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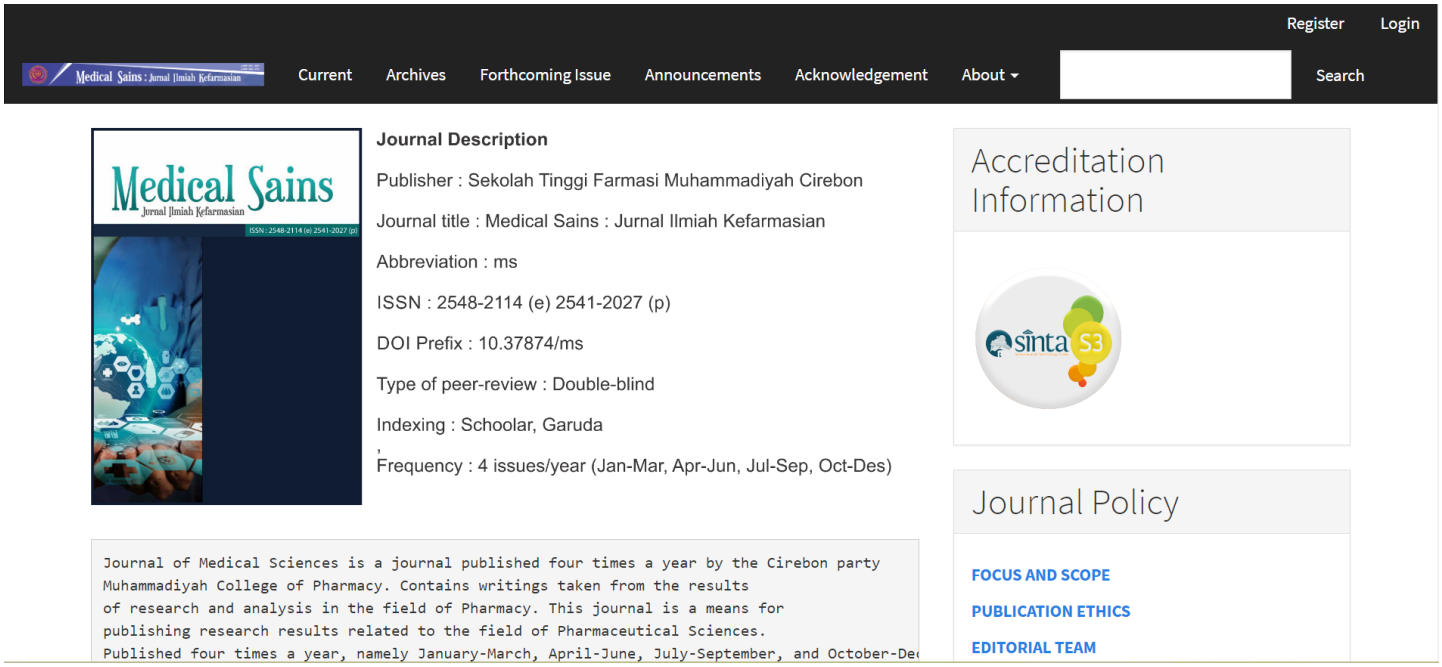

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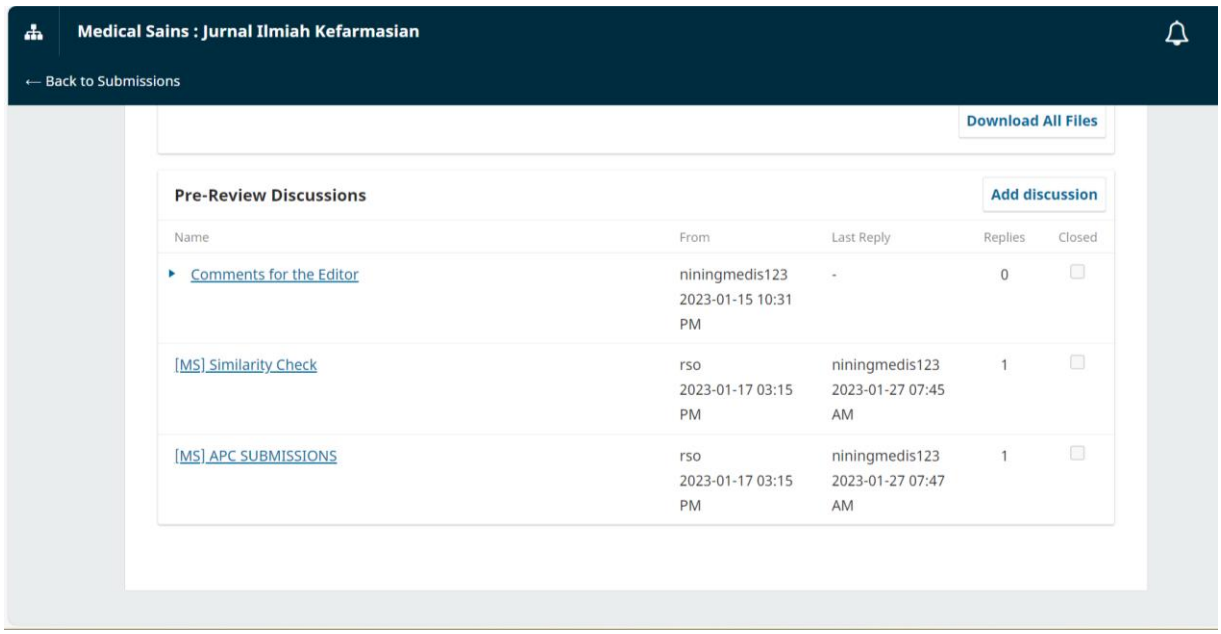

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DRUG REPURPOSING UNTUK *RHEUMATOID ARTHRITIS* MELALUI PEMANFAATAN DATA VARIASI GENETIK

