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Research Article

Computational Biology Approach for Deciphering Etiological Pathway of Polygenic Diseases: Rheumatoid Arthritis

Rubin M. Tamrakar[1*], Anushruti Sangami[1], Sunita Dangol[1], Pramod Aryal[2]

[1]Department of Biotechnology, SANN International College, Subarna Sumser Street, Gairidhara, Kathmandu 44600, Nepal
[2]Department of Biotechnology, Tribhuwan University, TU Road, Kirtipur 44618, Nepal

Abstract

Rheumatoid Arthritis (RA) (OMIM ID: 180300) is an inflammatory autoimmune disease caused by immune reaction against the proliferating synovial fibroblasts. Nonsuppurative proliferation of synoviocytes frequently progresses to destroy the articular cartilages and underlying bone, resulting in permanent disability. Using system biology approach, candidate genes obtained from OMIM (Online Mendelian Inheritance in Man) and its interacting proteins were prioritized on the basis of three Gene Ontology terms (Molecular Function, Cellular component, and Biological Process) employing FunSimMat (Functional Similarity Matrix). Among the many, four prioritized proteins NFκBIL1, HCST, MICA, and MICB were selected. Amongst the prioritized genes, literature review suggested that NFκBIL-1 (Nuclear Factor-κ of B-cell Inhibitor Like protein-1) (UniProtKB ID: Q9UBC1) competes against SR (Ser-Arg) protein, ASF/SF2, in negatively regulating CD45RO gene expression in CD4 T-cells, whose overproduction would lead to cytokine outburst, thus leading to an immunological attack. ASF/SF2 mediated splicing variant, CD45RA, otherwise would have prevented overproduction of these cytokines. Overproduced cytokines such as TNF-α, IL-6, IL-15 simultaneously induces inflammatory stress in the synovial membrane and activates stress induced MICA/B (UniProtKB ID: Q29983/Q29980) through downstream signaling following TNFR1-TRAF mediated signaling pathway. Synovial expression of MICA/B enables interaction with its ligand NKG2D associated with DAP10 (UniProt KB ID: Q9UBK5) amply present on CD8+ αβ T-cells, CD4+γδ T-cells, and NK cells, thus promoting cytolysis of MICA/B expressing synoviocytes along with further production of cytokines TNF-α and IL-15. Hence, alteration in NFκBIL-1 promoter induces MICA/B expression that leads in production of cytokines could be a probable cause of chronic RA.

Keywords: Rheumatoid Arthritis (RA); NFκBIL1; FunSimMat; Cytokines; Ontology

Introduction

Rheumatoid arthritis (OMIM ID: 180300) is a systemic, chronic inflammatory disease and is a long lasting autoimmune disorder occurring in women (3 to 5 times) commonly than in men. Generally, it has peak incidence period during second to fourth decade of one's life (Kumar *et al.,* 2007). This disease affects many tissues but principally attack the joint to produce non-suppurative proliferative synovitis that frequently progresses to destroy articular cartilage and underlying bone resulting in

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[1]*Corresponding author
Rubin M. Tamrakar,
Department of Biotechnology, SANN International College, Subarna Sumser Street, Gairidhara, Kathmandu 44600, Nepal
Email: rubintamrakar@gmail.com

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disabling arthritis. It is associated with increased morbidity causing deterioration in patients' health-related quality of life *(Kumar et al., 2007).*

Although the cause of Rheumatoid arthritis (RA) is unclear, it is suggested an equal contribution of genetic and environment factors (smoking, alcoholism and lifestyle) leads to disease genesis. Gene anomalies responsible for RA is believed to be pre-deposited in the patient, however the trigger, whether viral/bacterial infection or autoimmunity is yet unclear (*Holoshitz et al., 2010; Kumar et al., 2007)*. Vitamin D deficiency is observed in RA patients, however, whether it is a cause or a consequence is oblivious. Several medical treatment measures has been established over the years to keep the disease under control, however, they pose as a control measure rather than an effective cure (*Athanassiou et al., 2017*).

