ANALYSIS OF HLA AND RECEPTOR GENE POLYMORPHISMS IN MULTIPLE MYELOMA

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

HASAN YALIM AKIN

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
BIOCHEMISTRY

NOVEMBER 2024

Approval of the thesis:

ANALYSIS OF HLA AND RECEPTOR GENE POLYMORPHISMS IN MULTIPLE MYELOMA

submitted by **HASAN YALIM AKIN** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Biochemistry, Middle East Technical University** by,

Prof. Dr. Naci Emre Altun
Dean, **Graduate School of Natural and Applied Sciences** _____

Prof. Dr. Çağdaş Devrim Son
Head of the Department, **Biochemistry** _____

Prof. Dr. N. Tülün Güray
Supervisor, **Biochemistry, METU** _____

Prof. Dr. Meral Beksaç
Co-Supervisor, **Faculty of Medicine, İstinye University** _____

Examining Committee Members:

Prof. Dr. Orhan Adalı
Biological Sciences, METU _____

Prof. Dr. N. Tülün Güray
Biological Sciences, METU _____

Assoc. Prof. Dr. Yeşim Aydın Son
Bioinformatics, METU _____

Prof. Dr. Pınar Yurdakul Mesutoğlu
Faculty of Medicine, Istinye University _____

Assoc. Prof. Dr. Klara Dalva
Faculty of Medicine, Ankara University _____

Date: 06.11.2024

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name Last name : Hasan Yalım Akın

Signature :

ABSTRACT

ANALYSIS OF HLA AND RECEPTOR GENE POLYMORPHISMS IN MULTIPLE MYELOMA

Akın, Hasan Yalım
Doctor of Philosophy, Biochemistry
Supervisor: Prof. Dr. N. Tülün Güray
Co-Supervisor: Prof. Dr. Meral Beksaç

November 2024, 66 pages

Multiple myeloma (MM) is a treatable but rarely curable neoplastic plasma-cell disorder, primarily effecting elderly with a male predilection with a higher incidence rate in African ancestry. Natural killer (NK) cells are regulators of immune response against infections and cancer. Tumor cells are recognized through their human leukocyte antigen (HLA) ligands by killer cell immunoglobulin-like (KIR) receptors on NK cells. Due to their highly polymorphic structure, numerous combinations of KIR-HLA pairs result in underpowered statistical significance in KIR/HLA association studies. A few studies reported conflicting associations between KIR/HLA ligands and MM, due to the limited number of participants. In this study, an alternative approach was conducted to increase the sample size by using large existing data from various studies aiming to investigate the effect of KIR and HLA ligands on MM in different populations. Along with the local patients, KIR and HLA genes were imputed from the whole exome sequencing data belong to one of the largest MM datasets. The preliminary findings on a local cohort have revealed the protective role of activating KIR as well as an association between KIR/HLA ligands and age of MM onset. Remarkably, increasing the sample size uncovered the predisposing effect of Bw4 ligand (one of the public epitopes of HLA-B molecules),

and a bidirectional interaction between KIR genes 3DL1, 3DS1 and their cognate ligand Bw4, that had not been reported in prior studies. Moreover, low frequencies of the protective genotypes and high frequencies of the predisposing genotypes among African Americans suggests a relationship between KIR/HLA ligands and ethnic disparities in MM. Finally, this study provides a reference model for the association between KIR/ligand genotypes and MM. It also presents a validated methodology for the global integration of large genomic datasets, enabling accurate evaluation of KIR and HLA allotypes across various disease courses.

Keywords: Multiple Myeloma, Innate Immunity, Human Leukocyte Antigen, Killer Cell Immunoglobulin-Like Receptors, Imputation

ÖZ

MULTİPL MYELOMDA HLA VE RESEPTÖR GEN POLİMORFİZMLERİNİN ANALİZİ

Akın, Hasan Yalım
Doktora, Biyokimya
Tez Yöneticisi: Prof. Dr. N. Tülün Güray
Ortak Tez Yöneticisi: Prof. Dr. Meral Beksaç

Kasım 2024, 66 sayfa

Multipl miyelom (MM), özellikle yaşlıları ve erkekleri etkileyen ve Afrika kökenlilerde daha sık görülen, tedavi edilebilen ancak nadiren iyileştirilebilen bir neoplastik plazma hücre hastalığıdır. Doğal öldürücü (NK) hücreler, enfeksiyonlara ve kansere karşı bağışıklık yanıtında rol oynarlar. Tümörlerin tanınması, NK hücre üzerindeki KIR reseptörleri ile hedef hücrelerdeki ligandlar aracılığıyla gerçekleşir. Polimorfik yapıları nedeniyle çok sayıda olan KIR-HLA kombinasyonları nedeniyle ilişkilendirme çalışmalarında istatistiksel güç zayıf kalmaktadır. Birkaç çalışmada KIR/HLA ligandları ile MM yatkınlığı arasında çelişkili sonuçlar rapor edilmiştir. Bu çalışmada, alternatif bir yaklaşımla örneklem büyüklüğünü arttırmak için çeşitli çalışmaların mevcut genomik verileri kullanılarak KIR ve HLA ligandlarının MM hastalarına etkisinin farklı popülasyonlarda araştırılması amaçlanmıştır. Yerel MM hastalarına ek olarak, en büyük MM veri tabanlarından birine ait tüm ekzom dizileme verisinden KIR ve HLA genleri impute edilmiştir. Yerel hastalarda yapılan ön çalışmada aktivatör KIR'ların koruyucu etkisi ve KIR/HLA ligandları ile MM tanı yaşı arasında ilişki saptanmıştır. Arttırılan örneklem büyüklüğü sayesinde önceki çalışmalarda rastlanmayan Bw4 ligandının (HLA-B moleküllerinin genel epitoplarından biri) MM yatkınlığına etkisi ve KIR 3DL1 ve 3DS1 genlerinin

karşılık gelen HLA ligandı Bw4 ile iki yönlü bir etkileşim gösterdiği saptanmıştır. Dahası, Afrikalı Amerikalılarda koruyucu genotiplerin düşük frekansta ve yüksek riskli genotiplerin ise daha sık görülmesi, KIR/HLA ligandları ile etnik farklılıklar arasında bir ilişki olabileceğine işaret etmektedir. Son olarak, bu çalışmada KIR/HLA-MM ilişkilendirme çalışmaları için bir referans model sunulmakta, ilaveten KIR ve HLA allotiplerinin çeşitli hastalıklar üzerindeki etkilerini araştırmaya yönelik dünya genelindeki büyük genomik veri setlerinin kullanılabilmesinin önünü açan valide edilmiş bir metodoloji ortaya konmaktadır.

Anahtar Kelimeler: Multipl Miyelom, Doğal Bağışıklık, İnsan Lökosit Antijeni, Doğal Öldürücü Hücre İmmünoglobulin-Benzeri Reseptörler, İmputasyon

To my family, both near and far, whether by my side or in my memories.

ACKNOWLEDGMENTS

I would like to express my deepest gratitude to my supervisor, Prof. Dr. N. Tülün Güray, for her kind support throughout my doctoral studies and her considerate guidance on my research. I am grateful to her for accepting me as her student.

I would also like to thank my co-supervisor, Prof. Dr. Meral Beksaç, who supported me through the early stages of my academic career. She provided me with invaluable experiences and never withheld her support, knowledge, and expertise while I conducted my research.

I would like to extend my appreciation to Assoc. Prof. Dr. Yeşim Aydın Son for sharing her extensive knowledge and for her encouraging support throughout my bioinformatic analyses, as well as her guidance in resolving the technical issues I encountered during my studies.

I would also like to express my gratitude to Prof. Dr. Orhan Adalı for his kindest encouragement and unwavering support throughout the developmental stages of my thesis.

I am sincerely grateful to Assoc. Prof. Dr. Klara Dalva for her kind support and for making it easy for me to learn the key points, which are not covered in the literature but can only be gained through experience. I would also like to give special thanks to Rıdvan and Ezgi Anlıaçık for their unlimited technical support.

I would like to thank Prof. Dr. Pınar Yurdakul Mesutoğlu for providing invaluable guidance at the very beginning of my academic journey and for her continuous support.

I would like to express my appreciation to my fellow lab members for the support they provided to the best of their expertise, and special thanks to Dr. Güçlü Ongun for his assistance with computational processes during the early stages of my research.

Finally, my deepest gratitude goes to my family: To my wife, Sinem, for her unrestricted support throughout this journey, in my life and in relation to the scientific aspects of my research, and to my parents for their boundless support, which I have felt deeply throughout all aspects of my life, including my academic development. And of course, it would be remiss not to mention Yoldaş Lena, our cat.

TABLE OF CONTENTS

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

LIST OF ABBREVIATIONS

ABBREVIATIONS

BM: Bone Marrow

EMD: Extramedullary disease

GWAS: Genome-wide Association Studies

HLA: Human Leukocyte Antigen

KIR: Killer Cell Immunoglobulin-Like Receptors

MGUS: Monoclonal Gammopathy of Undetermined Significance

MM: Multiple Myeloma

MMRF: Multiple Myeloma Research Foundation

NGS: Next-Generation Sequencing

NK: Natural Killer

PCR: Polymerase Chain Reaction

PFS: Progression-Free Survival

SMM: Smoldering Multiple Myeloma

SNP: Single Nucleotide Polymorphism

SRA: Sequence Read Archive

VCF: Variant Call Format

WES: Whole Exome Sequencing

WGS: Whole Genome Sequencing

CHAPTER 1

INTRODUCTION

1.1 Multiple Myeloma

1.1.1 Disease Pathogenesis

Multiple myeloma (MM), the second most common hematological disease, is a monoclonal gammopathy disorder characterized by anemia, lytic lesions in the bone marrow (BM) and immunoglobulin residues in blood and urine as a result of over-secretion of monoclonal immunoglobulins by malign plasma cells proliferating at BM environment (Cowan et al., 2018; Dima et al., 2022; Kumar et al., 2017). MM is more common in males and being seen most frequently among people aged over 50 with a median of 69 years old and it accounts for 1.0-1.8% of all neoplastic diseases (Dimopoulos et al., 2021; Kumar & Rajkumar, 2018; *Cancer Stat Facts: Myeloma*, 2024). The incidence rates vary among worldwide populations with regard of the geographical location as well as the ethnicity (Figure 1.1). African Americans (AFA), for instance, show 2-3 times higher incidence and death rates compared to Caucasians or Asian populations (*Cancer Stat Facts: Myeloma*, 2024; Röllig et al., 2015).

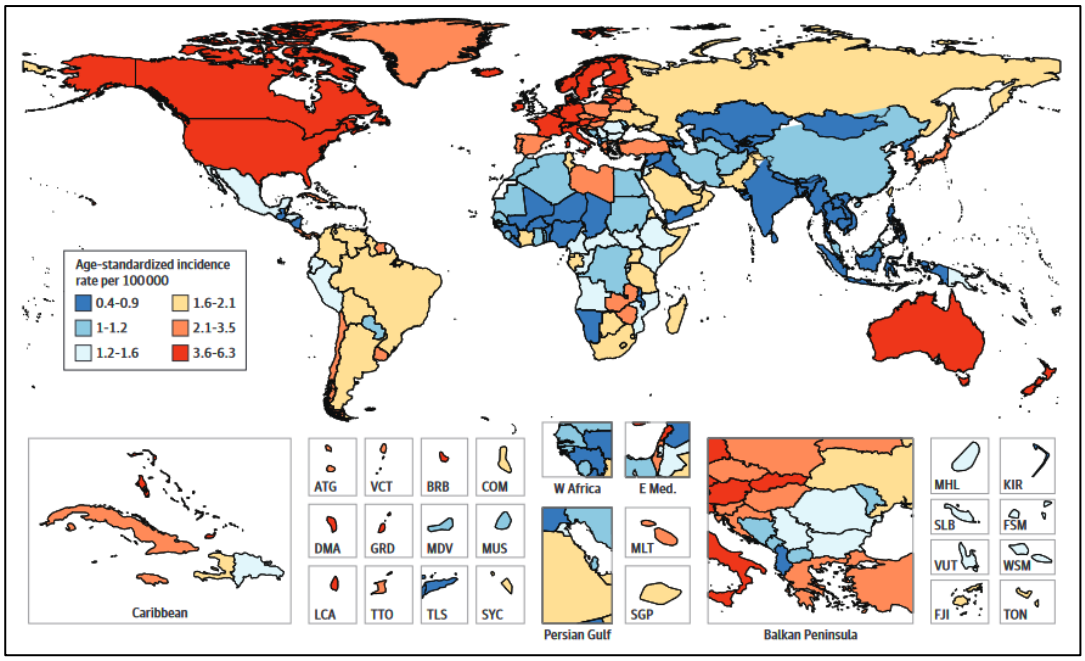

Figure 1.1. Age-standardized worldwide incidence rate of MM (Cowan et al., 2018).

MM pathogenesis is associated with acquired genetic mutations within the malignant plasma cells complicated with genomic instability leading to additional abnormalities. MM, carries a collection of numerical abnormalities plus several mutations or translocations evolving from pathologies such as smoldering myeloma (SMM) and monoclonal gammopathy of undetermined significance (MGUS) (Figure 1.2) (Kumar & Rajkumar, 2018; Pratt, 2002; van Nieuwenhuijzen et al., 2018). While some SMM/MGUS patients rapidly progress to myeloma, some patients can be followed up with indigenous precondition states for many years or even for a lifetime (Kyle et al., 2010).

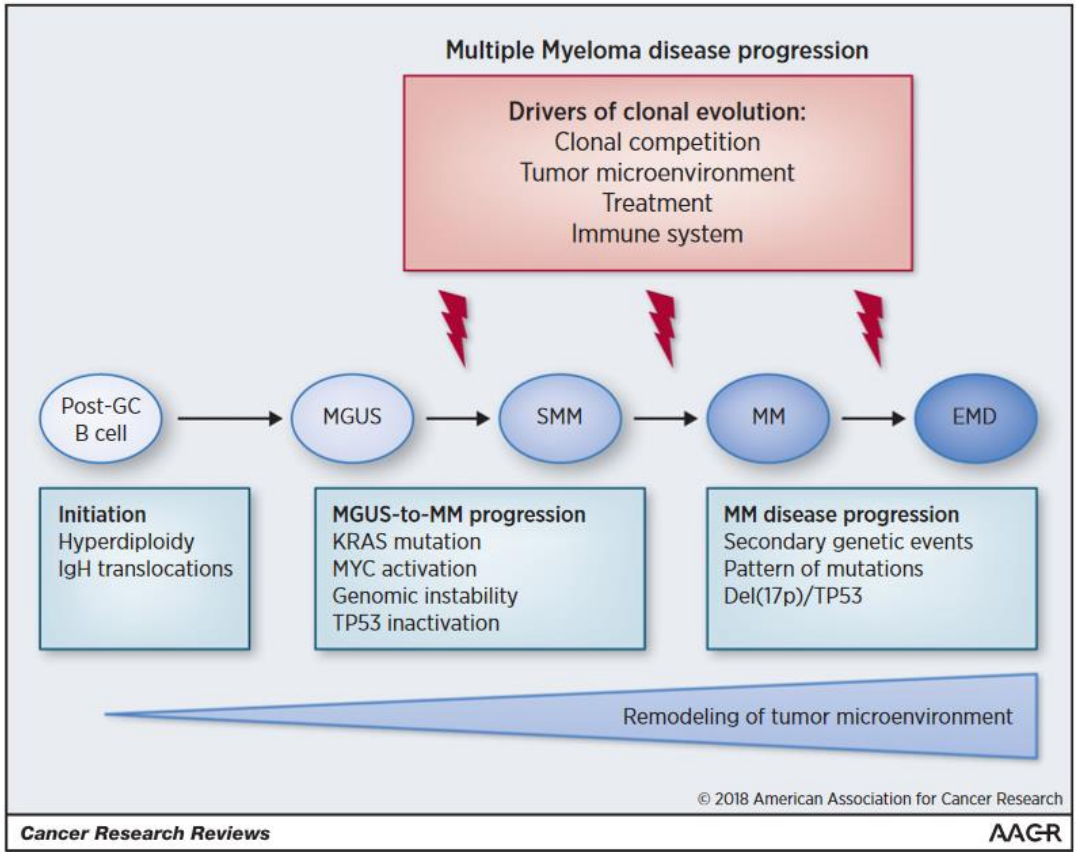

Figure 1.2. Clonal Evolution Stages of MM Premalignant Cells (van Nieuwenhuijzen et al., 2018).

MGUS: monoclonal gammopathy of undetermined significance; SMM: smoldering myeloma, MM: Multiple myeloma, EMD; extramedullary disease.

Progression of MM is regulated by multiple factors, such as cell signaling, certain cytokines and chemokines as well as the components of extracellular matrix and the tumor microenvironment, which includes the naturally occurring cell types in BM. The specificity of the immune microenvironment including the interactions between immunoreactive cells and malignant cells are of great importance in malignant plasma cell growth and escape from immune system and immunotherapies as well as the clonal evolution of the disease progression (Bila et al., 2021; García-Ortiz et al., 2021). Despite the numerous prognostic factors described for MM, such as age, comorbidities, tumor load, cytogenetic and molecular characteristics of tumor cells,

there is still need for a multiparameter characterization for understanding the dynamic risk factors affecting the outcome of MM patients (Aksenova et al., 2021; Corre et al., 2021; Dima et al., 2022).

Individual genetic disparities have long been studied in the context of genetic polymorphism and disease association studies (Cooper & Clayton, 1988). Definition of polymorphism has been widely recognized as one or more nucleotide changes in the DNA, similar to mutations; however with a minor allele frequency of greater than 1% in a population (Brookes, 1999). Although the earlier definitions of polymorphism and mutation are no longer consistent with the reality in population genomics, the fact that the polymorphic variations in the germ-line DNA have an impact on disease susceptibility as well as the prognosis and have been widely studied for many years (Chiarella et al., 2023; Karki et al., 2015). MM and its relationship with genetic polymorphisms have also been studied by many researchers around the globe. Genetic polymorphisms have been associated with not only susceptibility to MM, but also with their impact on the disease progression in response to certain therapies (Clavero et al., 2023; Sood et al., 2024). Among the many studies focusing on prognostic risk factors influenced by germ-line somatic alterations on predisposition to MM on an individual basis, Multiple Myeloma Research Foundation (MMRF) CoMMpass study holds one of the largest genomic MM datasets with the aim of understanding individual-level disease prognosis, risk stratification and exploring new targeting strategies., and it includes 1143 patients, mostly from the United States, Canada, Spain and Italy. Ancestry of the patients within the dataset was distributed as 81% “Caucasian”, 18% “African American or Black” and 2% Asian (Skerget et al., 2021). Moreover, recent studies focus on familial cases in order to unravel the genome-wide level risk factors (Akkus et al., 2024; Pawlyn & Davies, 2019; Samur et al., 2020).

Most of the MM patients are diagnosed by laboratory findings, including low blood count levels, renal disease, and/or increased protein levels. The differential diagnosis requires complete blood analysis; serum chemistries; creatinine, lactate dehydrogenase, and beta2-microglobulin tests; immunoglobulin studies; skeletal

survey; and evaluation of bone marrow (Dima et al., 2022; Kumar & Rajkumar, 2018). Following the diagnosis of MM, therapy strategies are proceeded according to disease specific parameters; specific gene mutations of cytogenetic indications (translocations, deletions, or certain insertions) on clonal plasma cells derived from BM aspirates and age of patients (mostly considered according to be older or younger than 60 years old).

MM has been recognized since 1800's, and with substantial development of many treatment strategies, including the use of proteosome inhibitors and immunomodulatory agents following autologous stem cell transplantation, it has become a treatable disease but not curable yet (Beksaç et al., 2008; Cowan et al., 2018; Rodriguez-Otero et al., 2021). According to SEER Statistics (2001-2021), 10 years of remission within the patients adjusted for age, sex and ethnicity has only been increased to 47.5% from 22.4% in ten years; however, the complete remission is still an important challenge for MM in overall (*Cancer Stat Facts: Myeloma*, 2024; Dimopoulos et al., 2021).

The classical and optimized approach to treat MM is autologous stem cell transplantation, if eligible, following a 2-3 drug myeloablative chemotherapy. While this optimized therapy provides long-term survival rate by 10%, older patients need to be reconsidered with regard to their biological fitness conditions. In addition to conventional therapies, patients need to be considered in their own conditions (Pawlyn & Davies, 2019). MM may progress accordingly with specific issues such as renal diseases, bone diseases, thromboembolic complications, neurologic diseases, and infections. Despite of these specific issues, MM itself a great challenge in diagnosis, follow-up of the disease progression as well as evaluation and understanding the reason underlying refractory/relapse state is crucial (Cowan et al., 2018; Dimopoulos et al., 2021; Rodriguez-Otero et al., 2021).

