A PREDICTIVE ANALYSIS TO EXPLORE COMMON KEY PATHWAYS BETWEEN APLASTIC'S ANEMIA AND FANCONI ANEMIA: A BIOINFORMATICS APPROACH

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This paperwork is submitted to the Deprtment of Software Engineering In qualify the prerequisites for the authorization of graduting B.Sc in Software Engineering

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APPROVAL

This thesis titled as “A PREDICTIVE ANALYSIS TO EXPLOR COMMON KEY PATHWAYS BETWEEN APLASTICS ANEMIA AND FANCONI ANEMIA: A BIOINFORMATICS APPROACH”, submitted by Sanjida Bijly, ID: 171-35-197 to software Engineering Department, Daffodil International University has been accepted qualify the prerequisistes for authorization of graduting B.Sc in Software Engineering and approved as to its style and contents.

BOARD OF EXAMINERS

DECLARATION

I am Sanjida Bijly do hereby declare that this report has been done by me under the supervision of Shariful Islam, Lecturer, Dept. of Software Engineering, Daffodil International University. I also declare that this report nor any part of this report has been submitted elsewhere for award of any degree.

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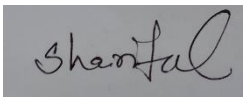

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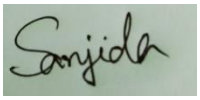

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I am only the front faces, but there are lots of guides behind me who are helping me in every steps. I am really thankfull to all.

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ABSTRACT

Aplastic's Anemia reduces the number of blood cells in the human body and is a serious disorder of the bone marrow. When oxygen-carrying tissue in the human body and blood clotting platelets, but sometimes bone marrow fail, one of the reasons for this is a genetic condition is called Fanconi Anemia. We collected all dataset from NCBI to identify common genes between Aplastic's Anemia (AA) and Fanconi Anemia (FA).We found 37 common genes by using VENNY online tools. We found Protein-Protein Interaction network from Cytoscape. We found three cluster network from Protein-Protein Interaction network. According to the degree score, we have identify 10 hub genes (DTL,BUB1,KIF14,FANCI,SKP2,PF4,PPBP,SPARC,ACTN1,RCHY1) from Protein-Protein Interaction network. These 10 hub genes are responsible for causing Aplastic's Anemia (AA) and Fanconi Anemia (FA) diseases. These all of hub genes are cured by applying drug it is possible to rid of these two diseases (Aplastic's Anemia and Fanconi Anemia) at the same time.

CHAPTER 01: INTRODUCTION

Aplastic's anemia is the formation of blood cells in the living body (especially in the bone marrow) failure, the number of blood cells very low, also it is fatal bone marrow distemper [1, 2]. Bone marrow transplantation or stem cell transplantation is a treatment used in some types of cancer particularly malignancies of the blood [1, 2]. Bone marrow is the soft, spongy area in the center of some larger bone of the body, which gives the scope to heal sick people [1, 2]. Paroxysmal Nocturnal Hemoglobinuria (PNH) [2]. A rare acquired, life-threating disease of the blood, the disease is characterized by destruction of red blood cells (hemolytic anemia), blood clots (thrombosis), and impaired bone marrow function (not making of the three blood component) , because the number of abnormal people in many patients with bone marrow failure has increased [2]. The studies have shown that the disease affects 1.4-14 people per million, which is much higher in Asian countries than in western countries and is more common in the third stage of human life [1].

Inside bones is a flexible, spongy network of connective tissue called bone marrow [3]. It makes oxygen-carrying red blood cells, disease-fighting white blood cells and blood clotting platelets, but sometimes bone marrow can fail, one of the reasons for this breakdown is a genetic condition is called Fanconi Anemia, also known as FA [3].The disease is rare an usually first diagnosed in children 2,5and 15, only 10% people that have it are diagnose in adulthood [3, 4]. Main and common syndromes of FA is abnormal genitalia Misshapen thumbs or forearms Short stature Small, or Misshapen, eyes Skeletal issue Smaller-than normal head, called micro cephalous patches of light-colored skin[3,4]. Fanconi Anemia occurs in about one per 130,000 births, with a higher frequency in

Ashkenazi Jews and Afrikaners in South Africa[5].FA is primarily an autosomal genetic disorder which means that two mutated alleles (one from each parent) are required to cause the disease, the risk is 25% that each subsequent child will have FA[4,5]. FA cases are X-linked recessive, which means that if the mother carries one mutated Fanconi anemia allele on one X chromosome, a 50% chance exists that male offspring will present Fanconi Anemia. In this analysis, we have identified the common genes between Aplastic's anemia and Fanconi Anemia where the gene similarities between the two diseases are 1.2%. From this common gene we have identified GO Analysis, Pathway Analysis, Protein-Protein Interaction (PPI) Network Analysis, Hub Gene, and Cluster Network.