In general, it is assumed that cytokines play prominent role to activate immune responses which is regulated by multiple genes. Inflammatory conditions like rheumatoid arthritis (RA), gout, lupus and psoriatic arthritis has been found to increase risk of developing heart disease (*Kahlenberg et al., 2011*). That includes heart attack, stroke, atrial fibrillation (irregular heartbeats), high blood pressure, heart failure and atherosclerosis (plaque in the arteries). More than 50 % of premature deaths in people with RA result from cardiovascular disease (*Kahlenberg et al., 2011*).

The mechanisms of immune response in RA possibly microorganisms or some self-antigen act as arthritogenic agent that activates T-cells to produce cytokines which activate macrophages or other cells in the joint releasing degrading enzymes and activate B cells to produce antibodies against self-antigens in the joint. This might lead to cytokines mediated stimulation of secretion of proteolytic and matrix degrading enzymes by synovial cells and chondrocytes (*Kumar et al., 2007*). 80% patients carry antibodies in blood against antigen causing RA, which can be detected through serological tests. Autoantibodies raised against the Fc portion of own citrullinated Ig, also called Anti-citrullinated protein Antibody (ACPA) form immune complexes are deposited in the joints and other tissues causing inflammation and tissue damage. Remaining 20% patients do not show antibodies in the bloodstream, hence showing negative result; this is known as seronegative or Rheumatoid Factor negative (*Aletaha et al., 2010*).

Initiation of RA is a huge dilemma for understanding the progression of the disease. Several hypothesis such as microbial infection (bacterial/viral) and auto antigen has been proposed. A number of association studies revealed the link of NFKBIL1 to autoimmune or inflammatory diseases. It has been demonstrated that a combination of sequence variation in the NFKBIL1 promoter, which results in the reduced expression of IkBL, may confer the susceptibility to RA (*Shibata et al., 2006*). NFκBIL1 expression in T-cells has quite a role to keep the expression level of CD45 at an optimum level. NFκBIL1, and CLK1 and SR protein, ASF/SF2, creates a different splice variant other than CD45, hence keeping the concentration of CD45 on the T-cells at optimum (*An et al., 2013;* Greetham *et al.*, 2007).

However, SNPs on the promoter sequence result in under expression or no expression of NFκBIL1 gene (*Allcock et al., 2001*). The resultant is an exceeding amount of CD45RO over CD45RA on T-cell surface, a Tyrosine phosphorylase enzyme which play a significant role in downstream signaling for activation of transcription factors (AP1, NF-κB, and NFAT) that express cytokines such as TNF-α (*An et al., 2013*). An increased amount of CD45RO result in higher signal transduction resulting in overproduction of cytokines. The result is inflammation along with infiltration of immune cells like macrophage, Natural Killer (NK) cells, $CD8^+$ T-cells, and B-cells (*Kirsten et al. 2009*).

Outburst of cytokines result in inflammation of synoviocytes and result in stress induced expression of MICA/B. These are MHC Class I polypeptide-related sequence A/B that act as stress induced self-antigen recognized by ligands of NKDG2D associated with DAP10 that are present in $CD4^+$ γδ T-cells and NK cells *(Bauer et al., 1999*). This triggers downstream signalling in the interacting lymphocytes, thus leading to cytolysis of MICA/B presenting cells along with further production of cytokines (*Groh et al. 2003*).

Using gene ontology to prioritize genes in etiological manifestation of disease (*Schlicker et al. 2007*), NFκBIL1, HCST, MICA, and MICB were top prioritized genes. Moreover, Genotypic variation at -62 region upstream of transcription start site of NFκBIL1 gene has been mentioned in Taiwanese and Caucasian population which is considered most susceptible allelic variation for disease genesis (*Chinoy et al., 2012; Ramasawmy et al., 2008*). Variation in NFκBIL1 promoter were also mentioned in Type I Diabates Mellitus, Takayasu's Arthritis, and chronic thromboembolic pulmonary hypertension (*An et al., 2013*).

Materials and Methods

Seed Protein Generation

OMIM (Online Mendelian Inheritance for Man) is a continuously updating archive for human genes and genetic disorder. It mostly focus on gene to phenotype relation which ultimately helps in disease identification. OMIM also helps to draw gene map of particular disease using disease ID number. Hence, seed proteins related to RA were retrieved from OMIM (Online Mendelian Inheritance in Man) by clicking disease ID number (180300) where all the genes published in PubMed are mentioned. A total of 147 OMIM entries were expanded into UniProtKB ID to bring

up isoforms and natural variants of the respective proteins using HGNC ID and Ensemble number. OMIM gene name was synchronized with HGNC number, and Ensemble number provided by HGNC was hyperlinked to generate UniProtKB ID.

