

GENETIC ASSOCIATIONS IN ACUTE LEUKEMIA PATIENTS AFTER
MATCHED UNRELATED DONOR ALLOGENEIC HEMATOPOIETIC STEM
CELL TRANSPLANTATION

Dissertation

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By

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ABSTRACT

HLA-matched unrelated donor (MUD) hematopoietic stem cell transplantation (HSCT) is used as a potential curative therapy for otherwise fatal hematological cancers, including but not limited to acute and chronic leukemia, Hodgkin and non-Hodgkin lymphoma, and myelodysplastic syndrome (MDS). MUD-HSCT involves the transfer of healthy individual (donor) bone marrow stem cells to an unhealthy patient (recipient). Despite improvements in human leukocyte antigen (HLA) matching algorithms, treatment protocols and patient care overall survival (OS) rates profoundly decrease in the first 1-year post-transplant. The largest predictor of mortality during this time frame is HLA matching, emphasizing the importance of genetic variation in determining survival. Despite the clear importance of genetics to survival following transplant, the relationship of non-HLA genetic variation remains underexplored. In this dissertation, we characterized and explored the independent and joint non-HLA genetic contributions to survival outcomes in donors and recipients with acute leukemias or MDS using pre-existing genome-wide association study (GWAS), *Determining the Influence of Susceptibility COneying Variants Related to one-Year mortality after BMT* (DISCOVeRY-BMT), a study of 3,532 donor-recipient pairs with survival, clinical and genomic data available.

We used DISCOVeRY-BMT to replicate or validate all previous literature that investigated survival outcomes after allogeneic HSCT. None of the previous literature was reproduced, likely due to the previous cohorts comprising heterogeneous samples (testing with multiple diseases) or small sample sizes. Next we developed an R/Bioconductor software package called gwasurvivr. This package takes input from popular genomics software and performs multivariate Cox regression sur-

vival analysis in a scalable and efficient manner. We integrated gwasurvivr into an automated pipeline that performs full survival GWAS analyses and conducts meta-analysis. We conducted this on numerous subsets of DISCOVeRY-BMT. Here, we report only post-HSCT genetic associations for acute lymphoblastic leukemia (ALL) subset of DISCOVeRY-BMT GWAS. We found SNPs associated to recipients for the outcomes overall survival (OS), disease related moratlity (DRM), and organ failure (OF). We also found donor SNPs associated with OS, transplant-related moratlity (TRM), DRM, and graft-versus host disease (GVHD). Lastly, we probed our findings for biological relevance (affecting gene expression or transcription factor binding) by annotating associated SNPs using publicly available databases.

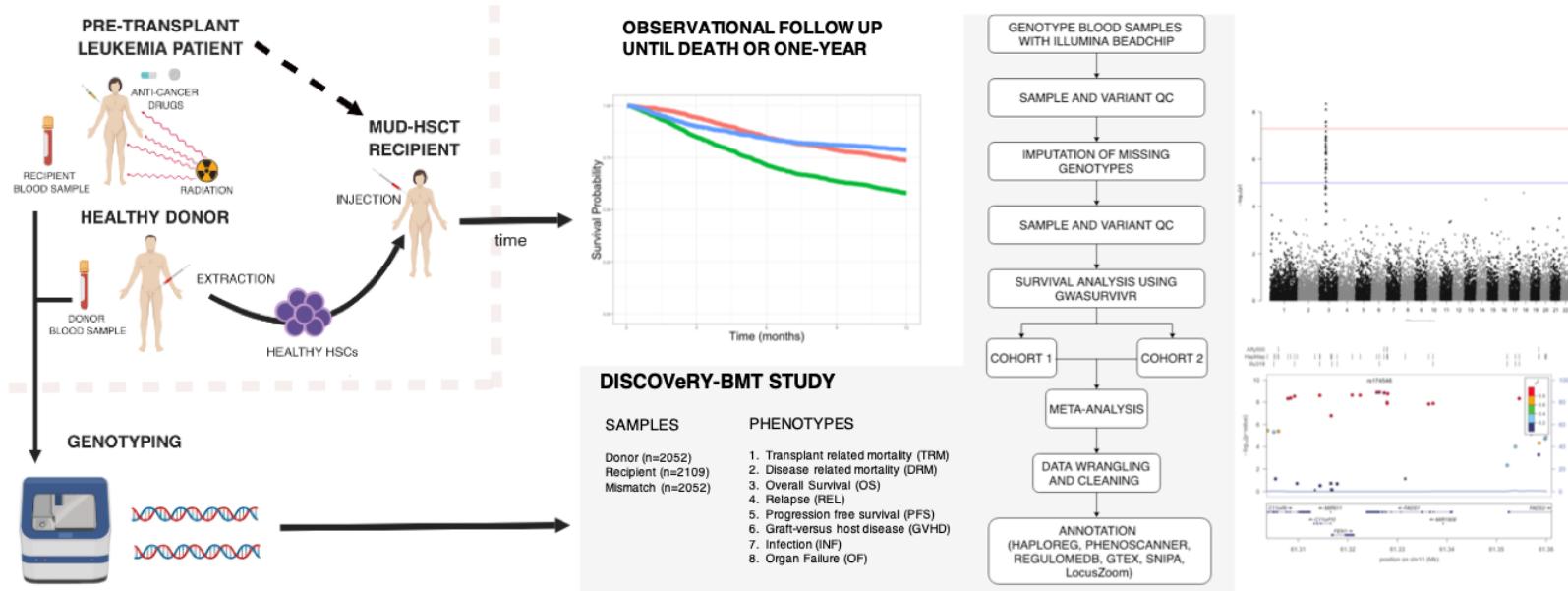


Figure 0.1: Graphical Abstract. The flow of the abstract begins on the upper left, illustrating the HSCT process of a patient receiving chemotherapy and/or radiation prior to kill all of the patients bone marrow cells prior to allogeneic HSCT. An HLA matched donor donates their healthy hematopoietic stem cells (HSCs) to the recipient. We genotyped both the donor and recipient blood samples for a GWAS testing for associations with survival outcomes. The gray boxes describe the DISCOVeRY-BMT phenotype and sample sizes, as well as our workflow pipeline. The graphical figures on the far right are typical output after GWAS (Manhattan plot and regional plot) to visualize results.

This is dedicated to Ezgi,
my parents, my siblings, and their families,
for their endless love and support.
And to my sweet little pooch Bernie.

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Fields of Study

Major Field: Pharmaceutical Sciences

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CHAPTER 1: Introduction

Broadly, this dissertation examines the role of germline genetic variation and survival outcomes in the matched unrelated donor (MUD) hematopoietic stem cell transplantation (HSCT) patients with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), or myelodysplastic syndrome (MDS) who received HSCT as curative therapy. In addition, this dissertation also seeks to enhance the computational workflows that are used in to identify genetic variants associated with worse (or better survival) after HSCT. The significance is three-fold, first, we can identify clinically relevant markers that may improve donor selection beyond traditional methods; second, we can characterize pre-transplant risk of disease or transplant related death within the first year; and third, we can help facilitate other researchers studying similar problems with similar data or data structures by developing open-source software. Six chapters comprise my dissertation. Chapter 1 first introduces genetic association studies, allogeneic HSCT and DISCOVeRY-BMT genome wide-association study (GWAS), including the corresponding clinical data in detail. Chapter 2 will discuss a replication and validation of genetic variants shown to be significantly associated with survival outcomes after transplant; this work is has been published. Chapter 3 discusses in detail the R package that we developed to perform genome wide survival analyses; this work has been published and the package freely available. Chapter 4 is the application of the R package and custom pipeline developed to perform large automated GWAS, specialized to our lab, but importantly generalizable in a broader (and larger context). Chapter 5 discusses the discovery and inference of markers of survival in ALL donor and recipient following transplant. This dissertation ends with Chapter 6, which briefly

reviews the contents of this dissertation, discusses ongoing research in our laboratory and the future directions that should be undertaken.

1.1 Genetic Association Studies

Genetic association studies test for correlations between genetic variation as it relates to disease risk or to physical quantitative traits (i.e. height or weight) (C. M. Lewis and Knight 2012). These studies have been successful in identifying variants associated disease susceptibility or drug response and have helped us understand that many diseases have complex genetic signatures that need to be further understood (Visscher et al. 2012). The human genome consists of over 3 billion base pairs, all of which are contained in every nucleated cell in the body. A genome sequence is the complete collection of all nucleotides (A, C, T, or G for DNA genomes) that make up all the chromosomes in individuals or species (Lander et al. 2001). The vast majority of nucleotides (>99.5%) are identical between individuals within a species, however, genetic variation arises within individuals and populations over time and different spaces. Indeed, the fundamental source of genetic variation is mutation, where permanent alterations occur to a single nucleotide or larger structural changes in the genome of a species. A variant at single position (locus) in a DNA sequence that occurs in at least 1% of a population is called a single nucleotide polymorphism (SNP). SNPs are the most widely used marker to describe genetic variation. Larger structural variations may include microsatellite regions, insertion/deletions (indels), copy number variations (CNVs), or variable-tandem repeats (VNTRs) all of which have importance in understanding the genetic architecture and disease etiology (Sudmant et al. 2015). For purposes of this document, unless otherwise specified, genetic variation will refer to single nucleotide polymorphisms (SNPs). SNPs will also be referred to as simply as polh-

morphisms, genetic markers, or markers interchangeably.

Different forms of the same variant are called alleles. For diploid organisms, one allele is passed from each parent. At a given locus in the genome, individuals can have two copies of the same allele (called homozygous) or two different copies of alleles (heterozygous). Sequence variations can occur coding regions of genes, non-coding regions of genes, or intergenic regions (between genes). SNPs in coding regions are synonymous, meaning the amino acid sequences are the same and therefore do not change protein structure, or non-synonymous SNPs, the change in basepair changes the amino acid sequence. Non-synonymous SNPs are further stratified into two types: missense SNPs, which result in codon changes that code for different amino acids (Z. Shi and Moult 2011) and nonsense SNPs, which result in a premature stop codon that often yield a non-functional or truncated protein product. Most polymorphisms are in non-coding regions, however despite the fact there is not an immediately obvious consequence they may alter important transcriptional properties such as gene splicing, transcription factor binding, or messenger RNA (mRNA) decay (Green et al. 2003). SNPs may affect gene expression (and may be upstream or downstream from a gene) are called expression quantitative trait loci (eQTL).

Over the past two decades, research has increasingly evolved from looking at specific regions of interest (candidate gene association studies) to more agnostic approaches that investigate larger portions of the genome, such as genome wide association studies (GWAS), whole-exome sequencing studies (WES studies) and whole-genome sequencing studies (WGS studies) (Timpson et al. 2018). This dissertation is primarily focused on GWAS, specifically the application of using GWAS in the context of identifying common variants in after hematopoietic stem cell transplantation (HSCT), where more details will be discussed in subsequent subsections

of this chapter.

GWAS employ genotyping microarrays to measure genetic variation – and they have become the standard platform in academic and industry to test for association of phenotype with common genetic variants. Common genetic variants are defined as those with a *minor allele frequency* (MAF) of $\geq 1\%$ and rare variants are defined as those with a MAF \leq than 1%. Genotyping microarrays are designed to contain common variants but optionally can contain rare variants. GWAS ask if the allele of a genetic variant is found more often than what would be expected than by random chance in individuals with the phenotype of interest (e.g. the disease being studied). If the variant (one allele) occurs more in those affected by the disease than those without the disease, then the variant deemed as being *associated* with the disease. Nonetheless, GWAS have been very successful at revealing new pathways involved in disease, but often the post-GWAS understanding of the associations is poorly understood. That is, identification of causal variants, biological relevance and the interaction that these associations have with other genetic or environmental factors.

1.1.1 Linkage Disequilibrium

GWAS are heavily based on the principal of linkage disequilibrium (LD) at the population level. LD is the non-random dependence of allele frequencies at two more loci in the general population (Jorde 2000). LD reflects the relationship between alleles at different loci. In other words, LD is a measure of two alleles or specific sequences being inherited together. The unit of measure for LD is r^2 (squared correlation coefficient) (J. K. Pritchard and Przeworski 2001). In general, loci that are in close proximity exhibit stronger LD (J. K. Pritchard and Przeworski 2001). Perfect LD ($r^2 = 1$) means that no recombination occurred on this chunk

of genome. Regions that are further apart on a chromosome exhibit weaker LD (D. E. Reich et al. 2001). Low LD means that recombination occurred and that there are lots of possible rearrangements that may have occurred during meiosis. LD decay influences the number of SNPs needed to “tag” a haplotype, and that number of SNPs is just a small subset of the number of segregating polymorphisms in the population (D. E. Reich et al. 2001). Knowledge of haplotype structure makes it possible to retrieve more information from GWAS. Tagging SNPs with known haplotype block structure can capture much of the genetic information in a region.

With the rapid growth of genetic association studies and statistical methods assessing genetic variation, researchers routinely exploit LD to map regions in the human genome. While costs of genotyping have lowered over the past decade, a major barrier to overcome when conducting large scale studies, was the expense of searching the entire genome for disease associations (International HapMap Consortium 2005). The International HapMap Project (International HapMap Consortium 2005) was the first attempt to address these challenges. HapMap was a large scale multi-institutional international project that finely mapped common genetic variation (or establish a “haplotype map”). HapMap demonstrated that genomic blocks are shared in common areas across continental population. HapMap alleviated high costs of studies by preferentially selecting ‘tag’ SNPs that covered the entire genome, and due to LD structure, inference could be drawn about nearby variants that were not genotyped (Bakker et al. 2005). HapMap ended with a catalogue across several populations for 420 haplotypes at 3.5 million SNPs (International HapMap Consortium et al. 2007). Afterwards, the 1000 Genomes Project, with similar ambitions to HapMap, aimed to create a more complete and thorough catalogue of human genetic variation, which could be leveraged for GWAS investigating disease (1000 Genomes Project Consortium 2015). The consortium aimed to dis-

cover >95% of variants with MAF as low as 1% across the genome, as well as estimate population specific allele frequencies, haplotype maps and LD patterns of alleles (1000 Genomes Project Consortium et al. 2010). The results provided a more comprehensive picture of human genetic variation than what was previously available (5,008 haplotypes at over 88 million SNPs in 26 worldwide populations) (1000 Genomes Project Consortium et al. 2010). And even more recently, the Haplotype Reference Consortium (HRC) has described nearly 65,000 human haplotypes at ~40 million SNPs via whole-gene sequence data from predominantly European ancestry (McCarthy et al. 2016). Other reference panels have been developed, particularly population specific ones, but are beyond the scope of this document.

1.1.2 Genetic Imputation

Genetic imputation (will also be simply referred to as imputation) has had significant contributions to genetic association studies. Imputation can be defined as predicting unobserved genotypes that were not directly assayed in a sample of individuals. The term refers to when a reference panel of haplotypes at set of a SNPs is used to impute SNPs that have been genotyped at a subset of that set of SNPs (Marchini et al. 2007). Genotype imputation is useful for three reasons: (1) by boosting the number of SNPs that can be tested for association, thus increasing the power of the study, (2) homogenizes variant sets for meta-analyses, and (3) help control false positive for which genotype calling is challenging (Marchini and Howie 2010).

Today, several reference panels are available, such as (but not limited to) HapMap2 (International HapMap Consortium 2005), 1000 Genomes Phase 3 (1000 Genomes Project Consortium 2015), HRC (McCarthy et al. 2016), which differ by the number of samples, sites (chromosomes 1-22, X), and number of haplotypes.

These reference panels are widely used to carry out accurate imputation in studies. HRC can impute SNPs with MAF as low as 0.1% (McCarthy et al. 2016). Most studies take a two step approach that first will impute the missing genotypes using the reference panel without consideration of the phenotype. The imputed genotypes are then tested for association with the phenotype in the second pass. Multiple phenotypes can be tested for association without the need for re-imputation.

Several imputation algorithms and software packages are available as stand-alone software, such as IMPUTE2 (B. N. Howie, Donnelly, and Marchini 2009), MaCH (Li et al. 2010), BEAGLE (B. L. Browning and Browning 2016) or from imputation web services, such as the Sanger imputation server (McCarthy et al. 2016, R. Durbin (2014)) or the University of Michigan imputation server (Das et al. 2016). The details of the imputation methods are beyond the scope of this dissertation, but very briefly, each algorithm is an extension of the hidden Markov model (HMMs) to carry out inference when modeling LD or haplotype estimation (also called phasing; N. Li and Stephens (2003)). The imputation methods vary in terms slight methodological differences in estimating haplotypes, computational performance and error rates.

1.1.3 Meta-analysis

Although GWAS have been successful at identifying novel loci that are associated to some disease or trait, the finding typically have modest effects and large sample sizes are needed to detect common variants with small effect sizes. In order to improve the power to detect variants with small effect sizes, meta-analyses have been used. Meta-analysis using summary statistics has been important for GWAS of complex genetic diseases and traits (Bakker et al. 2008). Researchers combine the effects of multiple studies without having to integrate both genotype and phe-

notype data. After imputation, meta-analysis is useful for different cohorts that are used on different genotyping chips to boost power.

A popular tool to perform meta-analysis on GWAS is METAL (Willer, Li, and Abecasis 2010). METAL can combine either test statistics and standard errors, or p-values across studies (while taking direction of effect and sample size into account). The results are combined using fixed-effects or random-effects models.

1.2 Hematopoietic Stem Cell Transplantation

Blood cells continuously go through a self-renewing maturation process from less differentiated precursor cells to mature cells in a process called hematopoiesis (Copelan 2006). The process begins with hematopoietic stem cells (HSCs) which are located in center of bone marrow. HSCs differentiate into either lymphoid or myeloid progenitor cells and further develop into one of three lineages: red blood cells (erythrocytes), lymphocytes (T-cells, B-cells, and natural killer (NK) cells), and myeloid cells (granulocytes, megakaryocytes, and macrophages). All of these blood cells have vital roles in the human body. Tumors arise from malignant stem cells that usually originate from normal stem cells but retain the self-renewal property. Leukemic cells are limited in their ability to proliferate and incessantly are replenished from leukemic stem cells. Acute leukemias are characterized by the rapid increase of immature blood cells, such that the bone marrow is unable to produce healthy blood cells. Immediate treatment is required. Acute leukemias can be treated with some combination of chemotherapy, radiation therapy or an HSCT. When all other treatment options have been exhausted, an HSCT is used as last resort.

HSCT is an established therapeutic procedure that is used as a potentially curative treatment for life-threatening congenital or acquired blood disorders

(malignant or non-malignant) (Henig and Zuckerman 2014). HSCT involves the intravenous infusion of autologous or allogeneic hematopoietic progenitor cells to restore normal function in patients whose bone marrow is compromised. Autologous HSCT involves self-donation of marrow stem cells, whereas allogeneic HSCT is when stem cells are transferred from a HLA-matched related donor (MRD) or a HLA-matched unrelated donor (MUD). Although a matched sibling donor is preferred, only approximately 30% of patients who may benefit from HSCT have such a donor available. In the United States, the number of allogeneic transplants yearly has dramatically risen over the past decade, across all diseases (M. Pasquini et al. 2013). Patients with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), or myelodysplastic syndrome (MDS), represent the largest group treated with allogeneic HSCT. While both patient care and matching has improved over the past few decades almost half of all high-resolution 10/10 HLA MUD-HSCT recipients die within one-year post-transplant due to either their disease or transplant-related causes (M. Pasquini et al. 2013). These trends also show transplant-related causes are a larger contributor to mortality within the first 100-days post-transplant and shift towards primary disease after approximately six months post-transplant (D’Souza et al. 2017). Reducing TRM without increasing risk of disease death and vice versa continue to represent a substantial clinical challenge.

The four elements of HSCT are:

1. Graft Source

The graft sources are either from bone marrow or peripheral blood stem cells (PBSCs). For bone marrow grafts, hematopoietic stem cells (HSCs) are typically extracted from the center of the posterior iliac crest (pelvis) using a large needle while the donor is under general anesthesia (Copelan 2006). HSCs are continually

going through a cycle of detaching from the bone marrow and entering circulation and back into the bone marrow, making it very convenient to use the peripheral blood as a source.

2. Graft Type

The graft can either be autologous, syngeneic, allogeneic, or from umbilical cord blood. Autologous HSCTs are self-donating, where a patient's marrow is taken and treated exogeneously. In that time the patient is treated with chemotherapy to reduce the tumor burden and then cells are donated back to the patient. Syngeneic transplants are when the donor is an identical twin. And allogeneic HSCTs are when a related or unrelated donor is the graft source. Umbilical cord blood transplants are rich in HSCs but limited in volume and are less common than the other forms of transplant.

3. HLA matching

HLA genes are closely linked on chromosome 6 and are inherited as haplotypes. HLA encodes for the major histocompatibility complex (MHC). MHC are class of molecules that are found on antigen presenting cells and are important for initiating immune response. MHC has two primary classes, class I and class II. MHC class I is encoded by HLA-A, -B, and -C. MHC class II is encoded by HLA-DP, -DM, -DOA, -DOB, -DQ, and -DR. The preferred HLA match for an allogeneic donor is high resolution typed 10/10, which means the matched alleles are at HLA-A, -B, -C, -DQ, and -DP. Survival varies considerably depending on which HLA alleles are matched. A single mismatch is a significant risk factor for development of GVHD and is associated with higher mortality and decreased survival (Hamilton and Copelan 2012).

4. Pre-transplant conditioning regimens

Patients are given pre-transplant chemotherapy to reduce tumor burden of the

leukemia. HSCTs are most successful when patients are in first complete remission (CR1). These can include myeloablative chemotherapy or reduced intensity therapy.

1.3 DISCOVeRY-BMT

Determining the Influence of Susceptibility COnveying Variants Related to one-Year mortality after Bone Marrow Transplant (DISCOVeRY-BMT) is a GWAS. This GWAS aims at identifying and characterizing non-human leukocyte antigen (HLA) genetic variation in the context of survival outcomes in acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS) patients and their matched unrelated donor (MUD) after hematopoietic stem cell transplantation (HSCT) (L. E. Sucheston-Campbell et al. 2015). HSCT is also called bone marrow transplantation (BMT) and hence both terms will be used interchangeably throughout this document. Additionally, the terms recipients and patients will be used interchangeably.

1.3.1 *Patient Characteristics*

DISCOVeRY-BMT investigates donor and recipient genetic factors that contribute to 1 year cause-specific mortality after MUD-HSCT (Hahn et al. 2015; L. E. Sucheston-Campbell et al. 2015; Clay-Gilmour et al. 2017). This GWAS comprises two independent cohorts. All patients in DISCOVeRY-BMT were reported to the Center for International Blood and Marrow Research (CIBMTR) and had banked biorepository samples (blood samples) from recipients and donors at the National Marrow Donor Program (NMDP). Data that were reported to CIBMTR was collected from 151 transplant centers in the USA. Furthermore, all patients were de-identified and approved by the Roswell Park Cancer Institute Institutional Review Board and signed informed consent for research. Although no patients were ex-

Table 1.1: Donor and Recipients Disease Proportions by Cohort in DISCOVeRY-BMT.

	Donor (N/Percent %)		Recipient (N/Percent %)	
	Cohort 1	Cohort 2	cohort 1	cohort 2
ALL	468 (23%)	93 (12%)	483 (23%)	94 (12%)
AML	1247 (61%)	478 (63%)	1282 (61%)	488 (63%)
MDS	337 (16%)	192 (25%)	345 (16%)	195 (25%)
Total	2052	763	2110	777

Shown here are disease proportions of ALL, AML and MDS in DISCOVeRY-BMT cohorts. The percentage in each cell is computed column-wise as the proportion of sample size (N) within a cohort across disease group.

cluded based on gender, age, or race, over 90% of DISCOVeRY-BMT cohorts were of self-reported European American ancestry. Additional exclusion criteria included: umbilical cord blood graft, *ex vivo* T-cell depleted graft or the patient underwent a prior autologous or allogeneic transplant.

Blood samples were genotyped and comprise the genotype data is the “genetic data” that is reported throughout this dissertation. In preparation for this GWAS, all post-mortem cause-specific deaths of the patients were reviewed and judged by a post-mortem by an expert panel (Hahn et al. 2015). The review and adjudicated of cause of death was conducted because many of the 151 centers the data are inconsistent in reporting and classification of outcome attributions. Thus the panel was established for concordance in cause of death, logic, and quality. Re-assessment by the panel was performed using autopsy reports and death certificates. The panel consisted of three HSCT oncologists and a HSCT clinical epidemiologist (Hahn et al. 2015).

Both cohorts comprise AML, ALL, or MDS patients who were T-cell replete and treated with myeloablative (MA) or reduced intensity (RIC) conditioning regimens prior to transplant. Cohort 1 included 2110 HSCT recipients and 2052 10/10 HLA matched unrelated donors (matched at HLA-A, -B, -C, -DRB1, and -DQB1) from 2000 to 2008 (Table 1.1). Cohort 2 included 763 donors and 777 recipients

from the years 2000 to 2011 (Table 1.1). A subset of patients ($N = 281$) in cohort 2 were 8/8 HLA matched (matched at HLA-A, -B, -C, -DRB1), while the remaining patients ($n=496$) had the same matching criteria as Cohort 1 and are 10/10 HLA matched.

The proportion of patients with AML is relatively consistent between cohorts at approximately 60% of patients (Table 1.1). However, the proportion of ALL and MDS patients shifts between cohorts. For ALL, in cohort 1 the proportion is 23%, while in cohort 2, the proportion is 12% (Table 1.1). Similarly, for MDS, in cohort 1 the proportion of patients with this disease is 16% and in cohort 2 it is 25% (Table 1.1). For all future analyses in this dissertation, MDS is never analyzed alone. MDS is either analyzed with ALL and AML (AML + ALL + MDS), or with AML (AML + MDS).

1.3.2 Survival Outcome Definitions

The survival outcomes are cause-specific deaths during the first year post-HSCT that were reviewed and re-assessed with high confidence (described above). The survival outcomes included are: overall survival (OS), disease related mortality (DRM), and transplant related mortality (TRM), progression-free survival (PFS) and relapse (REL) (Table 1.2).

TRM subtypes were further stratified into graft-versus-host disease (GVHD), organ failure (OF), infection (INF), or other. Other causes of death included more rare events, such as (but not limited to) secondary malignancies, primary or secondary graft failure, or hemorrhage. Despite being adjudicated, TRM subtypes are difficult to differentiate from one another, and some times more than one may present in the autopsy or report. The boundaries aren't necessarily clear so there may be overlap between these patients. Nevertheless, GVHD deaths included

Table 1.2: Definitions of Survival Outcomes

Outcomes	Definitions
Overall Survival (OS)	defined as any patient (recipient) that died at any point within the first 12 month window post-HSCT of this observational study.
Transplant Related Mortality (TRM)	defined as any cause of death except the underlying disease, pre-existing disease, accidental death or suicide, or death unrelated to the transplant.
Disease Related Mortality (DRM)	broadly defined as deaths relating to leukemia/MDS relapse/progression, including death attributed to toxicity or infection from anti-leukemic treatments post-HSCT.
Progression Free Survival (PFS)	is defined as the time to relapse. All patients were analyzed as time to progression of disease
Relapse (REL)	patients who were not in CR pre-HSCT and the disease returns (relapse) after HSCT.

acute or chronic GVHD, conditional upon a patient actively receiving treatment for GVHD at the time of death. Deaths that were considered to be infection were identified as arising from bacterial, fungal, viral, and/or protozoan organisms that caused organ damage. Of deaths were defined as organ toxicity relating to the transplant and not due to disease progression, GVHD, or infection.

The phenotypes that are tested in DISCOVeRY-BMT are stratified into different disease groups:

1. Mixed Disease – which includes AML, MDS, and ALL (the full cohort).
2. AML + MDS – the myeloid malignancies being grouped together, excluded the lymphoid lineage (ALL)
3. AML only – includes only AML
4. ALL only – includes only ALL
5. MDS only – includes only MDS. This will not be tested or discussed in

much detail.

In agreement with published CIBMTR statistics, about 40% of patients die after 1 year in both DISCOVeRY-BMT cohorts (Table 1.3, Figure 1.1). Patients dying from transplant related causes is about 22% between both DISCOVeRY-BMT cohorts (Table 1.3). Similarly, about 18-19% of patients die of their disease within the first year after transplant (Table 1.3). Dying due to disease is the leading cause of death in AML + MDS and AML only as well. Conversely, for ALL, transplant related mortality is a larger contributor to overall death than the other subgroups. Progression free survival is about 50% for the full cohort and disease subsets. AML alone has the most relapse compared to all other groups.