1.1 Background

Aplastic's Anemia is a rare and severe disease. Every year many people die of this disease. A research of Montane, Eva Montane, Luisa Ibanez, Xavier Vidal, Elena Ballarina, Ramon Puig, Nuria Garcia, and Joan-Ramon Laporte studied the disease in a population in Barcelona. They described the Aplastic's Anemia rate is lower in Barcelona but the death rate is much higher. They point out there have been allegations of drug involvement with Aplastic's Anemia [17].

Fanconi Anemia is a rare disease. A research of E Gluckman and AD Auerbach studied they described that the disease is genetic disorder. Which is mainly related to genetic abnormalities, bone marrow failure and increased risk of cancer. They compared transplant regimens by including only patients treated with the regimen. They mentioned that people with fanconi Anemia died 30 years ago. They point out bone marrow transplantation is an effective treatment [18].

1.2 Motivation of the research

In the work we have seen with Aplastic's Anemia, they have mentioned the number of disease attacks in a particular place, the mortality rate, why this disease occur. But they did not diagnose the disease in different countries of world. They did not specify which genes were responsible for Aplastic's Anemia. They did not find the Protein-Protein Interaction network of Aplastic's Anemia and. They did not find hub genes and cluster network of Aplastics Anemia. [17]

In the work we have seen with Fanconi anemia, they have mentioned the number of disease attacks in a particular place, the mortality rate, why this disease occur. But they did not find diagnose the disease in different countries of world. They did not specify which genes were responsible for Fanconi Anemia. They did not find the Protein-Protein Interaction network of Fanconi Anemia. They did not find hub genes and cluster network of Fanconi Anemia. [18]

We have found so many scopes to work with by Aplastic's Anemia and Fanconi Anemia with best performance.

1.3 Problem Statement

After learning about the previous related works, we have seen some gaps which can be take us to a next level of our research. The problem statement from those papers are,

i. Did not work about Protein-Protein Interaction network.
ii. Did not work about Hub Genes.
iii. Did not work about Cluster Network.
iv. Did not work about GO terms.
v. Did not work about Pathway enrichment analysis.

1.4 Research Questions

The fundamental steps of research is to investigate some answerable queries of an issue. That is called research questions. The list is given bellow.

i. Why Protein-Protein Interaction network needed and how it worked?
ii. Why Hub Genes find out?
iii. Why Cluster network find out?
iv. Why need GO terms.
v. Why need pathways enrichment analysis.

1.5 Research Objectives

We have researched to find some objectives.

i. Find out Protein-Protein interaction network.
ii. Find out Hub genes.
iii. Find out Cluster network.
iv. Find out common genes between Aplastics Anemia and Fanconi Anemia.
v. Find out GO terms.
vi. Find out pathways enrichment analysis.

1.6 Research Scopes

Research scopes describes the area which the researchers have been analyzed. Here is our research scopes.

i. Common genes.
ii. Protein-Protein interaction network.
iii. Hub genes.
iv. Cluster network.

v. GO terms.

vi. Pathway enrichment analysis.

1.7 Thesis Organization

We have organized the whole paper in some sections. Where is the section 2, we have discussed about the previous related works. The research method applied is discussed in section 3. We explained how we have worked to get our final result. In section 4, we have explained our findings and final result. All the results have been explained in that section. Finally, we have concluded our work with the section 5. We gave a short brief there and also discuss about the future works that can be done from our research.

CHAPTER 02: LITERATURE REVIEW

In the previous work I seen both of Aplastic's Anemia and Fanoni Anemia, author only focus the number of disease attacks in a particular place, the mortality rate, why this diseases occur. But they did not diagnose this both diseases in different countries of world. They did not specify which genes were responsible for both diseases. They did not find out Protein-Protein Interaction network, Hub Gene, Cluster Network.

In my work, I find common genes between Aplastic's Anemia and Fanconi Anemia. I find Protein-Protein Interaction network from common genes. I find hub genes from Protein-Protein Interaction network. I find cluster network from Protein-Protein interaction network.

CHAPTER 03: RESEARCH METHODOLOGY

3.1 Identify differential expressed genes from microarray dataset

We obtained the gene expression microarray dataset GSE3807 (Aplastic's anemia) and GSE16334 (Fanconi anemia) from NCBI-GEO database [6]. NCBI provides a healthy information for medicine and biological sciences [6]. At first, P-value < 0.05 and logFC value (Is getter than 0.75 or is less than -0.75) was filter than using the Venny online tool, we found common gene between Aplastic's anemia (GSE3807) and Fanconi Anemia (GSE16334) [7]. Venny is a drawing tools for comparing up to four lists of elements at a time [7].

3.2 Identify ontological terms and pathway from Enrichment Analysis.

We accomplished enrichment analysis by Enricher and String to find out ontological analysis and pathway analysis of common gene. This enrichment method uses to identify significantly depleted groups of genes, this can be done by comparing the input gene set to each of the terms in the gene ontology [8]. Ontological analysis has three part: biological process, molecular function, and cellular component, [8]. P-value < 0.05 was selected for all the enrichment analysis. Also identify log10 absolute value.