DRUG REPURPOSING FOR RHEUMATOID ARTHRITIS THROUGH THE UTILIZATION OF GENETIC VARIATION DATA

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ABSTRAK

Rheumatoid arthritis (RA) adalah penyakit autoimun progresif dengan inflamasi kronik yang menyerang sistem muskuloskeletal yang menyebabkan pembengkakan, nyeri sendi dan destruksi jaringan sinovial disertai gangguan pada sistem motorik. Faktor genetik berperan sangat penting dalam patogenesis RA. Pada saat ini pengobatan RA masih sangat terbatas sehingga perlunya upaya untuk menemukan obat baru. Memanfaatkan obat lama untuk indikasi baru atau *drug repurposing* dapat menjadi salah satu solusi terbaik pengobatan RA. Penelitian ini bertujuan untuk mengetahui variasi genetik yang berisiko terhadap RA dan memperoleh kandidat obat baru yang berpotensi terhadap RA dengan memanfaatkan database genomik dan analisis bioinformatika. *Single Nucleotide Polymorphisms* (SNPs) terkait RA diperoleh dari database GWAS *catalog* dengan *p-value* < 10^{-8} kemudian dikembangkan menggunakan HaploReg v4.1. dengan signifikansi $r^2 \geq 0.8$ untuk populasi Asia. Gen yang paling berpengaruh terhadap RA diprioritaskan berdasarkan anotasi fungsional. Kandidat gen yang berisiko terhadap patogenesis RA masing-masing diberikan skor 1. Gen yang memiliki skor ≥ 2 dikategorikan sebagai gen yang paling berisiko terhadap patogenesis RA. Semua gen yang berpotensi untuk RA dipetakan ke DrugBank. Dari hasil pencarian di DrugBank didapatkan 24 gen yang mengikat 60 obat. Diantara obat tersebut tiga obat yang disetujui FDA untuk RA : tocilizumab, sarilumab dan abatacept. Studi ini juga menemukan dua kandidat obat dengan indikasi lain yang berpotensi sebagai kandidat obat baru untuk RA: alpha-linolenic acid yang menargetkan jalur gen *FADS1*, belatacept yang menargetkan gen *CD80* dan *CD86*. Kedua kandidat obat ini memiliki potensi yang besar untuk digunakan pada RA. Strategi pengembangan obat untuk menemukan indikasi baru dari obat yang sudah ada atau kandidat obat yang potensial termasuk pengembangan klinis menawarkan keuntungan berharga dalam proses pengembangan obat seperti efisiensi waktu, biaya dan peningkatan keberhasilan pengobatan.

Kata kunci : Autoimun; bioinformatika; *drug repositioning*; *rheumatoid arthritis* ; variasi genetik

ABSTRACT

Rheumatoid arthritis (RA) is a progressive autoimmune disease with chronic inflammation that attacks the musculoskeletal system causing swelling, joint pain and destruction of synovial tissue accompanied by disturbances in the motor system. Genetic factors play a very important role in the pathogenesis of RA. At this time the treatment of RA is still very limited so efforts are needed to find new drugs. Utilizing old drugs for new indications or drug repurposing can be one of the best solutions for RA treatment. This study aims to determine the genetic variations that are at risk for RA and obtain potential new drug candidates for RA by utilizing genomic databases and bioinformatic analysis. SNPs related to RA were obtained from the GWAS catalog database with a p-value < 10^{-8} and then developed using HaploReg v4.1. with a significance of $r^2 \geq 0.8$ for Asian populations. Genes that influence RA most are prioritized based on functional annotations. Candidate genes that are at risk for RA pathogenesis are each given a score of 1. Genes with scores ≥ 2 are categorized as genes most at risk for RA pathogenesis. All potential drugs for RA are mapped to the DrugBank. From a search on DrugBank that included 24 genes and 60 drugs, the FDA approved three drugs for RA: tocilizumab, sarilumab and abatacept. This study also found two drug candidates with other indications as potential new drug candidates for RA: alpha-linolenic acid which targets the FADS1 gene pathway, belatacept which targets the CD80 and CD86 genes. Both of these drug candidates have great potential for use in RA. Drug development strategies to find new indications for existing drugs or potential drug candidates including clinical development offer valuable advantages in the drug development process such as time, cost efficiency and increased treatment efficacy.

Keywords *: Autoimmune; bioinformatics; drug repositioning; rheumatoid arthritis; genetic variation*

PENDAHULUAN

Rheumatoid arthritis (RA) adalah penyakit autoimun progresif dengan inflamasi kronik yang menyerang sistem muskuloskeletal menyebabkan pembengkakan, nyeri sendi dan destruksi jaringan sinovial disertai gangguan pada sistem motorik (Rodríguez-Elías *et al.*, 2016). RA merupakan kelainan heterogen yang disebabkan oleh respons autoimun abnormal yang dipicu karena adanya interaksi kompleks faktor genetik dan lingkungan (Zamanpoor, 2019). Peradangan sinovial kronis pada RA menimbulkan kerusakan sendi dan kecacatan. Penyebab pasti RA tidak diketahui, namun inisiasi penyakit merupakan hasil dari interaksi antara kerentanan genetik dan pemicu lingkungan (Deane *et al.*, 2017). Faktor risiko yang mempengaruhi RA adalah genetik, lingkungan dan autoimun (Karami *et al*., 2019).

Prevalensi RA mempengaruhi sekitar 0,5-1% dari populasi dunia (Laufer *et al*., 2019). Berdasarkan diagnosis nakes prevalensi RA di Indonesia (2013) sebanyak 11.9% dan berdasarkan diagnosis atau gejalanya 24.7% (Kemenkes Ri, 2013). Prevalensi tertinggi pada provinsi di Indonesia tahun 2013 terdapat di Nusa Tenggara Timur (33.1%), Jawa Barat (32.1%), Bali (30%). Prevalensi di Jawa Tengah berjumlah 26.9% dan berdasarkan diagnosis atau gejalanya sebesar 11.2% (Kemenkes RI, 2013). Penelitian Laufer *et al*., (2019) pada populasi Asia dan Eropa menunjukkan bahwa faktor risiko genetik pada lokus *Human Leukocyte Antigen* (HLA) berkontribusi pada etiologi RA (Laufer *et al*., 2019). Okada *et al.*, (2014) juga melakukan penelitian meta-analisis studi asosiasi genom pada lebih dari 100.000 subjek keturunan Eropa dan Asia (29.880 kasus RA dan 73.758 kontrol), mengevaluasi adanya 10 juta SNPs yang terkait RA (Okada *et al*., 2014).

Tujuan pengobatan RA yaitu untuk menghilangkan inflamasi, mencegah deformitas, mengembalikan fungsi sendi, dan mencegah kerusakan jaringan lebih lanjut (Bullock *et al*., 2018). Secara substansial pengobatan RA dapat mencegah dan memperlambat perkembangan kerusakan sendi hingga 90% (Aletaha & Smolen, 2018). Agen antiinflamasi, seperti obat antiinflamasi nonsteroid (OAINS) dan glukokortikoid adalah manajemen awal nyeri atau peradangan (Del Grossi Moura *et al*., 2018). Monoterapi dengan *Disease-Modifying Antirheumatic Drugs* (DMARDs) adalah langkah pertama dalam pengobatan RA (Lin *et al*.,

2020). Metotreksat adalah DMARDs pilihan paling umum sebagai agen lini pertama, mengingat kemanjurannya yang sangat baik dalam mencapai remisi dan efektif pada 25% pasien (Mysler *et al*., 2021). Leflunomide adalah DMARDs lini pertama alternatif yang menghambat dihydroorotate dehydrogenase, juga penting untuk sintesis DNA dan proliferasi limfosit (Alamri *et al*., 2021).