Feed Protein Generation

Each of the seed protein (UniProtKB ID) were used to generate interacting proteins from Protein-Protein Interaction (PPI) databases: STRING, MINT and I2D. All the interacting proteins were noted. OMIM proteins and their isoforms and interactants along with MINT and KEGG were compiled as Feed Protein. The reason behind using three different PPI databases was to cover all possible interacting protein since each database has different sources to derive interacting proteins. I2D and STRING were used as the main source of generating feed proteins while MINT and KEGG (Kyoto Encyclopedia of Gene and Genome) were used as control to show how exclusion of one of the PPI sources would give different results.

Prioritization

UniProtKB ID of all the feed proteins were converted into text format. The converted list was uploaded to the query of FunSimMat (Functional Similarity Matrix) of Max Planck Institute for Informatics Computational Biology and Applied Algorithms. The prioritized protein list was downloaded and top four prioritized proteins were selected for etiological pathway generation.

Etiological Pathway Generation

Top prioritized proteins, NFκBIL1, HCST, MICA, and MICB were studied in detail for an etiological pathway generation. This was done by literature mining from sources such as PubMed, and Reactome. The expanded seed proteins were then queried into three PPI database in order to obtain the interacting proteins. Total number of 19,432 interacting proteins were obtained from PPI database. Out of which, 9917 interacting proteins were obtained from I2D database, 8745from STRING and 770 proteins from MINT. The total interacting proteins from three PPI databases may contain repeated IDs that are removed and reduced to singular unit when uploaded to FunSimMat prioritizer tool.

Results and Discussion

Feed Protein Generation

Entry of term “RHEUMATOID ARTHRITIS” when queried into OMIM database, 147 entries were retrieved on 7th October 2015.The total seed proteins were converted into UniProtKB ID using HGNC (Hugo Gene Nomenclature Committee). So retrieved 147 gene entries were expanded to 2897 seed proteins which contained 368 isoforms and 2529 variants that might be involved in RA genesis. The expanded seed proteins were then queried into three PPI database in order to obtain the interacting proteins. Total number of 19,432 interacting proteins were obtained from PPI database. Out of which, 9917 interacting proteins were obtained from I2D database, 8745 from STRING and 770 proteins from MINT. The total interacting proteins from three PPI databases may contain repeated IDs that are removed and reduced to singular unit when uploaded to FunSimMat prioritizer tool.

Prioritization

The list of 19,432 feed proteins tabulated in .xlsx file was converted into .txt file format and uploaded on Disease Candidate Prioritization Query on 2nd November 2015. The top ten genes from prioritized result was taken under consideration to construct a putative etiological pathway of the disease (Fig. 1).

The result shows the top prioritized genes with functional similarity scores with different GO terms. The difference in the color reflects difference in similarity, white color shows no similarity and blue color shows high similarity. Out of total interacting proteins, NFκBIL1 was found to be the top gene for the disease genesis of RA followed by HCST, SELK, TDRD3, MIA, MICA, NTCP, IGHM, PF-4V1, and MICB (Fig. 2).

Pathway Generation

Understanding the etiological pathway of any disease help us to know the specific role of involved genes in disease genesis. The above enlisted gene obtained by gene prioritization have direct or indirect link with RA. These links are important to be generated to understand the disease etiology and to target therapeutic drugs to find a better cure for RA as no cure has yet been discovered. Four genes from top 10 prioritized genes were taken under consideration, namely, NFκBIL1, HCST, MICA, and MICB in order to develop a putative pathway to explain RA genesis.