In the scope of personalized medicine, alternative to the conventional treatment in refractory/relapse MM, individualized immunotherapeutic strategies have been frequently used within the last decade. T cell and Natural Killer (NK) cell-mediated

immunotherapies have the highest potential regarding their specific anti-tumoral cytotoxic effect. Anti-tumoral effects of NK cells have been thoroughly analyzed and being tested in various clinical applications (Bila et al., 2021; X. Chen et al., 2022; Clara & Childs, 2022; Marin et al., 2024). Although chimeric antigen receptor (CAR) T cell therapy show promising results, there are a number of challenges in use of this new therapy, such as the high risk of graft versus host disease, susceptibility of the cells to the inhibitory effects of the microenvironment and *in vivo* durability of the modified cells. Thus, NK cell mediated strategies remain as a safe and valuable choice for immunotherapeutic approaches (Daher & Rezvani, 2018; Lin et al., 2024).

The regulation of NK cell functions has been proven to play an important role in the pathogenesis of MM, and in addition to therapeutic strategies, it is also shown by few study groups that the NK cell biology may have an impact on age of disease onset and predisposition to MM (Beelen et al., 2024; M. Beksac et al., 2023; Hoteit et al., 2014; Martínez-Sánchez et al., 2015; Sun et al., 2021).

1.2 NK Cell Mediated Anti-Tumor Immunity

NK cells are one of the major components of the innate immune system and known by their role in immune response against virally infected cells or tumor cells. They reside in circulating blood with a ratio of 5%-15% and in various organs as well (Liu et al., 2021). NK cells, similar to T cells and B cells, originate from lymphoid progenitors; however, they differ from those antigen receptor-expressing similar subsets by their expression of receptors with activating or inhibitory properties in various combinations.

NK cells are characterized by their CD56 CD16 expressions and are classified in two main phenotypes as $CD56^{dim}CD16^{+}$ cells, which exhibit highly cytotoxic profile, and $CD56^{bright}CD16^{-}$ cells, which are responsible for cytokine secretion unless activated by external signals. In addition to their direct cytotoxicity on target cells, they are

also known as pro-inflammatory cytokine producers, by which they can induce adaptive immune responses, in addition to preventing angiogenic, proliferative abilities and initiate TNF-induced pro-apoptotic effects on target cells (Liu et al., 2021; Myers & Miller, 2021).

Among the known activating receptors of NK cells, CD16 is the most dominant one with the ability to be activated without requirement of any other receptor. When activated, it induces antibody-dependent cell-mediated cytotoxicity and subsequently downregulated by ADAM17, a metalloproteinase which removes CD16 from the cell surface. This process led to development of a novel treatment strategy targeting inhibition of ADAM17 and currently being tested on clinical trials (Cooley et al., 2018; Myers & Miller, 2021). Another approach regarding the CD16-dependent activation of NK cells is to generate high-affinity CAR-NK cells and to genetically modify CD16 to prevent its cleavage by ADAM17. Both strategies are currently being tested on phase I/II clinical trials NCT02141451 and NCT04023071, respectively (Myers & Miller, 2021).

Another group of activating receptors include the natural cytotoxicity receptors (NKp30, NKp44 and NKp46). NKp46 in particular has been recognized by its role in anti-MM NK cytotoxicity. NKp30 together with NKG2D receptor, which is another activating receptor expressed on NK cells, have been shown to be downregulated in the bone marrow niche in patients with monoclonal gammopathy disorders (Clara & Childs, 2022).

In addition to above mentioned NK cell receptors, killer cell immunoglobulin-like receptors (KIR) represent the highest polymorphic characteristics among all (Amorim et al., 2021). NK cell functionality highly depends on the balance between activating KIR (aKIR) and inhibitory KIR (iKIR), and their state of steadiness or the regulation of the cytotoxic response through the interactions with their corresponding ligands. This process is regulated by complex KIR/ligand combinations in different individuals through numerous variations in the germ-line encoded polymorphic characteristics of KIRs on NK cells and HLA ligands on target

cells (Liu et al., 2021). Not only therapeutic approaches, but also many association studies and donor-patient matching strategies have been conducted regarding the interactions of KIR/HLA ligand variations and are detailed in the section below.

1.2.1 KIRs and the Corresponding Ligands

The independently inherited KIR and Human Leukocyte Antigen (HLA) genes are located at chromosome 19 and chromosome 6, respectively. They both display significant variations, resulting in a great diversity in the number of KIR-HLA combinations among individuals. KIR gene family consists of aKIRs (3DS1, 2DS1, 2DS2, 2DS3, 2DS4 and 2SD5), iKIRs (2DL1, 2DL2, 2DL3, 2DL5A/B, 3DL1, 3DL2 and 3DL3) and pseudogenes 2DP1 and 3DP1 which are not expressed on the cell surface. Figure 1.3 represents the known/unknown binding interactions of KIRs and their corresponding HLA ligands.

Regarding the iKIR interactions with their corresponding ligands; 2DL1 interacts with HLA-C2 ligand, only with the exception of allele 2DL1*022, of which corresponding ligand is HLA-C1 instead of C2 due to the change in an amino acid residue at position 44 (methionine to lysine). 2DL2 and 2DL3 also binds with HLA-C1 ligand. On the other hand, 3DL1 recognizes Bw4 allotypes. HLA-ligands recognized by 2DL5A and 2DL5B remain unknown. Regarding the aKIR-ligand interactions; 2DS1 is known to be acceptor of C2 ligand and 2DS2 binds with C1 allotypes. There is no known ligand for 2DS3, and it is not expressed on the cell membrane. 2DS4 has two variants; one of which differs from the other with a deletion of 22 base pairs (truncated) and ends up in a soluble form instead of the transmembrane structure. Non-truncated form of 2DS4 recognizes both C1 and C2 ligands in certain allotypes (Hadjis & McCurdy, 2024; Jennifer Zhang, 2022; Rajalingam, 2016). The corresponding ligand for 2DS5 is reported to be unknown by many researchers, however there are few reports suggesting its interaction with HLA-C2 (Blokhuis et al., 2017; Hadjis & McCurdy, 2024). There is also an uncertainty regarding the corresponding ligand of 3DS1. It is well known that 3DL1

is a strong receptor for Bw4 ligand. However, even though 3DS1 and 3DL1 are located in the same locus, the assumptions regarding the 3DS1-Bw4 binding capability lack evidence (Figure 1.3) (*IPD-IMGT/HLA Database*, 2024; Parham, 2005; Pollock et al., 2022; Rajalingam, 2016). In addition to the ligands given in the figure, 3DS1 and 2DL4 receptors, which have only two allotypes for each, can also be presented by ligands from HLA-F and HLA-G molecules, although there is lack of evidence for those interactions (Pollock et al., 2022). Peptide-specific recognition by NK cell receptors has recently been studied in NK-cell mediated innate and adaptive responses to viral infections and cancer. Polymorphic heterogeneity between individuals determines the activation and expansion of NK cells, and the receptor-ligand binding specificities, which are altered by the substitutions of HLA peptides presented by tumor cells or viral factors in certain infectious diseases control the differential levels of immune response (Hammer et al., 2018; Sim et al., 2023).

Ligands of KIRs are mainly originated from class I HLA molecules (HLA-A, HLA-B and HLA-C) carrying more than 20.000 known alleles. HLA-A and HLA-B are known by their roles in diverse expression of T cell receptor ligands in the evolutionary process, and it seems that together with HLA-C they have their role in the evolution as the predictors of ligands of KIRs (Capittini et al., 2012; Older Aguilar et al., 2010).

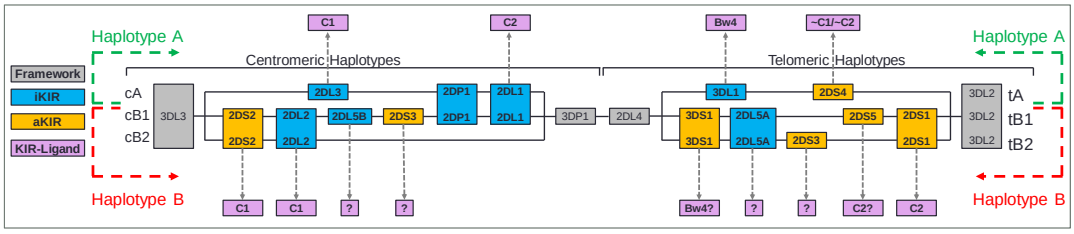

Figure 1.3. Representative illustration of distribution of KIR genes.

KIR haplotypes, based on both the content of A/B haplotypes and their chromosomal locations (telomeric or centromeric), and their KIR gene content with their binding ligands were illustrated. Grey boxes represent framework genes. Blue and orange

boxes show inhibitory and activating KIR receptors (aKIR and iKIR) and purple boxes represent the HLA ligands binding specifically with their corresponding receptors. Purple boxes with question marks represent the unknown or uncertain ligands.

As mentioned before, the functional state of NK cells is regulated by the balance between activating and inhibitory signals upon interfering with cognate ligands presented by other interacting cells. Conventional knowledge suggests an imbalance towards predominancy of activating signals which will result in cytotoxic response of NK cells. This may occur in case of a missing cognate ligand of an aKIR or in case of alterations in expression profile of class I HLA molecules of tumor cells (Hadjis & McCurdy, 2024; Liu et al., 2021; Parham, 2005).

Regarding the balance between aKIRs and iKIRs, KIR2DS1 is a known exception with its role in both activating an inhibitory effect depending on its interaction with HLA-C ligands, although it is classified within aKIRs. While 2DS1 and C2 ligand interaction results in a non-responsive state of the effector cells, 2DS1 interference with C1 ligand leads to interferon-gamma secretion and a cytotoxic response (Campbell & Hasegawa, 2013).

Such interaction led researchers to search for an ideal KIR-ligand match/mismatch between the transplant patients and their donors. Following a pioneering study in 2002 (Ruggeri et al., 2002), there have been numerous attempts to find an optimal KIR-ligand match/mismatch between donors and recipients (Beksaç & Dalva, 2012; Jennifer Zhang, 2022; Sahin et al., 2018). Donor selection algorithms have been developed for predicting NK cell alloreactivity to reduce relapse and increase overall survival rates for hematopoietic stem cell transplantations in hematologic malignancies and are being provided for public use in EMBL European Bioinformatics Institute website (*IPD-IMGT/HLA Database*, 2024). However, current donor selection strategies remain controversial as they do not consider individual-level KIR polymorphisms, but only focus on a single set of activating or inhibitory receptors (Jennifer Zhang, 2022). Thus, there is a need for a

comprehensive approach to understand NK cell alloreactivity in terms of KIR-ligand matching algorithms (Hadjis & McCurdy, 2024; Jennifer Zhang, 2022).

NK cell mediated immune-checkpoint inhibition strategies have been testing in clinical trials. iKIR antagonists, such as anti-2DL1/2/3 or anti-3DL2, had controversial outcomes and some of the trials have been terminated due to inefficacy in clinical outcomes. However, there are also ongoing trials with promising results so far (NCT01714739) (Myers & Miller, 2021). Additionally, KIRs arises as one of the targets of recently developing CAR-NK mediated treatment strategies (Daher & Rezvani, 2021). A combination of iKIRs are currently being identified as novel targets, and the reason was reported that it would be a challenge to specifying a target at both population and sequence-level considering the highly polymorphic structure of KIRs (Graham et al., 2023). Currently, the experts of the field meet at the point of the requirement of larger cohorts for validation of the above mentioned clinical trials and for a better understanding of the KIR-ligand interactions (Hadjis & McCurdy, 2024; Jennifer Zhang, 2022; Myers & Miller, 2021).

In addition to donor selection algorithms and therapeutic strategies, KIR and HLA variants have been widely explored in terms of their contribution to individual/population-level disease susceptibility association studies regarding resistance to viral infection, cancer, or autoimmune diseases.

1.3 KIR/HLA Ligand and MM Association Studies

The genetic diversities have been widely explored in the context of presence/absence of KIR genes (receptors with or without their cognate ligands) and individual gene frequencies have been associated with viral infections, autoimmune disorders, pregnancy related complications and malignant diseases (K. Beksac et al., 2015; M. Beksac et al., 2021; Castaño-Núñez et al., 2019; Hematian Larki et al., 2022; Mori et al., 2019; Nakimuli et al., 2015; Orgul et al., 2021). KIR and HLA polymorphisms show distinct profiles among worldwide populations and KIR haplotypes have been

described across the globe (Amorim et al., 2021; Maxwell et al., 2004; Nakimuli et al., 2015; Ozturk et al., 2012; Rajalingam et al., 2008). Currently, there are 274 published studies from 50 countries with a record of 1474 disease associations only within the Allele Frequency Net Database (Takeshita et al., 2013). However, KIR/HLA association studies reveal conflicting results, particularly among hematological malignancies (Augusto, 2016).

There are a limited number of KIR-MM association studies. According to the literature, the first KIR-MM association was studied in 2010 by Gabriel et al. They reported that 3DS1 and 3DL1 positivity along with absence of Bw4 ligand was associated with shorter progression-free survival (PFS) in MM patients (n=182) after autologous hematopoietic stem cell transplantation (Gabriel et al., 2010). Just a few months later, another group in Germany focused on KIR haplotypes and suggested that B haplotype was associated with improved PFS in a cohort study including 118 MM patients (Kröger et al., 2011). A comprehensive analysis published in 2015, proposed an impact of certain combinations of 2DL1, 2DL2 and 2DL3 genotypes and HLA-C ligands on susceptibility to MM as well as PFS of the patients. They have included 286 healthy control subjects and 164 Caucasian patients diagnosed with MGUS, SMM and MM, of which only 53 were MM and their results on PFS does not fully cover the findings from Gabriel et al (Gabriel et al., 2010; Martínez-Sánchez et al., 2015). Another study was conducted on Lebanese population including 120 control and 34 MM subjects. They have reported an association with 2DS4 and 2DS5 in favor of susceptibility to MM; however, the sample size significantly lowers the statistical power in their analysis (Hoteit et al., 2014). Sun et al. reported KIR-dependent individual-level inconsistencies regarding the efficacy of an anti-MM drug combination in a Phase II clinical trial (NCT01749969). Their findings show that 3DL2 and its corresponding ligand was associated with prolonged PFS, while 2DL1 and HLA-C2 homozygosity was associated with a shorter PFS and a reduced response to the drug therapy (Sun et al., 2021). The most recent study regarding KIR-MM association has been published in 2024 and included a cohort from Netherlands (172 MM and 195 control subjects). Their findings differ from

those of Hoteit et al. and Martínez-Sánchez et al., and they explain the possible causation of the dissimilarity with the relatively small sample size included in the previous studies. However, their suggested association (HLA ligands C1-C2+Bw4+ and 3DL1+Bw4+ genotype in favor of MM occurrence) lack statistical power due to the low number of cases distributed among genotyping groups resulting in a false significance (OR [95% CI]: 1.996 [0.992–4.014], P=0.049 and OR [95% CI]: 1.557 [0.999–2.427], P=0.050; respectively] (Beelen et al., 2024).

Several HLA-MM association studies have been carried out and the biggest cohort study was published using the Center for International Blood and Marrow Transplant Research (CIBMTR) database. This study reported associations of HLA-C*07:02 and HLA-B*07 with susceptibility to MM and HLA-C*05:01 and HLA-B*44:02 were reported to be as protective variants in a population including 3724 MM cases and 50.000 control subjects (M. Beksac et al., 2016). Considering HLA-C*07:02 belonging to C1 group ligands, HLA-C*05:01 belonging to C2 and HLA-B*44:02 to Bw4 ligands; these results are consistent with the previous findings from the local cohort of MM patients, which demonstrated an association between KIR and age of onset (M. Beksac et al., 2023).

Regarding the inconsistencies between the findings of all above-mentioned studies, the common interference clusters around the requirement of studies recruiting much higher number of patients due to the excessive number of variables resulting from KIR-HLA ligand combinations as well as the varying haplotypes among ethnically diverse populations, thus sample sizes remain small and most of the current studies are underpowered for statistical significance (Augusto, 2016; Gao et al., 2022; Jennifer Zhang, 2022; Myers & Miller, 2021).

KIR/HLA genotyping from high number of patients from a single center requires time to reach patients and also it is not cost effective, since the high-resolution KIR/HLA genotyping kits are relatively expensive. One possible way to increase the sample size is to use available genome-wide association studies (GWAS), whole genome sequencing (WGS) or whole exome sequencing (WES) datasets providing

in large databases, which have been being accumulating data for decades from various research studies (Chattopadhyay et al., 2019; MacArthur et al., 2014). Many tools have been developed by researchers for using polymorphic regions from WGS data effectively, in disease association studies. These include HLA and KIR imputation tools, which are still under development (J. Chen et al., 2021; Sakaue et al., 2023; Ustunkar & Aydin-Son, 2011; Vukcevic et al., 2015).

1.4 KIR and HLA Imputation

Given the significant importance of HLA and KIR variations in disease association studies, many researchers have been tended to expand their research on large-scale. However, high cost of conventional HLA/KIR genotyping methods led to development of other strategies. The increased availability of genome-wide association studies and whole genome/exome sequencing data draw attention to computational solutions.

HLA*IMP and KIR*IMP are among the most frequently used HLA and KIR imputation tools, and they were developed by implementation of statistical methods to benefit from linkage disequilibrium of single nucleotide polymorphisms (SNPs) neighboring to genomic region of HLA and KIR genes (Figure 1.4) in high resolution SNP arrays from GWAS (Dilthey et al., 2013; Vukcevic et al., 2015). There are several other imputation methods developed for both HLA (J. Chen et al., 2021; Motyer et al., 2016), and KIR imputation (Ahn et al., 2021; Ritari et al., 2022; Sakaue et al., 2022) from GWAS arrays. These methods generally require multi-ancestry reference panels or panels specific to a particular ancestry. They also require datasets constructed by Immunochip-like SNP panels, which are enriched in SNPs and they reveal higher coverage in the HLA or KIR regions (J. Chen et al., 2021).

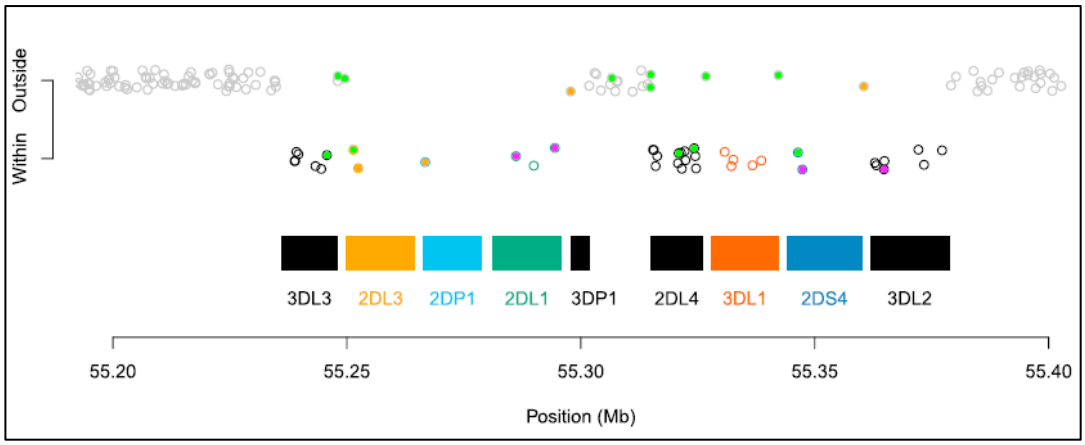

Figure 1.4. Informative clusters of SNPs aligned with genomic location of KIR genes according to the imputation algorithm of KIR*IMP (Vukcevic et al., 2015).

As high-throughput next-generation sequencing (NGS) technique become more cost-effective and widespread in genomic studies, imputation tools using short-read NGS or long-read WGS/WES data have been developed (J. Chen et al., 2021; Gao et al., 2022; Roe et al., 2020; Roe & Kuang, 2019). Even though the most recent imputation tool, which was introduced as a model for both HLA and KIR imputation as well as for calling SNPs using either WGS, WES or even RNA-sequencing data was published in June 2023 (Song et al., 2023), the KIR-disease association studies performing KIR imputation methods are still very limited (Ahn et al., 2021; Bao et al., 2018; Gao et al., 2022).

1.5 Scope of the Study

MM is the second most prevalent hematological malignancy showing differences in disease pathogenesis among different ethnic groups and gender types. Disease progression is known to be profoundly influenced by germ-line genetic disparities influencing immune-response mediators, which still remain elusive according to the current scientific literature.

NK cells are immune system regulators against viral infections and cancer, including MM. They recognize self-HLA missing tumor cells through the interaction between

their KIR receptors and HLA ligands on target cells. Polymorphic KIR and HLA genes display a wide range of variations resulting in an extensive diversity among individuals. There are few reports, including a published study by our study group, associating KIR/HLA variants with MM. Due to the high-level diversity in KIR-HLA pairs, most of the current studies remain small-scaled and underpowered in terms of statistical significance. Expensiveness of conventional KIR/HLA genotyping method is another limitation against conducting large cohort studies. Thus, alternative strategies are required for better understanding the relationship between KIR and MM.

