Biochemistry and Biophysics Reports 40 (2024) 101870

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Decoding differentially expressed genes to identify potential immunity associated biomarkers in Tuberculosis: An integrative bioinformatics approach

Ankur Datta, Divyanshi Gupta, Diya Waryani, George Priya Doss C *

Laboratory of Integrative Genomics, Department of Integrative Biology, School of BioSciences and Technology, Vellore Institute of Technology, Vellore, Tamil Nadu, 632014, India

ARTICLE INFO

Keywords:
Tuberculosis
NGS
India
Transcriptome
Biomarkers
Diagnostics

ABSTRACT

Tuberculosis (TB) poses a significant threat to the Indian population, with India accounting for 20 % of the global TB cases. The current study aims to identify molecular biomarkers for better diagnostics by comparing the transcriptome signatures of healthy individuals against TB-affected individuals. Next-generation sequencing (NGS) tools were used to identify critical differentially expressed genes (DEGs). 302 DEGs were identified based on a logFC threshold of |3| and adjusted p-value < 0.05. STRING database was used to plot the interactions amongst the 302 DEGs. The DEGs were functionally annotated, highlighting numerous physiological functions affected due to the dysregulation of the identified hub genes. *TLR4*, *FCGR1A*, *ITGAM*, *LTF,* and *CXCR2* were the hub genes identified and observed to dysregulate crucial physiological functions. *TLR4* has been implicated in the progression of TB in various populations, and the findings of this study will enable researchers to improve the current landscape of diagnostics for TB.

1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is a contagious disease primarily affecting the lungs and spreading via the respiratory route. Only about 10 % of those infected with Mtb develop active TB, while most harbor the bacteria dormant. Although TB typically manifests in the chest, it can lead to extra-pulmonary infections, which constitute approximately 15 % of all TB cases and are particularly challenging to diagnose and treat, especially in HIV patients [1,2]. The risk of developing active TB is highest shortly after initial infection and significantly increases in individuals with compromised immune systems, such as those with HIV/AIDS [3]. Consequently, TB is the leading cause of death among individuals with AIDS, accounting for around 13 % of AIDS-related deaths globally [4]. HIV-positive individuals have a 20 to 30 times higher risk of developing active TB [5], which can be managed with antiretroviral therapy (ART) alongside intensive TB medication. Diabetes mellitus (non-insulin-dependent) is another critical factor for TB patients, associated with higher rates of treatment failure, relapse, and mortality [6]. Diabetes adversely affects TB treatment outcomes through altered immune responses [7], increased insulin resistance induced by anti-TB drugs like rifampicin, and compromised immunity [8]. Accurate and timely TB diagnosis is crucial for reducing mortality and preventing premature death. Conventional diagnostic methods include sputum smear microscopy, cultures, tuberculin skin test (TST), chest X-ray, nucleic acid amplification tests, TB ELISA, and Interferon-gamma Release Assays (IGRAs) [9]. While sputum smear microscopy is cost-effective and convenient, it has poor sensitivity, with over 30 % of cases yielding false-negative results. Bacterial cultures, considered the gold standard for TB detection, are 100 times more sensitive than smear microscopy but require over two weeks for initial results [10].

Techniques such as TST, IGRAs, ELISA, and urinary lipoarabinomannan tests diagnose latent TB [11,12]. In contrast, phenotypic drug susceptibility testing, luciferase reporter assays, probe-based assays, molecular drug susceptibility testing, DNA microarrays, and loop-mediated isothermal amplification (LAMP) are used to detect multidrug-resistant TB. Conventional methods face significant limitations, including time consumption, low sensitivity, false negatives, poor

* Corresponding author. Laboratory of Integrative Genomics, Department of Integrative Biology, School of BioSciences and Technology, Vellore Institute of Technology (VIT), Vellore, 632014, Tamil Nadu, India.
E-mail addresses: ankur.datta2023@vitstudent.ac.in (A. Datta), divyanshi.gupta2022@vitstudent.ac.in (D. Gupta), diya.waryani2022@vitstudent.ac.in (D. Waryani), georgepriyadoss@vit.ac.in (G.P.D. C).

https://doi.org/10.1016/j.bbrep.2024.101870
Received 23 September 2024; Received in revised form 31 October 2024; Accepted 9 November 2024

efficiency, bacterial viability detection, and inadequate strain differentiation [11,12]. To address these challenges, advanced techniques like digital PCR have been developed, which are capable of detecting extra-pulmonary TB even with minimal DNA and can be combined with methods like the Xpert MTB/RIF assay to improve accuracy [13,14].

TB is treated with a six-month regimen of four drugs: isoniazid (INH), rifampin, ethambutol, and pyrazinamide, collectively known as HREZ, achieving cure rates exceeding 95 %. Each drug serves a specific role: isoniazid and rifampin are the most potent, ethambutol prevents additional resistance in INH-resistant Mtb strains, and pyrazinamide acts as a sterilizing agent [15]. Resistance to these drugs results in drug-resistant TB, often linked to single nucleotide polymorphisms in Mtb genes such as *gyrA*, *rpsL*, *rrs*, *embB*, *rpoB*, *inhA*, and *katG* [16]. Effective treatment relies on prescribing the optimal drug combination [17]. Whole-genome sequencing (WGS) can help healthcare workers interpret clinically relevant genetic variants [18], but a comprehensive catalog of resistance biomarkers is needed to distinguish resistant from non-resistant strains [19]. Advances in sequencing technology, particularly RNA sequencing, have significantly improved accuracy and computational capabilities [20,21]. This report examines and compares the differential expression of transcriptome profiles in peripheral blood mononuclear cells from healthy and TB-affected individuals using the DESeq2 package, which employs negative binomial generalized linear models.

2. Methodology

1 Dataset selection and retrieval of raw reads:

The National Center for Biotechnology Information (NCBI) provides a platform for researchers to upload and retrieve biomedical and genomic information from databases. Gene expression omnibus (GEO) is one such database for micro-array and RNA-sequencing technologies [22]. GEO was accessed to acquire the dataset that was analyzed in this report. Keywords such as "*Homo sapiens*," "Tuberculosis," and "Expression profiling by high throughput sequencing" were used to query the search. The dataset with ID "GSE198557″ was selected for analysis in the current study. SRAtoolkit was used to import the reads from the sequence read archive (SRA) database. The reads were then subjected to quality check using the FastQC tool. No clinical analysis was carried out during the study.

2 Processing of raw reads and differential expression analysis (DEA):

FastQC analysis confirmed the absence of adapter sequences; thus, the adapter trimming step was omitted. Subsequently, raw reads were aligned to the hg38 reference genome assembly using the HISAT2 tool [23]. The resulting alignments were stored in SAM format and processed with the HTSeq-count tool to generate gene count files. These files were imported into R to construct a count matrix for DESeq dataset creation. Principal Component Analysis (PCA) assessed sample similarity and clustering. Gene expression patterns were also visualized through heat maps and volcano plots, utilizing packages such as ggplot2 and pheatmap, among others [24]. Differential expression analysis (DEA) was conducted using the DESeq2 package in R [25].

3 Identification of DEGs and network analysis:

An adjusted p-value threshold of < 0.05 was established. Log_2Fold Change (logFC) thresholds were set at > 3 for up-regulated and < -3 for down-regulated genes. The differentially expressed genes (DEGs) were input into the STRING database to map their interaction patterns. Protein-protein interaction (PPI) networks were visualized using Cytoscape (v3.10.1). The CytoHubba plugin in Cytoscape identified the top 25 hub genes among the DEGs [26]. Various statistical algorithms were employed, including MCC, MNC, and DMMC.