Conventional research methodology in polygenic diseases like Rheumatoid Arthritis cannot address all the responsible genes in the disease genesis as it requires significant amount of time and resources. So, computational biology was applied in order to tackle these problems. Max Plank Functional Similarity Matrix was found to be an effective tool to carry out research as it covers different gene ontology terms (biological process, molecular function, cellular components) (*Schlicker et al. 2007*). The comparison between different genes on the basis of gene ontology terms is known as Semantic similarity, which is a valuable tool for validating the results drawn from biomedical studies such as gene clustering, gene expression data analysis, prediction and validation of molecular interactions, and disease gene prioritization (*Schlicker et al. 2007*). However, FunSimMat has some limitations as well. It limits the selection of proteins which are not reported in OMIM database. The selection of proteins in FunSimMat is based on maximum similarity with functional profile of particular disease. But, the crucial proteins which may not be reported in the involvement of disease genesis are excluded, nonetheless they might possess the properties to

regulate he process of disease genesis such as TNF-α and PTPN22 (*Kyogoku et al., 2004*).

NFκBIL1 belongs to Inhibitor of kappa B protein family, however, shows a rather different function from inhibition. Located in chromosome 6 central MHC locus within HLA-DRB1 region, this protein contributes as a negative regulator of CD45 tyrosine phosphatase mediated activation of T-lymphocytes *(An et al., 2013).* Protein IκBL encoded by this gene splices CD45RA isoform which is generally present on naïve and memory T-cells and prevent its transition to activated state in which CD45RA is replaced by CD45RO isoform. Knockdown of endogenous IκBL augments the expression of CD45RO isoform through which the T-cells tend to remain in active state. This state transition is linked with higher NFκB transcription factor activation upon antigen encounter resulting in outburst of cytokines such as TNF-α, IL-6, IL-15, and cell survival and proliferation inducing proteins. This phenomenon has been linked to proinflammatory disease such as RA for its exceeding amount of TNF-α production which can be linked to non-specific inflammation and tissue damage *(An et al., 2013).*