In this study, allele-level imputation of HLA and KIR receptor genes in MM patients was performed using WES data of MM patients available in Database of Genotypes and Phenotypes (dbGaP) from the CoMMpass dataset. Results were compared with a local KIR/HLA ligand dataset to be acquired from conventional KIR and HLA ligand genotyping on MM patients from Ankara University Department of Hematology. Validation of the KIR and HLA imputation accuracy was performed on a randomly selected subgroup of local MM patients, which were subjected to both WES and targeted genotyping.

Healthy subjects from Ankara University Donor Registry Database, of whom KIR and HLA ligand genotyping had been performed by conventional methods, were included as the local control group in this study. Healthy control group dataset was constructed using individual KIR/HLA ligand genotyping data from various countries provided in Allele Frequencies Net Database, so that the ethnic frequency distribution was matching with the patients from CoMMpass dataset.

Distribution of HLA and KIR variants, including the individual KIR genes and haplotypes in addition to their relationship with their corresponding HLA ligands, was evaluated among the patient subgroups in terms of gender, age, and ethnicity. Additionally, KIR and HLA ligand frequencies were compared between MM patients and healthy subjects in order to achieve a better understanding of the effects of KIR-associated immune-checkpoints on susceptibility to MM. The use of the

largest sample size currently available in literature will provide clarifying evidence in order to resolve the inconsistent results obtained in earlier KIR-MM association studies. The results from this study are expected to be a significant contribution to a better understanding of the individual genetic disparities in predisposition to MM.

CHAPTER 2

MATERIALS AND METHODS

2.1 MM Patients

Two separate groups of newly diagnosed MM patients were included in this study. One of the patient groups was selected among dbGaP controlled-access datasets and the other set of patients were recruited from Ankara University Hematology Department.

2.1.1 Dataset of CoMMpass Study

MM dataset has been queried from European Nucleotide Archive (ENA) and dbGaP. CoMMpass dataset provided by Multiple Myeloma Research Foundation (MMRF) includes well-documented patient characteristics and it is known as one of the largest MM datasets with a total of 978 participants including the data from WGS, WES and RNA sequencing assays.

Authorized access has been granted (Project ID: 132438-1) for CoMMpass study (PRJNA248539, dbGaP: phs000748.v7.p4) and paired-end FASTQ files containing WES reads belong to non-tumor germline DNA derived from peripheral blood of 707 MM patients have been downloaded in Sequence Read Archive (SRA) format, converted to FASTQ using SRA Toolkit (v3.1.0) and compressed by *pigz* (v2.6). Since it is known that the structural changes in the tumor DNA may occur in the locus where KIR gene sequences are located (Pratt, 2002), patients with a single data derived only from their bone marrows have been excluded.

2.1.2 Dataset of Local Patients from Ankara University

Peripheral blood from a total of 218 newly diagnosed MM patients from Ankara University Hematology Department have been collected with the informed consent in scope of a conducted research project (Project ID: 115S579) with the approval of Ethical Committee of Ankara University (Approval number: 06-421-18). All patients were genotyped for their KIR and HLA ligands, among whom twenty were also subjected to WES assay. DNA isolation and targeted genotyping of KIR/HLA ligands were performed at Ankara University Hematology Laboratories. WES has been performed by Mikrogen Diagnostics and raw sequencing data was received in FASTQ format.

2.2 Healthy Subjects

KIR and HLA ligand genotyping data of healthy subjects from Ankara University Donor Registry were used as the local control group representing the Turkish population.

Since the patients from CoMMpass dataset belong to different ethnic populations, individual KIR/HLA ligand genotyping data of ethnic groups from various countries has been collected from Allele Frequencies Net Database and included in this study as healthy controls for the CoMMpass dataset (Gonzalez-Galarza et al., 2011). Some of the datasets which do not include all KIR genes and HLA ligands were excluded from this study. Among 49 datasets from 31 different countries, the populations which can be classified as “Caucasians” have been selected and included in this study. The only dataset of Black individuals provided within the database belongs to South Africa Xhosa population. It was excluded from this study, as KIR and HLA ligand frequencies may differ within African American and African populations.

The Allele Frequencies Database does not distinguish between 2DL5A and 2DL5B genes; it only provides the information on the presence or absence of 2DL5 gene. Both 2DL5A and 2DL5B are of substantial importance in haplotype-level analyses,

and they are located at distinct locations in terms of telomeric and centromeric regions. Therefore, a formula for the calculation of subtypes was generated using the known KIR motifs within the telomeric and centromeric haplotypes.

2.3 Targeted Genotyping of KIR and HLA Ligands

2.3.1 DNA Isolation

DNA isolation from peripheral blood of MM patients was performed using EZ1 DNA Blood Kit (Qiagen, Netherlands) on the Easy1 Advanced XL (Qiagen, Netherlands) instrument according to the manufacturer's instructions. Quality and quantity of the isolated DNA were measured using Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, US).

2.3.2 KIR Genotyping

KIR genotyping was performed by polymerase chain reaction (PCR) using KIR specific primers included in Olerup SSP KIR Genotyping kit (104.101-12u; Olerup, Sweden), for the detection of KIR2DL1, 2DL2, 2DL3, 2DL4, 2DL5A/B, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1, 2DP1 and 3DP1 genes. KIR-specific PCR amplicons were run through the agarose gel electrophoresis and the bands were observed under UV light. Presence or absence of the KIR variants were determined according to the reference markers using manufacturer's instructions. Both negative and internal controls were included in each experiment.

2.3.3 HLA Ligand Genotyping

Targeted genotyping of HLA ligands was performed using KIR HLA Ligand PCR SSP Kit (104.201-12u; Olerup, Sweden), which is able to detect HLA-ABw4+, HLA-B Bw4+Thr80, HLA-B Bw4+Ile80, HLA-B Bw4+Asp77, Thr80, HLA-C

Asn80 (group C1), and HLA-C Lys80 (group C2). Similar to KIR genotyping, DNA samples were amplified using HLA ligand specific primers and run on agarose gels along with negative and internal controls. Bands detected under UV light have been evaluated according to the manufacturer's instructions.

2.4 Whole Exome Sequencing

In order to validate the KIR and HLA/HLA ligand imputation accuracy 20 MM patients from Ankara University, whose KIR and HLA ligands were determined by targeted KIR genotyping, were subjected to WES assay. WES was performed at Mikrogen. The assay was conducted on the Twist Bioscience technology platform using Twist Human Core Exome Plus Kit. Library construction was performed by ligation of adapters to the fragmented DNA samples. In order to amplify the exonic regions, oligonucleotide probes provided by the manufacturer were hybridized and captured by magnetic beads. Amplified fragments were sequenced on the high-throughput Illumina Novaseq platform. FASTQ and Variant Call Format (VCF) files were directly received from Mikrogen. VCF files were used for KIR imputation by KIR*IMP tool. FASTQ files were used to impute both KIR and HLA variants using T1K imputation tool.

2.5 KIR and HLA Imputation

The basis of choosing the optimal imputation algorithm depends on the quality of the sequencing data, the presence of short or long reads, the quality of the reads, and the assay type (WGS, WES, RNA-seq, high/low coverage SNP panels). In accordance with the purpose of this study, KIR/HLA imputation tools have been reviewed and further selected according to their reported performance on WGS or WES datasets.

KIR*IMP is the most frequently used and cited tool for KIR imputation and T1K is the latest tool published capable of imputing both KIR and HLA in a single platform.

Although it is reported by the developers that KIR*IMP requires the output of high coverage SNP panels, such as Immunochip assays, within the scope of this study only the WES dataset was used to test the imputation accuracy. Although T1K fits better in terms of the sequencing format of the datasets included in this study, both methods were practiced on a small set of patients.

2.5.1 KIR Imputation Using KIR*IMP Software

KIR*IMP software (version 1.2.0) requires phased SNP data in file formats HAPS and SAMPLE. WES outputs received from Mikrogen were imported to Galaxy platform (https://usegalaxy.eu) for data preparation steps (Galaxy Community, 2022). WES reads were mapped against reference genome (hg19/GRCh37) and variant calling was performed using FreeBayes after quality control steps. Annotating, deduplication, and SNP filtering steps were performed by BCFtools (Figure 2.2). All patients' data included in a VCF file was downloaded and subjected to the phasing step using SHAPEIT (O'Connell et al., 2014). HAPS and SAMLE files were uploaded to KIR*IMP (https://imp.science.unimelb.edu.au/kir/) and the results were recorded.

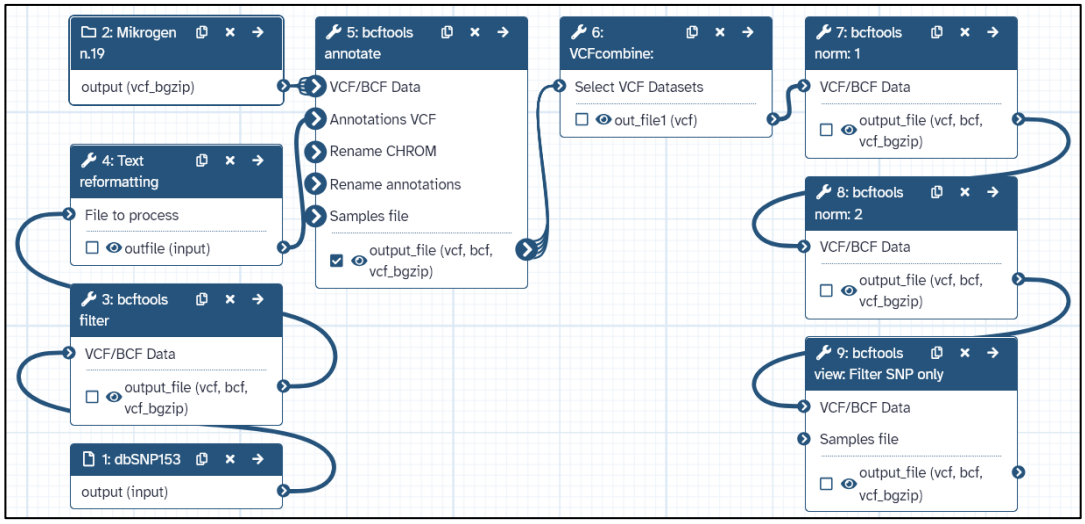

Figure 2.1. Data preparation workflow constructed in Galaxy platform.

2.5.2 KIR and HLA Imputation Using T1K Software

T1K is an open-source tool for allele level imputation of KIR and HLA genes from WGS, WES or RNA-seq data. FASTQ files are accepted as input without requiring pre-processing of the raw sequencing reads. KIR and HLA genes of all MM patients included in this study were imputed using T1K (version v1.0.6-r206).

2.5.2.1 KIR Imputation and Data Stratification

Paired-end sequencing reads in FASTQ format were introduced using preset parameters for WES data. Interpretation of the results in terms of presence or absence of KIR alleles were performed according to the quality score calculated from abundance level as per the instructions given by the developers. Two different variants of KIR2DS4 gene (normal and truncated) were determined according to the primary alleles. KIR2DS4*001, *011, *014 and *015 alleles were considered as normal variants, while KIR2DS4*003, *004, *006, *007, *008, *009, *010, *012 and *013 alleles were recorded as the truncated version. For further improvement of the imputation accuracy for KIR2DL5A/B genotypes, minimum alignment similarity (-s) and the effect of relative gene expression (--crossGeneRate) parameters have been adjusted to -s 0.05 and --crossGeneRate 0.08 (https://github.com/mourisl/T1K).

2.5.2.2 HLA Imputation and Classification of HLA Ligands

Similar to KIR imputation, paired-end read files were used without pre-processing and HLA imputation was performed using the preset parameters specifically provided for WES data (https://github.com/mourisl/T1K). Default parameters did not require any adjustment for HLA alleles. HLA ligands were calculated manually using the information of HLA-KIR pairs provided in the Allele Query Database (*IPD-IMGT/HLA Database*, 2024).

2.6 Validation of Imputation Results

KIR imputation results from KIR*IMP and T1K were compared with the targeted genotyping results of 20 MM patients from Ankara University. Optimal parameters for T1K were adjusted to reach the highest accuracy rate. The same parameters were used on CoMMpass dataset. HLA ligands were calculated according to the imputed HLA alleles and compared with the targeted genotyping results of 20 patients. Comparison was performed at both patient level and KIR/HLA ligand level in order to eliminate the effect of genetic disparities among individuals and the effect of diverse polymorphic characteristics of KIR/HLA genes.

2.7 Statistical Analysis

Statistical analyses were performed by IBM SPSS Statistics (version 26; IBM Corporation, Armonk, NY). Descriptive statistics of normally distributed data were given as mean (standard deviation), while the non-normally distributed data was expressed as median (min-max). Significance level of the difference between the mean or median values of two groups were analyzed by t-test or Mann Whitney test, respectively. Mean values among three or more groups were compared using ANOVA and median values were compared by Kruskal Wallis test. Post-hoc comparisons were performed by Tukey or Dunn's test for the mean and median values, respectively. $p < 0.05$ was considered as statistically significant, except for the cases where Bonferroni correction was applied for multiple hypothesis testing.

CHAPTER 3

RESULTS

This study is structured in three main stages: Data collection, imputation of KIR and HLA genes from WES data and validation of the imputation accuracy using targeted genotyping of KIR/HLA ligands, and statistical analyses.

3.1 MM Patients and Healthy Control Datasets

Two different groups of datasets were included in this study. One of the datasets containing the WES data from non-tumor sites of 707 newly diagnosed MM patients from CoMMpass study was pulled from the database of Genotypes and Phenotypes (dbGaP). Healthy control group consisting of the similar ethnic populations was collected from Allele Frequencies Net Database (n=1803), in which KIR and HLA ligands for each subject was provided as presence/absence of the genes. The second dataset of patients consisted of 218 MM patients recruited from Ankara University Hematology Department, all of which were genotyped for KIR and HLA ligands by targeted PCR. Twenty of those patients were also subjected to WES assay. The local control group of this dataset consisted of 424 healthy donors from Ankara University Unrelated Donor Registry. Their KIR/HLA ligand data was collected as presence/absence of the genes (Table 3.1).

Table 3.1 Patient and healthy control datasets included in this study.

Group	Data Origin	Assay Type	Number of Participants
Patients	dbGaP (phs000748.v7.p4)	Whole exome sequencing	707
Healthy Subjects	Allele Frequencies Net Database	Targeted genotyping	1803
Patients	Ankara University	Targeted genotyping	218
		Whole exome sequencing	20
Healthy Subjects	Ankara University	Targeted genotyping	424

3.2 Imputation and Validation of KIR and HLA Ligands

3.2.1 Imputation Using KIR*IMP

Data pre-processing steps are required for imputing KIR genes by KIR*IMP. SNP coverage data obtained after the processing steps of 20 patients was uploaded to KIR*IMP (https://imp.science.unimelb.edu.au/kir/). Outputs were provided in separate file formats containing various information, including the imputation results, KIR alleles, SNP alleles, posterior probabilities and the estimated accuracy of the imputation results (Figure 3.1).

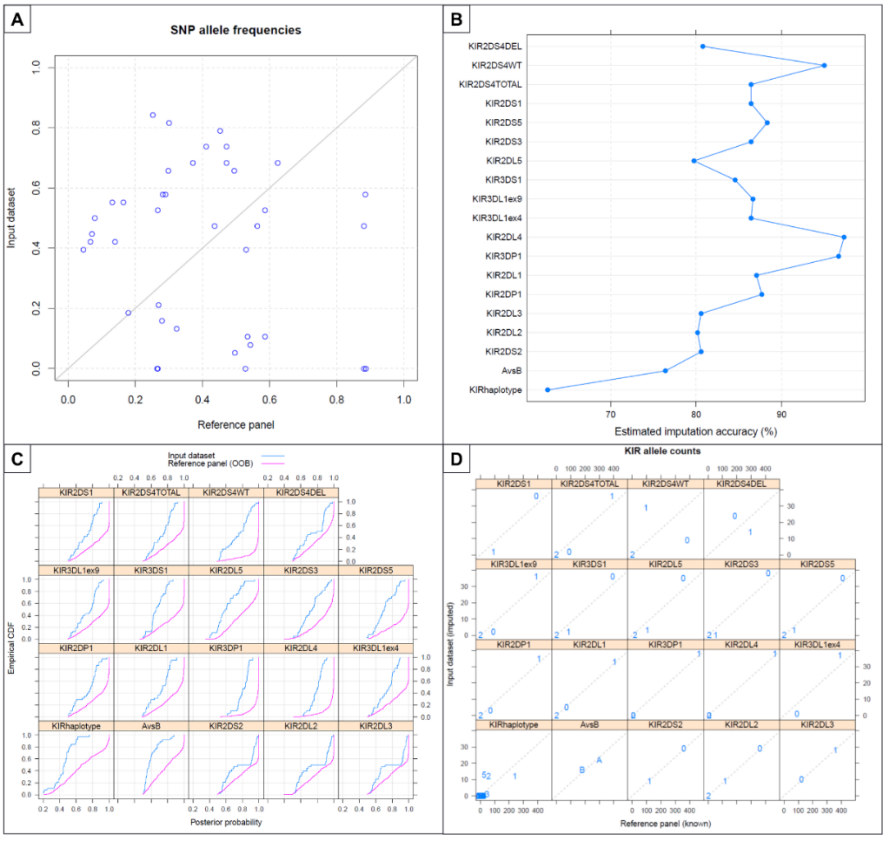

Figure 3.1. KIR imputation output graphics from KIR*IMP.

*A) SNP allele frequencies of the reference panel (KIR*IMP use UK reference panel as default) and input dataset, which includes 20 MM patients from Ankara University. B) Average imputation accuracy estimated for each locus. C) Posterior*

probabilities of the reference panel and the input dataset of patients. D) Intersecting allele frequencies of the reference panel and the patient dataset.

Validation of the imputation results was performed on 20 patients, who were subjected to both WES and targeted genotyping of KIR genes. Overall median accuracy of the imputed genes was found to be 86% (14%-100%). The lowest correct imputation rate was observed on the non-truncated form of 2DS4 gene. 2DL2 and 2DS2 showed the second lowest accuracy rate. Additionally, KIR*IMP was unable to impute the distinct A and B subtypes of the 2DL5 gene. Therefore, the comparison was made using the combined 2DL5A/B genotype with a correct imputation rate of 57%. Considering KIR*IMP is an imputation tool optimized for high-coverage SNP datasets, a low rate of imputation accuracy was expected, as the input data was derived from a WES assay. Therefore, no further analyses were performed using KIR*IMP, since all the datasets included in this study were constructed by WES.

3.2.2 Imputation Using T1K

T1K is an all-in-one tool for both KIR and HLA imputation and it can be run using the input files generated from WGS, WES, and RNA sequencing data. The local patient dataset was constructed using raw output files from WES assays performed on the patients from Ankara University. The collected data was used directly without any manipulation. To collect data from the CoMMpass dataset, SRA files of each patient filtered for WES assays were downloaded from dbGaP using SRA Toolkit and converted to FASTQ files, and finally compressed by *pigz* in order to save storage space. Paired-end read files from both datasets were introduced to T1K separately for KIR and HLA imputation.

3.2.2.1 KIR imputation

Each run for KIR imputation resulted in seven output files. Table 3.2 shows an example of the output table from a single patient. For each patient, **.genotype.csv*

files were used for interpretation of absence or presence of the KIR genes according to the instructions of the developers of T1K. For each KIR gene, a quality score less than or equal to 0 was interpreted as absence of the allele, while any score greater than 0 (out of 60) was considered as presence of the allele.

Table 3.2 Representative sample of a KIR imputation output from T1K.

gene_name	num_diff_alleles	allele_1	abundance_1	quality_1	allele_2	abundance_2	quality_2	secondary_alleles
KIR2DL1	1	KIR2DL1*003	222.912.282	60	.	0	-1	
KIR2DL2	1	KIR2DL2*001	210.258.543	45	.	0	-1	
KIR2DL3	1	KIR2DL3*001	236.244.937	59	.	0	-1	
KIR2DL4	1	KIR2DL4*011	280.839.934	60	.	-	-1	
KIR2DL5A	1	KIR2DL5A*027	0.827856	0	.	0	-1	
KIR2DL5B	2	KIR2DL5B*006	222.601.562	60	KIR2DL5B*008	70.189.960	38	
KIR2DP1	1	KIR2DP1*002	182.397.865	36	.	-	-1	
KIR2DS1	1	KIR2DS1*002	184.223.377	36	.	0	-1	
KIR2DS2	1	KIR2DS2*001	195.603.385	36	.	0	-1	
KIR2DS3	0	.	0	-1	.	0	-1	
KIR2DS4	1	KIR2DS4*010	165.619.572	38	.	0	-1	
KIR2DS5	1	KIR2DS5*002	194.464.150	46	.	0	-1	
KIR3DL1	1	KIR3DL1*005	245.923.015	60	.	0	-1	
KIR3DL2	2	KIR3DL2*001	218.926.759	60	KIR3DL2*007	230.558.916	60	
KIR3DL3	2	KIR3DL3*009	263.125.634	60	KIR3DL3*003	247.219.046	60	
KIR3DP1	1	KIR3DP1*003	173.470.559	60	.	0	-1	
KIR3DS1	1	KIR3DS1*013	1.136.333	0	.	0	-1	

Presence or absence of each KIR gene was determined based on to the quality scores of the corresponding alleles (columns "quality_1" and "quality_2"). Alleles with a quality greater than or equal to one were accepted as a positive (presence of the allele), according to the developers' instructions.