4 Functional annotation of DEGs:

Gene Set Enrichment Analysis (GSEA) was utilized to elucidate the physiological roles of the DEGs [27]. GSEA facilitates pathway-centric analysis of molecular expression data between groups. The analysis was performed on the DESeq dataset object, using the hallmark gene set as the reference. This set aggregates several MSigDB gene sets to highlight and study predefined cellular processes and functions, enhancing the ease of use and implementation in research. ClueGO was employed for functional validation, identifying specific DEGs targeting pathways [28].

3. Results

1 Data retrieval and data processing:

Raw reads from the GSE198557 dataset were obtained from the SRA database using sratoolkit. Quality assessment of these reads was performed with FastQC. The HISAT2 tool was employed to align the reads to the reference genome. Gene counts were generated using HTSeq-count, and the resulting count matrix was used as input for the DESeq package in R. Various statistical analyses were conducted, including PCA and Z-score normalization. Fig. 1A illustrates the PCA distribution among the samples in this study. Thresholds for adjusted p-value (<0.05) and logFC (<-3 for down-regulated and >3 for up-regulated genes) were applied. The heatmap in Fig. 1B displays the Z-score normalized expression of DEGs meeting these criteria. Fig. 1C presents the volcano plot distribution of the DEGs. All expression count files, and FastQC reports are supplementary files in the GitHub repository, detailed in the data availability section.

2 PPI network analysis and hub-gene interaction analysis:

The identified DEGs were input into the STRING database to map their interactions. The interaction data from STRING was imported into Cytoscape (v3.10.1), where PPIs were visualized using the GeneMANIA force-directed layout, shown in Fig. 2. Post PPI network generation, the DEGs were analyzed with the CytoHubba plugin to identify hub genes, applying the previously mentioned p-value and logFC thresholds. Various algorithms were employed to determine the hub genes, including MNC, MCC, and others. Fig. 3A presents an upset plot with a uniform set size of 25, showing the number of common DEGs per set. Fig. 3B illustrates the PPI network of the most significant hub genes.

3 Functional annotation:

Gene Set Enrichment Analysis (GSEA) was performed on the identified DEGs. GSEA identifies gene sets over-represented in each list compared to a background set, grouping genes based on their roles in known physiological pathways. Fig. 4A lists the hallmark pathways impacted by the DEGs, with a significance threshold of adjusted p-value <0.05. Fig. 4B illustrates the association of DEGs with these pathways and their log2FC expression patterns. Dendrogram clustering was applied to both DEGs and hallmark pathway terms. Table 1 presents functional enrichment results via GSEA, highlighting the KRAS signaling pathway as the most significantly affected. Functional validation was conducted using the ClueGO plugin in Cytoscape (v3.10.1), with results visualized using the yFiles circular layout in Fig. 5. *TLR4* and *FCGR3B* were identified as dysregulating processes like temperature homeostasis (GO:0001659) and response to bacteria (GO:0009617).

4. Discussion

TB, a contagious bacterial disease caused by Mtb, transmits among humans through the respiratory route and typically infects the lungs. Studies have tried to study the transcriptomic signature of individuals

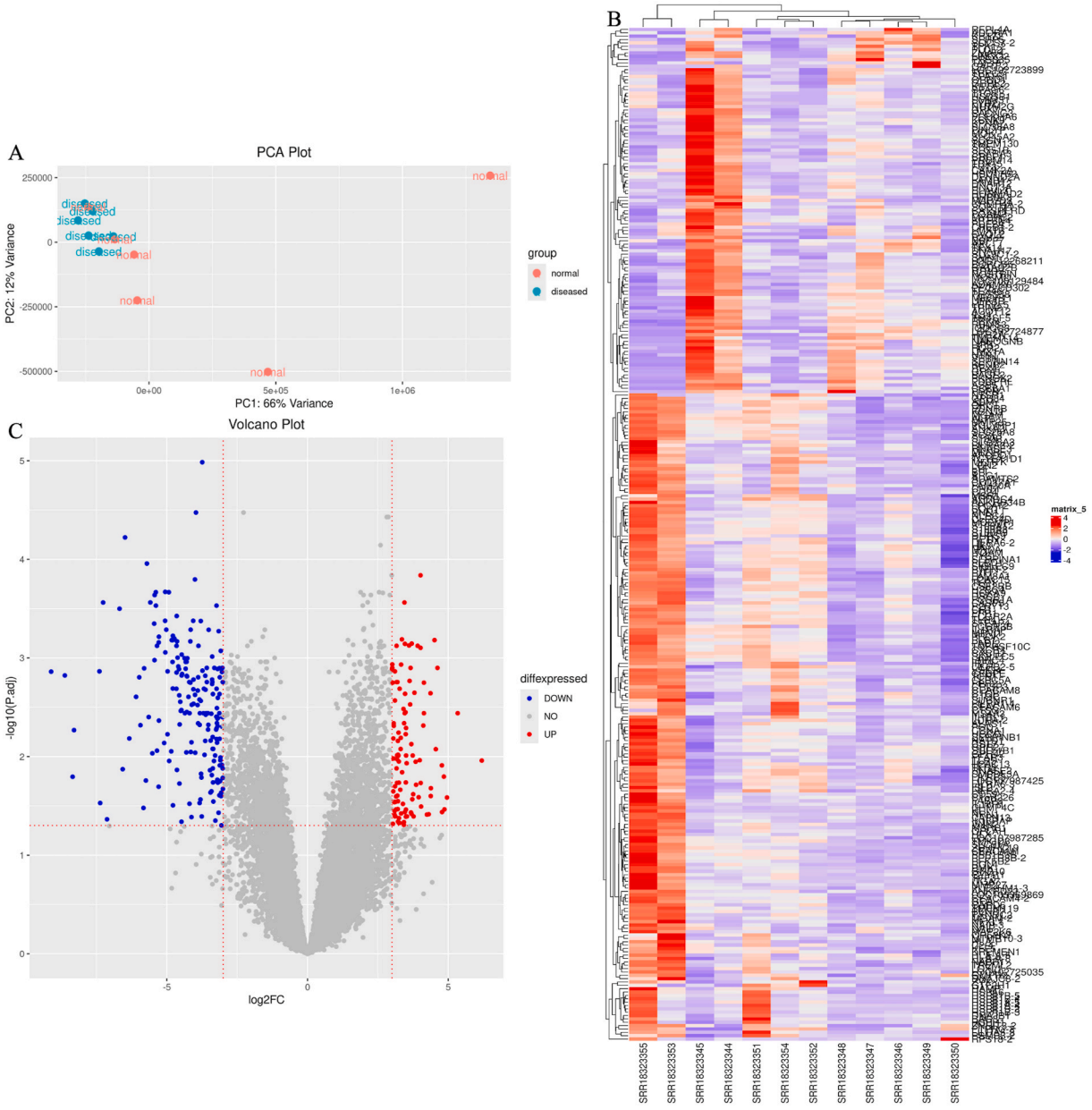

Fig. 1. Showcases the various statistical tools used; (A) Results of PCA plot, depicting the closeness amongst the patient samples; (B) Heatmap expression distribution of the DEGs identified using DESeq2 package; (C) Volcano plot distribution of the genes identified using DESeq2 package.

