RESEARCH ARTICLE

Identification of Differentially Expressed Hematopoiesis-associated Genes in Term Low Birth Weight Newborns by Systems Genomics Approach

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Abstract: *Background*: Low Birth Weight (LBW) (birth weight <2.5 Kg) newborns are associated with a high risk of infection, morbidity and mortality during their perinatal period. Compromised innate immune responses and inefficient hematopoietic differentiation in term LBW newborns led us to evaluate the gene expression status of hematopoiesis.

Materials and Methods: In this study, we compared our microarray datasets of LBW-Normal Birth Weight (NBW) newborns with two reference datasets to identify hematopoietic stem cells genes, and their differential expression in the LBW newborns, by hierarchical clustering algorithm using gplots and RcolorBrewer package in R.

Results: Comparative analysis revealed 108 differentially expressed hematopoiesis genes (DEHGs), of which 79 genes were up-regulated, and 29 genes were down-regulated in LBW newborns compared to their NBW counterparts. Moreover, protein-protein interactions, functional annotation and pathway analysis demonstrated that the up-regulated genes were mainly involved in cell proliferation and differentiation, MAPK signaling and Rho GTPases signaling, and the down-regulated genes were engaged in cell proliferation and regulation, immune system regulation, hematopoietic cell lineage and JAK-STAT pathway. The binding of down-regulated genes (LYZ and GBP1) with growth factor GM-CSF using docking and MD simulation techniques, indicated that GM-CSF has the potential to alleviate the repressed hematopoiesis in the term LBW newborns.

Conclusion: Our study revealed that DEHGs belonged to erythroid and myeloid-specific lineages and may serve as potential targets for improving hematopoiesis in term LBW newborns to help build up their weak immune defense against life-threatening infections.

Keywords: LBW and NBW newborns, hematopoietic-associated genes, genomics, molecular docking, systems genomics approach, neutropenia.

1. INTRODUCTION

Low birth weight (LBW) newborns defined as a birth weight below 2.5 kg, suffer from a higher rate of morbidity and mortality than normal birth weight (NBW) newborns [1-3]. Highest numbers of LBW newborns are born in India annually with nearly 7.5 million newborns being born with a birth weight less than 2.5 kg of which 60% are born at term, and 40% are born preterm [4]. Neonatal mortality rate for premature and LBW newborns has steadily risen from 12.3 per 1,000 live births in 2000 to 14.3 by 2015 [5]. Pneumonia, acute lower respiratory infections, hematological problem, hypocalcemia and hypoglycemia, hypo and hyperthermia are physiological problems associated with LBW newborns [6, 7]. LBW newborns have an increased tendency towards bacterial infection and mortality and need specialized medical consideration. The underlying mechanisms that lead to a high rate of infections in LBW newborns are not well understood.

White blood cells like lymphocytes and granulocytes are principal components of the immune system. Significantly lesser numbers of circulating lymphocytes and granulocytes in LBW newborns are one of the primary reasons for their inefficient defense system making them highly susceptible to life-threatening infections and high mortality rates [8]. It has been reported that the platelet counts are less (thrombocytopenia) in very LBW newborns (≤1000g birth weight). The newborns with thrombocytopenia have increased risk of intraventricular hemorrhage, mortality and adverse neurodevelopmental consequences [9, 10]. Low neutrophil counts (<1000/μl) (neutropenia), is mostly seen in 6-8% of all neonates admitted to NICUs (Neonatal Intensive Care Units) [11]. Neutropenia causes decreased neutrophil production, increased neutrophil destruction, copper deficiency, myelodysplastic syndrome and vitamins' deficiency. Lower levels of IgG [12-14], impaired early IgA and IgM synthesis [14], lower T and B lymphocyte percentage and lower innate cells

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are reported in LBW newborns as compared to NBW newborns [12, 15, 16]. Normocytic, normochromic anemia reported in term and preterm infants [17]. Multiple reasons for this increased severity of anemia have been described, including physiologic responses to decreased oxygen consumption, blood loss secondary to phlebotomy for laboratory studies related to clinical management in the first few weeks of life, a developmentally immature erythropoietic response to anemia, decreased survival of RBCs in preterm infants, and deficiencies of folate, vitamin B-12 or vitamin E [6]. As immune cell numbers are reduced in LBW newborns, we assumed that their hematopoiesis process might be deficient.

Earlier, we have studied global gene expression patterns in LBW and NBW newborns using Affymetrix Human Genome U133 Plus 2.0 Array platform, to obtain an insight of the impaired immunocompetence in the LBW newborns (NCBI GEO-databases, accession number-GSE29807) [18]. Several studies have addressed the association of birth weight and hematopoietic stem cell measurements in the cord blood samples [19-22]. But the process of hematopoiesis in full-term newborns, specifically in LBW newborns has not been adequately addressed till date and remains a poorly covered area. Therefore, the present study is aimed at studying the comparative gene expression analysis of hematopoiesis-associated genes to identify the defective checkpoints of hematopoiesis in full-term LBW newborns. Our approach involved using two 'reference' datasets extracted from Gene Expression Omnibus (GEO) (GSE64888 and GSE107497) on gene expression profiling of hematopoietic stem cells to identify the hematopoietic stem cells genes and their differential expression in our LBW-NBW newborns 'test' dataset (GSE29807). We compared LBW-NBW microarray dataset with hematopoietic microarray datasets and then identified differentially expressed hematopoiesis genes (DEHGs) between full-term LBW and NBW newborns dataset. Subsequently, using the identified DEHGs in the term LBW newborns we performed integrated protein-protein interaction, functional enrichment analysis, biological pathways, and molecular docking. We propose that differentially expressed erythroid and myeloid-lineage specific genes identified through this study may be employed as potential drug targets for improving the compromised hematopoiesis in term LBW newborns which could contribute towards a more strengthened immune defense against life-threatening infections. To the best of our knowledge, this is the first report on the defective expression of hematopoiesis genes in term LBW newborns compared to NBW newborns.

2. MATERIALS AND METHODS

2.1. Comparative Analysis of Datasets

Two microarray datasets associated with gene expression profiling of hematopoietic stem cells GSE64888 (reference dataset I) [23] and GSE107497 (reference dataset II) [24] were retrieved from the GEO database (https://www.ncbi.nlm.nih.gov/geo/) for comparative analysis of our microarray dataset GSE29807 (test dataset) on differential gene expression in term LBW-NBW newborns [18]. The reference datasets were chosen primarily as they were related to expression profiling of human hematopoietic stem/progenitor cells and their early myeloid and erythroid proge-

ny, using RNA samples derived from human cord blood from healthy newborns same as that for the test dataset. As our objective was to study the status of DEHGs in low birth weight (LBW) newborns so, using gene expression information from similar sample source (cord blood) was necessary. The gene expression profiling of the datasets was performed using Affymetrix Human Genome U133 Plus 2.0 Array [18, 23] or Affymetrix Human Transcriptome Array 2.0 [24]. A comparative analysis of the reference datasets with the test dataset was done to identify DEHGs between LBW and NBW newborns. The data pre-processing was done by gene expression average method using BiGGEsTS (Biclustering gene expression time series) tool [25]. Furthermore, a hierarchical clustering algorithm [26] followed by 'gplots' and 'RcolorBrewer' package in R was employed to deduce the differential gene expression. DEHGs were selected by two approaches, fold change >2 or false discovery rate (FDR) value of 0.5. Principal Component Analysis (PCA) was done by ClustVis tool [27]. The study selection protocol for comparative analysis is presented as a flowchart (Fig. **1**).