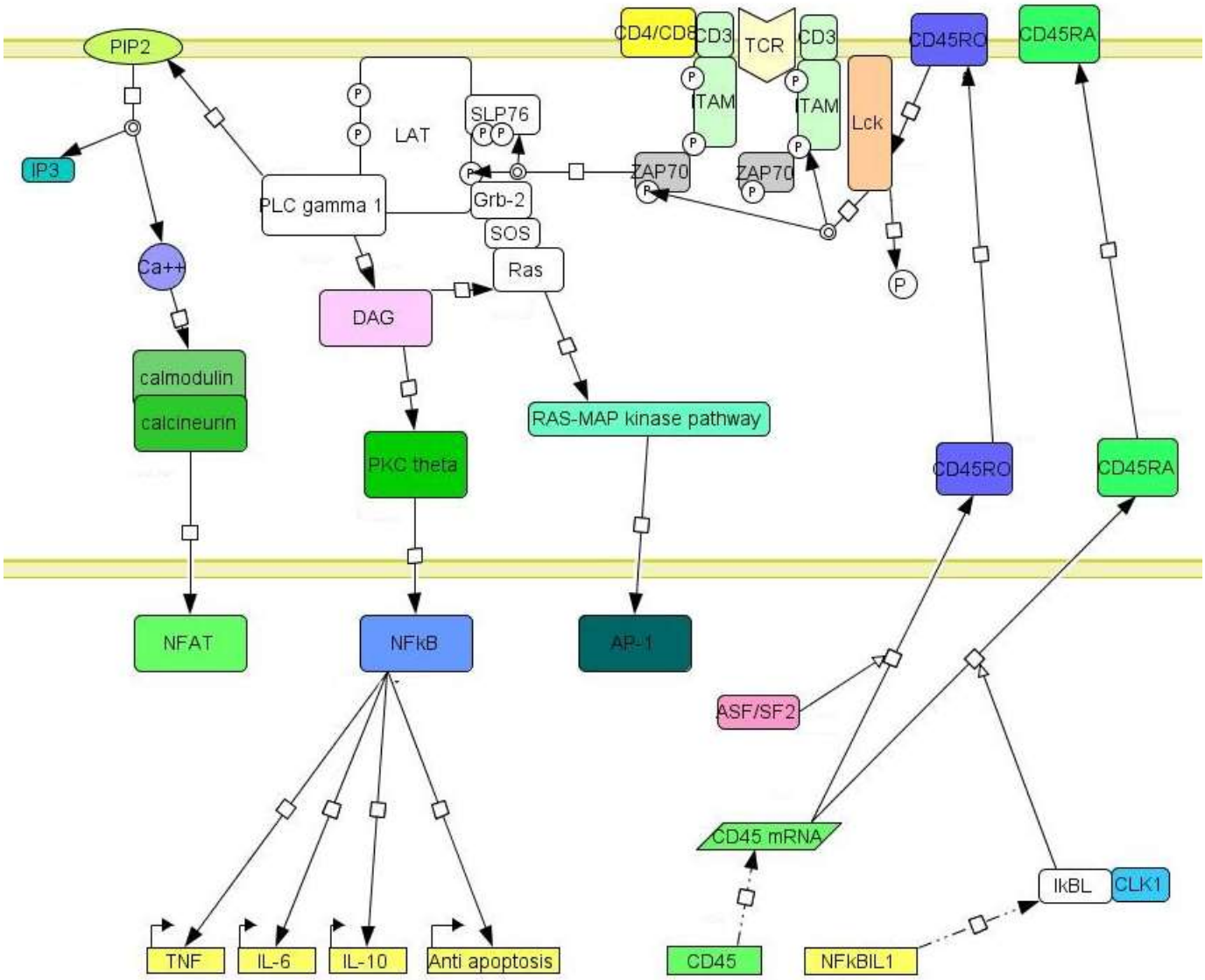
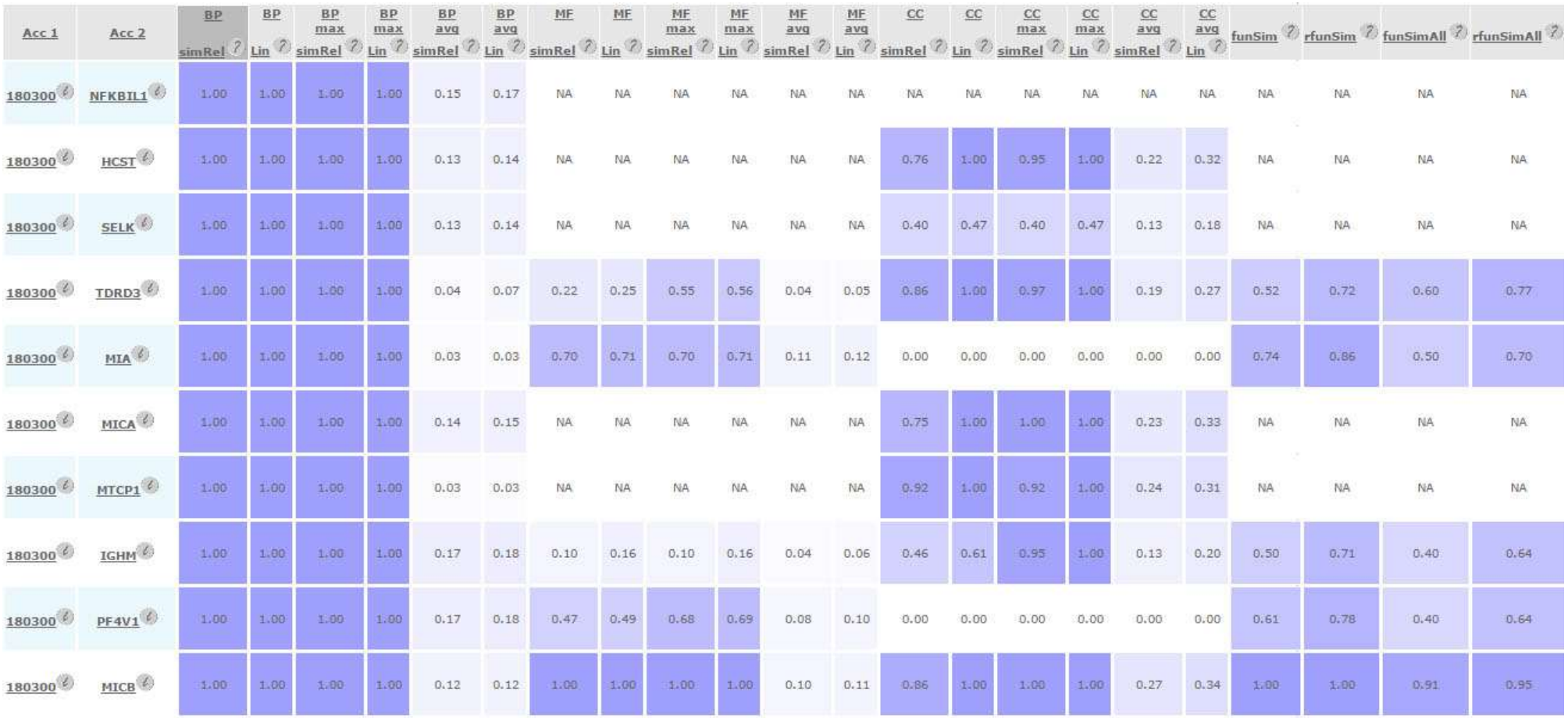


