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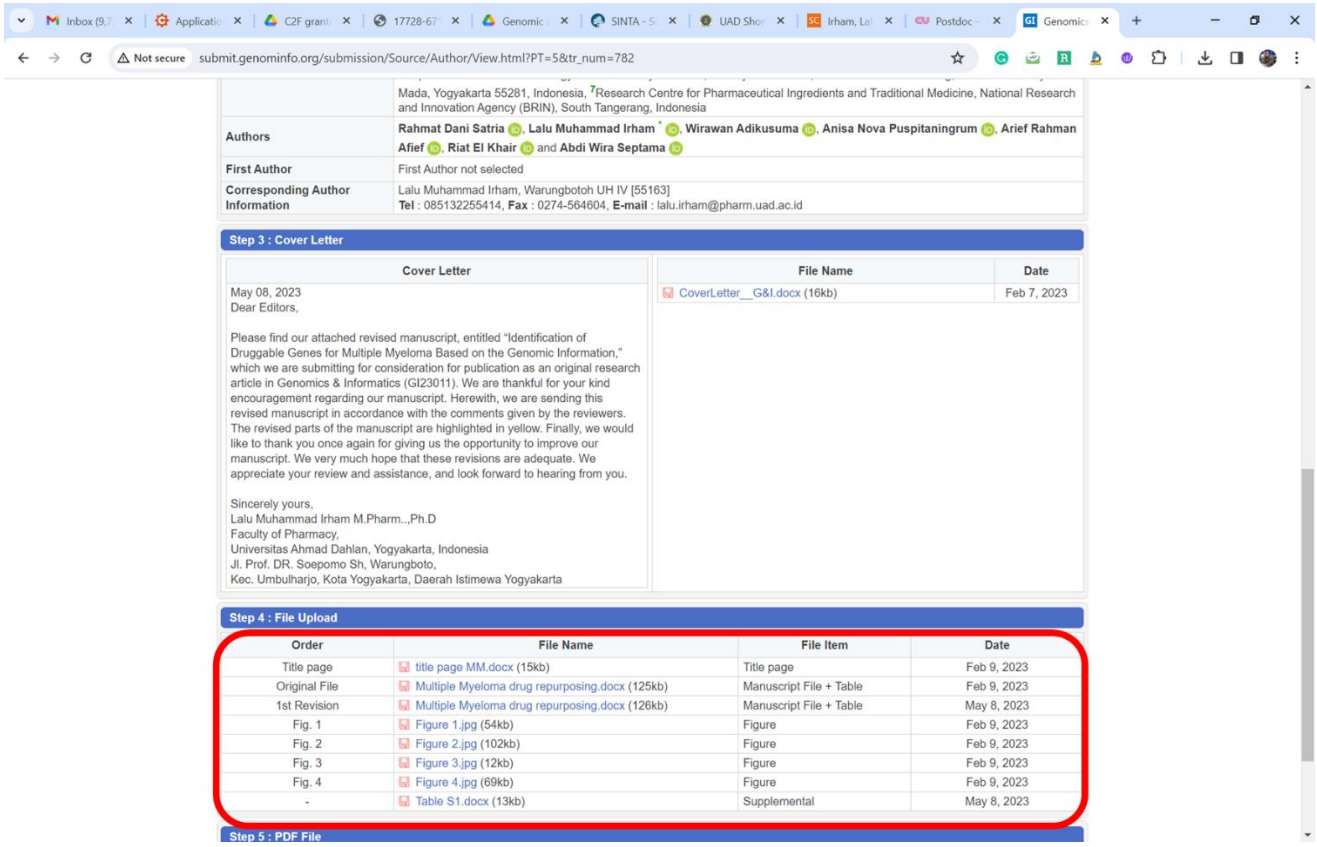

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Step 3 : Cover Letter

Cover Letter	File Name	Date
May 08, 2023 Dear Editors, Please find our attached revised manuscript, entitled "Identification of Druggable Genes for Multiple Myeloma Based on the Genomic Information," which we are submitting for consideration for publication as an original research article in Genomics & Informatics (GI23011). We are thankful for your kind encouragement regarding our manuscript. Herewith, we are sending this revised manuscript in accordance with the comments given by the reviewers. The revised parts of the manuscript are highlighted in yellow. Finally, we would like to thank you once again for giving us the opportunity to improve our manuscript. We very much hope that these revisions are adequate. We appreciate your review and assistance, and look forward to hearing from you. Sincerely yours, Lalu Muhammad Irham M.Pharm..,Ph.D Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia Jl. Prof. DR. Soepomo Sh, Warungboto, Kec. Umbulharjo, Kota Yogyakarta, Daerah Istimewa Yogyakarta	CoverLetter__G&I.docx (16kb)	Feb 7, 2023

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Order	File Name	File Item	Date
Title page	title page MM.docx (15kb)	Title page	Feb 9, 2023
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Fig. 1	Figure 1.jpg (54kb)	Figure	Feb 9, 2023
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Fig. 3	Figure 3.jpg (12kb)	Figure	Feb 9, 2023
Fig. 4	Figure 4.jpg (69kb)	Figure	Feb 9, 2023
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Step 5 : PDF File

Cover Letter

May 08, 2023

Dear Editors,

Please find our attached revised manuscript, entitled "**Identification of Druggable Genes for Multiple Myeloma Based on the Genomic Information**," which we are submitting for consideration for publication as an original research article in *Genomics & Informatics* (**GI23011**). We are thankful for your kind encouragement regarding our manuscript. Herewith, we are sending this revised manuscript in accordance with the comments given by the reviewers. The revised parts of the manuscript are highlighted in yellow. Finally, we would like to thank you once again for giving us the opportunity to improve our manuscript. We very much hope that these revisions are adequate. We appreciate your review and assistance, and look forward to hearing from you.

Sincerely yours,

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Universitas Ahmad Dahlan, Yogyakarta, Indonesia

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Kec. Umbulharjo, Kota Yogyakarta, Daerah Istimewa Yogyakarta

Identification of Druggable Genes for Multiple Myeloma Based on the Genomic Information

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Abstract

Multiple myeloma (MM) is a type of malignancy in hematology. Genetics is believed to be involved in MM development. Several studies have been conducted to clarify the genetics involved in MM. However, the use of genomic information for clinical purposes, both for diagnostic and prognostic biomarkers, is still limited in research. This research used genetic information reported in the genetic database for clinical trial studies on MM (Genomic Driven Clinical Implementation for Multiple Myeloma). Genetic information was collected from the Genome-Wide Association Studies (GWAS) catalog database. We prioritized genes that have the potential to cause MM disease based on established annotations. Furthermore, we prioritized biological risk genes for MM for drug target candidates. The DrugBank database was used to identify drug candidates with drug target genes. We discovered 14 MM biological risk genes and identified 10 drugs targeting three genes. Remarkably, only 1 out of 10 drugs, panobinostat has been approved for use in MM. Additionally, the two most promising genes, Calcium signal-modulating cyclophilin ligand (*CAMLG*), and Histone deacetylase 2 (*HDAC2*) were targeted by four drugs: cyclosporine, belinostat, vorinostat, and romidepsin with clinical evidence in the treatment of MM. Notably, there are 5 out of 10 drugs that have been approved for other indications which have not been reported for MM but may be also used for the treatment of MM. Accordingly, this study aimed to elucidate the genomic variants involved in the pathogenesis of MM and provide the benefits of genomic variants that can contribute to drug discovery.

Keywords: Multiple Myeloma, genomic variants, biological risk genes, drug repositioning

Introduction

Multiple Myeloma (MM) is a hematological malignancy caused by the uncontrolled proliferation of abnormal plasma cells in the bone marrow (BM). This abnormal proliferation of plasma cells causes damage to multiple organs throughout the body and manifests systemically. Systemic manifestations of MM include hypercalcemia, renal failure, anemia and bone lytic lesions [1], [2]. Over time, the number of MM cases is reported to be increasing. In 2020 the reported incidence of MM was 160,000 cases with 106,000 deaths [3]. This high mortality rate indicates that most of these MM cases end in death. In fact, preventing the worsening progression of the disease toward a poor prognosis requires an effective

diagnostic tool to detect the disease at an early stage. Currently, the diagnosis of MM uses bone marrow (BM) analysis to determine the percentage of plasma cells in the BM and serum protein electrophoresis for M-band and urinary Bence-Jones protein followed by the use of beta-2 microglobulin and serum albumin to determine the stage of MM [4], [5]. However, the use of these diagnostic tools is still not sufficient to detect the early stages of the MM disease, and most cases are detected in the late stages.

Recently, more accurate diagnostic tools have been developed to establish the MM diagnosis and prognosis. Karyotyping identification is one of the tools used to determine the prognosis and therapy of this disease [6]–[8]. However, the use of karyotyping is still not adequate because it can only detect abnormalities at the chromosomal level, and not at the gene level. Genomic detection is expected to detect early disease development before it progresses in a worse direction, and it is used to determine the accuracy of therapy. It can even be used for drug repurposing.

The Genome-Wide Association Study (GWAS) is one of genomic databases used to catalog the genomic variants associated with various diseases including for MM. Although GWAS data have provided valuable biological insights of the genomic variants associated with many diseases, however, the translation into the clinic situation has remained limited. Therefore, our study aimed at integrating the genomic variants from GWAS and the bioinformatics-based approach to drive more practical biological insights for MM treatment.

Methods

Study design

We started by identifying the genomic variants associated with MM or MM-associated single-nucleotide polymorphisms (SNPs) using data from the GWAS Catalog with criteria p value $< 10^{-8}$. Next, we obtained more SNPs which are known to encode these genes by utilizing the HaploReg version 4.1 in the Asian (ASN) population from the 1000 Genome Project Phase I data. In order to identify biological MM risk genes, we further utilized a genomic-driven drug repurposing approach based on the established criteria. These genes have been proposed as potential MM treatment targets. Finally, we determined the prospective drugs where the mechanisms and therapeutic targets overlapped.