2.2. Protein-Protein Interaction (PPI) Network

The PPI network was constructed with the DEHGs mapped into STRING (Search Tool for the Retrieval of Interacting Genes/Proteins database version 10.0) [28]. The active interaction sources were based on the seven parameters including experiments, co-expression, gene fusion, co-occurrence, databases, text mining, and neighbourhood. The species for PPI analysis was set as a human with the interaction score ≥ 0.4. Functional domain and motif pattern prediction were performed by InterPro scan [29] and MO-TIFSCAN tool [30].

2.3. Functional Characterization and Pathway Analysis

Functional annotation of DEHGs was performed using DAVID v6.7 (https://david.ncifcrf.gov) and REVIGO tool [31, 32]. DEHGs were analyzed using KEGG and Reactome database to illustrate intermediary metabolism, regulatory pathways and signal transduction [33, 34]. Selected GO terms and pathways were mainly enriched with a cutoff criteria of p-value < 0.05.

2.4. Protein-Protein Docking and MD Simulation to Identify Molecular Targets for Growth Factor

To identify whether some of the molecules under expressed in the LBW newborns group could serve as targets for growth factors known to induce differentiation to myeloid/granulocyte lineage molecular interaction study between the growth factor (GM-CSF) and human LYZ and GBP1 proteins were performed using ClusPro [35]. Further, the docked complexes were analyzed *via* binding energy, hydrogen bonds and visualizing the PPI interface using PyMol (v1.7.4) software [36]. After docking, MD simulation was applied to gain insight into the impact of docked complexes. The docked complex was subjected to simulation for a time period of 18ns on GROMACS 4.6.7 version [37] through a gromos53a6 force field [38]. The simulations were repeated twice, and each time the result was found to be steady. To avoid unwanted steric clashes in the system, we performed
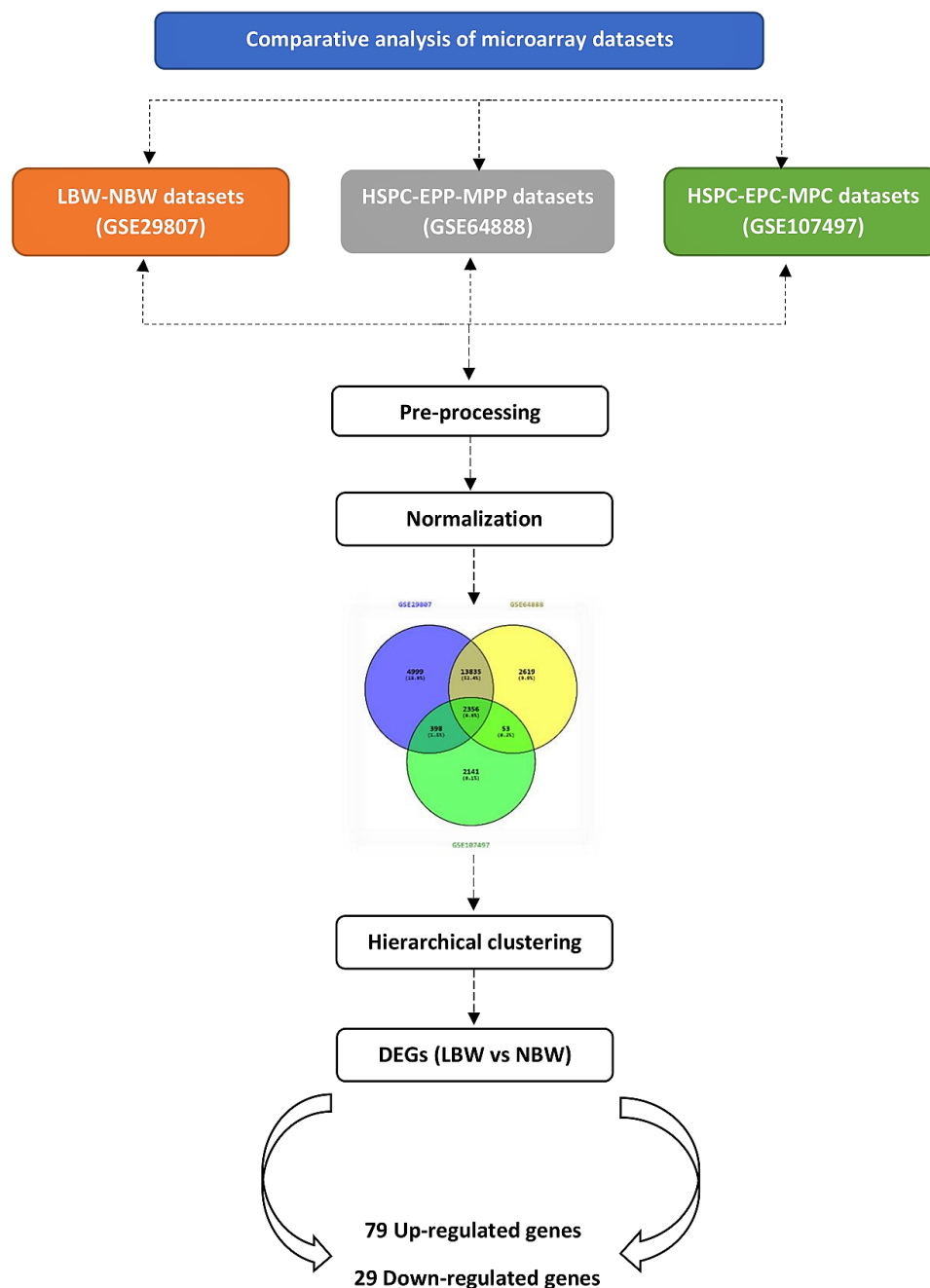
Fig. (1). A flowchart describing steps used in the comparative analysis of gene expression data from three microarray datasets. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

energy minimization for 50,000 steps utilizing the steepest-descent method, and Maxwell-Boltzmann distribution equivalent to 300K calculated the initial velocities. The stability of the trajectories was evaluated by the root-mean-square deviation (RMSD) and root mean square fluctuation (RMSF). We used the Xmgrace program to plot our data.