Most TRM events happen early and then DRM supersedes TRM as the year progresses (after about 6 months) for all diseases and myeloid subtypes (Figure 1.1A). The AML only curve shows disease contributing to death early on and that if AML patients die after 6 months, DRM begins to equilibrate with TRM (Figure 1.1A). For ALL

Additional information that is included from the CIBMTR were recipient/donor age, recipient/donor sex, recipient BMI, graft source, Karnofsky performance score, disease status (early, intermediate, advanced), year of HSCT, and conditioning regimens given prior to transplant. These meta-data will be incorporated in the statistical models that will be discussed in the next subsection as well as other chapters (Figure 1.2).

1.3.3 Genotyping and Quality Control

All samples were genotyped on a Illumina Human OmniExpress BeadChip whole-genome genotyping microarray. This chip had 637,655 tagged SNPs available that were strategically selected from all three phases of the HapMap project to cap-

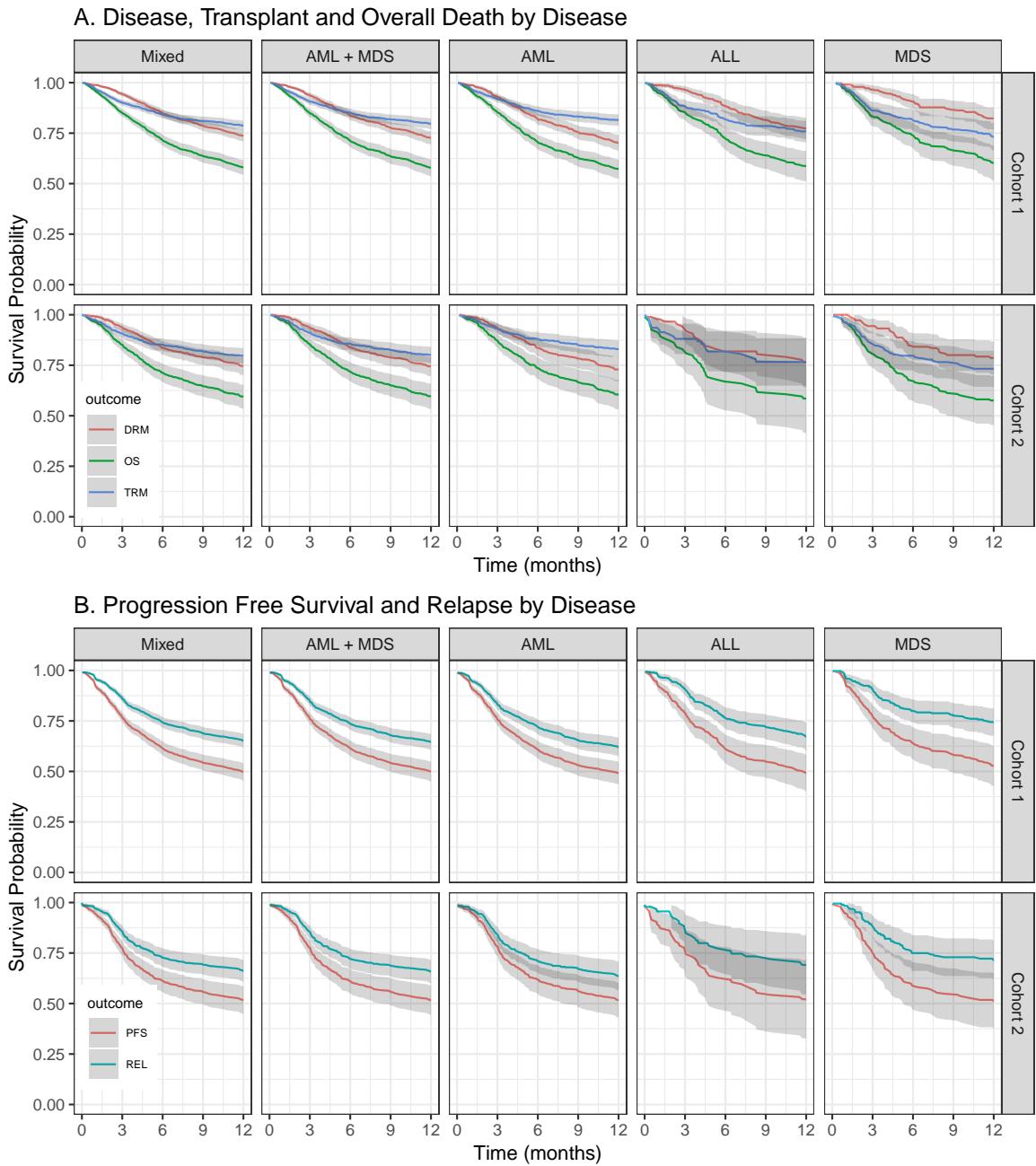


Figure 1.1: Survival curves for all disease groups being tested (Mixed disease, AML+MDS, AML only, and ALL only). The x-axis is survival probability. The y-axis is time in months. Cohorts 1 and 2 are shown in the left and right panel respectively. The gray shaded areas are 95 percent confidence intervals. (A) Red is disease-related mortality (DRM), green is overall survival (OS), and blue is transplant related mortality (TRM). (B) Cyan is relapse and red is progression free survival (PFS)

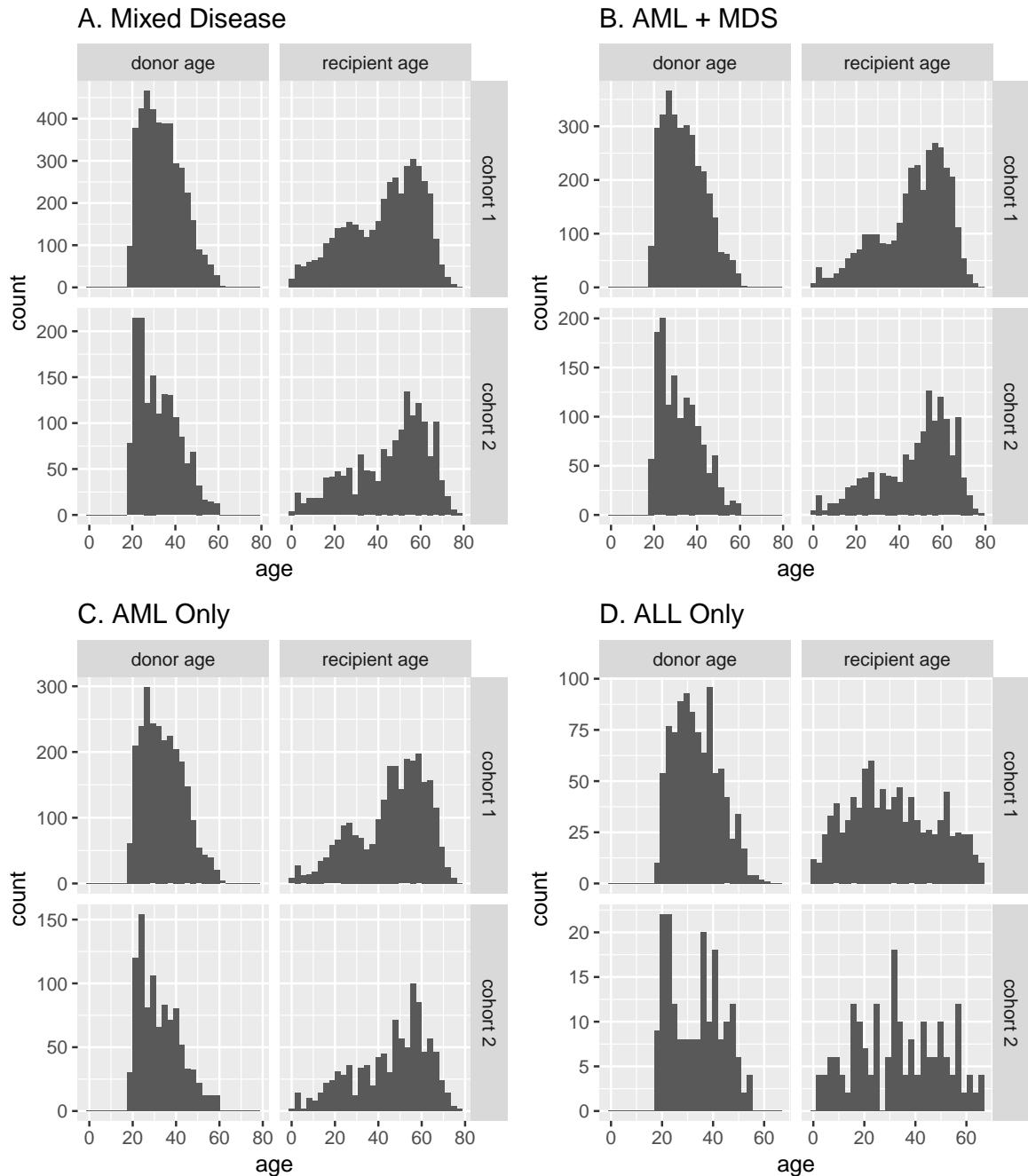


Figure 1.2: Histogram of Donor and Recipient Age Distributions in (A) Mixed Disease (AML, ALL, and MDS), (B) AML + MDS, (C) AML only, and (D) ALL only.

ture the greatest amount of common SNP variation (>5% MAF).

Samples were assigned to plates to ensure the even distribution of patient characteristics and potential confounding variables using Optimal Sample Assignment Tool (OSAT), an R/Bioconductor software package (Yan et al. 2012). Over 90% of DISCOVeRY-BMT patients self-reported as European American, caucasian or white and thus replication and validation analyses are performed on these recipient-donor pairs.

An important concept in statistical genetics is *population stratification* and the necessity to adjust for it when doing association studies. The association could be due to the underlying structure of the population and not a disease associated locus. QC is done to control from those using one of several different available software. Stringent quality control was performed on both samples and SNPs within this population. Population outliers were removed using EIGENSTRAT (A. L. Price et al. 2006) ($n = 73$). Additional sample quality control removed samples with missing call rate $\geq 2\%$ ($n = 54$), sex mismatch ($n = 9$), abnormal inbreeding coefficients ($n=20$), and evidence of cryptic relatedness ($n = 17$), yielding 2107 and 777 donor-recipient pairs in cohorts 1 and 2, respectively. Typed SNPs were removed if the call rate was $<98\%$, there was deviation from Hardy-Weinberg equilibrium proportions (C. C. Laurie et al. 2010) or discordance between duplicate samples was $> 2\%$.

Originally, DISCOVeRY-BMT was imputed using IMPUTE2 and 1000 Genomes Phase 3 data. Again, as the most of the population are of European ancestry, when HRC was released, DISCOVeRY-BMT was reimputed using HRC to have higher quality imputation specific to this population due large number of European samples in HRC.

1.4 Statistical Analysis

1.4.1 Cox Proportional Hazards Model

Survival models examine the time it takes for events to occur. Specifically, survival models examine the relationship between survival (time that passes before some event occurs) and one or more *covariates* (predictors) that may be associated with that quantity of time. The Cox proportional hazards regression model (Cox 1972) is used for survival analysis and is our main statistical model of choice. For GWAS, we conduct single SNP analysis such that each individual SNP is tested under the null hypothesis of no association. SNPs are converted from genotype probabilities to allele dosage and added to the model as a covariate.

Assumptions of the Cox model:

1. the regression coefficient (β) is constant over time (proportional hazards assumption)
2. linear combination of covariates
3. link function is exponential

1.4.1.1 Mathematical concepts and notations

The Cox model is as follows:

Consider a population of subjects, i , we observe either time to event or censoring. For the censored individuals, we know that time to event is greater than censoring time. So the survival function is $S(t)$. Let T represent survival time. T is a random variable:

Cumulative distribution function (CDF):

$$P(t) = \Pr(T \leq t) \tag{1.1}$$

Probability density function (PDF):

$$p(t) = \frac{dP(t)}{dt} \quad (1.2)$$

The survival function $S(t)$ is the complement of the CDF:

$$S(t) = Pr(T \geq t) = 1 - P(t) \quad (1.3)$$

and the hazard function is $\lambda(t)$ (or age specific failure rate). The hazard function, $\lambda(t)$, is the distribution of survival times, which assesses the instantaneous risk of dying at time t , conditional on survival to that time:

$$\begin{aligned} \lambda(t) &= \lim_{\Delta t \rightarrow 0} \frac{Pr[t \leq T < t + \Delta t] | t \geq T]}{\Delta t} \\ &= \frac{f(t)}{S(t)} \end{aligned} \quad (1.4)$$

Let $X_i = X_{i1}, \dots, X_{ip}$ be realized values of covariates for subject i . The hazard function for the Cox model has the form:

$$\begin{aligned} \lambda(t|X_i) &= \lambda_0(t) \cdot \exp(\beta_1 X_{i1} + \dots + \beta_p X_{ip}) \\ &= \lambda_0(t) \cdot \exp(X_i^T \cdot \beta) \end{aligned} \quad (1.5)$$

This expression gives us the hazard function at time t for subject i with covariate vector X_i . The baseline hazard is a nuisance parameter and is completely removed. For simplicity, we assume that there are no tied failure times, although there are methods for modifying the partial likelihood in the case of ties (Breslow 1974, Efron (1977)).

The probability of the event to be observed occurring with subject i at time Y_i :

$$\begin{aligned}
L_i(\beta) &= \frac{\lambda(Y_i|X_i)}{\sum_{j:Y_j \geq Y_i} \lambda(Y_j|X_j)} \\
&= \frac{\lambda_0(Y_i) \exp(X_i^T \cdot \beta)}{\sum_{j:Y_j \geq Y_i} \lambda_0(Y_j) \exp(X_j^T \cdot \beta)} \\
&= \frac{\exp(X_i^T \cdot \beta)}{\sum_{j:Y_j \geq Y_i} \exp(X_j^T \cdot \beta)}
\end{aligned} \tag{1.6}$$

where $L_i(\beta)$ is between 0-1. This is the partial likelihood function which estimates the beta coefficients without having to model a time-dependent hazard function.

Assume the subjects are independent of one another (Cox 1972), the joint probability of all realized events takes the partial likelihood form of (occurrence of event C is $C_i = 1$):

$$L_i(\beta) = \prod_{i:C_i=1} L_i(\beta) \tag{1.7}$$

We can take the log of this begin estimating the parameters via log partial likelihood:

$$\ell(\beta) = \sum_{i:C_i=1} \left(X_i^T \beta - \log \sum_{j:Y_j \geq Y_i} \exp(X_j^T \cdot \beta) \right) \tag{1.8}$$

We can estimate the model parameters by computing the first order derivative

of the log partial likelihood (score function):

$$\ell'(\beta) = \sum_{i:C_i=1} \left(X_i \frac{\sum_{j:Y_j \geq Y_i} \exp(X_j^T \cdot \beta) X_j}{\sum_{j:Y_j \geq Y_i} \exp(X_j^T \cdot \beta)} \right) \quad (1.9)$$

And the Hessian matrix is attainable by computing the second order derivative of the log partial likelihood:

$$\ell''(\beta) = - \sum_{i:C_i=1} \left(\frac{\sum_{j:Y_j \geq Y_i} \exp(X_j^T \cdot \beta) X'_j X_j}{\sum_{j:Y_j \geq Y_i} \exp(X_j^T \cdot \beta)} - \frac{[\exp(X_j^T \cdot \beta) X_j] [\exp(X_j^T \cdot \beta) X'_j]}{[\exp(X_j^T \cdot \beta)]^2} \right) \quad (1.10)$$

The partial likelihood is maximized using the Newton-Raphson method. Furthermore, standard errors estimates (β) can be approximated by computing inverse Hessian matrix (an approximation of the variance-covariance matrix) and taking the diagonal. The standard errors estimates compute the confidence intervals for the hazard ratio (HR).

$$\text{HR} [95\% \text{CI}_{\text{LB}}, 95\% \text{CI}_{\text{UB}}] = \exp(\hat{\beta}) [\exp(\hat{\beta} - 1.96 \cdot se), \exp(\hat{\beta} + 1.96 \cdot se)] \quad (1.11)$$

HRs are reported with every survival analysis result that we discuss throughout this dissertation. HRs describe the risk over the entire study period. The interpretation in statistical genetics is that, if $\text{HR} > 1$ we report that as risk of the effect allele, if $\text{HR} < 1$, we report the HR as protected for a given effect allele.

1.4.1.2 Model Diagnostics

The Cox model can be evaluated in two ways. The proportional hazards assumption can be tested using Schoenfeld residuals graphically or using a goodness-of-fit test (Schoenfeld 1982). The model itself can be validated by simulation. Here will we show both methods. Schoenfeld residuals are based on the effects of the predictor variables that are assumed to be independent of time, plotting these residuals versus time is done to visually assess the effect of the predictor variable and its relationship with time. A smooth line is fit to the plot of the residuals (Grambsch and Therneau 1994). If the smoothed line has a slope and intercept of approximately 0, then the proportional hazards assumption has been met (Grambsch and Therneau 1994). The Schoenfeld residuals were the diagnostic tool used in our lab and were performed and assessed prior to joining the lab (data not shown).

1.4.2 Power Calculations

We conducted meta-analyses to combine the effects of both cohorts (discussed in next section below), as such, power calculations were done considering the sample size of both cohorts combined. Minimum detectable hazard ratios of recipient and/or donor depend on three variables: 1.) proportion of individuals experiencing an event, 2.) frequency of a causal variant, and 3.) the quality of genotyping SNPs that capture the genetic variation underlying the hazard of an event. Events are measured at 1 year and at the most updated observation time (most recent phenotype data available is May 5th, 2017) post-HSCT. The events that will be measured are death due to transplant (TRM) and specific causes of death (organ failure, infection, GVHD) attributable to TRM. OS is a function of TRM and thus we will present minimum detectable hazard ratios for OS. The proportion of events

(Figure 3) ranges from infrequent events to (i.e. TRM subtypes) to frequent events (i.e. OS). We will assume lower and upper bound causal variant frequency between 5% and 40%, respectively. We assumed SNPs selected for genotyping capture 85% of the variation across each gene; thus, all power calculations are corrected by setting our effective total sample size equal to $0.85 \times 3532 = \sim 3000$. We present the range of hazard ratios detectable for varying proportions of events and allele frequencies in a univariate model assuming 80% power to detect genome-wide significance at 5×10^{-8} . With 3,532 recipient-donor pairs, the minimum detectable hazard ratio under these assumptions is identical for recipient genotype, donor genotype, and the mismatch between donor and recipients. Given the minimum proportion of events experienced in TRM subtypes and overall TRM are between 0.10 and 0.30 with a common allele (MAF=0.40), we have power to detect hazard ratios between 1.69 and 1.35, respectively. Under these same proportion of events, with more rare variants (MAF=0.05), we have the power to detect hazard ratios between 3.3 and 2.0, respectively. For OS models, assuming the overall rate of death is 0.50, we can detect SNPs with hazard ratios between 1.26 (MAF=0.40) and 1.7 (MAF=0.05). Lower bound is based off each TRM subtype being at least 0.10 (10%) of all patients.

1.5 Discussion

In this Chapter we laid the foundation for understanding the research and approaches that were conducted for this dissertation. The first principles of genetic association studies were discussed, where we introduced important concepts such as reference panels, population stratification, genetic imputation, meta-analyses. This was followed by detailed introduction to DISCOVeRY-BMT cohorts, modeling techniques/ diagnostics and power calculations that were used for the majority of this

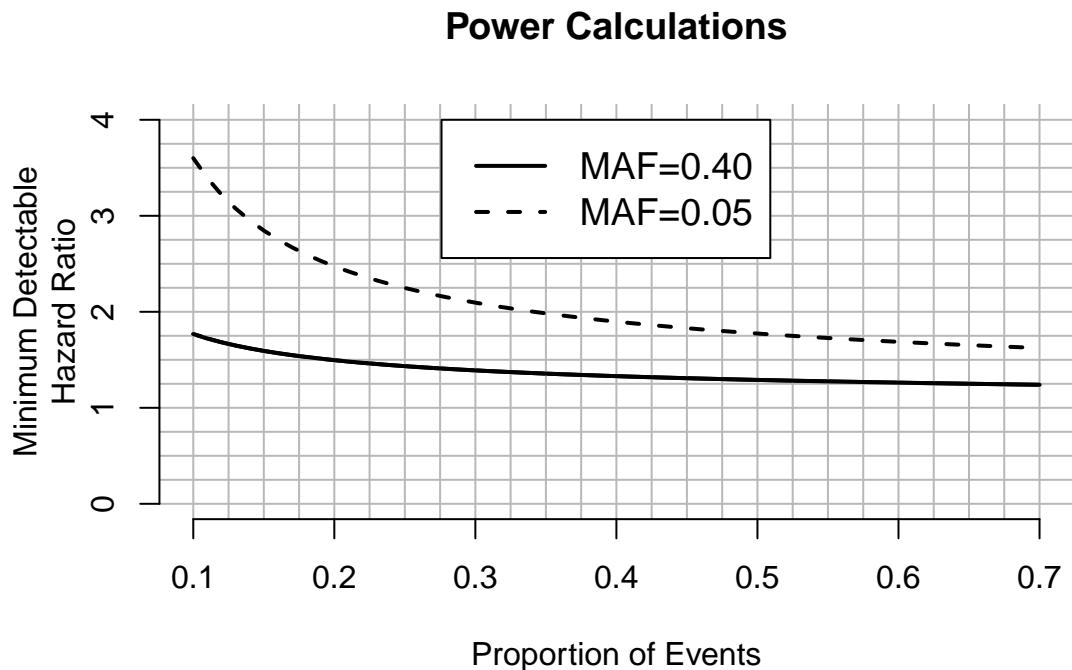


Figure 1.3: Power calculations for DISCOVeRY-BMT. Hazard ratios associated with survival models.

dissertation. DISCOVeRY-BMT is a rich trove of data and can be used for a multitude of problems, ranging from clinical to statistical challenges. This remainder of this document will set forth examples of important aspects of science leveraging these data.

Table 1.3: Proportion of Events by Survival Outcome

Outcome	Recipient (N/Percent %)	
	Cohort 1	Cohort 2
Mixed Disease		
Disease related mortality (DRM)	474 (22.46%)	168 (21.62%)
Transplant related mortality (TRM)	405 (19.19%)	141 (18.15%)
Overall survival (OS)	879 (41.66%)	309 (39.77%)
Relapse (REL)	639 (30.28%)	233 (29.99%)
Progression free survival (PFS)	1055 (50%)	374 (48.13%)
Graft-versus-host disease (GVHD)	134 (6.35%)	59 (7.59%)
Infection (INF)	122 (5.78%)	36 (4.63%)
Organ failure (OF)	104 (4.93%)	26 (3.35%)
AML + MDS		
Disease related mortality (DRM)	383 (23.54%)	149 (21.82%)
Transplant related mortality (TRM)	297 (18.25%)	121 (17.72%)
Overall survival (OS)	680 (41.79%)	270 (39.53%)
Relapse (REL)	506 (31.1%)	208 (30.45%)
Progression free survival (PFS)	810 (49.78%)	329 (48.17%)
Graft-versus-host disease (GVHD)	97 (5.96%)	55 (8.05%)
Infection (INF)	90 (5.53%)	27 (3.95%)
Organ failure (OF)	76 (4.67%)	21 (3.07%)
AML Only		
Disease related mortality (DRM)	333 (25.98%)	115 (23.57%)
Transplant related mortality (TRM)	211 (16.46%)	73 (14.96%)
Overall survival (OS)	544 (42.43%)	188 (38.52%)
Relapse (REL)	431 (33.62%)	161 (32.99%)
Progression free survival (PFS)	648 (50.55%)	234 (47.95%)
Graft-versus-host disease (GVHD)	61 (4.76%)	26 (5.33%)
Infection (INF)	69 (5.38%)	14 (2.87%)
Organ failure (OF)	59 (4.6%)	18 (3.69%)
ALL Only		
Disease related mortality (DRM)	91 (18.84%)	19 (20.21%)
Transplant related mortality (TRM)	108 (22.36%)	20 (21.28%)
Overall survival (OS)	199 (41.2%)	39 (41.49%)
Relapse (REL)	133 (27.54%)	25 (26.6%)
Progression free survival (PFS)	245 (50.72%)	45 (47.87%)
Graft-versus-host disease (GVHD)	37 (7.66%)	4 (4.26%)
Infection (INF)	32 (6.63%)	9 (9.57%)
Organ failure (OF)	28 (5.8%)	5 (5.32%)

2.1 Introduction

Genetic associations studies usually fall under two main subcategories, candidate gene association studies (CGAS) or genome wide association studies (GWAS). CGAS are association studies that investigate specific genes or regions of interest. Typically these are performed when researchers believe that the underlying biology is understood and they want to identify specific markers that contribute to genetic variation in these ‘known’ regions. For over a decade, researchers in the hematology and hematological transplant field have conducted CGAS that investigated the relationship between non-HLA genetics and survival outcomes after allogeneic transplant. The rationale for conducting the CGAS was to increase knowledge about clinical management or to serve as a potential target for novel therapeutics. We exhaustively searched PubMed for CGAS where the phenotype of interest was survival outcomes (DRM, TRM, OS, PFS) in patients with ALL, AML, or MDS after HLA-matched -related-donor (MRD) or -unrelated-donor (MUD) HSCT (Karaesmen et al. 2017). We identified 70 studies that reported 45 SNPs in 36 genes as significantly associated with survival outcomes after transplant. DISCOVeRY-BMT was used to replicate or validate these published studies (Karaesmen et al. 2017). The majority of these studies tested for associations in small datasets, ranging from a few dozen to a few hundred patients and donors, included heterogeneous diseases spanning benign to malignant hematological diseases, related and/or unrelated donors with various degrees of HLA-matching and patients treated across multiple decades, from the 1980s through early 2000s. In addition to reproducing previous

findings, we were interested in agnostically evaluating whether genes that had been previously reported upon had an aggregate effect that could be detected and contributed to survival after transplant.

2.2 Methods

2.2.1 Literature Review

An extensive literature search of PubMed was performed using to identify peer-reviewed scientific studies (published on or before December 30, 2016) that reported non-HLA genetic polymorphisms associated with survival outcomes after allogeneic BMT, including disease-related mortality (DRM), progression-free survival (PFS), transplant-related mortality (TRM) and/or overall survival (OS) (Karaesmen et al. 2017). The PubMed search terms, filtering approach are described below:

```
(SNP[Text Word] OR ("polymorphism, genetic"[MeSH Terms] OR  
("polymorphism"[All Fields] AND "genetic"[All Fields]) OR  
"genetic polymorphism"[All Fields] OR  
"polymorphism" [All Fields])) AND  
(allo-HSCT[All Fields] OR allo-HCT[All Fields] OR  
("unrelated"[All Fields] AND ("donor"[All Fields] OR  
"donors"[All Fields]) AND ("transplant"[All Fields] OR  
"transplantation"[All Fields])) OR ("allogeneic"[All Fields] AND  
("transplant"[All Fields] OR "transplantation"[All Fields])) OR  
("hematopoietic"[All Fields] AND ("transplant"[All Fields] OR  
"transplantation"[All Fields]))) AND ((("mortality"[Subheading] OR  
"mortality"[All Fields] OR "mortality"[MeSH Terms]) OR  
("mortality"[Subheading] OR "mortality"[All Fields] OR  
"survival"[All Fields] OR "survival"[MeSH Terms]) OR  
(non[All Fields] AND ("recurrence"[MeSH Terms] OR  
"recurrence"[All Fields] OR "relapse"[All Fields])) OR  
non-relapse[All Fields]) AND English[Language]
```

The Inclusion Criteria comprised of:

Inclusion criteria:

1. non-HLA genes
2. survival after BMT as phenotype

Excluded:

1. Non-English papers
2. Working group studies
3. Reviews
4. SNPs not in build hg19
5. Haplotypes
6. Chronic Lymphocytic Leukemia (CML) or multiple myeloma (MM) or lymphoma only papers
7. Autosomal only
8. Microsatellites, CNVs, VNTRs, or other variation markers

2.2.2 Definitions of Replication and Validation

In principle, results from CGAS or GWAS should be reproduced in an independent study to confirm findings (Colhoun, McKeigue, and Smith 2003; Martin et al. 2016). Two distinctive terms have gained popularity amongst researchers to describe reproducibility – specifying if there are differences between the original population that was studied and the confirmation study: replication and validation (Igl, Konig, and Ziegler 2009). Replication is deemed to be when the inclusion criteria are near or completely identical (i.e. same ancestral population), so that any differences between the samples in the study can attributed to random variation (Igl, Konig, and Ziegler 2009). Validation reproducibility is when the original and confirmation study have similar but slightly different inclusion criteria (i.e. different ancestral populations. In the validation case, the underlying differences between

the original and confirmation study can be attributed to systematic variation (Igl, Konig, and Ziegler 2009).