suffering from latent TB and active TB [29,30]. In the current report, we study the transcriptomic profiles of peripheral blood mononuclear cells (PBMCs) from 6 healthy individuals and 6 TB-infected patients in the Indian population. The raw reads were retrieved and analyzed using numerous high-throughput computational tools like FastQC, Hisat2, etc. Differential expressions of the protein-coding genes were implemented using the DESeq2 package in R. Numerous genes were aberrantly expressed, and a threshold value of logFC > 3 and logFC of < -3 and p-value of 0.05 for up-regulated and down-regulated genes. PPI networks were generated to study the interaction patterns of the DEGs identified amongst each other, depicted in Fig. 2. Furthermore, hub-genes were identified using numerous statistical algorithms via the cytohubba plug-in, integrated with Cytoscape (v.3.10.1), and the interactions amongst the most consistently identified hub-genes among all the algorithms have been visualized in Fig. 3B. GSEA has also performed to group the genes and discover the physiological pathways and functions affected by the grouped genes, as shown in Fig. 4. ClueGO was used to conduct functional validation and identify the dysregulated terms by each DEG. The results of the functional annotation revealed that the differential expression of *TLR4*, *FCGR1A*, *ITGAM*, *LTF*, and *CXCR2* was found to affect physiological functions such as response to a bacterium (GO:0009617), acute inflammatory response (GO:0002526), temperature homeostasis (GO:0001659) and several other processes, shown in Fig. 5.

Toll-like Receptor 4 (*TLR4*) belongs to the family of pattern recognition receptors (PRRs), which represent a crucial receptor protein and act as a mechanism to induce a pro-inflammatory response against infectious microbial disease [31]. As an integral member of the innate immunity response team, the TLR4 pathway works tirelessly to fight off microbes by inducing intracellular signals intervened by adaptor molecule Toll/IL-1R (TIR). This pathogen receptor acts as a binding agent for gram-negative lipopolysaccharides and intrinsic molecules produced due to tissue injury [32]. Numerous studies aimed at establishing *TLR4* relations with immunity against TB were executed in the

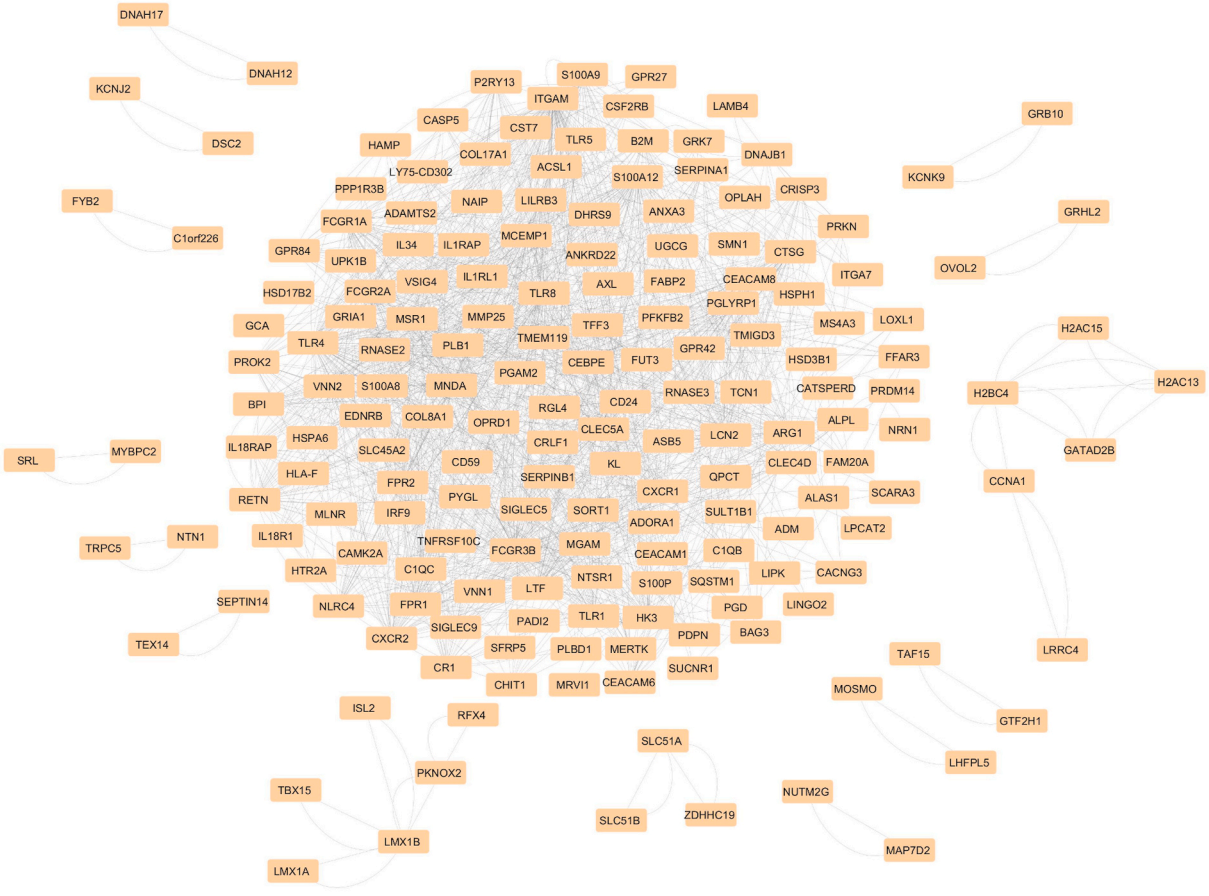

Fig. 2. PPI interaction plot generated for 302 DEGs using STRING and Cytoscape.

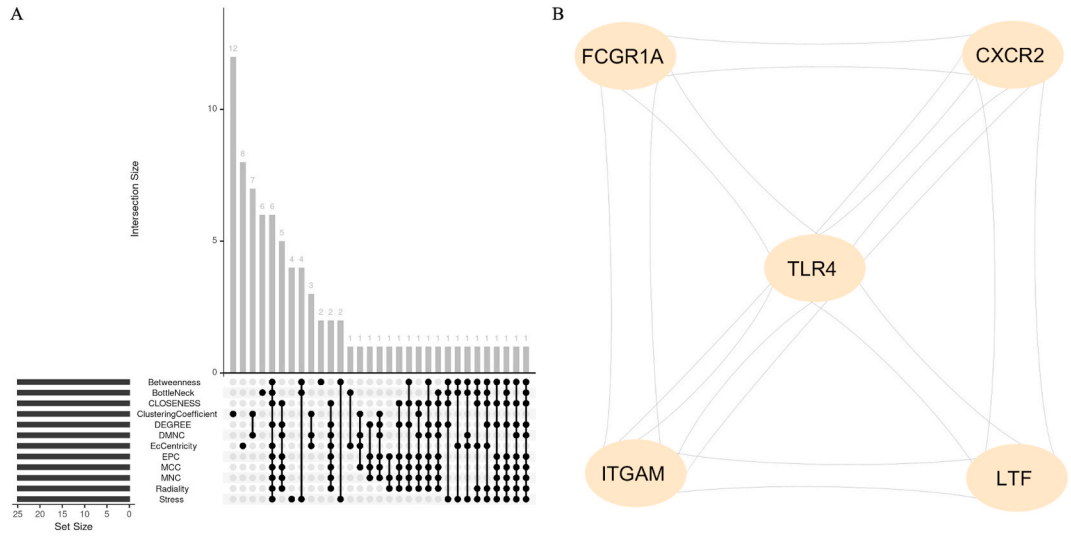

Fig. 3. Showcases relevant information of the hub-genes identified using Cytohubba plugin; (A) Upset plot showcasing the distribution of the hub-genes identified using various statistical algorithms; (B) Interaction patterns observed amongst the most relevant hub-genes.