Multiple myeloma risk genes

After widening the search using HaploReg version 4.1, SNPs encoding the genes were further examined to pinpoint the biological MM risk genes. In order to identify genes with greater likelihood and more solid supporting data, we strictly annotated the biological risk genes. The biological MM-risk genes were

ranked in this study using six criteria. Each criterion-compliant gene received one point (maximum six points per gene). Genes with higher scores have greater potential as biological risk genes. We applied the following six criteria to filter the biological MM risk genes: (1) missense mutation, HaploReg version 4.1 annotated missense mutations in genes containing MM risk SNPs with linkage disequilibrium (r^2 > 0.80); (2) Cis expression quantitative trait loci (*cis*-eQTL), MM risk SNP-containing genes with notable *cis*-eQTL effects in whole blood; (3) Biological process; (4) Cellular component; (5) Molecular function. Criteria 3, 4, and 5 are included in the Gene Ontology (GO) category. Genes were prioritized by using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) online tool version 6.8 accessed at: (https://david-d.ncifcrf.gov/tools.jsp) [9]. Finally, we added 6) Primary Immunodeficiency (PID): The PID was the final annotation to prioritize the MM risk genes. The International Union of Immunological Societies (IUIS) collected PID genes until 2013 [10]. A hypergeometric test was used to analyze the data for enrichment with a *p*-value of 0.05 considered significant.

Discovering new candidate drugs for multiple myeloma

The scoring system derived from the six criteria was used to prioritize biological MM risk genes. Genes with scores greater than or equal to 2 were regarded as biological MM risk genes. Unfortunately, there are only a couple few druggable drug target genes. Therefore, we further broadened the biological MM risk genes utilizing the STRING database (https://string-db.org/) accessed on September 12, 2022. After completing gene expansion based on protein-protein interactions (PPIs) information from the STRING database, we conducted the overlapping analysis using the DrugBank database accessed on September 12, 2022. In addition to these steps, to validate the finding, we used ClinicalTrial.gov (https://clinicaltrials.gov/; accessed on September 13, 2022) to check whether the drug target genes were undergoing clinical trials. We also used PubMed mining (https://pubmed.ncbi.nlm.nih.gov/; accessed on September 13, 2022) to check whether the candidate drugs were undergoing preclinical investigation.

Statistical analysis

Analytic workflows were performed using RStudio version 4.2.1 (RStudio, 250 Northern Ave, Boston, MA 02210). The haploR package was used to identify missense variants and Cis-eQTL (https://cran.r-project.org/web/packages/haploR/index.html). GO enrichment analyses, including BP, CC and MF were performed using the RDAVIDWebService, which is available as an *R* package from the Bioconductor project (www.bioconductor.org) [11].

Results

Identification of multiple myeloma-associated genes

In this study, 72 SNPs were identified, which were obtained from the GWAS catalog and fulfilled the inclusion criteria $p < 10^{-8}$ **(Table S1).** Next, we used HaploReg version 4.1 with criteria $r^2 > 0.8$ in the Asian population to extend the SNPs encoding the identified genes. The genomic variants associated with MM were further utilized to obtain the variants encoded these genes. We identified 2,555 SNPs that overlap with 63 genes associated with MM, and these genes were used for further analysis.

Identification of multiple myeloma biologic risk gene with functional annotation criteria

We used the six functional annotation criteria to prioritize genes at risk for the pathogenesis of MM with a scoring system for each gene if they met each criterion. Genes with missense variants (n=11); gene with cis-eQTL effect (n=19); genes that are prioritized by biological process (n=4); genes prioritized by cellular component (n=11), genes prioritized by molecular function (n=5), and genes prioritized by PID (n=2) **(Figure 2)**. The detailed information regarding the scoring system for each functional annotation is depicted in **Figure 3**. We found that out of 63 genes, 14 of them had a score of 2 or more and were categorized as MM biological risk genes. The top four genes are prioritized as the most biological risk genes because they have a score of 3 or more out of 6, including *RFWD3, HMGXB4, CDCA7L,* and *CCHCR1* **(Table 1)**. Furthermore, we expanded the 14 MM biological risk genes using the STRING database to derive more drug-targeted genes. In this step, we found 336 gene pairs of the protein-protein interaction network in the STRING database **(Table s2)**.

Candidates of Drug Repurposing for Multiple Myeloma

To identify genes targeted by drug candidates, we used the DrugBank database. Notably, not all genes that have targeted drugs have pharmacological activity. Remarkably, we identified 10 drugs that target 3 genes that are at risk for MM, and these drugs have been approved for use in other diseases **(Figure 4)**. There is only 1 among these 10 drugs, panobinostat, which is identified as an approved drug for MM, while 4 drugs are under clinical examination for MM, and 5 drugs have not been reported to treat MM.

This study focuses on drugs that have been approved based on clinical trials using the ClinicalTrial.gov database. Therefore, the target genes of the four drugs: cyclosporine (NCT04813653), belinostat (NCT00131261), vorinostat (NCT01502085), and romidepsin (NCT00765102) which are currently under clinical examination are considered the most promising target genes for MM. We identified two targeted genes, including Calcium signal-modulating cyclophilin ligand (*CAMLG*), and Histone deacetylase 2 (*HDAC2*). Among 5 new candidate drugs, 4 of them target the most promising targeted genes including, theophylline, aminophylline, oxtriphylline, and tixocortol, which may be also used for MM. The findings of this study emphasized that the human genomic variants not only drive the disease risk loci but also can drive novel biological insights for drug repurposing for MM.

Discussion

In this study, we extracted 72 SNPs associated with MM from the GWAS catalog database with inclusion criteria $p < 10^{-8}$ to search for candidate genes that have potential for drug reuse for MM treatment. Six functional annotations were used to assess and prioritize MM risk genes that may be associated with new drug targets. We found three drug target genes associated with 10 drugs. Among these 10 drugs, panobinostat is the only identified drug approved for MM, while there are 4 drugs under clinical examination for MM, and 5 drugs which have not been reported to treat MM. There are 2 genes (*CAMLG* and *HDAC2*) targeted by 4 drugs: cyclosporine (NCT04813653), belinostat (NCT00131261), vorinostat (NCT01502085), and romidepsin (NCT00765102) which are currently under clinical examination. Presently, *CAMLG* and *HDAC2* are considered the most promising target genes for MM treatment that have been studied and approved based on clinical trials using the ClinicalTrial.gov database.

Cyclosporine has been shown to be an immunosuppressive agent used to treat postoperative organ rejection [12]. A study conducted by Sonneveld *et al.* in 1994 demonstrated that cyclosporin can be used clinically to modulate multi-drug resistance (MDR) in patients with MM to vincristine, doxorubicin, and dexamethasone [13]. Among several target genes that have been identified, belinostat, vorinostat and romidepsin have been shown to be antineoplastic agents [14]–[16]. Belinostat and vorinostat are histone deacetylase (HDAC) inhibitors belonging to the hydroxamate group with the mechanism of stopping growth, affecting cell differentiation and producing malignant cell apoptosis [15].

In a clinical study conducted by Plumb *et al.* in 2003, belinostat was shown to have antitumor activity *in vitro* and *in vivo* studies against tumor cells [17]. Vorinostat is used in the FDA-approved management of cutaneous T-cell lymphoma (CTCL) [15]. In addition, other studies have shown that

vorinostat inhibits tumor growth, breast cancer, and lung cancer [18]–[20]. Romidepsin is also a new FDA-approved drug for the treatment of CTLC [21]. This was demonstrated in phase II studies with patients with recurrent or refractory CTLC, showing an overall response rate of 34-35% [22].

Drug repurposing has the advantage of exploiting gene variations by using the GWAS catalog database to determine potential new drug candidates for MM [23]. However, this research has limitations, including in this study, not all of the identified target genes had pharmacological activity. Thus, the identified genes could potentially miss the drug targets that have been found for MM. Therefore, further research is needed to verify the candidate drug effects in clinical applications in MM disease.

Conclusions

By utilizing the GWAS catalog database to map disease-gene-protein-drug relationships, we discovered three drug target genes that may be potential candidates for new drugs in the treatment of MM. We found 10 potential drug candidates for MM, and remarkably, there was only 1 identified drug approved for MM, panobinostat. Among the identified targets, 4 drugs are under clinical examination for MM, and 5 drugs have not been reported to treat MM. In the study, it was found that the two top biological MM risk genes were *CAMLG* and *HDAC2*. The evidence supports the possibility that these genes are significantly associated with MM, so further translational research is needed. Drug repurposing offers many advantages in the drug development process, such as shorter time required, lower costs, and higher success rates. In this study, we combined a drug repurposing approach with an integrative research methodology to identify drugs with new indications for MM.

Declaration of Competing Interest

The authors disclose no conflict of interest

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Table 1. Functional annotation applied to prioritize the biological risk genes for Multiple Myeloma

GENCODE_id	GENCODE_name	Misnon	ciseQTL	Biological Process	Cellular Component	Molecular Function	PID	Total score
ENSG00000168411	*RFWD3*	1	1	1	1	1	0	5
ENSG00000100281	*HMGXB4*	1	0	0	1	1	0	3
ENSG00000164649	*CDCA7L*	0	1	1	1	0	0	3
ENSG00000204536	*CCHCR1*	0	1	1	1	0	0	3
ENSG00000025770	*NCAPH2*	0	0	1	1	0	0	2
ENSG00000080603	*SRCAP*	0	0	0	1	1	0	2
ENSG00000100307	*CBX7*	0	1	0	1	0	0	2
ENSG00000138101	*DTNB*	0	0	0	1	1	0	2
ENSG00000156858	*PRR14*	1	1	0	0	0	0	2
ENSG00000168038	*ULK4*	1	1	0	0	0	0	2
ENSG00000182606	*TRAK1*	0	1	0	1	0	0	2
ENSG00000204525	*HLA-C*	1	1	0	0	0	0	2
ENSG00000204531	*POU5F1*	0	0	0	1	1	0	2
ENSG00000240505	*TNFRSF13B*	1	0	0	0	0	1	2

We set the threshold score >= 2 from the number of functional annotations ranged from 0-6, in which each gene was assigned one point for each annotation. Those genes with one functional annotation were awarded one point (score) and those genes with a score >= 2 were classified as “biological multiple myeloma genes”. Our study showed

that the higher the threshold of biological score applied, the smaller the number of biological genes identified, limiting the number of drug targets we could observe. (i.e., we found 1 biological multiple myeloma genes for threshold score >=5, 3 biological multiple myeloma genes for threshold score >=3 and 10 biological multiple myeloma genes for threshold score >= 2). The more biological multiple myeloma genes we find, the more candidate drug targets for multiple myeloma drug repurposing can be identified