Pengobatan dini dengan metotreksat yang dikombinasi dengan glukokortikoid dan DMARDs lain, seperti penghambat *TNF*, *IL-6*, atau *Janus kinase*, meningkatkan hasil dan mencegah kecacatan RA (Aletaha & Smolen, 2018). Perjalanan penyakit RA bervariasi, ditentukan oleh ketaatan pasien untuk berobat dalam jangka waktu yang lama sehingga perlunya dicarikan strategi pengobatan yang lebih efektif dengan biaya terjangkau dan waktu yang lebih cepat. Penggunaan kembali obat lama untuk mengobati penyakit umum dan langka semakin menjadi proposisi yang menarik karena melibatkan penggunaan senyawa yang tidak berisiko, dengan potensi biaya pengembangan keseluruhan yang lebih rendah dan jadwal pengembangan yang lebih pendek. Beberapa studi lain menjelaskan manfaat genetik digunakan sebagai target dari pengembangan obat baru pada beberapa penyakit, seperti, *colorectal cancer* (Irham *et al*., 2020), *atopic dermatitis* (Adikusuma *et al*., 2021), *chronic hepatitis B* (Irham, Adikusuma, Perwitasari, *et al*., 2022), *asthma* (Adikusuma *et al*., 2022), *tuberculosis* (Irham *et al*., 2022), *antidepressants* (Lesmana *et al*., 2022), dan *multiple sclerosis* (Afief *et al*., 2022).

Banyak database yang menyediakan tentang informasi hubungan suatu gen dengan patogenesis penyakit salah satunya GWAS *catalog*, yang merupakan database yang menyediakan informasi variasi genetik yang menghubungkan seluruh genom dengan fenotip yang telah dipetakan dengan *cis-expression quantitative trait loci* (cis-eQTL) yang mempengaruhi sifat alami dalam sifat fenotipe (Irham *et al*., 2020). GWAS *catalog* mengidentifikasi varian terkait penyakit umum dalam populasi yang dapat berkontribusi secara akumulatif terhadap patogenesis suatu penyakit seperti RA. Tujuan penelitian ini untuk mengetahui variasi genetik yang berisiko terhadap RA dengan memanfaatkan database genomik dan analisis bioinformatika untuk mendapatkan kandidat obat baru yang berpotensi terhadap RA.

METODE PENELITIAN

Single Nucleotide Polymorphism (SNPs) terkait RA diperoleh dari database GWAS *catalog* yang didownload (https://www.ebi.ac.uk/gwas/) diakses tanggal 06 Februari 2022. SNPs yang berisiko terhadap RA diidentifikasi dengan kriteria signifikansi *p-value* $<10^{-8}$ kemudian dikembangkan menggunakan HaploReg v4.1. dengan kriteria inklusi $r^2 \geq 0.8$ untuk populasi Asia. Gen yang paling berpengaruh terhadap RA diprioritaskan berdasarkan enam anotasi fungsional meliputi *mutasi missense/nonsense*, *cis-expression quantitative trait loci* (cis-eQTL), *protein-protein interaction* (PPI), *molecular pathway analyses*, *knockout mouse phenotype*, *dan primary immunodeficiency* (PID). Kandidat gen yang berisiko terhadap patogenesis RA yang memenuhi enam kriteria biologi masing-masing diberikan skor 1. Gen yang memiliki skor ≥ 2 dikategorikan sebagai gen yang paling berisiko terhadap patogenesis RA. Gen tersebut dianalisis menggunakan database STRING untuk memperluas daftar kandidat gen sebagai target obat. Semua obat yang berpotensi untuk RA dipetakan ke DrugBank untuk mengetahui aktivitas farmakologis, efektivitas pada manusia, uji klinis dan obat eksperimental. Selanjutnya semua obat dikonfirmasi ke ClinicalTrials.gov untuk mengetahui uji klinis obat RA atau penyakit lain.

ANALISIS DATA

Enam anotasi fungsional digunakan untuk membangun sistem penilaian yang mewakili kandidat gen yang paling mungkin menjadi target RA. Enam anotasi fungsional biologi terdiri dari : *missense atau nonsense* yang diperoleh dari perluasan HaploReg v4.1 (Ward & Kellis, 2016) dengan kriteria signifikansi $r^2 \geq 0.8$ untuk populasi Asia. HaploReg v4.1 menghubungkan varian genetik dengan *cis-expression quantitative trait loci* (cis-eQTLs) (Okada *et al*., 2014). Jika gen memiliki risiko terhadap RA dengan cis-eQTL di seluruh

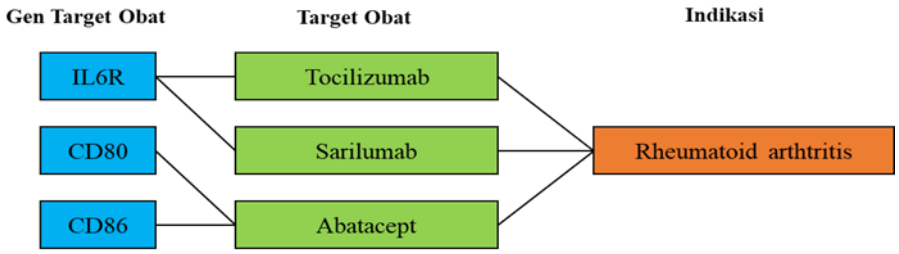
darah, maka gen tersebut diberi satu poin. Gen dari Ensembl ID manusia diubah menjadi Ensembl ID tikus menggunakan BioMart (Frankish *et al.*, 2019). Sumber data selanjutnya menggunakan *mammalian phenotype ontology* yang berisi informasi tentang tikus dan mamalia lainnya (Drabkin *et al*., 2012). Analisis proses biologis menggunakan gen ontologi dengan *False Discovery Rate* (FDR) < 0.05. Analisis pengayaan pada jalur molekuler menggunakan KEGG (Frankish *et al*., 2019) pada signifikansi FDR < 0,05. Kriteria anotasi terakhir adalah PID yang berfungsi untuk memprioritaskan gen yang berisiko terhadap RA. PID adalah keadaan dimana terjadinya defek sistem imun yang disebabkan oleh mutasi pada kode genetik yang mengkode komponen-komponen penyusun sistem imun tubuh (Mahendra, 2021). Analisis data dilakukan menggunakan uji hipergeometrik dengan kriteria signifikansi $p < 0,05$ (Adikusuma *et al*., 2021). Setiap anotasi fungsional diberi skor 1, dan gen dengan skor ≥ 2 dikategorikan sebagai gen yang berisiko terhadap RA.