Tibetan, Sudanese, and Brazillian populations [33–35]. In the Tibetan population, an SNP analysis study found two *TLR4* polymorphisms that affect the risk of developing pulmonary TB [33]. Subsequently, another survey of the Sudanese population identified four SNPs associated with TB, suggesting *TLR4's* involvement in inducing immunity against bacterial infection [34]. Similar results were observed in the Brazilian population study by analyzing the data from 536 individuals, out of which 177 were TB index cases [35].

Consequently, Fc gamma receptor 1a (*FCGR1A*) also plays a vital role in the immune system by encoding a glycoprotein called CD64 that acts as the only high-affinity receptor for immunoglobulin G (IgG) in humans [36]. Previous research reported on 99 Indian children strongly suggested the connection between *FCGR1A* and the risk of Mtb infection [37]. Another study aimed to identify novel biomarkers for

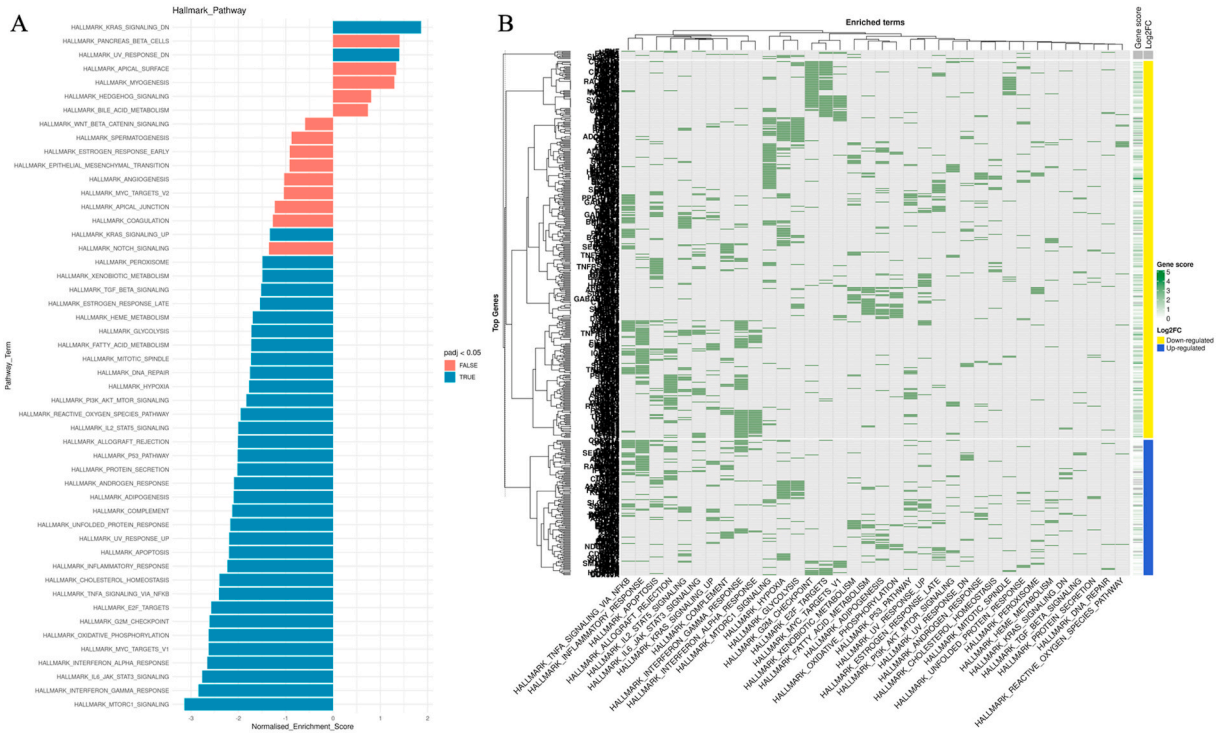

Fig. 4. Results of GSEA functional analysis; (A) Bar chart showcasing the normalized enrichment score (NES), plotted for the adjusted p-value score; (B) Heatmap distribution of the genes affecting the concerned Hallmark pathways, along with the log_2FC expression pattern.

Table 1
Full list of functional annotation terms generated by GSEA.

Pathway	P.Val	P.Adj	NES
HALLMARK_KRAS_SIGNALING_DN	2.85634006796169E-05	6.49168197264021E-05	1.860930877
HALLMARK_UV_RESPONSE_DN	0.026779694	0.03719402	1.397449278
HALLMARK_KRAS_SIGNALING_UP	0.024115372	0.034450531	−1.332394364
HALLMARK_PEROXISOME	0.012507085	0.018950129	−1.490742575
HALLMARK_XENOBIOTIC_METABOLISM	0.004869033	0.007607865	−1.49196214
HALLMARK_TGF_BETA_SIGNALING	0.017145791	0.025214398	−1.518478019
HALLMARK_ESTROGEN_RESPONSE_LATE	0.003447917	0.005561156	−1.542511719
HALLMARK_HEME_METABOLISM	0.000368859	0.000635963	−1.698098067
HALLMARK_GLYCOLYSIS	0.000104404	0.000200777	−1.723812578
HALLMARK_FATTY_ACID_METABOLISM	0.000333622	0.000595754	−1.726841615
HALLMARK_MITOTIC_SPINDLE	0.00010325	0.000200777	−1.729672408
HALLMARK_DNA_REPAIR	9.99661650714606E-05	0.000200777	−1.755830087
HALLMARK_HYPOXIA	3.03212362203994E-05	6.59157309139117E-05	−1.770472889
HALLMARK_PI3K_AKT_MTOR_SIGNALING	0.000124326	0.000230234	−1.832009312
HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY	0.000552481	0.000920802	−1.949346337
HALLMARK_IL2_STAT5_SIGNALING	5.93012468869746E-08	1.85316396521796E-07	−2.003168471
HALLMARK_ALLOGRAFT_REJECTION	1.16994522373431E-07	3.44101536392444E-07	−2.008355151
HALLMARK_P53_PATHWAY	1.65803625666448E-07	4.60565626851244E-07	−2.013900615
HALLMARK_PROTEIN_SECRETION	2.45447227701795E-06	5.84398161194751E-06	−2.020459962
HALLMARK_ANDROGEN_RESPONSE	1.03748947470839E-06	2.59372368677098E-06	−2.091068996
HALLMARK_ADIPOGENESIS	2.32297894055881E-08	7.74326313519603E-08	−2.098205498
HALLMARK_COMPLEMENT	1.7764140229198E-08	6.34433579614213E-08	−2.127827307
HALLMARK_UNFOLDED_PROTEIN_RESPONSE	2.38410418949043E-07	6.27395839339587E-07	−2.170111603
HALLMARK_UV_RESPONSE_UP	1.23951131124001E-08	4.76735119707698E-08	−2.193127348
HALLMARK_APOPTOSIS	6.62974592670303E-09	3.0135208757741E-08	−2.195852605
HALLMARK_INFLAMMATORY_RESPONSE	5.70664670673152E-10	2.85332335336576E-09	−2.230584066
HALLMARK_CHOLESTEROL_HOMEOSTASIS	1.09811680092141E-08	4.57548667050588E-08	−2.398691197
HALLMARK_TNFA_SIGNALING_VIA_NFKB	2.34098697229536E-13	1.67213355163954E-12	−2.408307245
HALLMARK_E2F_TARGETS	1.73095020346086E-15	1.44245850288405E-14	−2.570206274
HALLMARK_G2M_CHECKPOINT	1.36546486615453E-16	2.27577477692421E-15	−2.606894263
HALLMARK_OXIDATIVE_PHOSPHORYLATION	3.35134841717099E-16	3.53991541941918E-15	−2.624884615
HALLMARK_MYC_TARGETS_V1	3.53991541941918E-16	3.53991541941918E-15	−2.627006317
HALLMARK_INTERFERON_ALPHA_RESPONSE	5.3364448434789E-13	2.9646915797105E-12	−2.654884445
HALLMARK_IL6_JAK_STAT3_SIGNALING	5.1878492849839E-13	2.9646915797105E-12	−2.76206492
HALLMARK_INTERFERON_GAMMA_RESPONSE	4.18180965872624E-21	1.04545241468156E-19	−2.842444981
HALLMARK_MTORC1_SIGNALING	1.58562880184299E-28	7.92814400921497E-27	−3.14071737

