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Integration of genomic variants and bioinformatic-based approach to drive drug repurposing for multiple sclerosis

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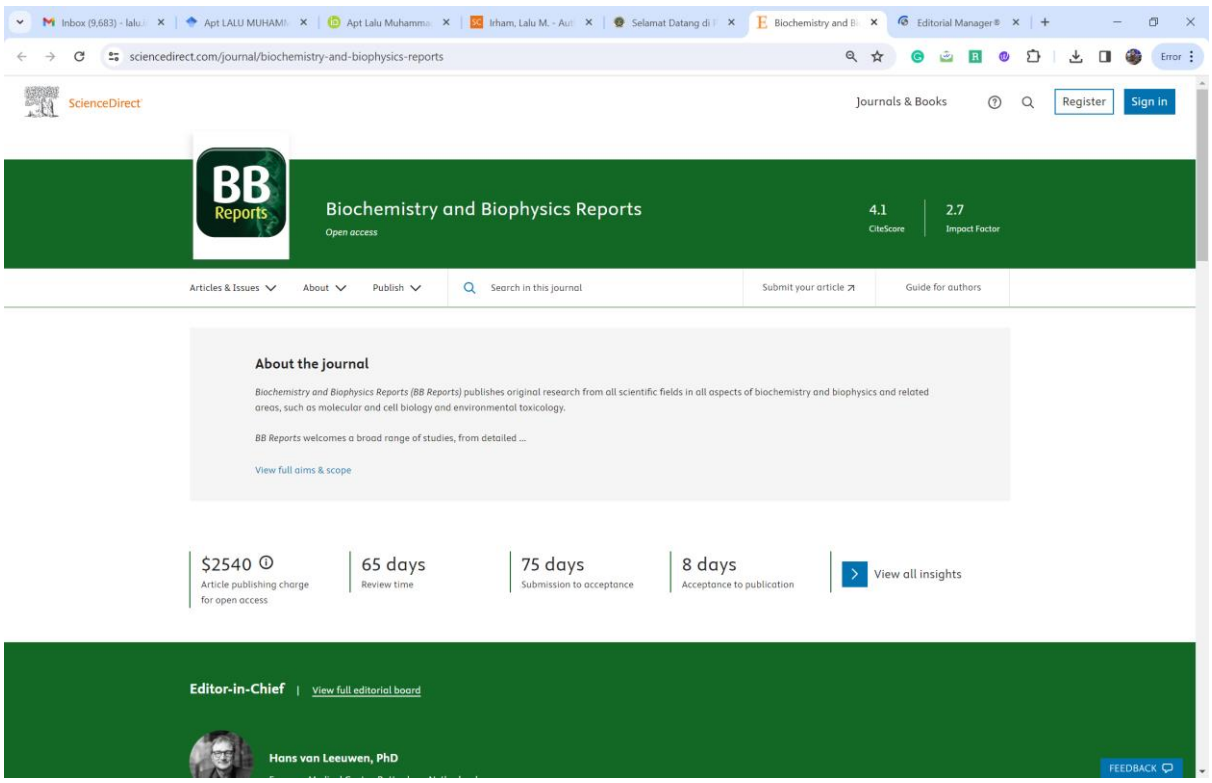

Biochemistry and Biophysics Reports

Integration of Genomic Variants and Bioinformatic Based Approach to Drive Drug Repurposing for Multiple Sclerosis

--Manuscript Draft--

Manuscript Number:	BBREP-D-22-00416R1
Article Type:	Research Paper
Keywords:	multiple sclerosis; drug repurposing; autoimmune; Genome; Bioinformatics; genetic variation
Corresponding Author:	Muhammad Irham, Ph.D INDONESIA
First Author:	Arief Rahman Afief
Order of Authors:	Arief Rahman Afief
	Ialu Muhammad Irham, M.Sc
	Wirawan Adikusuma
	Dyah Aryani Perwitasari
	Ageng Brahmadhi
	Rocky Cheung
Abstract:	Multiple sclerosis (MS) is a chronic disease that attacks the central nervous system (CNS) with symptoms of inflammation, demyelination, and axonal loss. Currently, available MS drugs have limitations, thereby calling for a strategy to speed up new drug discovery. The concept of drug repurposing, using existing drugs for new indications, has the potential for use in MS. The genetic factor is inseparable from MS disease. This genomic application might offer new insight into the disease's pathobiology and an expected to be of great use for new drug discovery. This research employed MS-associated single nucleotide polymorphisms (SNPs) extracted from the GWAS catalog (p-value < 5x10-8). We identified 421 MS-associated SNPs, and after expansion HaploReg v4.1 under the criteria r2 > 0.8, a total of 427 risk genes associated with MS an identified. MS the risk genes are then prioritized using bioinformatics analysis to identify biological MS risk genes, namely missense mutation, cis-expression quantitative trait locus (cis -eQTL), molecular pathway analysis, protein-protein interaction (PPI), overlap knockout mouse phenotype, and primary immunodeficiency (PID). We developed them based on the STRING for molecular interactions to find drug-target genes. As many as 144 biological MS risk genes were found and later mapped into 194 genes within an expanded PPI network. According to DrugBank and Therapeutic Target Database, 27 genes within the list the targeted by 68 new candidate drugs. In this analysis, we discovered one drug recognized and approved for clinical medication in MS named dimethyl fumarate. Based on data from ClinicalTrials.gov, eight drugs with nine drug-target genes are likely to have the potential for MS with clinical or pre-clinical evidence.
Suggested Reviewers:	Rahmat Dhani, Ph.D Taipei Medical University rahmat.dani.s@ugm.ac.id The candidate of reviewer expert in autimmune disease including for multiple sclerosis
	Ichtiarini NurullitaSantri, Ph.D Ahmad Dahlan University Ichtiarini.ns@gmail.com The Candidate of Reviewer is expert in Bioinformatic
	Nova Yuli Prasetyo, Ph.D Taipei Medical University d151109002@tmu.edu.tw The Candidate reviewer is expert in drug discovery

Cover Letter

August 25, 2022

Dear Editors,

Please find our attached manuscript entitled "Integration of Genomic Variants and Bioinformatic Based Approach to Drive Drug Repurposing for Multiple Sclerosis," which we submitted for consideration for publication as an Original Research article in ***Biochemistry and Biophysics Reports*** (BBREP-D-22-00416). We are thankful for your insightful suggestions regarding our manuscript. Here, we are attaching the revised manuscript in accordance with the comments from the five reviewers. We read through all the reviewers' suggestions carefully, and made the necessary revisions as detailed below in a point-by-point format, with the revised sections highlighted in yellow in the main manuscript. Finally, we would like to sincerely 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 assistance and look forward to hearing from you.

Sincerely yours,

Apt Lalu Muhammad Irham M.Farm.,Ph.D.

Faculty of Pharmacy,
Universitas Ahmad Dahlan, Yogyakarta, Indonesia
Jl. Prof. DR. Soepomo Sh, Warungboto,
Kec. Umbulharjo, Kota Yogyakarta, Daerah Istimewa Yogyakarta. Indonesia

August 25, 2022

Dear Editors,

Please find our attached manuscript entitled "Integration of Genomic Variants and Bioinformatic Based Approach to Drive Drug Repurposing for Multiple Sclerosis," which we submitted for consideration for publication as an Original Research article in ***Biochemistry and Biophysics Reports*** (BBREP-D-22-00416). We are thankful for your insightful suggestions regarding our manuscript. Here, we are attaching the revised manuscript in accordance with the comments from the five reviewers. We read through all the reviewers' suggestions carefully, and made the necessary revisions as detailed below in a point-by-point format, with the revised sections highlighted in yellow in the main manuscript. Finally, we would like to sincerely 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 assistance and look forward to hearing from you.

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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. Indonesia

Manuscript Number: BBREP-D-22-00416

Integration of Genomic Variants and Bioinformatic Based Approach to Drive Drug Repurposing for Multiple Sclerosis

Dear Dr Irham,

Thank you for submitting your manuscript to Biochemistry and Biophysics Reports.

I have completed my evaluation of your manuscript. The reviewers recommend reconsideration of your manuscript following minor revision and modification. I invite you to resubmit your manuscript after addressing the comments below. Please resubmit your revised manuscript by Sep 02, 2022.

When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully: please outline every change made in response to their comments and provide suitable rebuttals for any comments not addressed. Please note that your revised submission may need to be re-reviewed.

To submit your revised manuscript, please log in as an author at https://www.editorialmanager.com/bbrep/, and navigate to the "Submissions Needing Revision" folder under the Author Main Menu.

Biochemistry and Biophysics Reports values your contribution and I look forward to receiving your revised manuscript.

Kind regards,

Vladimir N. Uversky, Ph.D., D.Sc.

Executive Editor

Biochemistry and Biophysics Reports

Editor and Reviewer comments:

Reviewer #1: First of all, congratulations to the authors for their work, which opens up potential new treatment avenues for multiple sclerosis. This is a well-written and overall clear manuscript. However, I would like to highlight some improvements that I believe will enhance the article's focus.

Answer: We sincerely thank the reviewer for taking the time to review our work.

Q1: Introduction lines 118 - 119: the authors mention that Genome-wide association studies (GWASs) can potentially be leveraged for precision drug repurposing by applying functional annotation. Please mention one example of drug repurposing using GWAS data to emphasize the statement that GWAS can potentially be leveraged for precision drug repurposing.

A1: Thank you for the great suggestion. We have revised the manuscript and added the example of drug repurposing using GWAS-based approach accordingly in the manuscript. The revised sentences are as below:

*"Several studies were applied to the risk variants from GWAS, and have prioritized the biological risk genes based on the functional annotations to drive drug repurposing for various diseases, including chronic hepatitis B [12], atopic dermatitis [13], asthma [14], colorectal cancer [15] and the drug repurposing for rheumatoid arthritis [16]. [**Lines 121-125**]."*

Q2: Methods: Why do the authors use six functional annotations to prioritize the genes to find biological MS risk genes? Have the six functional annotations been adjusted based on MS diseases? Could the six functional annotations be used for other diseases?

A2: We appreciate this comment. It is a very important question. In the present study, we prioritized the genes disease and Multiple Sclerosis (MS) genetics genetics-driven genomic drug repurposing for MS and have not been applied yet previously. We hypothesized that MS genetic variants prioritization using six functional annotations will would enable us to translate the risk genes to meaningful insights on MS 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 *cis*-expression quantitative trait loci (*cis*-eQTL) are regions harbouring 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 protein-protein interactions (PPIs) to understand relationships between diseases and biological protein networks. If the genes involved in the biological protein networks are related in to MS pathogenesis, then it is important to inhibit the protein. The genes implicated in knockout mouse phenotype and Kyoto Encyclopedia of Genes and Genomes (KEGG) was also applied to determine the type of molecular pathways enriched on the MS-associated gene list and the genes involved. The last annotation is the Primary immuno- deficiency (PID) diseases which are innate immune diseases reported to be associated with autoimmune diseases. Genes overlapping with the PID play a causal role in MS pathogenesis. It is important to consider the

MS causal relationship and the drug target genes for MS 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. Furthermore, we found 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 MS genes". And these functional annotations are the potentially to be applied for to other diseases according to characteristic of disease and the genomic variants of the disease. In addition, the six functional annotations have been applied to other diseases such as Colorectal cancer and atopic dermatitis (Adikusuma et al., 2021), (Irham et al., 2020).

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.

W. Adikusuma, L.M. Irham, W.H. Chou, H.S.C. Wong, E. Mugiyanto, J. Ting, D.A. Perwitasari, W.P. Chang, W.C. Chang, Drug Repurposing for Atopic Dermatitis by Integration of Gene Networking and Genomic Information, Front. Immunol. 12 (2021). https://doi.org/10.3389/fimmu.2021.724277.

L.M. Irham, H.S.C. Wong, W.H. Chou, W. Adikusuma, E. Mugiyanto, W.C. Huang, W.C. Chang, Integration of genetic variants and gene network for drug repurposing in colorectal cancer, Pharmacol. Res. 161 (2020). https://doi.org/10.1016/j.phrs.2020.105203.

Q3: Results line 191: the authors use HaploReg v4.1 to extend the MS-associated SNPs to identify MS-associated genes under the criterion r2 > 0.8. Please clarify which population the author used to expand it by using HaploReg.

A3: Thank you for the suggestions. The following statements have now been added to the "Methods" section **[lines 138-141]**:

MS-associated single nucleotide polymorphisms (SNPs) were obtained from the GWAS catalog under the criterion p-value > 10^{-8} and expanded using HaploReg (v4.1) based on the criterion of $r^2 \geq 0.8$ in Asian (ASN) populations retrieved from the 1000 Genome Project Phase I data[18][19]**.**

Q4: Discussion

Line 266-267: "Targeting CD80 and CD86 might become novel 267 therapeutic options for MS therapy" should be rewritten. The authors should lower the expectations of their article, given that there have been no further in vitro or animal experiments conducted by the authors.

A4: Thank you for the suggestions. We have rewritten as follows [**Lines 279-284**]:

"In addition, we identified CD80- and CD86- targeting drugs, including anti-thymocyte immunoglobulin (rabbit), abatacept, and belatacept. Among these drugs, in fact, anti-thymocyte immunoglobulin (rabbit) (NCT03342638) and abatacept (NCT01116427) are currently under clinical investigation for MS. Therefore, from this perspective, targeting CD80 and CD86 might become novel therapeutic options for MS therapy. Further clinical evidence generation would be needed to validate these targets."

Q5: Line 269-270: Please add the references.

A5: Based on the reviewer's suggestions, we added the ClinicalTrials.gov identifier (NCT number) to represent the drugs are under clinical investigations [**Lines 279-284**] as below:

"In addition, we identified CD80- and CD86- targeting drugs, including anti-thymocyte immunoglobulin (rabbit), abatacept, and belatacept. Among these drugs, in fact, anti-thymocyte immunoglobulin (rabbit) (NCT03342638) and abatacept (NCT01116427) are currently under clinical investigation for MS. Therefore, from this perspective, targeting CD80 and CD86 might become novel therapeutic options for MS therapy. Further clinical evidence generation would be needed to validate these targets."