Gen yang berisiko terhadap RA diperluas menggunakann database STRING (http://string-db.org) untuk mendapatkan lebih banyak kandidat gen sebagai target obat. Tujuan database STRING adalah untuk mengintegrasikan interaksi fungsional yang terkait dengan ekspresi protein dengan memasukkan dan mengatur data yang terkait dengan interaksi protein-protein (Szklarczyk *et al.*, 2019). Gen target obat untuk RA dipetakan ke DrugBank (Wishart *et al*., 2018 ; Li *et al*., 2018). Database DrugBank berfungsi untuk mengetahui aktivitas farmakologis, efektivitas pada manusia, uji klinis dan obat eksperimental. Semua target obat untuk RA dikonfirmasi ke ClinicalTrials (http://clinicaltrials.gov/) merupakan database universal yang mendokumentasikan obat-obatan yang dalam penyelidikan klinis pada subjek manusia. Data dari ClinicalTrials.gov didownload tanggal 19 Maret 2022 untuk memeriksa apakah obat sedang dalam pemeriksaan klinis untuk RA atau penyakit lainnya. Selanjutnya,target obat dipetakan ke dalam DrugBank (http://www.drugbank.ca/) diakses pada tanggal 05 April 2022 untuk menemukan kandidat obat RA.

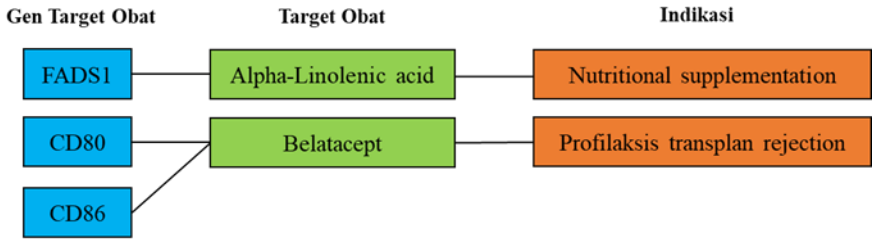
HASIL DAN PEMBAHASAN

Berdasarkan studi ini diperoleh 360 SNPs terkait RA (Tabel S1). Kemudian SNPs diperluas menggunakan HaploReg V4.1 berdasarkan kriteria $r^2 \geq 8$ pada populasi Asia. Enam anotasi fungsional dirancang untuk memprioritaskan gen yang berisiko terhadap RA berdasarkan kriteria biologinya. Dari enam anotasi fungsional, ditemukan :(1) gen dengan varian *mutase missense*/*nonsense* (n=39); (2) gen cis-eQTL(n=101); (3) gen yang terlibat dalam jalur gen fenotipe tikus *knockout mouse phenotype* (n=102); gen yang terlibat dalam GO yang digunakan untuk mengevaluasi PPI (n=122)); (3) gen yang terlibat dalam jalur KEGG (n=61); PID (n=16). Selanjutnya setiap gen diberikan skor berdasarkan jumlah kriteria yang sesuai (skor mulai dari 0 hingga 6 untuk setiap gen). Gen dengan skor 0 sebanyak 156 gen dengan skor 1 sebanyak 85 gen dengan skor 2 sebanyak 45, gen dengan skor 3 sebanyak 40, gen dengan skor 4 sebanyak 26, gen dengan skor 5 sebanyak 6, dengan skor 6 sebanyak 2. Diperoleh 119 gen dengan skor ≥2 didefinisikan sebagai gen yang berisiko terhadap RA.

Selanjutnya gen yang berisiko terhadap RA diperluas menggunakan database STRING. Tujuan menggunakan database STRING adalah untuk mendapatkan kandidat target obat yang lebih banyak lagi. Target obat dipetakan ke DrugBank (http://www.drugbank.ca/) untuk menemukan kandidat obat RA. Dari hasil pencarian di DrugBank didapatkan 24 gen yang mengikat 60 obat, diantaranya tiga obat untuk RA yaitu tocilizumab, sarilumab dan abatacept. Studi ini juga menemukan dua kandidat obat dengan indikasi lain yang berpotensi sebagai kandidat obat baru untuk RA yaitu alpha-linolenic acid dan belatacept yang menargetkan jalur gen *FADS1*, *CD80* dan *CD86* (**Gambar 2**). Obat ini sedang dalam penyelidikan untuk RA dalam *phase II* (NCT01179971) untuk alpha-linolenic acid dan *phase 1*, *phase II* (NCT) untuk belatacept. Kedua kandidat obat ini memiliki potensi yang besar untuk digunakan pada RA (**Gambar 1**).



Gambar 1. Hubungan Antara Gen yang Berisiko terhadap RA dan Obat yang Tersedia untuk RA



Gambar 2. Hubungan Antara Gen yang Berisiko terhadap RA dan Obat yang Tersedia untuk Indikasi Lain.

RA terkait dengan beberapa gen kerentanan penting, antara lain gen *FADS1* (Thalayasingam *et al.*, 2018) dan *CD80/CD86* (Marquez Pete *et al.*, 2020). *FADS1* telah diidentifikasi sebagai target potensial dalam penelitian terkait RA. Sebagai enzim yang terlibat dalam sintesis asam lemak omega-6 dan omega-3, *FADS1* memiliki potensi untuk modulasi jalur peradangan yang terlibat dalam perkembangan RA (Seifert *et al.*, 2012). Penelitian baru-baru ini telah menunjukkan bahwa alpha-linolenic acid dan belatacept, yang menargetkan jalur gen *FADS1*, mungkin menjadi kandidat potensial untuk terapi RA. Namun, perlu dilakukan penelitian lebih lanjut untuk memahami secara lebih mendalam mekanisme yang terlibat dan mengevaluasi efektivitas serta efek samping potensial dari penggunaan alpha-linolenic acid dan belatacept sebagai terapi RA. *CD80* adalah gen penyandi protein dan merupakan glikoprotein transmembran tipe I (Mir, 2015). *CD80/86* diekspresikan pada a*ntigen-presenting cells* (APCs), yang membantu melawan zat asing yang masuk ke dalam tubuh dimana sel-sel ini mengirimkan sinyal ke limfosit T *helper* (Patakas *et al.*, 2016). Fungsi *CD80* dalam aktivasi sel T adalah mengatur aktivasi sel B normal dan ganas. Sel B, sel T, dan sitokin pro inflamasi berperan penting dalam patogenesis RA (Bonelli *et al.*, 2016). Hal ini terjadi karena hasil diferensiasi dari sel T merangsang pembentukan *IL-17*, yaitu sitokin yang merangsang terjadinya sinovitis (Kondo *et al.*, 2021). Sinovitis merupakan peradangan pada membran sinovial, jaringan yang melapisi dan melindungi sendi (Burke *et al.*, 2019). Sedangkan sel B berperan melalui pembentukan antibodi, mengikat patogen, kemudian menghancurkannya (Darwin, 2021). Kerusakan sendi diawali dengan reaksi inflamasi dan pembentukan pembuluh darah baru pada membran sinovial akibatnya dapat menyebabkan terbentuknya pannus, yaitu jaringan granulasi yang terdiri dari sel fibroblas yang berproliferasi, mikrovaskular dan berbagai jenis sel radang (Choy, 2012). Pannus tersebut dapat mendestruksi tulang, melalui enzim yang dibentuk oleh sinovisit dan kondrosit yang menyerang kartilago. Reaksi sistemik dapat terjadi karena adanya pembentukan *c-reactive protein* (CRP), anemia akibat penyakit kronis, penyakit jantung, osteoporosis serta mampu mempengaruhi *hypothalamic-pituitaryadrenalaxis*, sehingga menyebabkan kelelahan dan depresi (Choy, 2012). *CD80* juga berinteraksi dengan *CD28* pada sel penyaji antigen, dan berfungsi dalam pensinyalan kostimulasi yang mengatur aktivitas sel T (Miura *et al.*, 2022). Dari interaksi gen ini berpotensi kuat dalam patogenesis RA sehingga dapat mengurangi inflamasi.