3.3 Protein-Protein Interaction network analysis

In this study, we find the PPI network from the STRING [9] database .In molecular biology, STRING (Search tool for the retrieval of interacting Gene/Proteins) is a biological database of known and predicated Protein-Protein-Interaction.

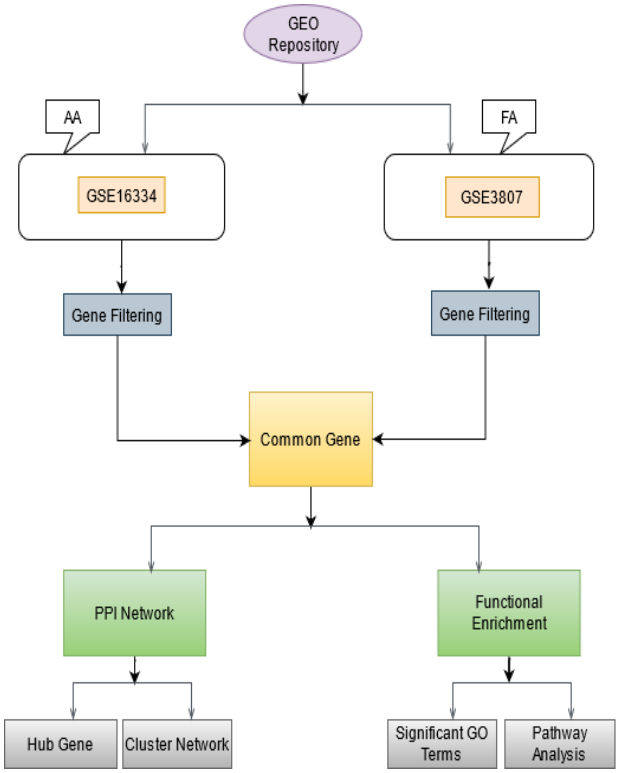

Fig 1: Flow chart of this analysis.

Figure 1. In this study, gene expression dataset of Aplastic's anemia and Fanconi anemia repository. The dataset were analyzed to identify common differentially expression gene between AA and FA. The significantly enriched pathways and Gene Ontology (GO) terms were identified enrichment analysis. Protein-Protein Interaction (PPI) was analyzed to identify hub gene and cluster network.

3.4 Identify hub genes and cluster network from Protein-Protein Interaction network

To identify hub protein and cluster network from Protein-Protein Interaction (PPI) analysis by using Cytoscape [10].hub gene is highly connected protein or gene in PPI network .Cluster network is the complex part of PPI network.

CHAPTER 04: RESULTS AND DISCUSSION

4.1 Identify Common Gene between Aplastic's Anemia and Fanconi Anemia:

In this Study, we Analyzed Aplastic's Anemia (AA) and Fanconi Anemia (FA) to identify common gene between AA and FA. In this analysis we have found similar expression of 37 common genes between AA and FA [Figure 2].

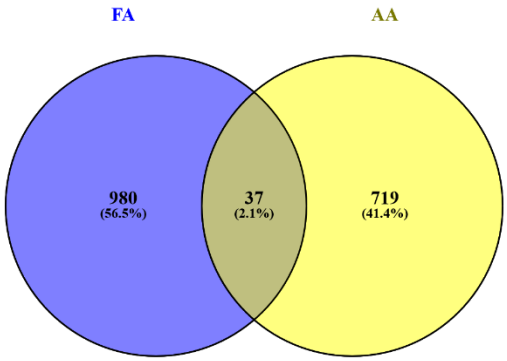

Fig 2: common genes between Aplastic's Anemia and Fanconi Anemia.

Figure 2: Common gene identification between Aplastic's Anemia and Fanconi Anemia. Find 37 common genes in between 756 of Aplastic's Anemia and 1017 of Fanconi Anemia.

We accomplished a gene set enrichment analysis to refine the biological significance pathways. The significant GO terms were biological process, molecular function, and cellular components [Table 1].The significant pathway is KEGG [Table 2].We find Log10 (P-value) both of GO terms and pathway.

Table 1. Gene ontological term analysis with their p-value.

Categories	P-value	term
BP	9.79E-05	platelet degranulation
	1.94E-04	regulated exocytosis
	2.75E-04	negative regulation of blood vessel morphogenesis
	3.29E-04	negative regulation of angiogenesis
	3.83E-04	regulation of angiogenesis
MF	0.001235389	CXCR chemokine receptor binding
	0.002929024	ubiquitin-protein transferase activity
	0.003632608	GTP binding
	0.003653035	chemokine activity
	0.004112314	purine ribonucleoside binding
CC	8.66E-06	platelet alpha granule lumen
	2.80E-05	platelet alpha granule
	3.18E-05	secretory granule lumen
	0.003717854	specific granule
	0.005244585	cullin-RING ubiquitin ligase complex

Table 2. Pathways enrichment analysis with KEGG.

categories	P-value	term
KEGG	0.002399397	Ubiquitin mediated proteolysis
	0.004973412	Fanconi anemia pathway
	0.01945621	Cytokine-cytokine receptor interaction
	0.024332249	Cell cycle
	0.027319817	FoxO signaling pathway

4.2 Identify Protein-Protein Interaction network from Enrichment analysis:

In this study, we find Protein-Protein Interaction network from enrichment analysis by using Cytoscape. This Protein-Protein Interaction network has 23 nodes and 27 edges [Figure 3].