3. RESULTS

3.1. Segregation of Hematopoiesis Genes

To segregate the hematopoiesis associated genes present in our test dataset, it was compared with two reference da-

tasets (GSE64888, and GSE107497) of hematopoietic stem progenitor cell and their committed erythroid and myeloid progeny [23, 24]. The comparison of our test dataset revealed that there were overall 16,589 genes present in our dataset, that are associated with hematopoiesis (D+E+G, Fig. **2A**). Among them, 13,835 hematopoiesis genes were common to test dataset and reference dataset I only (E, Fig. **2A**), 398 hematopoiesis genes were common to test dataset and reference dataset II only (D, Fig. **2A**), whereas 2356 hematopoiesis genes of test dataset commonly present in both reference datasets I and II (G, Fig. **2A**). Owing to higher
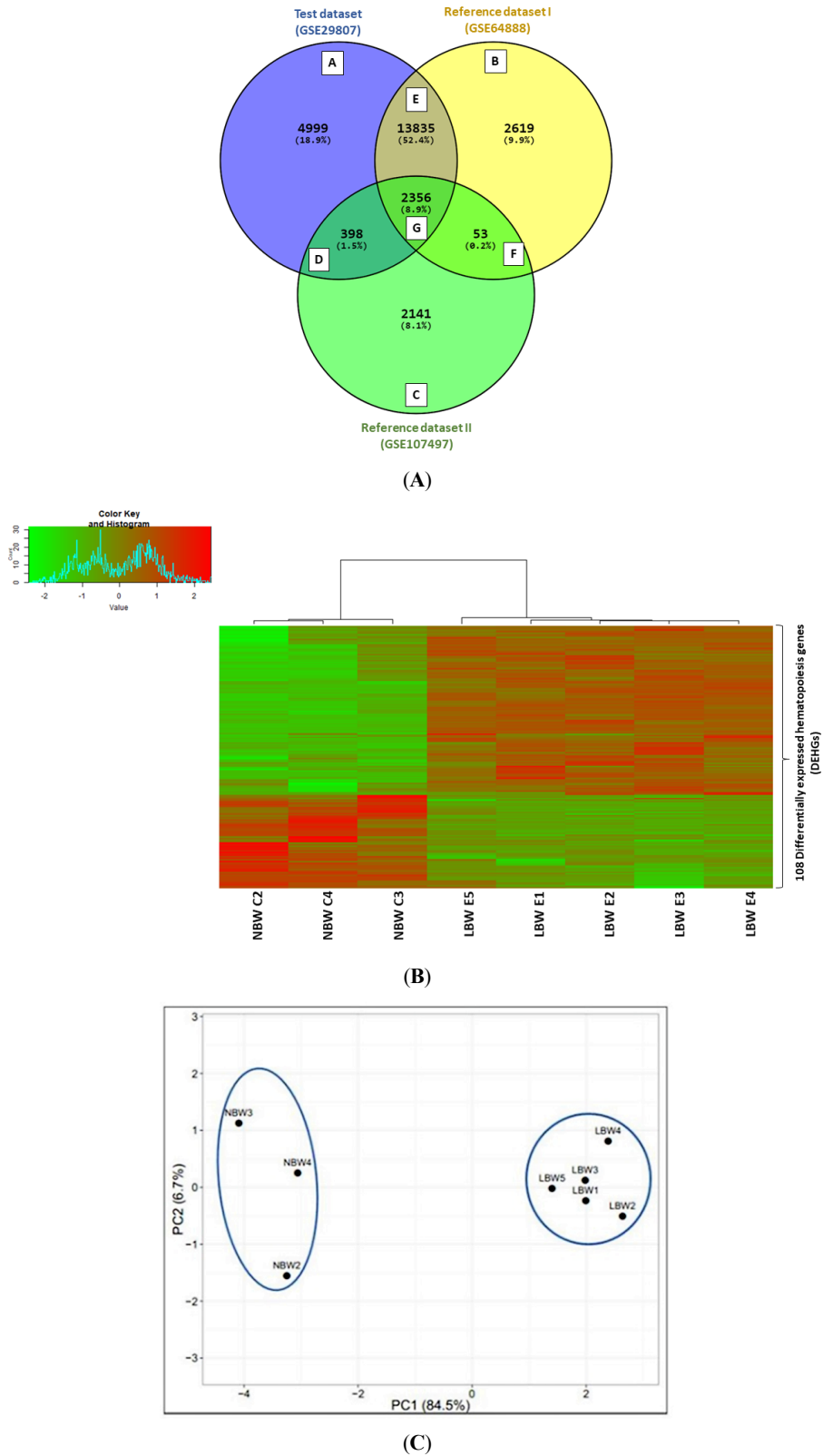
(A)

(B)

(C)

Fig. (2). (A) Venn diagram representing the overlapping genes between three microarray datasets. GSE29807 set denote term LBW-NBW newborns 'test dataset', GSE64888set symbolizes 'reference dataset I' and GSE107497set is 'reference dataset II'. **(B)** Expression profile of 108 DEHGs in LBW and NBW newborns. Hierarchical clustering heatmap showing the up-regulated (red) and down-regulated (green) genes in term LBW newborns *vs* NBW newborns. Each column represents one sample and each row represents one differential mRNA. The scale bar denotes expression value. **(C)** PCA shows two distinct clusters which correspond to NBW and LBW newborns samples. X-axis and Y-axis denote PC1 and PC2 that explain 84.5% and 6.7% of the total variance, respectively. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

confidence associated with the set 'G' genes being hematopoiesis related, we, therefore, chose the group 'G' for further analysis in this study. There were 2619 hematopoiesis genes (B, Fig. **2A**) present in reference dataset I alone, 2141 genes (C, Fig. **2A**) present in reference dataset II alone and 53 genes (F, Fig. **2A**) present only in the two reference datasets but not in the test dataset. In addition, 4999 genes (A, Fig. **2A**) of the test dataset were non-hematopoiesis genes. Therefore, the genes in sets (A, B, C and F) were not relevant for this study and hence not analyzed further.

3.2. Analysis of DEHGs

For further identification of DEHGs in LBW newborns, we analyzed the 2356 hematopoiesis genes of set G, by performing hierarchical cluster analysis using an unweighted pair group method with arithmetic mean (UPGMA) method. The result of hierarchical cluster analysis revealed that of 2356 genes, 108 hematopoiesis-associated genes were identified differentially expressed in LBW newborns as compared to the NBW newborns taken as control. The heatmap generated for 108 DEHGs using R package, revealed that 29 genes were down-regulated, and 79 genes were up-regulated in LBW newborns group (Fig. **2B**, Table **1**; Supplementary table **S1** and **S2**). The PCA plot on individual samples of LBW and NBW newborns on two principal components reveals significant variation between two newborns samples (Fig. **2C**).

3.3. Protein-Protein Interaction (PPI) Network

All the 108 DEHGs were used to construct the PPI network using the STRING database. After removing the disconnected genes, 90 nodes with 79 edges selected on the basis of *high confidence* and *k* means *clustering* were mapped in the PPI network. The selected proteins included AKIRIN1, KHDRBS1, PDCD4, DDX17, HNRNPA3, ACIN1, SNRNP200, PRPF40A, UHMK1, RBM8A, PPIG, ALOX5, CXCR4, IL6, GNB1, CD34, CD200, CD52, CD84, HBB, HBD, LYZ, ACTB, FCGR2A, WASF2, ARPC5, ACTR2, TAGLN2, ENO1, MYCBP, EPB41, CADM3, ARF1, ALDH4A1, RTN1, ALDH6A1, ACBD3, RTN4, EPRS, RAB10, EHBP1L1, SNAP23, YIPF4 and SYNCRIP (Fig. **3**). The analysis revealed that they were mainly involved in *Fc gamma R-mediated phagocytosis, regulation of IL-2 secretion, immune system, RHO GTPase signaling,* and *neutrophil-mediated immunity*. We also performed domain and motif analysis of these interacted proteins and found they were majorly involved in RNA-binding domain superfamily (IPR035979), Reticulon (IPR003388), Haemoglobin, beta type (IPR002337) and RNA recognition motif domain (IPR000504) (Supplementary Fig. **1**).