Database yang digunakan pada penelitian ini adalah GWAS *catalog* dimana database ini mudah diakses oleh siapapun, bersifat terstruktur dan komprehensif. GWAS *catalog* mengidentifikasi lokus genetik yang terkait dengan patogenesis suatu penyakit, termasuk memprioritaskan lokus gen kandidat suatu obat, mengeksplorasi mekanisme penyakit, memprediksi risiko penyakit, mengidentifikasi target obat baru, dan mengumpulkan informasi tentang sifat atau populasi (MacArthur *et al*., 2017). Namun, database GWAS *catalog* memiliki kekurangan yaitu tidak tersedianya data deskripsi fenotipik secara terperinci dari latar belakang etnis penelitian, dan tidak ada cara standar untuk membuat laporan etnis (Hart & Kranzler, 2015). Kekurangan database GWAS *catalog* yang lain yaitu biasanya hanya satu atau beberapa gen yang diperiksa, kemungkinan mengarah ke multitargeting, arah tindakan mungkin tidak jelas, dan informasi anotasi fungsional untuk beberapa SNPs mungkin terlewatkan (Gallagher & Chen-Plotkin, 2018).

Harapan untuk penelitian selanjutnya adalah mengembangkan penggunaan kriteria anotasi fungsional yang lebih luas dalam upaya mendapatkan hasil yang lebih banyak lagi untuk menentukan variasi genetik yang mempengaruhi patogenesis RA. Manfaat dari penelitian ini adalah untuk mengetahui variasi genetik yang mempengaruhi patogenesis RA dan memperoleh pemahaman dengan memanfaatkan variasi genetik yang dapat digunakan untuk pengembangan obat dengan menggunakan pendekatan genomik dan analisis bioinformatika. Peran farmasis dalam pemanfaatan data informasi genetik sangat krusial, bukan hanya dalam mengidentifikasi variasi genetik saja tetapi dapat dimanfaatkan untuk biomarker pada RA.

KESIMPULAN

Strategi untuk menemukan indikasi baru dari obat yang sudah ada termasuk pengembangan klinis menawarkan keuntungan berharga dalam proses pengembangan obat seperti efisiensi waktu, biaya dan peningkatan keberhasilan pengobatan. Dalam studi ini, kami menggabungkan *drug repurposing* dengan mengintegrasikan database genomik dan analisis bioinformatika untuk mengidentifikasi obat dengan indikasi baru untuk RA. Kami mengidentifikasi *FADSI*, *CD80* dan *CD86* sebagai kandidat target obat yang potensial untuk RA. Perlunya pemeriksaan lebih lanjut untuk mengetahui keterlibatan gen ini dengan RA. Alpha-linolenic acid dan belatacept dapat menjadi pilihan terapi yang menjanjikan untuk terapi RA, namun diperlukan uji klinis untuk memberikan jaminan kepastian manfaat dan keamanan suatu obat, memastikan efektivitas, keamanan dan gambaran efek samping yang sering timbul pada manusia akibat pemberian suatu obat.

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DAFTAR PUSTAKA

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HISTORY ARTIKEL KE 2

Mapping Rheumatoid Arthritis Susceptibility through Integrative Bioinformatics and Genomic

Sistem yang digunakan editorial dapat diakses melalui Link: http://www.journal.uad.ac.id/index.php/Media-Farmasi/author/submission/24912. Artikel di submite pada tanggal 25 September 2022. Artikel di review oleh 4 orang reviewer. Revisi artikel dilakukan pada tanggal 19 Oktober 2022 kemudian pada tanggal 17 Januari 2023, artikel dinyatakan diterima oleh Editor. Proses editing dan layout dilakukaan sekitar satu pekan, dan pada Maret 2023, artikel diterbitkan secara online. Untuk lebih jelasnya dapat dilihat pada gambar berikut. Dokumen perubahan dari artikel yang diterbitkan sejak proses submit, hasil review dari reviewer, artikel revisi, dan artikel versi terbit, terlampir.

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Mapping Rheumatoid Arthritis Susceptibility through Integrative Bioinformatics and Genomic

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ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune disease that influences several organs and tissues, especially the synovial joints, and is associated with multiple genetic and environmental factors. Numerous databases provide information on the relationship between a specific gene and the disease pathogenesis. However, it is important to further prioritize biological risk genes for downstream development and validation. This study aims to map RA-association genetic variation using genome-wide association study (GWAS) databases and prioritize influential genes in RA pathogenesis based on functional annotations. These functional annotations include missense/nonsense mutations, cis-expression quantitative trait locus (cis-eQTL), overlap knockout mouse phenotype (KMP), protein-protein interaction (PPI), molecular pathway analysis (MPA), and primary immunodeficiency (PID). 119 genetic variants mapped had a potential high risk for RA based on functional scoring. The top eight risk genes of RA are TYK2 and IFNGR2, followed by TNFRSF1A, IL12RB1 and CD40, C5, NCF2, and IL6R. These candidate genes are potential biomarkers for rheumatoid arthritis that can aid drug discovery and disease diagnosis.

1. Introduction

Rheumatoid arthritis (RA) is the most common autoimmune disease in the world. It is an an uncontrollable chronic inflammatory disease with various multi-system modalities followed by the proliferation of the synovial tissue that causes pain, joint erosion, and functional disorder (Garner et al., 2014). This synovial tissue proliferation can cause chronic impairments (Song & Lin, 2017). One sign of RA is persistent inflammation in the synovial joints. RA prevalence is within the age range of 40 – 60 which is between 0.5% and 1%, with greater prevalence in women than in men (Song & Lin,

2017). The main risk factors of RA are gender, family history, advanced age, exposure to silicate, and smoking cigarette (Garner et al., 2014) The primary influential determinants for RA vulnerability are genetic and environmental factors (Deane et al., 2017).