Thus, replication analyses were conducted when the original study included HLA MUD-HSCT in patients of European ancestry. Validation analyses were performed on studies of leukemia patients of non-European ancestry, patient populations who received MRD-HSCT, or patient populations that were mixed between those who received a MRD-HSCT and MUD-HSCT (Karaesmen et al. 2017). For studies of outcomes involving multiple hematologic malignancies, the entire DISCOVeRY-BMT study population was analyzed. If the original study population was specified as AML, ALL and/or MDS, the same disease inclusion criteria were applied so that the replication/validation study population aligned with that of the original study population.

2.2.3 Genotyping data

All samples were genotyped using the Illumina Human OmniExpress BeadChip and the Illumina HumanExome BeadChip (University of Southern California Genomics Facility). In total, 637,655 and 632,823 SNPs from the OmniExpress BeadChip were available for imputation in cohorts 1 and 2, respectively, using 1000 Genomes Project phase 3. The missing genotypes were imputed using IMPUTE2 software (B. N. Howie, Donnelly, and Marchini 2009). QCTOOL was used to remove imputed genotypes with an info score > 0.7 , certainty > 0.7 , and a minor allele frequency ≥ 0.005 . To test the joint effect of recipient and donor genetic variation, the recipient-donor (R-D) *mismatch* genome computed by taking the absolute value of the difference of minor alleles between recipient and donor at each SNP. For example, at a given SNP where the recipient is homozygous minor (2 minor alleles) and the donor is heterozygous (1 minor allele), the mismatch allele dosage

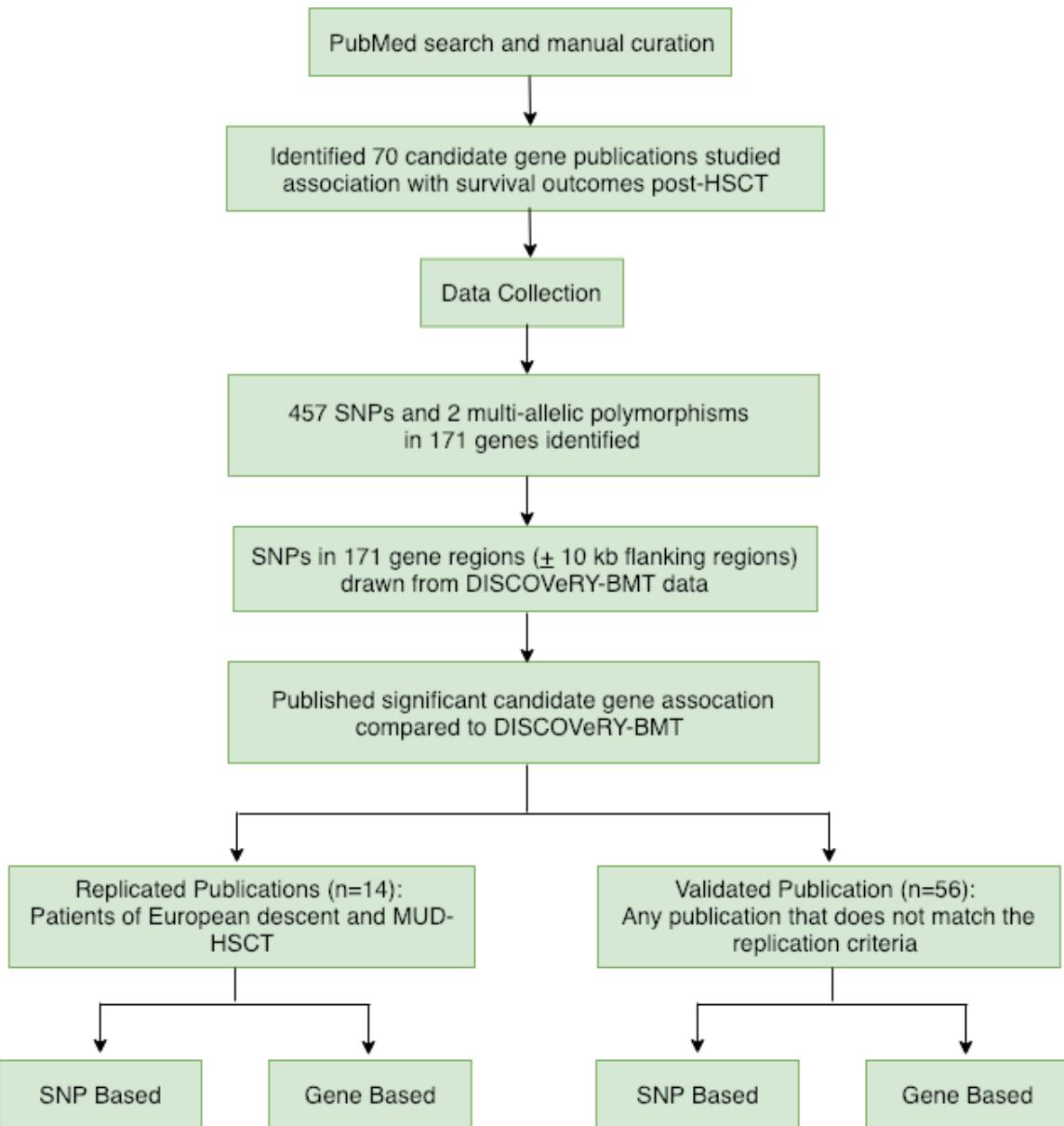


Figure 2.1: Pipeline performed for reproducing previous candidate gene association study literature

would be $|2 - 1| = 1$. Rs2066847 (SNP13) in *NOD2/CARD15* was the only variant analyzed from the Illumina HumanExome, as it was not typed on the OmniExpress chip or available following imputation.

2.2.4 Genetic Models

Prior to genetic analyses, clinical covariates for inclusion in genome-wide survival models were selected using bidirectional stepwise regression (Venables and Ripley 2002) on Cox proportional hazard models (Cox 1972) of OS, PFS, TRM and DRM using R statistical software (R Core Team 2018). Cox proportional hazard models of OS, TRM and DRM evaluated SNPs associated with time to death with all survivors censored at 1 year post-HSCT (Therneau and Grambsch 2013). PFS was defined as the time to disease progression or death. The covariates that were included in the model were (see appendix for R code):

1. OS – recipient age, disease status (early or advanced), graft source (bone marrow or peripheral blood)
2. DRM – recipient age, disease status (early or advanced)
3. TRM – recipient age, body mass index (BMI) (underweight, normal, overweight, obese), graft source (bone marrow or peripheral blood)
4. PFS – recipient age, disease status (early or advanced)

Depending on the disease group, disease types were adjusted for by creating an indicator variables. E.g. For analyses without ALL (AML and MDS), the indicator variable AML dummy and MDS dummy were added to the model. Competing risk models (Fine and Gray 1999) were used for TRM and DRM (because a patient can only die once). Dosage data accounting for the probability of each genotype were used in all analyses of imputed data. Effect size estimates and standard errors from DISCOVeRY-BMT Cohorts 1 and 2 were compared and combined using

a fixed-effects inverse variance meta-analyses in METAL (Willer, Li, and Abecasis 2010). For each SNP, heterogeneity of effect size estimates between cohorts 1 and 2 was assessed using p-values from significance tests of heterogeneity (P_{het}) and I^2 . Variants with $P_{het} < 0.05$ and $I^2 > 50$ were meta-analyzed with a random effects models using meta (Schwarzer 2007) in R.

2.2.4.1 Multi-allelic models

NOD2/CARD15 was the most common gene studied in the HSCT literature. The associations were based on a 3-variant R-D pair model (rs2066844 [SNP8], rs20666845 [SNP12], rs2066847 [SNP13]) and single SNP associations with SNP13 (Ernst Holler et al. 2004). The null type was when the R-D pair are homozygous common allele for all 3 SNPs and the effect allele combination was considered the presence of /geq 1 minor allele in any of the 3 SNPs within the R-D pair. *CCR5* haplotype was studied in one replication study (McDermott et al. 2010). The risk H1/H1 haplotype was defined as presence of homozgous genotype for the major allele at rs1799987 (AA), rs1800023 (AA), rs333 (ACAGTCAGTATCAATTCTG-GAAGAATTCCAG); individuals not homozygous common were considered null. rs333, a 32-basepair deletion, was not typed or imputed in the DISCOVeRY-BMT cohort, however we selected a proxy SNP (rs1133418) in strong linkage disequilibrium ($r^2 = 0.97$) with rs333. The presence of the G allele in the proxy SNP corresponds to ACAGTCAGTATCAATTCTGGAAGAATTCCAG in rs333. McDermott and colleagues (2010) defined 3 risk subgroups:

1. R-D Group 1 – R-D pairs lacked *CCR5* H1/H1 haplotype
2. R-D Group 2 – donors only had *CCR5* H1/H1 haplotype
3. R-D Group 3 – recipients only had *CCR5* H1/H1 haplotype

The imputed data for *CCR5* was in the IMPUTE2 output data chunk for chromo-

some 3 (BMT093013_forImpute.chr3-45000000-50000000.impute2). QCTOOL was used to extract the data out from the IMPUTE2 and output it into the a VCF file. The location for these files was stored on the University at Buffalo Computational Center Resource (UB CCR) supercomputer. See Appendix for full code on analysis.

```
qctool \
  -g ./BMT093013_forImpute.chr3-45000000-50000000.impute2 \
  -s ./BMT093013_forImpute.chr16-50000000-55000000.impute2_samples \
  -og ./ccr5_rep_dosages_threshold.vcf \
  -incl-rsids ccr5_snps.txt
```

The data wrangling to prepare the data for these models was done in R. The Cox survival models were written and automated with custom R code that leveraged the survival package (Therneau and Grambsch 2013) (see Appendix for code used for the full analysis).

2.2.5 Gene-based association testing

VErsatile Gene-based Association Study 2 (VEGAS2) software was used for gene-based association testing (Mishra and Macgregor 2015). VEGAS2 uses 10^6 Monte Carlo simulations to test the global significance of an association for sets of SNPs in defined genomic regions. VEGAS2 reports a gene-based P-value for each gene determined using individual SNP association P-values. Directional effects are not incorporated into analyses; thus, all SNPs can be aggregated without dampening an association signal. For the gene-based replication or validation analyses, the P-values from typed and imputed SNPs in DISCOVeRY-BMT (\pm a 10kb flanking region) meta-analyses of OS, PFS, TRM and DRM were used as input into the VEGAS2 software. Gene-based P-values were calculated for donor, recipient, and R-D mismatch analyses of the full cohort (ALL, AML and MDS patients) or homo-

geneous disease subgroups (ALL or AML or MDS patients) corresponding to the analyses performed in the original studies (Karaesmen et al. 2017).

To run VEGAS2, a flat text file is needed that has two unlabeled columns (rsid and GWAS P-value [P_{meta}]).

For example:

```
#snps.txt
rs6696752 0.827182998293298
rs72638700 0.874653327370856
```

And then a simple command prompting VEGAS2 is run on the command line:

```
vegas2 \
    snps.txt \
    -pop 1000GEURO \
    -subpop EURO \
    -genesize 10kbloc \
    -top 100 \
    -sex BothMnF \
    -max 1000000 \
    -out ./results/output_V2out
```

VEGAS2 analyses were using SNPs from all of the identified genes and p-values from DISCOVeRY-BMT (for all outcomes and 3 genomes) on UB CCR (see Appendix).

2.2.6 Functional Annotation

To gain a deeper understanding of genetic associations, often bioinformatics approaches leverage the plethora of curated, publically available datasets and databases (Gallagher and Chen-Plotkin 2018). The databases used in this study were RegulomeDB (Boyle et al. 2012), Blood expression quantitative trait loci (eQTL) Browser (Westra et al. 2013), and Variant Effect Predictor (VEP) (McLaren et al. 2010), which were used to annotate the SNPs in the candidate

genes. For RegulomeDB and Blood eQTL Browser, the full database consisting of raw data scores, P-values, and annotations were downloaded. VEP annotation was done directly on VEP’s website.

RegulomeDB stores variant annotation with known and predicted regulatory DNA elements in humans. The elements include DNase hypersensitivity, transcription factor (TF) binding sites, and promoter regions that have been characterized to alter transcription (Boyle et al. 2012). RegulomeDB database compiled these annotations using the publically available data sets from the Encyclopedia of DNA elements (ENCODE) project and the Roadmap Epigenome Consortium and Gene Expression Omnibus (GEO). RegulomeDB scores are the following: 1a-1f likely to affect TF binding and linked to altering expression of target genes; 2a-2c likely to affect TF binding; 3a-3b less likely to affect TF binding, and > 3 has minimal binding evidence. RegulomeDB DB scores are assigned (only one per SNP) based on the level of effect and evidence of functional modification that is attributable to the SNP across multiple cell lines from differing tissues. The scores are supported by experimental evidence, with scores from 1 to 7, with 1 having the greatest functional effect, and scores of 7 show no evidence of having modifying effects.

Blood eQTL database was built from a study ($N = 5000$) that investigated the correlation between genetic expression and genetic polymorphisms and replicated the results in an independent study ($N \approx 3000$), Westra et al. (2013)]. We only considered *cis*-eQTLs, defined as < 250KB distance between the SNP chromosomal position and the probe midpoint for gene expression. Furthermore, VEP was used to determine the hypothetical (predicted) functional importance of missense and nonsense variants based on SIFT, Mutation Taster and PolyPhen-2 software.

2.3 Results

2.3.1 Candidate Gene Studies of Survival Outcomes

The literature search identified 70 publications that studied a total 458 SNPs and 2 multi-allelic polymorphisms in 171 genes (Figure 2.1). Studies included patients who received a transplant from an MUD-HSCT (19 articles), a MRD-HSCT (23 articles), or both (28 articles) (Table 2.1). Study populations included patients and donors of European ancestry (53 articles), Asian ancestry (15 articles), or mixed genomic ancestry (2 articles) (Table 2.1).

A total of 14 articles assessed genetic variation in HLA MUD-HSCT patients of European ancestry, but only 7 of these articles reported significant associations ($P < 0.05$ or an author specified significance threshold) and thus comprise our replication study (Table 2.1, Figure 2.2). A total of 56 articles tested associations in either a combination of MRD and MUD-HSCT, only MRD and/or in non-European populations; 39 of these 56 articles reported at least one significant SNP association with survival outcome and we attempted to validate the significant findings from these 39 articles (Table 2.1, 2.3) (Karaesmen et al. 2017).

2.3.2 Replication

To reproduce previous reported results, DISCOVeRY-BMT was used to replicate findings that comprised of acute leukemia or MDS patients with European ancestry, treated with MUD-HSCT (Karaesmen et al. 2017). Seven articles were included in the replication analyses. Multi-allelic models, *CCR5* and *NOD2/CARD15*, were tested in 2 articles; single SNP associations in *CD274*, *CD40*, *HMGB1*, *IL1A*, *IL1B*, *NOD2/CARD15*, *TGFBI*, and *TNFSF4* were tested in 5 articles (Table 2.1, Figure 2.2) (Karaesmen et al. 2017).

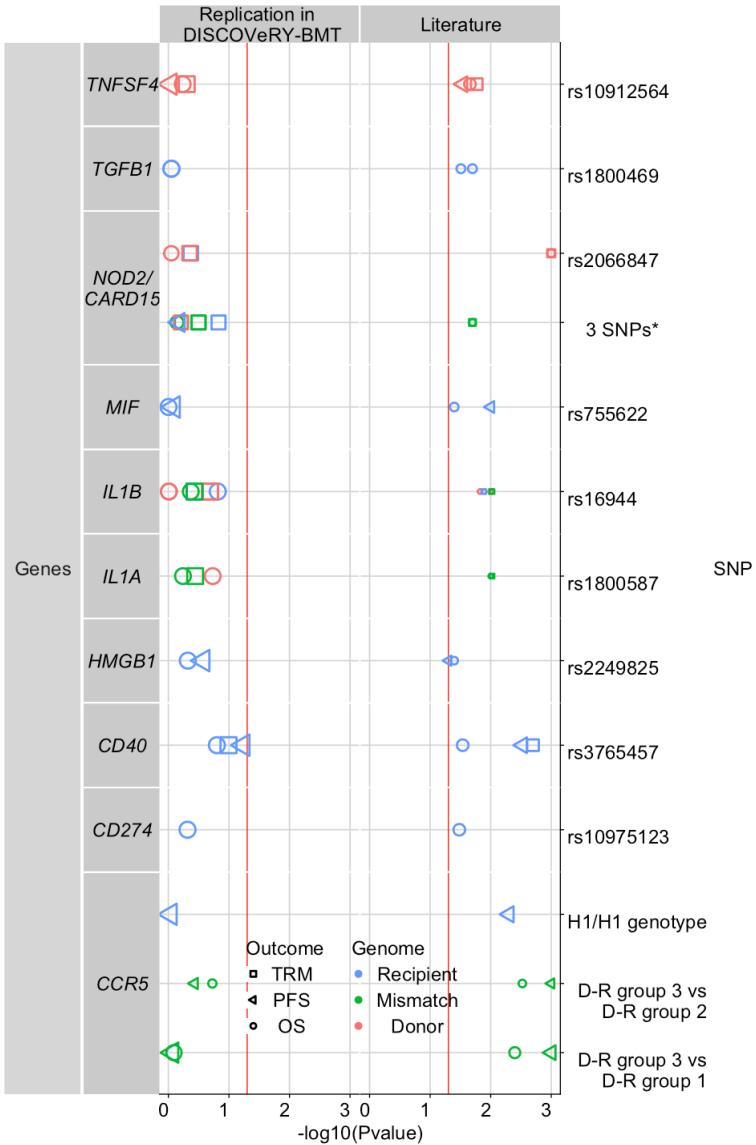


Figure 2.2: Replication attempts of previously reported significant candidate gene-association studies in DISCOVeRY-BMT. Survival association P values as reported in previous literature (Right panel) and replication attempts of these associations in DISCOVeRY-BMT cohort (Left panel) are shown as data points. Vertical panels indicate the genes that these polymorphisms and haplotypes are located in or close to as reported by the previous studies. Shapes represent associations with survival outcomes OS, PFS, or TRM; colors correspond to donor, recipient, or donor-recipient mismatch polymorphisms. The size of the point represents the sample size of the study, with larger points reflecting a bigger sample size. Shown on y-axis are the 9 polymorphisms from the literature reporting associations at $P < 0.05$ with OS, PFS, or TRM by 1 or more previously published studies; the x-axis is the $\log_{10}(P\text{-value})$. The red vertical lines in (left panel) and (right panel) indicate $P < 0.05$. Details on the haplotypes are described in “Results” under “Replication.”

Table 2.1: Count of reports with SNPs that were studied at least twice (in addition to CCR5 studies) that were attempted for replication or validation in DISCOVeRY-BMT.

SNP	Gene	Reports of SNPs with any HSCT outcome	Significant SNPs with any HSCT outcome	Reports of SNPs European ancestry MUD-HSCT populations	Significant SNPs in European ancestry MUD-HSCT populations	Reports of SNPs tested non-European ancestry and/or MRD-HSCT populations	Significant SNPs in non-European ancestry and/or MRD-HSCT populations
rs1045642	ABCB1	3	1	0	0	3	1
rs2032582	ABCB1	2	1	0	0	2	1
^ R-D Group 3 vs R-D Group 1	CCR5	1	1	1	1	0	0
^ R-D Group 3 vs R-D Group 2	CCR5	1	1	1	1	0	0
H1/H1 genotype	CCR5	1	1	1	1	0	0
rs2569190	CD14	2	1	0	0	2	1
rs231775	CTLA4	9	4	1	0	8	4
rs3087243	CTLA4	9	2	1	0	8	2
rs5742909	CTLA4	6	1	0	0	6	1
rs4553808	CTLA4	5	1	1	0	4	1
rs4244285	CYP2C19	2	1	0	0	2	1
rs8192917	GZMB	2	1	0	0	2	1
rs1800587	IL1A	3	1	0	0	3	1
rs16944	IL1B	4	1	0	0	4	1
rs1800587	IL1B	3	1	0	0	3	1
rs11209026	IL23R	3	2	0	0	3	2
rs1800795	IL6	5	3	0	0	5	3
rs1800797	IL6	3	1	0	0	3	1
rs1801133	MTHFR	4	3	0	0	4	3
rs1801131	MTHFR	3	1	0	0	3	1
3 SNPs*	NOD2/CARD15	9	4	2	1	7	3
rs2066847	NOD2/CARD15	4	2	1	1	3	1
rs2066842	NOD2/CARD15	3	1	0	0	3	1
rs9658254	NOS1	2	1	0	0	2	1
rs1800469	TGFB1	3	1	1	1	2	0
rs4986790	TLR4	2	1	0	0	1	1
rs731236	VDR	5	2	0	0	5	2
rs7975232	VDR	5	2	0	0	5	2

$\wedge\wedge\wedge$ CCR5 H1/H1 genotypes and risk groups defined using multiallelic models described in the original publication (McDermott et al 2010) and in the main text.

3 SNPs* – The NOD2/CARD15 3 SNP multiallelic model. Described in the text.

As previously discussed, candidate genes and candidate SNPs are selected due to *a priori* biological knowledge of the phenotype. The general thought in HSCT is that patients are either dying from their disease or from the transplant. And transplant related causes are mostly infection, organ failure, or GVHD. Relevant biological pathways that are included in these phenotypes are likely to involve immunological and inflammatory pathways. Indeed, the most frequently studied gene was *NOD2/CARD15*, which is a susceptibility gene for inflammatory bowel disease and may be involved in Crohn's disease (E Holler et al. 2008) (Table 2.1). The two *NOD2/CARD15* associations were based on a three-variant R-D pair model [rs2066844 (SNP8), rs2066845 (SNP12) and rs2066847 (SNP13)] and single SNP associations with SNP13 (E Holler et al. 2008). The null genotype is when the R-D pair are homozygous common allele for all 3 SNPs and the effect allele combination is the presence of 1 or more minor alleles at any of the 3 SNPs within the R-D pair. The *NOD2/CARD15* multi-SNP model was significantly associated with OS (RR: 1.6, 95% CI 1.1-2.4, $P = 0.02$) and TRM (RR: 1.6, 95% CI 1.1-2.4, $P = 0.02$) in a study of 196 patients who received a MUD-HSCT for AML or ALL. However, no associations were found for OS (HR: 1.03, 95% CI 0.9-1.2, $P = 0.72$) or TRM (HR: 1.1, 95% CI 0.8-1.4, $P = 0.6$) in DISCOVeRY-BMT, which is a larger study ($N = 1597$) with AML and ALL patients treated with MUD-HSCT (Figure 2.2). Furthermore, a study of 342 AML or ALL patients after MUD-HSCT (E Holler et al. (2008)) reported donor genotype rs2066847 (SNP13) significantly increased risk of TRM and OS approximately 3-fold ($P = 0.001$) and 2.5 ($P = 0.001$), respectively. When this SNP was tested in DISCOVeRY-BMT donors, no associations were found with either TRM (HR: 1.17, 95% CI 0.78-1.74, $P = 0.45$) or OS (HR: 0.98, 95% CI 0.73-1.31, $P = 0.89$, in ALL or AML patients) (Figure 2.2).

One of the largest CGAS ($N = 1370$) reported significant associations

for *CCR5* H1/H1 genotype ($N = 163$) in recipients (McDermott et al. 2010). McDermott and colleagues also defined genotype risk subgroups and OS (Figure 2.2; able 2.1). These associations were tested in DISCOVeRY-BMT and neither *CCR5* H1/H1 genotype ($N=294$) nor the genotype risk groups defined by H1/H1 status were significantly associated with PFS or OS (Figure 2.2, Table 2.1). The genotype risk groups (Group 3 vs Group 1 and Group 3 vs Group 2) were substantially smaller than the full cohort. We tested these in DISCOVeRY-BMT and found no associations. Considering the fact that DISCOVeRY-BMT cohorts were approximately twice as large as those in the original study and adequately powered to detect these associations (Figure 1.11), these risk group associations were not real (Karaesmen et al. 2017). In DISCOVeRY-BMT these subgroups were approximately twice as large as those in the original study and adequately powered to detect these associations.

DISCOVeRY-BMT was unable to replicate another large CGAS of 1170 patients (Jindra et al. 2016), which reported an association between rs10912564 (*TNFSF4*) and TRM ($P = 0.017$), OS ($P = 0.022$), and PFS (HR: 0.8, 95% CI [0.9-1.2], $P = 0.03$) (Figure 2.2). Similarly DISCOVeRY-BMT could not replicate rs2249825 in *HMGB1* (Kornblit et al. 2010, $N=276$), rs1800469 in *TGFB1* (Arrieta-Bolaños et al. 2016, $N=493$), rs755622 in *MIF* (Chang et al. 2009, $N=454$), nor SNPs in *IL1A* and *IL1B* (Figure 2.2).

2.3.3 Validation

Validation attempts were conducted on 36 genetic polymorphisms in 26 genes from 39 previously published CGAS (Table 2.1). The genes that were included: *ABCB1*, *CD14*, *CTLA4*, *CYP2C19*, *DAAM2*, *EP300*, *ESR1*, *GSTA2*, *GZMB*, *ICAM1*, *IL23R*, *IL6*, *IRF3*, *KLRK1*, *LIG3*, *MTHFR*, *MUTYH*, *NOD2/CARD15*,

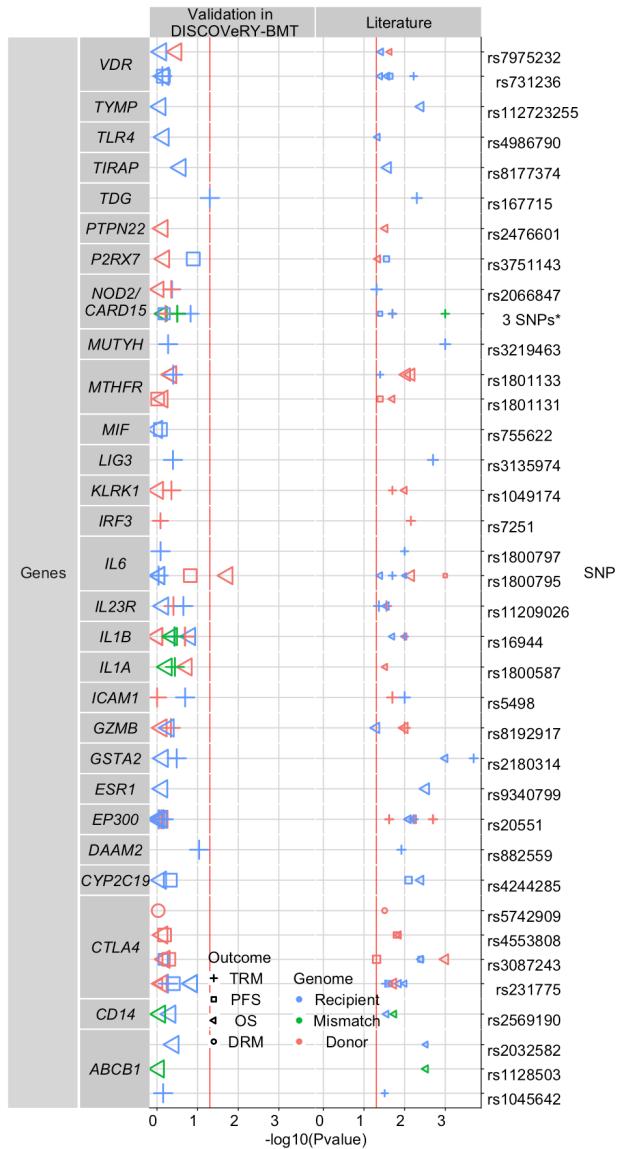


Figure 2.3: Validation attempts of previously reported significant candidate gene-association studies. Survival association P-values as reported in previous literature (Right panel) and validation attempts of these associations in DISCOVeRY-BMT cohort (Left panel) are shown as data points. Vertical panels indicate the genes that these 17 polymorphisms are located in or closest to as reported by the previous studies. Shapes represent associations with survival outcomes DRM, OS, PFS, and TRM; colors correspond to donor, recipient, or donor-recipient mismatch polymorphisms. The size of the point represents the sample size of the study, with larger points reflecting a bigger sample size. Shown on y-axis are the 17 polymorphisms from the literature reporting associations at $P < 0.05$ with OS, PFS, or TRM by 1 or more previously published studies; the x-axis is the $-\log_{10}(P\text{-value})$. The red vertical lines in (left panel) and (right panel) indicate $P < 0.05$. Details on the haplotypes are described in “Results” under “Validation.”

NOS1, *P2RX7*, *TDG*, *TIRAP*, *TLR4*, *TYMP*, and *VDR* (Figure 2.3) (Karaesmen et al. 2017). Each of these studies reported at least one significant genetic associations with survival in patients who received a HLA MRD-HSCT (19 articles) or had a study population including MRD- and MUD-HSCT patients, without stratification of results (17 articles). Validation attempts for survival associations reported in non-European leukemia patients who received an MUD-HSCT (3 articles) (Table 2.1). The variants that had been reported to be associated to survival outcomes after transplant and our attempts to validate the results in DISCOVeRY-BMT are shown in Figure 2.3.