In the first attempt on 20 patients, the mean (SD) quality scores of 2DL5A and 2DL5B genes were found to be lower than the others [15 (17) and 19 (24), respectively]. Therefore, the parameters of the imputation algorithm were adjusted to achieve the highest quality score possible for each allele. Optimal results were obtained by setting the minimum alignment similarity (-s) cutoff to 0.05 and by adjusting the effect of relative gene expression (--crossGeneRate) as 0.08. As a result, the mean (SD) quality scores of 2DL5A and 2DL5B were increased to 31 (2) and 35 (21), respectively. The comparison of the imputed KIR genes of 20 MM patients from Ankara University with the targeted KIR genotyping results of the same patients achieved a 100% accuracy for each KIR gene of each patient.

3.2.2.2 HLA imputation

Similar to the KIR imputation results, T1K resulted in seven output files and **.genotype.csv* was used to interpret the HLA genotyping results (Table 3.3).

Imputation of HLA-A, -B and -C alleles resulted in high quality scores of the abundance level of the genes for each patient. Therefore, the algorithm settings did not require any adjustment. According to the information given by Allele Query Database, cognate HLA ligands of KIR genes were calculated using the imputed HLA types (*IPD-IMGT/HLA Database*, 2024).

Table 3.3 Representative sample of an HLA imputation output from T1K.

gene_name	num_diff_alleles	allele_1	abundance_1	quality_1	allele_2	abundance_2	quality_2	secondary_alleles
HLA-A	2	HLA-A*33:01:01	301.629.079	60	HLA-A*11:01:01	301.171.486	60	
HLA-B	2	HLA-B*14:02:01	312.321.511	60	HLA-B*40:02:01	287.693.190	60	
HLA-C	2	HLA-C*02:02:02	331.366.885	60	HLA-C*08:02:01	262.960.250	60	
HLA-E	1	HLA-E*01:01:01	624.552.052	60	.	0	-1	
HLA-F	1	HLA-F*01:01:02	621.014.089	60	.	0	-1	
HLA-G	2	HLA-G*01:03:01	326.883.511	60	HLA-G*01:01:03	318.491.359	60	
HLA-H	2	HLA-H*02:12	288.538.252	60	HLA-H*02:07:01	258.506.139	60	
HLA-J	1	HLA-J*01:01:01	569.013.383	60	.	0	-1	
HLA-K	2	HLA-K*01:03	87.387.618	39	HLA-K*01:01:01	182.158.170	60	
HLA-L	2	HLA-L*01:01:01	266.463.432	60	HLA-L*01:02	169.855.897	60	
HLA-N	1	HLA-N*01:01:01	216.858.239	60	.	0	-1	
HLA-P	2	HLA-P*02:01:01	108.966.078	60	HLA-P*01:01:01,HLA-P*01:02	55.590.053	33	
HLA-S	1	HLA-S*01:02:01	253.639.236	60	.	0	-1	
HLA-T	1	HLA-T*03:01	208.921.335	60	.	0	-1	
HLA-U	2	HLA-U*01:04	175.537.686	60	HLA-U*01:01:01	127.333.940	60	
HLA-V	1	HLA-V*01:01:01	442.793.468	60	.	0	-1	
HLA-W	2	HLA-W*03:01:01	137.117.194	60	HLA-W*01:01:01	45.545.283	26	
HLA-Y	1	HLA-Y*02:01	271.681.464	60	.	0	-1	
HLA-DRA	1	HLA-DRA*01:01:01	590.593.045	60	.	0	-1	
HLA-DRB1	2	HLA-DRB1*14:01:01	235.843.095	60	HLA-DRB1*11:01:01	211.194.993	60	
HLA-DRB3	2	HLA-DRB3*02:24	178.213.881	60	HLA-DRB3*02:02:01	255.821.667	60	
HLA-DRB4	1	HLA-DRB4*01:03:01	11.293.195	2	.	0	-1	
HLA-DRB5	1	HLA-DRB5*02:02:01	1.781.896	0	.	0	-1	
HLA-DQA1	2	HLA-DQA1*01:04:01	260.037.811	60	HLA-DQA1*05:05:01	249.097.828	60	
HLA-DQA2	2	HLA-DQA2*01:01:02	335.695.820	60	HLA-DQA2*01:01:01	309.308.795	60	
HLA-DQB1	2	HLA-DQB1*05:03:01	221.793.491	60	HLA-DQB1*03:01:01	199.259.398	60	
HLA-DQB2	2	HLA-DQB2*01:01:01	229.448.459	60	HLA-DQB2*01:02:01	180.886.898	60	
HLA-DPA1	1	HLA-DPA1*01:03:01	583.460.944	60	.	0	-1	
HLA-DPA2	1	HLA-DPA2*01:01:01	56.864.864	50	.	0	-1	
HLA-DPB1	1	HLA-DPB1*04:01:01	583.935.243	60	.	0	-1	
HLA-DPB2	1	HLA-DPB2*03:01:01	274.531.054	60	.	0	-1	
HLA-DMA	2	HLA-DMA*01:01:01	315.151.159	60	HLA-DMA*01:01:02	283.653.184	60	
HLA-DMB	1	HLA-DMB*01:01:01	585.155.347	60	.	0	-1	
HLA-DOA	2	HLA-DOA*01:01:01	244.882.816	60	HLA-DOA*01:01:02	237.058.835	60	
HLA-DOB	1	HLA-DOB*01:01:01	648.517.941	60	.	0	-1	
HLA-HFE	2	HLA-HFE*001:01:02	230.636.655	60	HLA-HFE*001:01:01	186.220.180	60	
MICA	2	MICA*027:01:01	275.602.059	60	MICA*011:01:01	266.585.740	60	
MICB	2	MICB*005:03:01	311.642.818	60	MICB*013:01:01	208.357.185	60	
TAP1	1	TAP1*01:01:01	556.061.390	60	.	0	-1	
TAP2	2	TAP2*01:03:04	229.105.797	60	TAP2*02:01:02	253.165.314	60	TAP2*01:03:02;111.441878;60

Presence or absence of HLA alleles were determined based on to the quality scores of the corresponding alleles (columns "quality_1" and "quality_2"). Alleles with a

quality greater than or equal to one were accepted as a positive (presence of the allele), according to the developers' instructions.

In Table 3.4 the imputed HLA alleles and their corresponding HLA ligands are given. For each patient a complete match in HLA ligands was observed when the calculated HLA ligands of 20 patients were compared to the targeted genotyping results. Further validation was performed at 6-digit allele level on 10 patients by comparing the imputed HLA types with the laboratory results, which were generated by NGS for diagnostic purposes at Ankara University Hematology Laboratories.

Table 3.4 HLA alleles and the corresponding HLA ligands.

Patient ID	HLA-A1	HLA-A2	HLA-B1	HLA-B2	HLA-C1	HLA-C2	C2	C1	Bw4
126813_3221	24:02:01	24:02:01	08:01:01	48:01:01	08:01:01	07:02:01	0	1	1
MG107982_S38	03:01:01	26:01:01	38:01:01	18:01:01	12:03:01	12:03:01	0	1	1
MG107987_S39	03:02:01	02:01:01	35:03:01	18:01:01	07:01:01	04:01:01	1	1	0
MG107988_S40	02:01:01	02:11:01	58:01:01	52:01:01	03:02:02	12:02:02	0	1	1
MG108001_S42	32:01:01	32:01:01	35:01:01	56:01:01	01:02:01	04:01:01	1	1	1
MG112973_S25	26:01:01	29:01:01	38:01:01	41:02:01	17:03:01	12:03:01	1	1	1
MG112974_S24	29:02:01	30:01:01	45:01:01	13:02:01	06:02:01	06:02:01	1	0	0
V300114923_L02_64	11:01:01	02:01:01	40:02:01	51:01:01	02:02:02	04:01:01	1	0	1
V300114923_L03_65	33:01:01	11:01:01	14:02:01	40:02:01	02:02:02	08:02:01	1	1	0
V300114923_L03_66	32:01:01	24:02:01	50:01:01	51:01:01	14:02:01	06:02:01	1	1	1
V300114923_L03_67	03:01:01	24:02:01	07:02:01	51:01:01	07:02:01	14:02:01	0	1	1
V300114923_L03_68	32:01:01	02:01:01	50:01:01	51:01:01	06:02:01	04:01:01	1	0	1
V300114923_L03_69	32:01:01	24:02:01	50:01:01	51:01:01	14:02:01	06:02:01	1	1	1
V350080493_L02_91	02:01:01	26:01:01	08:01:01	35:215:02	04:01:01	05:01:01	1	0	0
V350080493_L02_92	02:01:01	24:02:01	51:01:01	35:57	04:01:01	07:02:01	1	1	1
V350080493_L04_125	68:02:01	02:01:01	46:01:01	15:73:01	01:02:01	03:03:01	0	1	0
V350080493_L04_126	68:02:01	02:01:01	13:02:01	15:73:01	03:03:01	06:02:01	1	1	0
V350160404_L02_90	11:01:01	30:04:01	35:01:01	35:01:01	04:01:01	16:02:01	1	0	0
V350160404_L02_91	11:01:01	68:01:01	35:03:01	35:01:01	04:01:01	04:01:01	1	0	0
mg107999	29:02:01	01:01:01	35:01:01	45:01:01	06:02:01	04:01:01	1	0	0

3.3 Association Between KIR/HLA Ligands and MM

A total of 925 MM patients and 2227 healthy subjects were included in this study. The patient cohort included 218 cases from Ankara University Hematology Department and 707 cases from CoMMpass dataset. Healthy control group was consisted of 424 subjects from Ankara University Donor Registry and 1803 subjects from Allele Frequency Net Database. Among all MM patients, 40.8% were female

and 59.2% were male, and among the healthy controls 52.9% were female and 47.1% were male. Median age of the patients at diagnosis was 61 (27-89) with a distribution of ISS I/II/III: 36.1%/31.8%/32.1%. Frequency distribution of KIR and HLA ligand genotypes between all MM patients and all healthy controls were evaluated using chi-square or 2-tailed Fisher's exact test as appropriate. Bonferroni correction was applied to multiple testing of aKIR and iKIR genes, KIR genotypes (A/B, centromeric and telomeric), HLA ligands and KIR-HLA ligand pairs (Table 3.5, Table 3.6, Table 3.7, Table 3.8).

Among iKIR genes, 2DL5AB and 2DL5A were found to be more frequent among the control group, however the significance level did not survive the Bonferroni correction (uncorrected P values: 0.032 and 0.019, respectively). Among aKIR, 2DS1, 2DS3 and 3DS1 were more frequent among healthy subjects compared to MM patients. Only 2DS1 (41.4% vs 35.7%, OR: 0.786 [0.671-0.922], Pc=0.042) and 3DS1 (40.9% and 34.5%, OR: 0.762 [0.649-0.894], Pc=0.012) remained significant after correcting for multiple testing. The mean number of aKIRs was slightly higher in the control group compared to MM patients (3.0 and 2.8, P=0.031), however there was no significant difference between the mean numbers of iKIRs (Table 3.5).

In order to analyze the clustered KIR genotypes, classification was performed based on the gene motifs used for defining the A/B haplotypes, centromeric haplotypes and telomeric haplotypes as illustrated in Figure 1.3. AX genotype represents individuals carrying AA or AB genotypes, while BX represents the individuals with BB or AB genotypes. There was no significant association between A/B genotypes and predisposition to MM. Centromeric or telomeric motifs were classified as AA, B1B1, B2B2, B1B2, AB1 and AB2 according to the information in Figure 1.3. There was no individual with cB1B1 genotype within the entire dataset. Only 9 subjects were tB2B2, and all of them were belong to the healthy control group. Frequency distribution of ambiguous genotypes, which include KIR motifs that do not fit any classification of centromeric or telomeric haplotypes, was similar between MM patients and healthy subjects (3.3% and 5.3%, respectively). Among centromeric genotypes, cAB1 and cAB2 were found to be significant in opposite directions (OR:

0.828 [0.685-1.000], P=0.050 and OR: 1.262 [1.054-1.511], P=0.011; respectively); however, the significance level did not survive the Bonferroni correction. tAB2 was the only genotype that survived the correction for multiple testing and was found to be more frequent among healthy controls compared to MM patients (7.9% and 5.1%, OR: 0.628 [0.451-0.874], Pc=0.033) (Table 3.6).

Table 3.5 KIR frequencies among control groups and MM patients.

Comparison of KIR Frequencies Among Healthy Subjects and MM Patient Datasets	Control Group Datasets		MM Patient Datasets		All Healthy Subjects vs All MM Patients			
	AF Healthy Subjects (n=1803)	AU Healthy Subjects (n=424)	AU MM Patients (n=218)	CoMMpass MM Patients (n=707)	All Healthy Subjects (n=2227)	All MM Patients (n=925)	P value*	P_c value§
Inhibitory KIR Genes								
2DL1	1735 (96.2%)	408 (96.2%)	214 (98.2%)	683 (96.6%)	2143 (96.2%)	897 (97.0%)	0,304	
2DL2	975 (54.1%)	264 (62.3%)	125 (57.3%)	404 (57.1%)	1239 (55.6%)	529 (57.2%)	0,423	
2DL3	1603 (88.9%)	392 (92.5%)	212 (97.2%)	623 (88.1%)	1995 (89.6%)	835 (90.3%)	0,561	
2DL4	1803 (100%)	424 (100%)	218 (100%)	707 (100%)	2227 (100%)	925 (100%)	*NA*	
2DL5AB	898 (49.8%)	262 (61.8%)	116 (53.2%)	327 (46.3%)	1160 (52.1%)	443 (47.9%)	**0,032**	*ns*
2DL5A	665 (36.9%)	209 (49.3%)	92 (42.2%)	230 (32.5%)	874 (39.2%)	322 (34.8%)	**0,019**	*ns*
2DL5B	509 (28.2%)	165 (38.9%)	64 (29.4%)	195 (27.6%)	674 (30.3%)	259 (28.0%)	0,205	
3DL1	1714 (95.1%)	396 (93.4%)	198 (90.8%)	680 (96.2%)	2110 (94.7%)	878 (94.9%)	0,842	
3DL2	1803 (100%)	424 (100%)	218 (100%)	707 (100%)	2227 (100%)	925 (100%)	*NA*	
3DL3	1803 (100%)	424 (100%)	218 (100%)	707 (100%)	2227 (100%)	925 (100%)	*NA*	
Activating KIR Genes								
2DS1	709 (39.3%)	212 (50.0%)	98 (45.0%)	232 (32.8%)	921 (41.4%)	330 (35.7%)	**0,003**	**0,042**
2DS2	975 (54.1%)	264 (62.3%)	127 (58.3%)	393 (55.6%)	1239 (55.6%)	520 (56.2%)	0,765	
2DS3	583 (32.3%)	175 (41.3%)	68 (31.2%)	211 (29.8%)	758 (34.0%)	279 (30.2%)	**0,035**	*ns*
2DS4 total	1715 (95.1%)	395 (93.2%)	199 (91.3%)	677 (95.8%)	2110 (94.7%)	876 (94.7%)	0,960	
2DS5	537 (29.8%)	160 (37.7%)	80 (36.7%)	211 (29.8%)	697 (31.3%)	291 (31.5%)	0,929	
3DS1	705 (39.1%)	205 (48.3%)	91 (41.7%)	228 (32.2%)	910 (40.9%)	319 (34.5%)	**0,001**	**0,012**
2DP1	1739 (96.5%)	409 (96.5%)	211 (96.8%)	682 (96.5%)	2148 (96.5%)	893 (96.5%)	0,903	
3DP1	1803 (100%)	424 (100%)	218 (100%)	707 (100%)	2227 (100%)	925 (100%)	*NA*	
Number of aKIR/iKIR genes								
Number of aKIRs¥	3 (0-6)	3 (1-6)	2 (1-6)	3 (0-6)	3 (0-6)	3 (1-6)	**0,031**	*ND*
Number of iKIRs¥	6 (4-7)	6 (4-7)	6 (4-7)	6 (4-7)	6 (4-7)	6 (4-7)	0,785	*ND*

*AF: Allele Frequencies Net Database, AU: Ankara University, MM: Multiple Myeloma, NA: Not applicable, ND: Not done, ns: non-significant. *Statistical analyses on differences in frequencies of KIR genes between all MM patients and all healthy subjects were performed using Pearson chi-square or two-tailed Fisher's exact tests as appropriate. §Bonferroni correction was applied for multiple testing of inhibitory and activating KIR genes. ¥Number of aKIR and iKIR genes of MM patients and healthy subjects were compared using Mann-Whitney U test. P<0.05 was accepted as statistically significant.*

Table 3.6 KIR genotype frequencies among control groups and MM patients.

Comparison of KIR Genotype Frequencies Among Healthy Subjects and MM Patient Datasets	Control Group Datasets		MM Patient Datasets		All Healthy Subjects vs All MM Patients			
	AF Healthy Subjects (n=1803)	AU Healthy Subjects (n=424)	AU MM Patients (n=218)	CoMMpass MM Patients (n=707)	All Healthy Subjects (n=2227)	All MM Patients (n=925)	P value*	P_c value§
KIR Genotypes (A/B)								
KIR AX	1508 (83.6%)	363 (85.6%)	187 (85.8%)	596 (84.3%)	1871 (84%)	783 (84.6%)	0,657	
KIR BX	1276 (70.8%)	326 (76.9%)	168 (77.1%)	486 (68.7%)	1602 (71.9%)	654 (70.7%)	0,485	
KIR AB	981 (54.4%)	265 (62.5%)	137 (62.8%)	375 (53%)	1246 (55.9%)	512 (55.4%)	0,758	
KIR genotypes (Centromeric)								
cAA	806 (44.7%)	156 (36.8%)	78 (35.8%)	300 (42.4%)	962 (43.2%)	378 (40.9%)	0,228	
cB1B1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	*NA*	
cB2B2	54 (3%)	9 (2.1%)	1 (0.5%)	23 (3.3%)	63 (2.8%)	24 (2.6%)	0,715	
cB1B2	126 (7%)	20 (4.7%)	1 (0.5%)	56 (7.9%)	146 (6.6%)	57 (6.2%)	0,682	
cAB1	376 (20.9%)	138 (32.5%)	45 (20.6%)	139 (19.7%)	514 (23.1%)	184 (19.9%)	**0,050**	*ns*
cAB2	380 (21.1%)	89 (21%)	66 (30.3%)	167 (23.6%)	469 (21.1%)	233 (25.2%)	**0,011**	*ns*
Ambiguous	61 (3.4%)	12 (2.8%)	27 (12.4%)	22 (3.1%)	73 (3.3%)	49 (5.3%)	*ND*	
KIR genotypes (Telomeric)								
tAA	1016 (56.4%)	211 (49.8%)	106 (48.6%)	433 (61.2%)	1227 (55.1%)	539 (58.3%)	0,102	
tB1B1	28 (1.6%)	9 (2.1%)	10 (4.6%)	10 (1.4%)	37 (1.7%)	20 (2.2%)	0,337	
tB2B2	8 (0.4%)	1 (0.2%)	0 (0%)	0 (0%)	9 (0.4%)	0 (0%)	0,053	
tB1B2	33 (1.8%)	18 (4.2%)	5 (2.3%)	17 (2.4%)	51 (2.3%)	22 (2.4%)	0,881	
tAB1	400 (22.2%)	128 (30.2%)	54 (24.8%)	146 (20.7%)	528 (23.7%)	200 (21.6%)	0,205	
tAB2	128 (7.1%)	47 (11.1%)	12 (5.5%)	35 (5%)	175 (7.9%)	47 (5.1%)	**0,006**	**0,033**
Ambiguous	190 (10.5%)	10 (2.4%)	31 (14.2%)	66 (9.3%)	200 (9%)	97 (10.5%)	*ND*	

*AF: Allele Frequencies Net Database, AU: Ankara University, MM: Multiple Myeloma, NA: Not applicable, ND: Not done, ns: non-significant. *Statistical analyses on differences in frequencies of KIR genotypes between all MM patients and all healthy subjects were performed using Pearson chi-square or two-tailed Fisher's exact tests as appropriate. §Bonferroni correction was applied for multiple testing of each cluster. P<0.05 was accepted as statistically significant.*

HLA-C1 and C2 ligands were evaluated according to their presence either in homozygous or in heterozygous genotype for each individual. C2C2 homozygous genotype was found to be more frequent in MM patients compared to control group (19.7% vs 16.4%, OR: 1.250 [1.026-1.522], P=0.027), however the significance was lost after applying correction for multiple testing of all ligand combinations. There was no significant association among other HLA-C ligands. Strikingly, Bw4 ligand was found to be significantly more frequent among MM patients (79.6% vs 65.7%, OR: 2.034 [1.695-2.440], Pc<0.001). Its frequency distribution showed an increasing trend towards MM patients. The trend was consistent either between the local

patients and its local control group (77.5% vs 73.1%) or between the CoMMpass dataset and its healthy control group (80.2% vs 63.9%) (Table 3.7).