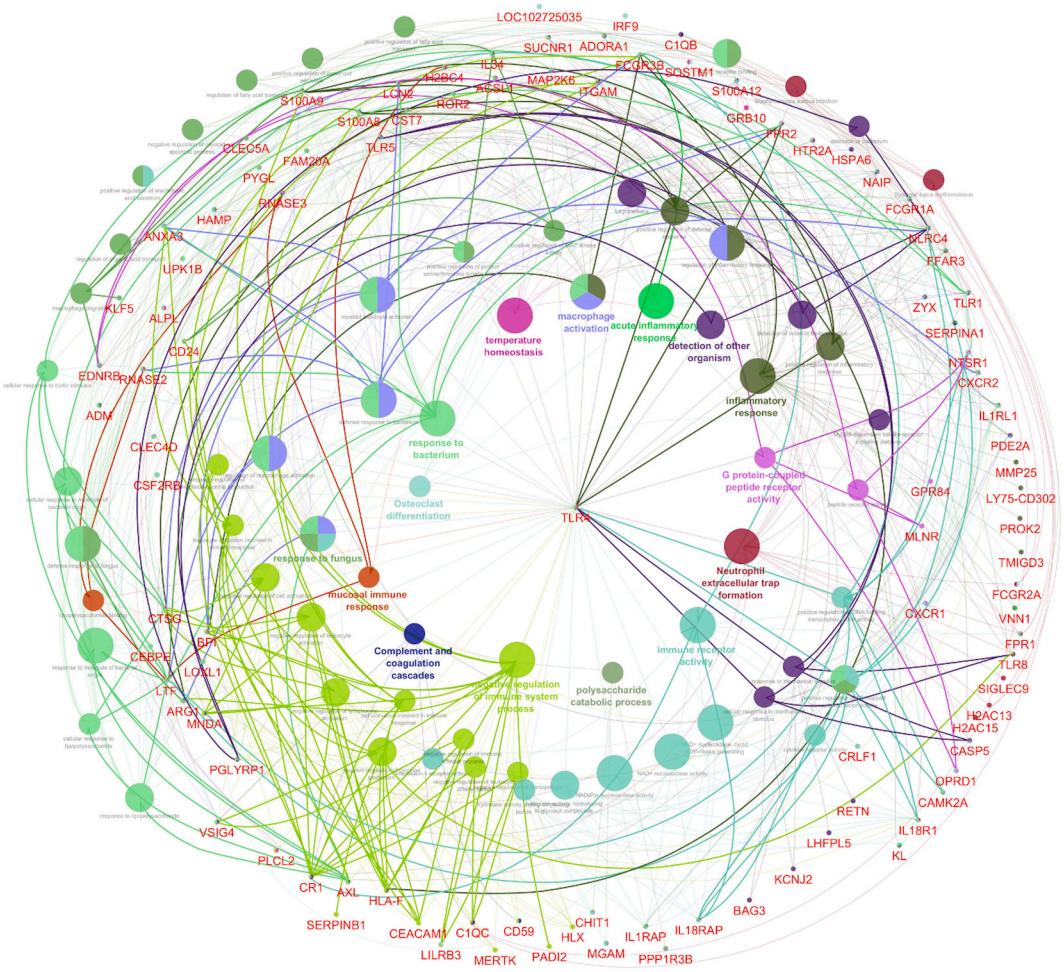

Fig. 5. Results of ClueGO functional analysis, wherein the nodes labeled in red signify the genes affecting the physiological function, and the colored nodes depict the physiological function. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

differentiating infection statuses of Mtb compared the DEGs of healthy controls, active and latent TB infection. It supported the relation of *FCGR1A* with different infection statuses of M. tuberculosis [38]. In the current dataset, *FCGR1A* was observed to be significantly downregulated.

Integrin subunit alpha M (*ITGAM*) is a part of the heterodimeric cell adhesion receptor family that mediates various biological functions. *ITGAM* acts as a macrophage biomarker regulating immune cell functions [39]. By facilitating adhesive interactions between monocytes, macrophages, and other cell types and the uptake of complement-coated particles and microbes [40], *ITGAM* can be regarded as playing a pivotal role in activating a diverse response of these cells, including phagocytosis, cell-mediated killing, chemotaxis, and cellular activation [41]. Integrins, including *ITGAM*, have been deemed vital for controlling a host of microbial infections, especially in the case of TB. In an experiment conducted on mice by comparing wild-type mice to the *ITGAM* knock-out one, it was seen that the latter has decreased T cells in the lungs, resulting in a lower capacity to fight against TB bacteria and a lesser survival rate [42]. In another study aimed to identify potential biomarkers of TB infection in diabetic patients, ITGAM is observed as a hub protein and has significant relevance in signaling pathways like toll-like receptors, RAP1 signaling routes, and many more [43].

The lactotransferrin (*LTF*) gene belongs to the transferrin family and is an integral part of the human non-specific immune system. The complementary protein of the *LTF* gene is present in secondary granules of neutrophils, which helps increase the immune system's efficiency against invasive or foreign disease-causing microbes by competing with them for iron due to the high affinity [44]. An experiment on a murine model revealed that TB susceptibility was significantly diminished by preventing iron overload using *LTF* [45]. Another study aimed at identifying potential biomarkers for TB by comparing gene expression profiles from TB patients and Mtb-infected healthy individuals supported the role of *LTF* in protection against TB [46]. C-X-C motif chemokine receptor 2 (*CXCR2*) belongs to the transmembrane G-coupled receptor protein group [47]. It is essential to fight against microbial infections by assisting in neutrophil infiltration by inducing late apoptotic cell injection [48]. In-depth studies around TB and *CXCR2* have suggested that *CXCR2* interacts with chemokine *CXCL5* to facilitate neutrophil accumulation [49]. By comparing *CXCR2* in healthy individuals against TB patients and HIV patients with TB infection [50], it was observed that an interesting correlation existed between neutrophil accumulation and infiltration using *CXCR2*. Similar results were observed in another study, where *CXCR2* was therefore down-regulated in TB-infected patients [51].

The current study is limited by a relatively small, homogenous sample group and lacks clinical and experimental validation. However, experimental validation using RT-PCR techniques in more extensive and diverse cohorts has yielded positive results, supporting the potential of candidate biomarkers as diagnostic transcriptional biomarkers for TB in the Indian population [52,53]. Additionally, alternative diagnostic strategies, such as automated chest radiograph interpretation tools based on advanced computational algorithms, C-reactive protein-based point-of-care systems, and other innovative methods, are being evaluated to enhance existing TB diagnostic approaches [54,55]. In

conclusion, this study provides preliminary insights into the transcriptomic profiles of TB patients within an Indian cohort, aiding in the development of diagnostic tests that can be effectively applied across different ethnicities and population groups.