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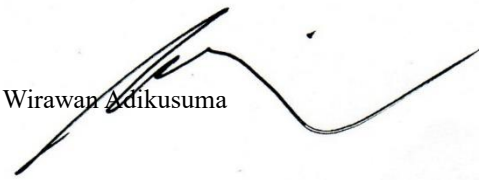
Author's signature	 Wirawan Adikusuma
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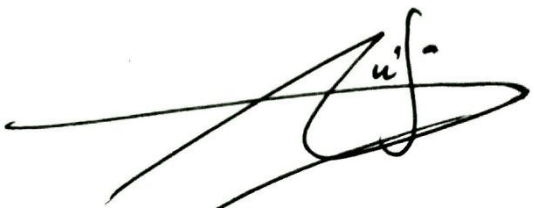
Author's signature	 **Arief Rahman Afief**
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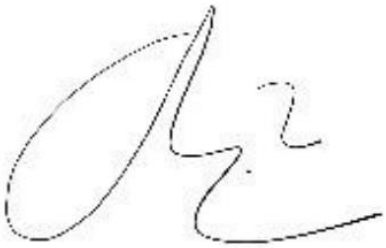
Author's signature	 Abdi Wira Septama
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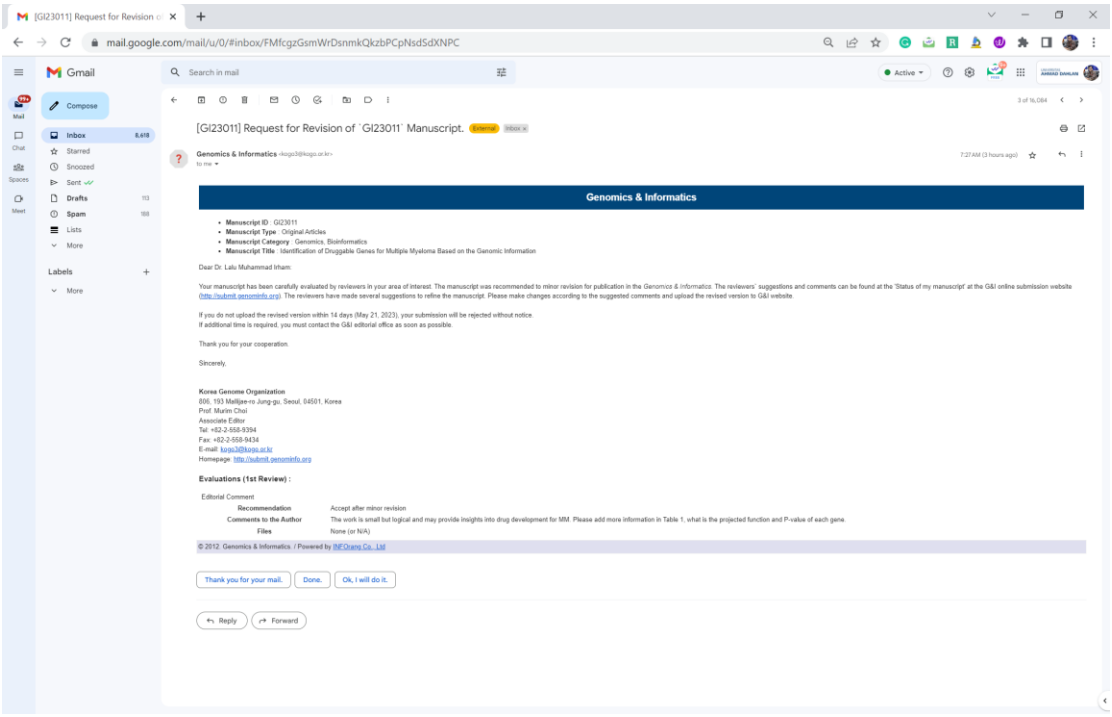

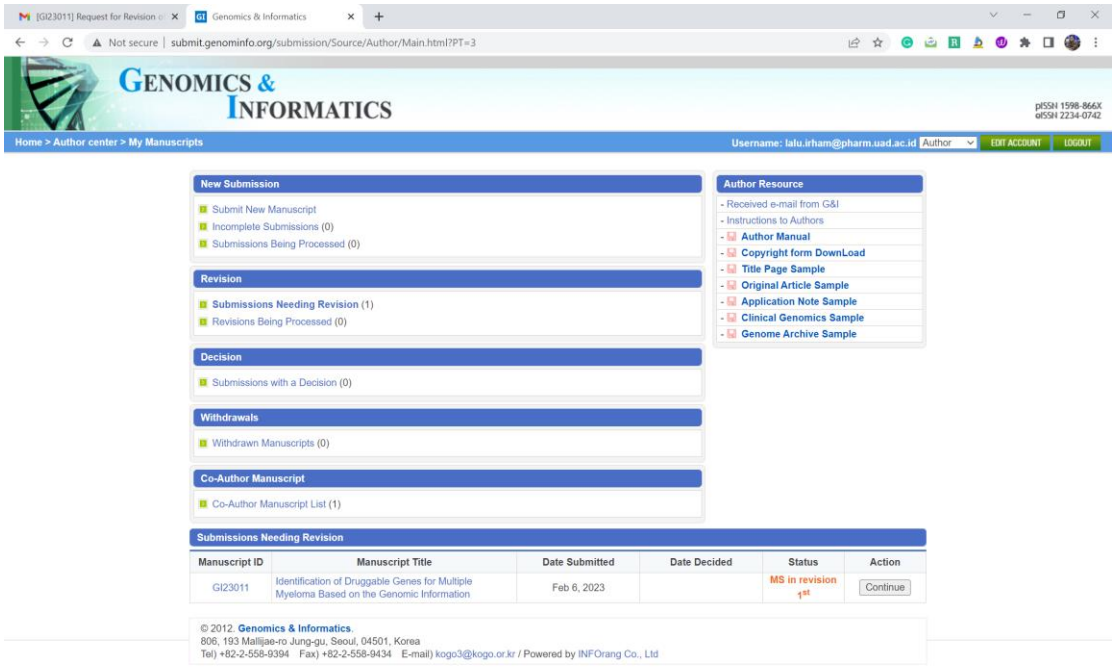

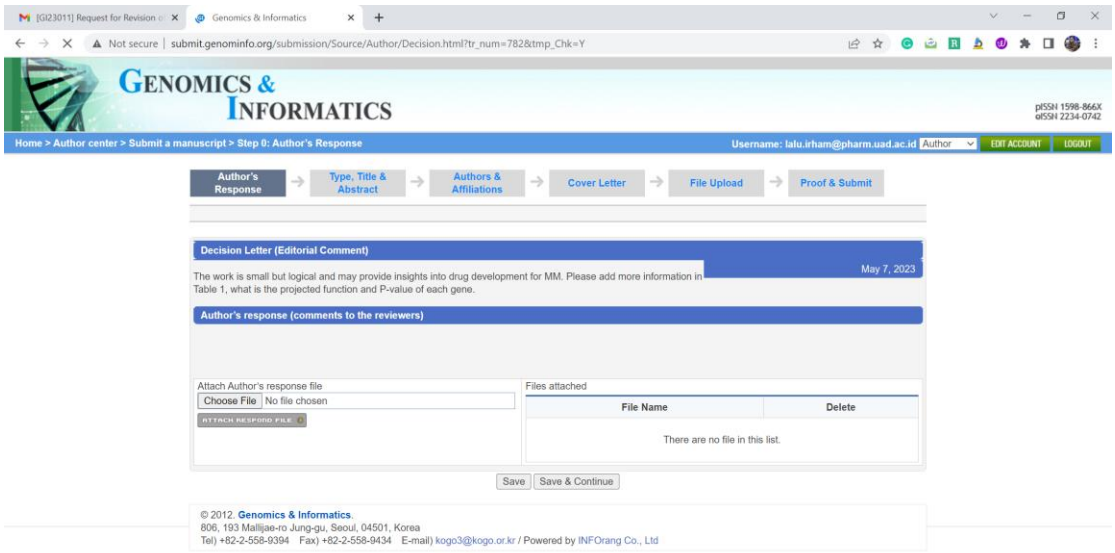

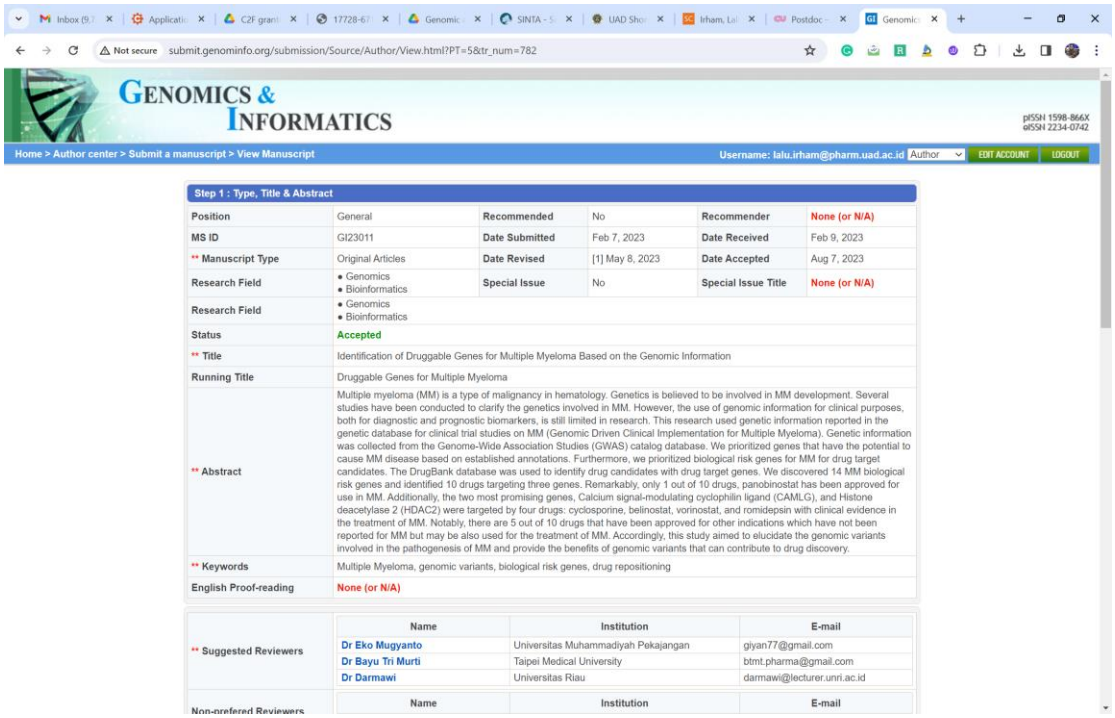

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Step 1 : Type, Title & Abstract