Only one of our validation attempts were successfully reproduced – where donor genotype rs1800795 (*IL-6*) was associated with OS (HR: 1.11, 95% CI 1.0-1.2, $P = 0.02$) (Figure 2.3, note: this did not reach genome-wide significance at the $P < 5 \times 10^{-8}$ threshold). The rs1800795 association to OS (HR: 1.29, 95% CI 1.07-1.55, $P = 0.007$) was reported in a single study in patients with acute leukemia, CML, or lymphoma (N=743) treated with MRD- or MUD-HSCT (Balavarca et al. 2015).

Similar to our replication set, *NOD2/CARD15* was the most frequently studied gene and reported the most of all CGAS in our validation set (Table 2.1). Three studies reported associations between the presence of *NOD2/CARD15* multi-SNP polymorphism and either TRM (Ernst Holler et al. 2004) or PFS. None of these studies were validated in DISCOVeRY-BMT cohorts (Figure 2.3). Likewise, DISCOVeRY-BMT was unable to validate single SNP analyses in *NOD2/CARD15* for rs2066842 in MRD- or MUD-HSCT donors with PFS or for TRM at rs2066847 (SNP13) in recipients of MRD- or MUD-HSCT (Figure 2.3).

CTLA4 is a member of the immunoglobulin superfamily that is expressed by activated T-cells and transmits inhibitory signal to T-cells. Due to these known

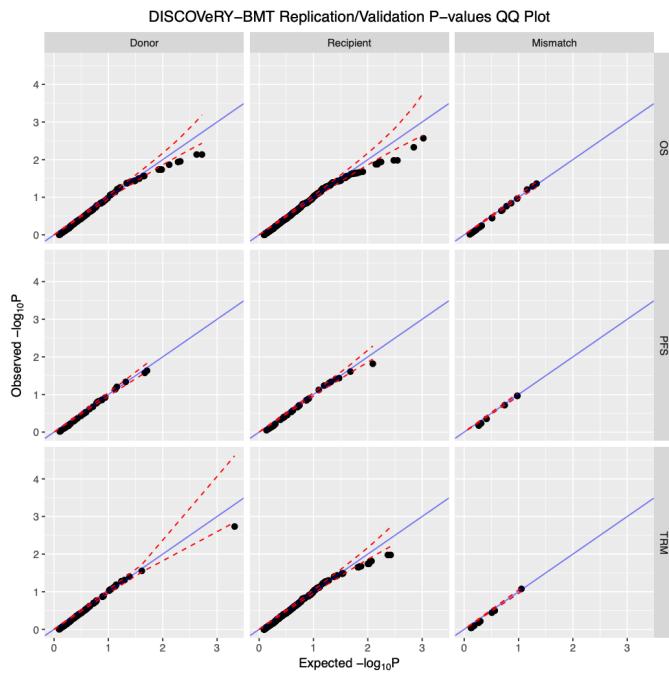


Figure 2.4: Quantile-quantile (QQ) plot of SNP p-values in DISCOVeRY-BMT for all previously studied SNPs. From left to right, vertical panels represent the donor, recipient, and mismatch genome, horizontal panel represents the survival outcome that was tested, from top to bottom overall survival (OS), progression free survival (PFS) and transplant related mortality (TRM). Dashed red lines indicate 95% confidence intervals

functions and perceived implications in transplant biology, associations with multiple SNPs in *CTLA4* have been tested in numerous transplant populations (Table 2.1; Pérez-García et al. (2007)). In previous CGAS, four *CTLA4* SNPs (rs3087243, rs231775, rs4553808, rs5742909) were significantly associated with survival after MRD- or MUD-HSCT in patients with acute leukemia, CML, lymphomas, MDS, and other hematological disorders (Table 2.1). Attempts to validate *CTLA4* SNPs with DRM, PFS, OS, and TRM were unsuccessful in the DISCOVeRY-BMT cohorts (Figure 2.3). The remaining results of the 25 additional candidate genes containing SNPs that were tested in the DISCOVeRY-BMT cohorts are summarized in Figure 2.3; no SNP associations were found at $P < 0.05$. Importantly, the P-value distribution of the single SNP associations showed no deviation from the null expectation with 95% confidence intervals (Figure 2.4), suggesting we cannot reject the null hypothesis of no association with survival outcome (Karaesmen et al. 2017).

2.3.4 Gene based replication and validation of previous studies

From the previous literature, candidates genes were first selected genes based on their hypothesized or known function, and subsequently authors selected variants within that gene for single SNP or haplotype testing (Karaesmen et al. 2017). Thus, while SNPs and haplotypes were tested individually for association, the hypotheses from the literature can be considered gene-based. The density of typed and imputed markers in the DISCOVeRY-BMT recipients and donors allows us to measure the aggregate effect of all SNPs within each candidate gene on survival. Genes were selected for testing from the same literature summarized above for the replication and validation SNP and haplotype analyses. VEGAS2 gene-based testing did not reveal any associations at $P < 0.05$ with any of the survival outcomes in either the replication or validation groups (Karaesmen et al. 2017).

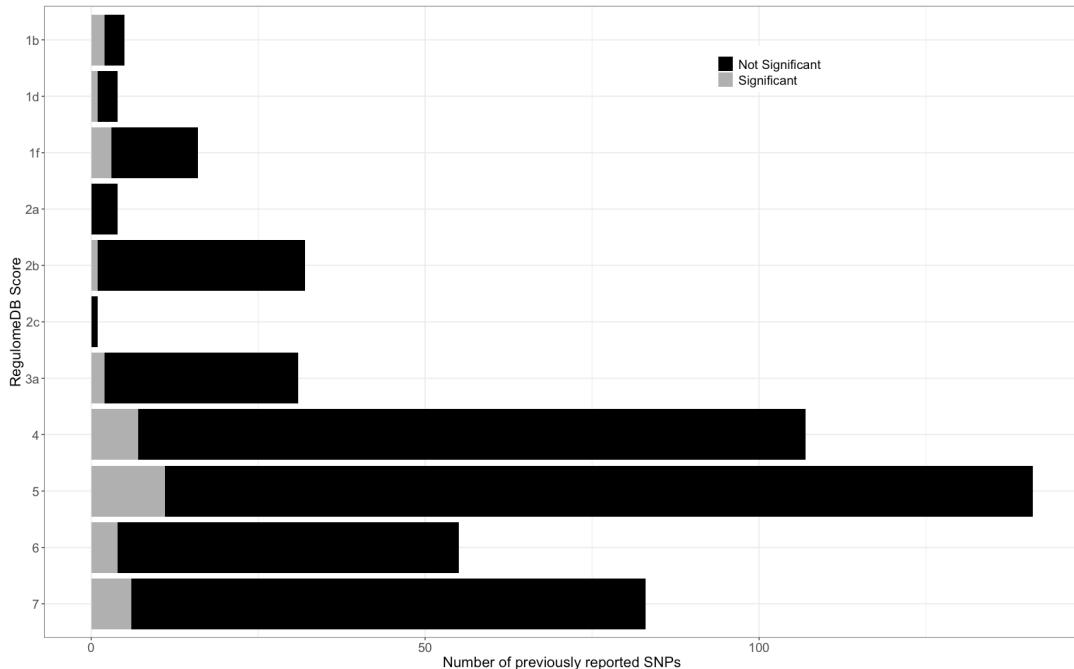


Figure 2.5: RegulomeDB score distribution of previously studied polymorphisms. RegulomeDB categories are shown on the x-axis; counts of SNPs falling into RegulomeDB score category are shown on the y-axis. Blue portion of the bar indicates the counts of SNPs that were tested but not reported significant; red portion shows the counts of SNPs that were reported significant at least once. Score descriptions are given below the image. 1b indicates eQTL 1 transcription factor (TF) binding 1 any motif 1 DNase footprint 1 DNase peak; 1d, eQTL 1 TF binding 1 any motif 1 DNase peak; 1f, eQTL 1 TF binding/DNase peak; 2a, TF binding 1 matched TF motif 1 matched DNase footprint 1 DNase peak; 2b, TF binding 1 any motif 1 DNase footprint 1 DNase peak; 2c, TF binding 1 matched TF motif 1 DNase peak; 3a, TF binding 1 any motif 1 DNase peak; 4, TF binding 1 DNase peak; 5, TF binding or DNase peak; 6, motif hit; 7, no evidence.

2.3.5 Candidate polymorphism annotation

Candidate gene SNPs were analyzed using the RegulomeDB, VEP and Blood eQTL Browser databases to assess their functional characteristics and better understand their biological framework. Eighty percent of previously reported SNPs had RegulomeDB scores greater than 3 (Figure 2.5), indicating that these SNPs have minimal to no effect on modifying transcription. This distribution aligns with the overall distribution of SNPs in the genome, thus the candidate SNPs are not

enriched for their impact on gene expression or transcription factor binding. Our replication and validation analyses includes 2 protein coding variants, VEP shows that only, rs2066845 (SNP12) in *NOD2/CARD15*, is predicted to be damaging and disease causing.

We were interested in better understanding the biological framework of candidate SNPs that had previously been identified. The candidate SNPs were analyzed using RegulomeDB, Blood eQTL Browser, and VEP to assess functional characteristics. The majority ($\geq 80\%$) candidate SNPs had RegulomeDB scores greater than 3 (Figure 2.5). This indicates that the SNPs had minimal to no evidence of modifying DNA transcription. The observed distribution coincides with the overall distribution of SNPs in the genome. Therefore, from our point of view, the candidate SNPs are not enriched in terms of impact on TF binding or gene expression. VEP analyses revealed that the candidate SNPs in our replication and validation analyses only included 2 protein coding variants, and that rs2066845 (SNP12) in *NOD2/CARD15* is predicted to be damaging and disease causing (Karaesmen et al. 2017).

We looked at the Blood eQTL browser to determine if the candidate SNPs played a roll in *cis* gene expression of the candidate genes. Rough half ($\approx 52\%$) of the 171 genes that are included from the literature search had at least one significant (probe level false discovery rate, FDR < 0.05) *cis*-eQTL. And when compared to the entire genome, approximately 44% of genes have blood *cis* eQTLs (FDR $P < 0.05$). However, when we look at the the candidate SNPs, only 13% are blood *cis*-eQTLs. So while blood eQTLs have indeed been identified in these genes, the SNPs that previous literature studied, were not the ones that affected expression. Furthermore, almost half of the eQTLs in the CGAS are correlated with expression, they alter expression in neighboring or nearby genes rather than the gene they

are located in. For example, rs7975232 (*VDR*) is an eQTL for *SLC48A1* while the *CTLA4* SNPs studied are eQTLs for *CD28* (Karaesmen et al. 2017). The remaining eQTLs were correlated with expression of the candidate gene of interest, but in most cases, were also significant eQTLs for several other nearby genes (Karaesmen et al. 2017).

2.4 Discussion

In this study, we aimed to replicate or validate all existing CGAS literature that investigated the relationship between non-HLA genetic effects on survival after allogeneic HSCT (Karaesmen et al. 2017). Considering that the main purpose of CGAS is to select SNPs due to known function at the gene level, we conducted single SNP analyses and gene-based analyses to determine the joint effects of marginally associated SNPs within candidate genes while considering the underlying genetic correlation structure due to LD.

Donor rs1800795 in *IL6* was the only association (OS) that was able to be reproduced using DISCOVeRY-BMT (only in the validation set; no candidate SNPs replicated). The original authors (Balavarca et al. 2015) studied *IL6* due to its known immunological function, as well as the fact that two prior studies reported significant associations in different phenotypes: to GVHD (Cavet et al. 2001) and chronic hepatitis C virus therapy (Yee et al. 2009). No evidence of an association of rs1800795 at $P < 0.05$ in DISCOVeRY-BMT with death due to GVHD and/or infection (data not shown). Furthermore, rs1800795 is found in an intronic region of *IL6*, and has no effect on *IL6* expression or levels, but rather is an eQTL for two other nearby genes (Arnold et al. 2015, GTEx Consortium (2013)). We made an extra effort to validate interesting CGAS findings, such as in *CCR5* and the H1/H1 risk groups from McDermott et al. 2010. These associations were found in the

largest study we attempted to validate, as well as having samples from CIBMTR (earlier years than DISCOVeRY-BMT), and the reported effects started two years after HSCT. We were not able to validate these associations with either outcome at $P < 0.10$ (the threshold McDermott 2010 used, Figure 2.3).

CTLA4 was another gene that was studied frequently (Table 2.1), this gene is a good example of the heterogeneity to studies of genetic polymorphisms in HSCT and may be a plausible explanation of why were unable to reproduce any of the previous associations. The SNP rs5742909 in *CTLA4* was studied in 6 independent studies of HLA MRD-HSCT donor-recipient pairs. For donors, rs5742909 was reported to be associated with DRM in a smaller study (N=120). The lack of consistency between studies is apparent – previously two *CTLA4* SNPs, rs231775 and rs231774 were reported associated in recipients for PFS ($P = 0.025$) – and before this study, only 1 of 9 studies tried to validate these results. These SNPs are like many candidate gene hypotheses such that they have not been tested in the same genome for the same outcome in similar populations, and if they have, the sample size is very small.

The inclusion criteria with respect to disease, donor relation, or to differences in our endpoint of 1-year survival versus longer-term survival could be attributed as to why previous results are not reproducible. As previous genetic associations were hypothesized to be independent of the underlying disease, one may expect that results should be reproducible in a homogeneous patient populations such as DISCOVeRY-BMT, which unfortunately was not the case. This study meticulously attempted to align the study population in DISCOVeRY-BMT to the original studies (i.e. subset the full population for this only with AML). DISCOVeRY-BMT primarily focuses on 1-year survival after HSCT and this may have different genetic effects than later survival, however, the survival curves from the CGAS often

showed separation of death occurring well into the first year. DISCOVeRY-BMT has a large sample size that is adequately powered (L. E. Sucheston-Campbell et al. 2015) to reproduce results from previously published CGAS. However, we were unable to reproduce almost all of the findings. This is in agreement with other studies in this field, Particularly in the context of death due to GVHD as an outcome (Chien et al. 2012; Martin et al. 2016). Several other reports have also concluded that published CGAS have presented false positive associations (Ioannidis et al. 2001).

Confirmatory studies are vital to identify true positive genetic variants that are associated to complex phenotypes. False positives may lead researchers to allocate resources and time to research that will lead to a dead end. In the long-term this may delay treatment to patients or discovery of markers that would be relevant in improving survival after HSCT. As only one SNP had a predicted damage or deleterious effect and that a only a small proportion of the previous 70 studies conducted research on SNPs that correlated with gene expression, we conclude that the transplant field can look agnostically at the entire genome to hone in on potential candidate genes and SNPs (Karaesmen et al. 2017). And while we could not reproduce previous associations, the SNPs selected are not linked to functional annotation nor are they clearly related to the candidate genes. This underscores a fundamental flaw with CGAS which are contingent on the scientific knowledge at the time. We assert that by using adequately powered testing (i.e. larger studies, GWAS) with transplant will be vital to identifying promising targets and further help us understand the underlying biological framework and how it relates to survival outcomes, with the global objective of improving patient outcomes (Karaesmen et al. 2017).

CHAPTER 3: gwasurvivr: an R Bioconductor package for genome wide survival analysis

3.1 Abstract

Summary: To address the limited software options for performing survival analyses with millions of SNPs, we developed gwasurvivr, an R/Bioconductor package with a simple interface for conducting genome-wide survival analyses using VCF (outputted from Michigan or Sanger imputation servers), IMPUTE2 or PLINK files. To decrease the number of iterations needed for convergence when optimizing the parameter estimates in the Cox model, we modified the R package survival; covariates in the model are first fit without the SNP, and those parameter estimates are used as initial points. We benchmarked gwasurvivr with other software capable of conducting genome-wide survival analysis (genipe, Survival-GWAS_SV and GWASTools). gwasurvivr is significantly faster and shows better scalability as sample size, number of SNPs and number of covariates increases.

Availability and implementation: gwasurvivr, including source code, documentation and vignette are available at: <http://bioconductor.org/packages/gwasurvivr>.

3.2 Introduction

Genome-wide association studies (GWAS) are population-level experiments that investigate genetic variation in individuals to observe single nucleotide polymorphism (SNP) associations with a phenotype. Genetic variants tested for association are genotyped on an array and imputed from a reference panel of sequenced genomes using 1000 Genomes Project or Haplotype Reference Consortium (HRC)

(Das et al. 2016; 1000 Genomes Project Consortium 2015). Imputation increases genome coverage from hundreds of thousands or a few million to upwards of 30 million SNPs, improves power to detect genetic associations, and/or homogenizes variant sets for meta-analyses (Das et al. 2016). Imputed SNPs can be tested for association with binary outcomes (case/control) and quantitative outcomes (i.e. height) using a range of available software packages including SNPTEST (Marchini et al. 2007) or PLINK (Purcell et al. 2007). Typically imputed SNPs are used in association testing for large studies.

As we learned from our candidate gene replication/validation project, researchers (including our lab) often opt to write custom survival analysis scripts using the survival package in R. This hinders reproducibility of results as unintentional errors may incur and propagate without effectively testing and/or reviewing the custom scripts. A solution to this would be a well-developed, flexible, and tested open source software package that performs survival analysis on GWAS data. Software options for performing survival analyses do indeed exist, such as, genipe (Lemieux Perreault et al. 2016), SurvivalGWAS_SV (Syed, Jorgensen, and Morris 2017), or GWASTools (S. M. Gogarten et al. 2012). These software are able to analyze millions of imputed SNPs but either require user interaction with raw output, were not initially designed for survival and/or have other limitations that could deter more introductory users (i.e. mapping patient meta data with the genetic data). To address these needs, we developed an R/Bioconductor package, gwasurvivr, for genome wide survival analyses of imputed data in multiple formats with flexible analysis and output options (A. A. Rizvi et al. 2018). This package uses output from the most popular imputation software and/or services as input, and unlike other packages, no additional data wrangling or file format conversion is needed. And then we wanted to test our package and compare it to the other

packages.

We wanted our package to perform and fulfill the following tasks:

- User friendly
- Propagate reproducible results
- Handle typed or imputed genetic data from common formats
- Scalable for very large datasets
- Allow exploration of covariates when modeling
- Interaction modeling, i.e. SNP \times drug, SNP \times age
- Compression of genetic data for less storage and faster computation

3.3 Methods

We detail our methodology and computational experiments conducted in the development of gwasurvivr. This also includes generation of the simulated data, survival analysis benchmarking and diagnostics. All of the code that was published can be found in the gwasurvivr manuscript repository. GitHub Large File Storage (LFS) is on GitHub Large File Storage (LFS) or in the Appendix.

The repository can be cloned by invoking the following command:

```
git lfs clone \
https://github.com/suchestoncampbelllab/gwasurvivr_manuscript.git
```

3.3.1 Data Structure

In a typical GWAS pipeline, raw genotyping data ('typed' data) is pre-processed with standard quality control (QC)/quality assurance (QA) and then delivered in PLINK (Purcell et al. 2007) formatted files (.bed, .bim, and .fam files). The primary representation of the genotype calls are .bed files. Bim files are a text file with no header containing more information about the variant

(chromosome, identified, position, base pair, and allele1 and allele2). The `.fam` file contains information about the samples (family ID, within-family ID, father ID, mother ID, sex code, and additional phenotype information). PLINK does not do survival analysis to test for association on its own and separate packages are needed. `Gwasurvivr` was designed to take PLINK ready files directly using `plinkCoxSurv`. For all survival analyses implemented in `gwasurvivr`, in addition to the genetic data, a phenotype file is needed, which contains survival time, survival status and additional covariates, both files are indexed by sample ID. Potential data wrangling that the user may have to do, however, is that they must convert categorical variable need to be convert to indicator (or dummy) variables and be class numeric (or integer).

Most GWAS, including DISCOVeRY-BMT, impute the typed data to obtain a greater number of SNPs for association testing. In preparation for imputation, PLINK files need to be converted into an appropriate format (genotype (`.gen`) format for IMPUTE2 or VCF for Sanger and Michigan imputation servers). The output format from these imputation programs is the same as the input format (i.e. VCF input, VCF output). After imputation, the VCF files contain both sample IDs and imputation quality metrics (INFO score [Sanger] or r^2 [Michigan]), while IMPUTE2 (B. N. Howie, Donnelly, and Marchini 2009) come in separate files (`.gen`, `.sample`, and `.info`). Data from each format are prepared in `gwasurvivr` by leveraging existing Bioconductor packages GWASTools (S. M. Gogarten et al. 2012), VariantAnnotation (Obenchain et al. 2014), or SNPRelate (Zheng et al. 2012) depending on the imputation/typed data file format.

IMPUTE2 (B. N. Howie, Donnelly, and Marchini 2009) format is a standard genotype (`.gen`) file which store genotype probabilities (GP). We utilized GWASTools in R to compress files into genomic data structure (GDS) format (S.

M. Gogarten et al. 2012). GDS format compresses large array-oriented datasets and allows for efficient, iterative access to subsets of the data, while simultaneously converting GP into dosages (DS) for use in survival analyses. The INFO score for IMPUTE2 (B. N. Howie, Donnelly, and Marchini 2009) results are not calculated in `gwasurvivr` internally, instead we use the INFO scores that are provided in a separate file after performing imputation (`.info` file). Users select SNPs from the `.info` file to remove based on preferred criterion (i.e. $\text{INFO} < 0.8$) these are then used in the argument `exclude.snps` in `impute2CoxSurv` to filter out the SNPs prior to analysis. VCF files generated from these Michigan or Sanger servers include a DS field and server-specific meta-fields (INFO score [Sanger] or r^2 [Michigan], as well as reference panel allele frequencies) that are iteratively read in by VariantAnnotation (Obenchain et al. 2014). For the Michigan imputation server, imputation is performed using the minimac3 algorithm (Das et al. 2016). minimac3 computes and outputs an imputation quality metric known as R^2 . R^2 is the estimated value of the squared correlation between imputed genotypes and true, unobserved genotypes (Das et al. 2016). The R^2 value is extracted directly from the Michigan imputation output VCF in `michiganCoxSurv`. For the Sanger imputation server, we extract the INFO field directly from the VCF file in `sangerCoxSurv`. The INFO field is the IMPUTE2 (B. N. Howie, Donnelly, and Marchini 2009) score as calculated by from posterior genotype probabilities using bcftools + impute-info plugin (McCarthy et al. 2016).

The `gwasurvivr` functions for IMPUTE2 (`impute2CoxSurv` or `gdsCoxSurv`) and VCF (`michiganCoxSurv` or `sangerCoxSurv`) include arguments for the survival model (event of interest, time to event, and covariates) and arguments for quality control that filter on minor allele frequency (MAF) or imputation quality (`michiganCoxSurv` and `sangerCoxSurv` only). INFO score filtering using `impute2CoxSurv`

can be performed by accessing the `.info` file from IMPUTE2 results and subsequently providing the list of SNPs to `exclude.snps` argument to `gwasurvivr`. Users can also provide a list of sample IDs for `gwasurvivr` to internally subset the data.

`Gwasurvivr` outputs two files:

- (1) `.snps_removed` file, listing all SNPs that failed QC parameters
- (2) `.coxph` file with the results from the analyses, including parameter estimates, p-values, MAF, the number of events and total sample N for each SNP.

Users can keep compressed GDS files after the initial run by setting `keepGDS=TRUE` when using `impute2CoxSurv`. On subsequent runs, `gdsCoxSurv` can then be used instead of `impute2CoxSurv` to avoid compressing the data on each GWAS run. This allows users to easily perform association testing on different subsets of data as well.

3.3.2 MAF Calculation

After imputation, many SNPs will have low allele frequencies, as newer imputation methods are able to impute SNPs with MAFs around 0.1% (McCarthy et al. 2016). As rare variant analysis is beyond the scope of `gwasurvivr` and its usage is only meant for common variant ($MAF > 0.1\%$) association testing. `Gwasurvivr` allows users to filter out SNPs with low MAFs. As such, we found it useful to calculate a MAF for each SNP specifically for the samples that are being analyzed, and that calculation is described below:

For a given SNP with alleles A and B , where n_{AB} and n_{BB} are the number of individuals with AB and BB genotype respectively, and N is the sample size, the expected allele frequency of allele B ($freq_B$) can be calculated as:

$$freq_B = \frac{n_{AB} + 2n_{BB}}{2N} \quad (3.1)$$

For individual i , the allele dosage of SNP j (D_{ij}) with alleles A and B , where allele B is the effect allele and p_{AB} and p_{BB} are the posterior genotype probabilities as computed by the imputation, is calculated as:

$$D_{ij} = p_{AB_{ij}} + 2 \cdot p_{BB_{ij}} \quad (3.2)$$

For SNP j The estimated allele frequency of an effect allele B (θ_{B_j}) can therefore be calculated as:

$$\theta_{B_j} = \frac{\sum_{i=1}^N D_{ij}}{2N} \quad (3.3)$$

In R, the genotypes are represented as a matrix allele dosages, where each column is a sample and each row is a SNP. The R code is shown below:

```
library(matrixStats)
exp_freq_A1 <- round(rowMeans2(genotypes) * 0.5,
  4)
MAF <- ifelse(exp_freq_A1 > 0.5, 1 - exp_freq_A1,
  exp_freq_A1)
```

3.3.3 Modifying survival package

`Gwasurvivr` implements a Cox proportional hazards regression model (Cox 1972) to test each SNP with an outcome with options for including covariates and/or SNP-covariate interactions. As an early proof of principal in the development of `gwasurvivr`, we assessed if we could speed up the survival package during the parameter estimation step of the Cox model by providing initial estimates covariates. Using the survival function as implemented in the survival package improves computational time, we tested a dataset of 500 individuals at 7255 SNPs with 1, 2, or 3 covariates. These data were simulated using HAPGENv2 (Su, Mar-

Table 3.1: Description of arguments that are built manually in gwasurvivr and passed directly to survival::coxph.fit, bypassing the main survival::coxph function

variables	description
X	matrix of predictors
Y	Surv object
STRATA	vector containing stratification, we set to NULL
OFFSET	offset vector, we set to NULL
INIT	initial values for coefficients
CONTROL	result of a call to survival:::coxph.control
WEIGHTS	vector of weights that we set to NULL
METHOD	efron method used for handling ties
ROWNAMES	rownames that we set to NULL

chini, and Donnelly 2011) and described in a subsequent subsection “Simulating Genotypes and Phenotypes”.

The helper function `gwasurvivr:::coxParam`, adjusted for this demonstration in this document is labeled `gcoxph`. In `gcoxph_model.R` we fit the model without the SNP and the parameter estimates are then used as initial points for all subsequent models and applied over all SNPs in the dataset (See manuscript GitHub, links in Appendix). To decrease the number of iterations needed for convergence when optimizing the parameter estimates in the Cox model we modified the R package `survival` and function `survival::coxph.fit` (Therneau and Grambsch 2013). Covariates in the model are first fit without the SNP, and those parameter estimates are used as initial points for analyses with each SNP. If no additional covariates are added to the model, the parameter estimation optimization begins with null initial value. This is implemented in `gwasurvivr` by manually creating the objects found in the helper function (`survival::coxph.fit`) that fits the Cox model. These R objects and object descriptions can be see in (Table 3.1). These variables are then passed to `survival::coxph.fit` (essentially the purpose of `gwasurvivr:::coxParam` function).

The function `coxph_model.R` implements a `survival` model (`survival`

package, Therneau and Grambsch 2013) without using the optimization starting point obtained from including covariates in the model. To test the package runtime over a pre-specified number of iterations and including 1, 2, or 3 covariates the `microbenchmark` package (Mersmann 2018) in R was used.

In order to maximize compute, `parallel::parApply` (R Core Team 2018) was used instead of `base::apply` (R Core Team 2018). The number of cores used during computation on Windows and Linux can be specified by the user.

3.3.4 Computational Experiments

Upon completion of `gwasurvivr`, we wanted to compare functionality and runtime with other available GWAS survival packages, specifically GWASTools, `SurvivalGWAS_SV` and `genipe`. None of these other packages directly take VCF data output from Sanger or Michigan imputation servers. `SurvivalGWAS_SV` does accept VCF files as an input but uncompressed and not explicitly the same format that Sanger and Michigan imputation servers output, rendering additional steps to be taken. Thus, the computational experiments comprised simulated genetic data were formatted as output from IMPUTE2 software (`.gen`). Computational runtimes for `gwasurvivr` were benchmarked against existing software comparing varying sample sizes and SNP numbers, increasing covariates, for a single chromosome with ranging 15,000-25,000 individuals. In addition, we evaluated time for `gwasurvivr` for a complete GWAS (~6 million SNPs) for 3000, 6000 and 9000 samples.