5. Conclusion

India, the country with the highest TB burden in the world, faces significant challenges in the accurate diagnosis of TB. The current study highlights the benefits of transcriptome sequencing for diagnosing TB by identifying potential biomarkers specific to the Indian population, along with corroborating previously published reports worldwide. Similar strategies would enable researchers and clinicians to distinguish and diagnose individuals with latent TB from healthy individuals, thereby providing more insight into the progression of the *Mycobacterium tuberculosis* species. *TLR4*, *FCGR1A*, *ITGAM*, *LTF*, *and CXCR2* genes were found to have significant interactions and associations with critical physiological functions and signaling pathways.

CRediT authorship contribution statement

Ankur Datta: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Divyanshi Gupta:** Writing – original draft, Visualization, Software, Methodology, Investigation, Conceptualization. **Diya Waryani:** Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **George Priya Doss C:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Declaration of competing interest

The authors declare that no competing interests, financial or personal, have influenced the work reported in the current study.

Acknowledgment

The authors would like to take this opportunity to thank the management of Vellore Institute of Technology (VIT), Vellore, Tamil Nadu, India, for providing the necessary facilities and encouragement to carry out this work.

Data availability

The supplementary information has been provided in the following link: https://github.com/ankur506/TB_GSE198557.

References

[1] M.A. Behr, P.H. Edelstein, L. Ramakrishnan, Is Mycobacterium tuberculosis infection life long? BMJ 367 (2019) l5770, https://doi.org/10.1136/bmj.l5770.
[2] D. Prapruttam, S.S. Hedgire, S.E. Mani, A. Chandramohan, N.K. Shyamkumar, M. Harisinghani, Tuberculosis–the great mimicker, Semin. Ultrasound CT MR 35 (2014) 195–214, https://doi.org/10.1053/j.sult.2014.02.002.
[3] B.R. Bloom, R. Atun, T. Cohen, C. Dye, H. Fraser, G.B. Gomez, G. Knight, M. Murray, E. Nardell, E. Rubin, J. Salomon, A. Vassall, G. Volchenkov, R. White, D. Wilson, P. Yadav, Tuberculosis, in: K.K. Holmes, S. Bertozzi, B.R. Bloom, P. Jha (Eds.), Major Infect. Dis., third ed., The International Bank for Reconstruction and Development/The World Bank, Washington (DC), 2017. http://www.ncbi.nlm.nih.gov/books/NBK525174/. (Accessed 19 August 2024).
[4] S. Bagcchi, WHO's global tuberculosis report 2022, Lancet Microbe 4 (2023) e20, https://doi.org/10.1016/S2666-5247(22)00359-7.
[5] S.M. Hermans, A.N. Kiragga, P. Schaefer, A. Kambugu, A.I.M. Hoepelman, Y. C. Manabe, Incident tuberculosis during antiretroviral therapy contributes to suboptimal immune reconstitution in a large urban HIV clinic in sub-saharan africa, PLoS One 5 (2010) e10527, https://doi.org/10.1371/journal.pone.0010527.
[6] K.E. Dooley, T. Tang, J.E. Golub, S.E. Dorman, W. Cronin, Impact of diabetes mellitus on treatment outcomes of patients with active tuberculosis, Am. J. Trop. Med. Hyg. 80 (2009) 634–639.
[7] K. Hodgson, J. Morris, T. Bridson, B. Govan, C. Rush, N. Ketheesan, Immunological mechanisms contributing to the double burden of diabetes and intracellular bacterial infections, Immunology 144 (2015) 171–185, https://doi.org/10.1111/imm.12394.
[8] P. Baghaei, M. Marjani, P. Javanmard, P. Tabarsi, M.R. Masjedi, Diabetes mellitus and tuberculosis facts and controversies, J. Diabetes Metab. Disord. 12 (2013) 58, https://doi.org/10.1186/2251-6581-12-58.
[9] F.A. Al-Zamel, Detection and diagnosis of Mycobacterium tuberculosis, Expert Rev. Anti Infect. Ther. 7 (2009) 1099–1108, https://doi.org/10.1586/eri.09.92.
[10] A. Gholoobi, A. Masoudi-Kazemabad, M. Meshkat, Z. Meshkat, Comparison of culture and PCR methods for diagnosis of Mycobacterium tuberculosis in different clinical specimens, Jundishapur J. Microbiol. 7 (2014) e8939, https://doi.org/10.5812/jjm.8939.
[11] K.K. Chopra, S. Singh, Newer diagnostic tests for tuberculosis, their utility, and their limitations, Curr. Med. Res. Pract. 10 (2020) 8–11, https://doi.org/10.1016/j.cmrp.2020.01.004.
[12] M. MacGregor-Fairlie, S. Wilkinson, G.S. Besra, P. Goldberg Oppenheimer, Tuberculosis diagnostics: overcoming ancient challenges with modern solutions, Emerg. Top. Life Sci. 4 (2020) 423–436, https://doi.org/10.1042/ETLS20200335.
[13] J. Luo, M. Luo, J. Li, J. Yu, H. Yang, X. Yi, Y. Chen, H. Wei, Rapid direct drug susceptibility testing of Mycobacterium tuberculosis based on culture droplet digital polymerase chain reaction, Int. J. Tuberc. Lung Dis. Off. J. Int. Union Tuberc. Lung Dis. 23 (2019) 219–225, https://doi.org/10.5588/ijtld.18.0182.
[14] J. Yang, X. Han, A. Liu, X. Bai, C. Xu, F. Bao, S. Feng, L. Tao, M. Ma, Y. Peng, Use of digital droplet PCR to detect Mycobacterium tuberculosis DNA in whole blood-derived DNA samples from patients with pulmonary and extra-pulmonary tuberculosis, Front. Cell. Infect. Microbiol. 7 (2017) 369, https://doi.org/10.3389/fcimb.2017.00369.
[15] W. Fox, D.A. Mitchison, Short-course chemotherapy for pulmonary tuberculosis, Am. Rev. Respir. Dis. 111 (1975) 325–353, https://doi.org/10.1164/arrd.1975.111.3.325.
[16] J. Domínguez, E.C. Boettger, D. Cirillo, F. Cobelens, K.D. Eisenach, S. Gagneux, D. Hillemann, R. Horsburgh, B. Molina-Moya, S. Niemann, E. Tortoli, A. Whitelaw, C. Lange, TBNET, RESIST-TB networks, Clinical implications of molecular drug resistance testing for Mycobacterium tuberculosis: a TBNET/RESIST-TB consensus statement, Int. J. Tuberc. Lung Dis. Off. J. Int. Union Tuberc, Lung Dis. 20 (2016) 24–42, https://doi.org/10.5588/ijtld.15.0221.
[17] S.D. Ahuja, D. Ashkin, M. Avendano, R. Banerjee, M. Bauer, J.N. Bayona, M. C. Becerra, A. Benedetti, M. Burgos, R. Centis, E.D. Chan, C.-Y. Chiang, H. Cox, L. D'Ambrosio, K. DeRiemer, N.H. Dung, D. Enarson, D. Falzon, K. Flanagan, J. Flood, M.L. Garcia-Garcia, N. Gandhi, R.M. Granich, M.G. Hollm-Delgado, T. H. Holtz, M.D. Iseman, L.G. Jarlsberg, S. Keshavjee, H.-R. Kim, W.-J. Koh, J. Lancaster, C. Lange, W.C.M. de Lange, V. Leimane, C.C. Leung, J. Li, D. Menzies, G.B. Migliori, S.P. Mishustin, C.D. Mitnick, M. Narita, P. O'Riordan, M. Pai, D. Palmero, S. Park, G. Pasvol, J. Peña, C. Pérez-Guzmán, M.I.D. Quelapio, A. Ponce-de-Leon, V. Riekstina, J. Robert, S. Royce, H.S. Schaaf, K.J. Seung, L. Shah, T.S. Shim, S.S. Shin, Y. Shiraishi, J. Sifuentes-Osornio, G. Sotgiu, M. J. Strand, P. Tabarsi, T.E. Tupasi, R. van Altena, M. Van der Walt, T.S. Van der Werf, M.H. Vargas, P. Viiklepp, J. Westenhouse, W.W. Yew, J.-J. Yim, Collaborative Group for Meta-Analysis of Individual Patient Data in MDR-TB, Multidrug resistant pulmonary tuberculosis treatment regimens and patient outcomes: an individual patient data meta-analysis of 9,153 patients, PLoS Med. 9 (2012) e1001300, https://doi.org/10.1371/journal.pmed.1001300.
[18] T.M. Walker, M. Merker, T.A. Kohl, D.W. Crook, S. Niemann, T.E.A. Peto, Whole genome sequencing for M/XDR tuberculosis surveillance and for resistance testing, Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis. 23 (2017) 161–166, https://doi.org/10.1016/j.cmi.2016.10.014.
[19] P. Miotto, B. Tessema, E. Tagliani, L. Chindelevitch, A.M. Starks, C. Emerson, D. Hanna, P.S. Kim, R. Liwski, M. Zignol, C. Gilpin, S. Niemann, C.M. Denkinger, J. Fleming, R.M. Warren, D. Crook, J. Posey, S. Gagneux, S. Hoffner, C. Rodrigues, I. Comas, D.M. Engelthaler, M. Murray, D. Alland, L. Rigouts, C. Lange, K. Dheda, R. Hasan, U.D.K. Ranganathan, R. McNerney, M. Ezewudo, D.M. Cirillo, M. Schito, C.U. Köser, T.C. Rodwell, A standardised method for interpreting the association between mutations and phenotypic drug resistance in Mycobacterium tuberculosis, Eur. Respir. J. 50 (2017) 1701354, https://doi.org/10.1183/13993003.01354-2017.
[20] K. Raza, S. Ahmad, Recent advancement in next-generation sequencing techniques and its computational analysis, Int. J. Bioinf. Res. Appl. 15 (2019) 191–220, https://doi.org/10.1504/IJBRA.2019.101205.
[21] X. Dai, L. Shen, Advances and trends in omics technology development, Front. Med. 9 (2022) 911861, https://doi.org/10.3389/fmed.2022.911861.
[22] T. Barrett, S.E. Wilhite, P. Ledoux, C. Evangelista, I.F. Kim, M. Tomashevsky, K. A. Marshall, K.H. Phillippy, P.M. Sherman, M. Holko, A. Yefanov, H. Lee, N. Zhang, C.L. Robertson, N. Serova, S. Davis, A. Soboleva, NCBI GEO: archive for functional genomics data sets—update, Nucleic Acids Res. 41 (2013) D991–D995, https://doi.org/10.1093/nar/gks1193.
[23] D. Kim, J.M. Paggi, C. Park, C. Bennett, S.L. Salzberg, Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype, Nat. Biotechnol. 37 (2019) 907–915, https://doi.org/10.1038/s41587-019-0201-4.
[24] H. Wickham, ggplot2: Elegant Graphics for Data Analysis, Springer, New York, NY, 2009, https://doi.org/10.1007/978-0-387-98141-3.