In this study, Genome-Wide Association Study (GWAS) is used to identify genetic variants that are at risk for disease pathogenesis by applying functional annotation criteria. This can also be useful in medicine through the bioinformatics-based approach (Narayanan, 2016; Reay & Cairns, 2021). GWAS is a research approach used in finding the genetic variation (Single sNucleotide Polymorphism, SNPs) associated with certain diseases, this involves the entire genome of several individuals. GWAS has been widely developed as a screening method not only in early-stage patients but also for people who are at risk based on family history. This research approach is useful for finding SNPs that contribute to complex diseases such as cancer, infectious diseases (AIDS, leprosy, hepatitis), autoimmune diseases, neuropsychiatric, and various diseases (Fareed & Afzal, 2013).

Meta-Analysis and GWAS have identified more than 100 RA loci, and GWAS is known as a database with genetic architecture also for RA (Kwon et al., 2020). GWAS is recognized as a powerful approach to map genes responsible for various diseases. For example, earlier studies have analyzed Chinese population SNPs that affect RA by using GWAS (Ren et al., 2014). In addition, the whole genome case-control of the linkage disequilibrium (LD) mapping was conducted by utilizing SNPs and RA-associated polymorphisms in PADI4 and SLC22A4/A5 cluster was identified (Ren et al., 2014). These findings showed that PADI4 is a risk gene for RA while SLC22A4/A5 has several polymorphisms associated with many autoimmune diseases. Thus, large-scale LD mapping seems effective at identifying RA polymorphisms (Ren et al., 2014).

Information on the relationship between a given gene and the pathogenesis of a disease is largely available on the GWAS database. The priority given to the most influential genes (biological risk genes) is scarcely examined. Thus, this research was conducted based on six functional annotations resulting in the prioritization of the most influential genes in RA pathogenesis.

2. Materials and Methods

2.1. RA-associated genes

Genomic variants RA risk genes were collected from the GWAS database, and the most influential genes were prioritized based on six functional annotations (Figure 1). Single nucleotide polymorphisms (SNPs) related to RA were expanded using the criterion $r2 > 0.8$ of HaploReg v4.1. The SNPs associated with RA were denoted as "RA-associated genes". The genomic data were then prioritized based on six functional annotations, where genes with scores ≥ 2 were classified as "biological RA risk genes".

2.2. Functional annotations of RA-associated genes

Candidate genes most likely to be RA targets were prioritized based on their biological function using six functional annotations. Missense or nonsense mutations are single amino acid changes that produce different protein functions and are selected as functional annotation criteria (Zhang et al., 2012). In accordance with HaploReg v4.1, the first functional annotation is missense or nonsense mutation because it contains functional annotations from the SNPs database (DB) (Leng et al., 2020). HaploReg v4.1 was used to link cis-expression quantitative trait loci (cis-eQTL) to genetic variants in blood target tissues (Ward & Kellis, 2016). We also used WebGestalt 2019 to understand the relationship between mutant genes and phenotypes (Wang et al., 2017). Phenotype information of mouse and other mammals was derived from the ontology of the Mammalian Phenotype (MP) (Smith & Eppig, 2009). To further understand the phenotypes of mouse, we used BioMart to convert genes with human Ensembl ID into mouse Ensembl ID (Drost & Paszkowski, 2017). Genes with False Discovery Rates (FDRs) in mouse phenotypes <0.05 were considered to be significant results. RA-associated genes from biological process networks were identified using protein-protein interactions (PPIs) (Qiu et al., 2021). In addition, we used WebGestalt 2019 to conduct reinforcement analysis and to investigate whether the genes were collected on certain functional annotations (Liao et al., 2019). Kyoto Encyclopedia of Genes and Genomes (KEGG) was used to determine what molecular pathways were gained from the list of RA-associated genes and what genes were involved. Further analysis of the biochemical pathway was carried out by WebGestalt 2019 database. The last annotation criterion was primary immunodeficiency (PID). PID refers to inborn immunity diseases and is often reported

to be associated with cancer (Mortaz et al., 2016). PID attacks the immune system and causes inflammation in RA (Dimitriades & Sorensen, 2016). The hypergeometric test was employed for reinforcement analysis of these data, with the significance criterion being p-value < 0.05.

Candidate genes most likely to be RA targets were prioritized based on their biological function using six functional annotations. Missense or nonsense mutations are single amino acid changes that produce different protein functions and are selected as functional annotation criteria (Z. Zhang et al., 2012). In accordance with HaploReg v4.1, the first functional annotation is missense or nonsense mutation because it contains functional annotations from the SNPs database (DB) (Leng et al., 2020). HaploReg v4.1 was used to link *cis*-expression quantitative trait loci (*cis*-eQTL) to genetic variants in blood target tissues (Ward & Kellis, 2016). We also used WebGestalt 2019 to understand the relationship between mutant genes and phenotypes (Wang et al., 2017). Phenotype information of mouse and other mammals was derived from the ontology of the Mammalian Phenotype (MP) (Smith & Eppig, 2009). To further understand the phenotypes of mouse, we used BioMart to convert genes with human Ensembl ID into mouse Ensembl ID (Drost & Paszkowski, 2017). Genes with False Discovery Rates (FDRs) in mouse phenotypes <0.05 were considered to be significant results. RA-associated genes from biological process networks were identified using protein-protein interactions (PPIs) (Qiu et al., 2021). In addition, we used WebGestalt 2019 to conduct reinforcement analysis and to investigate whether the genes were collected on certain functional annotations (Liao et al., 2019). Kyoto Encyclopedia of Genes and Genomes (KEGG) was used to determine what molecular pathways were gained from the list of RA-associated genes and what genes were involved. Further analysis of the biochemical pathway was carried out by WebGestalt 2019 database. The last annotation criterion was primary immunodeficiency (PID). PID refers to inborn immunity diseases and is often reported to be associated with cancer (Mortaz et al., 2016). PID attacks the immune system and causes inflammation in RA (Dimitriades & Sorensen, 2016). The hypergeometric test was employed for reinforcement analysis of these data, with the significance criterion being *p*-value < 0.05.