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MS ID	GI23011	Date Submitted	Feb 7, 2023	Date Received	Feb 9, 2023
** Manuscript Type	Original Articles	Date Revised	[1] May 8, 2023	Date Accepted	Aug 7, 2023
Research Field	• Genomics • Bioinformatics	Special Issue	No	Special Issue Title	None (or N/A)
Research Field	• Genomics • Bioinformatics				
Status	Accepted				
** Title	Identification of Druggable Genes for Multiple Myeloma Based on the Genomic Information				
Running Title	Druggable Genes for Multiple Myeloma				
** Abstract	Multiple myeloma (MM) is a type of malignancy in hematology. Genetics is believed to be involved in MM development. Several studies have been conducted to clarify the genetics involved in MM. However, the use of genomic information for clinical purposes, both for diagnostic and prognostic biomarkers, is still limited in research. This research used genetic information reported in the genetic database for clinical trial studies on MM (Genomic Driven Clinical Implementation for Multiple Myeloma). Genetic information was collected from the Genome-Wide Association Studies (GWAS) catalog database. We prioritized genes that have the potential to cause MM disease based on established annotations. Furthermore, we prioritized biological risk genes for MM for drug target candidates. The DrugBank database was used to identify drug candidates with drug target genes. We discovered 14 MM biological risk genes and identified 10 drugs targeting three genes. Remarkably, only 1 out of 10 drugs, panobinostat has been approved for use in MM. Additionally, the two most promising genes, Calcium signal-modulating cyclophilin ligand (CAMLG), and Histone deacetylase 2 (HDAC2) were targeted by four drugs: cyclosporine, belinostat, vorinostat, and romidepsin with clinical evidence in the treatment of MM. Notably, there are 5 out of 10 drugs that have been approved for other indications which have not been reported for MM but may be also used for the treatment of MM. Accordingly, this study aimed to elucidate the genomic variants involved in the pathogenesis of MM and provide the benefits of genomic variants that can contribute to drug discovery.				
** Keywords	Multiple Myeloma, genomic variants, biological risk genes, drug repositioning				
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May 07, 2023

Dear Editors,

Please find our attached revised manuscript, entitled "**Identification of Druggable Genes for Multiple Myeloma Based on the Genomic Information**," which we are submitting for consideration for publication as an original research article in *Genomics & Informatics* (**GI23011**). We are thankful for your kind encouragement regarding our manuscript. Herewith, we are sending this revised manuscript in accordance with the comments given by the reviewers. The revised parts of the manuscript are highlighted in yellow. Finally, we would like to thank you once again for giving us the opportunity to improve our manuscript. We very much hope that these revisions are adequate. We appreciate your review and assistance, and look forward to hearing from you.

Sincerely yours,

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Faculty of Pharmacy,

Universitas Ahmad Dahlan, Yogyakarta, Indonesia

Jl. Prof. DR. Soepomo SH, Warungboto,

Kec. Umbulharjo, Kota Yogyakarta, Daerah Istimewa Yogyakarta

Recommendation Reviewer 1:

Q1: The work is small but logical and may provide insights into drug development for MM. Please add more information in Table 1, what is the projected function and P-value of each gene.

We sincerely thank the reviewer for taking the time to review our work, and have addressed the critical comments point-by-point.

A1: We appreciate the reviewer's comments. We now revised the Table 1.

In response to the reviewer's question, we would like to provide a more detailed explanation of the methodology used in our study.

In the present study, we prioritized the genes disease and multiple myeloma genetics driven genomic drug repurposing for multiple myeloma. We hypothesized that multiple myeloma genetic variants prioritization using six functional annotations will enable us to translate the risk genes to meaningful insights on multiple myeloma pathogenesis. We first mapped the variants onto the corresponding genes with missense/nonsense mutations as one of the non-synonymous changes in the single base substitution of a different amino acid in the resulting protein. We utilized this annotation with the knowledge that functional rules of variants affect protein expression. Furthermore, we leveraged the fact that the expression quantitative trait loci (eQTL) are regions harboring nucleotides correlated with alterations in gene expression. Therefore, the variants may cause changes in gene expression in the direction of the tissues involved (i.e., our analyses focused on the whole blood). If the identified variants cause an upregulation of gene *X*, leading to an increased risk of a disease, then an inhibitor of its protein product may be considered a repositioning candidate. In addition, we applied Biological Process, Cellular Component and Molecular Function to understand relationships between diseases and biological protein networks. If the genes involved in these three biological process are related in multiple myeloma pathogenesis, then it is important to inhibit the protein. The last annotation is the Primary immuno-deficiency (PID) diseases which are innate immune diseases reported to be associated with cancer including multiple myeloma. Genes overlapping with the PID play a causal role in multiple myeloma pathogenesis. It is important to consider the multiple myeloma causal relationship and the drug target genes for multiple myeloma disease. In addition, these functional annotations have been validated by Yukinori Okada *et al* to prioritize the most likely causal gene relationships with Rheumatoid Arthritis and to find its candidate drugs. According to our analyses, we set the threshold of a biological score >= 2 to find a much higher number of genes as biological multiple myeloma genes and candidates of multiple myeloma drug targets. Our study showed that the higher the threshold of biological score applied, the smaller the number of biological genes identified, limiting the number of drug targets we could observe. (i.e., we found 1 biological multiple myeloma genes for threshold score >=*5*, *3* biological multiple myeloma genes for threshold score >=*3 and* 10 biological multiple myeloma genes for threshold score >= *2*). The more biological multiple myeloma genes we find, the more candidate drug targets for multiple myeloma drug repurposing can be identified.

Reference:

Okada Y, Wu D, Trynka G, et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature.* 2014;506(7488):376-381.

We added the information regarding table 1 as the following information.

"*We set the threshold score >= 2 from the number of functional annotations ranged from 0-6, in which each gene was assigned one point for each annotation. Those genes with one functional annotation were awarded one point (score) and those genes with a score >= 2 were classified as "biological multiple myeloma genes". Our study showed that the higher the threshold of biological score applied, the smaller the number of biological genes identified, limiting the number of drug targets we could observe. (i.e., we found 1 biological multiple myeloma genes for threshold score >=5, 3 biological multiple myeloma genes for threshold score >=3 and 10 biological multiple myeloma genes for threshold score >= 2). The more biological multiple myeloma genes we find, the more candidate drug targets for multiple myeloma drug repurposing can be identified*".

Regarding the P-Value of the genes. In this study, we begin with the leveraging of the variants associated with multiple myeloma. 72 SNPs were identified, which were obtained from the Genome wide association study (GWAS) catalog and fulfilled the inclusion criteria $p < 10^{-8}$ **(Table S1).** Next, we used HaploReg version 4.1 with criteria $r^2 > 0.8$ in the Asian population to extend the SNPs encoding the identified genes. The genomic variants associated with MM were further utilized to obtain the variants encoded these genes. We identified 2,555 SNPs that overlap with 63 genes associated with MM, and these genes were used for further analysis.

Table 1. Functional annotation applied to prioritize the biological risk genes for Multiple Myeloma

GENCODE_id	GENCODE_name	Misnon	ciseQTL	Biological Process	Cellular Component	Molecular Function	PID	Total score
ENSG00000168411	*RFWD3*	1	1	1	1	1	0	5
ENSG00000100281	*HMGXB4*	1	0	0	1	1	0	3
ENSG00000164649	*CDCA7L*	0	1	1	1	0	0	3
ENSG00000204536	*CCHCR1*	0	1	1	1	0	0	3
ENSG00000025770	*NCAPH2*	0	0	1	1	0	0	2
ENSG00000080603	*SRCAP*	0	0	0	1	1	0	2
ENSG00000100307	*CBX7*	0	1	0	1	0	0	2
ENSG00000138101	*DTNB*	0	0	0	1	1	0	2

ENSG00000156858	*PRR14*	1	1	0	0	0	0	2
ENSG00000168038	*ULK4*	1	1	0	0	0	0	2
ENSG00000182606	*TRAK1*	0	1	0	1	0	0	2
ENSG00000204525	*HLA-C*	1	1	0	0	0	0	2
ENSG00000204531	*POU5F1*	0	0	0	1	1	0	2
ENSG00000240505	*TNFRSF13B*	1	0	0	0	0	1	2

We set the threshold score >= 2 from the number of functional annotations ranged from 0-6, in which each gene was assigned one point for each annotation. Those genes with one functional annotation were awarded one point (score) and those genes with a score >= 2 were classified as “biological multiple myeloma genes”. Our study showed that the higher the threshold of biological score applied, the smaller the number of biological genes identified, limiting the number of drug targets we could observe. (i.e., we found 1 biological multiple myeloma genes for threshold score >=5, 3 biological multiple myeloma genes for threshold score >=3 and 10 biological multiple myeloma genes for threshold score >= 2). The more biological multiple myeloma genes we find, the more candidate drug targets for multiple myeloma drug repurposing can be identified

May 08, 2023

Dear Editors,

Please find our attached revised manuscript, entitled “**Identification of Druggable Genes for Multiple Myeloma Based on the Genomic Information**,” which we are submitting for consideration for publication as an original research article in *Genomics & Informatics* (**GI23011**). We are thankful for your kind encouragement regarding our manuscript. Herewith, we are sending this revised manuscript in accordance with the comments given by the reviewers. The revised parts of the manuscript are highlighted in yellow. Finally, we would like to thank you once again for giving us the opportunity to improve our manuscript. We very much hope that these revisions are adequate. We appreciate your review and assistance, and look forward to hearing from you.

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We sincerely thank the reviewer for taking the time to review our work, and have addressed the critical comments point-by-point.

A1: Thank you for your positive feedback on our study. We now revised the Table 1. In response to the reviewer's question, we would like to provide a more detailed explanations of the methodology used in our study.

In this study, we aimed to repurpose drugs for multiple myeloma by prioritizing disease-associated genes using six functional annotations. We hypothesized that multiple myeloma genetic variants prioritization these annotations would enable us to translate the risk genes to meaningful insights on multiple myeloma pathogenesis. To achieve this, we first mapped the variants onto their corresponding genes, with a focus on non-synonymous changes resulting in missense/nonsense mutations that affect protein expression. We also leveraged expression quantitative trait loci (eQTL) to identify variants that may cause changes in gene expression in relevant tissues, such as whole blood.