Reviewer #2: Title & Abstract
Q1: Do the title and abstract cover the main aspect of the work?
A1: Yes. We confirmed that the title and abstract cover the main aspect of the work.

Q2: Does the introduction provide background and information relevant to the study?
A2: Yes. We confirmed that the introduction provides background and information relevant to the study.

Material and Methods
Q3: Are the methods clear and replicable? Do all the results presented match the methods described?
A3: Yes.

Results

Q4: If relevant are the results novel? Does the study provide an advance in the field? Is the data plausible?
A4: Yes.

Discussion
Q5: Do the findings described by the author correlate with the results? Are the findings relevant?
A5: Yes.

Conclusion
Q6. Do the conclusions correlate to the results found?
A6: Yes.

Figures & Tables
Q7: If the author has provided figures and tables are the figures and tables clear and legible? Are the figures free from unnecessary modification?
A7: Yes.

Q8. Does the paper raise any concerns?
A8: We affirm that no concerns are flagged from the authors' end.

Competing interest
Q9: Do any of the authors' competing interests raise concerns about the validity of the study i.e. have the authors' competing interests created a bias in the reporting of the results and conclusions?
A9: “No competing interest” has now been indicated in the manuscript. Thank you for noting this issue.

Q10. Do you think the manuscript requires English editing to correct the grammar or flow?
A10: Thank you for the comment. We have revised the manuscript accordingly and requested professional proof-readers to improve the overall language quality.

Q11: In the abstract, the word GWAS first appears so it is necessary to explain the abbreviation.
A11: We now added the abbreviation of GWAS in line 47-48.

Reviewer #3: Review: BBREP-D-22-00416

Integration of Genomic Variants and Bioinformatic Based Approach to Drive Drug Repurposing for Multiple Sclerosis

The authors of this manuscript used various bioinformatic tools to identify drug-gene targets in MS in the hope of using the results in drug repurposing. They have screened multiple candidate genes and studied their interactions to find the best fit for the therapy. The research that went into this paper is commendable.
Answer: Thank you for noting the importance of our results and the appreciation of this manuscript.
There are some minor issues with the paper, which are noted below:

Q1: Please replace the abstract on the Title page with the abstract on Page 2.
A1: Thank you for bringing this to our attention. The abstract has been replaced on the Title page according to the reviewer’s suggestion.

Q2: The sentence "Thus, it will develop in genetically susceptible individuals who are exposed to a diversity of triggering environmental factors" is not the correct sentence to follow the previous sentence. Remove the word, 'thus".
A2: The word “Thus” has now been removed in **line 99**.

Q3. Lines 109 - 113: Please rewrite the sentence in a simple manner without a lot of punctuation. You can write two sentences instead.
A3: We modified the sentences to the following below as suggested in **Lines 109-115**:
So far, these medications can help people with MS that have fewer and less severe relapses. However, the problem is still arising from those medications, including resistance and toxicity [6]. Under such circumstances, drug repurposing emerges as one of the solutions to identify new candidate drugs for MS disease. In addition, further investigations such as clinical validation and in vivo experimental are needed to accelerate new discoveries for the treatment

of MS disease, which aims to maximize the likelihood of success during pre-clinical development and validation [7].

Q4: Figure 1: Include GO and KEGG analysis in the Biological risk gene.
A4: We appreciate this comment. We added the GO and KEGG analysis according to the reviewer's suggestion.

Q5: Supplemental file 2; All the scores under "Total score" are either 0 or 6, instead of the mentioned varied scores in the text and corresponding figures.
A5: We revised accordingly based on the reviewer's suggestion.

Q6: Line 217: Correct 2.904 to 2,904 gene pairs.
A6: Thank you for your comment. We revised the numbering of the gene pairs from "2.904" to "2,904" gene pairs according to the reviewer's suggestion **[Line 227]**.

Reviewer #4:

In the manuscript authors tried to find out the drug targets for repurposing old drugs in multiple sclerosis. Using GWAS database and bioinformatics approach focusing on six functional annotations, they have identified 144 biological MS risk genes. Which ultimately gave eight drug targets and eight drugs to have the potential for multiple sclerosis treatment. Still there are some concerns.
Q1: The quality of language is poor.
A1: We sincerely thank the reviewer for taking the time to review our work. We have revised the manuscript accordingly and requested professional proof-readers to improve the overall language quality.

Q2: Why authors used only these six functional annotations (missense mutation, cis-expression quantitative trait locus (cis-eQTL), molecular pathway 55 analysis, protein-protein interaction (PPI), genes overlap with knockout mouse phenotype, and 56 primary immunodeficiency (PID)) to build the assessment system? Authors should clarify the reason behind it.

A2: We sincerely thank the reviewer for taking the time to review our work. We appreciate this comment and in fact, a key question to this study. In the present study, we prioritized the genes disease and Multiple Sclerosis (MS) genetics driven genomic drug repurposing for MS and have not been applied yet previously. We hypothesized that MS genetic variants prioritization using six functional annotations will enable us to translate the risk genes to meaningful insights on MS 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 *cis*-expression quantitative trait loci (*cis*-eQTL) are regions harbouring 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 protein-protein interactions (PPIs) to

understand the relationships between diseases and biological protein networks. If the genes involved in the biological protein networks are related in MS pathogenesis, then it is important to inhibit the protein. The genes implicated in knockout mouse phenotype and Kyoto Encyclopedia of Genes and Genomes (KEGG) was also applied to determine the type of molecular pathways enriched on the MS-associated gene list and the genes involved. The last annotation is the Primary immuno- deficiency (PID) diseases which are innate immune diseases reported to be associated with autoimmune diseases. Genes overlapping with the PID play a causal role in MS pathogenesis. It is important to consider the MS causal relationship and the drug target genes for MS disease. In addition, these type of functional annotations have previously been validated by Okada *et al* to prioritize the most likely causal gene relationships with Rheumatoid Arthritis and to find its candidate drugs. Furthermore, we found the threshold score ≥ 2 (larger than or equal to 2) from the number of functional annotations ranged from 0-6, in which each gene was assigned one point for each annotation. The genes with one functional annotation were awarded one point (score) and those with a score ≥ 2 (larger than or equal to 2) were classified as "biological MS genes". These functional annotations can be potentially applied to other diseases according to the characteristics and the genomic variants of the disease.

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.

Q3: The quality of the figures is very poor. It looks like the graphs made by basic MS excel. Authors should use Graph Pad Prism or other good software to make representable figures.
A3: Many thanks to the reviewer's comments. We have revised the manuscript according to the reviewer's suggestions. The candidates for drug repurposing were visualization from R. Chord diagram was built using R with *circlize* package **[line 191-193]**. The drugs under clinical investigation for MS were built by using R (Chord diagram) with *circlize* package (RStudio 4.0.3 program).

Q4: Increase the font size of Figure 1 and Figure6. It is very hard to read.

A4: The font size in Figure 1 and Figure 6 has been increased as below:

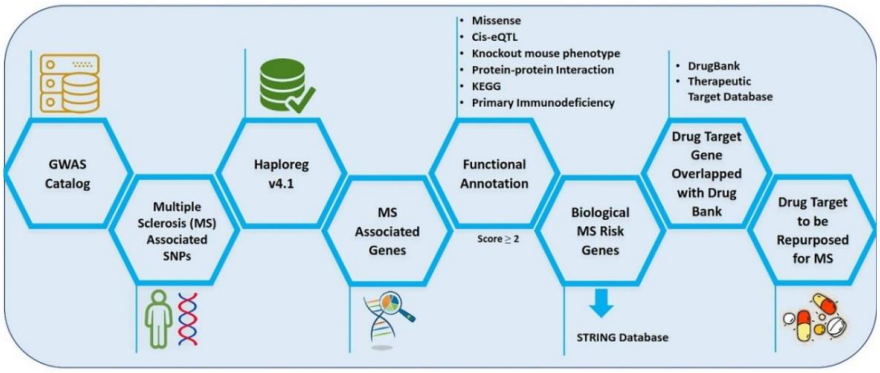

Figure 1. Scheme of drug repurposing using genomic database for multiple sclerosis (MS)

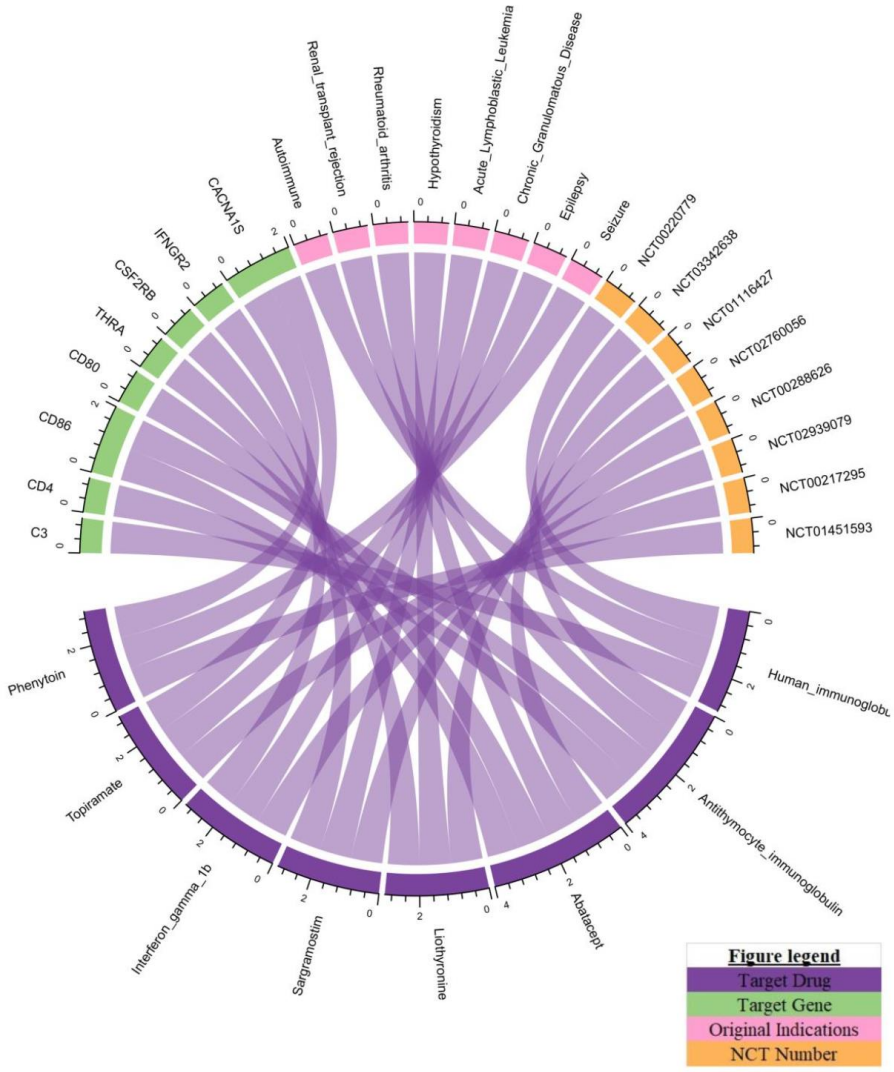

Figure 6. Relationship between biological MS genes, and drugs approved for other indications and under clinical investigation for MS

Q5: Figure 1 presentation is not great, it should be remade.
A5: We note the reviewer's comment and remade Figure 1 as requested.

Integration of Genomic Variants and Bioinformatic-Based Approach to Drive Drug Repurposing for Multiple Sclerosis

Arief Rahman Afief [1], Lalu Muhammad Irham [1]*, Wirawan Adikusuma[2]*, Dyah Aryani Perwitasari [1], Ageng Brahmadhi[3], Rocky Cheung[4]

[1]*Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia*

[2]*Departement of Pharmacy, University of Muhammadiyah Mataram, Mataram, Indonesia*

[3]*Faculty of Medicine, Universitas Muhammadiyah Purwokerto, Purwokerto, Central Java, Indonesia*

[4]*Department of Chemistry and Biochemistry, University of California, Los Angeles, USA*

*__Correspondence author:__ Lalu Muhammad Irham; email: lalu.irham@pharm.uad.ac.id; Wirawan Adikusuma; email: adikusuma28@gmail.com

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ABSTRACT

Multiple sclerosis (MS) is a chronic autoimmune disease in the central nervous system (CNS) marked by inflammation, demyelination, and axonal loss. Currently available MS medication is limited, thereby calling for a strategy to accelerate new drug discovery. One of the strategies to discover new drugs is to utilize old drugs for new indications, an approach known as drug repurposing. Herein, we first identified 421 MS-associated SNPs from the Genome Wide Association Study (GWAS) catalog (*p-value* < $5x10^{-8}$), and a total of 427 risk genes associated with MS using HaploReg version 4.1 under the criterion $r^2 > 0.8$. MS risk genes were then prioritized using bioinformatics analysis to identify biological MS risk genes. The prioritization was performed based on six defined categories of functional annotations, namely missense mutation, cis-expression quantitative trait locus (*cis*-eQTL), molecular pathway analysis, protein-protein interaction (PPI), genes overlap with knockout mouse phenotype, and primary immunodeficiency (PID). A total of 144 biological MS risk genes was found and mapped into 194 genes within an expanded PPI network. According to the DrugBank and the Therapeutic Target Database, 27 genes within the list targeted by 68 new candidate drugs were identified. Importantly, the power of our approach is confirmed with the identification of a known approved drug (dimethyl fumarate) for MS. Based on additional data from ClinicalTrials.gov, eight drugs targeting eight distinct genes are prioritized with clinical evidence for MS disease treatment. Notably, CD80 and CD86 pathways are promising targets for MS drug repurposing. Using *in silico* drug repurposing, we identified belatacept as a promising MS drug candidate. Overall, this study emphasized the integration of functional genomic variants and bioinformatic-based approach that reveal important biological insights for MS and drive drug repurposing efforts for the treatment of this devastating disease.