Table 3.7 HLA ligand frequencies among control groups and MM patients.

Comparison of HLA Ligand Frequencies Among Healthy Subjects and MM Patient Datasets	Control Group Datasets		MM Patient Datasets		All Healthy Subjects vs All MM Patients			
	AF Healthy Subjects (n=1803)	AU Healthy Subjects (n=424)	AU MM Patients (n=218)	CoMMpass MM Patients (n=707)	All Healthy Subjects (n=2227)	All MM Patients (n=925)	P value*	P_c value§
HLA ligands								
C1C1	658 (36.5%)	140 (33%)	69 (31.7%)	241 (34.1%)	798 (35.8%)	310 (33.5%)	0,214	
C2C2	301 (16.7%)	64 (15.1%)	62 (28.4%)	120 (17%)	365 (16.4%)	182 (19.7%)	**0,027**	*ns*
C1C2	844 (46.8%)	220 (51.9%)	87 (39.9%)	346 (48.9%)	1064 (47.8%)	433 (46.8%)	0,621	
Bw4	1153 (63.9%)	310 (73.1%)	169 (77.5%)	567 (80.2%)	1463 (65.7%)	736 (79.6%)	**<0.001**	**<0.001**

*AF: Allele Frequencies Net Database, AU: Ankara University, MM: Multiple Myeloma, ns: non-significant. *Statistical analyses on differences in frequencies of HLA ligands between all MM patients and all healthy subjects were performed using Pearson chi-square or two-tailed Fisher's exact tests as appropriate. §Bonferroni correction was applied for multiple testing of HLA ligands. P<0.05 was accepted as statistically significant.*

KIR genes are known for their binding capabilities with certain HLA ligands. In this study, KIR and HLA ligand pairs were constructed according to their known interactions given in the literature as shown in Figure 1.3. In addition to evaluating the matching pairs, individuals with KIR genes but without their cognate ligands were also assessed. 3DL1+Bw4+, an inhibitory KIR together with its HLA ligand, was found to be associated with a predisposing effect towards MM (73.2% vs 62.2%, 1.660 [1.402-1.965], Pc<0.001). As expected from an opposite interaction, subjects carrying 3DL1, but lacking Bw4 ligand were significantly more frequent among healthy subjects (19.7% vs 32.6%, OR: 0.507 [0.422-0.610], Pc<0.001). An activating KIR gene 2DS1 pairing with its ligand C2 was found to have a potential protective role against MM (21.8% vs 27.3%, OR: 0.744 [0.620-0.892], Pc=0.011). However, there was no significant association with the 2DS1 positive and C2 ligand negative genotype. The frequency of an activating KIR in the absence of its ligand (3DS1+Bw4-) was found to be significantly higher in the healthy control group compared to MM patients (8% vs 14.8%, OR: 0.502 [0.385-0.654], Pc<0.001).

Regarding the pair of KIR haplotype motifs and their ligands, only the AA genotype with its ligands was included in the analysis, as the BB genotype displays numerous different motif combinations, unlike the AA genotype, which is defined by a single combination of specific KIR genes (2DL1+2DL3+2DP1+3DL1+2DS4+). However, there was no significant difference in frequencies between MM patients and healthy subjects (Table 3.8).

Table 3.8 Frequencies of KIR genes with or without their cognate ligands among control groups and MM patients.

Comparison of KIR/HLA Ligand Frequencies Among Healthy Subjects and MM Patient Datasets	Control Group Datasets		MM Patient Datasets		All Healthy Subjects vs All MM Patients			
	AF Healthy Subjects (n=1803)	AU Healthy Subjects (n=424)	AU MM Patients (n=218)	CoMMpass MM Patients (n=707)	All Healthy Subjects (n=2227)	All MM Patients (n=925)	P value*	P_c value§
KIR genes and HLA ligand pairs (matching corresponding ligands)								
2DL1+C2+	1102 (61.1%)	273 (64.4%)	146 (67%)	455 (64.4%)	1375 (61.7%)	601 (65%)	0,088	
2DL2+C1+	819 (45.4%)	219 (51.7%)	92 (42.2%)	344 (48.7%)	1038 (46.6%)	436 (47.1%)	0,788	
2DL3+C1+	1328 (73.7%)	337 (79.5%)	152 (69.7%)	513 (72.6%)	1665 (74.8%)	665 (71.9%)	0,094	
3DL1+Bw4+	1100 (61%)	285 (67.2%)	152 (69.7%)	525 (74.3%)	1385 (62.2%)	677 (73.2%)	**<0.001**	**<0.001**
2DS1+C2+	467 (25.9%)	141 (33.3%)	66 (30.3%)	136 (19.2%)	608 (27.3%)	202 (21.8%)	**0,001**	**0,011**
2DS2+C1+	817 (45.3%)	219 (51.7%)	93 (42.7%)	336 (47.5%)	1036 (46.5%)	429 (46.4%)	0,942	
2DS5+C2+	356 (19.7%)	108 (25.5%)	54 (24.8%)	125 (17.7%)	464 (20.8%)	179 (19.4%)	0,347	
3DS1+Bw4+	430 (23.8%)	151 (35.6%)	70 (32.1%)	171 (24.2%)	581 (26.1%)	241 (26.1%)	0,984	
KIR genes and HLA ligand pairs (unmatching corresponding ligands)								
2DL1+C2-	633 (35.1%)	135 (31.8%)	68 (31.2%)	228 (32.2%)	768 (34.5%)	296 (32%)	0,179	
2DL2+C1-	156 (8.7%)	45 (10.6%)	33 (15.1%)	60 (8.5%)	201 (9%)	93 (10.1%)	0,366	
2DL3+C1-	275 (15.3%)	55 (13%)	60 (27.5%)	110 (15.6%)	330 (14.8%)	170 (18.4%)	**0,013**	*ns*
3DL1+Bw4-	614 (34.1%)	111 (26.2%)	46 (21.1%)	136 (19.2%)	725 (32.6%)	182 (19.7%)	**<0.001**	**<0.001**
2DS1+C2-	242 (13.4%)	71 (16.7%)	32 (14.7%)	96 (13.6%)	313 (14.1%)	128 (13.8%)	0,873	
2DS2+C1-	158 (8.8%)	45 (10.6%)	34 (15.6%)	57 (8.1%)	203 (9.1%)	91 (9.8%)	0,525	
2DS5+C2-	181 (10%)	52 (12.3%)	26 (11.9%)	86 (12.2%)	233 (10.5%)	112 (12.1%)	0,178	
3DS1+Bw4-	275 (15.3%)	54 (12.7%)	21 (9.6%)	53 (7.5%)	329 (14.8%)	74 (8%)	**<0.001**	**<0.001**

*AF: Allele Frequencies Net Database, AU: Ankara University, MM: Multiple Myeloma, ns: non-significant. *Statistical analyses on differences in frequencies of KIR/HLA genotypes between all MM patients and all healthy subjects were performed using Pearson chi-square or two-tailed Fisher's exact tests as appropriate. §Bonferroni correction was applied for multiple testing of each group. P<0.05 was accepted as statistically significant.*

The age of MM onset is known to vary among different subsets of patients, and the exact mechanism remains unknown. Age of all patients at diagnosis is known for all datasets included in this study, and the study cohorts included only the newly

diagnosed MM patients. Median age at diagnosis of the patients from Ankara University and CoMMpass datasets were 56 (28-85) and 63 (27-89), respectively. Distribution in this study mirrors the worldwide MM patient statistics 61 (27-89) according to SEER Statistics (*Cancer Stat Facts: Myeloma*, 2024). To evaluate the possible effects of KIR and HLA ligands on age at diagnosis, only the genotypes found to be associated with MM occurrence (without considering Bonferroni corrections) were included in the analysis of age of onset associations (Table 3.9 and Table 3.10).

Table 3.9 KIR genes significantly associated with MM occurrence and their effects on age of MM onset.

Association of KIR/HLA Ligand Frequencies Between MM Patients and Healthy Subjects and Their Effects on Age of MM Onset	**Healthy Subjects vs MM Patients**					**Age at Diagnosis**	
	All Healthy Subjects (n=2227)	**All MM Patients (n=925)**	**OR (95% CI)**	**P value***	**P_c value§**	**Age at Diagnosis**	**P value$^{\Phi}$**
Characteristics of MM Patients and Healthy Subjects							
Gender (male/female)	47.1%/52.9%	59.2/40.8%	*NA*				
Age at index (median [min-max])	42 (18-83)‡	63 (27 - 96)					
Age at Diagnosis (median [min-max])	*NA*	61 (27 - 89)					
ISS I/II/III	*NA*	36.1/31.8/32.1%					
Inhibitory KIR Genes							
2DL5AB	1160 (52.1%)	443 (47.9%)	0.845 (0.725 - 0.986)	0,032	*ns*	61 (27 - 89)	**0,024**
not 2DL5AB	1067 (47.9%)	482 (52.1%)				62 (29 - 88)	
2DL5A	874 (39.2%)	322 (34.8%)	0.827 (0.705 - 0.970)	0,019	*ns*	62 (28 - 89)	0,591
not 2DL5A	1353 (60.8%)	603 (65.2%)				61 (27 - 88)	
Activating KIR Genes							
2DS1	921 (41.4%)	330 (35.7%)	0.786 (0.671 - 0.922)	0,003	**0,042**	61 (28 - 89)	0,626
not 2DS1	1306 (58.6%)	595 (64.3%)				61 (27 - 88)	
2DS3	758 (34.0%)	279 (30.2%)	0.837 (0.709 - 0.988)	0,035	*ns*	61 (27 - 88)	0,156
not 2DS3	1469 (66.0%)	646 (69.8%)				62 (29 - 89)	
3DS1	910 (40.9%)	319 (34.5%)	0.762 (0.649 - 0.894)	<0.001	**0,008**	62 (28 - 89)	0,567
not 3DS1	1317 (59.1%)	606 (65.5%)				61 (27 - 88)	

MM: Multiple Myeloma, OR: Odds Ratio, NA: Not Applicable, ns: non-significant. **Statistical analyses on differences in frequencies of KIR/HLA genotypes between all MM patients and all healthy subjects were performed using Pearson chi-square or two-tailed Fisher's exact tests as appropriate.* *§Bonferroni correction was applied for multiple testing of inhibitory and activating KIR genes.* *‡Age at index of healthy subjects is provided only for Ankara University population.* *ϕSignificance of difference between the mean age at diagnosis of MM patients and KIR genotypes was analyzed using t test. P<0.05 was accepted as statistically significant.*

Among the KIR and HLA ligand genotypes associated with MM occurrence, only 4 were found to be significantly associated with age of MM onset. Three of which were found to be associated with a delay in age at diagnosis compared to patients lacking those genotypes: KIR 2DL5AB (62 [29-88] vs 61 [27-89]; P=0.024) (Table 3.9); HLA ligand C2C2 (62 [29-89] vs 59 [27-84]; P=0.047) and unmatching KIR-ligand pair 2DL3+ C1-genotype (62 [27-89] vs 59 s[28-84]; P=0.046). In contrary, genotype AA together with C1C1 ligands was found to be associated with an earlier age of MM onset (61 [27 – 89] vs 63 [39-87]; P=0.036) (Table 3.10).

Table 3.10 KIR genes with or without their cognate ligands significantly associated with MM occurrence and their effects on age of MM onset.

Association of KIR/HLA Ligand Frequencies Between MM Patients and Healthy Subjects and Their Effects on Age of MM Onset	Healthy Subjects vs MM Patients					Age at Diagnosis	
	All Healthy Subjects (n=2227)	All MM Patients (n=925)	OR (95% CI)	P value*	P_c value§	Age at Diagnosis	P value$^{\Phi}$
HLA ligands							
C2C2	365 (16.4%)	182 (19.7%)	1.250 (1.026 - 1.522)	0,027	*ns*	59 (27 - 84)	**0,047**
not C2C2	1862 (83.6%)	743 (80.3%)				62 (29 - 89)	
Bw4	1463 (65.7%)	736 (79.6%)	2.034 (1.695 - 2.440)	<0.001	**<0.001**	62 (28 - 89)	0,278
not Bw4	764 (34.3%)	189 (20.4%)				61 (27 - 84)	
KIR genes and HLA ligand pairs							
3DL1+Bw4+	1385 (62.2%)	677 (73.2%)	1.660 (1.402 - 1.965)	<0.001	**<0.001**	61 (28 - 89)	0,317
not 3DL1+Bw4+	842 (37.8%)	248 (26.8%)				62 (27 - 84)	
2DS1+C2+	608 (27.3%)	202 (21.8%)	0.744 (0.620 - 0.892)	0,001	**0,011**	62 (28 - 86)	0,673
not 2DS1+C2+	1619 (72.7%)	723 (78.2%)				61 (27 - 89)	
2DL3+C1-	330 (14.8%)	170 (18.4%)	1.294 (1.056 - 1.586)	0,013	*ns*	59 (28 - 84)	**0,046**
not 2DL3+C1-	1897 (85.2%)	755 (81.6%)				62 (27 - 89)	
3DL1+Bw4-	725 (32.6%)	182 (19.7%)	0.507 (0.422 - 0.610)	<0.001	**<0.001**	61 (27 - 84)	0,299
not 3DL1+Bw4-	1502 (67.4%)	743 (80.3%)				61 (28 - 89)	
3DS1+Bw4-	329 (14.8%)	74 (8.0%)	0.502 (0.385 - 0.654)	<0.001	**<0.001**	62 (29 - 84)	0,812
not 3DS1+Bw4-	1898 (85.2%)	851 (92.0%)				61 (27 - 89)	
KIR genotypes (Centromeric)							
cAB1	514 (23.1%)	184 (19.9%)	0.828 (0.685 - 1.000)	0,050	*ns*	62 (28 - 86)	0,539
not cAB1	1713 (76.9%)	741 (80.1%)				61 (27 - 89)	
cAB2	469 (21.1%)	233 (25.2%)	1.262 (1.054 - 1.511)	0,011	*ns*	61 (30 - 89)	0,697
not cAB2	1758 (78.9%)	692 (74.8%)				61 (27 - 88)	
KIR genotypes (Telomeric)							
tAB2	175 (7.9%)	47 (5.1%)	0.628 (0.451 - 0.874)	0,006	**0,033**	64 (34 - 86)	0,072
not tAB2	2052 (92.1%)	878 (94.9%)				61 (27 - 89)	
KIR Genotypes (A/B)							
KIR AA and C1C1	225 (10.1%)	90 (9.7%)	0.959 (0.741 - 1.241)	0,750	*ns*	63 (39 - 87)	**0,036**
not KIR AA and C1C1	2002 (89.9%)	835 (90.3%)				61 (27 - 89)	
KIR AA and C2C2	113 (5.1%)	63 (6.8%)	1.367 (0.995 - 1.880)	0,053	*ns*	59 (29 - 84)	0,196
not KIR AA and C2C2	2114 (94.9%)	862 (93.2%)				62 (27 - 89)	
KIR AA and C1C2	287 (12.9%)	118 (12.8%)	0.988 (0.786 - 1.243)	0,921	*ns*	61 (34 - 86)	0,982
not KIR AA and C1C2	1940 (87.1%)	807 (87.2%)				61 (27 - 89)	

MM: Multiple Myeloma, OR: Odds Ratio, ns: non-significant. [*]Statistical analyses on differences in frequencies of KIR/HLA genotypes between all MM patients and all healthy subjects were performed using Pearson chi-square or two-tailed Fisher's exact tests as appropriate. [§]Bonferroni correction was applied for multiple testing of each cluster. [ϕ]Significance of difference between the mean age at diagnosis of MM patients and KIR genotypes was analyzed using t test. P<0.05 was accepted as statistically significant.

It is known that African Americans (AFA) are more prone to MM, with an increased incidence rate compared to so-called Caucasians. CoMMpass dataset was grouped by race of MM patients, and included 449 Caucasians, 92 AFA, 11 Asians, 5 others and 150 unknowns. Our local population was also grouped in Caucasians, as populations from Turkey have been considered as Caucasians in similar association studies. In this study, analysis of KIR/HLA ligand distributions among ethnic populations was performed on 667 Caucasians and 92 AFA, and only the genotypes found to be associated with MM occurrence were included in the analyses. The median age at diagnosis was 62 (27-89) for Caucasians and 62 (32-87) for AFA, with a distribution of ISS I/II/III 35.6%/28.8%/35.6% and 26.1%/42.0%/31.8%, respectively. Gender distribution was also homogeneous across the patient datasets (male/female: 59.8/40.2% and 58.7/41.3%, respectively). A plausible association was found between KIR/HLA ligand genotypes and the predisposition of the AFA population to MM. Interestingly, all of the KIR and HLA ligand genotypes associated with a protective role against MM occurrence were found to have a lower frequency among AFA compared to Caucasians. Furthermore, all genotypes which were found to be associated with predisposition to MM, were found to be more prevalent among the AFA population (Table 3.11). Statistical significance was found in the frequency distribution between these populations for both protective genotypes (2DL5AB, 2DL5A, 2DS1, 3DS1 and 3DS1+Bw4) and genotypes associated with a risk of MM occurrence (Bw4 and 3DL1+Bw4+) (Table 3.11).

Table 3.11 Comparison of KIR/HLA ligand genotypes between Caucasian and African American patients.

Comparison of Significantly Associated Genotypes Between Caucasian and African-American Patients	Healthy Subjects vs MM Patients			All MM Patients (n=925)	Among All MM Patients		
	OR (95% CI)	P value*	P_c value§		Caucasian (n=667)	African American (n=92)	P value*
Characteristics of MM Patients and Healthy Subjects							
Gender (male/female)	*NA*			59.2/40.8%	59.8/40.2%	58.7/41.3%	0.837
Age at Diagnosis (median [min-max])				61 (27 - 89)	65 (27 - 89)	62 (32 - 87)	0.076
KIR/HLA ligand genotypes associated with protection against MM							
2DL5AB	0.845 (0.725 - 0.986)	0.032	*ns*	443 (47.9%)	328 (49,2%)	32 (34,8%)	**0.010**
2DL5A	0.827 (0.705 - 0.970)	0.019	*ns*	322 (34.8%)	253 (37,9%)	13 (14,1%)	**<0.001**
2DS1	0.786 (0.671 - 0.922)	0.003	**0.042**	330 (35.7%)	255 (38,2%)	18 (19,6%)	**<0.001**
2DS3	0.837 (0.709 - 0.988)	0.035	*ns*	279 (30.2%)	196 (29,4%)	27 (29,3%)	0.994
3DS1	0.762 (0.649 - 0.894)	<0.001	**0.008**	319 (34.5%)	252 (37,8%)	12 (13%)	**<0.001**
2DS1+C2+	0.744 (0.620 - 0.892)	0.001	**0.011**	202 (21.8%)	153 (22,9%)	15 (16,3%)	0.151
3DL1+Bw4-	0.507 (0.422 - 0.610)	<0.001	**<0.001**	182 (19.7%)	145 (21,7%)	12 (13%)	0.054
3DS1+Bw4-	0.502 (0.385 - 0.654)	<0.001	**<0.001**	74 (8%)	66 (9,9%)	1 (1,1%)	**0.005**
cAB1	0.828 (0.685 - 1.000)	0.050	*ns*	184 (19.9%)	132 (19,8%)	14 (15,2%)	0.297
tAB2	0.628 (0.451 - 0.874)	0.006	**0.033**	47 (5.1%)	36 (5,4%)	1 (1,1%)	0.072
KIR/HLA ligand genotypes associated with predisposition to MM							
C2C2	1.250 (1.026 - 1.522)	0.027	*ns*	182 (19.7%)	135 (20,2%)	19 (20,7%)	0.927
Bw4	2.034 (1.695 - 2.440)	<0.001	**<0.001**	736 (79.6%)	517 (77,5%)	80 (87%)	**0.038**
3DL1+Bw4+	1.660 (1.402 - 1.965)	<0.001	**<0.001**	677 (73.2%)	466 (69,9%)	80 (87%)	**0.001**
2DL3+C1-	1.294 (1.056 - 1.586)	0.013	*ns*	170 (18.4%)	125 (18,7%)	18 (19,6%)	0.850
cAB2	1.262 (1.054 - 1.511)	0.011	*ns*	233 (25.2%)	172 (25,8%)	18 (19,6%)	0.197

MM: Multiple Myeloma, OR: Odds Ratio, NA: Not Applicable, ns: non-significant. **Statistical analyses on differences in frequencies of KIR/HLA genotypes were performed using Pearson chi-square or two-tailed Fisher's exact tests as appropriate.* *§Bonferroni correction was applied for multiple testing of inhibitory and activating KIR genes. P<0.05 was accepted as statistically significant.*

Finally, to evaluate the association between gender and KIR/HLA ligands, females (n=377) and males (n=548) were analyzed as independent variables, and tested for any difference in frequencies across the genotypes which were associated with MM occurrence. There was no statistically significant difference among the genotypes in terms of gender (Table 3.12).