[25] M.I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2, Genome Biol. 15 (2014) 550, https://doi.org/10.1186/s13059-014-0550-8.
[26] C.-H. Chin, S.-H. Chen, H.-H. Wu, C.-W. Ho, M.-T. Ko, C.-Y. Lin, cytoHubba: identifying hub objects and sub-networks from complex interactome, BMC Syst. Biol. 8 (Suppl 4) (2014) S11, https://doi.org/10.1186/1752-0509-8-S4-S11.
[27] A. Subramanian, P. Tamayo, V.K. Mootha, S. Mukherjee, B.L. Ebert, M.A. Gillette, A. Paulovich, S.L. Pomeroy, T.R. Golub, E.S. Lander, J.P. Mesirov, Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles, Proc. Natl. Acad. Sci. USA 102 (2005) 15545–15550, https://doi.org/10.1073/pnas.0506580102.
[28] G. Bindea, B. Mlecnik, H. Hackl, P. Charoentong, M. Tosolini, A. Kirilovsky, W.-H. Fridman, F. Pagès, Z. Trajanoski, J. Galon, ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks, Bioinformatics 25 (2009) 1091–1093, https://doi.org/10.1093/bioinformatics/btp101.
[29] Y. Cai, Q. Yang, Y. Tang, M. Zhang, H. Liu, G. Zhang, Q. Deng, J. Huang, Z. Gao, B. Zhou, C.G. Feng, X. Chen, Increased complement C1q level marks active disease in human tuberculosis, PLoS One 9 (2014) e92340, https://doi.org/10.1371/journal.pone.0092340.
[30] J.G. Burel, A. Singhania, P. Dubelko, J. Muller, R. Tanner, E. Parizotto, M. Dedicoat, T.E. Fletcher, J. Dunbar, A.F. Cunningham, C.S. Lindestam Arlehamn, D.G. Catanzaro, A. Catanzaro, T. Rodwell, H. McShane, M.K. O'Shea, B. Peters, Distinct blood transcriptomic signature of treatment in latent tuberculosis infected individuals at risk of developing active disease, Tuberc. Edinb. Scotl. 131 (2021) 102127, https://doi.org/10.1016/j.tube.2021.102127.
[31] Y. Bulut, K.S. Michelsen, L. Hayrapetian, Y. Naiki, R. Spallek, M. Singh, M. Arditi, Mycobacterium tuberculosis heat shock proteins use diverse Toll-like receptor pathways to activate pro-inflammatory signals, J. Biol. Chem. 280 (2005) 20961–20967, https://doi.org/10.1074/jbc.M411379200.
[32] J. Kleinnijenhuis, M. Oosting, L.A.B. Joosten, M.G. Netea, R. Van Crevel, Innate immune recognition of Mycobacterium tuberculosis, Clin, Dev. Immunol. 2011 (2011) 405310, https://doi.org/10.1155/2011/405310.
[33] X. Xue, Y. Qiu, D. Jiang, T. Jin, M. Yan, X. Zhu, Y. Chu, The association analysis of TLR2 and TLR4 gene with tuberculosis in the Tibetan Chinese population, Oncotarget 8 (2017) 113082–113089, https://doi.org/10.18632/oncotarget.22996.
[34] H.Y. Zaki, K.H. Leung, W.C. Yiu, N. Gasmelseed, N.E.M. Elwali, S.P. Yip, Common polymorphisms in TLR4 gene associated with susceptibility to pulmonary tuberculosis in the Sudanese, Int. J. Tuberc. Lung Dis. Off. J. Int. Union Tuberc. Lung Dis. 16 (2012) 934–940, https://doi.org/10.5588/ijtld.11.0517.
[35] J.M. Cubillos-Angulo, M.B. Arriaga, E.C. Silva, B.L.A. Müller, D.M.P. Ramalho, K. F. Fukutani, P.F.C. Miranda, A.S.R. Moreira, A. Ruffino-Netto, J.R. Lapa e Silva, T. R. Sterling, A.L. Kritski, M.M. Oliveira, B.B. Andrade, Polymorphisms in TLR4 and tnfa and risk of Mycobacterium tuberculosis infection and development of active disease in contacts of tuberculosis cases in Brazil: a prospective cohort study, Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 69 (2019) 1027–1035, https://doi.org/10.1093/cid/ciy1001.
[36] J.J.M.L. Hoffmann, Neutrophil CD64: a diagnostic marker for infection and sepsis, Clin. Chem. Lab. Med. 47 (2009) 903–916, https://doi.org/10.1515/CCLM.2009.224.
[37] S. Jenum, R. Bakken, S. Dhanasekaran, A. Mukherjee, R. Lodha, S. Singh, V. Singh, M.C. Haks, T.H.M. Ottenhoff, S.K. Kabra, T.M. Doherty, C. Ritz, H.M.S. Grewal, BLR1 and FCGR1A transcripts in peripheral blood associate with the extent of intrathoracic tuberculosis in children and predict treatment outcome, Sci. Rep. 6 (2016) 38841, https://doi.org/10.1038/srep38841.
[38] Y. Xu, X. Li, L. Pu, C. Niu, AIM2 and FCGR1A, two potential diagnostic biomarkers for distinguishing infection statuses of Mycobacterium tuberculosis. https://doi.org/10.21203/rs.3.rs-3009175/v1, 2023.
[39] B.C. King, K. Kulak, U. Krus, R. Rosberg, E. Golec, K. Wozniak, M.F. Gomez, E. Zhang, D.J. O'Connell, E. Renström, A.M. Blom, Complement component C3 is highly expressed in human pancreatic islets and prevents β cell death via ATG16L1 interaction and autophagy regulation, Cell Metabol. 29 (2019) 202–210.e6, https://doi.org/10.1016/j.cmet.2018.09.009.