Keywords: Autoimmune disease; Bioinformatics; Drug repurposing; Genomic variants; Multiple sclerosis;

1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune disease in the central nervous system (CNS) marked by inflammation, demyelination, and axonal loss since the onset of the disease. The onset of MS usually occurs between 20 and 40 years of age and more predominantly in women [1]. MS also causes a series of other heterogeneous symptoms due to varying involvements of the motor, sensor, visual, and autonomous systems. It is characterized by optic neuritis (optic nerve inflammation), Uhthoff's phenomenon (temporary fluctuation and worsened MS symptoms with increased body temperature), and Lhermitte's sign (abnormal electrical-shocklike sensation over the spinal cord or body parts during neck flexion) [2], and tends to develop in genetically susceptible individuals who are exposed to a diversity of triggering environmental factors (e.g., Epstein-Barr virus, tobacco use, and vitamin D deficiency) [3]. The genes involved in MS have long been sought after. A number of approaches to this problem have been applied with varying degrees of success. The candidate gene approach has been in use over several decades, where potentially MS-associated genes are selected based on autoimmune MS prognosis, involving class I and II immune-response-gene-controlling human leukocyte antigen (HLA) [4].

Treatments for MS have been divided into three categories: 1) acute relapse management; 2) disease-modifying therapies; and 3) symptomatic treatments [2]. One MS treatment available and approved is dimethyl fumarate (Tecfi dera) [2][5]. So far, these medications can help people with MS that have fewer and less severe relapses. However, the problem is still arising from those medications, including resistance and toxicity [6]. Under such circumstances, drug repurposing emerges as one of the solutions to identify new candidate drugs for MS disease. In addition, further investigations such as clinical validation and in vivo experimental are needed to accelerate new discoveries for the treatment of MS disease, which aims to maximize the likelihood of success during pre-clinical development and validation [7].

The concept of drug repurposing is to find new indications for existing drugs that are already available on the market [8]. The drug repurposing approach has several advantages compared to the traditional such as time and cost-effectiveness [9], safety profile (drugs have previously passed clinical trials), dosage, and that the toxicity of existing drugs have already been vetted [10]. Genome-wide association studies (GWASs) can potentially be leveraged for precision drug repurposing by applying functional annotation [11]. Several studies were applied to the risk variants from GWAS, and have prioritized the biological risk genes based on the functional

annotations to drive drug repurposing for various diseases, including chronic hepatitis B [12], atopic dermatitis [13], asthma [14], colorectal cancer [15] and the drug repurposing for rheumatoid arthritis [16]. In addition, GWAS has revolutionized MS genetic analyses, including the MS variants. These variants consistently implicate genes associated with immunological processes, mostly lie in regulatory rather than coding areas, and are often associated with other autoimmune diseases [17]. This research aimed to implement the bioinformatics strategy and identify biological MS candidate genes through an integrated gene network. Six functional annotations (missense mutation, cis-expression quantitative trait locus (*cis*-eQTL), molecular pathway analysis, protein-protein interaction (PPI), overlap knockout mouse phenotype, and primary immunodeficiency (PID)) were used to find biological MS risk genes. Finally, we overlapped the biological MS risk genes with the drug database and prioritized the candidate drug to be repurposed for MS disease.

2. Methods

A detailed description of the study design of drug repurposing utilizing the genomic information for MS is provided in **Figure 1**. MS-associated single nucleotide polymorphisms (SNPs) were obtained from the GWAS catalog under the criterion *p-value* > 10^{-8} and expanded using HaploReg (v4.1) based on the criterion of $r^2 \geq 0.8$ in Asian (ASN) populations retrieved from the 1000 Genome Project Phase I data [18][19]**.** Genes matching MS-associated SNPs are denoted as "MS-associated genes". Then, genomic data were prioritized based on six functional annotation criteria. Every functional annotation is assigned a score of 1, and genes with a score ≥ 2 are defined as "biological MS risk genes". Biological MS risk genes were used in advanced analysis using the STRING database to extend the list of candidate genes as drug-target genes. This research mapped an approved expanded list of drug-target genes in the DrugBank and Therapeutic Target Database. The drug-target genes were checked with ClinicalTrials.gov to determine the clinical status.

2.1. Functional annotations of MS risk genes

Functional annotation describes a gene's biological identity by compiling the relevant biological information for a particular gene. Herein, six categories of functional annotations were used to build an assessment system representing the candidate genes most likely to be MS targets. The first category of annotation was missense or nonsense mutation according to HaploReg v4.1, which contains functional consequence annotations of the SNP database (db).

HaploReg v4.1 also connected genetic variants to cis-expression quantitative trait loci (*cis*eQTLs) [16]. If a gene has an MS risk SNP with *cis*-eQTL effect throughout the blood, the gene was assigned one point. Then, to gain an understanding of the relationship between a mutant gene and the phenotype, WebGestalt 2019 was used for functional enrichment analysis. The data source was the Mammalian Phenotype Ontology (MP), which contains information on the mouse and other mammalian phenotypes [15]. The genes from human Ensembl ID were converted into mouse Ensembl ID using BioMart. Clusters of genes with FDR < 0.05 in the enrichment analysis were considered significant. Specifically, the gene ontology (GO) biological process categories were analyzed for this stage. The result significance was set at FDR < 0.05. Enrichment analysis was performed on molecular pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGG). Genes enriched on the KEGG pathway (FDR < 0.05) were assigned a score of 1. Primary immunodeficiency (PID) was the last annotation criterion. It refers to inborn immunity diseases that are genetic disorders associated with increased severity [15][13]. Data enrichment analysis was performed using the hypergeometric test; $p < 0.05$ was used in this stage as the significance criterion [13]. It is important to note that each functional annotation is assigned a score of 1, and genes with a score ≥ 2 are defined as "biological MS risk genes". Biological MS risk genes were used in advanced analysis using the STRING database.

2.2. STRING database

The use of the STRING database (http://string-db.org) aimed to integrate functional interactions related to protein expressions by inputting and regulating data associated with the predicted protein-protein interactions [20][21]. The majority of protein networks in various diseases can be the targets of the diseases [22]. The biological MS risk genes were expanded using the STRING database to gain more candidate drug targets. This step emphasized that the genomic information of MS has given insight into the biological risk gene for MS.

2.3. Validation and drug discovery

The drug target gene's candidate was overlapped with drug databases such as the DrugBank (https://www.drugbank.ca/) and Therapeutic Target Database (TTD) (http://db.idrblab.net/ttd/) to find the candidate drug to be repurposed for MS disease. DrugBank and TTD are databases widely used to identify the drug target precisely. It also contributes to driving drug repurposing for various diseases [23]. Drug-target genes were used

to analyze the database based on several criteria, such as drugs with pharmacological activity, effectiveness in humans, and approved annotations in clinical trials or drug experiments [24]. Furthermore, the identified drugs were reviewed with ClinicalTrials.gov (https://clinicaltrials.gov) to identify clinical examinations for MS or other diseases. The drugs under clinical investigation for MS were built by using R language (Chord diagram) with the circlize package (R Studio 4.0.3 program).

3. Results

The susceptibility of various MS genomic variants was retrieved from genomic database. A variety of genomic databases can be used including GWAS databases. GWAS not only provides information on the susceptibility of diseases but also provides information on the biological insight of diseases. In this study, 420 MS-associated SNPs were obtained from the GWAS database **(Table S1)**. The expansion was then performed using HaploReg v4.1 under the criterion $r^2 > 0.8$, resulting in 427 MS-associated risk genes **(Table S2).**

3.1. Functional annotations of MS risk genes

Six biological functional annotations were applied to prioritize biological MS risk genes. One point was assigned to each functional annotation. The assessment of each of the 427 candidate genes under the six criteria was as follows: (1) genes with missense mutation MS risk variants (n = 29); (2) cis-eQTL genes (n = 134); (3) genes in knockout mouse phenotypes (n = 101); (4) engaged genes in terms of GO for evaluating PPI (n = 146); (5) genes overlapped with the KEGG pathways (n = 93); and (6) number of genes overlapped with the PID (n = 13) **(Figure 2** and **Figure 3)(Table S2)**. Biological scoring was conducted after data collection. There were 173 genes with a score of 0, 110 genes with a score of 1, 67 genes with a score of 2, 47 genes with a score of 3, 7 genes with a score of 5, and 2 genes with a score of 6. A total of 144 genes had a score > 2 (**Figure 4**). We found that interferon-gamma receptor 2 (IFNGR2) and interleukin 7 receptor (IL7R) were the top two biological MS risk genes, each with a score of 6.

3.2. Expansion of biological MS risk genes list

The STRING database was utilized to expand the investigation of biological MS risk genes. The expansion is based on the rationale that, the more biological MS risk genes we find, the more candidate drug targets for MS drug repurposing can be identified. We successfully

obtained 194 genes on the list (**Table S3**). These genes were included on the list of new candidate drug genes for further analysis.

3.3. MS drug targets finding

Finally, the drug target genes were prioritized based on the network analysis and the drug databases. Herein, we obtained 2,904 gene pairs from the PPI network (**Table S4**) and 27 genes targeted by 68 new candidate drugs based on DrugBank and TTD (**Tabel S5**). We found one drug, dimethyl fumarate that has been clinically approved for MS treatment (**Figure 5**). This drug is an effective medicinal option, administered twice a day in MS medication [25][26]. This study emphasized that the biological functional annotation we applied can be validated through the drug used in the clinic for MS disease.

This research also found eight drug-target genes bound to 8 drugs approved for other diseases and under clinical investigation for MS, including human immunoglobulin G, antithymocyte immunoglobulin (rabbit), liothyronine, abatacept, topiramate, and phenytoin (**Table S6**). These drugs can potentially repurpose MS medication (**Figure 6**). An example of a drug repurposed for MS is abatacept, which is approved for rheumatoid arthritis, targeting CD80 and CD86 gene pathways. This drug is currently under clinical investigation for MS in a phase II trial (NCT01116427) and has a considerable potential to be used for MS [27]. Thus, we would like to emphasize that integrating genomic variants and gene networking can potentially guide the drug repurposing for MS disease.

4. Discussion

The focus of our present work is to narrow down candidate drugs of a debilitating disease, multiple sclerosis, through leveraging large bioinformatics datasets together with human genetics data. More specifically, we use personalized genomic data and genetic mapping to guide drug repurposing for MS. In particular, this study focused on using new candidate drugs for MS by prioritizing candidate genes derived and identified from the GWAS databases. Six categories of functional annotations were used to build an assessment system, in order to prioritize the MS risk genes as leads for new candidate drugs. We hypothesized that the broad strategy of genetic variant prioritization, using the functional annotations described in this study, would enable us to translate the risk genes to meaningful, actionable insights on MS. According to our analyses, we ensure the sensitivity of our study results by setting the threshold

of a biological score ≥ 2 to find a much higher number of genes as biological MS risk genes, and thereby candidates for MS drug targets.

In this research, 27 drug-target genes were found to be bound to 68 drugs. In addition, 8 drug-target genes were found to be bound to 8 drugs, of which some were still under clinical testing for MS, namely human immunoglobulin G (NCT00220779), anti-thymocyte immunoglobulin (rabbit) (NCT03342638), liothyronine (NCT02760056), abatacept (NCT01116427), topiramate (NCT00217295), and phenytoin (NCT01451593). From the data collected, one available drug approved for MS is dimethyl fumarate (**Figure 5**). Dimethyl fumarate, also known as BG-12, was licensed as first-line therapy and oral therapy for MS in 2013. It is also known as neuroprotective and immunomodulatory [28][29]. The mechanism of action of dimethyl fumarate is to react with cysteine residues from KEAP1 (Kelch-like ECHassociated protein 1), which causes KEAP1 to be dissociated from the nuclear factor (erythroidderived 2)-like 2 (Nrf2) pathway toward Nrf2 nuclear translocation. Nrf2 then binds antioxidant response element (ARE) and drives antioxidant target gene expression toward neuronal protection, reduces astrocyte activation, and prolongs cell life [30].

We identified eight promising targets that overlapped with drugs that could potentially be repurposed to treat MS. These include C3, CD4, CD86, THRA, CSF2RB, CD80, IFNGR2, and CACNA1S. Among them, we highly proposed CD80 and CD86 as potential targets for MS, since these targets are closely related to IL7R as biological MS risk genes with high functional annotation scores **(Figure 6)**. The CD80/CD86 pathway is essential for controlling T cell activation and preserving immunological tolerance to self-antigens. The findings of functional and genome-wide investigations demonstrate that genes encode molecules that fit in. This pathway may increase the likelihood of developing autoimmune illnesses and may be viewed as a potential MS candidate gene [31][32][33]. In addition, we identified CD80- and CD86-targeting drugs, including anti-thymocyte immunoglobulin (rabbit), abatacept, and belatacept. Among these drugs, in fact, anti-thymocyte immunoglobulin (rabbit) (NCT03342638) and abatacept (NCT01116427) are currently under clinical investigation for MS. Therefore, from this perspective, targeting CD80 and CD86 might become novel therapeutic options for MS therapy. Further clinical evidence generation would be needed to validate these targets.

Combining results from genome-wide association studies and bioinformatic, gene-level annotation of human genetic variants is a powerful approach to identify candidate new drugs for MS. However, it is important to consider that this approach is not without limitations, such

as not all the identified target genes can either be targeted and/or demonstrate pharmacological activity with the desired profile for use in clinic. The genes identified in this manner would therefore miss the drug targeting window for the particular disease. Further investigation is thus required to determine the effect of the candidate drugs towards the clinic. Hence, we suggest that the use of current finding with subsequent investigation in functional studies to ascertain the role of drug target genes discovered in this study. The validation from existing results is necessary and important to ensure whether our drug candidates produced the desired interaction (intended from the study), any undesired side effects, or ineffective effects.

5. Conclusions

Our study utilizes MS functional genomic variants to open up additional avenues for the repurposing of existing drugs. Herein, we identify CD80 and CD86 as potential targets for MS treatment. The involvement of these genes with MS might be highly significant, thereby requiring further examination. By targeting CD80 and CD86, belatacept could be a promising therapy option for MS therapy. However, more studies from animal models and clinical trials are needed to confirm the biological mechanisms of the drug for new diseases. In this research, we combined the drug repurposing approach with integrated bioinformatics methodology to identify drugs with new indications for MS. Finally, this study emphasizes the vast potential of utilizing functional genomic variants as a basis to drive repurposing for MS disease.