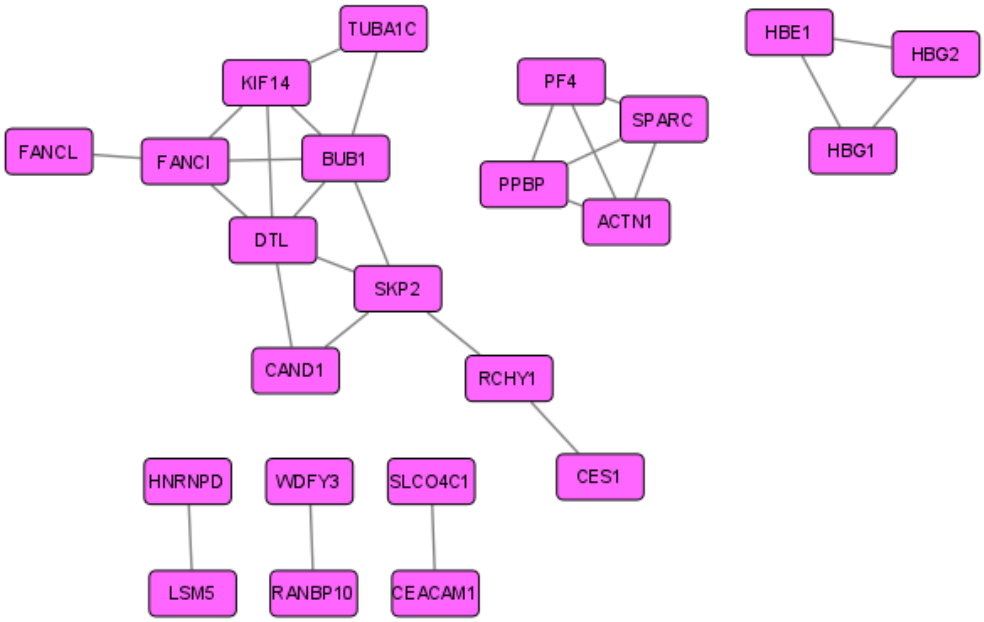

Fig 3. Protein-Protein Interaction network.

Figure 3: Protein-Protein Interaction network from enrichment analysis. All of pink color nodes are PPI genes. This Protein-Protein Interaction network has 23 nodes and 27 edges.

4.3 Identify hub genes and cluster networks from Protein-Protein Interaction network:

In this study, the degree has been selected to find hub genes from Protein-Protein Interaction network by using CytoHubba. Also selected top 10 hub genes, that will help get the drug. Top 10 hub genes are DTL,BUB1,KIF14,FANCI,SKP2,PF4,PPBP,SPARC,ACTN1,HBG2 [Figure 4], also there has 10 nodes an 15 edges. We find most complex cluster network from Protein-Protein Interaction network by using MCODE through Cytoscape. We find three cluster network from Protein-Protein Interaction network. First cluster network pathway is ACTN1-PPBR-SPARC-PF4 [Figure 5]. Second cluster network pathway is FANCI-DTL-BUB1-KIF14 [Figure 6]. Third cluster network pathway is HBE1-HBG2-HBG1 [Figure 7].

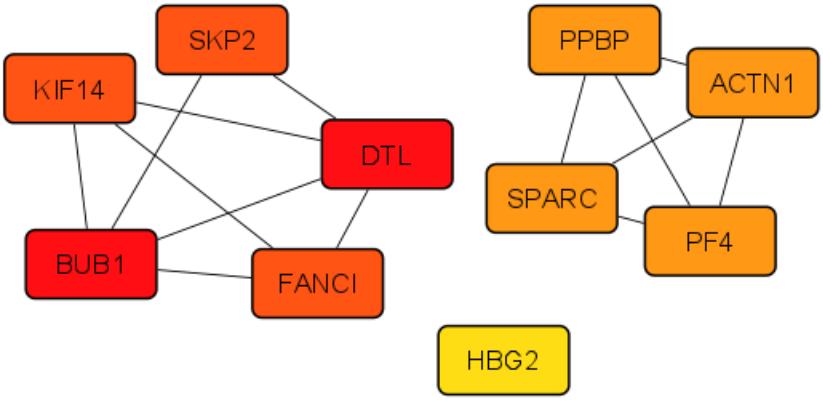

Fig 4. Hub genes from Protein-Protein Interaction network

Figure 4: Identify hub gene from Protein-Protein Interaction network. The highlighted nodes KIF14, SKP2, TL, BUB1, FANCI (red), PPBP, ACTN1, SPARC, PF4 (orange), HBG2 (yellow) are regarded as highly interconnected nodes, considered s hub nodes. The network is made up of 10 nodes and 15 edges.