3.3.4.1 Simulating Genotypes and Phenotypes

HAPGENv2 (Su, Marchini, and Donnelly 2011) was used to generate simulated genotype datasets from 1000 Genomes Project CEU data (NCBI Build 36)

(1000 Genomes Project Consortium 2015) for all benchmarking experiments. To replicate simulations the 1000 Genomes Project CEU data should be downloaded in its entirety.

For each sample size tested, survival events (alive/dead) were simulated as two separate datasets. For the dead dataset, time to event and covariates were simulated using a normal distribution. For the alive dataset, time was simulated by randomly sampling weighted probabilities for times to simulate few samples being censored, covariates were simulated from a normal distribution. Principal components (Zheng et al. 2012) were simulated using random normal distributions with decreasing variance for each additional PC. Furthermore, the `.sample` file from IMPUTE2 includes 4 columns (ID_1, ID_2, missing, and sex) which link individuals with their respective genotypes. For SurvivalGWAS_SV and GWASTools, the simulated phenotypes were appended to column 5 onward in the `.sample` file. The code for all of these analyses is available (or linked to) in the appendix or from the manuscript GitHub.

3.3.4.2 Running analysis

We used the University at Buffalo Computational Center for Research (UB CCR) academic cluster for our benchmarking analyses. Each analysis was run exclusively on node CPU-L5520 with the same system specifications, controlling the computational resources for each run. The UB CCR uses Simple Linux Utility for Resource Management (SLURM) scheduling for jobs. SLURM scripts to run the analyses were generated using shell scripts below. Benchmarking was performed using identical CPU constraints, 1 node (2.27 GHz Clock Rate) and 8 cores with 24 GB of RAM, on the University at Buffalo Center for Computational Research supercomputer. With the exception of the larger sample size tests, these were run

using the same node but 12 CPUs. `genipe` (Lemieux Perreault et al. 2016), `SurvivalGWAS_SV` (Syed, Jorgensen, and Morris 2017), and `GWASTools` (S. M. Gogarten et al. 2012) were performed as specified by the authors on available online documentation.

We performed the following benchmarking simulations with varying sample sizes (n) and SNP numbers (m):

Simulation 1. Compare `gwasurvivr` against `genipe`, `GWASTools` and `SurvivalGWAS_SV` - varying sample sizes ($n = 100$, $n = 1000$, $n = 5000$) and 100,000 SNPs ($m = 100,000$ from chromosome 18) and 3 non-genetic covariates.

Simulation 2. Comparison of `gwasurvivr`, `genipe`, `GWASTools` and `SurvivalGWAS_SV` with $n = 5,000$ and 100,000 SNPs ($m = 100,000$) with 4 covariates (age, drug treatment, sex and 1 PC), 8 covariates (age, drug treatment, sex and 5 PCs) and 12 covariates (age, drug treatment, sex and 9 PCs).

Simulation 3. `gwasurvivr` runtime for increasingly larger sample sizes ($n = 15,000$, $20,000$ and $25,000$) tested on chromosome 22.

Simulation 4. Full autosomal GWAS with varying sample sizes using `gwasurvivr` ($n = 3,000$, $n = 6,000$ and $n = 9,000$).

Additionally, to maximize the performance of `SurvivalGWAS_SV`, these jobs were run using “array” jobs as recommended by the authors. An example batch script, provided in the `SurvivalGWAS_SV` documentation, was converted from PBS to SLURM. 24GB of ram was not needed on all runs, however was used to ensure each run remained uniform. The jobs were split into array sets of 1000 SNPs for $m = 100,000$, totaling 100 batched jobs in a single array. Thus, we define *rate-limiting array* as the array index that had the longest runtime. This is an important caveat and bears consideration when using `SurvivalGWAS_SV`. Depending on availability on the computing cluster, the analyses could be completed as quickly as

the longest individual array job, or potentially the entire runtime could be equal to the summation runtime of all of the array indices if these cannot be run simultaneously (or if there are failures with any of the array indices).

Custom scripts were written in R or bash to streamline the analyses. For `gwasurvivr` and GWASTools, a ‘run’ script was used that involved passing variables that were assigned in bash into R variables and passing them into `gwasurvivr` or GWASTools within an R session. Both genipe and Survival-GWAS_SV can be invoked directly from the command line, so bash scripts were written to automate these processes for the different experiments. Please see the Appendix or manuscript GitHub for the code.

3.4 Results

3.4.1 Cox Model Modifications

Speeding up the original Cox model implemented in the survival package. By leveraging an initialization point from the analyses with covariates `gwasurvivr` (`gcoxph`) is several seconds faster than the survival analyses function as implemented in `survival` (`coxph`, Therneau and Grambsch (2013)) in R (Figure 3.1). While this is a small test dataset, in practice this would be an appreciable difference when testing across several thousands of samples and millions of SNPs.

3.4.2 Benchmarking Analyses

3.4.2.1 Differing Sample Size Runtime: `gwasurvivr` vs. other software

The computational benchmarking experiments comparing `gwasurvivr` with genipe, GWASTools, and SurvivalGWAS_SV were done using a sample size of $n = 5,000$ and $m = 100,000$ SNPs. The coefficient estimates from the Cox model

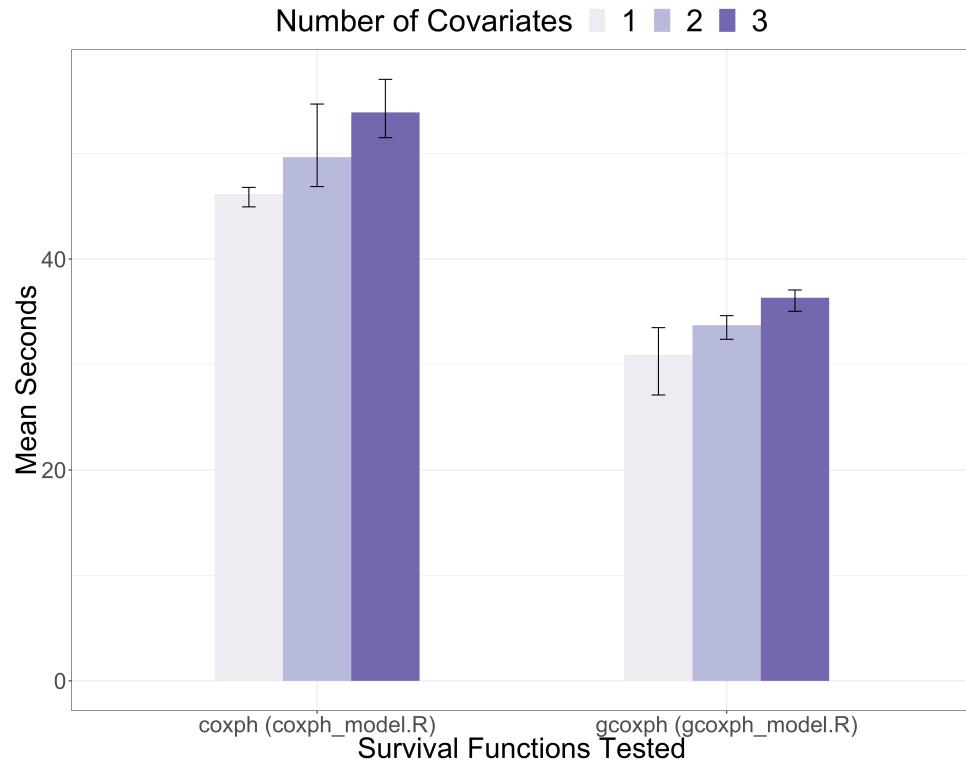


Figure 3.1: Time to convergence of original R survival package implementation vs modified implementation using simulated data. The x-axis are the survival functions tests, coxph and gcoxph. The y-axis are mean seconds from three iterations of each function. The error bars represent the maximum and minimum run time from three iterations with the barplots showing the mean runtimes of either coxph or gcoxph for 1, 2, or 3 covariates. gcoxph, which fits initial points based on parameter estimates from covariates alone, runs on average faster than the traditional coxph model for 1, 2, and 3 non-genetic covariates and shows a decreasing min to max range as the number of covariates increase.

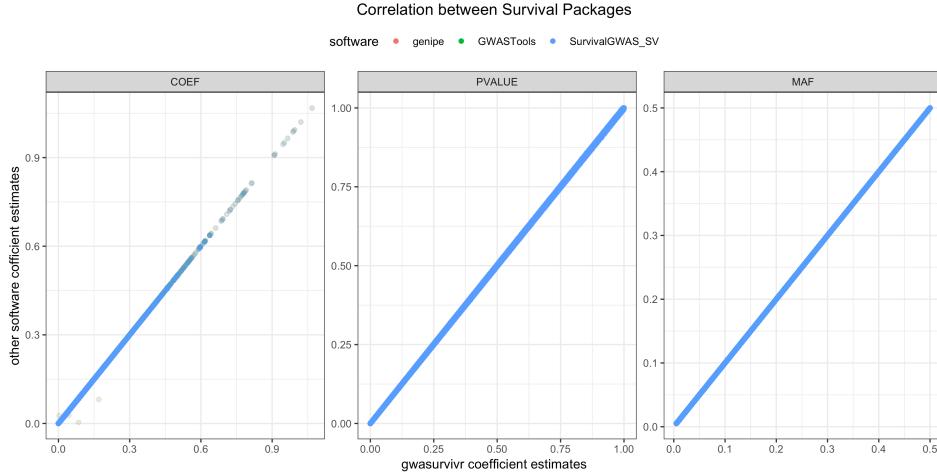


Figure 3.2: Correlation of parameter estimates and minor allele frequency across survival analysis packages. Shown here are $n = 5000$ and $m = 100,000$. The x-axes (from left-to-right) are gwasurvivr coefficient estimates, pvalues, and minor allele frequency (MAF). The y-axes (from left-to-right) are coefficient estimates, pvalues, and minor allele frequency (MAF) from the other software, respectively. The points are colored to indicate the software being used where red is genipe, green is GWASTools alone, and blue is SurvivalGWAS_SV. The estimates are near perfectly correlated and thus not all colors are visible in each plot.

and p-values are perfectly correlated between the packages (Figure 3.2, left and center panels). The `gwasurvivr` sample MAF calculation (Equation 3.3) is perfectly correlated with the MAFs calculated from the other software (Figure 3.2, right panel).

Scalability is a key component in software design, particularly in contemporary times with growth of data. As such, we wanted to benchmark how well `gwasurvivr` (and the other software) scaled as sample size increased from $n = 100$, $n = 1000$, and $n = 5000$. `gwasurvivr` was faster than `genipe` (Lemieux Perreault et al. 2016), `SurvivalGWAS_SV` (Syed, Jorgensen, and Morris 2017), and `GWASTools` (S. M. Gogarten et al. 2012) for $m = 100,000$ SNPs at $n = 100$, and $n = 5000$, with the exception of `SurvivalGWAS_SV` at $n = 1000$ (Figure 3.3). To reiterate, the reported time is the rate-limiting of `SurvivalGWAS_SV` (the shortest runtime for 1 of 10 arrays), meaning this may not necessarily reflect on how quickly the analysis

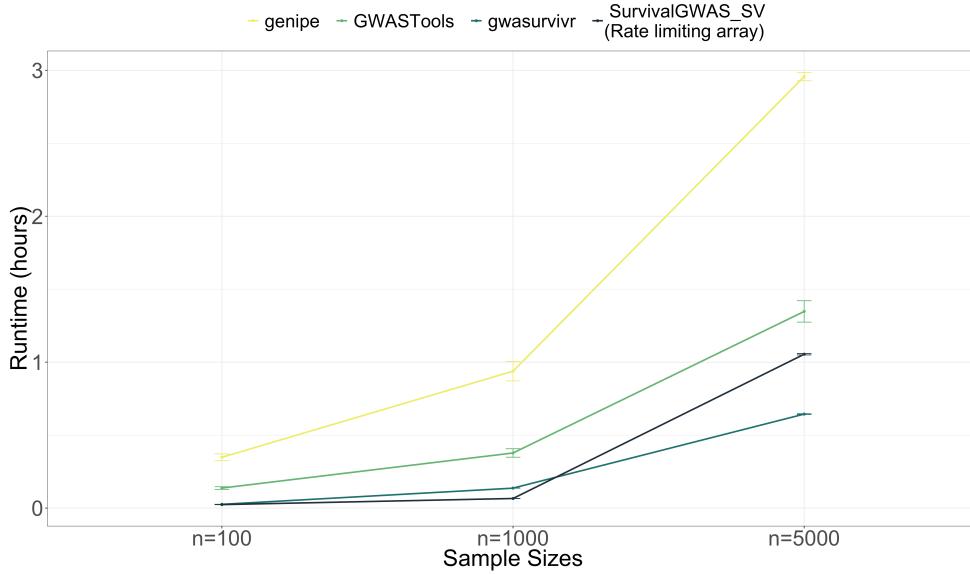


Figure 3.3: Runtime for survival analyses between different survival software. The x-axis shows the three sample sizes with 100,000 SNPs. The y-axis is the total runtime in hours. Mean and 95% confidence intervals (CI) are show for genipe (yellow), GWASTools (light green), SurvivalGWAS_SV (dark blue) and gwasurvivr (dark green). Confidence intervals were calculated for 3 simulations for each n and m combination.

will complete, as it could vary between one to all arrays. **Gwasurvivr** is orders of magnitudes faster than the other software when the sample size is $n = 5000$ (Figure 3.3).

3.4.2.2 Increasing sample size tests with gwasurvivr

As a single GWAS may include tens of thousands of individuals, the next question was to what extent could the sample size be increased. We tested **gwasurvivr** on the smallest chromosome (chromosome 2) available in our simulated dataset. **Gwasurvivr** is able to handle large sample sizes up to $n = 25,000$, however, compression time increases with increasing sample size, and likely will be limited by available RAM on a machine or cluster (Figure 3.4).

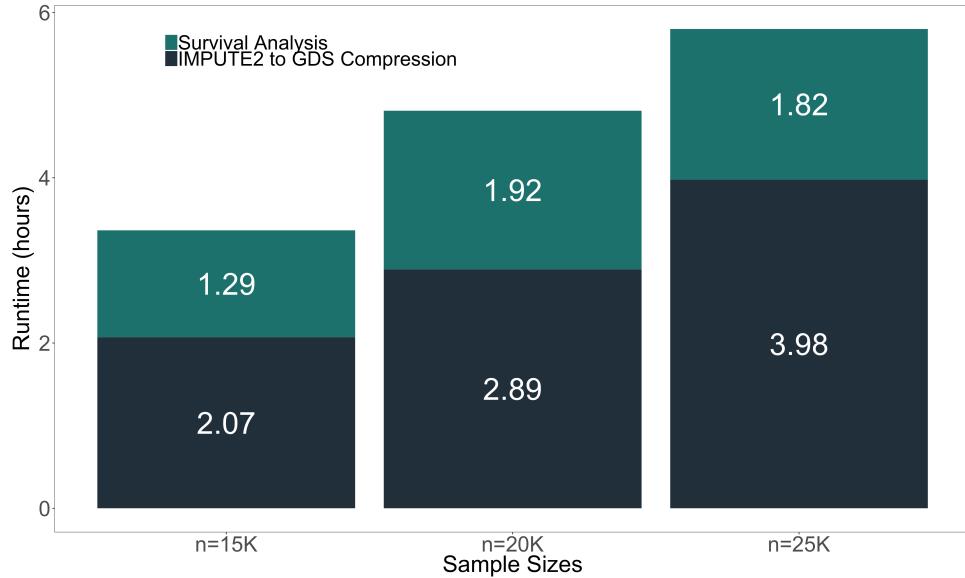


Figure 3.4: Runtimes for survival analyses with on chromosome 22 with increasing sample size. Gwasurvivr was run on IMPUTE2 data simulated from chromosome 22 ($m \approx 117,000$ SNPs) for $n = 15,000$, $n = 20,000$ and $n = 25,000$. The dark blue is elapsed time for compressing to GDS format and dark green is the computational time to run the survival analysis alone.

3.4.2.3 Additional Covariate Runtime: *gwasurvivr* vs. other software

We were then interested in how well each software scaled as the number of additional covariates increased and if a substantial increase in runtime would occur. Increasing the number of covariates for gwasurvivr has minimal effects on runtime versus other software (Figure 3.5).

3.4.2.4 Running full GWAS with different sample sizes using *gwasurvivr*

And the final and most practical question to answer was to determine the run time for a complete GWAS. A ~6 million SNP GWAS can be run in < 10 hours for 9000 samples when using separately scheduled jobs on a supercomputer (Figure 3.6). The `keepGDS` argument helps address this and results in reduced run times (Figures 3.4 and 3.6), i.e. < 3 hours for a GWAS of $n = 9,000$.

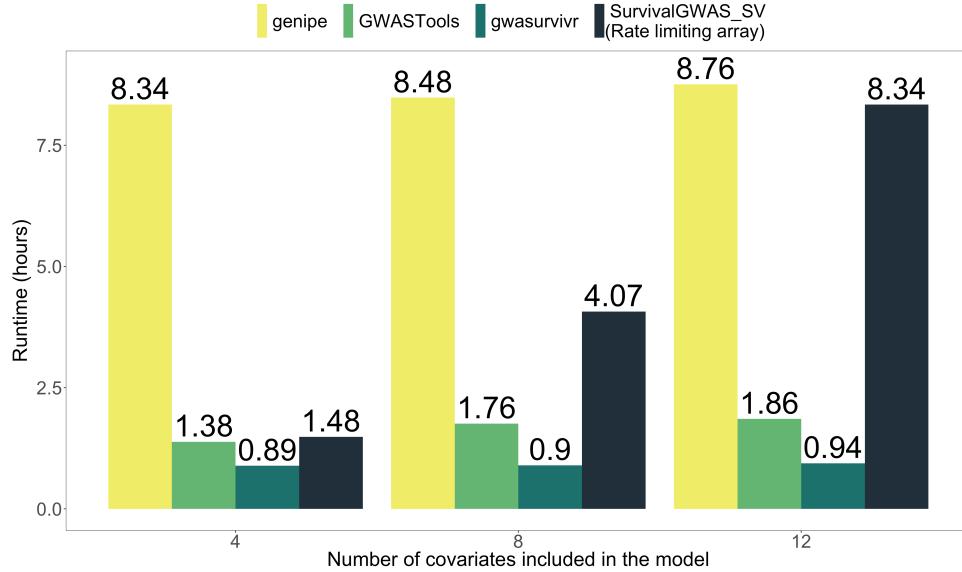


Figure 3.5: Runtime for survival analyses with increasing number of covariates. Genipe (yellow), GWASTools (light green), SurvivalGWAS_SV (dark blue) and gwasurvivr (dark green) run with 4, 8 and 12 covariates ($n = 5000$, $m = 100,000$).

3.5 Discussion

Large-scale GWAS that study phenotypes that result in death are becoming prevalent, particularly in the field of pharmacogenomics. Research should not be disrupted due to lack of workflows and software that permit error-free. To put this in perspective, we ran hundreds of survival analyses in our lab on GWAS data. Often, we would need to double check analyses before presenting findings at a conference or in a publication. We lacked reliable software that would enable straightforward reproducibility, and assumed other labs were experiencing this as well. A strategy to improve analyses would be to convert all genetic data into GDS and then for subsequent analyses the survival analyses would complete in a fraction of the time. We could improve this package by adding visualizations and also by leveraging SeqVarTools, a new R bioconductor package that uses GDS format and implements row wise computations. While other software already existed with the in-

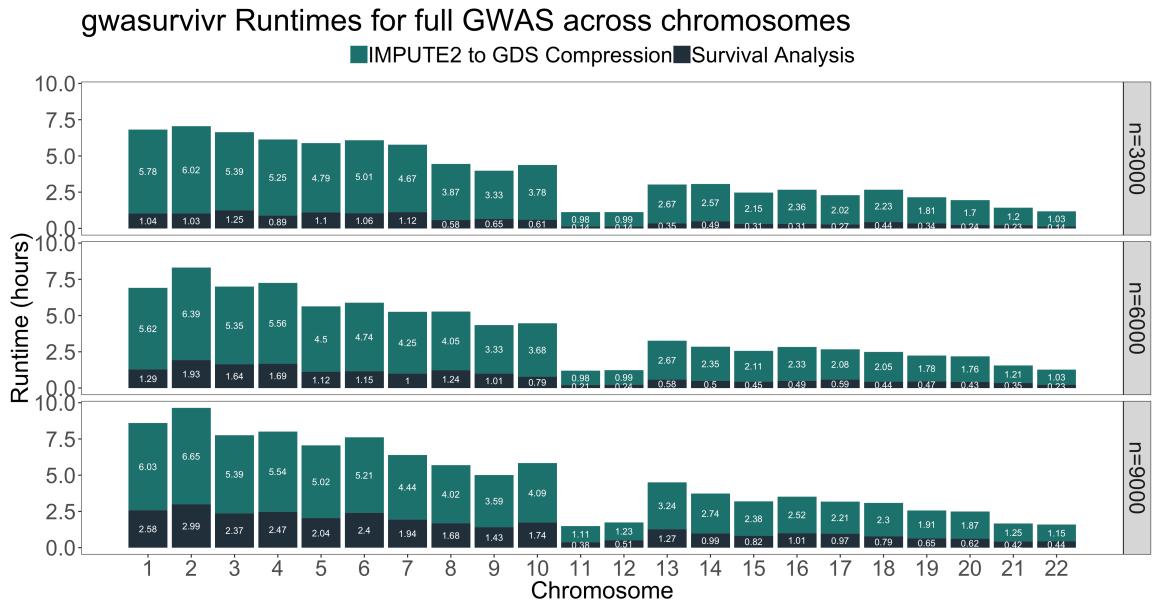


Figure 3.6: Runtime for survival analyses for full GWAS with increasing sample size using gwasurvivr impute2CoxSurv. The three panels represent different sample sizes of $n = 3000$ (top), $n = 6000$ (middle) and $n = 9000$ (bottom). The dark blue shaded area is elapsed time for compressing IMPUTE2 to GDS format and the dark green shaded areas are the computational time to run the survival analysis alone. On a computing cluster, each chromosome can be scheduled to run as individual jobs for best performance. Each GWAS was run on the UB CCR supercomputer with on the same node with 24 GB of RAM and 4 CPUs per node.

tent of addressing these needs, we developed `gwasurvivr` to be in the R/Bioconductor ecosystem and be flexible such that it can be easily integrated into workflows and pipelines, as well as being easily adjusted for advanced users.

CHAPTER 4: Application and Pipeline

4.1 Introduction

Automation of large scale studies is essential to the reproducibility of analyses. Robust workflows that have minimal user interaction decrease the possibility of continual, unnoticed errors from propagating. When modeling and analyzing DISCOVeRY-BMT, we often have to compute hundreds of analyses, i.e. for three genomes (donor, recipient and mismatch), different disease stratification, two cohorts, and different survival outcomes. The effects of both cohorts are combined using meta-analyses. In order to overcome this potentially cumbersome process, we developed an automated pipeline that uses imputed genotype data from the Sanger imputation server as input, runs user specified Cox regression models using gwasurvivr, performs a meta-analysis, and subsequently reshapes the data into a clean format. Here we show an overview of the pipeline (known as DISCOVeRY-BMT Meta-analysis Pipeline) we created and the analyses that were performed after re-imputing DISCOVeRY-BMT using the Sanger Imputation Server and describe the functional annotation that succeeding the initial GWAS.

4.2 Methodology

4.2.1 Data

4.2.1.1 Genetic Data (Imputation Results)

DISCOVeRY-BMT was first imputed using IMPUTE2 (B. N. Howie, Donnelly, and Marchini 2009) and 1000 Genomes Project (1kGP) Phase 3 (1000

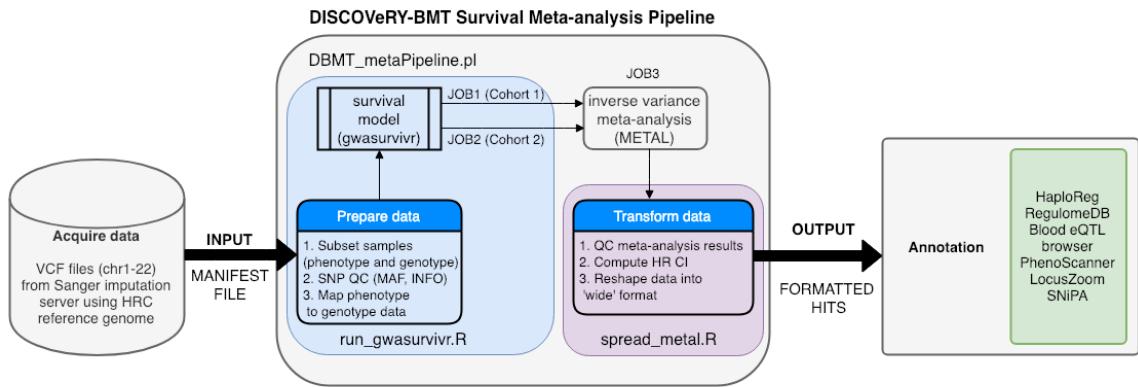


Figure 4.1: Process diagram of automated survival meta-analysis pipeline DISCOVeRY-BMT. The pipeline is written in `DBMT_metaPipeline.pl`. It submits three jobs on UB CCR SLURM, job 1 (cohort 1 survival analysis), job 2 (cohort 2 survival analysis), and job3 (meta-analysis) which waits for (dependency) job 1 and 2 to complete before starting. To elaborate, a manifest file passed to `DBMT_metaPipeline.pl` as input that defines the VCF file (chromosome), outcome, patient subset, and memory allocation. `run_gwasurvivr.R` captures the command line Rscript that is invoked in the perl script and prepares the data. `run_gwasurvivr.R` uses the outcome and patient subset from the manifest file and defines these outcomes and affiliated covariates and runs `gwasurvivr` survival analysis. After both cohort 1 and cohort 2 are complete, meta-analysis is performed. After meta-analysis is complete, `spread_metal.R`, filters out heterogeneous SNPs between cohorts, statistics are computed, and data is reformatted such that cohort 1, cohort 2, and meta-analysis are all shown in a single line corresponding to a single variant. The output it then used for annotation to further characterize associations.

Table 4.1: Broad Overview of DISCOVeRY-BMT Clinical Characteristics

Genome	Description
Donor	age, sex, race group, blood type (ABO)
Recipient	age, sex, race group, blood type (ABO), graft source, causes of death (levels 1-7), conditioning regimen and intensity, TBI fractionation, GVHD prophylaxis, current disease type, current disease progression, prior disease type, cytogenetics status, time to death, time to relapse, AML type, transplant year, HLA match (8/8 or 10/10), cytomegalovirus, Lansky Score, Karnovsky Score, Principal Components from EIGENSTRAT

Genomes Project Consortium 2015) reference panel. Although new reference panels have not updated to a newer genome release than Genome Reference Consortium Human Build 37 (GRCh37; same as 1kGP), the number of samples included in the development of the reference panel itself have increased substantially (i.e. from ≈ 1000 to $\approx 65,000$. As such we decided that it would be appropriate to update DISCOVeRY-BMT GWAS data to increase the quality of imputation.

As mentioned in Chapter 1 and Chapter 3, several imputation software and servers exist. Two imputation web services are publicly available, Michigan imputation server (Das et al. 2016) from University of Michigan and Sanger imputation server from Wellcome Sanger Institute (McCarthy et al. 2016). The largest, non-subtle, differences between the servers are related to the imputation algorithms used. Michigan imputation server uses minimac3 (Das et al. 2016). Sanger imputation server using the position Burrows-Wheeler transform (PBWT) algorithm (R. Durbin 2014). Both imputation services offer user friendly platforms and offer the most up to date reference panels, including HRC release 1.1. HRC reference panel combines data from over 20 different studies. The majority of samples in HRC are low coverage sequencing data and predominantly European ancestry, and HRC includes 1kGP Phase 3 as well. HRC has $\approx 65,000$ haplotypes and ≈ 40

million SNPs. Prior to imputation, it is common practice to pre-phase (haplotype estimation) before imputation, as previous studies have demonstrated estimating haplotypes prior to imputation substantially speeds up the imputation process (O’Connell et al. 2016). EAGLE2 (P.-R. Loh et al. 2016) or SHAPEIT2 (Delaneau et al. 2013) are commonly used pre-phasing algorithms. SHAPEIT2 pre-phasing and PBWT imputation were used for DISCOVeRY-BMT, returning unphased, imputed genotypes.