Acknowledgments

The author would like to thank Universitas Ahmad Dahlan for providing the resources and opportunities for this study.

Author's Contribution

A.R.A., L.M.I., and W.A. conceived and designed the study. A.R.A. and W.A. performed the computational analysis. A.R.A. wrote the manuscript. A.R.A., L.M.I., W.A., D.A.P., A.B. and R.C. revised the manuscript. L.M.I and W.A. supervised and coordinated this study. All authors have read and approved the manuscript and made significant contributions to this study.

Competing interests

The authors declare no conflict of interest.

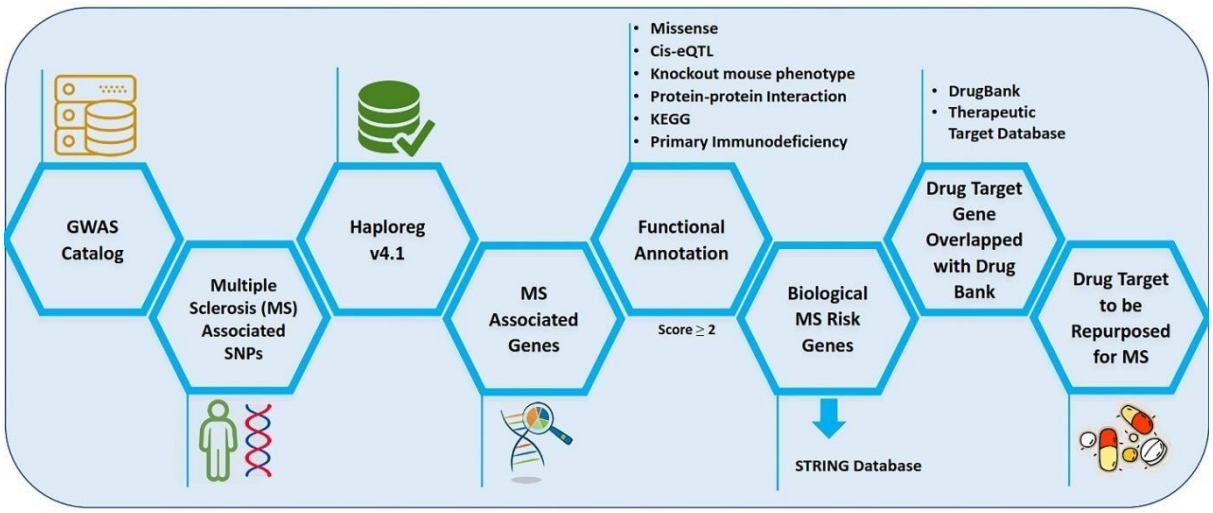

Figure 1. Scheme of drug repurposing using genomic database for multiple sclerosis (MS)

GENCODE_id	GENCODE_name	Missense/ Nonsense	cis-eQTL	KO mice	PPI	KEGG	PID	Total score
ENSG00000159128	IFNGR2	1	1	1	1	1	1	6
ENSG00000168685	IL7R	1	1	1	1	1	1	6
ENSG00000013725	CD6	1	1	1	1	1	0	5
ENSG00000067182	TNFRSF1A	0	1	1	1	1	1	5
ENSG00000101017	CD40	0	1	1	1	1	1	5
ENSG00000105397	TYK2	1	0	1	1	1	1	5
ENSG00000121966	CXCR4	0	1	1	1	1	1	5
ENSG00000131323	TRAF3	0	1	1	1	1	1	5
ENSG00000168610	STAT3	0	1	1	1	1	1	5
ENSG00000020633	RUNX3	1	0	1	1	1	0	4
ENSG00000081059	TCF7	0	1	1	1	1	0	4
ENSG00000102882	MAPK3	0	1	1	1	1	0	4
ENSG00000104432	IL7	0	1	1	1	1	0	4
ENSG00000107485	GATA3	0	1	1	1	1	0	4
ENSG00000109320	NFKB1	0	1	1	1	1	0	4
ENSG00000111252	SH2B3	1	1	1	0	1	0	4
ENSG00000114013	CD86	0	1	1	1	1	0	4
ENSG00000117090	SLAMF1	0	1	1	1	1	0	4
ENSG00000124181	PLCG1	1	0	1	1	1	0	4
ENSG00000125730	C3	0	0	1	1	1	1	4

Figure 2. Biological annotations prioritized for multiple sclerosis (MS) genes with score ≥ 2.

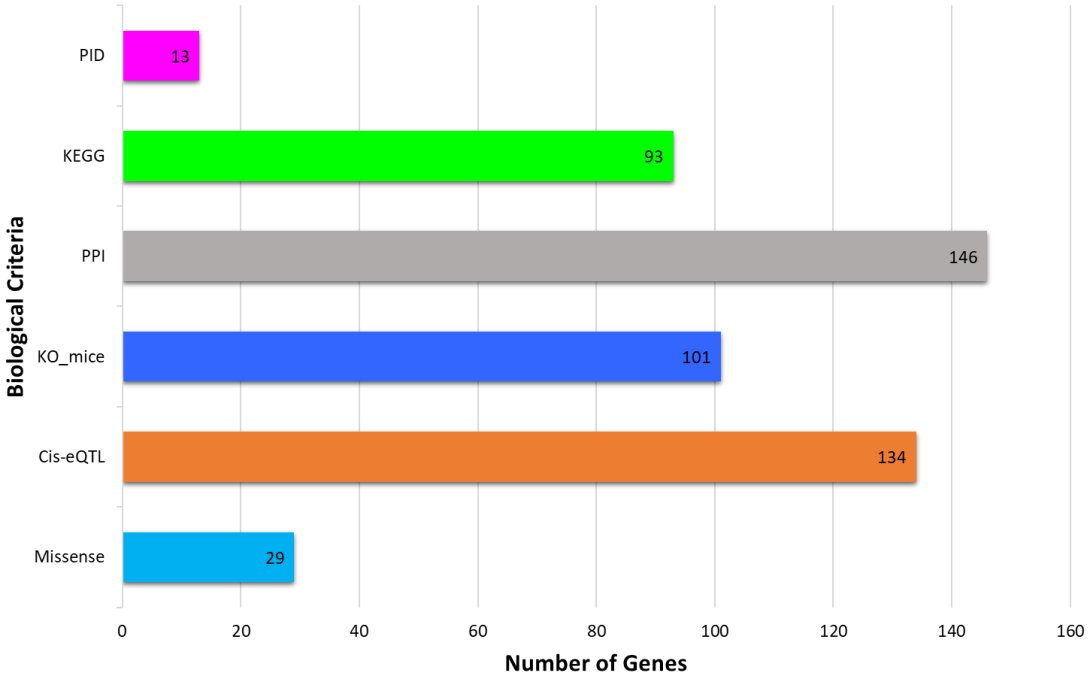

Figure 3. Histogram of the number of genes (y-axis) meeting each of the six biological criteria (x-axis) for drug prioritization..

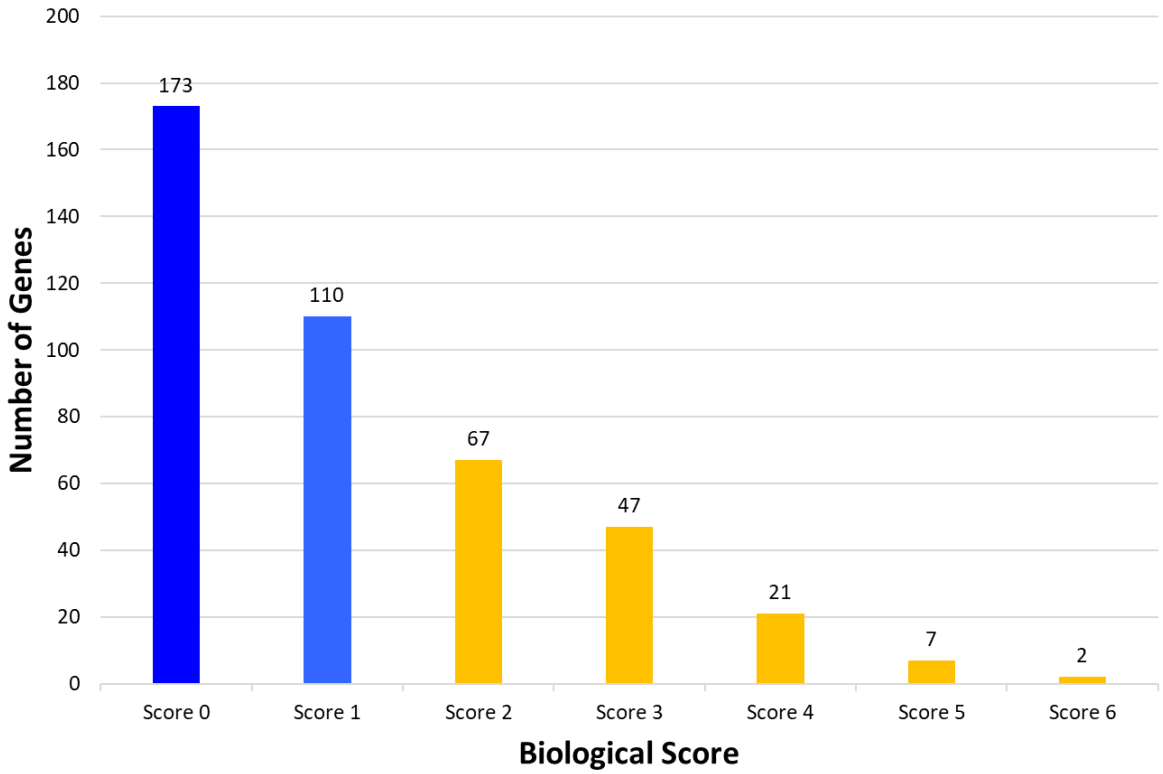

Figure 4. Histogram of the number of genes (y-axis) meeting each of the six biological criteria (x-axis) for drug prioritization. It is shown that there were 173 genes with score 0, 110 genes with score 1, and 144 (67+47+21+7+2) genes with total score ≥ 2, denoted as "biological MS risk genes".

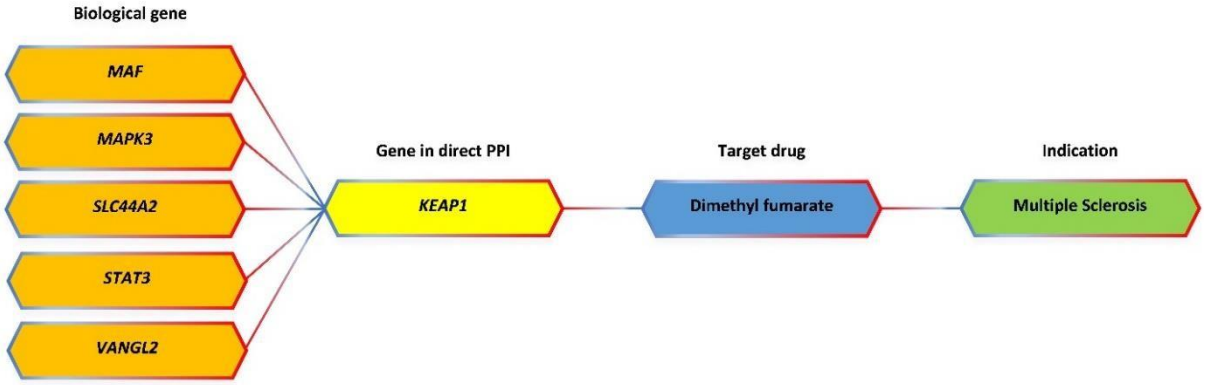

Figure 5. Relationship between biological MS risk genes and available drugs for MS.

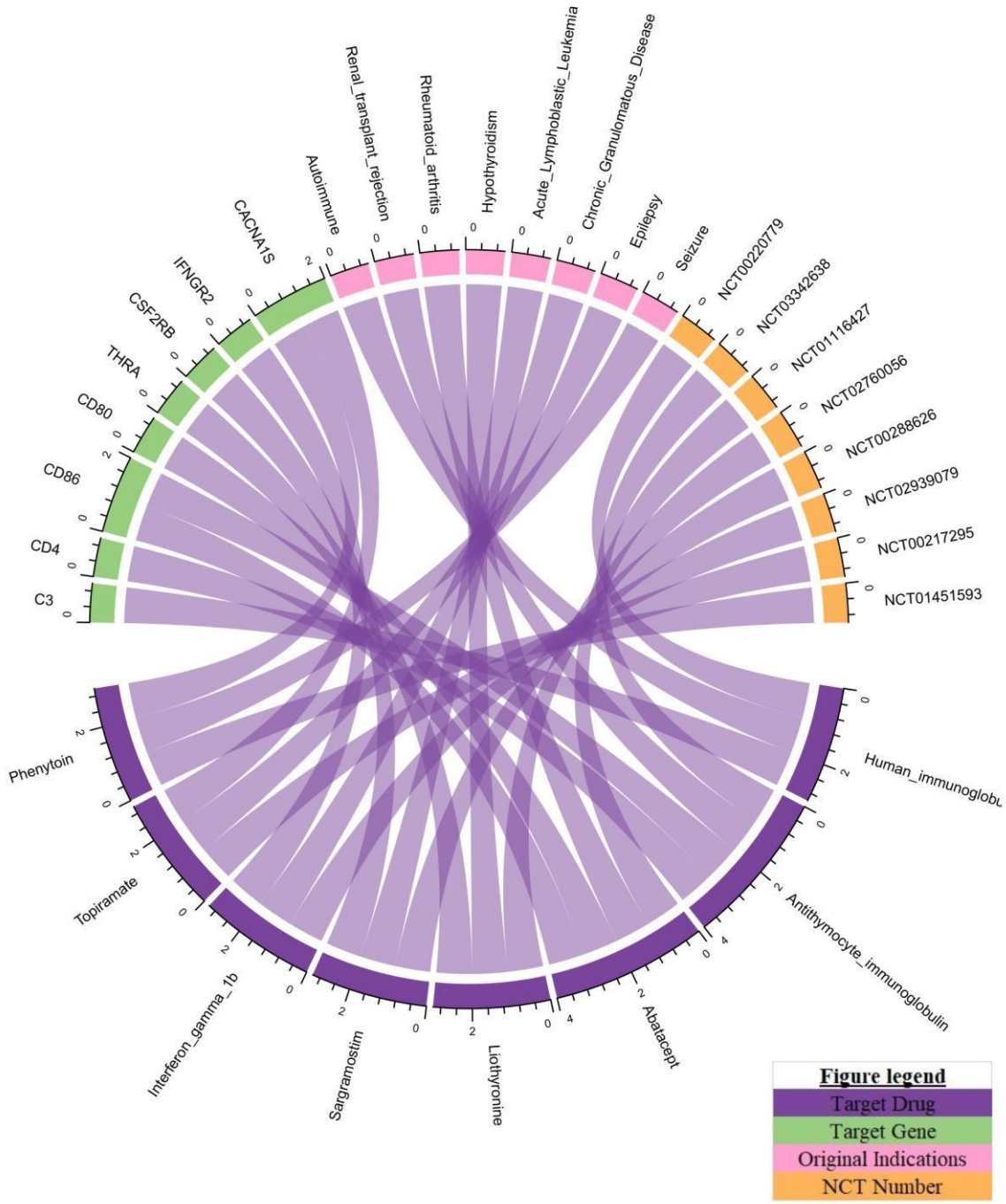

Figure 6. Relationship between biological MS genes, and drugs approved for other indications and under clinical investigation for MS.