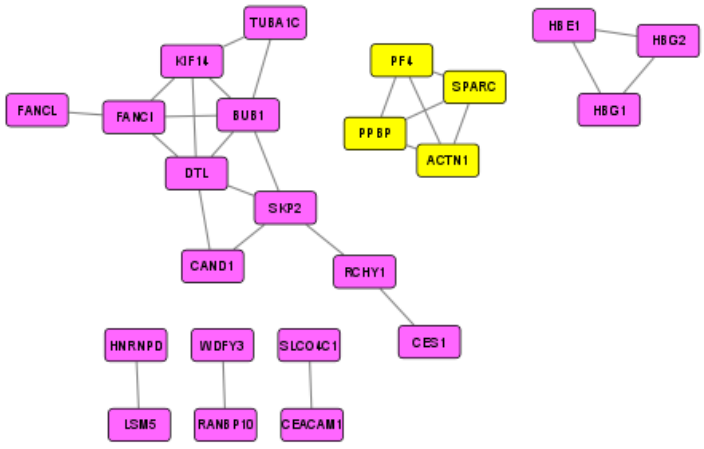

Fig 5. First Cluster Network from Protein-Protein Interaction network.

Figure 5: First cluster network from Protein-Protein Interaction network. All of yellow color nodes are cluster nodes. In this cluster network has 4 nodes and 6 edges.

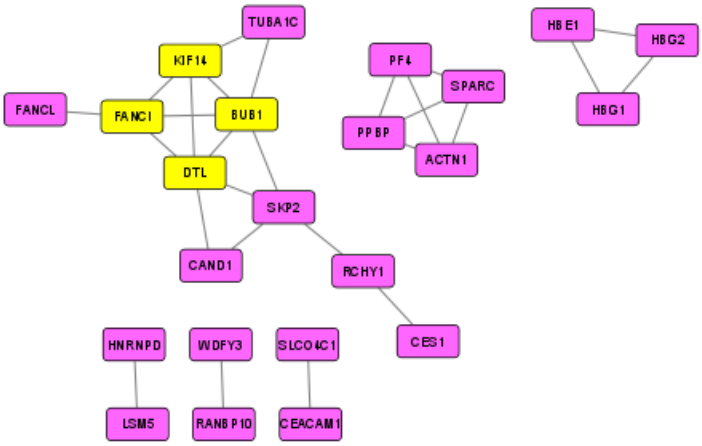

Fig 6. Second Cluster Network from Protein-Protein Interaction network.

Figure 6: Second cluster network from Protein-Protein Interaction network. All of yellow color nodes are cluster nodes. In this cluster network has 4 nodes and 6 edges.

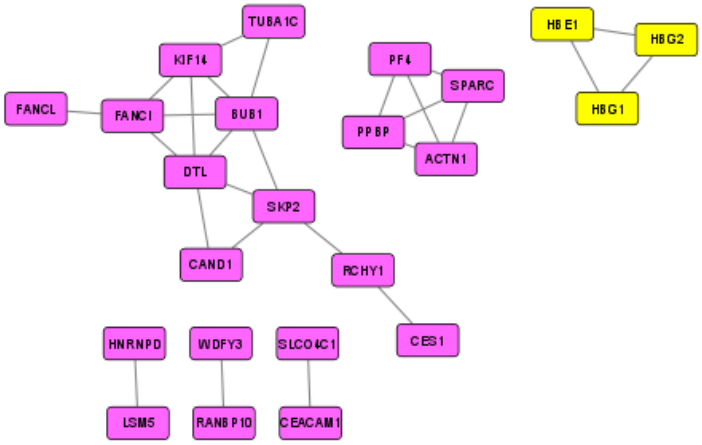

Fig 7. Third Cluster Network from Protein-Protein Interaction network.

Figure 7: Third cluster network from Protein-Protein Interaction network. All of yellow color nodes are cluster nodes. In this cluster network has 3 nodes and 3 edges.