Table 3.12 Comparison of KIR/HLA ligand genotypes between female and male patients.

Comparison of Significantly Associated Genotypes Between Female and Male Patients	Healthy Subjects vs MM Patients			All MM Patients (n=925)	Among All MM Patients		
	OR (95% CI)	P value*	P_c value§		Female (n=377)	Male (n=548)	P value*
Characteristics of MM Patients and Healthy Subjects							
Age at Diagnosis (median [min-max])	*NA*			61 (27 - 89)	62 (27 - 89)	62 (32 - 87)	0.790
KIR/HLA ligand genotypes associated with protective against MM							
2DL5AB	0.845 (0.725 - 0.986)	0.032	*ns*	443 (47.9%)	175 (46.4%)	268 (48.9%)	ns
2DL5A	0.827 (0.705 - 0.970)	0.019	*ns*	322 (34.8%)	130 (34.5%)	192 (35%)	ns
2DS1	0.786 (0.671 - 0.922)	0.003	**0.042**	330 (35.7%)	133 (35.3%)	197 (35.9%)	ns
2DS3	0.837 (0.709 - 0.988)	0.035	*ns*	279 (30.2%)	110 (29.2%)	169 (30.8%)	ns
3DS1	0.762 (0.649 - 0.894)	<0.001	**0.008**	319 (34.5%)	128 (34%)	191 (34.9%)	ns
2DS1+C2+	0.744 (0.620 - 0.892)	0.001	**0.011**	202 (21.8%)	84 (22.3%)	118 (21.5%)	ns
3DL1+Bw4-	0.507 (0.422 - 0.610)	<0.001	**<0.001**	182 (19.7%)	71 (18.8%)	111 (20.3%)	ns
3DS1+Bw4-	0.502 (0.385 - 0.654)	<0.001	**<0.001**	74 (8%)	27 (7.2%)	47 (8.6%)	ns
cAB1	0.828 (0.685 - 1.000)	0.050	*ns*	184 (19.9%)	73 (19.4%)	111 (20.3%)	ns
tAB2	0.628 (0.451 - 0.874)	0.006	**0.033**	47 (5.1%)	23 (6.1%)	24 (4.4%)	ns
KIR/HLA ligand genotypes associated with predisposition to MM							
C2C2	1.250 (1.026 - 1.522)	0.027	*ns*	182 (19.7%)	72 (19.1%)	110 (20.1%)	ns
Bw4	2.034 (1.695 - 2.440)	<0.001	**<0.001**	736 (79.6%)	302 (80.1%)	434 (79.2%)	ns
3DL1+Bw4+	1.660 (1.402 - 1.965)	<0.001	**<0.001**	677 (73.2%)	275 (72.9%)	402 (73.4%)	ns
2DL3+C1-	1.294 (1.056 - 1.586)	0.013	*ns*	170 (18.4%)	67 (17.8%)	103 (18.8%)	ns
cAB2	1.262 (1.054 - 1.511)	0.011	*ns*	233 (25.2%)	100 (26.5%)	133 (24.3%)	ns

*MM: Multiple Myeloma, OR: Odds Ratio, ns: non-significant. *Statistical analyses on differences in frequencies of KIR/HLA genotypes were performed using Pearson chi-square or two-tailed Fisher's exact tests as appropriate. §Bonferroni correction was applied for multiple testing of inhibitory and activating KIR genes. P<0.05 was accepted as statistically significant.*

CHAPTER 4

DISCUSSION AND CONCLUSION

4.1 Discussion

Multiple myeloma is the second most prevalent hematologic malignancy and treatment options are still limited due to several factors leading to refractory relapses. Individualized immunotherapeutic approaches have become prominent due to several genetic factors influencing the disease progression differently for each patient (Pawlyn & Davies, 2019; Skerget et al., 2021). KIR variants and their corresponding HLA ligands have been extensively studied in almost all cancers in terms of the interactions between NK cells and tumor cells. Effect of KIR genes on MM occurrence and transplantation success has been studied by several research groups (Beelen et al., 2024; Beksaç & Dalva, 2012; Jennifer Zhang, 2022; Kröger et al., 2011; Sahin et al., 2018). However, frequential disparities among different ethnic populations and the high number of combinations of polymorphic haplotypes result in conflicting results among studies (Augusto, 2016; Gao et al., 2022; Myers & Miller, 2021). High cost of KIR and HLA ligand genotyping by conventional methods and difficulties in reaching a large number of patients in limited local cohort studies result in relatively small-scaled and statistically under-powered results in KIR/HLA–disease association studies. Out of 14 KIR genes, 8 are known for their interactions with HLA ligands. Besides, certain combinations of the genes have been defined as haplotypes, and the frequencies of these gene motifs vary among worldwide populations (Liu et al., 2021). These high number of combinations involving KIRs, haplotypes and their presence with or without the cognate HLA ligands eventually result in a very small number of patients per genotype.

Over the years there has been an extensive accumulation of the whole genome/exome sequencing data, which were primarily used for genome-wide association studies.

These large datasets include valuable information about HLA or KIR genes, which were not the focus of the original studies. A few strategies have been developed within the last decade for imputation of KIR and HLA genes from genomic or transcriptomic sequencing data. HLA*IMP and KIR*IMP are among the most frequently used imputation tools to this end (Dilthey et al., 2013; Vukcevic et al., 2015). These tools were mainly developed for datasets enriched in SNP panels, and a high coverage rate within the genomic region where the KIR and HLA genes are located is required (J. Chen et al., 2021). As high-throughput NGS data accumulated in genomic studies, imputation tools that can use data directly from WGS/WES assays have been developed. Recently, a tool capable of imputing both KIR and HLA alleles from WGS, WES or RNA-seq data was introduced (Song et al., 2023).

In this study, KIR genes with or without their corresponding HLA ligands were analyzed using a large cohort of MM patients and healthy control subjects. A local cohort of MM patients from Ankara University Hematology Department was included in the study. Additionally, KIR/HLA genes were imputed from WES data of patients from CoMMpass study, which is one of the largest MM datasets. In addition to a worldwide-level analysis of KIR/HLA genotypes among MM patients, population-level distribution of these polymorphic genes was included in the analysis and the effect of immunomodulatory KIR genes on age of disease onset has been investigated. The aim of this study is to provide clarifying evidence on the relationship between KIR/HLA ligands and MM by integrating the methods for imputation of KIR and HLA genes from genomic studies and finally to resolve the conflicting results in the literature. Additionally, this study aims to investigate KIR/HLA-MM interactions on a population-level basis and to discuss the role of KIR-associated immune checkpoints on susceptibility to MM.

Despite the availability of HLA and KIR imputation tools for a considerable amount of time, only a few studies have used these tools to investigate the effects of KIR and HLA ligands across different types of cancer. While most of these studies focus on autoimmune diseases and viral infections, a few of them have integrated imputation methods to investigate cancer predisposition or transplantation outcomes (Ahn et al.,

2021; Diaz-Peña et al., 2020; Matzaraki et al., 2017). GWAS studies hold great potential for post-GWAS analyses, and many researchers focus on HLA types instead of NK-cell specific KIR genotypes. Moreover, imputing KIR genes has been a challenge because of their highly polymorphic character. T1K is the most recent tool with promising accuracy rates and has been implemented in a few immuno-oncology studies (Bonfiglio et al., 2024; Li et al., 2024; Song et al., 2023). However, to the best of our knowledge, this is the first study to use the KIR and HLA imputation method in a cohort of MM patients.

The role of KIR and HLA ligands in susceptibility to MM has been reported in a few studies. In one of the earliest studies in this regard, 34 MM patients were compared to 120 healthy controls in terms of KIR genotypes within the Lebanese population (Hoteit et al., 2014). However, HLA ligands were not included in their study. They reported an association of 2DS4 and 2DS5 with susceptibility to MM (52.9% vs 28.3%, P=0.007). These genes were reported to be present in only 11 and 18 patients, respectively. Thus, the inadequate number of patients per genotype lowers confidence in the statistical power. In our study neither 2DS4 nor 2DS5 were significantly differ in frequency between the MM patients and healthy controls (31.3% vs 31.5%, respectively; P=0.929). In fact, the frequency distribution of these genes was similar within the local cohort as well as in the CoMMpass dataset when compared to their corresponding healthy control subsets (Table 3.5). In addition to the low number of participants in the study by Hoteit et al., the reported significance level would not have survived an appropriate correction for multiple testing. Therefore, their study should not be considered comparable to our results.

In another study published in 2015, KIR/HLA ligands were associated with both susceptibility to MM and PFS among MM patients (Martínez-Sánchez et al., 2015). They showed an association of 2DL1, 2DL3, 3DL1 and 2DS4 genes as well as the 2DL1+2DL2+2DL3+ genotype with a decreased risk of MM occurrence. They also reported a significant association between the 2DL1-2DL2+2DL3- genotype and susceptibility to MM. In our study, neither of the individual KIR genes given in this report were found to be differ between MM patients and healthy controls. Instead of

analyzing inhibitory or activating receptors in random clusters, we preferred to perform a haplotype-level analysis. Nevertheless, there was no evidence to show a difference in frequency distribution of KIR2D combinations. To see if we could replicate their results, we analyzed the genotypes 2DL1+2DL2+2DL3+ and 2DL1-2DL2+2DL3- within the "Caucasian" population in our dataset. However, there was no significant difference between the MM patients and healthy control subjects (44.9% vs 47.1%; P=0.323 and 3.2% vs 2.8%; P=0.658, respectively). There were 7/53 patients and 8/286 healthy controls with 2DL1-2DL2+2DL3- within their study. In return, there were 19/668 patients and 71/2227 healthy controls with the same genotype in our dataset. Instead of randomly analyzing KIR2DL combinations, haplotype-level assessment would cover a wider range of populations.

A comprehensive analysis was performed on 182 MM patients by Gabriel *et al*. in 2010. They associated shorter PFS with the 3DS1+3DL1+ genotype along with the absence of their cognate ligand Bw4, although they did not analyze any effect of KIR/HLA ligand on MM susceptibility (Gabriel et al., 2010). Despite not being exactly comparable, their findings were in the opposite direction from our results, which suggest that both 3DL1+Bw4- and 3DS1+Bw4- genotypes were associated with a significantly reduced risk of susceptibility to MM (OR: 0.507 [0.422-0.610]; P<0.001 and 0.502 [0.385-0.654]; P<0.001, respectively). These results may seem conflicting due to the different immune-modulatory effects of KIR-ligand interactions on susceptibility to MM and PFS of patients after autologous stem cell transplantation. However, unlike KIR 2D receptors, alleles of 3DL1 and 3DS1 genes are encoded within the same locus. 3DL1 is an inhibitory receptor and it exhibits a stronger interaction with its corresponding ligand Bw4 compared to the activating receptor 3DS1. Upon binding with Bw4, 3DL1 transmits signals through the immunoreceptor tyrosine-based inhibitory motifs (ITIMs) on its cytoplasmic tail. These signals lead to dephosphorylation of the key molecules in the activation pathways of NK cells, resulting in a reduced cytotoxic response against target cells. 3DS1, on the other hand, is a receptor with short cytoplasmic tail, which does not contain ITIMs, similar to any other activating DS receptor, but interacts with

immunoreceptor tyrosine-based activation motifs (ITAMs). This interaction requires an adaptor protein, namely DAP12. Although it is known that 3DS1 also interacts with Bw4, this type of indirect interaction may result in a weaker association compared to 3DL1 (O'Connor & McVicar, 2013). The directional imbalance between the ligand interactions of 3DL1 and 3DS1 may explain the conflicting results from Gabriel et al. and our study. In our study, the 3DL1+Bw4+ genotype was associated with susceptibility to MM. This can be explained by NK cells remaining inactive due to the strong inhibitory effects of 3DL1 in the presence of its ligand. In contrast, the frequency of 3DL1+Bw4- genotype was higher in healthy controls. This association with a reduced risk of MM occurrence may be due to the activation of NK cells due to lack of inhibition in the absence of the ligand. 3DS1+Bw4- genotype was also significantly associated with reduced susceptibility to MM. Since the binding affinity of Bw4 is stronger with the inhibitory 3DL1 receptor, the absence of Bw4 in the presence of 3DS1 may contribute to enhancement in immune surveillance without being obstructed by the inhibitory effects of 3DL1. Gabriel et al. also reported that 3DS1+3DL1+Bw4- genotype was associated with shorter PFS compared to the patients with 3DS1+3DL1+Bw4+ genotype (Gabriel et al., 2010). This may be explained by overactivation of the immune response due to insufficient inhibition from the lack of interaction between the 3DL1 receptor and its ligand in case of 3DS1+3DL1+Bw4- genotype. Conversely, the balance of inhibitory and activating signals in patients with 3DS1+3DL1+Bw4+ genotype may lead to improved PFS. Comparison of these findings once again highlights the importance of the balance and complexity in the interplay between inhibitory and activating signals mediated by KIR and HLA ligand interactions. It also provides evidence for possible associations of KIRs with either susceptibility to MM or the progression of the disease course.

In another KIR/HLA ligand-PFS association study, 3DL2 and its corresponding ligand HLA-A3/11 was reported to be associated with improved survival in MM patients, while 2DL1 receptor along with C2C2 genotype was significantly associated with a reduced PFS (Sun et al., 2021). In our study, 3DL2 was present in

all cases including MM patients and healthy controls, and this was an expected result as it is known to be a framework gene. Within our patient cohort, although not significant, allele-level assessment of 2DS1+C2+ genotype was slightly more frequent among MM patients compared to healthy controls (65% vs 61.7%, P=0.088). Replicating the exact genotype analyzed by Sun et al., our study found that 2DS1+C2C2+ genotype was more frequent among MM patients compared to healthy controls (18.9% vs 15.8%, P=0.033). However, the significance level would not survive the correction for multiple testing. As previously discussed regarding the results from Gabriel et al., KIR and HLA ligands may act differently as risk factors for susceptibility to MM compared to their role as prognostic factors.

The most recent KIR-MM association study was conducted on a cohort from Netherlands including 172 MM patients and 195 healthy controls (Beelen et al., 2024). They have reported no differences in frequency of individual KIR genes or HLA ligands between MM patients and healthy controls. Among HLA ligands and KIR-ligand pairs, C1-C2+Bw4+ and 3DL1+Bw4+ genotypes were found to be associated with MM occurrence, although the reported significance level was borderline (OR [95% CI]: 1.996 [0.992–4.014], P=0.049 and OR [95% CI]: 1.557 [0.999–2.427], P=0.050; respectively]. Our study did not include the combinations of C1, C2 and Bw4 ligands, namely C1-C2+Bw4+ genotype in their analyses; however, our findings confirm their reported association between 3DL1+Bw4+ genotype and MM occurrence with a high level of statistical significance (OR: 1.660 [1.402-1.965], Pc<0.001).

According to the results from the preliminary study conducted by using the local cohort only (204 MM patients and 404 healthy controls), among the individual KIR genes, 2DL3 was found to be more frequent among MM patients, while 2DL5B and 2DS3 were less frequent compared to healthy controls (M. Beksac et al., 2023). Analysis of HLA ligands alone revealed a significant association between C2C2 and susceptibility to MM (OR: 2.128 [1.417-3.196], P<0.001), and a protective effect of C1C2 against MM (0.623 [0.444-0.874], P=0.006). Moreover, AA genotype in the presence of homozygous C2 ligands were more frequent among MM patients (OR:

2.509 [1.171-5.378], P=0.015). Frequency distribution of centromeric and telomeric genotypes cAB1, cAB2, cB1B2 and tAB2 was found to be different between the patients and healthy controls (M. Beksac et al., 2023). In this study, the total number of patients and healthy controls was increased to 925 and 2227, respectively. Among the individual KIR genes, association of 2DS3 with MM occurrence was common with the previous findings. However, it did not survive the Bonferroni correction in this study. Our extended sample size revealed additional findings, with 2DS1 and 3DS1 being significantly more frequent among the control group. Although the frequency distribution of C2C2 ligand was consistent between the studies and it was more frequent among MM patients, the significance level was not maintained after correction for multiple testing. In this study, Bw4 was the most prominent finding associated with high risk of MM occurrence (2.034 [1.695-2.440], P<0.001), whereas it was not observed in the previous report. Among the A/B and the telomeric/centromeric KIR genotypes; cAB1, cAB2 and tAB1 were common with the previous study, but only showed a borderline level of significance. Previous results also suggested that KIR-ligand genotypes have an effect on age of MM onset. Among the local cohort from Turkey, AA genotype in the presence of C1C1 ligands was found to be associated with a delay in age of MM onset, while genotype AA with C2C2 was associated with earlier onset of MM (M. Beksac et al., 2023). Delaying effect of genotype AA with C1C1 genotype was also confirmed in this study (median age of onset: 63 [39-87] vs 61 [27-89], P=0.036). Although the frequency distribution of genotype AA with C2C2 was not statistically significant, C2C2 ligands alone were found to be associated with an earlier onset of MM, consistent with the previous findings (59 [27-84] vs 62 [29-89], P=0.047). Although the significance levels were borderline, an association between KIR/HLA ligands and age of MM onset was still observed in our extended patient cohort.

One of the most striking findings of this study appeared when the KIR and HLA ligand genotypes, which were significantly associated with MM occurrence, were compared between Caucasian and AFA patients. It is known that MM occurs in 2-3 times higher incidence rates in the AFA/Black population compared to

Caucasian/White populations (*Cancer Stat Facts: Myeloma*, 2024; Röllig et al., 2015). In our study, all genotypes significantly associated with a high-risk of MM occurrence were also found to be significantly more frequent among the AFA population. Additionally, all genotypes significantly associated with a reduced risk of MM predisposition were less frequent in the AFA population. This trend was observed among all genotypes, but only certain genotypes were statistically significant (protective genotypes: 2DL5AB, 2DL5A, 2DS1, 3DS1 and 3DS1+Bw4-; high risk genotypes: Bw4 and 3DL1+Bw4+). The frequency distribution of these genotypes is in line with the worldwide differences in MM incidence rates between AFA and other populations. As a widely recognized fact, Bw4 is more prevalent in AFA compared to other populations in worldwide studies, and our results might contribute to explaining the high rates of MM incidence among AFA (Nemat-Gorgani et al., 2019). Moreover, a study from 2016 has reported a strong association between 3DL1+Bw4+ genotype and Multiple Sclerosis (MS). MS is more common in European Americans compared to African Americans (Hollenbach et al., 2016). In MM patients, we found an opposite effect of the 3DL1+Bw4+ genotype on susceptibility to MM. The inverse relationship between the prevalence rates of MM and MS among different populations might support the true effect of 3DL1 and Bw4 on both MM and MS, but in the opposite directions.

African Americans are also known to experience MM onset at earlier ages (*Cancer Stat Facts: Myeloma*, 2024). Our findings regarding the age at diagnosis of the populations in our study were consistent with the findings from the original CoMMpass study (Manojlovic et al., 2017). Although not statistically significant, the median age at diagnosis was higher in Caucasians compared to AFA (65 [27-89] and 62 [32-87], P=0.076). Statistical insignificance may be due to the heterogeneous distribution of the number of patients among ethnic populations. Although the CoMMpass study provides one of the largest MM datasets, population-level inequalities in accessing healthcare services, especially for AFA, remains to be a problem in constructing a homogeneous patient cohort. Increasing the number of African American participants may contribute to achieving statistical significance in

our findings, which are already consistent with the worldwide statistics and previously reported data.

Gender is another universal factor influencing the incidence rates of MM. In this study, we have finally analyzed the frequency distribution of significant genotypes between male and female patients in order to eliminate any misleading effect originating from gender. There was no significant difference between males and females in the frequencies of the significant genotypes, suggesting that our patient cohort had a homogeneous distribution, allowing for a reliable association.

4.2 Conclusion

- Most of the findings from earlier studies lack statistical power and generate inconsistency and conflicting results among each other due to the limited number of participants included in their studies and the high-level diversity of KIR/HLA ligands. This study expanded the sample size using KIR/HLA imputation from genomic datasets in order to maintain the statistical confidence and can be used as a reference model for defining the effects KIR/HLA ligand on MM.
- With the inclusion of the largest dataset of MM patients and healthy controls in the literature, most of the risk-associated genes reported in earlier studies (2DL1-2DL2-2DL3, 2DS4 and 2DS5) were found to be non-significant. 3DL1 and group-C HLA ligands along with the AA genotype were confirmed to be significantly associated with MM occurrence and age of disease onset, respectively.
- This study uncovered additional KIR and HLA ligands significantly contributing to susceptibility to MM. 3DS1 receptor was found to be significantly associated with MM occurrence, possibly through the bidirectional interaction of 3DL1 and 3DS1 with their cognate ligand Bw4, which was found to exhibit the most significant association among all KIR genes and HLA ligands.