4.2.1.2 Phenotype file

DISCOVeRY-BMT has clinical characteristics and survival times/events corresponding to recipients and donors provided by NMDP and CIBMTR. The phenotype file will interchangeably be referred to as “covariate file”. The survival times and events are for recipients only. Characteristics are shown in Table 4.1. In the phenotype file that is used for our analyses – many clinical covariates been recoded into dummy/indicator variables or stratified into joint groupings.

4.2.2 Perl Script for Pipeline

The perl script resides on UB CCR and is very specific to DISCOVeRY-BMT and folder structure on our accounts. The central idea is that the pipeline consists of three jobs (JOB1, JOB2, and JOB3) in a single Slurm Workload Manager submission. JOB1 and JOB2 are perform survival analysis using gwasurvivr on VCF files for one chromosome (Figure 4.1). JOB3 is the meta-analysis of cohorts 1 and 2 using METAL (Willer, Li, and Abecasis 2010) software. JOB3 also reshapes the data such that results from cohort 1, cohort 2, and meta-analysis are all in one row. JOB3 has a dependency on JOB1 and JOB2 completion before initiating (Figure 4.1).

Table 4.2: Manifest file column descriptions

Column name	Description
path_to_vcf_file	Chromosomes 1-22 VCF files (include full directory)
output_name	Output file name
patient_subset	Patient subset - i.e. ALL only, AML only, Mixed
genome	Donor, recipient, or mismatch
outcome	Outcome - i.e. DRM, TRM, OS, PFS, INF, OF, REL
memory	Random access memory (RAM) in megabytes (MB) allocated to the job

4.2.2.1 *Manifest file*

The imputed files can be directly passed to gwasurvivr. Manifest files are defined as a file that contains columns (for this pipeline, manifest files are tab-separated) that describe specific arguments that are passed to other functions (i.e. `run_gwasurvivr.R` or SLURM scripts). Manifest file fields can be found in Table 2.

4.2.2.2 *Execution of script*

Essentially the script facilitates submission of survival analysis for JOB1, JOB2, and JOB3.

Required commands:

```
-m      path to the manifest file
-e      e-mail address for SLURM status updates
        (completion of run)
-w      walltime in format 00:00:00
-rscript The R script (run_gwasurvivr.R) that internalizes
        arguments from the manifest file, assigns outcomes
        and corresponding covariates. And then invokes
        gwasurvivr for both cohort 1 and cohort 2.
```

For example, shown below is the command to execute the pipeline for recipients with AML when testing for DRM:

```
perl /projects/rpc1/lbuchest/lbuchest/DBMT_metaPipeline/\
    Meta_pipeline.pl \
```

Table 4.3: DISCOVeRY-BMT Analyses Run Using DBMT metaPipeline.

Genomes	Patient Subset	Survival Outcomes	Censoring Time	Analyses Run
Donor, Recipient, Mismatch	AML	DRM, TRM, OS, PFS	1 year	12
Donor, Recipient, Mismatch	AML + MDS	DRM, TRM, OS, PFS	1 year	12
Donor, Recipient, Mismatch	Mixed	DRM, TRM, OS, PFS	1 year	12
Donor, Recipient	ALL	DRM, TRM, OS, PFS, REL, OF, GVHD, INF	1 year	16
Donor, Recipient	B-ALL	DRM, TRM, OS, PFS	1 year	8
Donor, Recipient	T-ALL	DRM, TRM, OS, PFS	1 year	8
Donor, Recipient, Mismatch	ALL	DRM, TRM, OS, PFS, REL, INF, OF, GVHD	100 days	24
Donor, Recipient, Mismatch	AML	DRM, TRM, OS, PFS, REL, INF, OF, GVHD	100 days	24
Donor, Recipient, Mismatch	AML + MDS	DRM, TRM, OS, PFS, REL, INF, OF, GVHD	100 days	24
Donor, Recipient, Mismatch	Mixed	DRM, TRM, OS, PFS, REL, INF, OF, GVHD	100 days	24
Donor, Recipient	ALL	OS, REL	3 years	4
Total				168

```
-m D_AMLonly_DRM.manifest \
-e rizvi.33@osu.edu \
-w 08:00:00 \
-rscript /projects/rpc1/lbuchest/lbuchest/DBMT_metaPipeline/\
    run_gwasurvivr.R
```

4.2.3 GWAS Analyses Run

We ran 168 different analyses (504 analysis jobs total) (Table 4.3). The clinical covariates that we included in our models are in Table 4.4. The clinical covari-

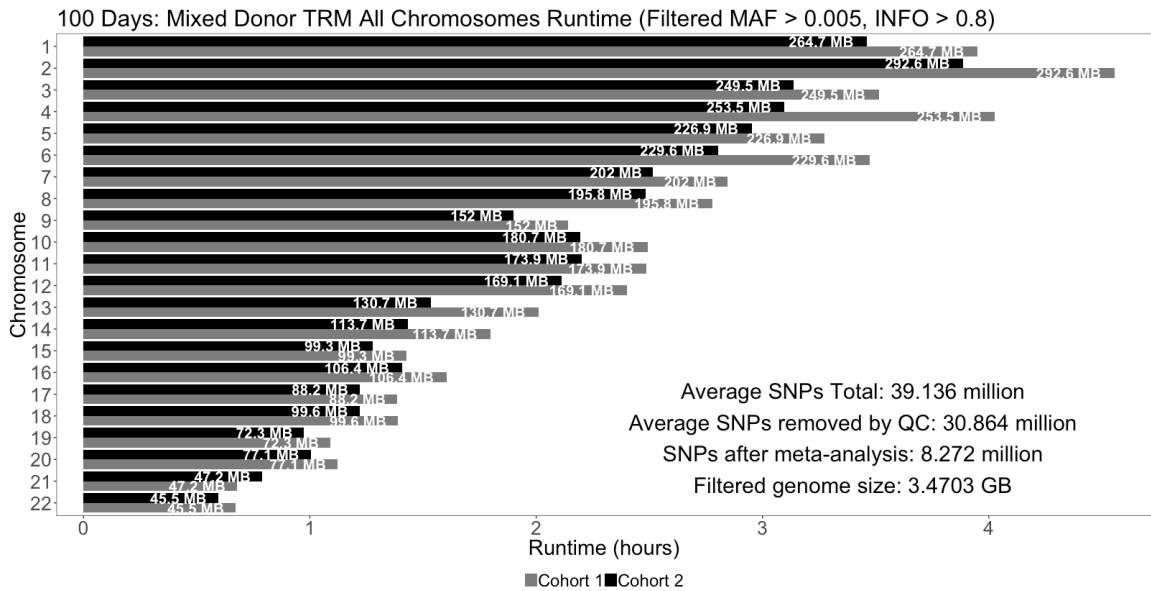


Figure 4.2: Runtime diagnostics of full GWAS using DBMT Meta analysis Pipeline. Shown here is just one example of all analyses run. This example is donor genotypes in mixed diseases (AML + ALL + MDS) recipients, testing for TRM. The y-axis is chromosomes 1-22 and the x-axis is computational runtime in hours. Each chromosome was run separately and all began at the same time, such that the total time for this GWAS was equivalent to chromosome 2 in cohort 1 runtime (4.66 hours). The white text inside each bar is the file size of the chromosomes after gwasurvivr survival analysis. The SNPs were filtered for MAF > 0.005 and INFO > 0.8.

Table 4.4: Survival Models Analyzed

Survival Outcomes	Time Interval	Covariates
TRM	time to death	recipient age, BMI, graft source
DRM	time to death	recipient age, disease status
OS	time to death	age, disease status, graft source
PFS	time to relapse	recipient age, disease status
REL	time to relapse	conditioning regimen and intensity
GVHD	time to death	recipient age, donor age, BMI
OF	time to death	disease status, graft source
INF	time to death	age, BMI, CMV status

ates were selected as they pertained to clinical relevance. For covariates in REL, the model was selected using bidirectional stepwise regression. Although we ran hundreds of analyses, we will only discuss ALL analyses censored at 1-year in Chapter 5. Each GWAS took just over 4 hours when submitting entire GWAS at the same time on UB CCR (Figure 4.2). Instances of delay of analysis due to backlog in Slurm Workload queue were rare. An entire genome began at \approx 40 million SNPs and after QC (INFO > 0.8, MAF > 0.005, filtering out SNPs that did not converge from Cox model), the final genome was \approx 8 million SNPs, or the equivalent to 3.47 GB of storage (Figure 4.2).

4.2.4 Post-GWAS Analyses

While GWAS in and of itself is a powerful method to identify disease-associated SNPs, it does not necessarily address the underlying biology the association signals. After the meta-analysis GWAS are complete, we first looked at our association signals. To visualize this, we created our own custom Manhattan plot in *ggplot2* (Wickham 2016), that labels the SNPs that are below $P_{meta} < 5 \times 10^{-8}$ threshold (code available on dissertation GitHub repository). The next step was to use publicly available data resources to annotate SNPs that reached the suggestive significance threshold $P_{meta} < 5 \times 10^{-5}$. Annotation aids

researchers develop mechanistic hypotheses to further characterize the impact of non-coding variants on clinical phenotypes and/or normal genetic variation. Annotation was done by leveraging publicly available databases that have compiled data from large scale studies (ENCODE, GTEx) using HaploReg (L. D. Ward and Kellis 2015), RegulomeDB (Boyle et al. 2012), and single nucleotide polymorphisms annotator (SNiPA) (Arnold et al. 2015).

4.2.4.1 Annotation Resources

HaploReg leverages LD information from the 1000 Genomes Project to compile chromatin states and protein binding annotation from ENCODE and Roadmap to Epigenome projects (L. D. Ward and Kellis 2015). HaploReg also collects and curates data from sequence conservation studies, as well as studies that determine effects of SNPs and consequences on regulatory motifs and/or gene expression (eQTL) (L. D. Ward and Kellis 2015). HaploReg data was accessed using haploR (Zhbannikov, Arbeev, and Yashin 2019).

RegulomeDB is a collection of annotation databases that reports SNPs with known and/or predicted regulatory elements in intergenic regions of the human genome (Boyle et al. 2012). Known and predicted regulatory DNA elements include regions of DNAase hypersensitivity, binding sites of transcription factors (TFs), and promoter regions that have been experimentally/biochemically characterized to regulate transcription (Boyle et al. 2012). The entire RegulomeDB database was downloaded and stored on UB CCR for ease of access when annotating regions of interest.

SNiPA annotations come from Ensembl87 (v3.2). It comprises variant-phenotype associations and annotations contained in Ensembl 92 release, as well as pQTL data. pQTL association data is obtained from the pGWAS study in

blood (B. B. Sun et al. 2018). SNiPA includes more than 16,500 cis- and trans- associations with blood protein levels (Arnold et al. 2015). The genome assembly for SNiPA is GRCh37.p13 and it uses 1000 Genomes Project Phase 3 (v5). SNiPA obtains data from HGMD, dbGaP, ClinVar, OMIM variation, UniProt, GWAS Catalog, DrugBank, DECIPHER, and GTEx eQTL associations (Arnold et al. 2015). Flanking regions of +/- 150kb from the sentinel SNP (snp of interest) are pulled from DISCOVeRY-BMT results.

The R code and SLURM scripts for data manipulating and pulling regions for HaploReg, RegulomeDB, and SNiPA are available on this dissertation’s GitHub repository.

4.3 Discussion

In this chapter we described the fulcrum to all of analyses run in DISCOVeRY-BMT Sanger imputation results. We developed a pipeline that facilitated automation of hundreds of survival analyses that ensured ease of implementation and reproducibility. We also described post-GWAS analyses that have yet to be integrated into this pipeline. An ongoing project is underway that will unite an annotation (SNiPA, HaploReg, and RegulomeDB) workflow with our current pipeline. As the phenotype of DISCOVeRY-BMT is quite complex, this will allow our lab to uncover insights in a much more reproducible and less cumbersome manner. Additional databases such as PhenoScanner (Staley et al. 2016) and Blood eQTL Browser (Westra et al. 2013) will also be implemented into the annotation portion of this pipeline.

CHAPTER 5: Death within 1 year after MUD-HSCT for Acute Lymphoblastic Leukemia (ALL) GWAS

5.1 Introduction

Acute lymphoblastic leukemia (ALL) is a type liquid cancer that originates in the lymphoid line of hematopoietic stem cells. It is the most common type of childhood cancer (25% of all cases) and about 60% of cases for those under 20 years old worldwide (Pui 2011). Childhood ALL is highly treatable with a combination of standard chemotherapy protocols with a 5-year survival upwards of 90% (Pui et al. 2015). Historically adolescents and young adults (AYA) have had poorer prognosis when it came to treatment from ALL, however, over the past several years major improvements have occurred as treatment protocols have shifted from treating AYA with adult regimens towards pediatric regimens (Huguet et al. 2009; Siegel et al. 2018).

While therapeutic regimens have been quite successful, ALL remains the second most common hematological malignancy for which patients will undergo HLA-matched unrelated donor hematopoietic stem cell transplantation (MUD-HSCT) (D'Souza et al. 2017). The number of transplants among ALL patients has steadily been increasing yearly (D'Souza et al. 2017). As recently as 2017, there were approximately 6,000 new cases of ALL and approximately 1500 patients underwent MUD-HSCT (SEER Database 2018; D'Souza et al. 2017). ALL patients receive HSCT as a curative therapy after reaching complete remission (CR) and disease status at time of diagnosis play a major role in disease prognosis (Pui 2011). Patients receive HSCT as a curative therapy after reaching complete remission (CR) and disease

status at time of diagnosis play a major role in disease prognosis. Nearly 60% of adult (\geq 40 years old) patients with advanced disease die within the first year after transplant (D’Souza et al. 2017). MUD-HSCT has slightly improved survival in childhood ALL (\leq 15 years old) with 40% of advanced disease patients dying in the first year (D’Souza et al. 2017). These large proportions of patients dying within the first year present a strong need to discover genetic markers that can inform clinicians on patient longevity and/improvements in donor selection.

Here, we use DISCOVeRY-BMT GWAS to identify and characterize germline genetic markers contribution to survival in ALL patients and their matched unrelated donor after MUD-HSCT. While this study is not specifically asking questions about age, over 60% the ALL patient population in DISCOVeRY-BMT are in childhood and AYA populations, presenting an excellent cohort to uncover markers that may contribute to poor outcomes in young and adult patients after transplant. Additionally, targeted therapeutics can be discovered from SNPs, particularly those that alter sequences of transcription factor binding or result in epigenetic remodeling. In this chapter we go through associated regions for recipient survival outcomes when considering either recipient or their unrelated donor genetics. Furthermore, we performed post-GWAS annotation to develop mechanistic and biologically relevant hypotheses of how these SNPs contribute to survival phenotypes.

5.2 Methods

Chapter 1 provides details on DISCOVeRY BMT cohorts and survival outcome definitions. Chapter 4 describes the clinical covariates included in all analyses and provides an in depth description of analytic methods. Acute lymphoblastic leukemia patients in DISCOVeRY-BMT are cytogenetically normal. We use Cox regression (Cox 1972; A. A. Rizvi et al. 2018) to test the significance of genetic vari-

ation in: overall survival (OS), disease related mortality (DRM), transplant related mortality (TRM), relapse (REL), progression-free survival (PFS) and TRM subtypes [graft versus host disease (GVHD), infection (INF), and organ failure (OF)].

All analyses were performed on one SNP (donors or recipients dosage across all patients) at a time, adjusted for clinically relevant covariates. Meta-analysis was done using a fixed effects model via METAL software (Willer, Li, and Abecasis 2010). Additional QC was performed on the SNPs (i.e. INFO score > 0.8, MAF > 0.05 in both cohorts, required to be same direction between cohorts and heterogeneous SNPs were filtered out). We define genome-wide significance as $P_{meta} < 5 \times 10^{-8}$. Reported significant results were filtered $P_{cohort1} < 5 \times 10^{-5}$ and $P_{cohort2} < 0.2$. Meta-hazard ratios were filtered $\frac{1}{15} \leq HR_{meta} \leq 15$.

Post-GWAS annotations were performed by leveraging publicly available databases including HaploReg v4.1 (L. D. Ward and Kellis 2015), RegulomeDB (Boyle et al. 2012), and SNiPA (Arnold et al. 2015). These annotation repositories were described in detail in Chapter 4.

5.3 Results

5.3.1 DISCOVeRY-BMT ALL Summary

DISCOVeRY-BMT has 483 patients in cohort 1 and 94 patients in cohort 2 (Table 5.1). The proportion of patients that die within the first year (overall survival) is on average 41.35% between both cohorts (Table 5.1). Slightly more patients die from TRM than from DRM at 21.85% and 19.5% between both cohorts, respectively (Table 5.1). About 27% of patients experience relapse after transplant and between 5-7% of patients are dying from TRM subtypes (Table 5.1).

Age is adjusted as a continuous variable in our Cox regression model, however,

Table 5.1: Proportion of Events at 1-year in DISCOVeRY-BMT ALL Cohorts

Outcome	Cohort 1	Cohort 2
DRM	91/483 (0.188)	19/94 (0.202)
TRM	108/483 (0.224)	20/94 (0.213)
OS	199/483 (0.412)	39/94 (0.415)
PFS	245/483 (0.507)	45/94 (0.479)
REL	133/483 (0.275)	25/94 (0.266)
GVHD	37/483 (0.077)	4/94 (0.043)
INF	32/483 (0.066)	9/94 (0.096)
OF	28/483 (0.058)	5/94 (0.053)

Proportion of Events (Death or Relapse) are displayed as the sample size of event (N_{event}) divided by the total number of patients (N) in the ALL cohort. Overlapping events are not shown here.

Table 5.2: Proportion of ALL Patient Age Groups Stratified by DISCOVeRY-BMT Cohorts

Age Category	Cohort 1	Cohort 2
Childhood	0.188	0.160
AYA	0.501	0.468
Adult	0.261	0.319
Senior Adult	0.050	0.053

Senior Adult: ≥ 60 years old; Adult: ≥ 40 years old and < 60 ; Adolescent or Young Adult (AYA): ≥ 15 and < 40 ; Childhood < 15

for characterization purposes we looked at the age distribution by standard clinical stratification groups. Those groups include senior adults (≥ 60), adult (≥ 40 years old < 60), adolescent and young adults (AYA; ≥ 15 and < 40 years old), and children (< 15 years old) (Table 5.2). Childhood and AYA comprise 69% of cohort 1 and 63% of cohort 2 (Table 5.2).

5.3.2 Post-HSCT associations with recipient SNPs

We tested recipient (patient) SNPs for association with OS, PFS, DRM, TRM and GVHD in ALL patients. While all the subgroups of TRM are of interest, for the purpose of this chapter the main focus are on these more frequent outcomes

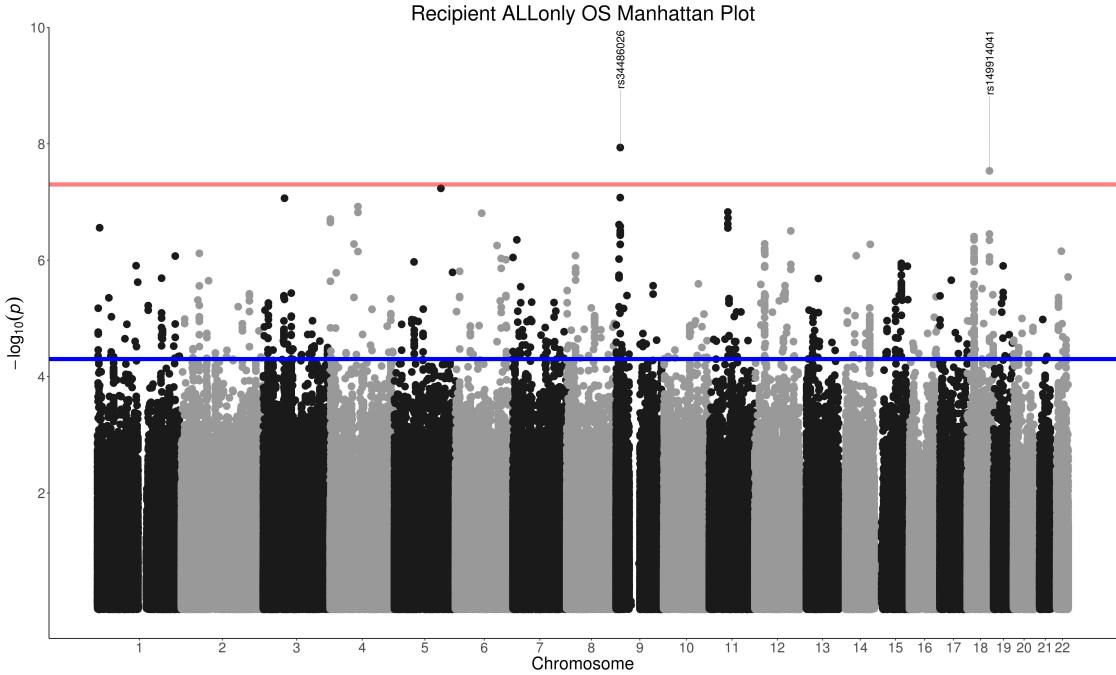


Figure 5.1: Recipient OS 1 Year Manhattan Plot. The x-axis is chromosome 1-22 and the y-axis is the $-\log_{10}(P_{meta})$. Each dot is a SNP. The red line is genome wide significance at $P_{meta} < 5 \times 10^{-8}$. The blue line is suggestive significance at $P_{meta} < 5 \times 10^{-5}$. Labeled SNPs are associated hits that have passed genome-wide threshold.

Table 5.3: DISCOVeRY-BMT Recipients Overall Survival Associations at 1-Year

RSID	CHR:POS	REF/ EF- FECT	Ref. Panel/Cohort 1/ Cohort 2 MAF	P-value	HR (95% CI)	INFO
rs34486026	9:12585285	C/T	0.0086/0.0112/0.009	1.159e-08	7 (3.59-13.67)	0.94
rs149914041	18:65924660	G/A	0.0128/0.0215/0.017	2.923e-08	4.24 (2.54-7.06)	0.90

All SNPs shown here are imputed. Genomic positions are GRCh37 reference genome.

while the rest are exploratory. We detected genome-wide associated regions with DRM and OS outcomes. Some of these regions were also genome-wide significant for patients diagnosed with B-ALL subtype. Identifying such recipient SNPs can be helpful in distinguishing individuals that have a higher risk of mortality following transplant and treatment strategies can be modified accordingly.

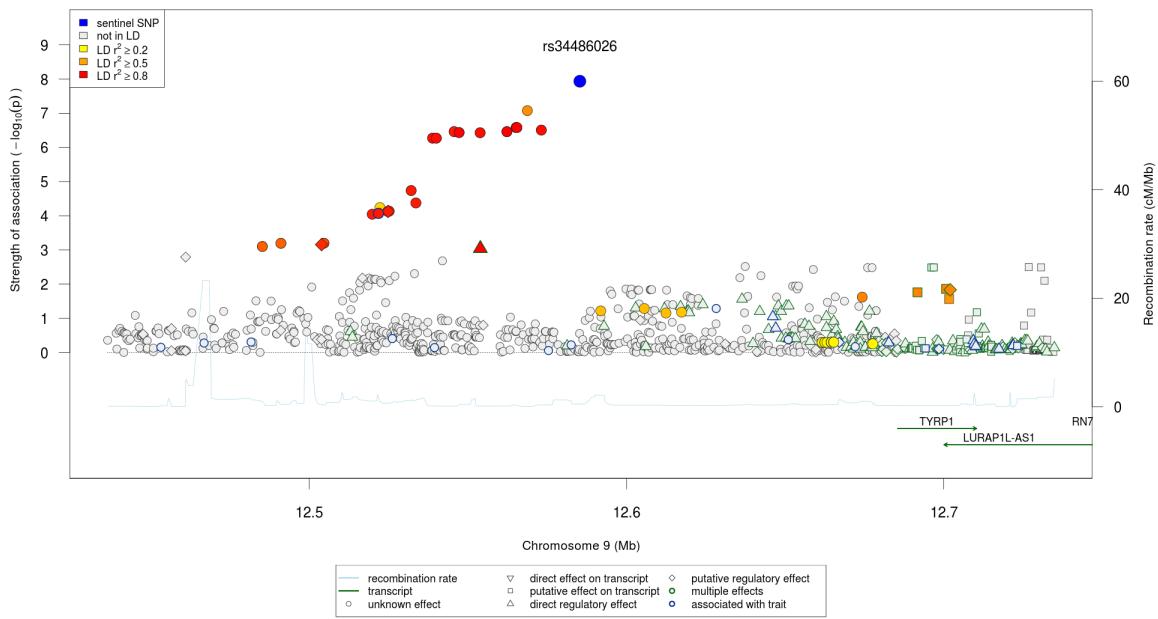


Figure 5.2: Regional association plot of recipient SNP rs34486026 with DRM in ALL patients. The x-axis is genomic position on chromosome 2 and the y-axis is the $-\log_{10}(P_{meta})$. Sentinel SNP is rs34486026 and colored blue. The region comprises +/- 150 kb window from the sentinel SNP. The red-yellow color gradient represents LD strength. Shapes represent various regulatory or functional effects that the SNP has been reported to have in this region from independent studies.

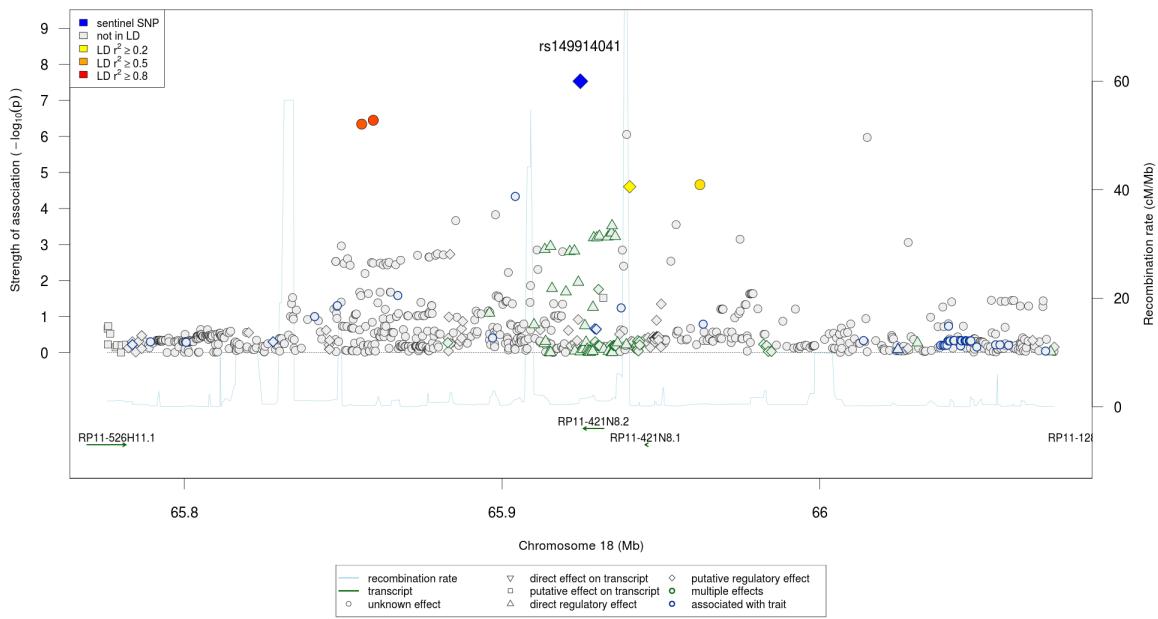


Figure 5.3: Regional association plot of recipient SNP rs149914041 with DRM in ALL patients. The x-axis is genomic position on chromosome 2 and the y-axis is the $-\log_{10}(P_{meta})$. Sentinel SNP is rs149914041 and colored blue. The region comprises +/- 150 kb window from the sentinel SNP. The red-yellow color gradient represents LD strength. Shapes represent various regulatory or functional effects that the SNP has been reported to have in this region from independent studies.

5.3.3 Recipient SNPs Overall Survival Associations

Association tests of recipient SNPs with overall survival after MUD-HSCT revealed two loci on chromosomes 9 and 18 that reached genome-wide significance (Figure 5.1; Table 5.7).

Association on chromosome 9 was detected for rs34486026 (Figure 5.2), which changes binding motifs for 6 proteins (Kheradpour and Kellis 2014). rs34486026 is also in LD ($r^2 = 0.82$) with rs16929156, a protein quantitative trait loci (pQTL) for lysozyme (Lyz) protein. Lyz is a protein with antibacterial activity found in multiple tissues and body fluids. Associated loci on chromosome 18 was rs149914041, which alters motif for Foxa proteins, a subclass of Fox family of transcription factors involved in cell growth, proliferation and longevity.

We also screened recipient SNPs with suggestive OS associations ($P < 5 \times 10^{-5}$) and detected two regions of interest on chromosomes 6 and 12.