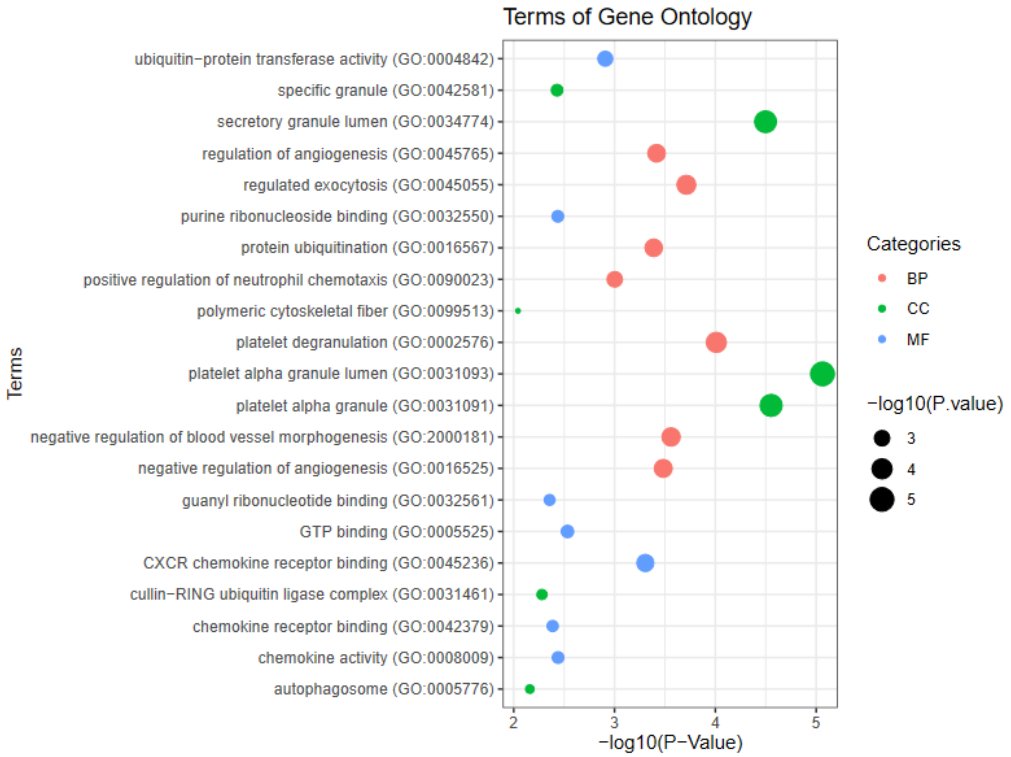

Fig 8: Bubble plot for GO terms.

Figure 8: Bubble plot for Go terms. GO terms has 3 categories. In this picture red color is BP, green color is CC and blue color is MF categories with –log10(p-value).

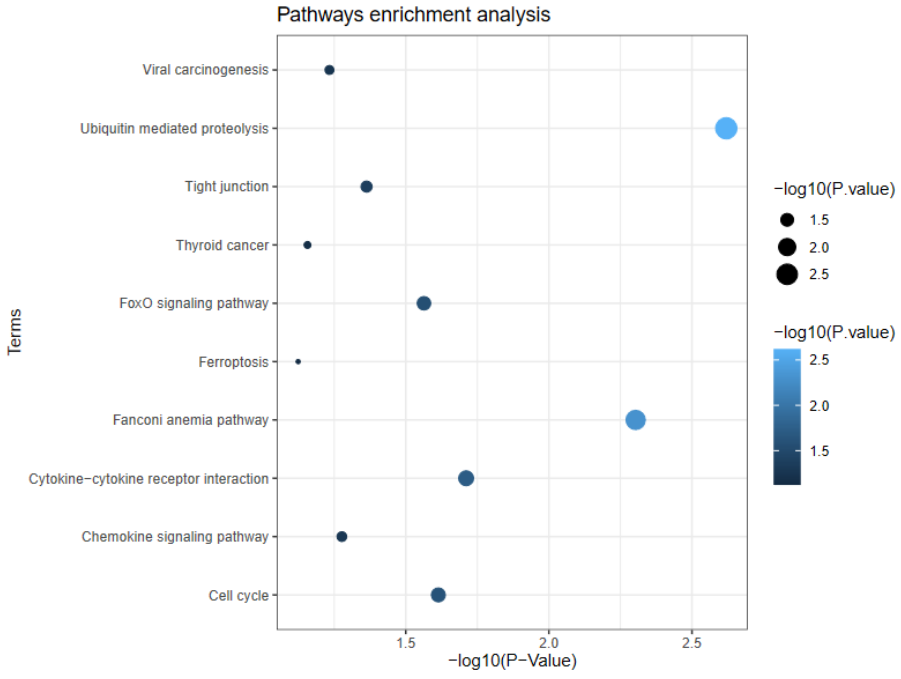

Fig 9: Bubble plot for pathways enrichment analysis.