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Highlights

- Utilizing genomic data from GWAS catalog to provide novel biological insight for drug repurposing for multiple sclerosis.
- The leveraging of genomic information can be translated into clinical implementation and guide the drug discovery for multiple sclerosis.
- Our findings suggested the plausibility of multiple sclerosis genomic variants-driven drug repurposing for multiple sclerosis.

Supplementary Files
Conflict of Interest

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Supplementary Files

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Supplementary Files

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Supplementary Files

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Tabel S3. Multiple sclerosis related genes.xlsx

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Tabel S4. STRING PPI.xlsx

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Supplementary Files

Tabel S5. List all drug target.xlsx

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Supplementary Files

Tabel S6. Drugs.xlsx

Integration of Genomic Variants and Bioinformatic-Based Approach to Drive Drug Repurposing for Multiple Sclerosis

Arief Rahman Afief [1], Lalu Muhammad Irham [1*], Wirawan Adikusuma[2*], Dyah Aryani Perwitasari [1], Ageng Brahmadhi[3], Rocky Cheung[4]

[1]*Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia*

[2]*Departement of Pharmacy, University of Muhammadiyah Mataram, Mataram, Indonesia*

[3]*Faculty of Medicine, Universitas Muhammadiyah Purwokerto, Purwokerto, Central Java, Indonesia*

[4]*Department of Chemistry and Biochemistry, University of California, Los Angeles, USA*

[*]**Correspondence author:** Lalu Muhammad Irham; email: lalu.irham@pharm.uad.ac.id; Wirawan Adikusuma; email: adikusuma28@gmail.com

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ABSTRACT

Multiple sclerosis (MS) is a chronic autoimmune disease in the central nervous system (CNS) marked by inflammation, demyelination, and axonal loss. Currently available MS medication is limited, thereby calling for a strategy to accelerate new drug discovery. One of the strategies to discover new drugs is to utilize old drugs for new indications, an approach known as drug repurposing. Herein, we first identified 421 MS-associated SNPs from the Genome Wide Association Study (GWAS) catalog (*p-value* < $5x10^{-8}$), and a total of 427 risk genes associated with MS using HaploReg version 4.1 under the criterion r^2 > 0.8. MS risk genes were then prioritized using bioinformatics analysis to identify biological MS risk genes. The prioritization was performed based on six defined categories of functional annotations, namely missense mutation, cis-expression quantitative trait locus (*cis*-eQTL), molecular pathway analysis, protein-protein interaction (PPI), genes overlap with knockout mouse phenotype, and primary immunodeficiency (PID). A total of 144 biological MS risk genes was found and mapped into 194 genes within an expanded PPI network. According to the DrugBank and the Therapeutic Target Database, 27 genes within the list targeted by 68 new candidate drugs were identified. Importantly, the power of our approach is confirmed with the identification of a known approved drug (dimethyl fumarate) for MS. Based on additional data from ClinicalTrials.gov, eight drugs targeting eight distinct genes are prioritized with clinical evidence for MS disease treatment. Notably, CD80 and CD86 pathways are promising targets for MS drug repurposing. Using *in silico* drug repurposing, we identified belatacept as a promising MS drug candidate. Overall, this study emphasized the integration of functional genomic variants and bioinformatic-based approach that reveal important biological insights for MS and drive drug repurposing efforts for the treatment of this devastating disease.

Keywords: Autoimmune disease; Bioinformatics; Drug repurposing; Genomic variants; Multiple sclerosis;

1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune disease in the central nervous system (CNS) marked by inflammation, demyelination, and axonal loss since the onset of the disease. The onset of MS usually occurs between 20 and 40 years of age and more predominantly in women [1]. MS also causes a series of other heterogeneous symptoms due to varying involvements of the motor, sensor, visual, and autonomous systems. It is characterized by optic neuritis (optic nerve inflammation), Uhthoff's phenomenon (temporary fluctuation and worsened MS symptoms with increased body temperature), and Lhermitte's sign (abnormal electrical-shocklike sensation over the spinal cord or body parts during neck flexion) [2], and tends to develop in genetically susceptible individuals who are exposed to a diversity of triggering environmental factors (e.g., Epstein-Barr virus, tobacco use, and vitamin D deficiency) [3]. The genes involved in MS have long been sought after. A number of approaches to this problem have been applied with varying degrees of success. The candidate gene approach has been in use over several decades, where potentially MS-associated genes are selected based on autoimmune MS prognosis, involving class I and II immune-response-gene-controlling human leukocyte antigen (HLA) [4].

Treatments for MS have been divided into three categories: 1) acute relapse management; 2) disease-modifying therapies; and 3) symptomatic treatments [2]. One MS treatment available and approved is dimethyl fumarate (Tecfi dera) [2][5]. So far, these medications can help people with MS that have fewer and less severe relapses. However, the problem is still arising from those medications, including resistance and toxicity [6]. Under such circumstances, drug repurposing emerges as one of the solutions to identify new candidate drugs for MS disease. In addition, further investigations such as clinical validation and in vivo experimental are needed to accelerate new discoveries for the treatment of MS disease, which aims to maximize the likelihood of success during pre-clinical development and validation [7].

The concept of drug repurposing is to find new indications for existing drugs that are already available on the market [8]. The drug repurposing approach has several advantages compared to the traditional such as time and cost-effectiveness [9], safety profile (drugs have previously passed clinical trials), dosage, and that the toxicity of existing drugs have

already been vetted [10]. Genome-wide association studies (GWASs) can potentially be leveraged for precision drug repurposing by applying functional annotation [11]. Several studies were applied to the risk variants from GWAS, and have prioritized the biological risk genes based on the functional annotations to drive drug repurposing for various diseases, including chronic hepatitis B [12], atopic dermatitis [13], asthma [14], colorectal cancer [15] and the drug repurposing for rheumatoid arthritis [16]. In addition, GWAS has revolutionized MS genetic analyses, including the MS variants. These variants consistently implicate genes associated with immunological processes, mostly lie in regulatory rather than coding areas, and are often associated with other autoimmune diseases [17]. This research aimed to implement the bioinformatics strategy and identify biological MS candidate genes through an integrated gene network. Six functional annotations (missense mutation, cis-expression quantitative trait locus (*cis*-eQTL), molecular pathway analysis, protein-protein interaction (PPI), overlap knockout mouse phenotype, and primary immunodeficiency (PID)) were used to find biological MS risk genes. Finally, we overlapped the biological MS risk genes with the drug database and prioritized the candidate drug to be repurposed for MS disease.

2. Methods

A detailed description of the study design of drug repurposing utilizing the genomic information for MS is provided in **Figure 1**. MS-associated single nucleotide polymorphisms (SNPs) were obtained from the GWAS catalog under the criterion *p-value* $> 10^{-8}$ and expanded using HaploReg (v4.1) based on the criterion of $r^2 \geq 0.8$ in Asian (ASN) populations retrieved from the 1000 Genome Project Phase I data [18][19]**.** Genes matching MS-associated SNPs are denoted as "MS-associated genes". Then, genomic data were prioritized based on six functional annotation criteria. Every functional annotation is assigned a score of 1, and genes with a score ≥ 2 are defined as "biological MS risk genes". Biological MS risk genes were used in advanced analysis using the STRING database to extend the list of candidate genes as drug-target genes. This research mapped an approved expanded list of drug-target genes in the DrugBank and Therapeutic Target Database. The drug-target genes were checked with ClinicalTrials.gov to determine the clinical status.

2.1. Functional annotations of MS risk genes

Functional annotation describes a gene's biological identity by compiling the relevant biological information for a particular gene. Herein, six categories of functional

annotations were used to build an assessment system representing the candidate genes most likely to be MS targets. The first category of annotation was missense or nonsense mutation according to HaploReg v4.1, which contains functional consequence annotations of the SNP database (db). HaploReg v4.1 also connected genetic variants to cis-expression quantitative trait loci (*cis*eQTLs) [16]. If a gene has an MS risk SNP with *cis*-eQTL effect throughout the blood, the gene was assigned one point. Then, to gain an understanding of the relationship between a mutant gene and the phenotype, WebGestalt 2019 was used for functional enrichment analysis. The data source was the Mammalian Phenotype Ontology (MP), which contains information on the mouse and other mammalian phenotypes [15]. The genes from human Ensembl ID were converted into mouse Ensembl ID using BioMart. Clusters of genes with FDR < 0.05 in the enrichment analysis were considered significant. Specifically, the gene ontology (GO) biological process categories were analyzed for this stage. The result significance was set at FDR < 0.05. Enrichment analysis was performed on molecular pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGG). Genes enriched on the KEGG pathway (FDR < 0.05) were assigned a score of 1. Primary immunodeficiency (PID) was the last annotation criterion. It refers to inborn immunity diseases that are genetic disorders associated with increased severity [15][13]. Data enrichment analysis was performed using the hypergeometric test; $p < 0.05$ was used in this stage as the significance criterion [13]. It is important to note that each functional annotation is assigned a score of 1, and genes with a score ≥ 2 are defined as "biological MS risk genes". Biological MS risk genes were used in advanced analysis using the STRING database.

2.2. STRING database

The use of the STRING database (http://string-db.org) aimed to integrate functional interactions related to protein expressions by inputting and regulating data associated with the predicted protein-protein interactions [20][21]. The majority of protein networks in various diseases can be the targets of the diseases [22]. The biological MS risk genes were expanded using the STRING database to gain more candidate drug targets. This step emphasized that the genomic information of MS has given insight into the biological risk gene for MS.

2.3. Validation and drug discovery

The drug target gene's candidate was overlapped with drug databases such as the

DrugBank (https://www.drugbank.ca/) and Therapeutic Target Database (TTD) (http://db.idrblab.net/ttd/) to find the candidate drug to be repurposed for MS disease. DrugBank and TTD are databases widely used to identify the drug target precisely. It also contributes to driving drug repurposing for various diseases [23]. Drug-target genes were used to analyze the database based on several criteria, such as drugs with pharmacological activity, effectiveness in humans, and approved annotations in clinical trials or drug experiments [24].

Furthermore, the identified drugs were reviewed with ClinicalTrials.gov (https://clinicaltrials.gov) to identify clinical examinations for MS or other diseases. The drugs under clinical investigation for MS were built by using R language (Chord diagram) with the circlize package (R Studio 4.0.3 program).

3. Results

The susceptibility of various MS genomic variants was retrieved from genomic database. A variety of genomic databases can be used including GWAS databases. GWAS not only provides information on the susceptibility of diseases but also provides information on the biological insight of diseases. In this study, 420 MS-associated SNPs were obtained from the GWAS database **(Table S1)**. The expansion was then performed using HaploReg v4.1 under the criterion $r^2 > 0.8$, resulting in 427 MS-associated risk genes **(Table S2).**

3.1. Functional annotations of MS risk genes

Six biological functional annotations were applied to prioritize biological MS risk genes. One point was assigned to each functional annotation. The assessment of each of the 427 candidate genes under the six criteria was as follows: (1) genes with missense mutation MS risk variants (n = 29); (2) cis-eQTL genes (n = 134); (3) genes in knockout mouse phenotypes (n = 101); (4) engaged genes in terms of GO for evaluating PPI (n = 146); (5) genes overlapped with the KEGG pathways (n = 93); and (6) number of genes overlapped with the PID (n = 13) **(Figure 2** and **Figure 3)(Table S2)**. Biological scoring was conducted after data collection. There were 173 genes with a score of 0, 110 genes with a score of 1, 67 genes with a score of 2, 47 genes with a score of 3, 7 genes with a score of 5, and 2 genes with a score of 6. A total of 144 genes had a score > 2 (**Figure 4**). We found that interferon-gamma receptor 2 (IFNGR2) and interleukin 7 receptor (IL7R) were the top two biological MS risk genes, each with a score of

6.

3.2. **Expansion of biological MS risk genes list**

The STRING database was utilized to expand the investigation of biological MS risk genes. The expansion is based on the rationale that, the more biological MS risk genes we find, the more candidate drug targets for MS drug repurposing can be identified. We successfully obtained 194 genes on the list (**Table S3**). These genes were included on the list of new candidate drug genes for further analysis.

3.3. MS drug targets finding

Finally, the drug target genes were prioritized based on the network analysis and the drug databases. Herein, we obtained 2,904 gene pairs from the PPI network (**Table S4**) and 27 genes targeted by 68 new candidate drugs based on DrugBank and TTD (**Tabel S5**). We found one drug, dimethyl fumarate that has been clinically approved for MS treatment (**Figure 5**). This drug is an effective medicinal option, administered twice a day in MS medication [25][26]. This study emphasized that the biological functional annotation we applied can be validated through the drug used in the clinic for MS disease.