We used Gene Ontology (Biological Process, Cellular Component, and Molecular Function) to identify relationships between diseases and biological protein networks. If the genes involved in these processes are related to multiple myeloma pathogenesis, then inhibiting the corresponding proteins may be a viable drug repurposing strategy. We also considered primary immunodeficiency (PID), which are innate immune disease associated with cancer, including multiple myeloma. Genes overlapping with PID play a causal role in multiple myeloma pathogenesis and could be potential drug targets.

We set a threshold of a biological score >= 2 to identify candidate drug targets for multiple myeloma. Our study showed that the higher the threshold applied, the smaller the number of biological genes identified, limiting the number of drug targets. For instance, we found 1 biological multiple myeloma gene for a threshold score >=5, 3 biological multiple myeloma genes for a threshold score >=3, and 10 biological multiple myeloma genes for a threshold score >=2. The more biological multiple myeloma genes we find, the more candidate drug targets for multiple myeloma drug repurposing can be identified. These functional annotations have been validated by Yukinori Okada *et al*. to prioritize the most likely causal gene relationships with Rheumatoid Arthritis and to find its candidate drugs.

Reference:

Okada Y, Wu D, Trynka G, et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature.* 2014;506(7488):376-381.

In Table 1, we have added information on the projected function and P-value of each gene, as follows:

This study used variants associated with multiple myeloma to identify potential drug targets. Specifically, we identified 72 SNPs from the GWAS catalog that met the inclusion criteria of $p < 10^{-8}$ (**Table S1**). To further identify genes associated with multiple myeloma, we used HaploReg version 4.1 with a threshold of $r^2 > 0.8$ in the Asian population to extend the SNPs encoding the identified genes. This allowed us to identify 2.555 SNPs that overlap with 63 genes associated with multiple myeloma.

We then assigned each gene a functional annotation score ranging from 0-6, with one point awarded for each annotation. Genes with a score of >=2 were classified as "biological multiple myeloma genes". Our analysis showed that increasing the threshold score resulted in fewer biological genes identified, which could limit the number of potential drug targets for multiple myeloma drug repurposing. For instance, we found one biological multiple myeloma gene for a threshold score >=5, three genes for a threshold score >=3, and ten genes for a threshold score >=2. Identifying more biological multiple myeloma genes could increase the number of potential drug targets for drug repurposing.

Table 1. Functional annotation applied to prioritize the biological risk genes for Multiple Myeloma

GENCODE_id	GENCODE_name	Misnon	ciseQTL	Biological Process	Cellular Component	Molecular Function	PID	Total score
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ENSG00000240505	*TNFRSF13B*	1	0	0	0	0	1	2

We set the threshold score >= 2 from the number of functional annotations ranged from 0-6, in which each gene was assigned one point for each annotation. Those genes with one functional annotation were awarded one point (score) and those genes with a score >= 2 were classified as "biological multiple myeloma genes". Our study showed that the higher the threshold of biological score applied, the smaller the number of biological genes identified, limiting the number of drug targets we could observe. (i.e., we found 1 biological multiple myeloma genes for threshold score >=5, 3 biological multiple myeloma genes for threshold score >=3 and 10 biological multiple myeloma genes for threshold score >= 2). The more biological multiple myeloma genes we find, the more candidate drug targets for multiple myeloma drug repurposing can be identified

Table S1. 72 SNPs associated Multiple Myeloma obtained from GWAS Catalog

SNPs	***P-value***
rs57104699	4,00E-08
rs57104699	2,00E-08
rs6919908	6,00E-10
rs6919908	4,00E-10
rs73071352	3,00E-08
rs57968458	3,00E-10
rs57968458	6,00E-11
rs1050976	6,00E-08
rs3132535	3,00E-17
rs10936600	6,00E-15
rs1052501	4,00E-09
rs34562254	4,00E-17
rs34562254	2,00E-08
rs6595443	1,00E-08
rs4325816	7,00E-09
rs17507636	9,00E-09

rs2790457	2,00E-08
rs58618031	3,00E-08
rs7193541	5,00E-12
rs1948915	4,00E-11
rs11086029	7,00E-11
rs13338946	1,00E-13
rs2811710	2,00E-13
rs877529	1,00E-09
rs56219066	4,00E-08
rs10936599	3,00E-08
rs2285803	1,00E-11
rs1423269	2,00E-11
rs9372120	9,00E-15
rs138740	6,00E-08
rs6746082	2,00E-10
rs7781265	1,00E-08
rs138747	3,00E-08
rs7781265	3,00E-10
rs139402	5,00E-26
rs7577599	1,00E-16
rs56219066	2,00E-10
rs56219066	1,00E-09
rs6599192	9,00E-18
rs4487645	5,00E-15
rs6066835	1,00E-13
rs4273077	3,00E-14
rs1052501	2,00E-08
rs200203825	8,00E-12
rs139371	2,00E-09
rs34229995	1,00E-08
rs4487645	3,00E-14
rs200203825	3,00E-10
rs4487645	1,00E-09
rs2272007	2,00E-09
rs6599175	1,00E-09
rs603965	8,00E-11
rs72773978	7,00E-09
rs603965	2,00E-11
rs2285803	1,00E-10
rs877529	8,00E-16
rs4273077	8,00E-09
rs10936599	9,00E-14
rs12711846	3,00E-14

rs4525246	3,00E-14
rs210143	7,00E-12
rs6763508	8,00E-12
rs12638862	2,00E-11
rs6546149	6,00E-10
rs11715604	2,00E-09
rs9392017	6,00E-09
rs9880772	7,00E-09
rs1875968	9,00E-09
rs51471313	4,00E-08
rs4916473	5,00E-08
rs2720680	7,00E-08
rs131821	7,00E-08

Rahmat Dani Satria[1,2], Lalu Muhammad Irham[3,6]*, Wirawan Adikusuma[4,5]*, Anisa Nova Puspitaningrum[3], Arief Rahman Afief[3], Riat El Khair[1,2], Abdi Wira Septama[6]

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[2]*Clinical Laboratory Installation, Dr. Sardjito Central General Hospital, Yogyakarta 55281, Indonesia*

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Abstract

Multiple myeloma (MM) is a type of malignancy in hematology. Genetics is believed to be involved in MM development. Several studies have been conducted to clarify the genetics involved in MM. However, the use of genomic information for clinical purposes, both for diagnostic and prognostic biomarkers, is still limited in research. This research used genetic information reported in the genetic database for clinical trial studies on MM (Genomic Driven Clinical Implementation for Multiple Myeloma). Genetic information was collected from the Genome-Wide Association Studies (GWAS) catalog database. We prioritized genes that have the potential to cause MM disease based on established annotations. Furthermore, we prioritized biological risk genes for MM for drug target candidates. The DrugBank database was used to identify drug candidates with drug target genes. We discovered 14 MM biological

risk genes and identified 10 drugs targeting three genes. Remarkably, only 1 out of 10 drugs, panobinostat has been approved for use in MM. Additionally, the two most promising genes, Calcium signal-modulating cyclophilin ligand (*CAMLG*), and Histone deacetylase 2 (*HDAC2*) were targeted by four drugs: cyclosporine, belinostat, vorinostat, and romidepsin with clinical evidence in the treatment of MM. Notably, there are 5 out of 10 drugs that have been approved for other indications which have not been reported for MM but may be also used for the treatment of MM. Accordingly, this study aimed to elucidate the genomic variants involved in the pathogenesis of MM and provide the benefits of genomic variants that can contribute to drug discovery.

Keywords: Multiple Myeloma, genomic variants, biological risk genes, drug repositioning

Introduction

Multiple Myeloma (MM) is a hematological malignancy caused by the uncontrolled proliferation of abnormal plasma cells in the bone marrow (BM). This abnormal proliferation of plasma cells causes damage to multiple organs throughout the body and manifests systemically. Systemic manifestations of MM include hypercalcemia, renal failure, anemia and bone lytic lesions [1], [2]. Over time, the number of MM cases is reported to be increasing. In 2020 the reported incidence of MM was 160,000 cases with 106,000 deaths [3]. This high mortality rate indicates that most of these MM cases end in death. In fact, preventing the worsening progression of the disease toward a poor prognosis requires an effective diagnostic tool to detect the disease at an early stage. Currently, the diagnosis of MM uses bone marrow (BM) analysis to determine the percentage of plasma cells in the BM and serum protein electrophoresis for M-band and urinary Bence-Jones protein followed by the use of beta-2 microglobulin and serum albumin to determine the stage of MM [4], [5]. However, the use of these diagnostic tools is still not sufficient to detect the early stages of the MM disease, and most cases are detected in the late stages.

Recently, more accurate diagnostic tools have been developed to establish the MM diagnosis and prognosis. Karyotyping identification is one of the tools used to determine the prognosis and therapy of this disease [6]–[8]. However, the use of karyotyping is still not adequate because it can only detect abnormalities at the chromosomal level, and not at the gene level. Genomic detection is expected to

detect early disease development before it progresses in a worse direction, and it is used to determine the accuracy of therapy. It can even be used for drug repurposing.

The Genome-Wide Association Study (GWAS) is one of genomic databases used to catalog the genomic variants associated with various diseases including for MM. Although GWAS data have provided valuable biological insights of the genomic variants associated with many diseases, however, the translation into the clinic situation has remained limited. Therefore, our study aimed at integrating the genomic variants from GWAS and the bioinformatics-based approach to drive more practical biological insights for MM treatment.

Methods

Study design

We started by identifying the genomic variants associated with MM or MM-associated single-nucleotide polymorphisms (SNPs) using data from the GWAS Catalog with criteria p value < 10^{-8}. Next, we obtained more SNPs which are known to encode these genes by utilizing the HaploReg version 4.1 in the Asian (ASN) population from the 1000 Genome Project Phase I data. In order to identify biological MM risk genes, we further utilized a genomic-driven drug repurposing approach based on the established criteria. These genes have been proposed as potential MM treatment targets. Finally, we determined the prospective drugs where the mechanisms and therapeutic targets overlapped.