3.4. Functional Characterization of DEHGs Identifies Cellular Regulation, Proliferation and Differentiation

Functional annotation of DEHGs for the biological processes, cellular components, and molecular functions was performed. The GO categories for up-regulated and down-regulated genes were enriched (p-value < 0.05).

3.4.1. Biological Process: Down-regulated DEHGs

Main biological functions of down-regulated genes were *cell differentiation* (GO:0030154), *regulation of immune system* (GO:0002682), *cell-cell adhesion* (GO:

Table 1.   **The list of 108 DEHGs (29 down-regulated genes and 79 up-regulated genes) in LBW newborns.**

| DEHGs	Gene Name
Down-regulated genes	C1orf56, RAB10, SNAP23, EPHA8, HBB, GBP1, YIPF4, PPAP2A,
	IL9R, ATP7B, RTN1, CD34, JAZF1, HBD, HNMT, EPHA8, CHAC2,
	LGALS8, CD200, CADM3, CD84, ALDH6A1, MR1, ALDH4A1,
	GLI2, ATPIF1, C1orf116, LYZ, MYCBP
Up-regulated genes	RABGAP1L, EPB41, HNRNPA3, PIGC, ACTR2, IRF2BP2, MALAT1,
	SOX4, ARPC5, EML4, ALKBH5, UHMK1, RSBN1, PRPF40A, EPRS,
	CD52, PPIG, IL6R, RBM8A, USP16, UHMK1, ZNF281, ELOVL1,
	ZNF205, ACIN1, NR4A2, KHDRBS1, NR4A2, PRPF40A, PDCD4,
	TNRC6B, SLC35D1, NUCKS1, VCAN, SNRNP200, PLEKHB2,
	SNX17, ZNF22, ENO1, MALAT1, GPATCH2L, ALOX5, ACBD3,
	ZFP36L2, AKIRIN1, SYNCRIP, ARF1, CXCR4, PDE4B, LIMS1,
	RTN4, ENSA, TAGLN2, PDCD4, NUCKS1, MALAT1, CCDC88A,
	DDX17, ENO1, SRGAP2C, VCAN, UBXN4, ACTB, EHBP1L1,
	SYNCRIP, TAGLN2, MALAT1, GNB1, FCGR2C, ACTR2, PTP4A2,
	EML4, C1orf43, PKN2, CD52, SPEN, WASF2, CXCR4, ACTB
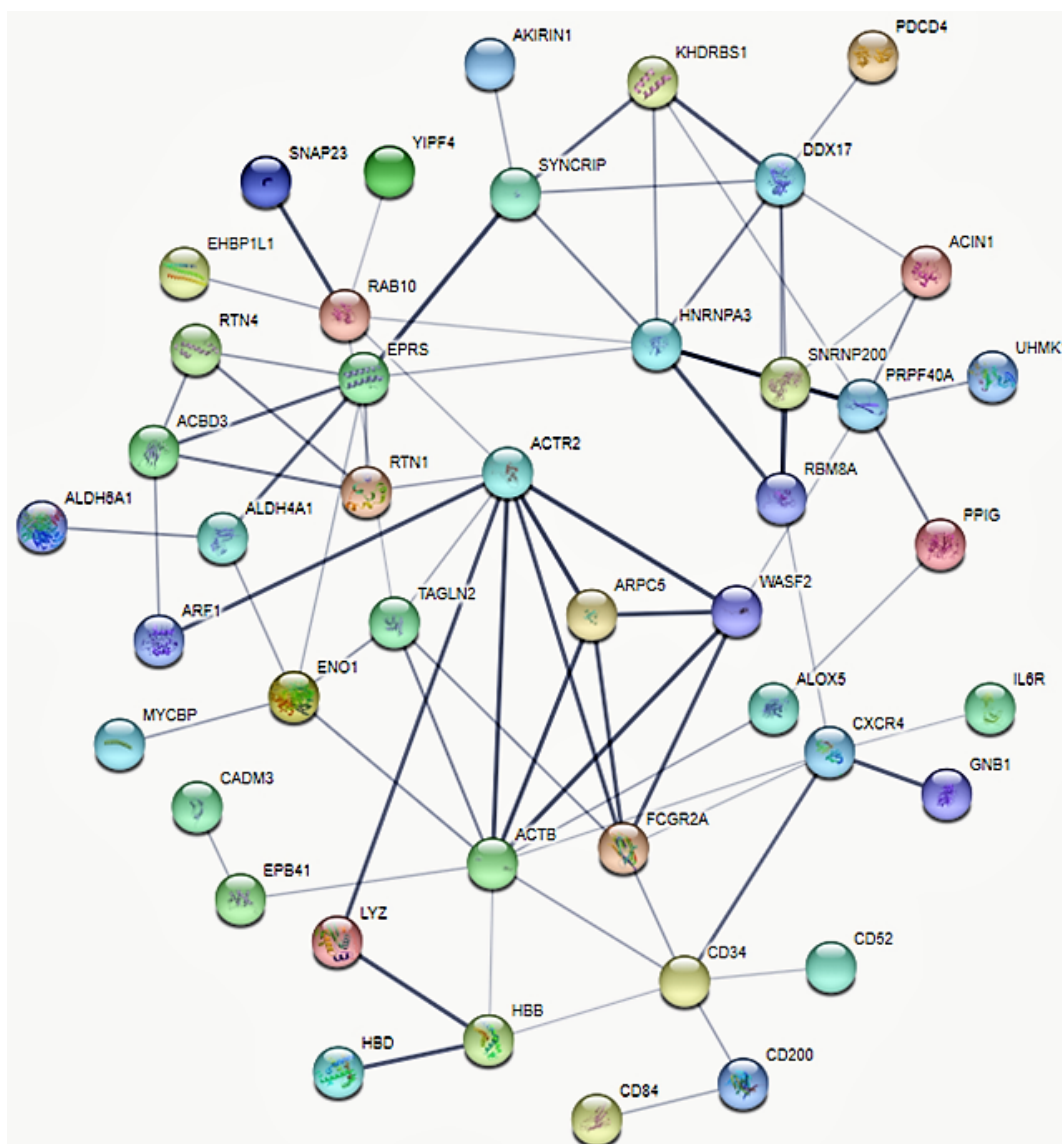
Fig. (3). The protein-protein interaction network performed by STRING software. Nodes represent proteins and an edge represents an association between proteins. The thickest edge indicates the highest confidence in protein-protein association. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

0098609), *peptide cross-linking* (GO:0018149), *cellular modified amino acid metabolic process* (GO:0006575), *regulation of IL-2 secretion* (GO:0032663), *neuron cell differentiation* (GO:0048667), *epidermal cell differentiation* (GO:0009913) and *mammary gland development* (GO:0030879) (Fig. **4A**).

3.4.2. Molecular Functions: Down-regulated DEHGs

The chief molecular functions associated with down-regulated genes were *oxygen transport activity* (GO: 0005344), *thiol-dependent ubiquitin-specific protease activity* (GO:0004843), *Ras GTPase binding* (GO:0017016), *Rho GTPase binding* (GO:0017048) and *enzyme binding* (GO:0019899) (Fig. **4A**).