This research also found eight drug-target genes bound to 8 drugs approved for other diseases and under clinical investigation for MS, including human immunoglobulin G, antithymocyte immunoglobulin (rabbit), liothyronine, abatacept, topiramate, and phenytoin (**Table S6**). These drugs can potentially repurpose MS medication (**Figure 6**). An example of a drug repurposed for MS is abatacept, which is approved for rheumatoid arthritis, targeting CD80 and CD86 gene pathways. This drug is currently under clinical investigation for MS in a phase II trial (NCT01116427) and has a considerable potential to be used for MS [27]. Thus, we would like to emphasize that integrating genomic variants and gene networking can potentially guide the drug repurposing for MS disease.

4. Discussion

The focus of our present work is to narrow down candidate drugs of a debilitating disease, multiple sclerosis, through leveraging large bioinformatics datasets together with human genetics data. More specifically, we use personalized genomic data and genetic mapping to guide drug repurposing for MS. In particular, this study focused on using new candidate drugs for MS by prioritizing candidate genes derived and identified from the GWAS databases. Six categories of functional annotations were used to build an assessment

system, in order to prioritize the MS risk genes as leads for new candidate drugs. We hypothesized that the broad strategy of genetic variant prioritization, using the functional annotations described in this study, would enable us to translate the risk genes to meaningful, actionable insights on MS. According to our analyses, we ensure the sensitivity of our study results by setting the threshold of a biological score ≥ 2 to find a much higher number of genes as biological MS risk genes, and thereby candidates for MS drug targets.

In this research, 27 drug-target genes were found to be bound to 68 drugs. In addition, 8 drug-target genes were found to be bound to 8 drugs, of which some were still under clinical testing for MS, namely human immunoglobulin G (NCT00220779), anti-thymocyte immunoglobulin (rabbit) (NCT03342638), liothyronine (NCT02760056), abatacept (NCT01116427), topiramate (NCT00217295), and phenytoin (NCT01451593). From the data collected, one available drug approved for MS is dimethyl fumarate (**Figure 5**). Dimethyl fumarate, also known as BG-12, was licensed as first-line therapy and oral therapy for MS in 2013. It is also known as neuroprotective and immunomodulatory [28][29]. The mechanism of action of dimethyl fumarate is to react with cysteine residues from KEAP1 (Kelch-like ECHassociated protein 1), which causes KEAP1 to be dissociated from the nuclear factor (erythroidderived 2)-like 2 (Nrf2) pathway toward Nrf2 nuclear translocation. Nrf2 then binds antioxidant response element (ARE) and drives antioxidant target gene expression toward neuronal protection, reduces astrocyte activation, and prolongs cell life [30].

We identified eight promising targets that overlapped with drugs that could potentially be repurposed to treat MS. These include C3, CD4, CD86, THRA, CSF2RB, CD80, IFNGR2, and CACNA1S. Among them, we highly proposed CD80 and CD86 as potential targets for MS, since these targets are closely related to IL7R as biological MS risk genes with high functional annotation scores **(Figure 6)**. The CD80/CD86 pathway is essential for controlling T cell activation and preserving immunological tolerance to self-antigens. The findings of functional and genome-wide investigations demonstrate that genes encode molecules that fit in. This pathway may increase the likelihood of developing autoimmune illnesses and may be viewed as a potential MS candidate gene [31][32][33]. In addition, we identified CD80- and CD86- targeting drugs, including anti-thymocyte immunoglobulin (rabbit), abatacept, and belatacept. Among these drugs, in fact, anti-thymocyte immunoglobulin (rabbit) (NCT03342638) and abatacept (NCT01116427) are

currently under clinical investigation for MS. Therefore, from this perspective, targeting CD80 and CD86 might become novel therapeutic options for MS therapy. Further clinical evidence generation would be needed to validate these targets.

Combining results from genome-wide association studies and bioinformatic, gene-level annotation of human genetic variants is a powerful approach to identify candidate new drugs for MS. However, it is important to consider that this approach is not without limitations, such as not all the identified target genes can either be targeted and/or demonstrate pharmacological activity with the desired profile for use in clinic. The genes identified in this manner would therefore miss the drug targeting window for the particular disease. Further investigation is thus required to determine the effect of the candidate drugs towards the clinic. Hence, we suggest that the use of current finding with subsequent investigation in functional studies to ascertain the role of drug target genes discovered in this study. The validation from existing results is necessary and important to ensure whether our drug candidates produced the desired interaction (intended from the study), any undesired side effects, or ineffective effects.

5. Conclusions

Our study utilizes MS functional genomic variants to open up additional avenues for the repurposing of existing drugs. Herein, we identify CD80 and CD86 as potential targets for MS treatment. The involvement of these genes with MS might be highly significant, thereby requiring further examination. By targeting CD80 and CD86, belatacept could be a promising therapy option for MS therapy. However, more studies from animal models and clinical trials are needed to confirm the biological mechanisms of the drug for new diseases. In this research, we combined the drug repurposing approach with integrated bioinformatics methodology to identify drugs with new indications for MS. Finally, this study emphasizes the vast potential of utilizing functional genomic variants as a basis to drive repurposing for MS disease.

Acknowledgments

The author would like to thank Universitas Ahmad Dahlan for providing the resources and opportunities for this study.

Author's Contribution

A.R.A., L.M.I., and W.A. conceived and designed the study. A.R.A. and W.A. performed the computational analysis. A.R.A. wrote the manuscript. A.R.A., L.M.I., W.A., D.A.P., A.B. and R.C. revised the manuscript. L.M.I and W.A. supervised and coordinated this study. All authors have read and approved the manuscript and made significant contributions to this study.

Competing interests

The authors declare no conflict of interest.

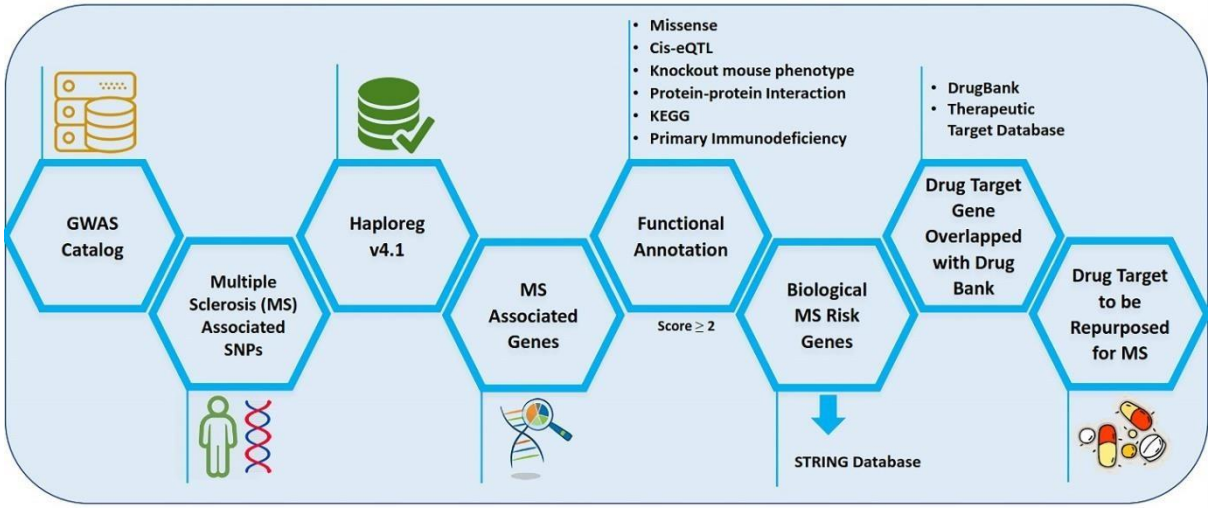

Figure 1. Scheme of drug repurposing using genomic database for multiple sclerosis (MS)

GENCODE_id	GENCODE_name	Missense/ Nonsense	cis-eQTL	KO mice	PPI	KEGG	PID	Total score
ENSG00000159128	IFNGR2	1	1	1	1	1	1	6
ENSG00000168685	IL7R	1	1	1	1	1	1	6
ENSG00000013725	CD6	1	1	1	1	1	0	5
ENSG00000067182	TNFRSF1A	0	1	1	1	1	1	5
ENSG00000101017	CD40	0	1	1	1	1	1	5
ENSG00000105397	TYK2	1	0	1	1	1	1	5
ENSG00000121966	CXCR4	0	1	1	1	1	1	5
ENSG00000131323	TRAF3	0	1	1	1	1	1	5
ENSG00000168610	STAT3	0	1	1	1	1	1	5
ENSG00000020633	RUNX3	1	0	1	1	1	0	4
ENSG00000081059	TCF7	0	1	1	1	1	0	4
ENSG00000102882	MAPK3	0	1	1	1	1	0	4
ENSG00000104432	IL7	0	1	1	1	1	0	4
ENSG00000107485	GATA3	0	1	1	1	1	0	4
ENSG00000109320	NFKB1	0	1	1	1	1	0	4
ENSG00000111252	SH2B3	1	1	1	0	1	0	4
ENSG00000114013	CD86	0	1	1	1	1	0	4
ENSG00000117090	SLAMF1	0	1	1	1	1	0	4
ENSG00000124181	PLCG1	1	0	1	1	1	0	4
ENSG00000125730	C3	0	0	1	1	1	1	4

Figure 2. Biological annotations prioritized for multiple sclerosis (MS) genes with score ≥ 2.

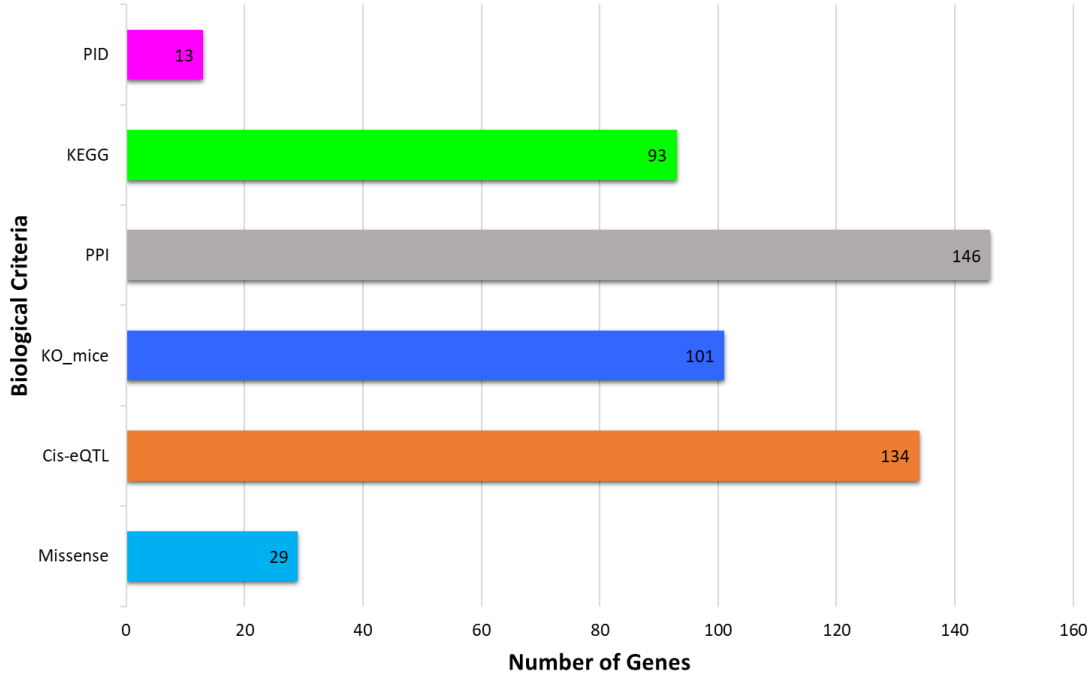

Figure 3. Histogram of the number of genes (y-axis) meeting each of the six biological criteria (x-axis) for drug prioritization..

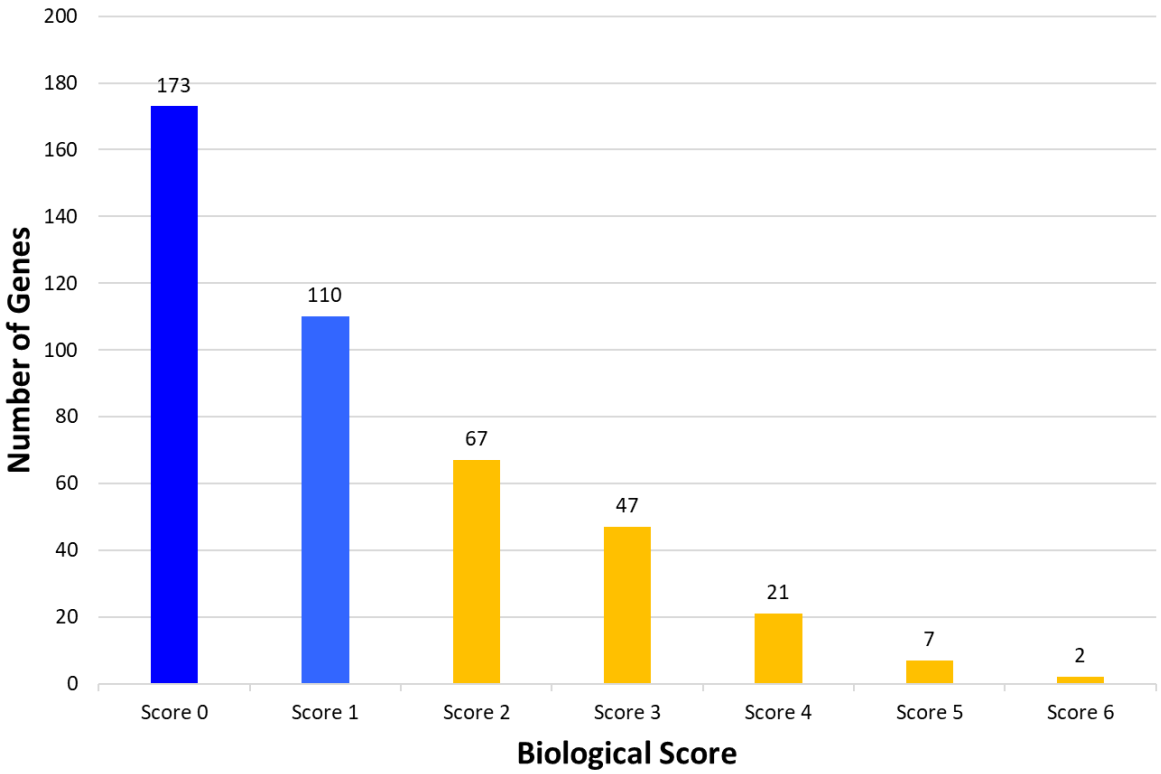

Figure 4. Histogram of the number of genes (y-axis) meeting each of the six biological criteria (x-axis) for drug prioritization. It is shown that there were 173 genes with score 0, 110 genes with score 1, and 144 (67+47+21+7+2) genes with total score ≥ 2, denoted as "biological MS risk genes".