Multiple myeloma risk genes

After widening the search using HaploReg version 4.1, SNPs encoding the genes were further examined to pinpoint the biological MM risk genes. In order to identify genes with greater likelihood and more solid supporting data, we strictly annotated the biological risk genes. The biological MM-risk genes were ranked in this study using six criteria. Each criterion-compliant gene received one point (maximum six points per gene). Genes with higher scores have greater potential as biological risk genes. We applied the following six criteria to filter the biological MM risk genes: (1) missense mutation, HaploReg version 4.1 annotated missense mutations in genes containing MM risk SNPs with linkage disequilibrium (r^2 > 0.80); (2) Cis expression quantitative trait loci (*cis*-eQTL), MM risk SNP-containing genes with notable *cis*-eQTL effects in whole blood; (3) Biological process; (4) Cellular component; (5) Molecular function. Criteria 3, 4, and 5 are included in the Gene Ontology (GO) category. Genes were prioritized by using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) online tool version 6.8 accessed at: (https://david-d.ncifcrf.gov/tools.jsp) [9]. Finally, we added 6) Primary Immunodeficiency

(PID): The PID was the final annotation to prioritize the MM risk genes. The International Union of Immunological Societies (IUIS) collected PID genes until 2013 [10]. A hypergeometric test was used to analyze the data for enrichment with a *p*-value of 0.05 considered significant.

Discovering new candidate drugs for multiple myeloma

The scoring system derived from the six criteria was used to prioritize biological MM risk genes. Genes with scores greater than or equal to 2 were regarded as biological MM risk genes. Unfortunately, there are only a couple few druggable drug target genes. Therefore, we further broadened the biological MM risk genes utilizing the STRING database (https://string-db.org/) accessed on September 12, 2022. After completing gene expansion based on protein-protein interactions (PPIs) information from the STRING database, we conducted the overlapping analysis using the DrugBank database accessed on September 12, 2022. In addition to these steps, to validate the finding, we used ClinicalTrial.gov (https://clinicaltrials.gov/; accessed on September 13, 2022) to check whether the drug target genes were undergoing clinical trials. We also used PubMed mining (https://pubmed.ncbi.nlm.nih.gov/; accessed on September 13, 2022) to check whether the candidate drugs were undergoing preclinical investigation.

Statistical analysis

Analytic workflows were performed using RStudio version 4.2.1 (RStudio, 250 Northern Ave, Boston, MA 02210). The haploR package was used to identify missense variants and Cis-eQTL (https://cran.r-project.org/web/packages/haploR/index.html). GO enrichment analyses, including BP, CC and MF were performed using the RDAVIDWebService, which is available as an *R* package from the Bioconductor project (www.bioconductor.org) [11].

Results

Identification of multiple myeloma-associated genes

In this study, 72 SNPs were identified, which were obtained from the GWAS catalog and fulfilled the inclusion criteria $p < 10^{-8}$ **(Table S1).** Next, we used HaploReg version 4.1 with criteria $r^2 > 0.8$ in the Asian population to extend the SNPs encoding the identified genes. The genomic variants associated with MM were further utilized to obtain the variants encoded these genes. We identified 2,555 SNPs that overlap with 63 genes associated with MM, and these genes were used for further analysis.

Identification of multiple myeloma biologic risk gene with functional annotation criteria

We used the six functional annotation criteria to prioritize genes at risk for the pathogenesis of MM with a scoring system for each gene if they met each criterion. Genes with missense variants (n=11); gene with cis-eQTL effect (n=19); genes that are prioritized by biological process (n=4); genes prioritized by cellular component (n=11), genes prioritized by molecular function (n=5), and genes prioritized by PID (n=2) **(Figure 2)**. The detailed information regarding the scoring system for each functional annotation is depicted in **Figure 3**. We found that out of 63 genes, 14 of them had a score of 2 or more and were categorized as MM biological risk genes. The top four genes are prioritized as the most biological risk genes because they have a score of 3 or more out of 6, including *RFWD3, HMGXB4, CDCA7L,* and *CCHCR1* **(Table 1)**. Furthermore, we expanded the 14 MM biological risk genes using the STRING database to derive more drug-targeted genes. In this step, we found 336 gene pairs of the protein-protein interaction network in the STRING database **(Table s2)**.

Candidates of Drug Repurposing for Multiple Myeloma

To identify genes targeted by drug candidates, we used the DrugBank database. Notably, not all genes that have targeted drugs have pharmacological activity. Remarkably, we identified 10 drugs that target 3 genes that are at risk for MM, and these drugs have been approved for use in other diseases **(Figure 4)**. There is only 1 among these 10 drugs, panobinostat, which is identified as an approved drug for MM, while 4 drugs are under clinical examination for MM, and 5 drugs have not been reported to treat MM.

This study focuses on drugs that have been approved based on clinical trials using the ClinicalTrial.gov database. Therefore, the target genes of the four drugs: cyclosporine (NCT04813653), belinostat (NCT00131261), vorinostat (NCT01502085), and romidepsin (NCT00765102) which are

currently under clinical examination are considered the most promising target genes for MM. We identified two targeted genes, including Calcium signal-modulating cyclophilin ligand (*CAMLG*), and Histone deacetylase 2 (*HDAC2*). Among 5 new candidate drugs, 4 of them target the most promising targeted genes including, theophylline, aminophylline, oxtriphylline, and tixocortol, which may be also used for MM. The findings of this study emphasized that the human genomic variants not only drive the disease risk loci but also can drive novel biological insights for drug repurposing for MM.

Discussion

In this study, we extracted 72 SNPs associated with MM from the GWAS catalog database with inclusion criteria $p < 10^{-8}$ to search for candidate genes that have potential for drug reuse for MM treatment. Six functional annotations were used to assess and prioritize MM risk genes that may be associated with new drug targets. We found three drug target genes associated with 10 drugs. Among these 10 drugs, panobinostat is the only identified drug approved for MM, while there are 4 drugs under clinical examination for MM, and 5 drugs which have not been reported to treat MM. There are 2 genes (*CAMLG* and *HDAC2*) targeted by 4 drugs: cyclosporine (NCT04813653), belinostat (NCT00131261), vorinostat (NCT01502085), and romidepsin (NCT00765102) which are currently under clinical examination. Presently, *CAMLG* and *HDAC2* are considered the most promising target genes for MM treatment that have been studied and approved based on clinical trials using the ClinicalTrial.gov database.

Cyclosporine has been shown to be an immunosuppressive agent used to treat postoperative organ rejection [12]. A study conducted by Sonneveld *et al.* in 1994 demonstrated that cyclosporin can be used clinically to modulate multi-drug resistance (MDR) in patients with MM to vincristine, doxorubicin, and dexamethasone [13]. Among several target genes that have been identified, belinostat, vorinostat and romidepsin have been shown to be antineoplastic agents [14]–[16]. Belinostat and vorinostat are histone deacetylase (HDAC) inhibitors belonging to the hydroxamate group with the mechanism of stopping growth, affecting cell differentiation and producing malignant cell apoptosis [15].

In a clinical study conducted by Plumb *et al.* in 2003, belinostat was shown to have antitumor activity *in vitro* and *in vivo* studies against tumor cells [17]. Vorinostat is used in the FDA-approved management of cutaneous T-cell lymphoma (CTCL) [15]. In addition, other studies have shown that vorinostat inhibits tumor growth, breast cancer, and lung cancer [18]–[20]. Romidepsin is also a new FDA-approved drug for the treatment of CTLC [21]. This was demonstrated in phase II studies with patients with recurrent or refractory CTLC, showing an overall response rate of 34-35% [22].

Drug repurposing has the advantage of exploiting gene variations by using the GWAS catalog database to determine potential new drug candidates for MM [23]. However, this research has limitations, including in this study, not all of the identified target genes had pharmacological activity. Thus, the identified genes could potentially miss the drug targets that have been found for MM. Therefore, further research is needed to verify the candidate drug effects in clinical applications in MM disease.

Conclusions

By utilizing the GWAS catalog database to map disease-gene-protein-drug relationships, we discovered three drug target genes that may be potential candidates for new drugs in the treatment of MM. We found 10 potential drug candidates for MM, and remarkably, there was only 1 identified drug approved for MM, panobinostat. Among the identified targets, 4 drugs are under clinical examination for MM, and 5 drugs have not been reported to treat MM. In the study, it was found that the two top biological MM risk genes were *CAMLG* and *HDAC2*. The evidence supports the possibility that these genes are significantly associated with MM, so further translational research is needed. Drug repurposing offers many advantages in the drug development process, such as shorter time required, lower costs, and higher success rates. In this study, we combined a drug repurposing approach with an integrative research methodology to identify drugs with new indications for MM.

Declaration of Competing Interest

The authors disclose no conflict of interest

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Table 1. Functional annotation applied to prioritize the biological risk genes for Multiple Myeloma

GENCODE_id	GENCODE_name	Misnon	ciseQTL	Biological	Cellular Component	Molecular	PID	Total score

ENSG00000168411	*RFWD3*	1	1	1	1	1	0	5
ENSG00000100281	*HMGXB4*	1	0	0	1	1	0	3
ENSG00000164649	*CDCA7L*	0	1	1	1	0	0	3
ENSG00000204536	*CCHCR1*	0	1	1	1	0	0	3
ENSG00000025770	*NCAPH2*	0	0	1	1	0	0	2
ENSG00000080603	*SRCAP*	0	0	0	1	1	0	2
ENSG00000100307	*CBX7*	0	1	0	1	0	0	2
ENSG00000138101	*DTNB*	0	0	0	1	1	0	2
ENSG00000156858	*PRR14*	1	1	0	0	0	0	2
ENSG00000168038	*ULK4*	1	1	0	0	0	0	2
ENSG00000182606	*TRAK1*	0	1	0	1	0	0	2
ENSG00000204525	*HLA-C*	1	1	0	0	0	0	2
ENSG00000204531	*POU5F1*	0	0	0	1	1	0	2
ENSG00000240505	*TNFRSF13B*	1	0	0	0	0	1	2

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Identification of druggable genes for multiple myeloma based on genomic information

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Multiple myeloma (MM) is a hematological malignancy. It is widely believed that genetic factors play a significant role in the development of MM, as investigated in numerous studies. However, the application of genomic information for clinical purposes, including diagnostic and prognostic biomarkers, remains largely confined to research. In this study, we utilized genetic information from the Genomic-Driven Clinical Implementation for Multiple Myeloma database, which is dedicated to clinical trial studies on MM. This genetic information was sourced from the genome-wide association studies catalog database. We prioritized genes with the potential to cause MM based on established annotations, as well as biological risk genes for MM, as potential drug target candidates. The DrugBank database was employed to identify drug candidates targeting these genes. Our research led to the discovery of 14 MM biological risk genes and the identification of 10 drugs that target three of these genes. Notably, only one of these 10 drugs, panobinostat, has been approved for use in MM. The two most promising genes, calcium signal-modulating cyclophilin ligand (*CAMLG*) and histone deacetylase 2 (*HDAC2*), were targeted by four drugs (cyclosporine, belinostat, vorinostat, and romidepsin), all of which have clinical evidence supporting their use in the treatment of MM. Interestingly, five of the 10 drugs have been approved for other indications than MM, but they may also be effective in treating MM. Therefore, this study aimed to clarify the genomic variants involved in the pathogenesis of MM and highlight the potential benefits of these genomic variants in drug discovery.