Figure 9: Bubble plot for pathways enrichment analysis with –log10 (p-value). In this picture 10 KEGG terms define

4.4 Discussion

In this present study, we analyzed two diseases Aplastic's Anemia (AA) and Fanconi Anemia (FA) to identify common gene between them. We got 37 common genes or proteins between Aplastic's Anemia (AA) and Fanconi Anemia (FA).We Applied GO terms and Pathway enrichment to all common genes or proteins. GO analysis have three terms, Biological Process (BP), Cellular Component (CC), Molecular Function (MF).BP is the large process or biological programs accomplished by multiple molecular activities. Example of broad BP term is DNA repair. DNA repair is a collection of processes by which a cell identifies and corrects damage to the DNA molecules that encode it genome. In this present analysis, we got 445 BP terms, top 10 terms we selected for Bubble plot [Figure 8]. First BP term is Platelet Degranulation (GO: 0002576). Platelet Degranulation within a PTCA-damaged vessel would be increased by a nonionic contrast medium, releasing procoagulant molecules and platelet-derived growth factors into the damaged vessel lumen, which might contribute to acute thrombosis and the initiation of the restenosis process [11, 12].Cellular Component (CC) is the locations relative to cellular structures in which a gene product performs a function either cellular component. Unlike the other aspects of GO cellular component classes refer not to processes but rather a cellular anatomy. In this present analysis, we got 79 Cellular Component terms, top 10 terms we selected for Bubble Plot [Figure 8].First term of CC is Platelet Alpha Granule Lumen (GO: 0031093).Granule contents contribute to the propagation of platelet activation as well as

other processes such a coagulation, inflammation, angiogenesis, arteriosclerosis and wound healing Alpha-granules are major contributors to the protein component of the platelet released [13, 14]. Molecular Function (MF) terms describe the activities that occur the molecular level, such as catalysis or transport. GO molecular terms represent activities rather than the entities (molecules or complex) that perform the action and do not specify where when or what context the action takes place. In this analysis, we got 97 MF terms, top 10 terms we selected for Bubble Plot [Figure 8]. First MF term is CXCR Chemokine Receptor Binding (GO: 0045236). The N-terminal end of a chemokine receptor binds to chemokines and is important for ligand specificity-proteins couple to the C-terminal end ,which is important for receptor signaling following legend binding, Chemokine receptors belong to the G-protein-coupled seven-transmembrane receptor superfamily, which has more than 600 members that use the heterotrimeric G protein to initiate signals. Chemokines have been classified into four main subfamilies: CXC,CC,CX3C and C. All of these protein exert their biological effects by integrating with G protein-linked transmembrane receptors called chemokine receptors, that are selectively found on the surfaces of their target cells [15].In this present study, we analyzed KEGG pathway enrichment. KEGG is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies. In this analysis, we got 35 KEGG terms, top 10 terms selected for Bubble plot [Figure 9].

First term of KEGG is Ubiquitin Mediated Proteolysis. Ubiquitin Mediated Proteolysis is the process by which ubiquitin binds covalently to the target protein and degrades the target

protein. Proteasomes are larger complexes that perform crucial roles in many cellular pathways by degrading proteins in the cytosol and nucleus of eukaryotic cells to enforce quality control and regulate many basic cellular processes [16].In this analysis, we find three cluster network from Protein-Protein Interaction Analysis. First cluster network pathway is ACTN1-PPBR-SPARC-PF4 [Figure 5], also in this cluster network has 4 nodes and 6 edges. Second cluster network pathway is FANCI-DTL-BUB1-KIF14 [Figure 6], also in this cluster network has 4 nodes and 6 edges. Third cluster network is HBE1-HBG2-HBG1 [Figure 7], also in this cluster has 3 nodes and 3 edges. In this analysis, we got 10 hub genes from Protein-Protein Integration network by using CytoHubba [figure 3]. All of 10 hub genes are DTL,BUB1,KIF14,FANCI,SKP2,PF4,PPBP,SPARC,ACTN1,RCHY1 [figure 4]. This 10 hub genes or proteins ,the first 5 genes are more responsible for Aplastic's Anemia (AA) and Fanconi Anemia (FA).In additions to the first 5 genes ,subsequence genes also effect of causing Aplastic's Anemia and Fanconi Anemia diseases, but less than the first five. If these all of hub genes or proteins are cured by applying drug, it is possible to get rid of these two diseases (AA, FA) at the same time.

CHAPTER 05: CONCLUSIONS AND RECOMMENDATIONS

5.1 Findings and Contributions

In this investigation, we analyzed Aplastic's Anemia (AA) and Fanconi Anemia (FA) to identify common genes or protein. We found 37 common genes in this analysis. We applied GO terms and pathway enrichment all of 37 common genes or proteins. In this analysis, we found Protein-Protein Interaction network from enrichment analysis. We find two

Cluster network from Protein-Protein Interaction analysis. We found top 10 hub genes from Protein-Protein Interaction network by using CytoHubba. In this top 10 hub genes, top 5 genes are more responsible for Aplastic's Anemia (AA) and Fanconi Anemia (FA).If these all of hub genes or proteins are cured by applying drug, it is possible to get rid of these two diseases (Aplastic's Anemia and Fanconi Anemia) at the same time.

Recommendations for Future Works.

5.2 Recommendations for future Works

The researchers who want to work future, can work in the following sectors.

I have worked with common genes between Aplastic's Anemia and Fanconi Anemia, Protein-Protein Interaction network, hub genes, cluster network. But I can't find drug for this analysis.