Identified loci on chromosome 6 were eQTLs for *CDCL5* gene (Figure 5.4). *CDCL5* encodes for Cell division cycle 5-like protein, which acts as a transcription activator. Interestingly PRP19-CDC5L complex may also play a role in the response to DNA damage. (Lei et al. 2000)

5.3.4 Recipient SNPs Disease Related Mortality Associations

We found genome-wide recipient SNP associations ($P_{meta} < 5 \times 10^{-8}$) with DRM on chromosomes 2, 11, and 17 (Figure 5.5).

Among the genome-wide associated (GWA) SNPs on chromosome 2, rs4663706 had the strongest association (Table 5.4). Three SNPs (rs55671334, rs4663709, rs76276644) in the region are in LD with rs4663706 at $r^2 > 0.8$. These SNPs are located in the upstream of *COL6A3* gene and this intergenic region

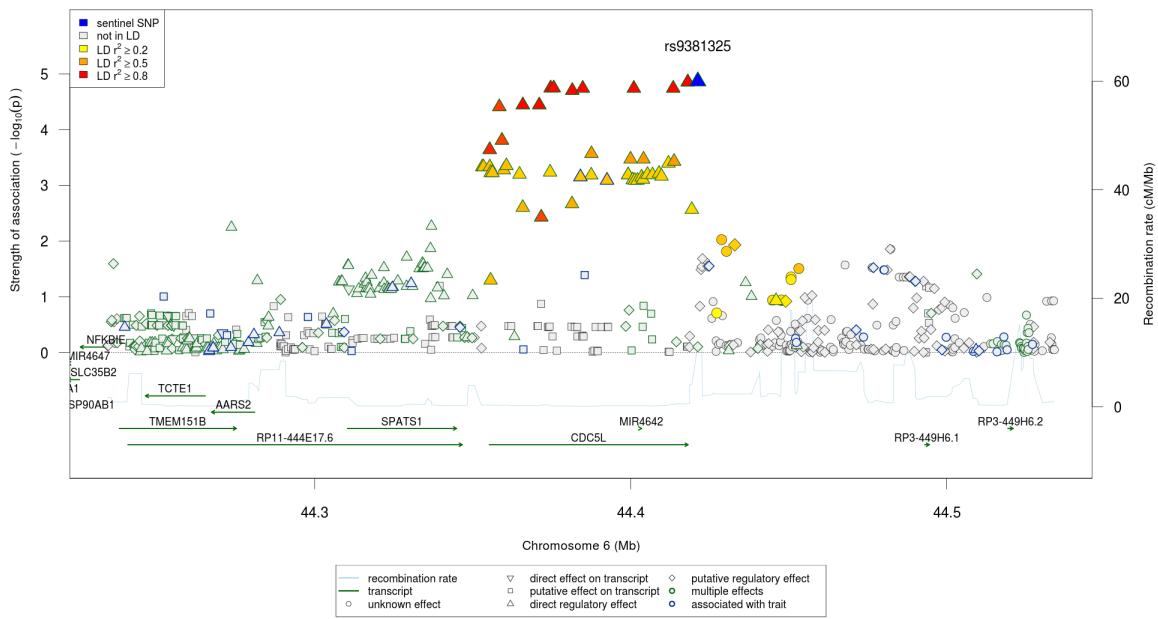


Figure 5.4: Regional association plot of recipient SNP rs9381325 with DRM in ALL patients. The x-axis is genomic position on chromosome 2 and the y-axis is the $-\log_{10}(P_{meta})$. Sentinel SNP is rs9381325 and colored blue. The region comprises +/- 150 kb window from the sentinel SNP. The red-yellow color gradient represents LD strength. Shapes represent various regulatory or functional effects that the SNP has been reported to have in this region from independent studies.

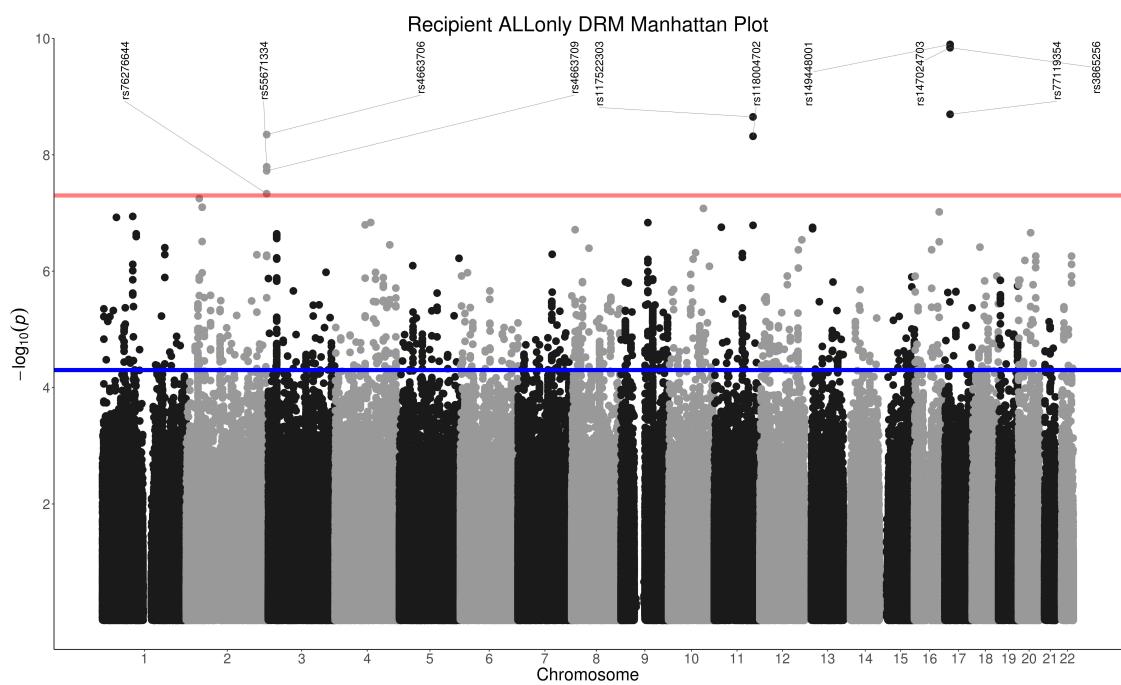


Figure 5.5: Manhattan Plot of Recipient ALL DRM GWAS. The x-axis is chromosome 1-22 and the y-axis is the $-\log_{10}(P_{meta})$. Each dot is a SNP. The red line is genome wide significance at $P_{meta} < 5 \times 10^{-8}$. The blue line is suggestive significance at $P_{meta} < 5 \times 10^{-5}$. Labeled SNPs are associated hits that have passed genome-wide threshold.

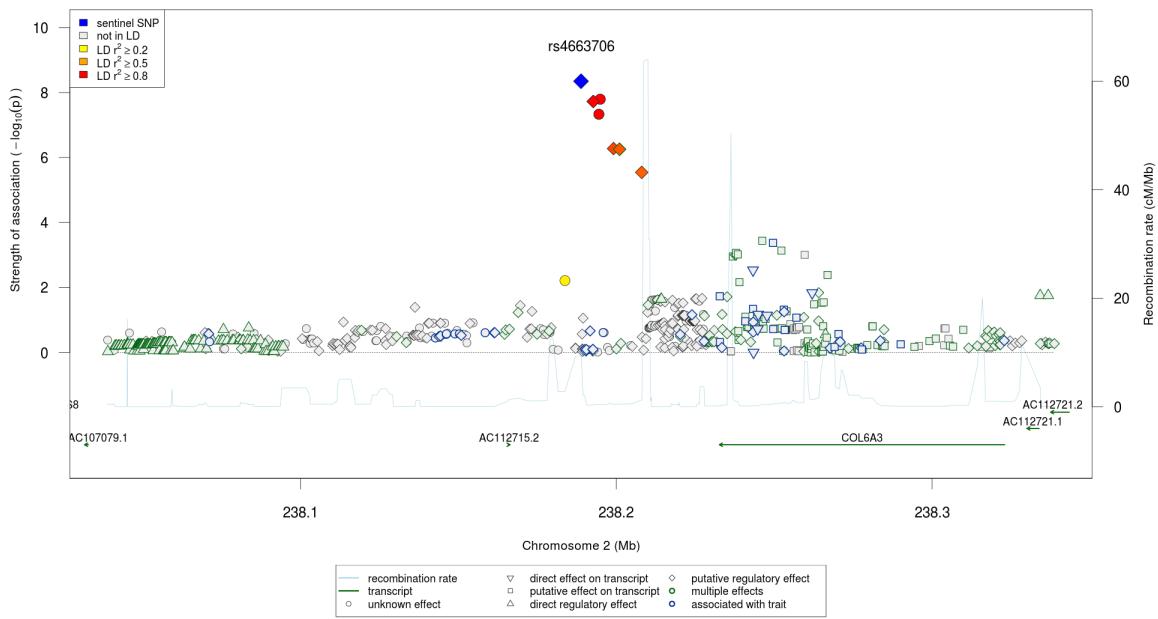


Figure 5.6: Regional association plot of recipient SNP rs4663706 with DRM in ALL patients. The x-axis is genomic position on chromosome 2 and the y-axis is the $-\log_{10}(P_{meta})$. Sentinel SNP is rs4663706 and colored blue. The region comprises +/- 150 kb window from the sentinel SNP. The red-yellow color gradient represents LD strength. Shapes represent various regulatory or functional effects that the SNP has been reported to have in this region from independent studies.

Table 5.4: DISCOVeRY-BMT recipient genotypes associated with disease related mortality at 1-year.

RSID	CHR:POS	REF/ EF- FECT	Ref. Panel/Cohort 1/ Cohort 2 MAF	P-value	HR (95% CI)	INFO
rs4663706	2:238188818	C/T	0.0208/0.0166/0.0152	4.495e-09	7.67 (3.88-15.15)	0.83
rs55671334	2:238194894	C/G	0.0212/0.0152/0.0114	1.609e-08	7.37 (3.68-14.73)	0.93
rs4663709	2:238192685	T/A	0.0192/0.0151/0.0112	1.885e-08	7.32 (3.66-14.65)	0.91
rs76276644	2:238194465	C/T	0.0215/0.016/0.0112	4.667e-08	6.96 (3.47-13.97)	0.92
rs117522303	11:114863319	C/T	0.014/0.0141/0.0202	2.233e-09	9.88 (4.66-20.94)	0.81
rs118004702	11:114910112	C/T	0.0152/0.0138/0.0221	4.804e-09	8.79 (4.25-18.2)	0.88
rs149448001	17:15490739	T/C	0.0148/0.0104/0.0261	1.269e-10	9.47 (4.77-18.78)	0.82
rs3865256	17:15489677	C/T	0.0185/0.0106/0.0261	1.444e-10	9.29 (4.7-18.37)	0.83
rs147024703	17:15487520	T/G	0.0146/0.0106/0.0261	1.445e-10	9.29 (4.7-18.37)	0.83
rs77119354	17:15495401	A/G	0.017/0.011/0.0266	2.015e-09	7.35 (3.83-14.12)	0.84

All SNPs shown are imputed. Genomic positions are GRCh37 reference genome.

has been predicted as an enhancer region in multiple tissues, including primary hematopoietic stem cells by Roadmap Epigenome project (Figure 5.6). It is important to note however, that all four SNPs including rs4663706 are imputed (INFO > 0.8). Furthermore, while rs55671334, rs4663709, rs76276644 had genome-wide significant P_{meta} values, (Figure 5.5) the associations appear to be driven by cohort 1, as $P_{cohort2} > 0.1$ (Table 5.4). Thus, p-values in cohort 2 weaken confidence in the genome-wide association in this region.

The region on chromosome 11, includes two genome-wide associated SNPs, rs118004702 and rs117517252 ($r^2 = 0.94$) (Table 5.4; Figure 5.5; Figure 5.7). ENCODE ChIP-Seq experiments report that SNPs in this region alter regulatory motifs on *HNF4* and *TAL1* (Kheradpour and Kellis 2014). *TAL1* has been reported as an oncogenic transcription factor in T-cell ALL subtype (Sanda and Leong 2017).

Chromosome 17 has four recipient SNPs (rs149448001, rs3865256, rs147024703, rs77119354) associated with DRM within the first year transplant (Table 5.4; Figure 5.4; Figure 5.8). All four SNPs are located in the intronic region of CDRT1 and alter binding motifs for various proteins known to play important roles in leukemias or other cancers (i.e. *BRCA1*, *CDX*, *E2A*, *STAT*

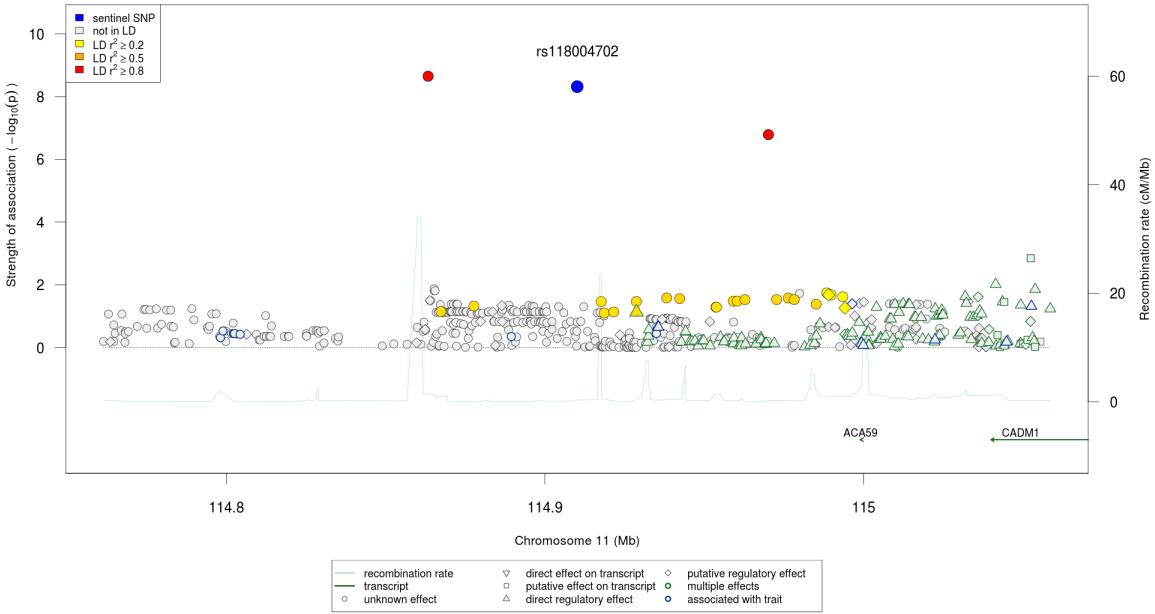


Figure 5.7: Regional association plot of recipient SNP rs118004702 with DRM in ALL patients. The x-axis is genomic position on chromosome 2 and the y-axis is the $-\log_{10}(P_{meta})$. Sentinel SNP is rs118004702 and colored blue. The region comprises +/- 150 kb window from the sentinel SNP. The red-yellow color gradient represents LD strength. Shapes represent various regulatory or functional effects that the SNP has been reported to have in this region from independent studies.

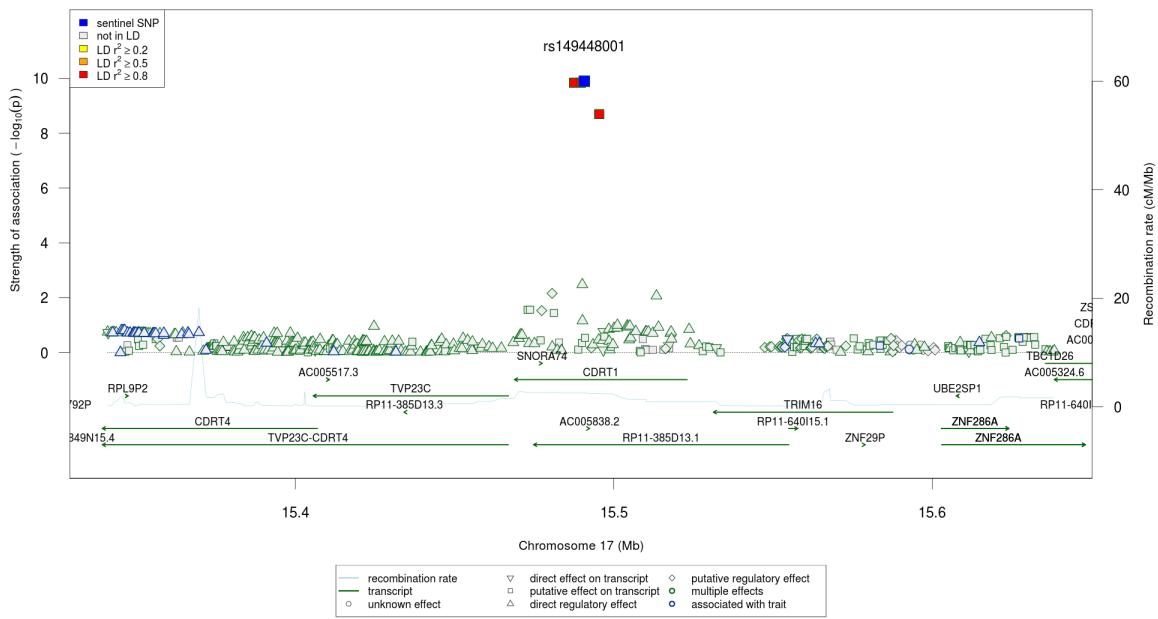


Figure 5.8: Regional association plot of recipient SNP rs149448001 with DRM in ALL patients. The x-axis is genomic position on chromosome 2 and the y-axis is the $-\log_{10}(P_{meta})$. Sentinel SNP is rs149448001 and colored blue. The region comprises +/- 150 kb window from the sentinel SNP. The red-yellow color gradient represents LD strength. Shapes represent various regulatory or functional effects that the SNP has been reported to have in this region from independent studies.

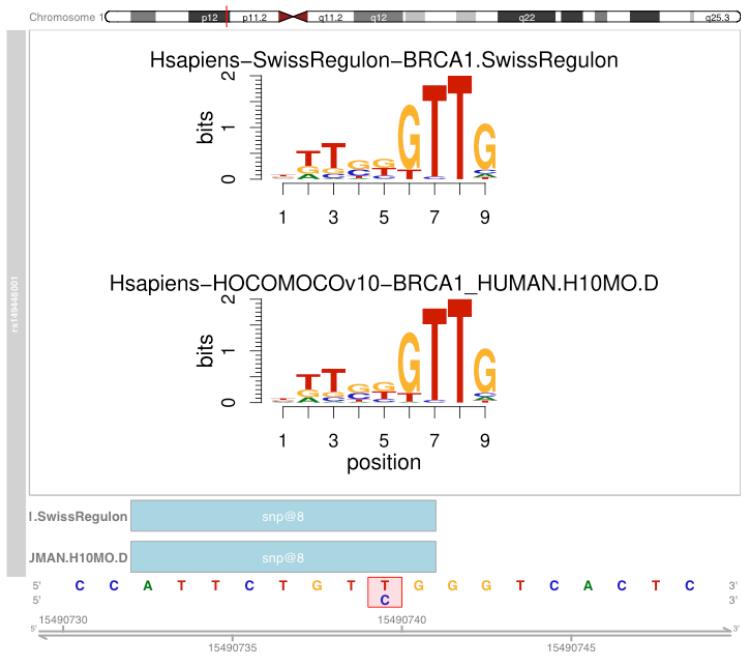


Figure 5.9: Recipient DRM Chromosome 17 rs149448001 motif. A schematic of chromosome 17 is at the top. The red vertical line is the genomic location of this motif. The two panels show BRCA1 binding motif on chromosome 7 from two different human motif databases. rs149448001 is located at position 8 from T to C. The coordinate is highly predicted to be a T, and therefore a C allele will likely alter the binding properties of proteins to this region.

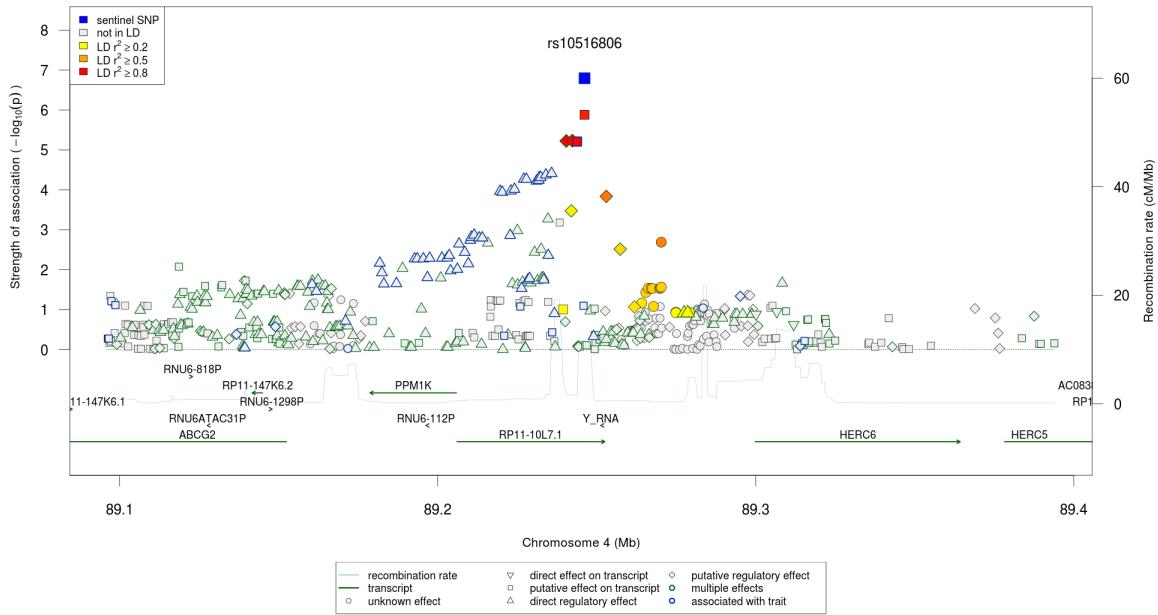


Figure 5.10: Regional association plot of recipient SNP rs10516806 with DRM in ALL patients. The x-axis is genomic position on chromosome 2 and the y-axis is the $-\log_{10}(P\text{-value})$. Sentinel SNP is rs10516806 and colored blue. The region comprises ± 150 kb window from the sentinel SNP. The red-yellow color gradient represents LD strength. Shapes represent various regulatory or functional effects that the SNP has been reported to have in this region from independent studies.

and *GATA* families) (Perez-Andreu et al. 2015). The SNP rs149448001 had the strongest association in this region and significantly changes the binding motif of *BRCA1*, a well known tumor suppressor. In fact rs149448001 changes the only unambiguous 8th position in the motif from T to C (Figure 5.9; S. G. Coetze, Coetze, and Hazelett (2015)). The Roadmap Epigenome Project, places one of the associated SNPs, rs77119354, in an enhancer region in almost all primary T-cell and hematopoietic stem cell lines (L. D. Ward and Kellis 2015).

We also inspected recipient SNPs with suggestive DRM associations ($P < 5 \times 10^{-5}$) for previously reported regulatory function. This revealed a region on chromosome 4 where blood metabolite levels associations (rs10022462; Shin et al. 2014) (Figure 5.10) had been reported. The blood metabolite GWAS hit is in LD

with rs10516806 ($r^2 = 0.82$; $P = 1.6 \times 10^{-7}$; HR: 0.474 [0.359-0.627] for T allele) in recipient DRM. Rs10516806 is in perfect LD ($r^2 = 1$) with rs2869930 which has been identified as protein bound in ChIP-Seq experiments for *FOXA1* (in liver cell line Hep G2) and *ER α* (also known as *NR3A1*; in T-47D human breast cancer cells) (ENCODE Project Consortium 2012). *FOXA1* is a transcription factor that belongs to the *FOX* family – of which another member of this family, *FOXP1*, when down-regulated is associated with deficient B-cells (H. Hu et al. 2006; Wlodarska et al. 2005). *FOXA1* and *FOXP1* are transcription factors that are known to modulate activity of *ER α* in breast cancer (Ijichi et al. 2013). It may be plausible to further interrogate *FOXA1* status in patients who die from their original disease.

rs10516806 and SNPs that are in LD ($r^2 > 0.82$) have enhancer and promoter histone marks in blood and transplant related tissues (i.e. skin, GI). Rs10516806 is associated with chromatin marks (H3K4me1 for primary T cells from peripheral blood). SNPs in this region alter regulatory motifs on *CEBPB* family genes, *ZPF410*, *FOXP3*, *HOXD8*, and *PAX5*. *ZPF410* is a *PAX5* activated gene (S. Yang et al. 2015). *PAX5* encodes for transcription factors that are essential for progression of adult B lymphogenesis from early B cell progenitor cells (Nutt et al. 1998; S. Shah et al. 2013). This region has been reported to be associated to gene expression or quantitative traits in several studies. The locus rs10516806 is an eQTL and effects *PPM1K* gene expression in liver and breast cell lines (Q. Li et al. 2014). Furthermore, a recent study *PPM1K* is involved in amino acid catabolism in HSCs and plays a major role in maintaining stem cell status and HSC repopulation in mice bone marrow (X. Liu et al. 2018). This study suggests that *PPM1K* deletion in murine models improve survival (X. Liu et al. 2018).

While this could manifest different phenotypes in humans, it suggests that investigating the direction (risk or protective) rs10516806 alters *PPM1K* gene ex-

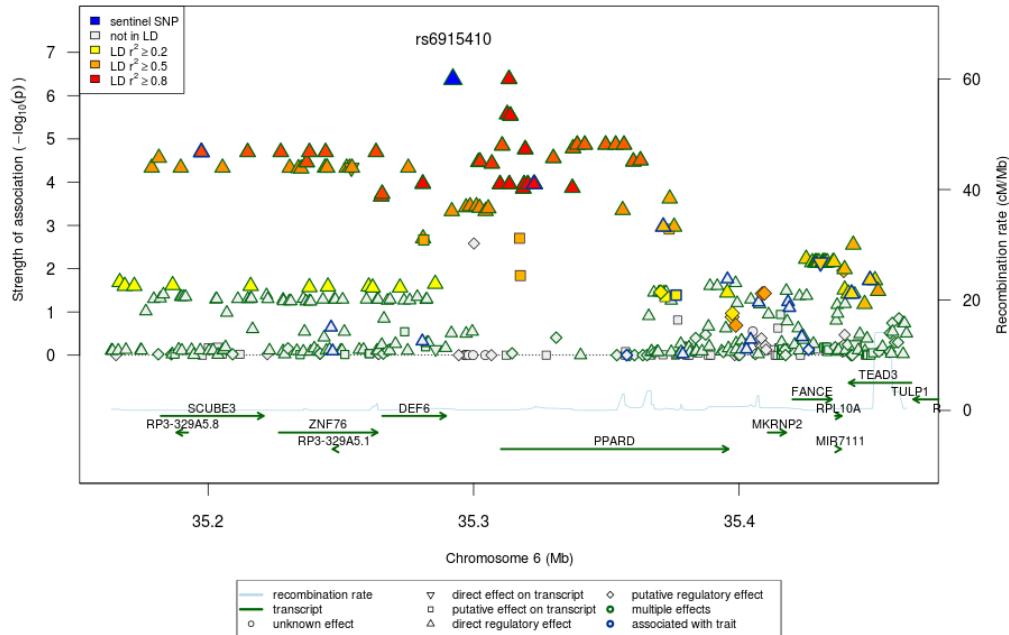


Figure 5.11: Organ failure in recipient on chromosome 6. Sentinel SNP is rs6915410 and colored blue. The region comprises +/- 150 kb window from the sentinel SNP. The red-yellow color gradient represents LD strength. Shapes represent different effects that the SNP has been reported to have in this region from independent studies.

pression is reasonable.

5.3.5 Exploratory analyses: Recipient SNP associations with Organ Failure

While more recipients died due to transplant in the first year compared to death due to disease (Table 5.1), no genome-wide associations with recipient SNPs were uncovered from TRM survival analyses (Manhattan Plot not shown).

However, upon when we scanned suggestive associations organ failure in DISCOVeRY-BMT, we identified rs6915410 ($P = 4.17 \times 10^{-7}$; HR: 4.64 [2.56-8.38]; C effect allele) on chromosome 6 as an interesting locus for death by organ failure (OF) in recipients. This SNP is in a very active region, where many SNPs that are in moderate to high LD have direct regulatory effects on *DEF6* and *PPARD*

(Figure 5.11). In addition to rs7744392 being *DEF6* eQTL in peripheral blood monocytes (Zeller et al. 2010) and blood (Fehrman et al. 2011), it is an eQTL for *MPO* in peripheral monocytes (Zeller et al. 2010) as well. These eQTLs were identified in lymphoblastoid and whole blood derived cell lines (Zeller et al. 2010; Fehrman et al. 2011). Blood is the most abundant tissue that has histone marks on enhancers and promoter regions for rs6915410 and corresponding correlated SNPs (L. D. Ward and Kellis 2015). Experimental ChIP-seq studies have shown that *ERα* protein binds to this region. Notably, this region has evidence of altered regulatory motifs including known leukemia genes *E2A*, *AR3A*, and *CEBPB*, *FOXO*, *GATA*, *STAT* families (Iacobucci and Mullighan 2017).