3.4.3. Biological Process: Up-regulated DEHGs

The same way with biological functions of up-regulated genes were mainly enriched in *cell morphogenesis*

(GO:0000902), *regulation of catalytic activity* (GO:0043085), *regulation of gene expression* (GO:0010608), *cell proliferation* (GO:0008284), *neuron differentiation* (GO:0048667), *ubiquitin-protein ligase activity during mitotic cell cycle* (GO:0051437), *cell-cycle process* (GO:0022402) and *macromolecule catabolic process* (GO:0009057) (Fig. **4B**).

3.4.4. Molecular Functions: Down-regulated DEHGs

Immunoglobin binding (GO:0019865), *transcription factor activity* (GO:0003700), *enzyme inhibitor activity* (GO:0004857) and *GTPase activator activity* (GO:0005096) mainly participated in molecular function of up-regulated genes (Fig. **4B**).

4. PATHWAY ANALYSIS

Based on KEGG and REACTOME pathway analysis performed for the DEHGs data, the down-regulated genes were mainly involved in *megakaryocyte development* and
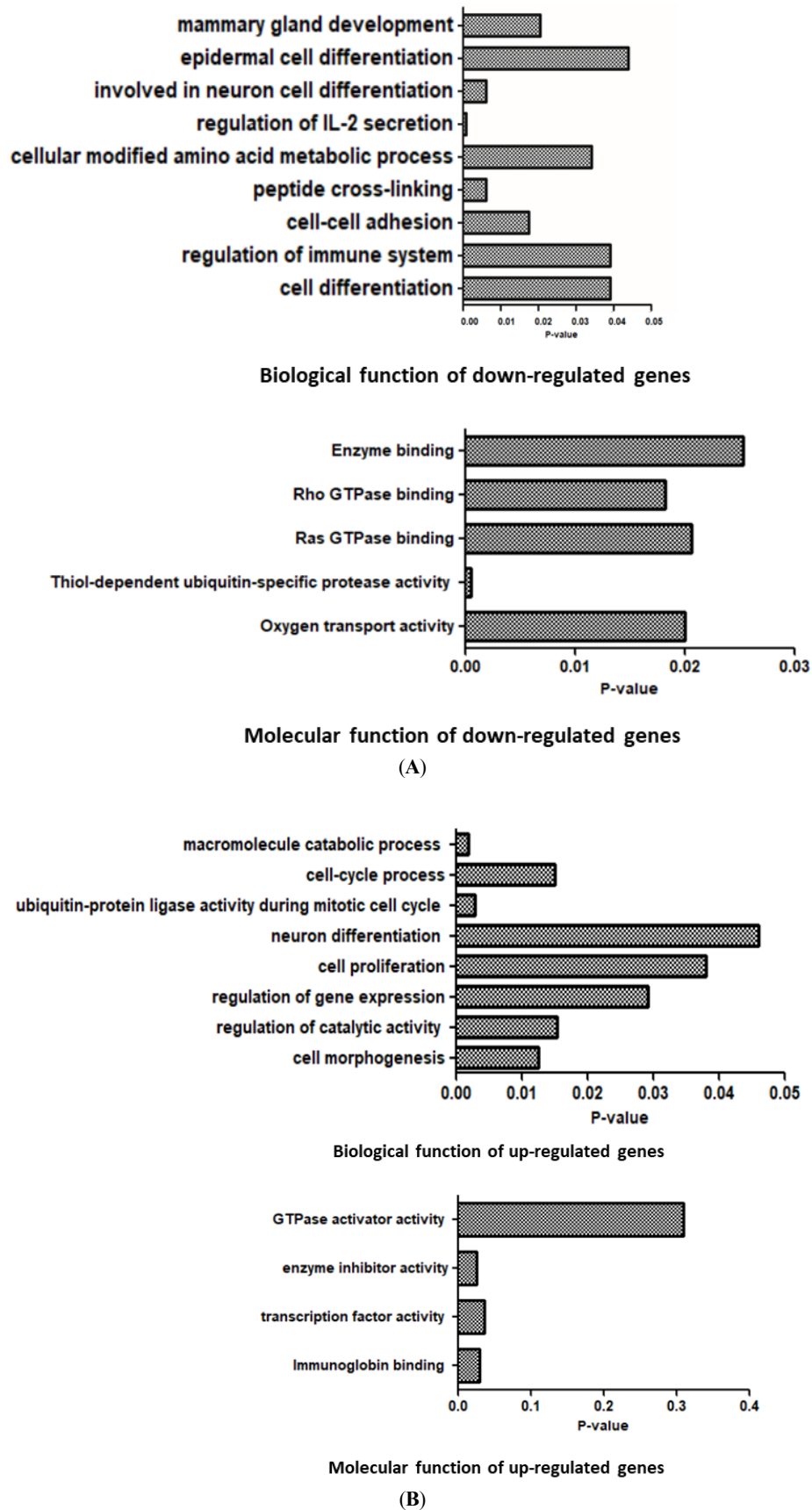
Fig. (4). (A) Functional enrichment analysis. Most significant biological processes and molecular functions of down-regulated genes. **(B)** Biological and Molecular function of up-regulated genes. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

platelet production, interferon gamma signaling, cell adhesion molecules, JAK-STAT signaling, Wnt signaling, Hedgehog signaling hematopoietic cell lineages and *PTEN regulation* (Fig. **5A**).

The up-regulated genes mainly participated in *Fc gamma R-mediated phagocytosis, MAPK family signaling cascades, Interleukin-12 signaling, Jak-STAT signaling after IL-12 stimulation, RHO GTPase signaling*, and *SLIT-ROBO signaling and deubiquitination* (Fig. **5B**) (Supplementary Table **3**).

5. PROTEIN-PROTEIN DOCKING

Protein-protein docking studies were planned with the objective of identifying molecular targets that could be employed therapeutically for rescuing the impact of under expressed hematopoiesis genes in the LBW newborns group. For these two molecules from the under expressed list of DEHGs, human LYZ and GBP1 proteins were selected based on their important role in immune system. Briefly, LYZ is associated with innate immunity (monocyte-macrophage system) which forms the first line of defense against microbial infection. It is an antimicrobial agent that breaks down the carbohydrates in bacterial cell walls [39]. Similarly, GBP1 exhibits antiviral activity and endorse oxidative killing and delivers antimicrobial peptides to autophagolysosomes, providing broad host protection against different pathogen classes. Guanine-nucleotide-binding protein involved in signaling pathways, such as Interferon gamma signaling, cytokine signaling and innate immune system [40]. Cytokine growth factor GM-CSF was chosen as a docking ligand as it is known to have growth-promoting activities on hematopoietic cell lineages. It induces differentiation of monocytes, macrophages, and granulocytes [41]. Molecular interaction study performed between the growth factor (GM-CSF) and human LYZ and GBP1 proteins using STRING database revealed that both the molecules were showing interaction with CSF2 (GM-CSF) based on functional annotation and immune system pathway (Supplementary Fig. **2**).