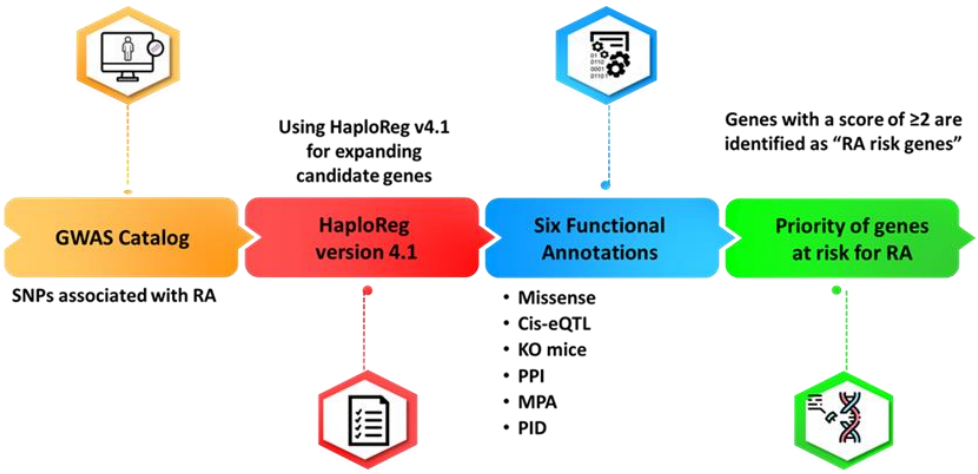

Fig 1. Schematic model showing how genome-based information can be integrated to map susceptibility genes for rheumatoid arthritis (RA). [*cis*-expression quantitative trait locus (cis-eQTL); protein-protein interaction (PPI); molecular pathway analysis (MPA); and primary immunodeficiency (PID).]

3. Results and Discussion

3.1. Prioritized SNPs potentially cause RA

2,732 RA-associated SNPs were dictated in the GWAS database (Table S1) and 2012 SNPs were obtained after filtering based on p-value < 10-8. 396 SNPs were obtained after checking for duplicates, and further filtering based on the inclusion criterion OR >2, led to a total of 360 RA-associated SNPs (Table S2). The SNPs collected from GWAS were expanded using HaploReg v4.1 while the RA-associated genes were prioritized based on six functional annotations, namely missense/nonsense

mutation, cis-eQTL, knockout mouse phenotype (KMP), protein-protein interaction (PPI), molecular pathway analysis (MPA), and primary immunodeficiency (PID).

3.2. RA risk SNPs Functional Annotations

This study was designed to prioritize the best candidate genes by using six functional annotations with a scoring system. In the functional annotation process, candidate genes with higher scores represent greater biological influences on RA pathogenesis (Figure 2). Following the six annotations, we discovered 39 SNPs of missense/nonsense mutation variants from the total 360 SNPs (Figure 3). We further examined the cis-eQTL effect using HaploReg v4.1, 101 SNPs were gained from the total 360 SNPs (Figure 3). Phenotype data was taken from the MP ontology which contains information about the phenotype of mice and other mammals. WebGestalt 2019 was applied to perform over-representation analysis (ORA) and we found 102 genes overlapping with RA risk genes (FDR <0.05). Gene Ontology (GO) annotations derived from WebGestalt 2019 were used to evaluate PPIs. Following the analysis on PPIs, 122 genes overlap with other RA risk genes (FDR < 0.05). KEGG was used to perform ORA on the molecular pathway. Thus, over 61 RA-associated genes on KEGG pathways were identified. We also used the PID data from IUIS to analyze and confirm the overlapping genes. Sixteen overlapping genes were statistically significant ($p < 0.05$) from PID analysis.

We also scored the risk genes from 0 to 6 and obtained 156 genes with a score of 0. 85 genes have a score of 1, 45 genes have a score of 2, 40 genes have a score of 3, 26 genes have a score of 4, 6 genes have a score of 5, and 2 genes have a score of 6 (Figure 4). In total, we obtained 119 genes with a score of ≥ 2, that were categorized as 'biological RA risk genes' (Table S2). Using the aforementioned socring criteria, the top eight RA risk genes are: tyrosine kinase 2 (TYK2), followed by interferon-gamma receptor 2 (IFNGR2). Members of the tumor necrosis factor receptor superfamily 1A (TNFRSF1A), interleukin receptor subunit 12 beta 1 (IL12RB1) and mass differentiation 40 (CD40), complement 5 (C5), neutrophil cytosolic factor 2 (NCF2), and interleukin receptor 6 (IL6R).

GENCODE_id	GENCODE_name	missense	cis-eQTL	KO mice	PPI	KEGG	PID	Total Score
ENSG00000105397	TYK2	1	1	1	1	1	1	6
ENSG00000159128	IFNGR2	1	1	1	1	1	1	6
ENSG00000067182	TNFRSF1A	0	1	1	1	1	1	5
ENSG00000096996	IL12RB1	0	1	1	1	1	1	5
ENSG00000101017	CD40	0	1	1	1	1	1	5
ENSG00000106804	C5	0	1	1	1	1	1	5
ENSG00000116701	NCF2	1	0	1	1	1	1	5
ENSG00000160712	IL6R	1	1	1	1	1	0	5
ENSG00000003400	CASP10	0	1	0	1	1	1	4
ENSG00000020633	RUNX3	1	0	1	1	1	0	4
ENSG00000056558	TRAF1	0	1	1	1	1	0	4
ENSG00000064012	CASP8	0	0	1	1	1	1	4
ENSG00000076662	ICAM3	1	1	0	1	1	0	4
ENSG00000081237	PTPRC	0	0	1	1	1	1	4
ENSG00000081985	IL12RB2	0	1	1	1	1	0	4
ENSG00000100906	NFKBIA	0	0	1	1	1	1	4
ENSG00000103313	MEFV	1	0	1	1	0	1	4
ENSG00000103811	CTSH	1	1	0	1	1	0	4
ENSG00000110448	CD5	1	0	1	1	1	0	4
ENSG00000112486	CCR6	0	1	1	1	1	0	4
ENSG00000115267	IFIH1	1	0	1	1	1	0	4
ENSG00000115604	IL18R1	0	1	1	1	1	0	4
ENSG00000117560	FASLG	0	0	1	1	1	1	4
ENSG00000118503	TNFAIP3	1	0	1	1	1	0	4

Fig. 2. Functional annotations for rheumatoid arthritis (RA) with scores ≥ 2 based on defined scoring criteria

Tyrosine kinase 2 (TYK2) is a member of the Janus kinase (JAK) family of transforming growth factors and cytokines. The TYK2 loss analysis function reveals its importance toward immunity against infections, inflammation (automatic), and immunity (automatic). Many genome-wide association studies in humans propose relationships between TYK2 genetic variants and many autoimmune diseases, inflammatory diseases, and tumors. Hence, TYK2 emerges as a target of interest for therapeutic interventions (Leitner et al., 2017). The human interferon-gamma receptor is a multimer of IFN-γR1 (coded by IFNGR1) and IFN-γR2 (two chains). Interferon-gamma receptor 2

(IFN-γR2) is a gene containing a protein and in humans, this gene is coded by the IFNGR2 gene. The IFNGR2 gene codes for beta chains that are not bound to the ligands of the IFN-γR.