5.3.6 Post-HSCT Associations with Donor SNPs

All eight patient outcomes (DRM, TRM, OS, PFS, REL, GVHD, INF and OF) in ALL patients were tested for association with their matched unrelated donors SNPs. We detected genome-wide associated regions with OS, DRM, TRM and GVHD outcomes. Identifying such donor SNPs can be helpful in providing insight on genes and pathways involved in post-transplant events. More importantly upon rigorous replication, these SNPs can help clinicians improve donor selection and ultimately post-transplant survival outcomes.

5.3.7 Donor SNPs Associated with Recipient Overall Survival

Donor SNPs were associated to recipient overall survival (OS) on chromosomes 2, 5, 9, 13, and 18 (Figure 5.12 and Table 5.5).

There are two associated regions on chromosome 2 (near 71Mb and 154 Mb). The former has two associated SNPs in perfectly correlated (rs116220248, rs76480148), both of these SNPs are in perfect LD with a SNP that has protein

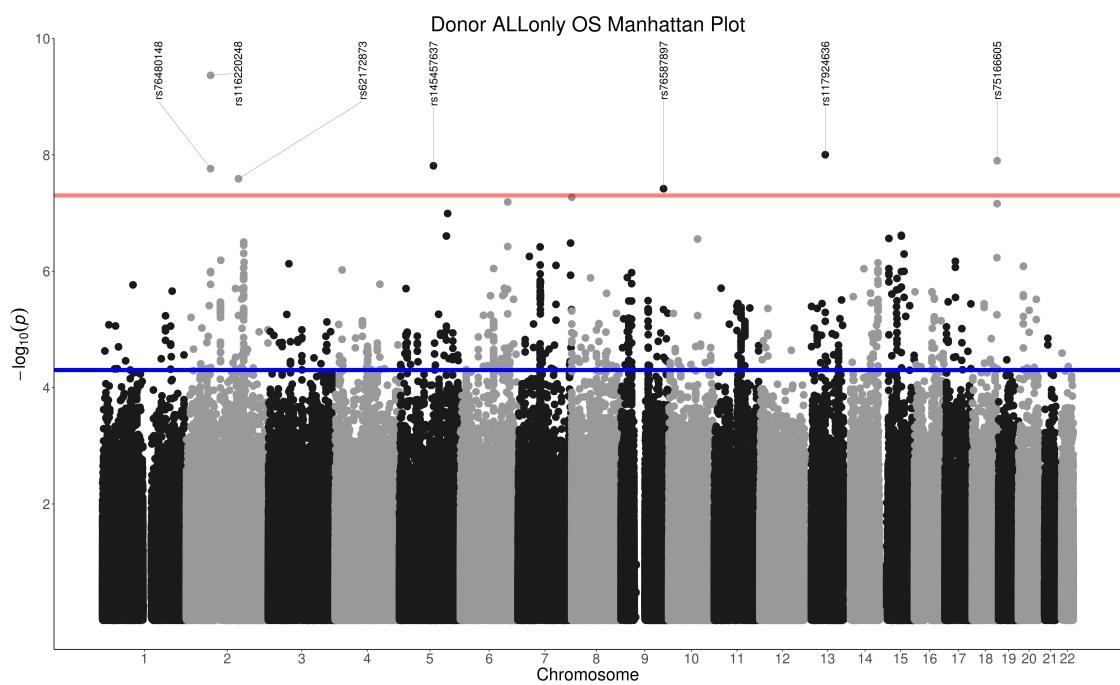


Figure 5.12: Manhattan Plot of Donor ALL OS GWAS. The x-axis is chromosome 1-22 and the $-\log_{10}(P_{meta})$. Each dot is a SNP. The red line is genome wide significance at $P_{meta} < 5 \times 10^{-8}$. The blue line is suggestive significance at $P < 5 \times 10^{-5}$. Labeled SNPs are associated hits that have passed genome-wide threshold.

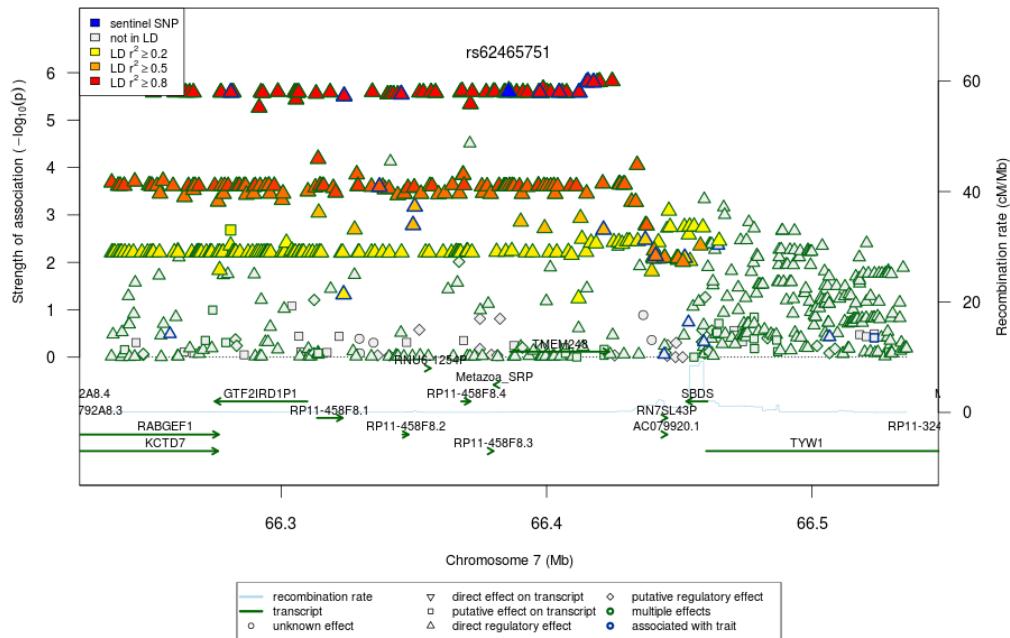


Figure 5.13: Donor OS Region Chromosome 7. Sentinel SNP is rs62465751 and colored blue. The region comprises +/- 150 kb window from the sentinel SNP. The red-yellow color gradient represents LD strength. Shapes represent different effects that the SNP has been reported to have in this region from independent studies.

Table 5.5: DISCOVeRY-BMT Donor SNPs Associated to Recipient Overall Survival at 1-Year

RSID	CHR:POS	REF/ EFF	Ref. Panel/Cohort 1/ Cohort 2 MAF	P-value	HR (95% CI)	INFO
rs116220248	2:71934021	C/A	0.0055/0.0065/0.0054	4.322e-10	12.57 (5.68-27.84)	0.87
rs76480148	2:71935916	A/G	0.0077/0.0096/0.0099	1.731e-08	6.96 (3.54-13.65)	0.85
rs62172873	2:154571794	G/C	0.007/0.0076/0.0121	2.585e-08	8.25 (3.92-17.35)	0.87
rs145457637	5:100869997	G/T	0.0123/0.0137/0.0204	1.546e-08	5.73 (3.13-10.48)	0.82
rs76587897	9:126156904	T/C	0.0227/0.0259/0.0196	3.826e-08	3.82 (2.37-6.16)	0.91
rs117924636	13:60333297	A/G	0.0131/0.01/0.007	9.976e-09	7.13 (3.64-13.97)	0.89
rs75166605	18:73806958	G/A	0.0067/0.0065/0.0199	1.267e-08	8.68 (4.12-18.26)	0.96

All SNPs shown here are imputed. Genomic positions are GRCh37 reference genome. REF is reference allele and EFF is effect allele.

binding evidence for CEBPB, a well known leukemia target gene (Akasaka et al. 2007; Guerzoni et al. 2006). The other region (\approx 154 Mb region) does not have any noteworthy functional effects. Similarly, the regions on 5, 9, 13, and 18 also provided little evidence of having any known eQTLs, promoters Histones, or relevant alterations to cancer related motifs (L. D. Ward and Kellis 2015; Arnold et al. 2015).

Upon screening suggestive associations of donor SNPs with patient OS, we detected rs62465751, a highly regulatory SNP located on chromosome 7, upstream of *TMEM248* (Figure 5.13). This location encompassing rs62465751 has been predicted to be an active transcription start site in almost all cell lines included in Roadmap Epigenome project. Consequently, rs62465751 was detected for 60 eQTL associations, involving multiple genes across multiple tissues included in GTEx project. rs62465751 belongs to a LD block consisting of 73 SNPs ($r^2 > 0.8$), 22 of which located in the intronic region of *TMEM248*. SNPs in this LD block alter more than 22 binding motifs and are all reported eQTLs for various tissues, including CEU ancestry lymphoblastoid cell lines (Stranger et al. 2007; Lappalainen et al. 2013).

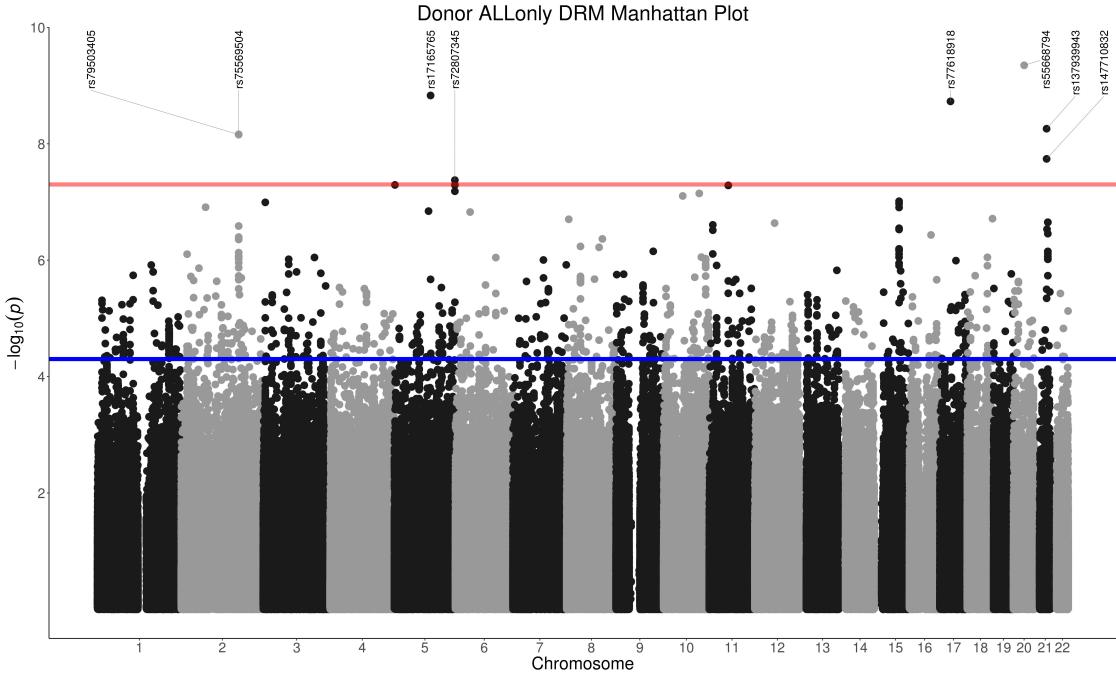


Figure 5.14: Manhattan Plot of Donor SNPs Contribution to Recipient DRM. The x-axis is chromosome 1-22 and the y-axis is the $-\log_{10}(P_{meta})$. Each dot is a SNP. The red line is genome wide significance at $P_{meta} < 5 \times 10^{-8}$. The blue line is suggestive significance at $P_{meta} < 5 \times 10^{-5}$. Labeled SNPs are associated hits that have passed genome-wide threshold.

5.3.8 Donor SNPs Associated with Recipient Disease Related Mortality

We found 5 regions associated with increased risk for DRM when donor SNPs had the risk allele in single regions on 17, 20, and 21, and two regions on chromosomes 2 and 5 (Figure 5.14; Table 5.6).

On chromosome 2, rs75569504 (Table 5.6) is in strong LD with SNPs that are intronic for *MYO3B*. This marker is in perfect LD with SNPs that bind proteins TFs Setdb1 and Hae2f1, however, little evidence exists relating these TFs to ALL. The other region on chromosome 2, rs79503405 was associated with increased risk in ALL DRM and in perfect LD with an eQTL (in testes cell lines) a synonymous variant on *LRP2* (rs35114151). Synonymous mutations may affect DNA replication

Table 5.6: DISCOVeRY-BMT Donor Genotypes Associated with Recipient Disease Related Mortality at 1-year.

RSID	CHR:POS	REF/ EFF	Ref. Panel/Cohort 1/ Cohort 2 MAF	P-value	HR (95% CI)	INFO
rs75569504	2:170066550	A/G	0.05/0.0421/0.0272	6.897e-09	3.87 (2.45-6.12)	0.96
rs79503405	2:170065565	G/T	0.0367/0.0312/0.0164	6.960e-09	5.17 (2.96-9.02)	0.96
rs17165765	5:107206623	C/T	0.0111/0.0123/0.0142	1.478e-09	10.6 (4.93-22.78)	0.83
rs72807345	5:179244672	C/T	0.0146/0.0173/0.0099	4.218e-08	6.04 (3.18-11.49)	0.93
rs77618918	17:31497097	C/T	0.0142/0.0166/0.0059	1.868e-09	8.57 (4.25-17.27)	0.91
rs55668794	20:31654699	A/G	0.0161/0.0174/0.0051	4.482e-10	9.91 (4.82-20.38)	0.80
rs137939943	21:35231995	G/A	0.0097/0.0082/0.0124	5.509e-09	10.24 (4.68-22.38)	0.86
rs147710832	21:35266776	C/T	0.0097/0.0091/0.0126	1.817e-08	9.66 (4.38-21.27)	0.83

All SNPs shown are imputed. Genomic positions are GRCh37 reference genome. REF is reference allele and EFF is effect allele.

processes such as transcription, splicing or translation, any of which can have implications on phenotype.

Chromosome 5 marker rs72807345 is intronic for *SQSTM1*, a gene that has shown to be involved in somatic chromosomal aberrations in adult T-cell leukemias (Gorello et al. 2010). The markers are on 17 is intronic for *ASIC2* and binds proteins *GR* (a highly targetable nuclear receptor and associated with T-ALL resistance) (McMaster and Ray 2008; Piovan et al. 2013) and *STAT3* (a well known leukemia target gene) (Adamaki et al. 2015; Adamaki et al. 2015). When looking at the regional association plots for the aforementioned DRM associations, SNiPA database did not reveal any notable effects on transcriptional regulation (Arnold et al. 2015).

The regions on chromosomes 20 and 21 showed no evidence of affecting transcriptional processes from functional studies (L. D. Ward and Kellis 2015; Arnold et al. 2015).

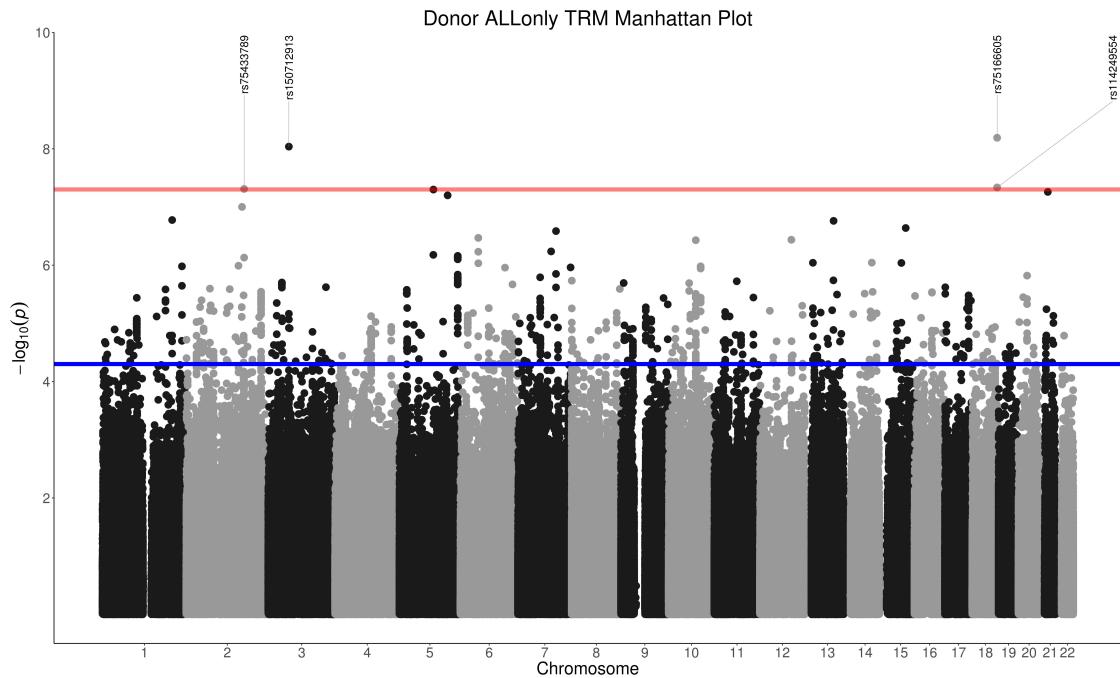


Figure 5.15: Manhattan Plot of Donor SNPs Contribution to Recipient TRM. The x-axis is chromosome 1-22 and the y-axis is the $-\log_{10}(P_{meta})$. Each dot is a SNP. The red line is genome wide significance at $P_{meta} < 5 \times 10^{-8}$. The blue line is suggestive significance at $P_{meta} < 5 \times 10^{-5}$. Labeled SNPs are associated hits that have passed genome-wide threshold.

Table 5.7: DISCOVeRY-BMT Donor Genotypes Associated with Recipient Transplant Related Mortality at 1-year.

RSID	CHR:POS	REF/ EFF	Ref. Panel/Cohort 1/ Cohort 2 MAF	P-value	HR (95% CI)	INFO
rs75433789	2:171353397	C/T	0.0096/0.0059/0.0054	4.897e-08	12.31 (4.99-30.33)	0.92
rs150712913	3:60957357	G/C	0.0137/0.0122/0.0167	9.181e-09	8.12 (3.97-16.58)	0.84
rs117595188	15:63517319	G/T	0.0078/0.0107/0.0129	2.376e-08	7.99 (3.85-16.56)	0.85
rs75166605	18:73806958	G/A	0.0067/0.0065/0.0199	6.470e-09	13.03 (5.48-31.01)	0.96
rs114249554	18:73779508	T/G	0.006/0.0059/0.0161	4.615e-08	13.66 (5.35-34.87)	0.93

All SNPs shown are imputed. Genomic positions are GRCh37 reference genome. REF is reference allele and EFF is effect allele.

5.3.9 Donor SNPs Associated with Recipient Transplant Related Mortality

Our analyses revealed TRM associations with donor SNPs on chromosomes 2, 3, 15, 18 and 20 (Table 5.7; Figure 5.15). The associated SNPs in chromosome 2 are in LD and are located in the intronic region of *MYO3B* gene (L. D. Ward and Kellis 2015). The chromosome 2 associations are driven by disease death, as we also observed significant SNPs in this region for DRM. Although lack of *FHIT* at the protein level has been shown to induce leukemia (U. R. Peters et al. 1999), the associated intronic SNP on chromosome 3 had minimal evidence of transcriptional regulation from existing literature based on experimental evidence (Arnold et al. 2015). Similarly, the associated chromosome 18 region for donor SNPs showed effect on transcription (L. D. Ward and Kellis 2015; Arnold et al. 2015).

5.3.10 Donor SNPs Associated with Graft-versus-Host Disease

We scanned suggestive SNP regions ($P < 5 \times 10^{-5}$) for regions that may affect transcription factor binding. Donor GVHD SNP rs9783053 (typed SNP; $P = 1.41 \times 10^{-6}$; HR: 3.27 [2.02-5.29]; G effect allele) was found to have a regulomeDB score of 1A (the highest score). This means based off of previous literature, there is strong evidence that rs9783053 affects transcription factor binding (Boyle et al.

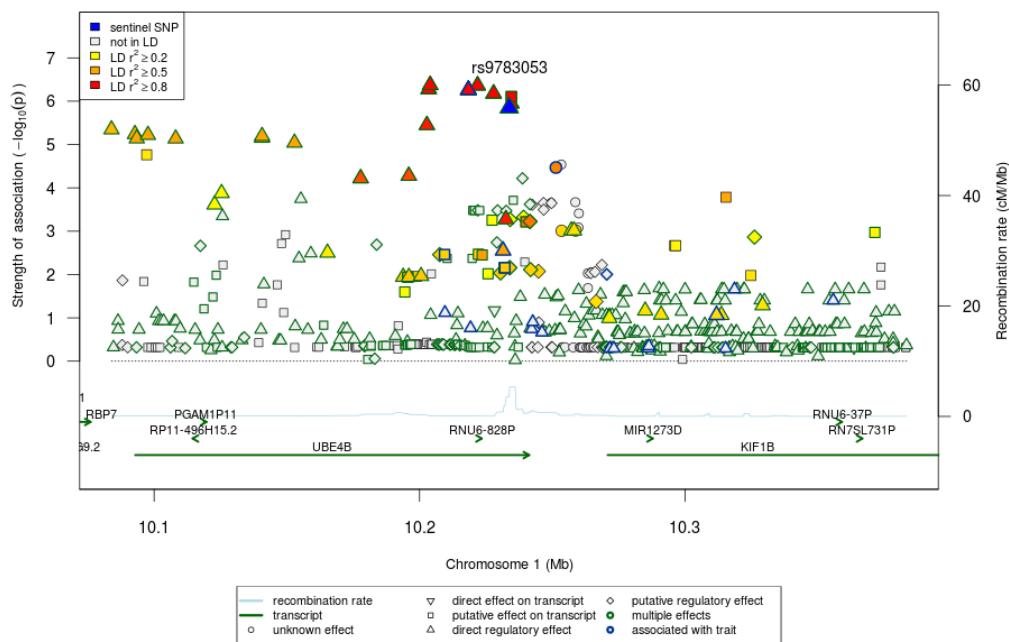


Figure 5.16: Donor GVHD Region Chromosome 1. Sentinel SNP is rs9783053 and colored blue. The region comprises +/- 150 kb window from the sentinel SNP. The red-yellow color gradient represents LD strength. Shapes represent different effects that the SNP has been reported to have in this region from independent studies.

2012). This region has many SNPs that directly regulate transcription on *UBE4B* (Figure 5.16). This polymorphism has five proteins (Ctcf, Ebf1, Elf1, Nfk κ b, Pu1) that bind to this SNP in lymphoblastoid cell lines (ENCODE Project Consortium 2012). This variant is in LD with synonymous variant rs2273299 ($r^2 = 0.88$). Experimental cell line studies from ENCODE provide evidence suggesting that this region alters regulatory motifs for cancer and leukemia related genes (L. D. Ward and Kellis 2015). Variants that alter gene expression were found in blood and lymphoblast derived CEU cell lines. Thus, while donor GVHD SNPs did not reach genome-wide significance, there is some evidence that suggests this region may transcriptional or epigenetic on effects on ALL related genes.

5.4 Discussion

Previously the relationship between survival outcomes and non-HLA donor-recipient genetics after MUD-HSCT had not been studied at the genome-wide level. Here we presented the first GWAS investigating this objective. The cohorts of DISCOVeRY-BMT span the entire ALL patient pool, ranging from childhood, AYA, adults and senior adults. Genome-wide associations to survival outcomes after 1-year post-transplant were identified in both recipient and donor genomes. Specifically, recipient DRM, OS, and OF. And for donors, associations were found for DRM, TRM, GVHD, and OS. Often our genome-wide associations provided only a small insights of possible biological mechanisms that may contribute to these observed death related phenotypes.

We attempted to gain additional insight by leveraging publicly available databases. We inspected regions that were below genome-wide significance at $P < 5 \times 10^{-5}$. Often we found regions that were in fact not genome-wide significant, but regional association plots revealed that there may be some effect on transcription

and that previous studies have provided evidence that these regions have many interactions. Future directions for this project include implementing PrediXcan which is currently underway in the lab and will be added to this work before publication. PrediXcan always assessment of gene-based expression levels in our data and thus will help us prioritize our GWAS associations. Future directions for this project include implementing PrediXcan (Barbeira et al. 2018) which is currently underway in the lab and will be added to this work before publication. PrediXcan allows assessment of gene-based expression levels in our data (using blood tissue samples from GTEx) and thus will help us prioritize our GWAS associations.

Although DISCOVeRY-BMT is the largest cohort to date investigating ALL recipient-donor genetic variation after transplant, sample size remains a large limitation. The next step will be replicate these findings in an independent study. Namely using University of Washington ALL cohort study for replication of relapse and overall survival. If available, in addition to replication using University of Washington cohorts, meta-analysis combining DISCOVeRY-BMT may provide stronger signal for overall survival and/or relapse outcomes. In terms of improving our survival models, alterations to our current workflow may improve signal by adjusting for age by stratifying by clinical age groups, or by modeling the age as a non-linear function and smoothing the distribution using splines (Shepherd, Rebeiro, and Caribbean, Central and South America network for HIV epidemiology 2017). Challenges in improving long-term survival after MUD-HSCT remain, however, we believe that this study provides unique insights in this field and is a positive step towards improving outcomes.

CHAPTER 6: Conclusion and Future Work

Modern science progresses through corroboration, computing, and collaboration. *Corroboration* by the need to continually seek answers and verify results. Reproducibility is necessary for robust ideas and expansion of knowledge. In genomics, traditional biomedical researchers often study one gene for one phenotype for numerous years, possibly decades. More often than not, these hypotheses propagate from a single study years ago or from using *a priori* information of a specific system in hypothesis development. Unfortunately, often, the original studies were never reproduced in well-designed independent studies. Constant pursuit of false leads may impede the rate at which important problems are solved. Agnostic approaches through usage of genome-wide technologies (such as GWAS), allow researchers to inspect phenotypes and gain biological insights at a global level. As technology continues to advance, data collection and data resources inherently grow. Multiple rich data resources are available from all aspects of the genome, the -*omics* (i.e. genomics, transcriptomics, metabolomics, proteomics, methylation), as well as clinical trials. As such, large scale *computing* is imperative to the success of modern studies. Broader insights can be leveraged to develop hypotheses that can investigate and characterize complex disease etiology. And the last element in order to accomplish anything is *collaboration* by conducting team science. Bringing together multiple talented individuals and groups together and enriching each other with combined expertise to achieve a common goal.

This dissertation was an attempt to exemplify these principles of modern science to the best of our ability. I was very fortunate to join a lab that had an incredibly rich clinically relevant data source, the DISCOVeRY-BMT GWAS.

DISCOVeRY-BMT is a fine example of collaborative team science. The team is made up of scientists, clinicians, and statisticians (and some who belong to more than one of those groups) from multiple institutions. With this GWAS, we attempted to corroborate researchers that continually pursued candidate SNPs in candidate genes that they believed held some biological relevance to survival in recipients or donors after transplant. We were unable to reproduce any of their results using DISCOVeRY BMT's much larger and more homogeneous study population. While we published on a negative result, we were able to make an important contribution to the transplant field by providing strong evidence that those studies should begin looking elsewhere in the genome for hidden insights.

We were able to address a computational challenges in genomics, specifically when it comes to survival analysis. We developed an R/Bioconductor package, gwasurvivr, that can directly take data from online imputation web services. As large studies continue to get funded with well-documented follow up data – survival studies will continue to become more prevalent. Gwasurvivr will be an important computational resource for the next several years. To date, over 200 unique IPs have downloaded gwasurvivr. We were also able to develop a pipeline that enhanced our workflow and reproducibility efforts. The pipeline should be expanded to automate annotation using SNiPA database and HaploReg. Construction of a centralized database (PostgreSQL database) is underway that will continue to enhance our workflow.

We were able to use our own software and pipeline to perform many GWAS using DISCOVeRY-BMT. While we reported recipient and donor SNP associations to survival outcomes post-HSCT, there remains the entire DISCOVeRY-BMT 100 day censoring analyses for ALL (and our other disease groups). These results have yet to be analyzed, curated, and reported. These results are very important and

relevant to the transplant field as patients that die within the first 100 days may present very different genetic profiles than those who survive until at least 1 year. This research will continue after my departure from our lab and I believe it will be quite impactful.

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