[40] D. Pisu, L. Huang, V. Narang, M. Theriault, G. Lê-Bury, B. Lee, A.E. Lakudzala, D. T. Mzinza, D.V. Mhango, S.C. Mitini-Nkhoma, K.C. Jambo, A. Singhal, H. C. Mwandumba, D.G. Russell, Single cell analysis of M. tuberculosis phenotype and macrophage lineages in the infected lung, J. Exp. Med. 218 (2021) e20210615, https://doi.org/10.1084/jem.20210615.
[41] D.A. Solovjov, E. Pluskota, E.F. Plow, Distinct roles for the alpha and beta subunits in the functions of integrin alphaMbeta2, J. Biol. Chem. 280 (2005) 1336–1345, https://doi.org/10.1074/jbc.M406968200.
[42] T.O. Bose, Q.-M. Pham, E.R. Jellison, J. Mouries, C.M. Ballantyne, L. Lefrançois, CD11a regulates effector CD8 T cell differentiation and central memory development in response to infection with Listeria monocytogenes, Infect. Immun. 81 (2013) 1140–1151, https://doi.org/10.1128/IAI.00749-12.
[43] G.T. Selvan, P. Gollapalli, P. Shetty, N.S. Kumari, Exploring key molecular signatures of immune responses and pathways associated with tuberculosis in comorbid diabetes mellitus: a systems biology approach, Beni-Suef Univ. J. Basic Appl. Sci. 11 (2022) 77, https://doi.org/10.1186/s43088-022-00257-5.
[44] P.P. Ward, S. Uribe-Luna, O.M. Conneely, Lactoferrin and host defense, Biochem. Cell Biol. Biochim. Biol. Cell. 80 (2002) 95–102, https://doi.org/10.1139/o01-214.
[45] U.E. Schaible, H.L. Collins, F. Priem, S.H.E. Kaufmann, Correction of the iron overload defect in β-2-microglobulin knockout mice by lactoferrin abolishes their increased susceptibility to tuberculosis, J. Exp. Med. 196 (2002) 1507–1513, https://doi.org/10.1084/jem.20020897.
[46] M. Jacobsen, D. Repsilber, A. Gutschmidt, A. Neher, K. Feldmann, H.J. Mollenkopf, A. Ziegler, S.H.E. Kaufmann, Candidate biomarkers for discrimination between infection and disease caused by Mycobacterium tuberculosis, J. Mol. Med. Berl. Ger. 85 (2007) 613–621, https://doi.org/10.1007/s00109-007-0157-6.
[47] J. Korbecki, P. Kupnicka, M. Chlubek, J. Gorący, I. Gutowska, I. Baranowska-Bosiacka, CXCR2 receptor: regulation of expression, signal transduction, and involvement in cancer, Int. J. Mol. Sci. 23 (2022) 2168, https://doi.org/10.3390/ijms23042168.
[48] T. Iyoda, Y. Kobayashi, Involvement of MIP-2 and CXCR2 in neutrophil infiltration following injection of late apoptotic cells into the peritoneal cavity, Apoptosis 9 (2004) 485–493, https://doi.org/10.1023/B:APPT.0000031450.95188.e7.
[49] L. Monin, S.A. Khader, Chemokines in tuberculosis: the good, the bad and the ugly, Semin. Immunol. 26 (2014) 552–558, https://doi.org/10.1016/j.smim.2014.09.004.
[50] S. Meddows-Taylor, D.J. Martin, C.T. Tiemessen, Reduced expression of interleukin-8 receptors A and B on polymorphonuclear neutrophils from persons with human immunodeficiency virus type 1 disease and pulmonary tuberculosis, J. Infect. Dis. 177 (1998) 921–930, https://doi.org/10.1086/515232.
[51] N.P. Juffermans, P.E. Dekkers, M.P. Peppelenbosch, P. Speelman, S.J. van Deventer, T. van Der Poll, Expression of the chemokine receptors CXCR1 and CXCR2 on granulocytes in human endotoxemia and tuberculosis: involvement of the p38 mitogen-activated protein kinase pathway, J. Infect. Dis. 182 (2000) 888–894, https://doi.org/10.1086/315750.
[52] L.T. Hoang, P. Jain, T.D. Pillay, M. Tolosa-Wright, U. Niazi, Y. Takwoingi, A. Halliday, L.C. Berrocal-Almanza, J.J. Deeks, P. Beverley, O.M. Kon, A. Lalvani, Transcriptomic signatures for diagnosing tuberculosis in clinical practice: a prospective, multicentre cohort study, Lancet Infect. Dis. 21 (2021) 366–375, https://doi.org/10.1016/S1473-3099(20)30928-2.
[53] S.-W. Lee, L.S.-H. Wu, G.-M. Huang, K.-Y. Huang, T.-Y. Lee, J.T.-Y. Weng, Gene expression profiling identifies candidate biomarkers for active and latent tuberculosis, BMC Bioinf. 17 (Suppl 1) (2016) 3, https://doi.org/10.1186/s12859-015-0848-x.
[54] T. Pande, C. Cohen, M. Pai, F. Ahmad Khan, Computer-aided detection of pulmonary tuberculosis on digital chest radiographs: a systematic review, Int. J. Tuberc. Lung Dis. Off. J. Int. Union Tuberc. Lung Dis. 20 (2016) 1226–1230, https://doi.org/10.5588/ijtld.15.0926.
[55] C. Yoon, F.C. Semitala, E. Atuhumuza, J. Katende, S. Mwebe, L. Asege, D. T. Armstrong, A.O. Andama, D.W. Dowdy, J.L. Davis, L. Huang, M. Kamya, A. Cattamanchi, Point-of-care C-reactive protein-based tuberculosis screening for people living with HIV: a diagnostic accuracy study, Lancet Infect. Dis. 17 (2017) 1285–1292, https://doi.org/10.1016/S1473-3099(17)30488-7.