Tumor necrosis factor receptor 1A (TNFRSF1A) is a CD120a which is a member of the superfamily tumor necrosis factor alpha-binding membrane receptor. The TNFRSF1A gene instructs the making of tumor necrosis factor receptor 1 (TNFR1) protein (Chen et al., 2015). This protein is found stretching on the cell membrane, both extracellularly and intracellularly (Chen et al., 2015). Extracellularly, the TNFR1 protein enchains tumor necrosis factor/TNF. The TNF and the TNFR1 protein interaction catalyzes the latter to chain to two other TNFR1 proteins, constituting trimer, a three-protein complex (Chen et al., 2015). This formation of the trimer is important for the TNFR1 protein to function. The chains of the protein of TNF and TNFR1 prompt the latter to deliver a signal into the cell (Chen et al., 2015). The TNFR1 protein signal can stimulate inflammation or cell self-destruction (apoptosis). The signal into the cell starts a routeway activating the nuclear factor kappa B protein triggering inflammation and directing to the cytokine production, the immune system protein (Chen et al., 2015).

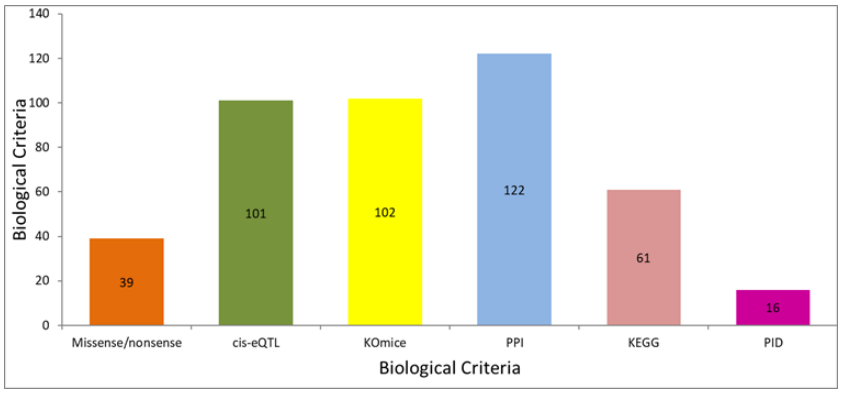

Fig. 3. Number of genes for each functional annotation that is prioritized for rheumatoid arthritis. [cis-expression quantitative trait locus (cis-eQTL); Knockout mice (KOmice); protein-protein interaction (PPI); Kyoto Encyclopedia of Genes and Genomes (KEGG); and primary immunodeficiency (PID).]

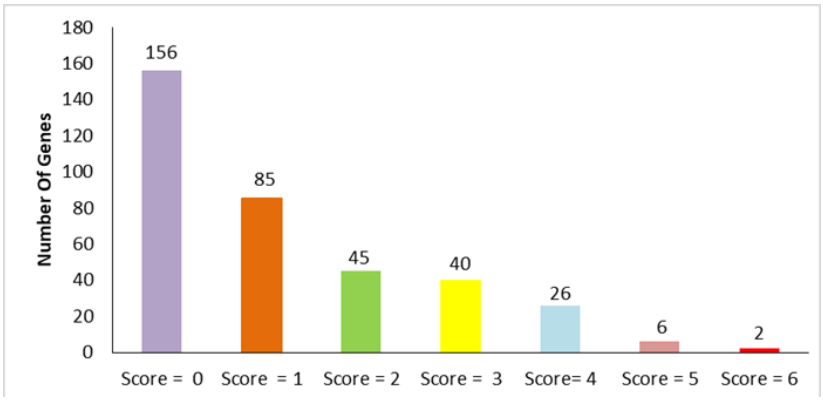

Fig. 4. Gene frequency distribution across defined functional scores for rheumatoid arthritis, showing 156 genes with a score of 0.85, genes with a score of 1, and 119 (45 + 40 + 26 + 6 + 2) genes with a total score of ≥ 2 are classified as RA risk genes.

Interleukin-12 receptor beta-1 subunit (IL12RB1) functions as an interleukin receptor that binds to interleukin-12 with low affinity which is involved in IL12 transduction (Floss et al., 2016). IL12RB2 makes a functional high-affinity receptor for IL12 (Chen et al., 2015). IL23R forms the receptor of interleukin-23 functioning in IL23 signal transduction via JAK/STAT signaling cascade activation (Floss et al., 2016). CD40 is a member of the tumor necrosis factor (TNF) superfamily found in antigen-presenting cells, including dendritic cells (DC), B cells, monocytes, and macrophages, which play an important role in activating the immune system (Karnell et al., 2019). The expression expands

to various non-hematopoietic cells, including nerve cells, epithelial cells, endothelial cells, and fibroblasts (Karnell et al., 2019). Typical genetic variants at the TRAF1-C5 locus on the chromosome correlate with an increased risk of anti-ccp-positive RA (Huang et al., 2019).

Genetic variants rs7021206 and rs3761847 at the TRAF1-C5 locus are associated with RA in the Han Chinese population, indicating that TRAF1-C5 may play an important role in the development of RA thereby reducing the pathogenic role of TRAF1-C5 in ethnic variations (van Steenbergen et al., 2015). NCF2 is carried to the membrane of the cell to be combined with other components to build a NOX system that is active through microbial stimulation. In other studies, the polymorphism NCF2 rs10911363 was linked to SLE risk in Sweden and US populations. The variant of NCF2 rs789181 was discovered to have a relationship with mild RA in men (T.-P. Zhang et al., 2020). IL6 is an important cytokine in mediating inflammation and RA systemic overview, including synovitis, fatigue, anemia, anorexia, and osteoporosis (Narazaki et al., 2017).

In this study, we used the GWAS database to obtain the genomic variants for RA. The GWAS database is easily accessible and effective because it is diverse and structured. The GWAS method effectively identifies genetic loci associated with the pathogenesis of a disease, including prioritizing candidate loci, exploring disease mechanisms, predicting disease risk, identifying new drug targets, and gathering information about the desired trait or population (Caliskan et al., 2021). However, the GWAS database has drawbacks in that detailed phenotypic descriptions of the study's ethnic background are not provided by many GWAS papers, and even nowadays, there is no standard way to make an ethnicity report (Hart & Kranzler, 2015). The prospect for further research is to develop a wider use of functional annotation criteria to get more results to determine genetic variations that affect RA pathogenesis and identify drug candidates that target biological genes for RA risk. The impact of this research is to find out the genetic variation that influences the pathogenesis of RA and gain an understanding by utilizing genetic variation that can be used for drug development by using a bioinformatics approach.

4. Conclusion

Our research showed that genomic information is highly important for mapping RA genomic variants based on defined functional annotations from this study. Two candidate genes, TYK2 and IFNGR2, were obtained based on defined scoring criteria, and we propose these genes as potential RA biomarkers to be prioritized for downstream clinical development and validation.

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Competing Interests

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