5.1. Protein-protein Docking Studies of GM-CSF and LYZ

Three-dimensional structure of LYZ protein (PDB id: -4I0C) was docked with GM-CSF (PDB id: -1CSG) by the help of FFT-based protein-protein docking using ClusPro server that is based on rigid-body docking (produce billions of conformation), root-mean-square deviation (RMSD) and refinement of selected structures using energy minimization. It provided two types of docking energies: the lowest energy among the conformations within a cluster of conformations, and the center energy of a cluster of conformations [35]. E-value was taken into consideration to represent protein-protein complex into the lowest energy state.

$$E = 0.40 Erep + -0.40 Eattr + 600 Eelec + 1.00 EDARS.$$

Eattr and Erep denote the attractive and repulsive contributions to the van der Waals interaction energy, Eelec and EDARS denote electrostatic energy and decoys as the reference state. An E-value score of the docked complex (LYZ: GM-CSF) was -826.5. Nine hydrogen bonds involved in this interaction are ARG^{23}-ASP^{49}, ARG^{24}-GLN^{58}, ARG^{62}-VAL^{116}, ARG^{62}-ILE^{117}, ARG^{23}-TYR^{63}, ARG^{98}-GLN^{11}, ASN^{17}-GLN^{104}, ARG^{113}-GLU^{21} and LYS^{72}-ASN^{114}. Interacting interface residues are essential for recognition and binding to other proteins. This small subset of essential residues

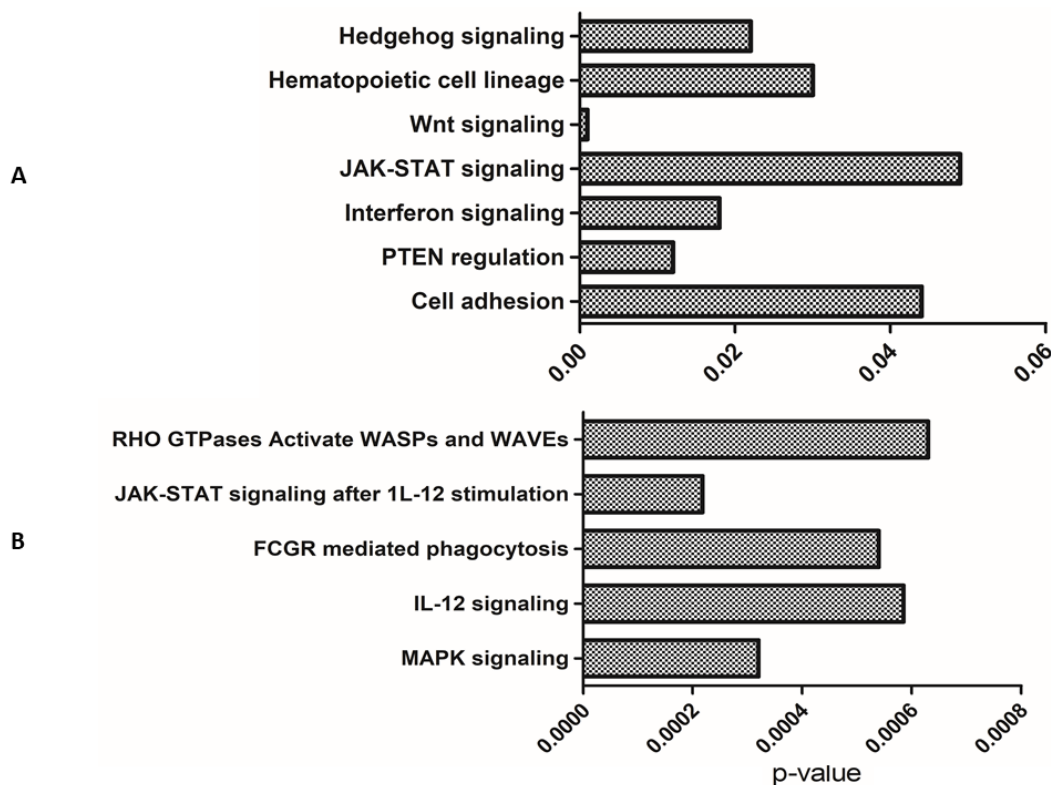**Fig. (5).** Pathway enrichment analysis represents the top most significant pathways. **(A)** Down-regulated pathways. **(B)** Up-regulated pathways. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

is referred to as "hotspots". Prediction of hotspots of dock complex identified GLU[35], ASP[49], ASP[53] and ASP[102] residues as interface residues of LYZ protein and GLU[21], ASP[112] and GLU[123] as interface residues of GM-CSF protein (Fig. **6A**). Hence, docking results suggested that GM-CSF bound strongly with LYZ receptor.

5.2. Protein-protein Docking Studies of GM-CSF and GBP1

We also performed protein-protein docking for GBP1 protein and GM-CSF. Five hydrogen bonds were involved in this interaction, such as GLN[36]-CYS[121], LYS[330]-GLU[14], ARG[23]-GLY[285], SER[5]-GLN[329] and ARG[24]-ASP[538]. Hotspots prediction of dock complex found GLU[323], GLU[533], GLU[536], ASP[538] and GLU[547] residues present as interface residues of GBP1 protein and GLU[14], GLU[21], ASP[48], ASP[120] and GLU[123] residues as interface residues of GM-CSF protein (Fig. **6B**). The lowest energy score of the most stable docked complex was -817.5. Thus, the data suggested that GM-CSF and GBP1 bound strongly with each other on the conserved domain.

6. TRAJECTORY ANALYSIS

Following the docking studies, the complex was further analyzed using MD simulation approach for more accuracy. RMSD value-based analyses of equilibration of MD trajectories and measurement of the backbone atoms of the complex system provide insights into the conformational stability. During the simulation, the LYZ: GM-CSF complex system reached a stable state after the initial fluctuation, there was a slight increase in the RMSD value to 0.57 nm and then down to 0.42 nm and finally reached 0.41 nm at 18 ns. After that, there were not many deviations in the RMSD value of GBP1: GM-CSF complex and it shows 0.37 nm at 18 ns. Hence, the RMSD value of both the docked complex was observed 0.4 nm and 0.3 nm at 18 ns (Fig. **7A**).

The RMSF per residue was calculated to determine higher flexibility in the complex system. High RMSF value indicates more flexibility, while low RMSF value indicates limited movements. The high RMSF value in GBP1: GM-CSF complex was more than 0.6nm and in case of LYZ: GM-CSF, higher fluctuation of residues was 0.4nm (Fig. **7B**). The analysis, when applied to the complex system, suggested that the following simulation docked complexes were relaxed and reached an equilibrium state.

7. DISCUSSION

In this study, the microarray datasets of the LBW-NBW newborns were compared with hematopoiesis-associated datasets to identify the DEHGs. Comparative microarray analysis revealed that 108 hematopoietic-associated genes were dysregulated in LBW newborns compared to NBW newborns. Of 108 differential genes, 79 genes were up-regulated, and 29 genes were down-regulated in LBW newborns compared to NBW newborns. Our results that hematopoiesis-associated genes are dysregulated in the LBW newborns suggests that this dysregulation could have the potential to contribute to their impaired hematopoietic status, especially pertaining to low WBCs counts and therefore need to be evaluated through *in-vitro/ in-vivo* lab studies.