Fig. 1: Etiological pathway for possible disease genesis. (Prepared using Cell Designer 4.4)



Acc 1	Acc 2	BP simRel	BP Lin	BP max simRel	BP max Lin	BP avg simRel	BP avg Lin	MF simRel	MF Lin	MF max simRel	MF max Lin	MF avg simRel	MF avg Lin	CC simRel	CC Lin	CC max simRel	CC max Lin	CC avg simRel	CC avg Lin	funSim	rfunSim	funSimAll	rfunSimAll
180300	NFKBIL1	1.00	1.00	1.00	1.00	0.15	0.17	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
180300	HCST	1.00	1.00	1.00	1.00	0.13	0.14	NA	NA	NA	NA	NA	NA	0.76	1.00	0.95	1.00	0.22	0.32	NA	NA	NA	NA
180300	SELK	1.00	1.00	1.00	1.00	0.13	0.14	NA	NA	NA	NA	NA	NA	0.40	0.47	0.40	0.47	0.13	0.18	NA	NA	NA	NA
180300	TDRD3	1.00	1.00	1.00	1.00	0.04	0.07	0.22	0.25	0.55	0.56	0.04	0.05	0.86	1.00	0.97	1.00	0.19	0.27	0.52	0.72	0.60	0.77
180300	MIA	1.00	1.00	1.00	1.00	0.03	0.03	0.70	0.71	0.70	0.71	0.11	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.74	0.86	0.50	0.70
180300	MICA	1.00	1.00	1.00	1.00	0.14	0.15	NA	NA	NA	NA	NA	NA	0.75	1.00	1.00	1.00	0.23	0.33	NA	NA	NA	NA
180300	MTCP1	1.00	1.00	1.00	1.00	0.03	0.03	NA	NA	NA	NA	NA	NA	0.92	1.00	0.92	1.00	0.24	0.31	NA	NA	NA	NA
180300	IGHM	1.00	1.00	1.00	1.00	0.17	0.18	0.10	0.16	0.10	0.16	0.04	0.06	0.46	0.61	0.95	1.00	0.13	0.20	0.50	0.71	0.40	0.64
180300	PF4V1	1.00	1.00	1.00	1.00	0.17	0.18	0.47	0.49	0.68	0.69	0.08	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.61	0.78	0.40	0.64
180300	MICB	1.00	1.00	1.00	1.00	0.12	0.12	1.00	1.00	1.00	1.00	0.10	0.11	0.86	1.00	1.00	1.00	0.27	0.34	1.00	1.00	0.91	0.95

Fig. 2: FunSimMat result generated using feed proteins (http://funsimmat.bioinf.mpi-inf.mpg.de/)