- One of the most striking results from this study is the association of KIR/HLA ligands with the increased prevalence of MM in African Americans. 3DL1 and/or Bw4 might be associated with higher incidence rates of MM among African Americans.
- Imputation accuracy of KIR and HLA alleles from WES data using T1K was validated in this study. However, further validation is required on other datasets constructed using different reference panels.
- This study highlights the importance of the evolutionary role of selection pressure on KIR/HLA genotypes and provides a universal level association model for predisposition to MM.
- A major limitation of this study is that it did not include ethnic populations other than Europeans and African Americans, which can be overcome by imputing KIR/HLA genotypes from other sources of MM datasets.
- Another limitation of this study is that it did not include expression-level analysis of KIR/HLA genes, which can be performed by expanding the data using RNA sequencing datasets.

REFERENCES

Ahn, R., Vukcevic, D., Motyer, A., Nititham, J., Squire, D. M. G., Hollenbach, J. A., Norman, P. J., Ellinghaus, E., Nair, R. P., Tsoi, L. C., Oksenberg, J., Foerster, J., Lieb, W., Weidinger, S., Franke, A., Elder, J. T., Jorgenson, E., Leslie, S., & Liao, W. (2021). Large-Scale Imputation of KIR Copy Number and HLA Alleles in North American and European Psoriasis Case-Control Cohorts Reveals Association of Inhibitory KIR2DL2 With Psoriasis. *Frontiers in Immunology*, *12*, 684326. https://doi.org/10.3389/fimmu.2021.684326

Akkus, E., Tuncalı, T., Akın, H. Y., Aydın, Y., Beşışık, S. K., Gürkan, E., Ratip, S., Salihoğlu, A., Sargın, D., Ünal, A., Turcan, A., Sevindik, Ö. G., Demir, M., & Beksac, M. (2024). Germline genetic variants in Turkish familial multiple myeloma/monoclonal gammopathy of undetermined significance cases. *British Journal of Haematology*, *204*(3), 931–938. https://doi.org/10.1111/bjh.19271

Aksenova, A. Y., Zhuk, A. S., Lada, A. G., Zotova, I. V., Stepchenkova, E. I., Kostroma, I. I., Gritsaev, S. V., & Pavlov, Y. I. (2021). Genome instability in multiple myeloma: Facts and factors. *Cancers*, *13*(23), 5949. https://doi.org/10.3390/cancers13235949

Amorim, L. M., Augusto, D. G., Nemat-Gorgani, N., Montero-Martin, G., Marin, W. M., Shams, H., Dandekar, R., Caillier, S., Parham, P., Fernández-Viña, M. A., Oksenberg, J. R., Norman, P. J., & Hollenbach, J. A. (2021). High-Resolution Characterization of KIR Genes in a Large North American Cohort Reveals Novel Details of Structural and Sequence Diversity. *Frontiers in Immunology*, *12*(May), 1–13. https://doi.org/10.3389/fimmu.2021.674778

Augusto, D. G. (2016). The impact of KIR polymorphism on the risk of developing cancer: Not as strong as imagined? *Frontiers in Genetics*, *7*(JUN), 121. https://doi.org/10.3389/fgene.2016.00121

Bao, X., Hanson, A. L., Madeleine, M. M., Wang, S. S., Schwartz, S. M., Newell, F., Pettersson-Kymmer, U., Hemminki, K., Tiews, S., Steinberg, W., Rader, J. S., Castro, F., Safaeian, M., Franco, E. L., Coutlée, F., Ohlsson, C., Cortes, A., Marshall, M., Mukhopadhyay, P., … Leo, P. J. (2018). *HLA* and *KIR* Associations of Cervical Neoplasia. *The Journal of Infectious Diseases*, *218*(12), 2006–2015. https://doi.org/10.1093/infdis/jiy483

Beelen, N. A., Molenbroeck, S. J. J., Groeneveld, L., Voorter, C. E., Bos, G. M. J., & Wieten, L. (2024). HLA class I NK-epitopes and KIR diversities in patients with multiple myeloma. *Immunogenetics*. https://doi.org/10.1007/s00251-024-01336-w

Beksac, K., Beksac, M., Dalva, K., Karaagaoglu, E., & Tirnaksiz, M. B. (2015). Impact of “killer immunoglobulin-like receptor/ligand” genotypes on outcome following surgery among patients with colorectal cancer: Activating KIRs are associated with long-term disease free survival. *PLoS ONE*, *10*(7), e0132526. https://doi.org/10.1371/journal.pone.0132526

Beksac, M., Akin, H. Y., Cengiz Seval, G., Yurdakul Mesutoglu, P., Anliacik, R. G., Anliacik, E., Gurman, G., Karaagaoglu, E., & Dalva, K. (2023). A Novel Hypothesis: Certain KIR/Cognate Ligand Containing Genotypes Differ in Frequency Among Patients With Myeloma and Have an Effect on Age of Disease Onset. *Clinical Lymphoma, Myeloma and Leukemia*, *23*(5), 394-400.e1. https://doi.org/10.1016/j.clml.2023.02.007

Beksac, M., Akin, H. Y., Gencer-Oncul, E. B., Yousefzadeh, M., Cengiz Seval, G., Gulten, E., Akdemir Kalkan, I., Cinar, G., Memikoglu, O., Karaagaoglu, E., & Dalva, K. (2021). A model integrating Killer Immunoglobulin-like Receptor (KIR) haplotypes for risk prediction of COVID-19 clinical disease severity. *Immunogenetics*, *73*(6), 449–458. https://doi.org/10.1007/s00251-021-01227-4

Beksaç, M., & Dalva, K. (2012). Role of Killer Immunoglobulin-Like Receptor and Ligand Matching in Donor Selection. *Bone Marrow Research*, *2012*, 1–6. https://doi.org/10.1155/2012/271695

Beksaç, M., Delforge, M., & Richardson, P. (2008). The evolving treatment paradigm of multiple myeloma: From past to present and future. *Turkish Journal of Haematology: Official Journal of Turkish Society of Haematology*, *25*(2), 60–70.

Beksac, M., Gragert, L., Fingerson, S., Maiers, M., Zhang, M. J., Albrecht, M., Zhong, X., Cozen, W., Dispenzieri, A., Lonial, S., & Hari, P. (2016). HLA polymorphism and risk of multiple myeloma. *Leukemia*, *30*(11), 2260–2264. https://doi.org/10.1038/leu.2016.199

Bila, J., Katodritou, E., Guenova, M., Basic-Kinda, S., Coriu, D., Dapcevic, M., Ibricevic-Balic, L., Ivanaj, A., Karanfilski, O., Zver, S., Beksac, M., Terpos, E., & Dimopoulos, M. A. (2021). Bone Marrow Microenvironment Interplay and Current Clinical Practice in Multiple Myeloma: A Review of the Balkan Myeloma Study Group. *Journal of Clinical Medicine*, *10*(17), Article 17. https://doi.org/10.3390/jcm10173940

Blokhuis, J. H., Hilton, H. G., Guethlein, L. A., Norman, P. J., Nemat-Gorgani, N., Nakimuli, A., Chazara, O., Moffett, A., & Parham, P. (2017). KIR2DS5 allotypes that recognize the C2 epitope of HLA-C are common among Africans and absent from Europeans. *Immunity, Inflammation and Disease*, *5*(4), 461–468. https://doi.org/10.1002/iid3.178

Bonfiglio, F., Lasorsa, V. A., Aievola, V., Cantalupo, S., Morini, M., Ardito, M., Conte, M., Fragola, M., Eva, A., Corrias, M. V., Iolascon, A., & Capasso, M.

(2024). Exploring the role of HLA variants in neuroblastoma susceptibility through whole exome sequencing. *HLA*, *103*(5), e15515. https://doi.org/10.1111/tan.15515

Brookes, A. J. (1999). The essence of SNPs. *Gene*, *234*(2), 177–186. https://doi.org/10.1016/S0378-1119(99)00219-X

Campbell, K. S., & Hasegawa, J. (2013). Natural killer cell biology: An update and future directions. *Journal of Allergy and Clinical Immunology*, *132*(3), 536–544. https://doi.org/10.1016/j.jaci.2013.07.006

Cancer Stat Facts: Myeloma. (2024). SEER. https://seer.cancer.gov/statfacts/html/mulmy.html

Capittini, C., Tinelli, C., Guarene, M., Pasi, A., Badulli, C., Sbarsi, I., Garlaschelli, F., Cremaschi, A. L., Pizzochero, C., Monti, C., Salvaneschi, L., & Martinetti, M. (2012). Possible KIR-driven genetic pressure on the genesis and maintenance of specific HLA-A,B haplotypes as functional genetic blocks. *Genes and Immunity*, *13*(6), 452–457. https://doi.org/10.1038/gene.2012.14

Castaño-Núñez, Á., Montes-Cano, M. A., García-Lozano, J. R., Ortego-Centeno, N., García-Hernández, F. J., Espinosa, G., Graña-Gil, G., Sánchez-Bursón, J., Juliá, M. R., Solans, R., Blanco, R., Barnosi-Marín, A. C., Gómez de la Torre, R., Fanlo, P., Rodríguez-Carballeira, M., Rodríguez-Rodríguez, L., Camps, T., Castañeda, S., Alegre-Sancho, J. J., … González-Escribano, M. F. (2019). Association of Functional Polymorphisms of KIR3DL1/DS1 With Behçet's Disease. *Frontiers in Immunology*, *10*. https://doi.org/10.3389/fimmu.2019.02755

Chattopadhyay, S., Thomsen, H., Yadav, P., da Silva Filho, M. I., Weinhold, N., Nöthen, M. M., Hoffman, P., Bertsch, U., Huhn, S., Morgan, G. J., Goldschmidt, H., Houlston, R., Hemminki, K., & Försti, A. (2019). Genome-wide interaction and pathway-based identification of key regulators in multiple myeloma. *Communications Biology*, *2*(1), 1–10. https://doi.org/10.1038/s42003-019-0329-2

Chen, J., Madireddi, S., Nagarkar, D., Migdal, M., Vander Heiden, J., Chang, D., Mukhyala, K., Selvaraj, S., Kadel, E. E., Brauer, M. J., Mariathasan, S., Hunkapiller, J., Jhunjhunwala, S., Albert, M. L., & Hammer, C. (2021). In silico tools for accurate HLA and KIR inference from clinical sequencing data empower immunogenetics on individual-patient and population scales. *Briefings in Bioinformatics*, *22*(3), 1–11. https://doi.org/10.1093/bib/bbaa223

Chen, X., Jiang, L., & Liu, X. (2022). Natural killer cells: The next wave in cancer immunotherapy. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.954804

Chiarella, P., Capone, P., & Sisto, R. (2023). Contribution of Genetic Polymorphisms in Human Health. *International Journal of Environmental Research and Public Health*, *20*(2), 912. https://doi.org/10.3390/ijerph20020912

Clara, J. A., & Childs, R. W. (2022). Harnessing natural killer cells for the treatment of multiple myeloma. *Seminars in Oncology*, *49*(1), 69–85. https://doi.org/10.1053/j.seminoncol.2022.01.004

Clavero, E., Sanchez-Maldonado, J. M., Macauda, A., Ter Horst, R., Sampaio-Marques, B., Jurczyszyn, A., Clay-Gilmour, A., Stein, A., Hildebrandt, M. A. T., Weinhold, N., Buda, G., García-Sanz, R., Tomczak, W., Vogel, U., Jerez, A., Zawirska, D., Wątek, M., Hofmann, J. N., Landi, S., … Sainz, J. (2023). Polymorphisms within Autophagy-Related Genes as Susceptibility Biomarkers for Multiple Myeloma: A Meta-Analysis of Three Large Cohorts and Functional Characterization. *International Journal of Molecular Sciences*, *24*(10), Article 10. https://doi.org/10.3390/ijms24108500

Cooley, S., Parham, P., & Miller, J. S. (2018). Strategies to activate NK cells to prevent relapse and induce remission following hematopoietic stem cell transplantation. *Blood*, *131*(10), 1053–1062. https://doi.org/10.1182/blood-2017-08-752170

Cooper, D. N., & Clayton, J. F. (1988). DNA polymorphism and the study of disease associations. *Human Genetics*, *78*(4), 299–312. https://doi.org/10.1007/BF00291724

Corre, J., Munshi, N. C., & Avet-Loiseau, H. (2021). Risk factors in multiple myeloma: Is it time for a revision? *Blood*, *137*(1), 16–19. https://doi.org/10.1182/blood.2019004309

Cowan, A. J., Allen, C., Barac, A., Basaleem, H., Bensenor, I., Curado, M. P., Foreman, K., Gupta, R., Harvey, J., Hosgood, H. D., Jakovljevic, M., Khader, Y., Linn, S., Lad, D., Mantovani, L., Nong, V. M., Mokdad, A., Naghavi, M., Postma, M., … Fitzmaurice, C. (2018). Global Burden of Multiple Myeloma: A Systematic Analysis for the Global Burden of Disease Study 2016. *JAMA Oncology*, *4*(9), 1221–1227. https://doi.org/10.1001/jamaoncol.2018.2128

Daher, M., & Rezvani, K. (2018). Next generation natural killer cells for cancer immunotherapy: The promise of genetic engineering. *Current Opinion in Immunology*, *51*, 146–153. https://doi.org/10.1016/j.coi.2018.03.013

Daher, M., & Rezvani, K. (2021). Outlook for New CAR-Based Therapies with a Focus on CAR NK Cells: What Lies Beyond CAR-Engineered T Cells in the Race against Cancer. *Cancer Discovery*, *11*(1), 45–58. https://doi.org/10.1158/2159-8290.CD-20-0556

Diaz-Peña, R., Mondelo-Macía, P., Molina de la Torre, A. J., Sanz-Pamplona, R., Moreno, V., & Martín, V. (2020). Analysis of Killer Immunoglobulin-Like

Receptor Genes in Colorectal Cancer. *Cells*, *9*(2), Article 2. https://doi.org/10.3390/cells9020514

Dilthey, A., Leslie, S., Moutsianas, L., Shen, J., Cox, C., Nelson, M. R., & McVean, G. (2013). Multi-Population Classical HLA Type Imputation. *PLOS Computational Biology*, *9*(2), e1002877. https://doi.org/10.1371/journal.pcbi.1002877

Dima, D., Jiang, D., Singh, D. J., Hasipek, M., Shah, H. S., Ullah, F., Khouri, J., Maciejewski, J. P., & Jha, B. K. (2022). Multiple Myeloma Therapy: Emerging Trends and Challenges. *Cancers*, *14*(17), 4082. https://doi.org/10.3390/cancers14174082

Dimopoulos, M. A., Moreau, P., Terpos, E., Mateos, M. V., Zweegman, S., Cook, G., Delforge, M., Hájek, R., Schjesvold, F., Cavo, M., Goldschmidt, H., Facon, T., Einsele, H., Boccadoro, M., San-Miguel, J., Sonneveld, P., & Mey, U. (2021). Multiple myeloma: EHA-ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. *Annals of Oncology*, *32*(3), 309–322. https://doi.org/10.1016/j.annonc.2020.11.014

Gabriel, I. H., Sergeant, R., Szydlo, R., Apperley, J. F., DeLavallade, H., Alsuliman, A., Khoder, A., Marin, D., Kanfer, E., Cooper, N., Davis, J., MacDonald, D., Bua, M., Foroni, L., Giles, C., Milojkovic, D., Rahemtulla, A., & Rezvani, K. (2010). Interaction between KIR3DS1 and HLA-Bw4 predicts for progression-free survival after autologous stem cell transplantation in patients with multiple myeloma. *Blood*, *116*(12), 2033–2039. https://doi.org/10.1182/blood-2010-03-273706

Galaxy Community. (2022). The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2022 update. *Nucleic Acids Research*, *50*(W1), W345–W351. https://doi.org/10.1093/nar/gkac247

Gao, G. F., Liu, D., Zhan, X., & Li, B. (2022). Analysis of KIR gene variants in The Cancer Genome Atlas and UK Biobank using KIRCLE. *BMC Biology*, *20*, 191. https://doi.org/10.1186/s12915-022-01392-2

García-Ortiz, A., Rodríguez-García, Y., Encinas, J., Maroto-Martín, E., Castellano, E., Teixidó, J., & Martínez-López, J. (2021). The role of tumor microenvironment in multiple myeloma development and progression. *Cancers*, *13*(2), 1–22. https://doi.org/10.3390/cancers13020217

Gonzalez-Galarza, F. F., Christmas, S., Middleton, D., & Jones, A. R. (2011). Allele frequency net: A database and online repository for immune gene frequencies in worldwide populations. *Nucleic Acids Research*, *39*(Database issue), D913-919. https://doi.org/10.1093/nar/gkq1128

Graham, L. V., Fisher, J. G., Khakoo, S. I., & Blunt, M. D. (2023). Targeting KIR as a novel approach to improve CAR-NK cell function. *Journal of*

Translational Genetics and Genomics, *7*, 230–235. https://doi.org/10.20517/jtgg.2023.25

Hadjis, A. D., & McCurdy, S. R. (2024). The role and novel use of natural killer cells in graft-versus-leukemia reactions after allogeneic transplantation. *Frontiers in Immunology*, *15*, 1358668. https://doi.org/10.3389/fimmu.2024.1358668

Hammer, Q., Rückert, T., Borst, E. M., Dunst, J., Haubner, A., Durek, P., Heinrich, F., Gasparoni, G., Babic, M., Tomic, A., Pietra, G., Nienen, M., Blau, I. W., Hofmann, J., Na, I.-K., Prinz, I., Koenecke, C., Hemmati, P., Babel, N., … Romagnani, C. (2018). Peptide-specific recognition of human cytomegalovirus strains controls adaptive natural killer cells. *Nature Immunology*, *19*(5), 453–463. https://doi.org/10.1038/s41590-018-0082-6

Hematian Larki, M., Ashouri, E., Barani, S., Ghayumi, S. M. A., Ghaderi, A., & Rajalingam, R. (2022). KIR-HLA gene diversities and susceptibility to lung cancer. *Scientific Reports*, *12*(1), 17237. https://doi.org/10.1038/s41598-022-21062-1

Hollenbach, J. A., Pando, M. J., Caillier, S. J., Gourraud, P.-A., & Oksenberg, J. R. (2016). The killer immunoglobulin-like receptor KIR3DL1 combination with HLA-Bw4 is protective against multiple sclerosis in African Americans. *Genes and Immunity*, *17*(3), 199–202. https://doi.org/10.1038/gene.2016.5

Hoteit, R., Bazarbachi, A., Antar, A., Salem, Z., Shammaa, D., & Mahfouz, R. (2014). KIR genotype distribution among patients with multiple myeloma: Higher prevalence of KIR 2DS4 and KIR 2DS5 genes. *Meta Gene*, *2*, 730–736. https://doi.org/10.1016/j.mgene.2014.09.008

IPD-IMGT/HLA Database. (2024). https://www.ebi.ac.uk/ipd/imgt/hla/matching/

Jennifer Zhang, Q. (2022). Donor selection based on NK alloreactivity for patients with hematological malignancies. *Human Immunology*, *83*(10), 695–703. https://doi.org/10.1016/j.humimm.2022.07.006

Karki, R., Pandya, D., Elston, R. C., & Ferlini, C. (2015). Defining "mutation" and "polymorphism" in the era of personal genomics. *BMC Medical Genomics*, *8*(1), 37. https://doi.org/10.1186/s12920-015-0115-z

Kröger, N., Zabelina, T., Berger, J., Duske, H., Klyuchnikov, E., Binder, T., Stübig, T., Hilde-Brandt, Y., Atanackovic, D., Alchalby, H., Ayuk, F., Zander, A. R., Bacher, U., & Eiermann, T. (2011). Donor KIR haplotype B improves progression-free and overall survival after allogeneic hematopoietic stem cell transplantation for multiple myeloma. *Leukemia*, *25*(10), 1657–1661. https://doi.org/10.1038/leu.2011.138

Kumar, S. K., & Rajkumar, S. V. (2018). The multiple myelomas—Current concepts in cytogenetic classification and therapy. *Nature Reviews Clinical Oncology*, *15*(7), 409–421. https://doi.org/10.1038/s41571-018-0018-y

Kumar, S. K., Rajkumar, V., Kyle, R. A., van Duin, M., Sonneveld, P., Mateos, M.-V., Gay, F., & Anderson, K. C. (2017). Multiple myeloma. *Nature Reviews Disease Primers*, *3*(1), 1–20. https://doi.org/10.1038/nrdp.2017.46