To understand the signaling and interactive network in-depth, we analyzed the functional characterization of DEHGs which suggested that the down-regulated genes were mainly involved in *cell differentiation, immune system regulation* and *cell-cell adhesion.* The up-regulated genes were functionally enriched in *cell morphogenesis, cell proliferation* and *differentiation*.

The pathway enrichment analysis of down-regulated genes was majorly enriched in *Hedgehog signaling, Wnt signaling, JAK-STAT signaling, interferon gamma signaling* and *hematopoietic cell lineage.* Several studies suggest that the Hedgehog signaling pathway regulates hematopoietic stem cells' function [42]. Suppression of the signaling pathway has resulted in inhibition of hematopoietic stem cells proliferation [43]. Wnt family of signaling proteins are capable of controlling multiple stages of embryonic hematopoietic stem cell development [44]. Additionally, the JAK/STAT pathway is known to be involved in multiple effector mechanisms like the stimulation of cell proliferation, differentiation, migration, and apoptosis. These cellular events have a significant role in hematopoiesis and immune development [45, 46]. Therefore, down-regulation of the above pathways in LBW newborns could impact hematopoiesis.

The up-regulated genes were involved in a *MAPK signaling* pathway which plays a critical role in controlling the balance between cell expansion, cell survival and differentiation of hematopoietic stem cells [47]. *Fc gamma R-mediated phagocytosis* pathway is involved in the host-defense mechanism through the uptake, degranulation, antigen presentation, and destruction of infectious pathogens [48]. The pathway enrichment analysis also revealed *Rho GTPases signaling, SLIT-ROBO signaling, JAK-STAT signaling*, and *IL-12 signaling*. All of these are essential pathways involved in immune response, growth, and development. The study results, therefore, indicate the requirement of optimum activation of these pathways for normal hematopoiesis.

Protein-protein interaction study indicates that a regulatory relationship exists between up-regulated and down-regulated genes and that many of DEHGs are functionally related to each other in driving various pathways like *Fc gamma R-mediated phagocytosis, cytokine signaling pathway, immune system, RHO GTPase signaling,* and *neutrophil degranulation.*

Of the most significantly down-regulated genes in LBW newborns, such as LYZ, MYCBP, ATPIF1, GLI2, and GBP1, LYZ is a precursor of lysozyme C involved in bacteriolytic function that catalyzes the hydrolysis of (1->4)-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in peptidoglycan [39, 49]. It is also associated with the immune system as it enhances the activity of the monocyte-macrophage immune cells [49]. According to Ye *et al.* (2003), myeloid-specific lysozyme gene retains long-term, multilineage repopulation potential in hematopoietic stem cells, thus ruling out the possibility that expression of a lineage-affiliated marker gene necessarily commits them towards differentiation [50]. It also acts as an antimicrobial agent which breaks down the carbohydrates in bacterial cell walls killing the bacteria. It has been reported that runx1, c/ebp1 and pu1 transcription factor plays an essential role in myelopoiesis and these potential transcriptional regulatory elements bind to the LYZ promoter in zebrafish myelopoiesis [51].
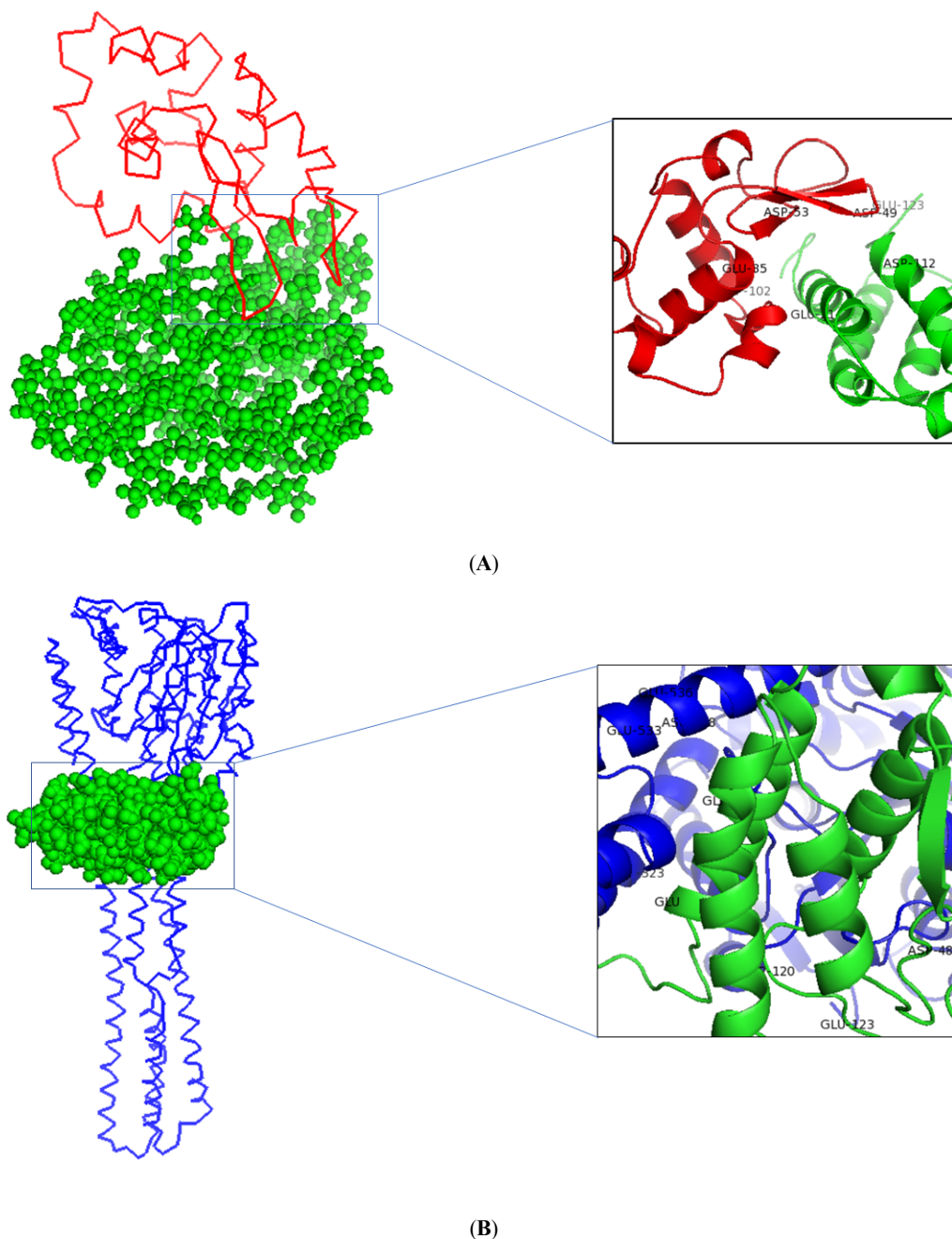
(A)

(B)

Fig. (6). (A) Structural model of the LYZ: GM-CSF complex. LYZ is in red, and GM-CSF is in green colour. Interacting amino acid residues at the binding interface are marked in red and green. **(B)** GBP1: GM-CSF complex visualized by PyMOL software. GBP1 is in blue, and GM-CSF is in green. Interacting amino acid residues at the binding interface are marked in blue and green. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Further, GBP1, a Guanine-nucleotide-binding protein, exhibits antiviral activity against influenza virus and also endorse oxidative killing and deliver antimicrobial peptides to autophagolysosomes, providing broad host protection against different pathogen classes. Guanine-nucleotide-binding protein is involved in signaling pathways, such as *Interferon gamma signaling* and other *immune system signaling pathways*. IFN-gamma has either a stimulating or inhibitory effect on hematopoietic specific lineages. It does not affect cell-cycle entry, differentiation, or apoptosis of hematopoietic stem cells but decreases the number of self-renewing cell divisions [52]. IFN- induced GBP1 acts as a

marker and intracellular regulator of the inhibition of proliferation, migration, and invasion of endothelial cells induced by several pro-inflammatory cytokines. Besides, GBP1 is actively secreted by endothelial cells and promotes oxidative killing and delivers antimicrobial peptides to autophagolysosomes [40]. Further studies suggested that GBP1 may serve as a target for mesenchymal stem cells homing [53] but the role in hematopoietic stem cells has not been mentioned yet.