Keywords: biological risk genes, drug repositioning, genomic variants, multiple myeloma

Introduction

Multiple myeloma (MM) is a hematological malignancy characterized by the uncontrolled proliferation of abnormal plasma cells in the bone marrow (BM). This abnormal growth of plasma cells inflicts damage on multiple organs throughout the body, resulting in systemic manifestations. These manifestations include hypercalcemia, renal failure,

anemia, and bone lytic lesions [1,2]. The number of MM cases has been reported to be on the rise. In 2020, the reported incidence of MM was 160,000 cases, with 106,000 resulting in death [3]. This high mortality rate suggests that the majority of MM cases are fatal.

Therefore, to prevent a poor prognosis, it is crucial to have an effective diagnostic tool that can detect the disease at an early stage. Currently, the diagnosis of MM involves a BM analysis to determine the percentage of plasma cells in the BM. This is followed by serum protein electrophoresis for M-band and urinary Bence-Jones protein detection. Subsequently, β-2 microglobulin and serum albumin are used to determine the stage of MM [4,5]. However, these diagnostic tools have proven insufficient for detecting the early stages of MM, with most cases only being identified in the late stages.

More accurate diagnostic tools have recently been developed for the diagnosis of MM and the prediction of its prognosis. One such tool is karyotyping identification, which is utilized to determine the prognosis and treatment plan for this disease [6-8]. However, the application of karyotyping has its limitations, as it can only detect abnormalities at the chromosomal level, not at the gene level. Genomic detection, on the other hand, holds promise for identifying early disease development before it worsens, and it is employed to determine the effectiveness of therapy. Furthermore, it can even be utilized for drug repurposing.

The genome-wide association study (GWAS) Catalog is a database containing the genomic variants associated with various diseases, including MM. While GWAS data have provided valuable biological insights into the genomic variants associated with many diseases, the translation of these insights into clinical situations has remained limited. Therefore, our study aimed to integrate the genomic variants from the GWAS catalog with a bioinformatics-based approach to derive more practical biological insights for MM treatment.

Methods

Study design

We began by identifying the genomic variants or single-nucleotide polymorphisms (SNPs) associated with MM using data from the GWAS catalog, with the criterion of a p-value < 10^{-8}. Subsequently, we obtained additional SNPs known to encode these genes by leveraging HaploReg version 4.1, focusing on Asian population data from the 1000 Genome Project Phase I. To identify potential MM risk genes, we employed a genomic-driven drug repurposing approach based on established criteria. These genes have been suggested as potential targets for MM treatment. Lastly, we identified prospective drugs where the mechanisms and therapeutic targets intersected.

MM risk genes

After widening the search parameters with HaploReg version 4.1, we further scrutinized SNPs that encoded genes to identify the biological MM risk genes more precisely. To pinpoint genes with a higher probability and more robust supporting data, we meticulously annotated the biological risk genes. In this study, we ranked the biological MM-risk genes using six distinct criteria. Each gene that met a criterion was awarded 1 point, with a maximum of 6 points per gene. Genes with higher scores were considered to have a greater potential as biological risk genes. We employed six criteria to filter the biological MM risk genes. The first five were as follows: (1) missense mutation, where HaploReg version 4.1 annotated missense mutations in genes containing MM risk SNPs with linkage disequilibrium ($r^2 > 0.80$); (2) cis expression quantitative trait loci (cis-eQTL), where MM risk SNP-containing genes exhibited significant cis-eQTL effects in whole blood; (3) biological processes; (4) cellular components; and (5) molecular functions. Criteria 3, 4, and 5 relate to Gene Ontology (GO) categories. We prioritized genes using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) online tool version 6.8 (https://david-d.ncifcrf.gov/tools.jsp) [9]. The sixth criterion was primary immunodeficiency (PID), which was the final annotation used to prioritize the MM risk genes. The International Union of Immunological Societies (IUIS) has compiled PID genes until 2013 [10]. A hypergeometric test was used to analyze the data for enrichment, with a p-value of 0.05 considered significant.

Discovering new candidate drugs for MM

We utilized a scoring system derived from six criteria to prioritize potential biological MM risk genes. Any genes with scores of 2 or higher were considered candidates. Regrettably, there are only a few druggable target genes. To address this, we expanded our search for biological MM risk genes using the STRING database (https://string-db.org/), accessed on September 12, 2022. After expanding our gene pool based on protein-protein interaction information from the STRING database, we performed an overlap analysis using the DrugBank database, also accessed on September 12, 2022. To validate our findings, we used ClinicalTrials.gov (https://clinicaltrials.gov/; accessed on September 13, 2022) to verify whether the drug target genes were currently under clinical trials. Additionally, we conducted PubMed mining (https://pubmed.ncbi.nlm.nih.gov/; accessed on September 13, 2022) to

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ascertain whether the candidate drugs were under preclinical investigation.

Statistical analysis

Analytic workflows were executed using RStudio version 4.2.1 (RStudio, Boston, MA, USA). The haploR package was utilized to identify missense variants and cis-eQTL (https://cran.r-project.org/web/packages/haploR/index.html). GO enrichment analyses, encompassing biological processes, cellular components, and molecular function, were conducted using the RDAVIDWebService. This service is accessible as an R package from the Bioconductor project (www.bioconductor.org) [11].

Results

Identification of multiple myeloma-associated genes

In this study, we identified 72 SNPs from the GWAS catalog that met the inclusion criteria of $p < 10^{-8}$ (Supplementary Table 1). We then utilized HaploReg version 4.1, applying a criterion of $r^2 > 0.8$ within the Asian population, to expand the SNPs encoding the identified genes. The genomic variants associated with MM were subsequently used to derive the variants encoding these genes. This process led to the identification of 2,555 SNPs that overlapped with 63 genes associated with MM. These genes were then used for further analysis.

Identification of MM biological risk genes with functional annotation criteria

We utilized six functional annotation criteria to identify genes potentially implicated in the pathogenesis of MM. Each gene was scored based on whether it met each criterion. The criteria included genes with missense variants (n=11), genes with a cis-eQTL effect (n=19), genes categorized as involving a biological process (n=4), genes categorized as involving a cellular component (n=11), genes categorized as involving a molecular function (n=5), and genes categorized as related to PID (n=2) (Fig. 2). Detailed information about the scoring system for each functional annotation is illustrated in Fig. 3. Out of 63 genes, we found that 14 had a score of 2 or more and were thus classified as MM biological risk genes. The top four genes, *RFWD3*, *HMGXB4*, *CDCA7L*, and *CCHCR1*, were identified as the most significant biological risk genes due to their score of 3 or more out of 6 (Table 1). We further expanded our analysis of the 14 MM biological risk genes using the STRING database to identify additional drug-targeted genes. This process yielded 336 gene pairs from the protein-protein interaction network in the STRING database (Supplementary Table 2).

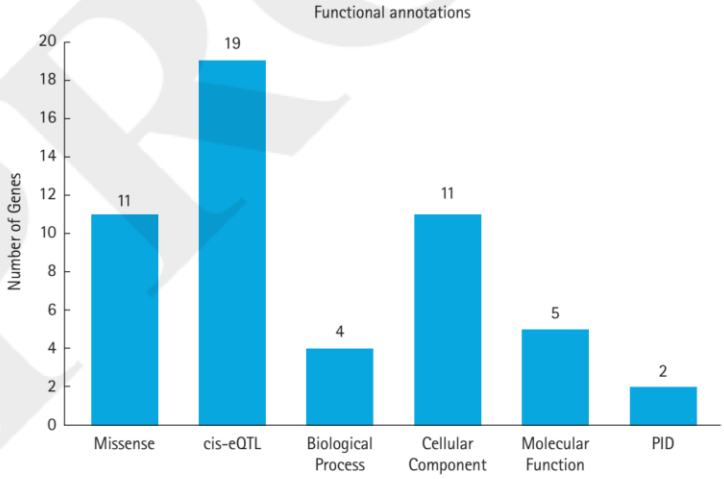

Fig. 2. Several functional annotations to prioritize the biological risk genes for multiple myeloma. cis-eQTL, cis expression quantitative trait loci; PID, primary immunodeficiency.

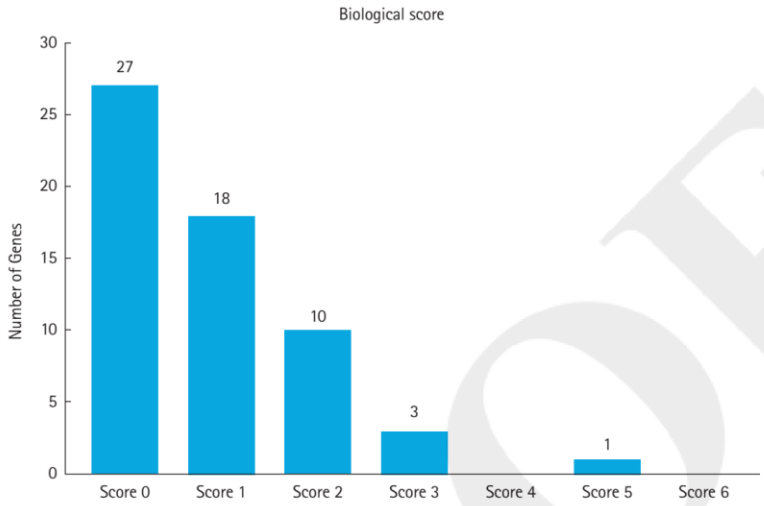

Fig. 3. Scoring system for each functional annotation applied.