Kyle, R. A., Durie, B. G. M., Rajkumar, S. V., Landgren, O., Blade, J., Merlini, G., Kröger, N., Einsele, H., Vesole, D. H., Dimopoulos, M., San Miguel, J., Avet-Loiseau, H., Hajek, R., Chen, W. M., Anderson, K. C., Ludwig, H., Sonneveld, P., Pavlovsky, S., Palumbo, A., … on behalf of the International Myeloma Working Group. (2010). Monoclonal gammopathy of undetermined significance (MGUS) and smoldering (asymptomatic) multiple myeloma: IMWG consensus perspectives risk factors for progression and guidelines for monitoring and management. *Leukemia*, *24*(6), 1121–1127. https://doi.org/10.1038/leu.2010.60

Li, Y., Wu, X., Sheng, C., Liu, H., Liu, H., Tang, Y., Liu, C., Ding, Q., Xie, B., Xiao, X., Zheng, R., Yu, Q., Guo, Z., Ma, J., Wang, J., Gao, J., Tian, M., Wang, W., Zhou, J., … Xiao, T. (2024). IGSF8 is an innate immune checkpoint and cancer immunotherapy target. *Cell*, *187*(11), 2703-2716.e23. https://doi.org/10.1016/j.cell.2024.03.039

Lin, Y., Qiu, L., Usmani, S., Joo, C. W., Costa, L., Derman, B., Du, J., Einsele, H., Fernandez de Larrea, C., Hajek, R., Ho, P. J., Kastritis, E., Martinez-Lopez, J., Mateos, M.-V., Mikhael, J., Moreau, P., Nagarajan, C., Nooka, A., O'Dwyer, M., … Martin, T. (2024). Consensus guidelines and recommendations for the management and response assessment of chimeric antigen receptor T-cell therapy in clinical practice for relapsed and refractory multiple myeloma: A report from the International Myeloma Working Group Immunotherapy Committee. *The Lancet Oncology*. https://doi.org/10.1016/S1470-2045(24)00094-9

Liu, S., Galat, V., Galat4, Y., Lee, Y. K. A., Wainwright, D., & Wu, J. (2021). NK cell-based cancer immunotherapy: From basic biology to clinical development. *Journal of Hematology & Oncology*, *14*(1), 7. https://doi.org/10.1186/s13045-020-01014-w

MacArthur, D. G., Manolio, T. A., Dimmock, D. P., Rehm, H. L., Shendure, J., Abecasis, G. R., Adams, D. R., Altman, R. B., Antonarakis, S. E., Ashley, E. A., Barrett, J. C., Biesecker, L. G., Conrad, D. F., Cooper, G. M., Cox, N. J., Daly, M. J., Gerstein, M. B., Goldstein, D. B., Hirschhorn, J. N., … Gunter, C. (2014). Guidelines for investigating causality of sequence variants in human disease. *Nature*, *508*(7497), 469–476. https://doi.org/10.1038/nature13127

Manojlovic, Z., Christofferson, A., Liang, W. S., Aldrich, J., Washington, M., Wong, S., Rohrer, D., Jewell, S., Kittles, R. A., Derome, M., Auclair, D., Craig, D. W., Keats, J., & Carpten, J. D. (2017). Comprehensive molecular profiling of 718 Multiple Myelomas reveals significant differences in mutation

frequencies between African and European descent cases. *PLoS Genetics*, *13*(11), e1007087. https://doi.org/10.1371/journal.pgen.1007087

Marin, D., Li, Y., Basar, R., Rafei, H., Daher, M., Dou, J., Mohanty, V., Dede, M., Nieto, Y., Uprety, N., Acharya, S., Liu, E., Wilson, J., Banerjee, P., Macapinlac, H. A., Ganesh, C., Thall, P. F., Bassett, R., Ammari, M., … Rezvani, K. (2024). Safety, efficacy and determinants of response of allogeneic CD19-specific CAR-NK cells in CD19+ B cell tumors: A phase 1/2 trial. *Nature Medicine*, *30*(3), 772–784. https://doi.org/10.1038/s41591-023-02785-8

Martínez-Sánchez, M. V., Periago, A., Legaz, I., Gimeno, L., Mrowiec, A., Montes-Barqueros, N. R., Campillo, J. A., Bolarin, J. M., Bernardo, M. V., López-Álvarez, M. R., González, C., García-Garay, M. C., Muro, M., Cabañas-Perianes, V., Fuster, J. L., García-Alonso, A. M., Moraleda, J. M., Álvarez-Lopez, M. R., & Minguela, A. (2015). Overexpression of KIR inhibitory ligands (HLA-I) determines that immunosurveillance of myeloma depends on diverse and strong NK cell licensing. *Oncoimmunology*, *5*(4), e1093721. https://doi.org/10.1080/2162402X.2015.1093721

Matzaraki, V., Kumar, V., Wijmenga, C., & Zhernakova, A. (2017). The MHC locus and genetic susceptibility to autoimmune and infectious diseases. *Genome Biology*, *18*(1), 76. https://doi.org/10.1186/s13059-017-1207-1

Maxwell, L. D., Ross, O. A., Curran, M. D., Rea, I. M., & Middleton, D. (2004). Investigation of KIR diversity in immunosenecence and longevity within the Irish population. *Experimental Gerontology*, *39*(8), 1223–1232. https://doi.org/10.1016/j.exger.2004.05.003

Mori, M., Leitman, E., Walker, B., Ndung'u, T., Carrington, M., & Goulder, P. (2019). Impact of HLA Allele-KIR Pairs on HIV Clinical Outcome in South Africa. *Journal of Infectious Diseases*, *219*(9), 1456–1463. https://doi.org/10.1093/infdis/jiy692

Motyer, A., Vukcevic, D., Dilthey, A., Donnelly, P., McVean, G., & Leslie, S. (2016). *Practical Use of Methods for Imputation of HLA Alleles from SNP Genotype Data*. https://doi.org/10.1101/091009

Myers, J. A., & Miller, J. S. (2021). Exploring the NK cell platform for cancer immunotherapy. *Nature Reviews Clinical Oncology*, *18*(2), 85–100. https://doi.org/10.1038/s41571-020-0426-7

Nakimuli, A., Chazara, O., Hiby, S. E., Farrell, L., Tukwasibwe, S., Jayaraman, J., Traherne, J. A., Trowsdale, J., Colucci, F., Lougee, E., Vaughanh, R. W., Elliott, A. M., Byamugisha, J., Kaleebu, P., Mirembe, F., Nemat-Gorgani, N., Parham, P., Norman, P. J., & Moffett, A. (2015). A KIR B centromeric region present in Africans but not Europeans protects pregnant women from pre-eclampsia. *Proceedings of the National Academy of Sciences of the United*

States of America, *112*(3), 845–850. https://doi.org/10.1073/pnas.1413453112

Nemat-Gorgani, N., Guethlein, L. A., Henn, B. M., Norberg, S. J., Chiaroni, J., Sikora, M., Quintana-Murci, L., Mountain, J. L., Norman, P. J., & Parham, P. (2019). Diversity of KIR, HLA Class I, and Their Interactions in Seven Populations of Sub-Saharan Africans. *The Journal of Immunology*, *202*(9), 2636–2647. https://doi.org/10.4049/jimmunol.1801586

O'Connell, J., Gurdasani, D., Delaneau, O., Pirastu, N., Ulivi, S., Cocca, M., Traglia, M., Huang, J., Huffman, J. E., Rudan, I., McQuillan, R., Fraser, R. M., Campbell, H., Polasek, O., Asiki, G., Ekoru, K., Hayward, C., Wright, A. F., Vitart, V., … Marchini, J. (2014). A General Approach for Haplotype Phasing across the Full Spectrum of Relatedness. *PLOS Genetics*, *10*(4), e1004234. https://doi.org/10.1371/journal.pgen.1004234

O'Connor, G. M., & McVicar, D. W. (2013). The Yin-Yang of KIR3DL1/S1: Molecular Mechanisms and Cellular Function. *Critical Reviews in Immunology*, *33*(3), 203–218.

Older Aguilar, A. M., Guethlein, L. A., Adams, E. J., Abi-Rached, L., Moesta, A. K., & Parham, P. (2010). Coevolution of Killer Cell Ig-Like Receptors with HLA-C To Become the Major Variable Regulators of Human NK Cells. *The Journal of Immunology*, *185*(7), 4238–4251. https://doi.org/10.4049/jimmunol.1001494

Orgul, G., Dalva, K., Dalva-Aydemir, S., Alniacik, R. G., Donmez, H. G., Cakar, A. N., Beksac, M., & Beksac, M. S. (2021). Significance of inhibitory maternal killer-cell immunoglobulin-like receptor (KIR) and fetal KIR ligand genotype combinations in placenta related obstetric complications. *Journal of Reproductive Immunology*, *148*, 103425. https://doi.org/10.1016/j.jri.2021.103425

Ozturk, O. G., Polat, G., & Atik, U. (2012). Diversity of killer cell immunoglobulin-like receptor genes in Southern Turkey. *Molecular Biology Reports*, *39*(2), 1989–1995. https://doi.org/10.1007/s11033-011-0945-5

Parham, P. (2005). MHC class I molecules and kirs in human history, health and survival. *Nature Reviews Immunology*, *5*(3), 201–214. https://doi.org/10.1038/nri1570

Pawlyn, C., & Davies, F. E. (2019). Toward personalized treatment in multiple myeloma based on molecular characteristics. *Blood*, *133*(7), 660–675. https://doi.org/10.1182/blood-2018-09-825331

Pollock, N. R., Harrison, G. F., & Norman, P. J. (2022). Immunogenomics of Killer Cell Immunoglobulin-Like Receptor (KIR) and HLA Class I: Coevolution and Consequences for Human Health. *Journal of Allergy and Clinical*

Immunology: In Practice, *10*(7), 1763–1775. https://doi.org/10.1016/j.jaip.2022.04.036

Pratt, G. (2002). Molecular aspects of multiple myeloma. *Molecular Pathology*, *55*(5), 273–283.

Rajalingam, R. (2016). The Impact of HLA Class I-Specific Killer Cell Immunoglobulin-Like Receptors on Antibody-Dependent Natural Killer Cell-Mediated Cytotoxicity and Organ Allograft Rejection. *Frontiers in Immunology*, *7*. https://doi.org/10.3389/fimmu.2016.00585

Rajalingam, R., Du, Z., Meenagh, A., Luo, L., Kavitha, V. J., Pavithra-Arulvani, R., Vidhyalakshmi, A., Sharma, S. K., Balazs, I., Reed, E. F., Pitchappan, R. M., & Middleton, D. (2008). Distinct diversity of KIR genes in three southern Indian populations: Comparison with world populations revealed a link between KIR gene content and pre-historic human migrations. *Immunogenetics*, *60*(5), 207–217. https://doi.org/10.1007/s00251-008-0286-2

Ritari, J., Hyvärinen, K., Partanen, J., & Koskela, S. (2022). KIR gene content imputation from single-nucleotide polymorphisms in the Finnish population. *PeerJ*, *10*, e12692. https://doi.org/10.7717/peerj.12692

Rodriguez-Otero, P., Paiva, B., & San-Miguel, J. F. (2021). Roadmap to cure multiple myeloma. *Cancer Treatment Reviews*, *100*, 102284. https://doi.org/10.1016/j.ctrv.2021.102284

Roe, D., & Kuang, R. (2019). *Accurate and Efficient KIR Gene and Haplotype Inference from Genome Sequencing Reads with Novel K-mer Signatures*. https://doi.org/10.1101/541938

Roe, D., Williams, J., Ivery, K., Brouckaert, J., Downey, N., Locklear, C., Kuang, R., & Maiers, M. (2020). Efficient Sequencing, Assembly, and Annotation of Human KIR Haplotypes. *Frontiers in Immunology*, *11*(October), 1–11. https://doi.org/10.3389/fimmu.2020.582927

Röllig, C., Knop, S., & Bornhäuser, M. (2015). Multiple myeloma. *The Lancet*, *385*(9983), 2197–2208. https://doi.org/10.1016/S0140-6736(14)60493-1

Ruggeri, L., Capanni, M., Urbani, E., Perruccio, K., Shlomchik, W. D., Tosti, A., Posati, S., Rogaia, D., Frassoni, F., Aversa, F., Martelli, M. F., & Velardi, A. (2002). Effectiveness of Donor Natural Killer Cell Alloreactivity in Mismatched Hematopoietic Transplants. *Science*, *295*(5562), 2097–2100. https://doi.org/10.1126/science.1068440

Sahin, U., Dalva, K., Gungor, F., Ustun, C., & Beksac, M. (2018). Donor-recipient killer immunoglobulin like receptor (KIR) genotype matching has a protective effect on chronic graft versus host disease and relapse incidence following HLA-identical sibling hematopoietic stem cell transplantation.

Annals of Hematology, *97*(6), 1027–1039. https://doi.org/10.1007/s00277-018-3274-0

Sakaue, S., Gurajala, S., Curtis, M., Luo, Y., Choi, W., Ishigaki, K., Kang, J. B., Rumker, L., Deutsch, A. J., Schönherr, S., Forer, L., LeFaive, J., Fuchsberger, C., Han, B., Lenz, T. L., de Bakker, P. I. W., Okada, Y., Smith, A. V., & Raychaudhuri, S. (2023). Tutorial: A statistical genetics guide to identifying HLA alleles driving complex disease. *Nature Protocols*, *18*(9), 2625–2641. https://doi.org/10.1038/s41596-023-00853-4

Sakaue, S., Hosomichi, K., Hirata, J., Nakaoka, H., Yamazaki, K., Yawata, M., Yawata, N., Naito, T., Umeno, J., Kawaguchi, T., Matsui, T., Motoya, S., Suzuki, Y., Inoko, H., Tajima, A., Morisaki, T., Matsuda, K., Kamatani, Y., Yamamoto, K., … Okada, Y. (2022). Decoding the diversity of killer immunoglobulin-like receptors by deep sequencing and a high-resolution imputation method. *Cell Genomics*, *2*(3), 100101. https://doi.org/10.1016/j.xgen.2022.100101

Samur, M. K., Aktas Samur, A., Fulciniti, M., Szalat, R., Han, T., Shammas, M., Richardson, P., Magrangeas, F., Minvielle, S., Corre, J., Moreau, P., Thakurta, A., Anderson, K. C., Parmigiani, G., Avet-Loiseau, H., & Munshi, N. C. (2020). Genome-Wide Somatic Alterations in Multiple Myeloma Reveal a Superior Outcome Group. *Journal of Clinical Oncology*, *38*(27), 3107–3118. https://doi.org/10.1200/JCO.20.00461

Sim, M. J. W., Brennan, P., Wahl, K. L., Lu, J., Rajagopalan, S., Sun, P. D., & Long, E. O. (2023). Innate receptors with high specificity for HLA class I–peptide complexes. *Science Immunology*, *8*(87), eadh1781. https://doi.org/10.1126/sciimmunol.adh1781

Skerget, S., Penaherrera, D., Chari, A., Jagannath, S., Siegel, D. S., Vij, R., Orloff, G., Jakubowiak, A., Niesvizky, R., Liles, D., Berdeja, J., Levy, M., Wolf, J., Usmani, S. Z., The MMRF CoMMpass Network, Christofferson, A. W., Nasser, S., Aldrich, J. L., Legendre, C., … Keats, J. J. (2021). *Genomic Basis of Multiple Myeloma Subtypes from the MMRF CoMMpass Study*. https://doi.org/10.1101/2021.08.02.21261211

Song, L., Bai, G., Liu, X. S., Li, B., & Li, H. (2023). Efficient and accurate KIR and HLA genotyping with massively parallel sequencing data. *Genome Research*, *33*(6), 923–931. https://doi.org/10.1101/gr.277585.122

Sood, N., Hamide, A., Dubashi, B., Nisha, Y., Mathaiyan, J., Munirajan, A. K., Ganesn, P., & Kayal, S. (2024). Impact of Genetic Polymorphisms in NF-κB2 and TRAF3 Genes on Response to Bortezomib-Based Therapy in Multiple Myeloma Patients. *Asian Pacific Journal of Cancer Prevention: APJCP*, *25*(3), 829–837. https://doi.org/10.31557/APJCP.2024.25.3.829

Sun, H., Martin, T. G., Marra, J., Kong, D., Keats, J., Macé, S., Chiron, M., Wolf, J. L., Venstrom, J. M., & Rajalingam, R. (2021). Individualized genetic makeup

that controls natural killer cell function influences the efficacy of isatuximab immunotherapy in patients with multiple myeloma. *Journal for ImmunoTherapy of Cancer*, *9*(7). https://doi.org/10.1136/jitc-2021-002958

Takeshita, L. Y. C., Gonzalez-Galarza, F. F., Santos, E. J. M. D., Maia, M. H. T., Rahman, M. M., Zain, S. M. S., Middleton, D., & Jones, A. R. (2013). A database for curating the associations between killer cell immunoglobulin-like receptors and diseases in worldwide populations. *Database*, *2013*. https://doi.org/10.1093/database/bat021

Ustunkar, G., & Aydin-Son, Y. (2011). METU-SNP: An Integrated Software System for SNPComplex Disease Association Analysis. *Journal of Integrative Bioinformatics*, *8*(2), 204–221. https://doi.org/10.1515/jib-2011-187

van Nieuwenhuijzen, N., Spaan, I., Raymakers, R., & Peperzak, V. (2018). From MGUS to Multiple Myeloma, a Paradigm for Clonal Evolution of Premalignant Cells. *Cancer Research*, *78*(10), 2449–2456. https://doi.org/10.1158/0008-5472.CAN-17-3115

Vukcevic, D., Traherne, J. A., Næss, S., Ellinghaus, E., Kamatani, Y., Dilthey, A., Lathrop, M., Karlsen, T. H., Franke, A., Moffatt, M., Cookson, W., Trowsdale, J., McVean, G., Sawcer, S., & Leslie, S. (2015). Imputation of KIR Types from SNP Variation Data. *American Journal of Human Genetics*, *97*(4), 593–607. https://doi.org/10.1016/j.ajhg.2015.09.005

CURRICULUM VITAE

PERSONAL INFORMATION

Surname, Name: Akın, Hasan Yalım

EDUCATION

Degree	Institution	Year of Graduation
MS	Ankara University Biotechnology	2017
BS	Istanbul University Molecular Biology and Genetics	2014
High School	METU Development Foundation High School, Ankara	2006

WORK EXPERIENCE

2019-Present	Ankara University School of Medicine, Cord Blood Bank Quality Specialist
2014-Present	Ankara University School of Medicine, Cord Blood Bank Molecular Biologist, Scientific Project Specialist Ankara
2014	Başkent University School of Medicine Department of Medical Genetics Long-term Internship

FOREIGN LANGUAGES

Advanced English

PUBLICATIONS

1. Gencer E.B., Akin H.Y., Toprak S.K., Turasan E., Yousefzadeh M., Yurdakul-Mesutoglu P., Cagan M., Seval M.M., Katlan D.C., Dalva K., Beksac M.S., Beksac M. “In vivo and in vitro effects of cord blood hematopoietic stem and progenitor cell (HSPC) expansion using valproic acid and/or nicotinamide”, Current Research in Translational Med.”, 72 (3), 103444 (2024), doi: 10.1016/j.retram.2024.103444.

2. Akkus E., Tuncalı T., Akın H.Y., Aydın Y., Beşışık S.K., Gürkan E., Ratip S., Salihoğlu A., Sargın D., Ünal A., Turcan A., Sevindik Ö.G., Demir M., Beksac M. “Germline genetic variants in Turkish familial multiple myeloma/monoclonal gammopathy of undetermined significance cases”, British Journal of Haematology. 204 (3), 931-938 (2024), doi: 10.1111/bjh.19271.

3. Beksac M., Akin H.Y., Cengiz Seval G., Yurdakul-Mesutoğlu P., Anliacik RG., Anliacik E., Gurman G., Karaagaoglu E., Dalva K. “A novel hypothesis: Certain KIR/cognate ligand containing genotypes differ in frequency among patients with myeloma and have an effect on age of disease onset”, Clinical Lymphoma Myeloma and Leukemia, 23 (5), 394-400 (2023), doi: 10.1016/j.clml.2023.02.007.

4. Yurdakul-Mesutoğlu P., Gencer Öncül E.B., Akın H.Y., Beksaç M. "Manual versus Automated Volume Reduction of Cord Blood", European Journal of Therapeutics. 28 (2) (2022), doi: 10.54614/eurjther.2021.0029.

5. Beksac M., Akin H.Y., Gencer-Oncul E.B., Yousefzadeh M., Cengiz Seval G., Gulten E., Akdemir Kalkan I., Cinar G., Memikoglu O., Karaagaoglu E., Dalva K. " A model integrating Killer Immunoglobulin-like Receptor (KIR) haplotypes for risk prediction of COVID-19 clinical disease severity", Immunogenetics, 73 (6):449-458 (2021), doi: 10.1007/s00251-021-01227-4.

6. Akin H.Y. “Serological Detection of Cytokine Levels in Cord Blood, Maternal Plasma and Amniotic Fluid and Their Impact on in vitro Cord Blood Colony Forming Potential”. M.Sc. Thesis, Ankara University Biotechnology Institute, Ankara, 2017.