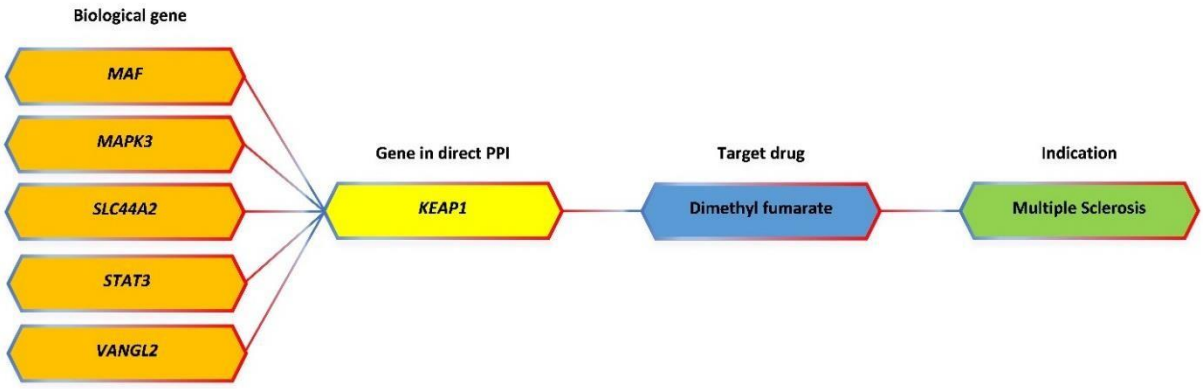

Figure 5. Relationship between biological MS risk genes and available drugs for MS.

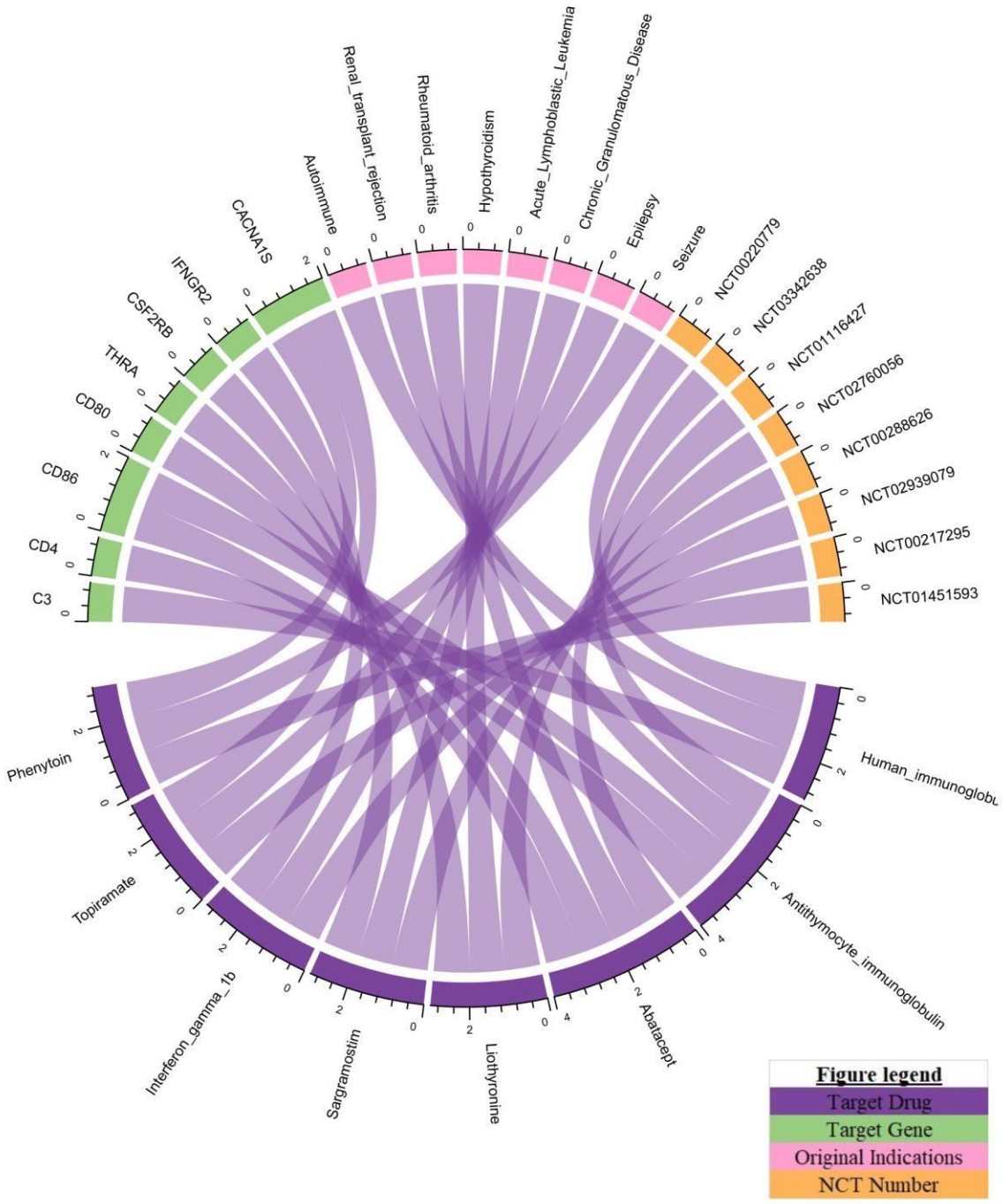

Figure 6. Relationship between biological MS genes, and drugs approved for other indications and under clinical investigation for MS.

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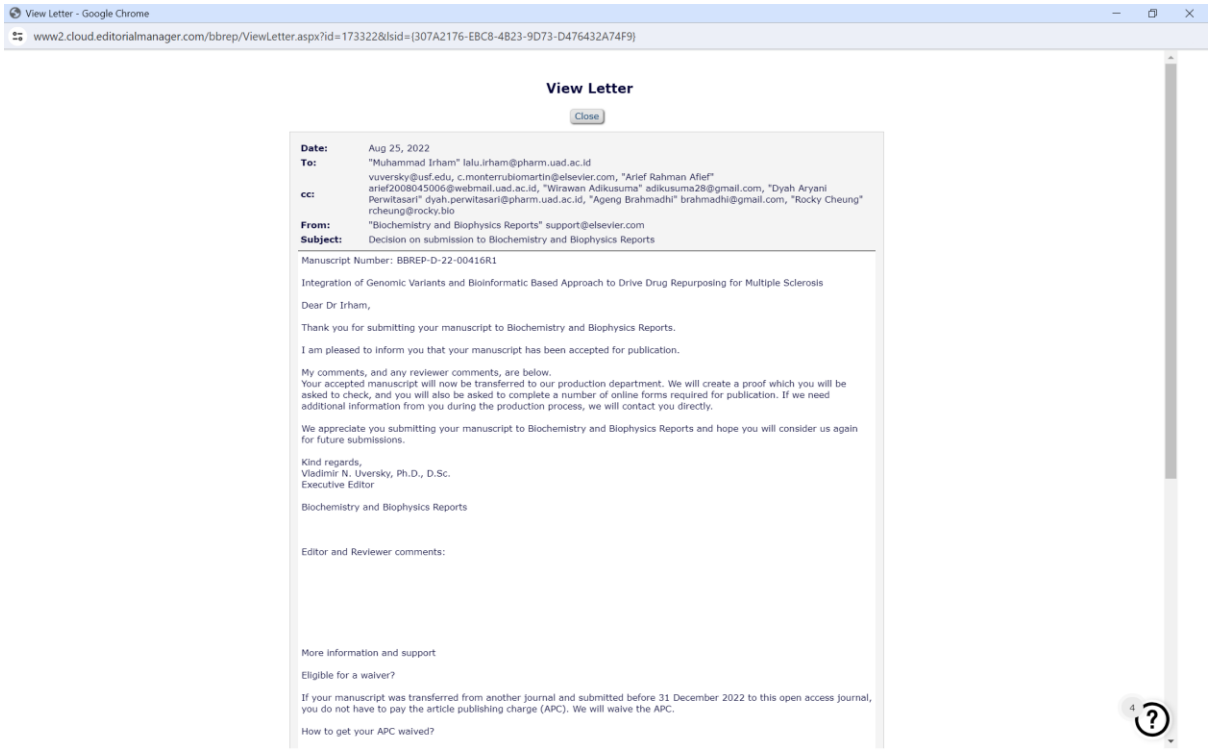

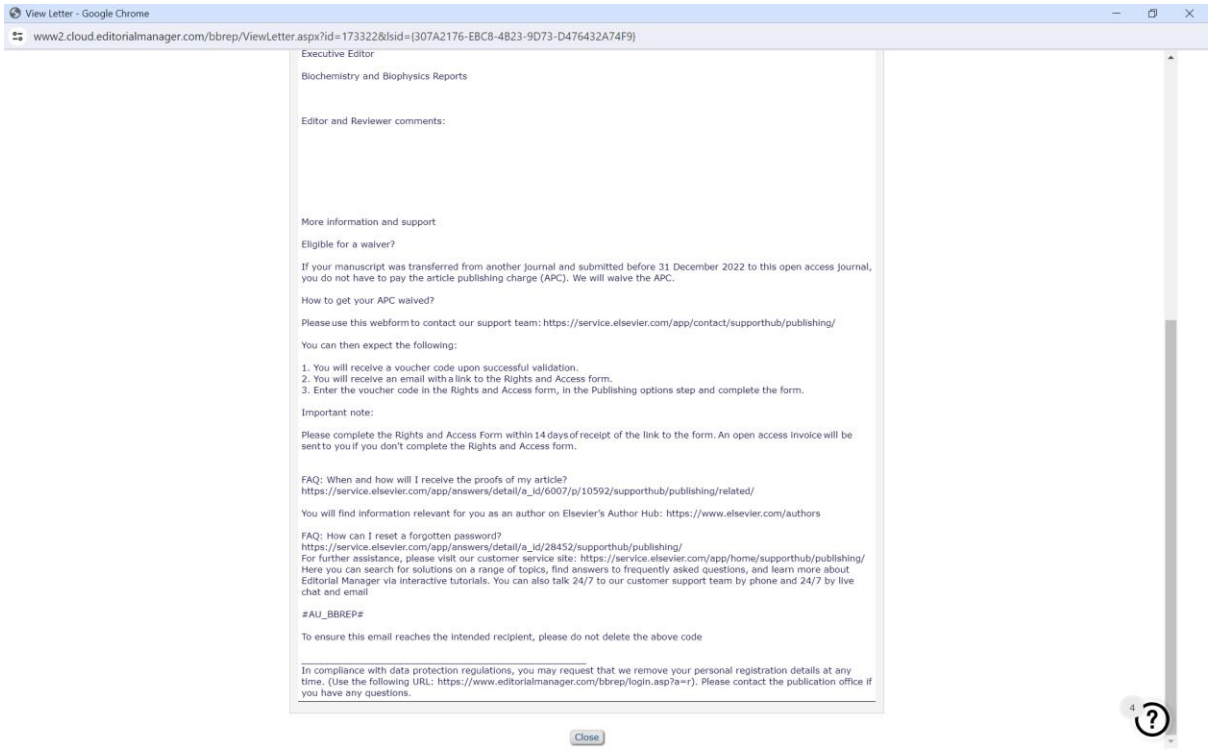

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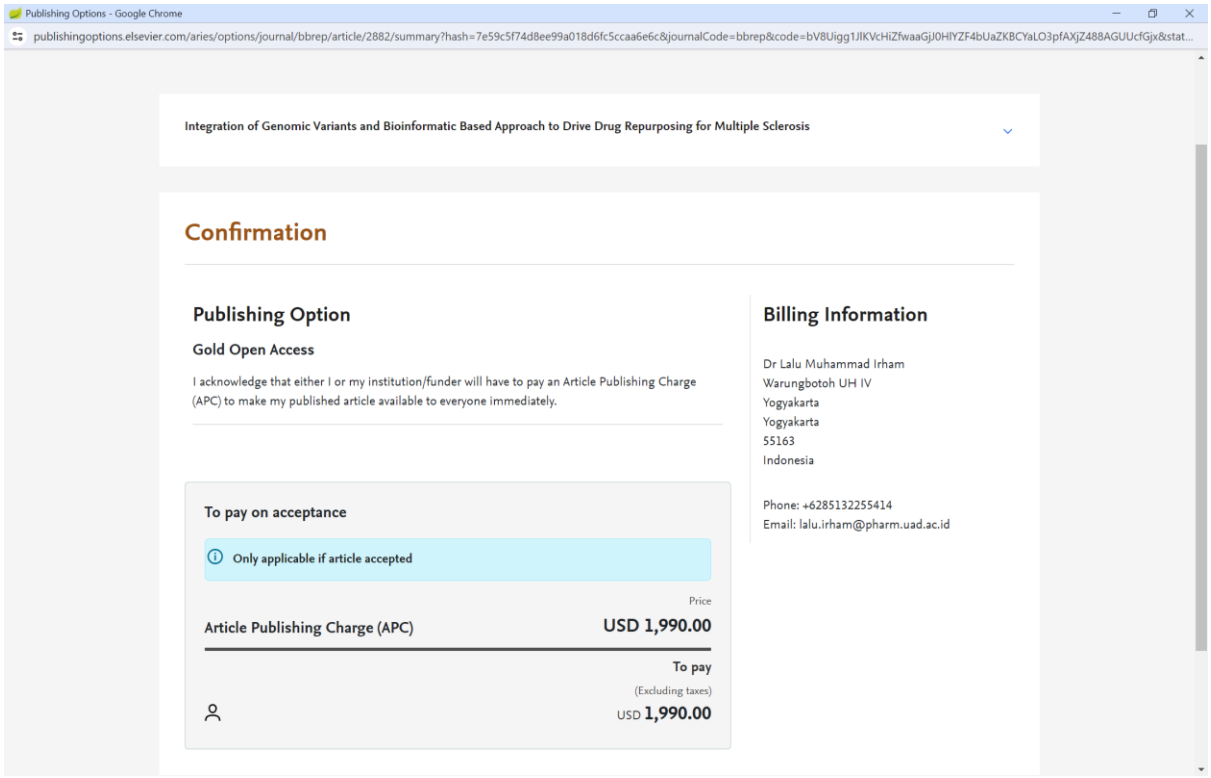