Researcher can work with drug finding using others tools and database.

Reference

1. Young NS, Scheinberg P, Calado RT. Aplastic anemia. *Curr Opin Hematol*. 2008; 15(3):162-168. doi:10.1097/MOH.0b013e3282fa7470.
2. Young, Neal S., Acquired Aplastic Anemia, doi: 10.7326/0003-4819-136-7-200204020-00011.
3. Grover C. Bagby, Blanche P. Alter, Fanconi Anemia, doi.org/10.1053/j.seminhematol.2006.04.005
4. Jean Soulier; Fanconi Anemia. *Hematology Am Soc Hematol Educ Program* 2011; 2011 (1): 492–497. doi: https://doi.org/10.1182/asheducation-2011.1.492.

5. Rosenberg PS, Tamary H, Alter BP. How high are carrier frequencies of rare recessive syndromes? Contemporary estimates for Fanconi Anemia in the United States and Israel. *Am J Med Genet A*. 2011;155A(8):1877-1883. doi:10.1002/ajmg.a.34087.
6. Tanya Barrett, Stephen E. Wilhite, Pierre Ledoux, Carlos Evangelista, Irene F. Kim, Maxim Tomashevsky, Kimberly A. Marshall, Katherine H. Phillippy, Patti M. Sherman, Michelle Holko, Andrey Yefanov, Hyeseung Lee, Naigong Zhang, Cynthia L. Robertson, Nadezhda Serova, Sean Davis, Alexandra Soboleva, NCBI GEO: archive for functional genomics data sets—update, *Nucleic Acids Research*, Volume 41, Issue D1, 1 January 2013, Pages D991–D995, https://doi.org/10.1093/nar/gks1193.
7. L. Cong, Y. Zhu, G. Tu, A bioinformatic analysis of microRNAs role in osteoarthritis, Osteoarthritis and Cartilage, https://doi.org/10.1016/j.joca.2017.03.012.
8. Subramanian, Aravind, et al. "Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles." *Proceedings of the National Academy of Sciences* 102.43 (2005): 15545-15550, https://doi.org/10.1073/pnas.0506580102.
9. Damian Szklarczyk, John H Morris, Helen Cook, Michael Kuhn, Stefan Wyder, Milan Simonovic, Alberto Santos, Nadezhda T Doncheva, Alexander Roth, Peer Bork, Lars J. Jensen, Christian von Mering, The STRING database in 2017: quality-controlled protein–protein association networks, made broadly accessible,

Nucleic Acids Research, Volume 45, Issue D1, January 2017, Pages D362–D368, https://doi.org/10.1093/nar/gkw937.

10. Michael E. Smoot, Keiichiro Ono, Johannes Ruscheinski, Peng-Liang Wang, Trey Ideker, Cytoscape 2.8: new features for data integration and network visualization, *Bioinformatics*, Volume 27, Issue 3, 1 February 2011, Pages 431–432, https://doi.org/10.1093/bioinformatics/btq675.
11. David M. Dohan, Joseph Choukroun, Antoine Diss, Steve L. Dohan, Anthony J.J. Dohan, Jaafar Mouhyi, Bruno Gogly, Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet-related biologic features, Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, https://doi.org/10.1016/j.tripleo.2005.07.009.
12. Yujie Zheng, Samantha J. Montague, Yean J. Lim, Tao Xu, Tienan Xu, Elizabeth E. Gardiner, Woei Ming Lee, Label-free multimodal quantitative imaging flow assay for intrathrombus formation in vitro, Biophysical Journal, 10.1016/j.bpj.2021.01.015, **120**, 5, (791-804), (2021).
13. Hezder van Nispen tot Pannerden, Felix de Haas, Willie Geerts, George Posthuma, Suzanne van Dijk, Harry F. G. Heijnen; The platelet interior revisited: electron tomography reveals tubular α-granule subtypes. *Blood* 2010; 116 (7): 1147–1156. doi: https://doi.org/10.1182/blood-2010-02-268680.
14. Blair P, Flaumenhaft R. Platelet alpha-granules: basic biology and clinical correlates. *Blood Rev*. 2009;23(4):177-189. doi:10.1016/j.blre.2009.04.001.
15. Hughes CE, Nibbs RJB. A guide to chemokines and their receptors. *FEBS J*. 2018;285(16):2944-2971. doi:10.1111/febs.14466.

16. Ciechanover A, Orian A, Schwartz AL. Ubiquitin-mediated proteolysis: biological regulation via destruction. Bioessays. 2000 May; 22 (5):442-51. doi: 10.1002/ (SICI) 1521-1878(200005)22:5<442::AID-BIES6>3.0.CO;2-Q. PMID: 10797484.
17. Montané, Eva, et al. "Epidemiology of aplastic anemia: a prospective multicenter study." *haematologica* 93.4 (2008): 518-523.
18. Gluckman, Eliane, et al. "Bone marrow transplantation for Fanconi anemia." (1995): 2856-2862.