Hematopoietic Stem Cell Signal Transducer (HCST) is a membrane protein actively expressed in both myeloid and lymphoid lineages (beside CD4 T-cells). This protein plays a crucial role in innate immunity for its downstream signaling and activation of immune cells to carry out cytotoxicity, cell mediated killing, and cytokine production. Located on chromosome 19q13.1, it associates with NKG2D receptor protein that recognizes a specific ligand which are expressed in stress induced tumorigenic cells (*Kelley et al., 2005*). HCST (DAP10/KAP10/PIK3AP) carry out downstream signaling in NK and CD8 T-cells after a confirmation change in NKG2D receptor due to ligand binding. This activation result in cytotoxicity by release of perforin and matrix degrading enzymes that lead to tissue damage in RA. The cytoplasmic region of DAP10 consist of Tyr-X-X-Met (YXXM) motif (*Wu et al., 1999),* a potential binding site of the SH2 domain for p85 subunit of PI3 kinase, as that of the CD3 and DAP12 molecule. DAP10-NKG2D complex are important as a defense mechanism and surveillance for tumor cells that could lead to cancer. DAP10 molecule has also been found to be associated with IL15 receptor in NK cells that regulate cell proliferation through JAK/STAT signaling pathway, cellular survival and cytotoxicity during RA genesis *(Horng et al., 2007).*

MHC class I related Chain A and B (MICA/B) are MHC class I like β2 microglobulin deficient surface molecules expressed on stress induced cells such as virus infected cells, and tumorigenic cells. Located on chromosome 6, these genes are highly polymorphic and also have a different role in immune system (*Stephens, 2001*). Unlike MHC class I molecules, MICA/B have no role in antigen presentation but rather act as flag tags on stress induced cells for NK and CD8 T-cells. Expression of MICA/B on synoviocytes are triggered by over production of proinflammatory cytokines by T-cells *(Groh et al., 2003).* Heat generated in cells due to inflammation trigger activation of heat shock proteins that also act as transcription factor for expression of these genes. MICA/B are recognized by its specific ligand NKG2D associated with DAP10 on lymphocytes (beside CD4 T-cells) which carry out cytolysis of such cells along with further cytokine production and proliferation of lymphocytes (*Jinushi et al., 2003*; *Steinle et al. 2001).*

Based on literature mining, NFκBIL1 gene regulates proper T-cell signaling by maintaining the CD45 isoforms, CD45RA and CD45RO. As shown in Fig. 1, IκBL protein expressed by this gene creates CD45RA isoform by splicing CD45 mRNA which is abundantly present in naïve and memory T-cells that negatively regulates T-cell signaling *(An et al., 2013*). Down regulation of NFκBIL1 gene due to combination of Single Nucleotide Polymorphisms enhances CD45RO isoforms which is abundantly expressed in activated T-cells by a Serine-Arginine protein (SR protein), ASF/SF2, upon competition with the negative regulator IκBL protein *(An et al., 2013*). Upon activation of T-cell by CD45RO, through phosphorylation and dephosphorylation of various signaling proteins which ultimately activates suppressed NFκB transcription factor, thus producing higher amount of proinflammatory cytokines such as TNF-α (*Christian et al., 2016).* So produced TNF-α induces inflammation in surrounding synoviocytes leading to expression of stress induced MICA and MICB proteins which interacts with its specific ligand NKG2D and trigger innate immune response leading to tissue damage *(Bauer et al. 2018).*

DAP10 (HCST) associates with NKG2D which are both expressed in NK cells and CD8 γδ T-cells upon encountering with proinflammatory cytokines like TNF-α. MICA/B is also expressed in synoviocytes upon encountering with TNF-α. Recognition of MICA/B by NKG2D triggers downstream signaling in NK and T-cells for cell survival and cytotoxicity (*Billadeau et al., 2003; Zafirova et al. 2011)* along with further TNF-α production. IL-15 is also found to be a key molecule in triggering cell proliferation and cytotoxicity through JAK/STAT signaling pathway *(Mishra et al., 2015).*

RA has shown an epidemic like pattern across the world among every age group. But research has not been done to the level to find out the definite cure of the disease. Mostly conventional research methods are applied that mainly focus on ways to control the disease progression, however, finding a cure to be rid of the disease should also be considered a primary objective. Many researches have been done to slow down the progression of disease, prevent bone deformity, and to slow down other symptoms, yet the curative measures have not been discovered. Computational system biology narrow down the targeted proteins having significant role in disease genesis, which in turn helps discover promising New Medical Entities (NME) ultimately to cure the disease. Integration of computational system biology with pharmacokinetics, pharmacodynamics along with toxicological data could be beneficial to find out the root causes and to treat them completely. Thus, computational system biology approach has been applied to identify the crucial genes, and the mutation responsible for occurrence of the disease.

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