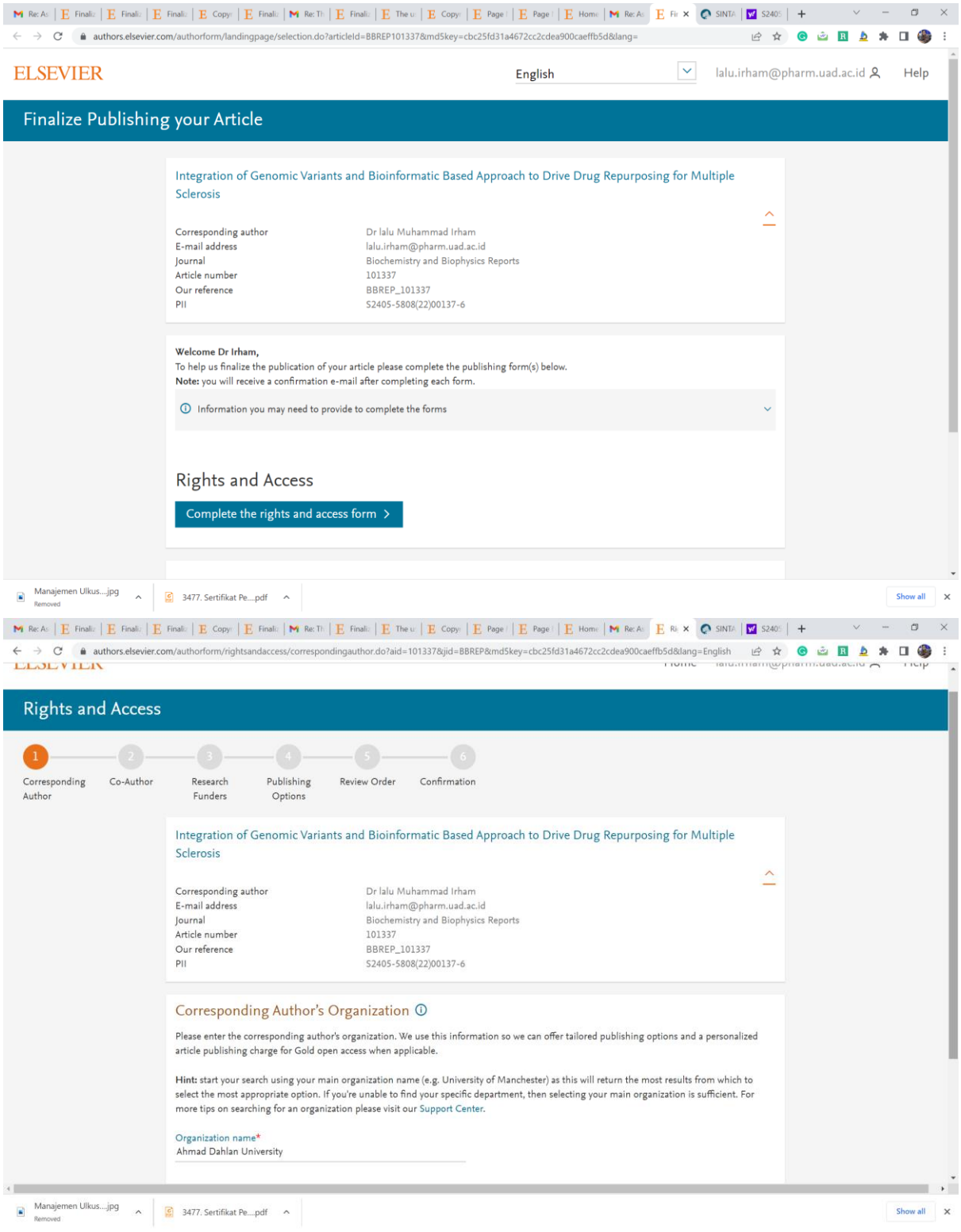
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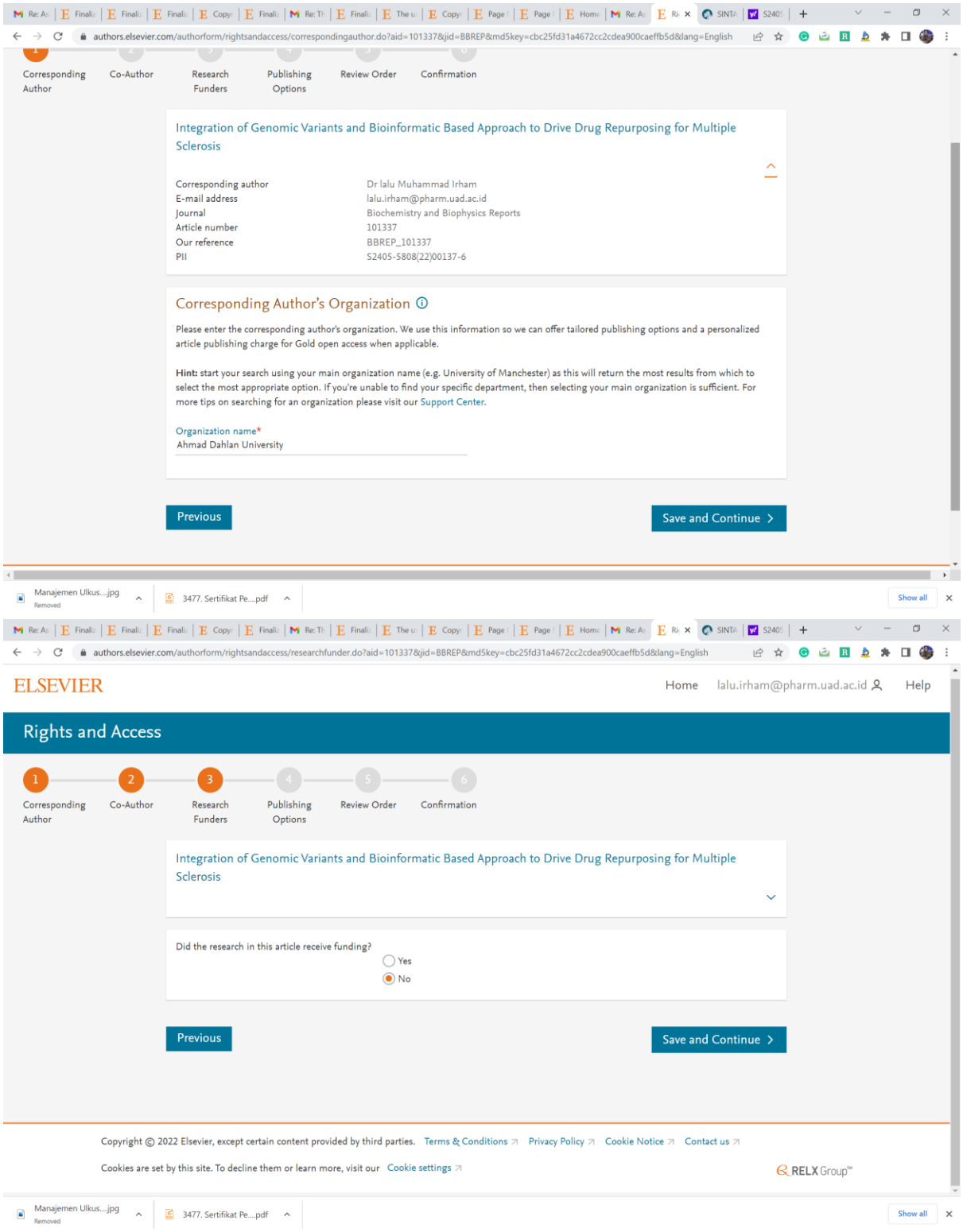
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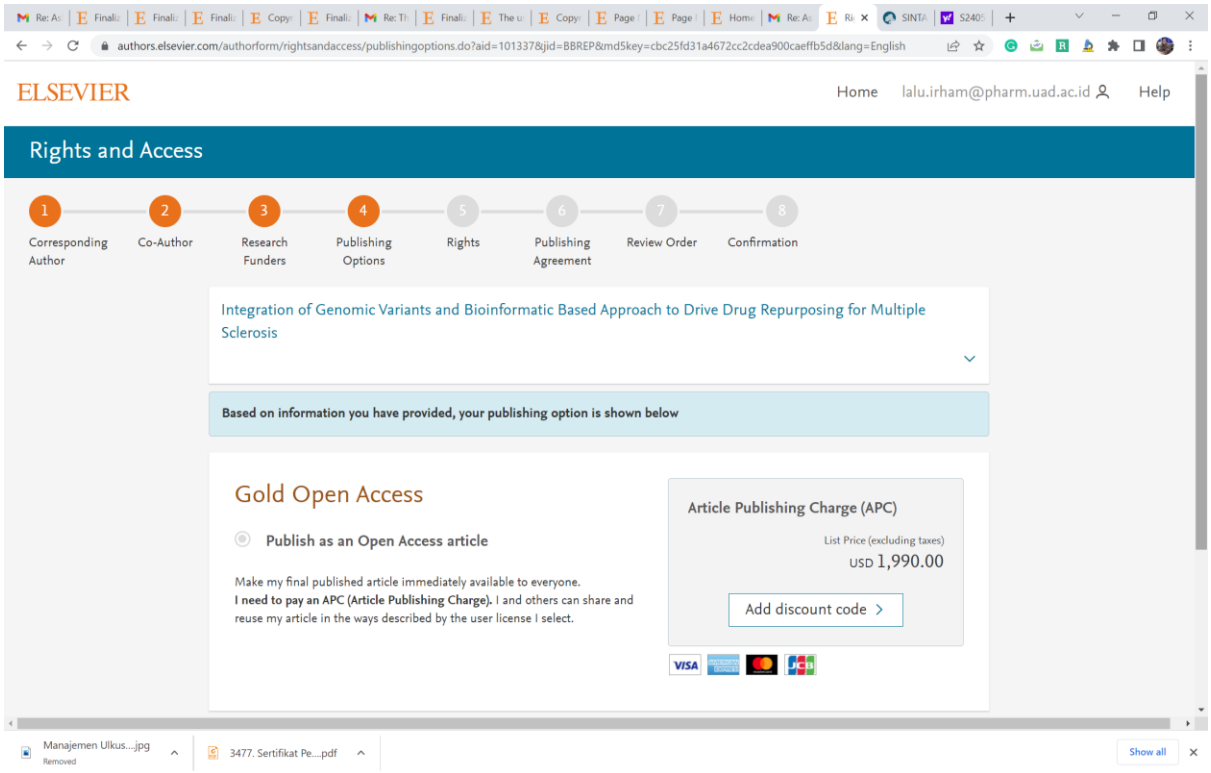

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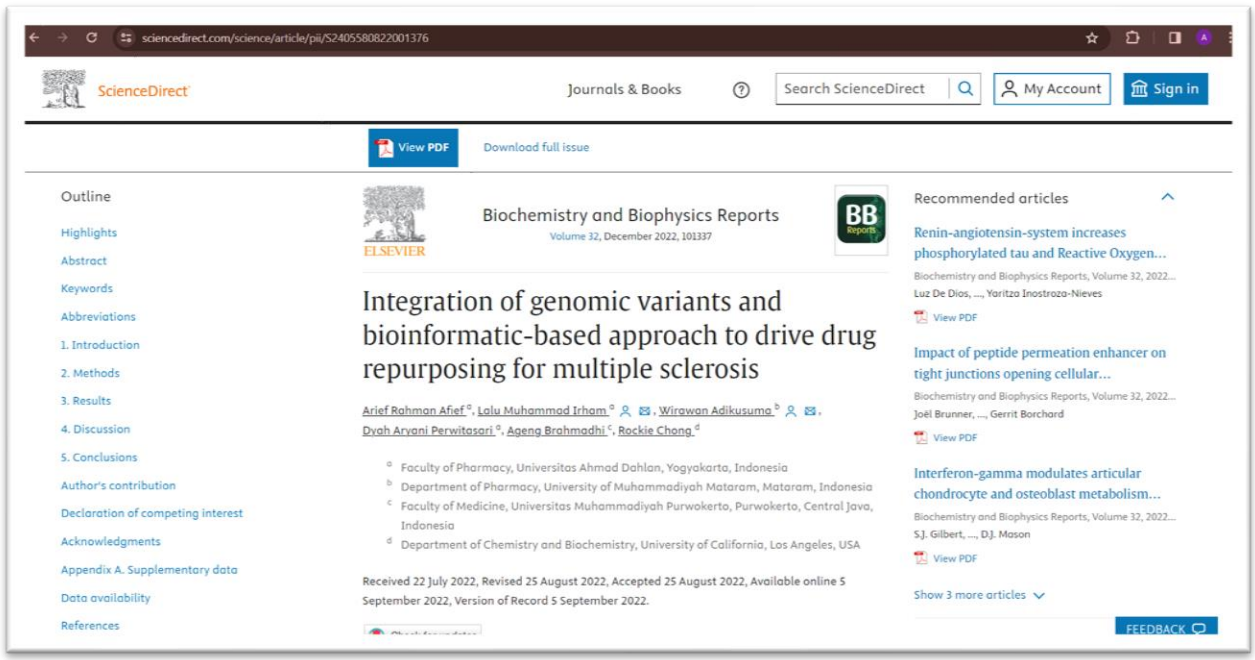

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