640	53769677	G	0.05
rs7203521	53769293	G	0.05

SNP=single nucleotide polymorphism, BP=base-pair, A1=tested risk allele, significance (0.0004), borderline significance (0.05), Meta=meta-analysis of cohort 1 and cohort 2. **Bold=Significant**

CHAPTER 5

DISCUSSION

Acute Myeloid Leukemia (AML) is a rare blood cancer with low survival rates for which there is limited understanding of etiology. Current treatments include aggressive chemotherapy, radiation, and blood and marrow transplants. Identification of *FTO* SNPs associated with risk of AML can lead to better risk stratification and further etiological understanding of AML. Our hypothesis states that the presence of *FTO* SNPs would increase the risk of AML. We did not find many SNPs that passed the multiple testing significance level except those found among obese participants. Our main results show that there are at least borderline significantly associated *FTO* SNPs related to increased risk of AML in all other categories tested, but none were significantly associated within African Americans or Hispanics or those who are under-weight, normal-weight, or overweight. We also identified two SNPs that showed significant inverse associations with AML.

We used cases aged 0-70 years and unrelated healthy adult controls from the DISCOVeRY-BMT cohorts to examine inherited germline risk of AML through *FTO* SNPs. In our statistical analysis approach, we used two significant values to identify borderline significant associations or significant associations already discussed within our results section. We found 63 borderline significant SNPs associated with increased risk of

AML and 13 significant SNPs (rs9939973, rs1121980, rs12149832, rs11642841, rs1421085, rs17817449, rs7193144, rs8050136, rs9922619, rs9922708, rs9930333, rs9936385, rs10521307, rs3751812) associated with increased risk of AML. The significant SNPs were all associated with AML only among obese European Americans.

In our interaction term analysis, we found 18 borderline significant and 12 significant *FTO* SNPs (rs9922708, rs9939973, rs9930333, rs1121980, rs1421085, rs12149832, rs9922619, rs17817449, rs8050136, rs3751812, rs8061518, rs7193144) associated with risk of AML. This supports building evidence that BMI may play a role in risk of AML. SNPs that significantly interacted with BMI included: rs1421085, rs17817449, rs3751812, rs9939973, rs9930333, rs1121980, rs12149832, rs9922619, rs8050136, rs8061518, rs9922708, and rs7193144. Many significant SNPs found in this study correspond with recent literature as being related to early onset or childhood risk of obesity (rs3751812, rs1121980, rs9939973),[54,56,66] increased risk of obesity in European Americans (rs9930333, rs17817449, rs7193144, rs1421085, rs1121980, rs3751812, rs8050136),[57,58,60,62,64,66] and increased risk of obesity in other races and ethnicities including Mexican (rs1121980, rs17817449, rs9930506),[59] Spanish (rs9930333),[56] Asian (rs8050136),[61] and Japanese(rs1121980).[63] *FTO* seems to have an innate effect on body weight and risk of obesity which is a risk factor for AML. More studies are needed to highlight potential links between the *FTO* gene and risk of AML in higher adiposity individuals.

Additional findings include four more significant *FTO* SNPs associated with increased risk of AML which were all found within obese European Americans and were not supported by any current literature. We also found 26 borderline significant SNPs

associated with AML. The identified SNPs could provide insights into the pathology of AML and lead to targets for treatment or prevention of this blood cancer. Further research on the *FTO* SNPs found in this study would be beneficial in the studying and advancement of AML etiology.

Strengths of our study include using the Center for International Blood and Marrow Transplant Research (CIBMTR). Since this is a national database, it is more generalizable and provides good external validity by allowing us to capture 99% of all AML diagnoses who received an unrelated donor blood transplant within the United States.[52] Also, our second cohort of the DISCOVeRY-BMT would be best for replication and helps with internal validity. One limitation includes the limited sample size of Hispanic, African American, and Asian American populations within our cohorts. This limited our ability to further stratify by BMI categories within these races/ethnicities. Another limitation is we do not have any comorbidity information pertaining to our study subjects meaning we were not able to control for it nor the genetic susceptibility that comes with said comorbidities. We also did not have a continuum of BMI measurements, we only had BMI taken at diagnosis where BMI could have changed throughout treatment, thus individuals BMI classification may have misclassification bias. While the focus of this study is not survival, we want to highlight the obesity paradox which states that obesity in older subjects or those with chronic diseases may be protective or experience decreased mortality.[67] Another limitation includes our controls not including pediatrics nor older adults. BMT controls must go through a rigorous health screening making them above average in health making older adults less likely to be able to participate as a control. There were no pediatric controls compared to cases since legally

individuals cannot donate blood marrow until they are 18 years old. Future research is needed with a more generalizable set of controls.

This study has shown that some *FTO* SNPs may have an impact on the etiology of AML, especially ones related to increased adiposity. Obesity has been linked to many different cancers, but not has not been strongly linked to AML. *FTO* is mainly expressed in adipose and skeletal tissues as well as the hypothalamus. SNP rs9939609 A/T is a common *FTO* SNP associated with obesity where 16% of adults who carried this risk allele gained 3kg of weight and risk of obesity increased by 1.67 times.[21]*FTO* proteins are involved in both adipogenesis and tumorigenesis by m6-A dependent demethylase activity which influences RNA processing.[29,70,71,72] *FTO* is shown to be highly expressed in cancer tissues playing a role of an oncogene regulating malignant phenotypes of cancer cells as well as having an impact on therapeutic responses of cancer.[29, 68,70] These studies support possible reasons behind why we only saw significance within our obese populations. By evaluating the base-pair range of *FTO* we were able to identify key SNPs associated with inherited risk of AML. Having one of the first studies researching multiple SNPs of the *FTO* gene in relation to AML, this study underlines possible SNPs never studied in relation to AML. More research is needed studying the inherited germline risk of AML to further help with clinical risk stratification, AML etiology, and thus future prevention of this rare and deadly disease.

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