MYCBP gene encoded a binding protein which binds to oncogenic protein c-Myc at N-terminus and activates the
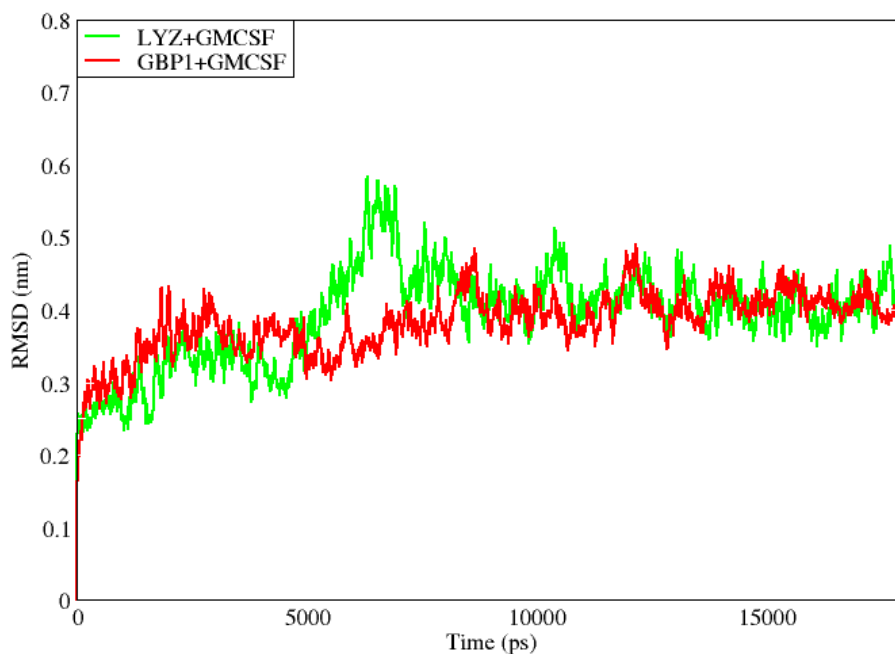
(A)
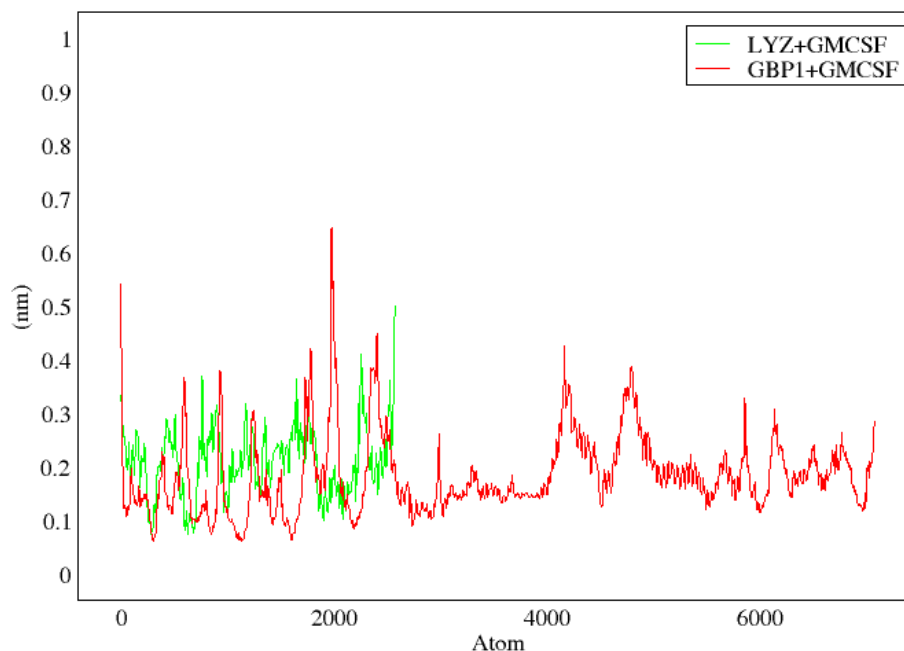
(B)

Fig. (7). Structural analysis of the MD simulation results. (**A**) Backbone RMSD of the docked complex. LYZ: GM-CSF is in green and GBP1: GM-CSF is in red colour. (**B**) The RMSF analysis of LYZ: GM-CSF and GBP1: GM-CSF complex. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

E-box-dependent transcription [54]. c-Myc regulates proliferation and differentiation in hematopoietic stem cells [55]. ATPIF1 gene reported as a regulator of energy metabolism and cell survival [56]. It is an inhibitor of ATP synthase and has an emerging context in metabolic reprogramming in cancer [57]. GLI2 acts as a transcriptional activator to provoke Hedgehog signaling in mice [58]. However, none of them has a role that is issued in hematopoietic stem cells.

We performed molecular docking of down-regulated genes encoded protein (LYZ and GBP1) with GM-CSF, a growth factor known to induce monocyte, macrophages and granulocytes cells. Docking suggested that the interaction surface of LYZ: GM-CSF complex and GBP1: GM-CSF complex was bound tightly and precisely. MD simulation analysis of the docked complex (LYZ: GM-CSF and GBP1: GM-CSF) suggested that the complex-system reached an

equilibrium state thus providing validation of docking results. Molecular docking and MD simulation results suggested that growth factor GM-CSF could stimulate the expression of LYZ and GBP1 proteins in impaired hematopoiesis of LBW newborns. Ongoing genetic and proteomic benchtop experiments in the lab would help further validate and evaluate these findings.

CONCLUSION

Having a compromised innate immune system, the mortality and morbidity rates in LBW newborns are higher than that in NBW newborns. Based on the results of this *in-silico* study an in-depth investigation of the impact of DEHGs on hematopoiesis status in LBW newborns has been initiated in the lab using cord blood-derived hematopoietic stem cells. We hope that the functional investigations and pathway analysis of the identified DEHGs would provide valuable information on the underlying mechanisms of increased susceptibility to infection in the term LBW newborns. Besides, therapeutic interventions targeting these dysregulated genes may be developed that can help rescue the repressed hematopoiesis in the term LBW newborns resulting in improved immune efficiency.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The following link has been created to allow review of record GSE29807, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE29807.

FUNDING

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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