Table 1. Functional annotations applied to prioritize the biological risk genes for multiple myeloma

GENCODE_id	GENCODE_name	Missense	Cis-eQTL	Biological process	Cellular component	Molecular function	PID	Total score
ENSG00000168411	*RFWD3*	1	1	1	1	1	0	5
ENSG00000100281	*HMGXB4*	1	0	0	1	1	0	3
ENSG00000164649	*CDCA7L*	0	1	1	1	0	0	3
ENSG00000204536	*CCHCR1*	0	1	1	1	0	0	3
ENSG00000025770	*NCAPH2*	0	0	1	1	0	0	2
ENSG00000080603	*SRCAP*	0	0	0	1	1	0	2
ENSG00000100307	*CBX7*	0	1	0	1	0	0	2
ENSG00000138101	*DTNB*	0	0	0	1	1	0	2
ENSG00000156858	*PRR14*	1	1	0	0	0	0	2
ENSG00000168038	*ULK4*	1	1	0	0	0	0	2
ENSG00000182606	*TRAK1*	0	1	0	1	0	0	2
ENSG00000204525	*HLA-C*	1	1	0	0	0	0	2
ENSG00000204531	*POU5F1*	0	0	0	1	1	0	2
ENSG00000240505	*TNFRSF13B*	1	0	0	0	0	1	2

We established a threshold score of ≥2 from a range of functional annotations numbered from 0 to 6. Each gene was assigned one point for each annotation. Genes with a single functional annotation received one point (score), and those with a score of ≥2 were categorized as "biological multiple myeloma genes". Our research indicated that as the threshold of the biological score increased, the quantity of identified biological genes decreased, thereby reducing the number of observable drug targets. For instance, we identified 1 biological multiple myeloma gene for a threshold score of ≥5, 3 biological multiple myeloma genes for a threshold score of ≥3, and 10 biological multiple myeloma genes for a threshold score of ≥2. The more biological multiple myeloma genes we discover, the more potential drug targets for multiple myeloma drug repurposing we can identify.
PID, primary immunodeficiency; cis-eQTL, cis expression quantitative trait loci.

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Candidates for drug repurposing to treat multiple myeloma

To identify genes targeted by potential drug candidates, we utilized the DrugBank database. It is important to note that not all drugs that target these genes exhibit pharmacological activity. We identified 10 drugs targeting three genes associated with an increased risk for MM. These drugs have already received approval for use in treating other diseases (Fig. 4). Among these 10 drugs, only panobinostat is recognized as an approved drug for MM. Meanwhile, four drugs are currently undergoing clinical trials for MM, and five drugs have not yet been investigated as treatments for MM.

This study focused on drugs that have received approval based on clinical trials, as documented in the ClinicalTrial.gov database. Consequently, the target genes of four drugs currently under clinical investigation—cyclosporine (NCT04813653), belinostat (NCT00131261), vorinostat (NCT01502085), and romidepsin (NCT00765102)—were deemed the most promising for MM treatment. We identified two such target genes: calcium signal-modulating cyclophilin ligand (*CAMLG*) and histone deacetylase 2 (*HDAC2*). Of the five new candidate drugs, four—namely, theophylline, aminophylline, oxtriphylline, and tixocortol—target these promising genes and may also be applicable for MM treatment. The results of this study underscore that human genomic variants not only influence disease risk loci, but can also provide new biological insights for drug repurposing in MM treatment.

Discussion

In this study, we extracted 72 SNPs associated with MM from the GWAS catalog database, using an inclusion criterion of $p < 10^{-8}$ to search for candidate genes with the potential for drug reuse for MM treatment. We utilized six functional annotations to evaluate and prioritize MM risk genes that could be associated with new drug targets. Our findings revealed three genes targeted by 10 drugs. Of these 10 drugs, panobinostat is the only one currently

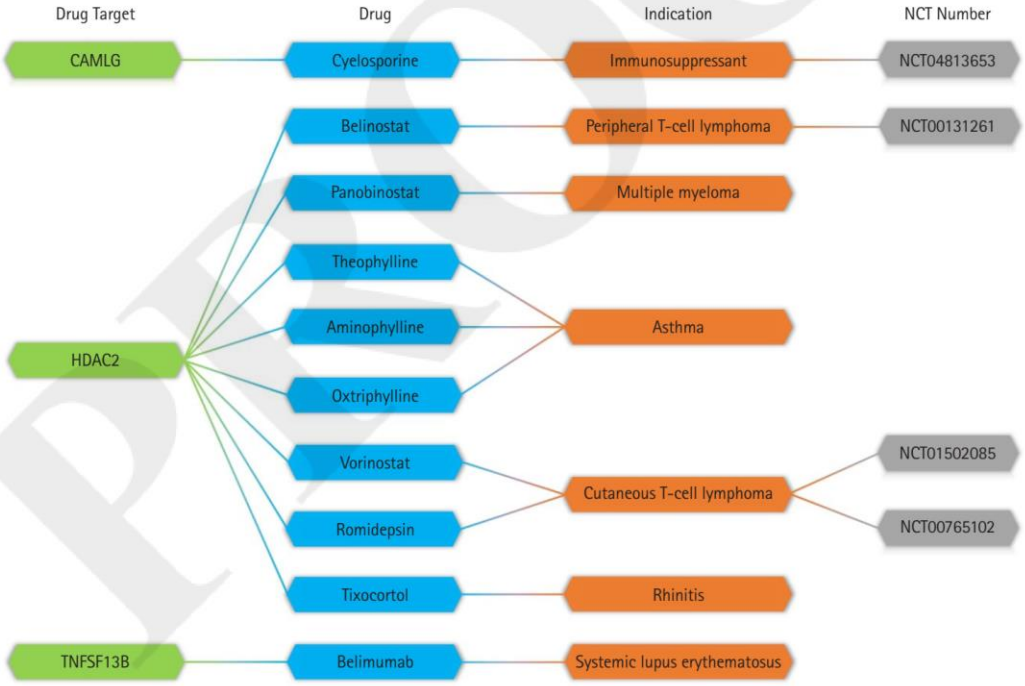

Fig. 4. Connections between drug targets and drug candidates for multiple myeloma. CAMLG, calcium signal-modulating cyclophilin ligand; HDAC2, histone deacetylase 2.

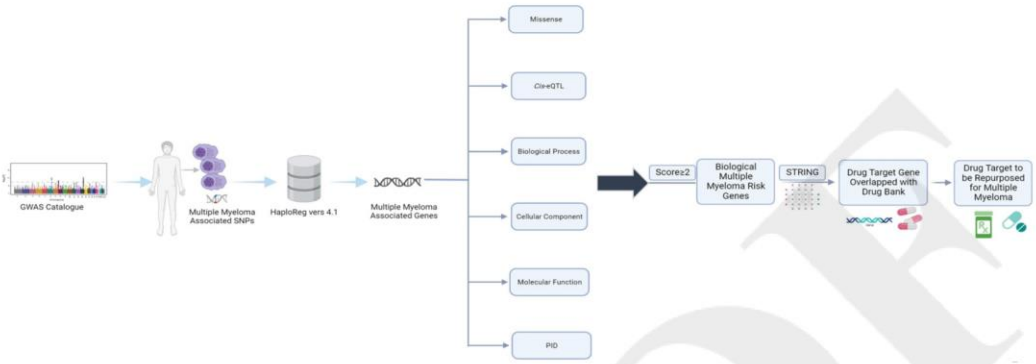

Fig. 1. Process of identification for multiple myeloma single nucleotide polymorphisms (SNPs) and the encoded genes driven drug repositioning for multiple myeloma. PID, primary immunodeficiency.

approved for MM treatment. Meanwhile, four drugs are under clinical investigation for MM, and five drugs have not yet been reported for MM treatment. Two genes, *CAMLG* and *HDAC2*, are targeted by four drugs: cyclosporine (NCT04813653), belinostat (NCT00131261), vorinostat (NCT01502085), and romidepsin (NCT00765102), all of which are currently under clinical investigation. At present, *CAMLG* and *HDAC2* are considered the most promising target genes for MM treatment, as determined by studies and approvals based on clinical trials from the ClinicalTrial.gov database.

Cyclosporine has been shown to be an immunosuppressive agent used in the treatment of postoperative organ rejection [12]. A study by Sonneveld et al. in 1994 [13] demonstrated the clinical utility of cyclosporin in modulating multi-drug resistance in patients with MM, specifically to vincristine, doxorubicin, and dexamethasone. Several target genes have been identified, with belinostat, vorinostat, and romidepsin shown to be effective antineoplastic agents [14-16]. Both belinostat and vorinostat are HDAC inhibitors from the hydroxamate group. Their mechanism of action includes inhibiting growth, influencing cell differentiation, and inducing apoptosis in malignant cells [15].

A clinical study conducted by Plumb et al. in 2003 [17] demonstrated that belinostat exhibits antitumor activity against tumor cells in both *in vitro* and *in vivo* studies. Vorinostat is utilized in the U.S. Food and Drug Administration (FDA)- approved treatment of cutaneous T-cell lymphoma (CTCL) [15]. Furthermore, various studies have indicated that vorinostat can inhibit the growth of tumors, as well as breast and lung cancers [18-20]. Romidepsin is another newly FDA-approved drug for the treatment of CTCL [21]. This was evidenced in phase II studies involving patients with recurrent or refractory CTCL, which showed an overall response rate of 34%–35% [22].

Drug repurposing offers the benefit of exploiting gene variations, utilizing the GWAS catalog database to identify potential new drug candidates for MM [23]. However, this research is not without limitations. In this study, not all the identified target genes exhibited pharmacological activity. Consequently, the identified genes may potentially overlook drug targets previously discovered for MM. Therefore, additional research is necessary to confirm the effects of these candidate drugs in clinical applications for MM disease.

By utilizing the GWAS catalog database to map the relationships between diseases, genes, proteins, and drugs, we identified three drug target genes that could potentially serve as candidates for new MM treatments. We discovered 10 potential drug candidates for MM, and notably, only one approved drug for MM, panobinostat, was identified. Among the targets identified, four drugs are currently undergoing clinical trials for MM, while five drugs have not been reported as MM treatments. Our study revealed that the two most significant biological risk genes for MM are calcium signal-modulating cyclophilin ligand (*CAMLG*) and histone deacetylase 2 (*HDAC2*). The evidence suggests a significant association between these genes and MM, warranting further translational research. Drug repurposing presents numerous advantages in the drug development process, including reduced time and costs, and increased success rates. In this study, we merged a drug repurpos-

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ing approach with an integrative research methodology to identify drugs with new potential applications for MM.

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Writing – review & editing: RDS, LMI, WA, ANP, ARF, AWS, REK.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Supplementary Materials

Supplementary data can be found with this article online at http://www.genominfo.org.

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